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Present Concepts of Menstrual Physiology: A Critical Appraisal

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In the last decade, general descriptions of normal and abnormal menstrual physiology have emphasized a very few highly selected and seemingly uncontroversial observations, and have assembled on this foundation an oversimplified, mechanistic concept which ignores many fundamental problems and debatable issues. The acceptance of such glib hypotheses has been furthered by attractive schematic illustrations (all remarkably similar) of pituitary-gonadal-uterine relationships which pepper textbooks and commercial publications. The fact that these drawings have changed relatively little during the last ten years or so seems to have attracted no attention.

Much of this tendency toward oversimplification can be traced back to the fundamental work of Markee and his group on intraocular endometrial transplants. The classical simplicity of these experiments has encouraged the unwary to extract the carefully restricted findings bodily from their experimental setting and to apply them, unchanged and sometimes dogmatically, to women troubled by functional menstrual disturbances. The fallacy of such a procedure becomes obvious with its statement. It would seem timely, on this occasion, to recall just a few of the facts generally forgotten or ignored, but nevertheless highly important to an understanding of the pituitary-gonadal-uterine relationship.

RELEVANCE OF ANIMAL EXPERIMENTS

Perhaps it seems trite to reiterate the point that a great deal of our knowl-

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edge concerning such physiology is based on animal experimentation, and that we very often overlook the fact that observations in animals cannot be translated unreservedly into human terms. Nowhere in physiology is there a mechanism more complex and variable from species to species in its finer details than the reproductive process. If we reflect for a moment on the relative simplicity of the pituitary-thyroid or the pituitary-adrenal axis, both of which have been studied successfully in animals and in man, and then consider the pituitary-gonadal-uterine mechanism, it is apparent that we have here a problem of vastly greater magnitude and complexity.

One of the outstanding tasks of future menstrual research will be to re-examine our so-called facts and to separate those which are pertinent only to animal physiology from those which do in fact apply to the human being. *Only in the past few years has there been an increasing tendency for courageous* workers to apply what are still considered daring technics to these questions as they exist in the human. The greater availability of patients who have been adrenalectomized or hypophysectomized, and the access to institutionalized patients who have been castrated in their reproductive years, makes clinical research much simpler than it was a decade ago.

VALIDITY OF MATERIALS AND METHODS

Another fundamental question which must be raised is the validity of the materials and methods that have been used to study the production, influence, and fate of the various hormones implicated in menstrual physiology. After all, the understanding of any given process cannot be more reliable than the technics by which it is measured. One insuperable obstacle has been the lack of pure human pituitary gonadotrophins; it is most unfortunate that recent studies with gonadotrophins from menopausal urine[12] have failed to stimulate the commercial manufacture of such material. With products of animal origin there are major difficulties in terms of foreign protein reactions, antigonadotrophins, and so on. There are, however, several rays of hope. The concerted attack on the chemical nature of ACTH has yielded much information of more general scope and applicability. Furthermore, in 1954 two pituitary hormones were specifically characterized as to their chemical structure, and have turned out to be relatively simple octapeptides.[27, 28] Newer preparations of ACTH also show low molecular weights, and similar success with gonadotrophins could be anticipated if an equal effort were to be expended in that direction.

Gonadotrophins

The availability of such gonadotrophic material, however, would be only a partial solution, as it has been amply shown that the effects of experimentally administered gonadotrophins depend not only on the type and amount given, but also on the past history and priming of the responding tissue, the presence or absence of other gonadotrophins, the quantitative ratios of one gonadotrophin to the other, and the sequence in which they act on the organism. In fact, the difficulties of obtaining from such experiments data which reflect truly physiologic events in the human are staggering.

The assay of urinary gonadotrophins, as performed with current technics, is extremely unsatisfactory in both its qualitative and quantitative aspects. Without a detailed discussion of the technicalities involved, it may be worthwhile simply to recall the experiment of Greep and his coworkers in which a castrate animal was joined in parabiosis to a hypophysectomized male on one side and to a hypophysectomized female on the other. The pituitary of the castrate animal obviously stimulated both sets of gonads equally, yet the ovaries showed changes indicative only of F.S.H. activity, whereas the testes showed evidence of response to both F.S.H. and I.C.S.H. Such findings cast serious doubt on the validity of many data which are part of the basis of our concepts of menstrual physiology. Important improvements in technic, such as recent work on the chromatographic separation of urinary gonadotrophins on calcium phosphate columns,[3] lends hope that it may be possible to separate and characterize urinary gonadotrophins with greater accuracy in the future. In addition, Astwood has pointed out the feasibility of developing an assay for luteotrophin based on histologic and chemical changes in suitably prepared corpora lutea, in a manner similar to standard ACTH assay procedures (which use the adrenal cortex and are based on adrenal ascorbic acid or adrenal cholesterol depletion).

Steroid Hormones

An entirely new field has been opened with the finding that gonadotrophin production may not be regulated exclusively by the ovarian steroid hormones. Some time ago, Sohval reported an increase in urinary gonadotrophin excretion in patients under treatment with cortisone or ACTH; this most important observation was verified by McGavack and Goldzieher during the course of metabolic balance studies on hydrocortisone and corticosterone as well as with cortisone itself. The implications of these observations are considerable, but apparently no further work has been done along these lines. It is also possible that adrenal steroid hormones play a "permissive" role in the function of the pituitary-gonadal axis, just as they do in so many other fields of physiology. There is already evidence that the presence of adrenocortical hormones is necessary for the occurrence of the ovarian hyperemia which follows the injection of chorionic gonadotrophin.[23]

Estrogens and Progestogens. On inspecting the production and metabolism of the steroid hormones, the estrogens and progestogens, the problem might seem easy in comparison to the gonadotrophins, but unfortunately such is not the case. Earlier work with estrogens was done with bioassay procedures which biometricians have since shown to be woefully inadequate. Many of the allegedly significant changes in blood estrogen levels or in urinary estrogen excretion will not stand the test of modern statistical evaluation.

Even the best bioassay procedures have grave disadvantages. In addition to the large investment in animals and time required for results of even a low order of accuracy, these technics are highly subject to the influence of uncontrolled or unknown variables. To illustrate, there is the report of Bates and Cohen[22] of a nonsteroid urinary augmentor which increases the biologic estrogen effect of products eliminated in the urine, to far above their equivalent in terms of chemically measured steroid estrogen. For such reasons, it is axiomatic that we try to replace bioassay methods by chemical or physical technics wherever possible. However, at the steroid meeting of the Laurentian Hormone Conference in May, 1953, it was the consensus that no contemporary chemical method is adequate for the separation and determination of estrogens in urine, to say nothing of estrogens in blood. All the previous procedures, with the possible exception of Engel's countercurrent distribution[5] and fluorescence method, are open to serious technical objections.

It is very fortunate that two relatively simple methods, capable of separating and measuring the urinary estrogens at levels as low as 8 μg. per day with a precision of 10–15 per cent, are shortly to be published.[20] This would be a considerable improvement in simplicity and sensitivity over Engel's method, which is reliable only with aliquots of urine containing about 100 μg. of estrogen. It appears, therefore, that we may soon have the tools to re-evaluate all the data on estrogen excretion and its relation to gonadal function and the menstrual cycle. One of the foremost problems in this field will be to check, by methods which are technically beyond reproach, the theories of Smith and Smith regarding the influence of progesterone on estrogen metabolism.

One further complication must be emphasized. It is known that only about 10 per cent of the metabolites of estrogen appear in the urine, and that a far larger proportion, perhaps 60 per cent, is excreted (via the enterohepatic circulation) in the feces. Possibly we have been examining the wrong excretory channel in past studies on estrogen.

Hooker-Forbes Bioassay and New Technics. The development of the Hooker-Forbes bioassay for the measurement of progesterone has been a great forward step in our approach to this aspect of menstrual physiology. In view of the previous remarks on bioassay procedures generally, it is no surprise that there are now appearing in the literature certain studies which question the specificity of the Hooker-Forbes procedure, and others which indicate that its quantitative aspect may be significantly influenced by synergism or antagonism of other steroid hormones.[21, 34] Pearlman has recently pointed out the large discrepancy between chemical and bioassay measurement of progesterone levels. Workers in England are about to publish a new technic which is reliable even at the low pregnandiol concentrations found in normal male urine as a product of adrenal steroid metabolism. Such procedures promise much useful and precise information regarding the luteal phase of menstrual physiology.

Finally, one must mention the important fact that pregnandiol is not the only excretory product of progesterone, although it is true that there is about five times as much pregnandiol-3β, 20β as there is of the next most important metabolite (in terms of quantity) of the other three identified so far. In fact, it has been shown[10] that these breakdown products of progesterone exert a significant influence on some of the actions of progesterone itself; this would indicate that pregnandiol, the pregnanolones, and pregnanetriol may possibly play a role in normal luteal physiology.

These remarks are not intended to imply that all the past work which has been done on steroid hormone levels is simply to be discarded. Quite to the contrary: with these new technics it may now be possible to explain, for example, the apparent contradictions between Markee's studies on the influence of the estrogen level and its fluctuation on intraocular endometrial transplants, and certain problems of in vivo endometrial bleeding. These contradictory facts may be summarized as follows:

1. *Studies of estrogens at low levels.* The investigations of Zuckerman and others[14, 35] have shown that monkeys maintained on constant low levels of estrogen show intermittent and perhaps cyclic bleeding. The proponents of Markee's theory attempted to explain this phenomenon with the suggestion that the adrenals of these castrated animals produced estrogen and that this output fluctuated enough to meet Markee's postulates. However, Zuckerman has shown that this intermittent bleeding also occurs in hypophysectomized or adrenalectomized animals. Furthermore, Phelps,[25] in 1946, showed that, in castrated monkeys treated with estrogen and progesterone, there was no relationship between the height or fluctuation of the estrogen level and the menstrual response. Similar observations on intermittent bleeding at low estrogen levels have been made in humans as well.[30]

2. *High estrogen levels.* These can be observed clinically in children or in postmenopausal women with granulosa cell tumors. Such patients bleed more or less intermittently. While it is entirely possible that fluctuations in the estrogen output of the tumor do occur at these high levels, a sudden drop of 50 per cent or more would seem to be unlikely. However, this is mere speculation, and there is less speculative evidence. Zondek, for example, implanted estrogen pellets in amenorrheic females and produced intermittent bleeding. Finally, Zuckerman has observed intermittent bleeding in castrate monkeys maintained on high estrogen levels. This author has also pointed out that the withdrawal of progesterone is an important but often neglected part of the process of endometrial regression, and Krohn has been able to induce and maintain cyclic bleeding in amenorrheic[15, 16] or freshly castrated[17] monkeys by the administration of small amounts of progesterone alone.

While on the subject of endometrial regression, it may be timely to call attention to the work of Phelps.[26] It was shown that the steroid therapy given to a castrate monkey in one cycle exerts a profound effect on menstrual bleeding in the *subsequent* cycle or cycles. Not only must the presence or absence of progesterone be considered, but changes in the dosage level of administered estrogen and progesterone also influence the response for two or more cycles. Confirmatory observations appear in the studies of Brewer and Jones. It follows from these experiments that changes in the endometrium persisting beyond menstruation must have taken place. The implication of these findings in judging the validity of many clinical studies is quite obvious.

Thyroid

There has been an enormous amount of speculation and amazingly little controlled study of the role of the thyroid in menstruation. Experimentally, it has been shown that thyroidectomy decreases corpus luteum formation in rats;[7] in rabbits thyroidectomy decreases production of follicles.[4] Most other studies are rendered invalid by the fact that animals with intact thyroids and pituitaries were used. These glands, responding as they do to exogenous thyroid administration, introduce a factor which cannot be measured or controlled satisfactorily. The thyrogonadal axis is indeed a fertile field for investigation, particularly by the use of hypophysectomized-thyroidectomized monkeys with and without castration. The meager investigations just mentioned do not in any way detract from numerous clinical observations on the efficacy of thyroid administration in menstrual disorders and sterility. Such observations are, however, frequently and incorrectly used as data in discussions of thyro-ovarian interrelationships. The fallacy here is twofold: first, the diagnosis of hypothyroidism in such cases usually rests on the most inadequate criteria imaginable, often no more than a determination of the basal metabolic rate; and second, the belief that the alleged effectiveness of thyroid administration implies the existence of a hypothyroid state.

It is hardly necessary to reiterate the fact that a low basal metabolic rate is no proof of thyroid hypofunction. It requires more information, both clinical and biochemical, before the level of thyroid activity can be evaluated. In addition to a specifically oriented history and physical examination, a spectrum of tests—including serum cholesterol, galactose tolerance, protein-bound iodine, and some measure of radioactive-iodine metabolism—is needed for adequate clinical research in this problem. By these criteria, there are few studies in the literature where clinical evaluation of the thyrogonadal axis has been adequate.

A most important point, and one that is often forgotten, is that the administration of thyroid substance does not necessarily imply substitution therapy. After all, cortisone in the treatment of asthma or insulin in the treatment of schizophrenia does not imply deficiency of the adrenals or the pancreas. In the same sense, thyroxin (taking into account the normal compensatory mechanism of the body) may exert pharmacologic effects on the reproductive tract of which we are not aware.

Neural Factors

Finally, it may be timely to mention the importance of neural factors in the menstrual process. Everett, Sawyer, and their group, using cyclic rats, have made many interesting observations on the hypothalamus and the chain of events which are involved in the release of gonadotrophins and the timing of ovulation. Whether or not these findings apply in the human remains to be demonstrated; to date, none of the drugs acting on the hypothalamus have been shown to exert consistent effects on ovulation or hormone production in man.

Psychogenic Factors

Psychogenic amenorrhea following anxiety, emotional crisis, or other stresses is a very common phenomenon, but one which has received relatively little endocrine appraisal. Klinefelter and coworkers described 4 cases in which normal "F.S.H. excretion" and "hypoestrinism" were found. These results must be regarded with considerable reservation in view of the assay technics on which these findings were based. Nevertheless, it was postulated that psychogenic influences blocked completely the hypothalamic stimuli of L.H. release. In the absence of L.H., the "pure" F.S.H. of the pituitary could not cause estrogen secretion, hence the hypoestrinism in the presence of normal F.S.H. titers. One might consider this a rather substantial theory to erect on such slim evidence. As a matter of fact, bilateral hypothalamic lesions abolish only cyclic L.H. discharge, but permit the constant secretion of moderate amounts of both F.S.H. and L.H.[9] Moreover, this theory provides no explanation for certain instances of amenorrhea following wartime conditions or other severe psychic stresses,[8, 11, 29] in which large amounts of steroid hormones failed to produce uterine withdrawal bleeding. The elucidation of this phenomenon awaits further investigation; only a few pertinent facts are known at this time.

Szego and Roberts have shown that a combination of stress and the injection of ACTH decrease the uterine response to estrogen. Another lead may have been provided by the studies in 2 such cases of hormone-resistant amenorrhea.[8] During the amenorrheic phase, no detectable urinary excretion followed a test injection of a large amount of estrone. At a subsequent time, when the psychologic situation had resolved itself and the patient had developed cyclic, ovulatory menses spontaneously, a similar test dose produced the usual level of urinary excretion. Interestingly enough, when the psychologic situation deteriorated once more and amenorrhea developed, one could no longer detect urinary excretory products of injected estrone. While this isolated observation must be regarded with the greatest caution, it suggests the possibility that neurologic influences may play a role in the hepatic or possibly other intermediate metabolism of steroid hormones. These hypothalamic and other neural influences are among the most important, most unexplored, and most fascinating problems in the study of menstrual physiology today.

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DISCUSSION

DR. J. T. VELARDO, *Harvard Medical School, Boston, Mass.:* Dr. Goldzieher has summarized many important facts for us. One very important area of investigation, however, has escaped his attention, and this perhaps might well be due to the vast amount of data encompassed in his report. I should like to call attention to the enzyme studies performed by Bener, Velardo and Hisaw; these show a very definite influence of estradiol-17β and progesterone on the lactic dehydrogenase diphosphopyridinenucleotide system in the uteri of a closely inbred strain of albino rats. This, we believe, is the newest approach that has been focused on the way exogenously administered hormones effect very important changes in enzyme systems in the endometrium and myometrium. These studies were made by biologists and it is quite conceivable that this basic research has paved the road in both facts and experimental methodology so that research on primates and on the human female could be effected with facility and dispatch.

My second thought is in reference to the complex role of the way in which estradiol-17β, estrone, and estriol work in concert to produce what is known as an estrogenic (or proliferative effect). On the progestational side, there are further complexities in the complex interactions of progesterone, pregnane-3α,20α-diol and its isomers as well as related pregnanes. It might be quite helpful to think of these reactions during the menstrual cycle in terms of the hormones-enzymes pathways, and in so doing we must dissuade ourselves from thinking that estradiol and progesterone are the only two available drugs in the clinicians' armamentaria. We must look ahead, and examine the effects of certain metabolites of estradiol-17β and progesterone. It might be likened to the administration of predigested hormones.

These facts when put all together might give us more of an insight into the more direct action of the various endometrial counterparts which participate in the phenomenon of menstruation.

Some Aspects of the Chemical Nature of Human Ovarian Follicular Fluid

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IT WAS NOTED by Kurzrok *et al.*, and Farris and his co-workers that human ovarian follicular fluid increased the speed of spermatozoa. Stimulated by these observations, we have recently begun an evaluation of some of the chemical and biologic properties of this material. Fifteen specimens were collected by us, or for us by cooperating gynecologists,* from various types of ovarian cysts or normally developing follicles. When the volume of aspirated fluid was sufficient, pregnancy tests were performed. In a few instances, enough fluid was available to determine the content of estrogens, gonadotrophins, and 17-ketosteroids. The concentration of proteins and electrophoretic components was ascertained in 13 specimens, and it is these data which constitute the body of this report. In addition, a few preliminary comments will be made concerning the nature of the pigment which lends color to ovarian fluid.

METHOD

Total proteins were determined by the Kjeldahl semimicro technic and a method recently described by Lowry. Samples of 0.01 and 0.005 cc. were

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taken for filter paper electrophoresis as described by Kunkel and Tiselius, and the protein was developed on the paper with bromphenol blue after 18 hours, as suggested by Durrum. The developed paper was dried and read in a photovolt densitometer at 0.5 cm. intervals from the origin, and the optical density was plotted against distance. From the plot, the areas of each protein were measured with a planimeter and the percentage of

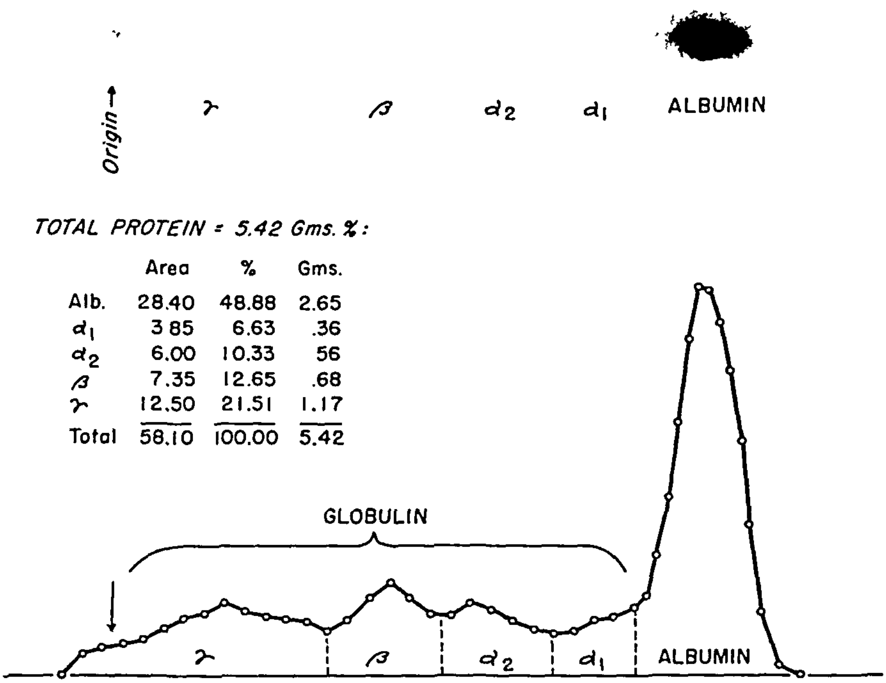

Fig. 1. Protein electrophoretic pattern (Specimen No. 7).

each component was calculated. These area percentage values indicated the percentage of total serum protein represented by each electrophoretic component (Fig. 1).

RESULTS

Ovarian Cysts

Table 1 lists the type of cyst from which the respective specimens were removed and the total protein content of these fluids. All fluids were tested against seminal specimens and in each instance they increased sperm motility. The concentration of protein in these ovarian fluids was remarkably constant, varying from 4.6 to 6.7 per cent in 10 samples. Of the remaining 3 fluids which contained lower protein concentrations, specimen No. 1 was from a tubal cyst, specimen No. 3 was from a simple ovarian cyst of a pregnant woman, and specimen No. 10 was removed from a patient with follicular cysts which expanded so rapidly that she had to be subjected to

TABLE 1. Protein Content of Ovarian Fluid

Specimen No.	*Source*	*Total % protein*
1.	Hydrosalpinx	0.24
2.	Simple cyst	5.89
3.[a]	Simple cyst	1.48
4.	Simple cyst	6.34
5.	Foll. cyst	4.56
6.	Foll. cyst	4.92
7.	Simple cyst	5.42
8.	Foll. cyst	5.24
9.	Foll. cyst	5.42
10.	Foll. cyst	1.72
11.	Foll. cyst	6.70
12.	Normal follicle	5.60
13.	Normal follicle	6.64
14.[b]	Foll. cyst	..
15.	Foll. cyst	..

[a] 14 weeks pregnant.
[b] Chorio-epithelioma.

an abdominal operation 6 weeks after the first ovarian cyst was emptied by culdoscopy.

Comparison with Blood Serum

Table 2 compares the values of the ovarian fluid protein fractions with the average distribution found in the serum of 7 normal individuals. The general resemblance of the constituent proteins is obvious, but certain differences of possible significance are apparent. The albumin percentage in ovarian fluid is somewhat higher than that in serum, as evidenced not only in the final analysis of the data, but also in the developed electrophoretic paper strips where the relative intensity of the albumin stands out more clearly than the globulin. This distinction is also confirmed in the diagram obtained after reading the pattern in the densitometer.

Examination of the data pertaining to the comparison of globulin concentration in ovarian fluid with serum reveals the possibly interesting fact that there is a significant lowering of this type of protein in ovarian fluid, particularly in the α-2 fraction. Whether this indicates that the fluid is an exudate of serum from which certain proteins were specifically removed is not clear, but it is possible that the follicle or cyst has selectively removed some of the globulins. It is interesting that chorionic gonadotrophin is a mucoprotein which in serum is found in the α globulins.[7] Of interest too, in this connection, is the observation by Zondek that gonadotrophins may be extracted from the wall of ovarian cysts during pregnancy. Since normal human serum contains approximately 6–8 Gm. of protein, the globulin values

TABLE 2. Comparison of Proteins of Serum, Cystic Fluid, and Follicular Fluid

Specimen No.	*Source*	*Total % protein*	*Albumin*[a]	*Globulins*[a]			
				α-1	*α-2*	*β*	*γ*
12.	Foll.[b]	5.60	48.6	5.94	6.27	11.61	27.6
13.	Foll.	6.64	41.6	7.24	7.34	15.90	28.0
7.	S. C.[c]	5.42	48.9	6.63	10.31	12.65	21.5
8.	F. C.[d]	5.24	44.7	5.61	12.13	12.82	24.7
9.	F. C.	5.42	48.2	5.27	8.00	15.4	23.1
	Average	5.66	46.4	6.14	8.81	13.67	25.0
	Serum[e]		40.2	6.45	11.90	14.80	26.9
					GRAMS % PROTEIN		
12.	Foll.		2.72	0.33	0.35	0.65	1.55
13.	Foll.		2.76	0.48	0.48	1.05	1.85
7.	S. C.		2.65	0.36	0.56	0.68	1.16
8.	F. C.		2.33	0.29	0.63	0.67	1.29
9.	F. C.		2.60	0.29	0.43	0.83	1.25
	Average		2.61	0.35	0.49	0.78	1.42
	Serum	7.17	2.88	0.46	0.85	1.06	1.92

[a] Per cent of total protein.
[b] Foll.—normal follicle.
[c] S. C.—simple cyst.
[d] F. C.—follicle cyst.
[e] Average of 7 normal humans.

for follicular fluid would be, on the whole, about 10 to 20 per cent lower than serum, and the albumin levels would be about the same.

Pregnancy Tests

Pregnancy tests were performed with 4 specimens (Nos. 1, 2, 3, and 14). Negative findings were noted with fluids from the hydrosalpinx and simple cysts, whereas positive tests were obtained in the patient who was pregnant and the one with chorio-epithelioma. The occurrence of a positive pregnancy test with fluid from an ovary of a pregnant woman has been noted previously.[8] Follicle-stimulating hormone was determined in specimen Nos. 1, 2, and 3. No activity was observed in Nos. 1 and 2, but 13 mouse units/100 cc. of fluid were recovered from the pregnant patient. Fifty-eight m.u. of estrogen/100 cc. were also found in the latter fluid. Patient No. 10 showed 250 m.u. of estrogen per 100 cc. and patient No. 15 showed 200 m.u./100 cc. of fluid. Both these cysts were of the follicular type. 17-Ketosteroids were ascertained in only 1 specimen, that of patient No. 3 who was pregnant, and was noted to be 573 γ/100 cc. of fluid.

Hemoglobin

The original fluid colors varied from red to green. Table 3 indicates the results of the spectroscopic observations on 6 specimens. Oxyhemoglobin

TABLE 3. Spectroscopic Observations

Specimen No.	Source	Original color	Spectral absorption: Native	Reduced	Reduced + CO	Reduced + pyridine
12.	N. F.[a]	Red	540–570[d]	550	535–565	550–525
13.	N. F.	Gr.-yellow				
7.	S. C.[b]	Red-br.	540–570	550	540–560	550–525
8.	F. C.[c]	Red-br.	540–570	550	535–565	550–525
10.	F. C.	Green				(550)[g]
11.	F. C.	Green	E. A. 500[e]	Yellow	U. V.[f]	(550)

[a] N. F.—normal follicle.
[b] S. C.—simple cyst.
[c] F. C.—follicle cyst.
[d] Boldface—stronger band.
[e] E. A.—end absorption.
[f] U. V.—ultraviolet.
[g] ( )—weak band.

shows absorption at 540 and 570. On reduction with hydrosulfite, a single diffuse band is formed at 550. The introduction of carbon monoxide splits this band to 2 bands at 540 and 560. Adding pyridine to a reduced solution of hemoglobin gives 2 bands at 550 and 525. On this basis, the data in Table 3 would indicate that the red color is due to hemoglobin. The green color, however, does not show any evidence of being a modified hemoglobin such as methemoglobin, but its marked absorption in the ultraviolet would be consistent with a more completely metabolized hemeprotein. It is possible that the green color is present in all the fluids but is masked by the red color in some.

Hemeprotein compounds are generally capable of catalase-like activity and liberate oxygen from hydrogen peroxide. Qualitative tests show that a rapid evolution of oxygen took place in the red fluids but that this did not occur in the green solutions, indicating the absence of hemeproteins in the latter specimens. We must therefore conclude that the red color is due to the presence of hemoglobin and that the green color is due to a pigment of as yet undetermined nature.

SUMMARY

Although the concentration and distribution of proteins in fluid from ovarian cysts and normal follicles are generally similar, there is enough variation to suggest that it may be incorrect to assume that fluid from cysts is identical with that obtained from a normal follicle. The increased sperm speed produced by these fluids cannot be used as a method of differentiation, since all fluids employed in this study, regardless of the source, showed such an accelerating effect.

Ovarian fluids demonstrate a protein pattern similar to that of normal serum but the globulin component, particularly the α-2 fraction, is somewhat reduced. This suggests the possibility that these fractions may be selectively withdrawn by the cyst or follicle.

The fluids studied varied from red to green. The red color appears to be due to hemoglobin. The nature of the green color is still not clear, although it does not appear to be a hemeprotein.

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DISCUSSION

Dr. Charles Birnberg: At the First World Congress on Fertility held in May, 1953, a paper by Dr. Raphael Kurzrok and the discussants on the role of follicular fluid was presented. It was then propounded by them that follicular fluid had a much more active role in fertilization than had previously been suspected.

It had been known that spermatozoa depended on extracellular nutriment for sustenance, which they received from the prostate gland and seminal vesicles.

The authors suggested that follicular fluid was passed into the tubes and uterus and possibly through the cervix, the fluid serving as a channel and a source of nutriment to the spermatozoa in their migration upwards. It was also noted that spermatozoa survived and remained actively motile in follicular fluid for a long period of time, many specimens remaining actively motile for 48 hours or more. This appeared to be the best medium for sperm survival.

Analysis of the follicular fluid revealed that it contained, aside from the hormonal contents, minimal amounts of fructose. A suggestion for a more critical determination of its contents was advised.

Dr. Perloff and his coworkers have demonstrated (1) protein concentration; (2) probable reason for the color; (3) presence of 17-ketosteroids in the fluid from the cyst of a pregnant woman; and (4) increased speed of spermatic movement.

These observations are essential in furthering our knowledge of follicular fluid. However, Dr. Kurzrok and I had hoped that the authors might come up with some method for the detection of follicular fluid, as this would be of great help in evaluating the existence of ovulation.

DR. RUTHERFORD: The paper is now open for a discussion from the floor.

DR. S. E. ROSS, JR., *Reno, Nev.*: I would like to ask Dr. Perloff what criteria were used in distinguishing the normal follicle.

DR. JOSEPH W. GOLDZIEHER, *San Antonio, Texas*: I am sorry to get up again. I would like to ask whether Dr. Perloff's determination of F.S.H. in the fluid was done for hypophysectomized animals or not and, second, whether his ketosteroid determination took into account the fact that some of the C21 steroids give colors with a Zimmerman-like pregnandiol.

DR. Q. E. FORTIER, *Minneapolis, Minn.*: Does serum alone speed them up?

DR. RUTHERFORD: Will you answer these questions, Dr. Perloff?

DR. PERLOFF: The diagnosis was made essentially by the pathologist and, as far as the normal follicle was concerned, we tried to get that by taking a patient at about the ninth or tenth day of the cycle, and in each instance a section was taken so that, at least from the point of view of histology, we were reasonably certain we were dealing with this particular type of entity.

I made a note here: "How about F.S.H.?"

DR. GOLDZIEHER: I asked you if the assay was done for hypophysectomized animals.

DR. PERLOFF: No. As to the color of pregnandiols, we did not mark a differential on that score. We don't think it is necessary. I won't go on to why.

Yes, serum does increase the motility of spermatozoa. As a matter of fact, every body fluid that was tested against the speed of spermatozoa showed an increase, and it was for that reason we became slightly discouraged with the importance of that phenomenon and switched our effort toward more of the chemical aspects, rather than the physiologic effect. You definitely see it with follicular fluid. But serum does it, and all sorts of fluids will do it.

Study of the Inadequate Secretion Phase Endometrium

John S. Gillam, M.D.

ENDOMETRIAL BIOPSIES from infertile patients are usually classified as "normal secretion phase endometrium," "anovulatory endometrium," or "abnormal mixed secretion phase endometrium." This is a preliminary report of a clinical investigation of the "mixed" endometrium or "inadequate secretion phase endometrium," which perhaps is a better term.

Recently much of the current thought regarding conception and early embryonic development has been consolidated.[3] Studies of normal[1, 13, 14, 17] and abnormal endometria[2, 4, 5, 8, 9, 20] and experiences with basal body temperature (B.B.T.) and other means of determining ovulation[7, 16, 17, 19, 21] have contributed much. The studies of irregular shedding of the endometrium by McKelvey and Samuels, and others,[2, 10, 11] specifically stimulated our investigations of the endometrium by bringing to light certain unexplained features of this condition. They showed normal levels of pregnanediol excretion in patients with irregular shedding of the endometrium both before and after therapy. Therefore, the abnormal persistence of the secretion phase endometrium late into the bleeding period may more likely be due to an abnormal endometrial response to progesterone than due to a prolongation of corpus luteum activity. It seemed important to determine whether the condition was due to abnormalities wholly within the endometrium. The pathologic diagnostic features as seen microscopically, such as abnormal, thickened vessels, would support this concept.

From the Department of Obstetrics and Gynecology, Fargo Clinic, Fargo, North Dakota.
Presented at the Conference on Sterility, University of Minnesota Continuation Center, February 19, 1954.

Irregular Shedding of Endometrium

Two of our infertility patients and two normally fertile patients who had a history typical of that seen in irregular shedding of the endometrium were placed on B.B.T. charts prior to curettage. In every patient bleeding began by the eleventh postovulatory day (P.O.D.), dating ovulation as the day of the low point of the temperature shift. The B.B.T. remained elevated 2–4 days following the onset of abnormal bleeding. After therapy by curettage, progesterone, or both, the temperature remained elevated 11–14 days and dropped premenstrually in 3 patients. The fourth immediately became pregnant. If the condition recurred, as it did in 2 patients, biopsies obtained on the first day of menses—eleventh P.O.D. and twelfth P.O.D., respectively—showed endometrium dated as eighth P.O.D. and tenth P.O.D., respectively. These and other patients with this condition have all shown a fairly consistent interval between the day of ovulation and the end of bleeding, regardless of the length of the bleeding phase. These data support the contention that the condition as seen clinically may represent "breakthrough" bleeding due to inadequate maintenance by the corpus luteum or to a decreased response by the endometrium.

In the data of McKelvey and Samuels it was noted that pregnanediol, when it was measured, appeared 10, 7, and 7 days prior to the onset of abnormal bleeding, and 12, 12, 10, 11, and 12 days prior to the onset of normal bleeding. In other words, it appeared that regardless of the normality of the corpus luteum function in any one patient, the endometrium of that patient was not being supported by the existing blood levels of progesterone. Curettage or adequate exogenous progesterone very early in the secretion phase will usually eliminate the condition. Such an endometrium, when present, and regardless of what it might have been previously, would probably be unable to support an implantation. Even though the clinical condition of irregular shedding of the endometrium was rarely encountered in infertility studies, it was felt that it might be relatively common in its subclinical phases and materially affect a significant number of infertile patients.

Classification of Endometrial Biopsies

Most reports pertaining to endometrial biopsies of infertility patients classify the biopsies as anovulatory or normal secretion phase and many also report a number of mixed secretion phase biopsies.[4, 5, 8, 13, 14, 20] However, the only logical way to date endometrial biopsies is to classify them by the most advanced stage of the endometrium. Were it not for the heterogeneous development seen in many samples of endometria, such a criterion would need not be stated. But it would seem that an endometrium with 80 per cent or more of its superficial layers immature would not likely be a highly fertile milieu, regardless of the normality of the remaining glands and stroma. For this reason the concept of endometrial adequacy was designated; its validity must meet the following test:

1. The endometrium must be adequately stimulated by estrogens and progesterone to accept the blastocyst and assist in its implantation; that is, to have reached normal maturation for the sixth to eighth P.O.D. within that period of time.
2. The endometrial stroma must continue to mature for another 5 days should it be required to nourish a conceptus.
3. The more homogeneous the superficial layers of the endometrium are, the more accurate is the analysis of adequacy.
4. The more heterogeneous the superficial layers of the endometrium are, the less likely it is that the endometrium has responded adequately.

MATERIAL AND METHOD

Biopsies

As a preliminary to this study, 100 random endometrial biopsies from infertile patients were examined. From this sample several factors were noted that required consideration. Biopsies from the lower uterine segment had to be guarded against since, for our purposes, they were of no value. Except to demonstrate irregular shedding of the endometrium or to demonstrate the simple existence of secretion phase endometrium, the same was true of biopsies taken during the menses. Using the criteria for dating suggested by Noyes *et al.*, biopsies were dated both as to the most advanced stage and the average stage of maturation of glands and stroma as seen in 8 of 10 fields and then compared with the B.B.T. curve of that cycle. In 60 random samples where these data were available, as judged by the adequacy and preparation of the sample and by an accompanying B.B.T. record, 40 per cent showed at least 8 of 10 $\times$ 100 fields lagging behind expected maturation by more than 2 days. Seventy-five per cent of these deficient biopsies showed no evidence in any field of further maturation. Thus 30 per cent of the total were fully 2 days or more immature and an additional

10 per cent were immatuie by that amount in 80 per cent or more of the areas examined. In 1 instance of the 60 the endometrium was dated 2 days in advance of the postovulatory date and in 3 it was dated 1 day in advance.

With this preliminary review and our experience with irregular shedding of the endometrium, the study program on private infertility patients was designed using the least expensive and simplest available means of testing this further. Since all our infertility patients could not be included, the program obviously had to be planned with certain limiting factors. Statistically, comparing the means of paired samples offers tremendous advantages in significance over comparing the averages of sample groups. Therefore, multiple biopsies on each patient were necessary, with pretherapy controls and posttherapy biopsies as well.

B.B.T. Curve

The low point of the B.B.T. curve shift was used throughout this study to designate the day of ovulation. Vaginal smears, fern tests, and *Spinnbarkeit* were quite often used, but rarely varied significantly from B.B.T. rises. If they did differ, it was usually on the side of dating ovulation earlier, rather than later, as determined by B.B.T. rise. If this was the case and if ovulation did occur prior to the assigned date, it would enhance the significance of the immaturity seen in any abnormal specimen, since the assigned date was the later rising B.B.T. Thus it was felt that by using B.B.T. we were more likely giving any endometrium the benefit of time.

Much of the criticism of the B.B.T. as a means of determining date of ovulation has been on the basis of a poor correlation of the endometrial biopsy. With this lack of correlation, we will agree; but we feel that the endometrial response and not the B.B.T. rise may be at fault. It seems fairly safe to assume that B.B.T. rise reflects the 24-hour period ± 18 hours of ovulation (a possible 60-hour period). In spite of noted deficiencies in our knowledge, it may be fairly safely assumed that the B.B.T. curve also reflects corpus luteum activity to a certain degree. If these assumptions are true, patients with immature endometria who are given adequate progesterone should develop normal endometria. This is accomplished by raising progesterone to normal blood levels in cases of corpus luteum deficiency or by raising levels above normal to overcome inherent endometrial resistance to maturation.

In this study, when a deficiency was suspected, only progesterone therapy was used. It was supplied as oral or vaginal tablets in daily doses of 10–30 mg., depending upon the degree of deficiency. Pretherapy and posttherapy biopsies were obtained to determine whether there was a significant difference in maturation. Finally, after determining the proper dosage, the number of normal pregnancies occurring after therapy were noted. The work of Hughes *et al.* was similar in principle. Their laboratory procedures were much superior to our simple biopsy interpretations, but were beyond the scope of any but an institutional study program.

Criteria for Selection of Subjects

The initial exclusion of a large group of patients was unavoidable. Couples with absolute sterility were omitted. Abortions were not considered as successful pregnancies. Since they are a subject of a separate study, they have been excluded except in rare instances as noted. Our experience in this group supports the work of Hughes. Since 30 per cent of our patients lived long distances away, accurate control was difficult, and many of these patients were not included in the investigation. Patients who cooperated poorly were excluded. Since some feel a 2-year infertility is necessary before inclusion in a study, the analysis encompasses only patients who have been infertility problems for at least 24 months. Thus 13 studied and treated patients who had periods of infertility ranging from 15 to 23 months have had to be eliminated. Seven of these patients became pregnant following therapy. Patients whose biopsies showed no secretion phase endometrium were excluded because this was not a study of anovulatory endometrium. No biopsies on patients were included where there was insufficient tissue for diagnosis.

Study of 123 Patients

Considering these exclusions and limitations, there remained a group of 123 infertility patients who were included in the endometrial study program. Ideally, biopsies were taken on the seventh P.O.D., the thirteenth P.O.D., and where indicated, on the fifth day of bleeding. Biopsies were obtained in at least one and two cycles where B.B.T. curves were also available. Nine had concomitant daily vaginal smears. If the endometrium showed a deficiency on at least 2 biopsies, 10–30 mg. of progesterone daily, sublingually or vaginally, was prescribed beginning as soon as the patient could recognize ovulation. Progesterone was continued until the temperature dropped premenstrually or until menses supervened. Biopsies were again taken in these treated cycles on the seventh and thirteenth P.O.D.

Occasionally, for the purpose of determining the reality of changes, subsequent cycles in which progesterone was not taken were biopsied. These were cycles where it was impossible, for various reasons, for the couple to have coitus on ovulation day ± 24 hours. There were 17 such control cycles, of which 11 showed reversion to the previous state and 6 in which the biopsy showed improvement over that taken before therapy. Four of these 6 appeared perfectly normal. These numbers are too few to be significant but imply that in some patients the deficient endometrial response appears chronic. Once a normal response had been obtained by therapy, the patient was maintained on that dosage. Repeat biopsies were obtained when the temperature fell premenstrually below 98.4° F. or, if one could be obtained, within 4 hours of onset of menses. Early in the study many were biopsied also 4 days after onset. Obviously, this ideal method of control could not be obtained in all patients. Furthermore, some other factors played a role which had to be considered in the analysis.

RESULTS

The 123 patients whose 295 biopsies showed secretion phase endometrium were classified as in Table 1.

Group I patients showed normal endometrial biopsies. These 66 patients with 105 normal biopsies had 9 pregnancies at or near term and 3 abortions. More biopsies in more cycles might show that some of these patients do not belong in this group.

The 17 patients in Group II showed progesterone-deficient biopsies, but complete evaluation has not yet been made. Group III patients represent the nucleus of this report. They were divided to eliminate possible sources of error. Abortive patients were excluded from analysis since such an end result is unsatisfactory. In Group III A there were 15 treated patients. Five had successful pregnancies. Even though results were good, inclusion of these 15 patients could be criticized for the reasons listed.

In Group III B there were 10 patients who, it was felt, had excellent biopsy control and treatment control. Each had other factors existent which might have contributed to her sterility. Part of this group was treated for 3 months prior to hormone therapy by local therapeutic methods to obtain a normal Sims-Huhner response. The remainder had homologous cup inseminations for 3 months before institution of hormone therapy. Employing the conception data as determined by Guttmacher and others,[18] if the sperm or local factors had been eliminated, 60 per cent should have become pregnant within these 3 months. Since none became pregnant prior to proges-

TABLE 1. Analysis of the 295 Biopsies Showing Progesterone Effect in 123 Infertility Patients

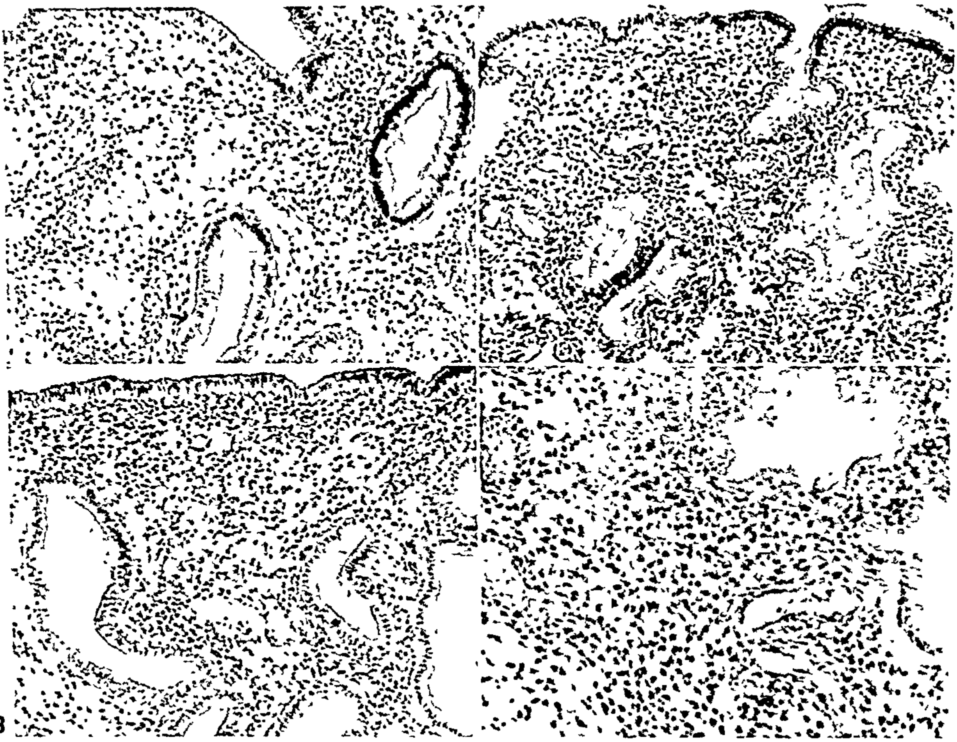
Group	*Patients*	*Biopsies*	*Pregnancies at or near term*
I. Probable normal endometrium	66	105	19
II. Probable progesterone deficiencies; less than 3 months' evaluation	17	38	2
III. Progesterone-deficient endometrium			
A. Treatment started but unsatisfactory control	15	25	5
1. Insufficient data	..	..	5
2. Therapy prescribed at a distance without further biopsy control	9	..	..
3. Multiple factors without 3 months' control of other factors	8	..	..
	(incl. some	..	..
	of 1. and 2.)	..	..
B. Satisfactory control, other contributing factors	10	35	5
Poor Sims-Huhner test	2	..	..
Oligospermia or oligotaxia	8	..	..
C. Satisfactory control, no other demonstrable factors	18	92 (3 during pregnancy—1 aborted)	12 (followed therapy)

terone therapy and since 5 of 10 have already become pregnant after its addition, this finding is approaching significance.

Because our object was to study adequately a particular deficiency and because the introduction of other factors would produce unlike samples, only the group of III C patients will be further analyzed. These 18 patients had no other demonstrable factor as a cause of their infertility. This group had been under study prior to therapy for 5 months. The period of infertility ranged from 2 to 13 years with a median of 3.5 years and an average of 4.3 years. Twelve patients, 2 of whom also had abortions, have pregnancies near term or have delivered. Figures 1–12 are typical microphotographs of endometrial biopsies from these patients.

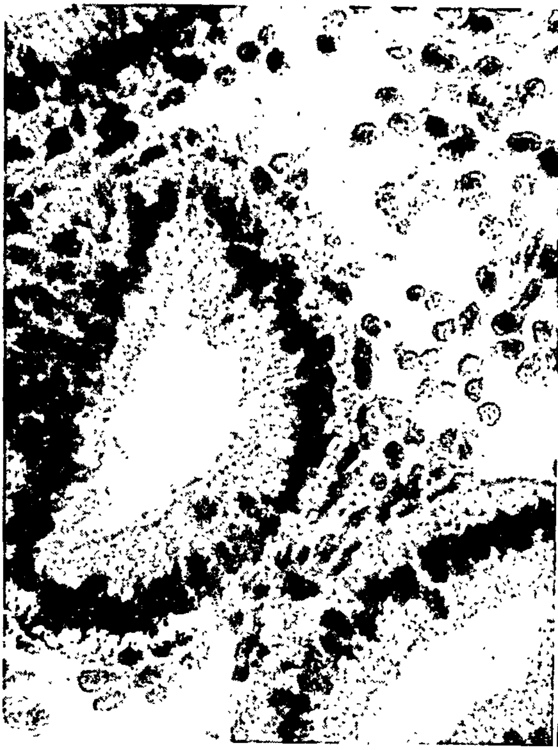
Interval Between Progesterone Therapy and Pregnancy

The average duration of time from the start of adequate progesterone



1 2 3 4

Fig. 1. Mrs. S. P., 150566, age 31, 5-year infertility. A biopsy obtained on the tenth P.O.D. showed typical fourth P.O.D. glands throughout and a cleared stroma of the seventh P.O.D. Quite marked inadequacy. Fig. 2. Same patient as in Fig. 1. Biopsy obtained 2 hours after the onset of menses on the fourteenth P.O.D. Glands are atrophying and stroma is almost normal premenstrual stroma. This represents the only encountered example of the "sudden shift." Subsequent cycles were same until therapy and then normal. No pregnancies. Fig. 3. Mrs. C. F., 141415, age 24, para 0; 18 months infertility when first seen, 26 months when became pregnant. A biopsy on the eighth P.O.D. showed glands appearing as those of the third to fourth P.O.D. and stroma perhaps of the sixth P.O.D. Most cells of the glands had basal vacuoles but occasional vacuoles were passing the nucleus. Fig. 4. Same patient as in Fig. 3. Patient was taking progesterone 20 mg. daily and the tenth P.O.D. biopsy appears normal with nearly exhausted glands and numerous stromal mitoses. Continuing therapy, patient became pregnant the following cycle.



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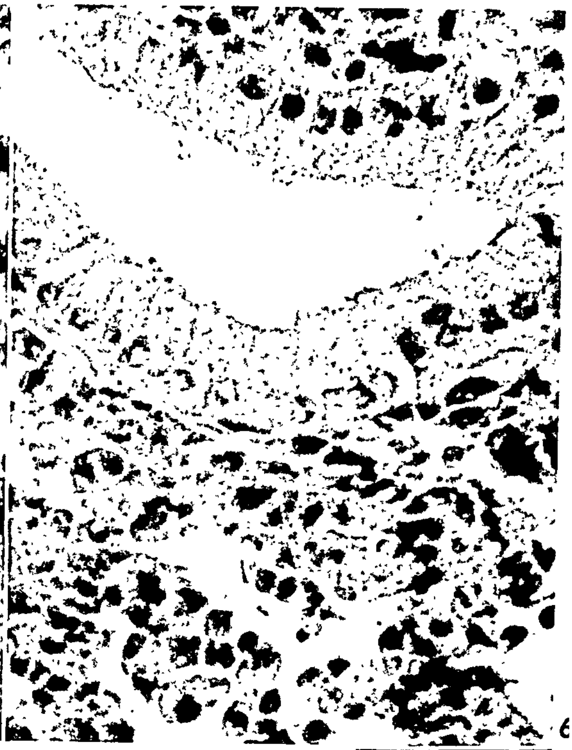

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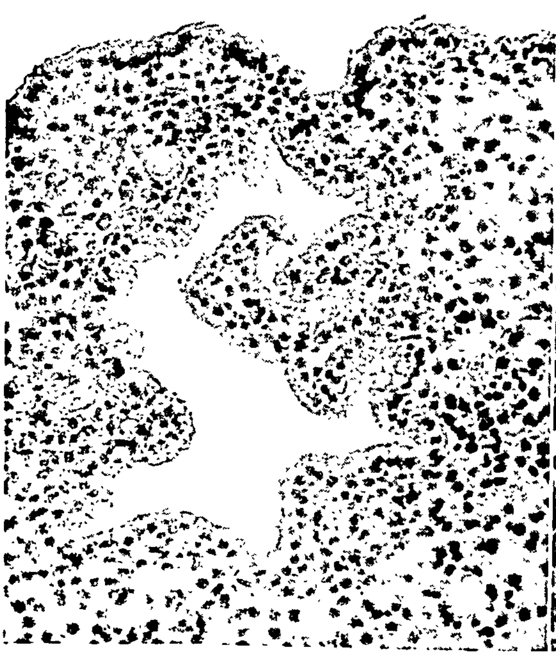

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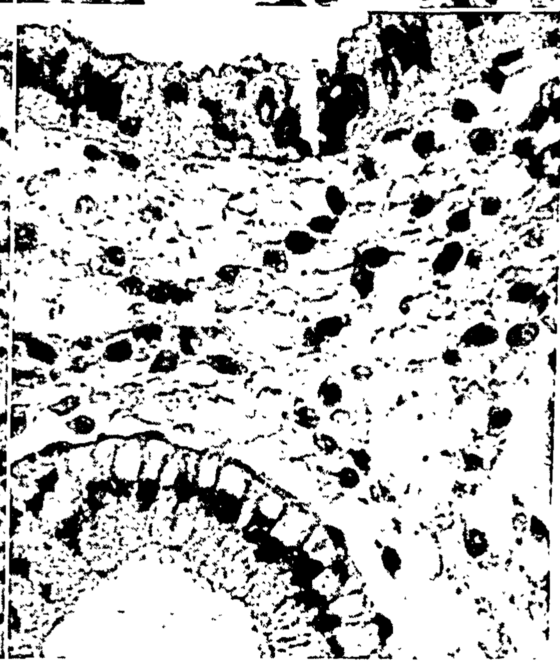

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Fig. 5. Mrs. D. B., 143487, age 29, 2-year infertility. Eighth P.O.D. biopsy showed glands as above throughout, with very early basal vacuole formation of second P.O.D. Note surface epithelium at upper left. **Fig. 6.** Same patient as Fig. 5, same cycle taken 3 hours after onset of menses on fourteenth P.O.D. Biopsy shows under high power a surface gland with the active secretion of sixth to eighth P.O.D. and stroma perhaps

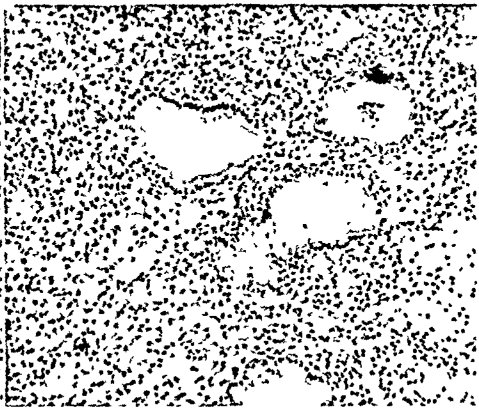
9   10

Fig. 9. Mrs. J. D., age 26, para 0, 2-year infertility. Biopsy taken on the ninth P.O.D. after 2 years of Type V B.B.T. curves. Menses on tenth P.O.D. as usual. Biopsy appeared normal ninth P.O.D. here as in next cycle, but certainly not in view of menses next day. Same curve followed without biopsy or therapy. Became pregnant and aborted at 6 weeks. Became pregnant again on progesterone 20 mg. daily and delivered normal female at term. Fig. 10. Mrs. O. S., age 33, 12-year infertility. Frequent Type IV B.B.T. curves. Biopsy prior to therapy on twelfth P.O.D., 2 hours after the onset of spotting, shows glands and stroma with a typical eighth P.O.D. maturation. After 12 months of varying dosages of progesterone and numerous biopsies, conceived, and delivered 3380-Gm. daughter.

therapy (as determined by multiple biopsies) until pregnancy occurred was 3.6 months, with a range from 0 months (3 biopsied while pregnant and under therapy) to 13 months. Nine of the 12 patients were pregnant before the end of the fourth month of treatment. Excluded from the average periods of time were 2 months in each of 4 patients where vaginal placebos instead of progesterone were used. No pregnancies resulted in this minimal

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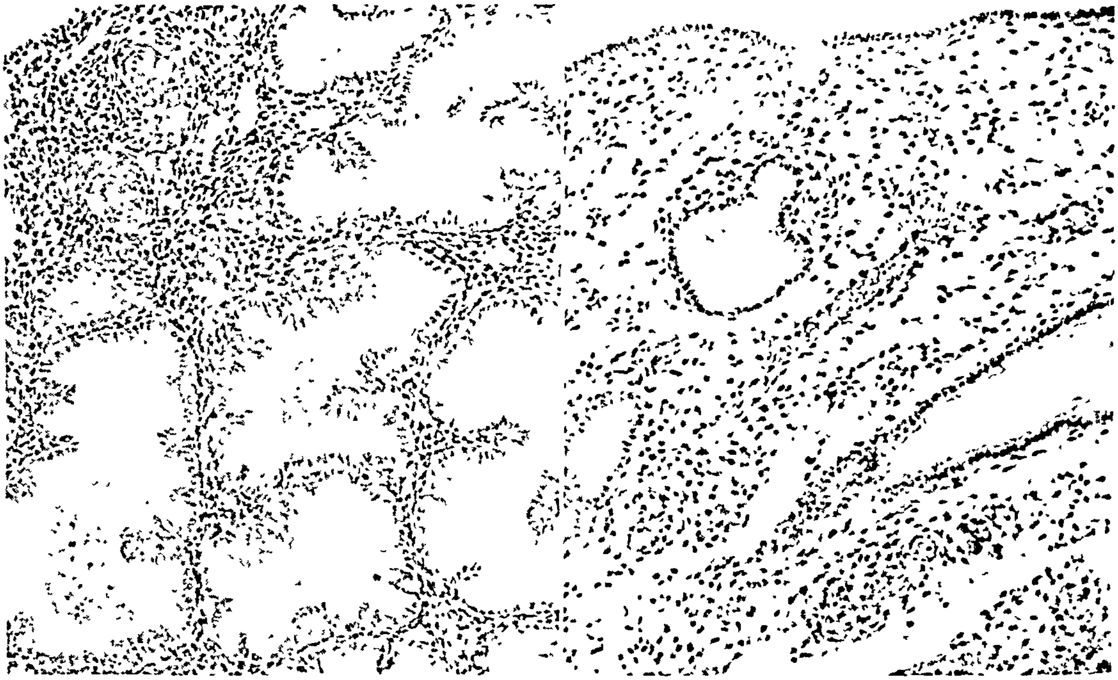
as advanced as eleventh P.O.D. Fig. 7. Same patient as Figs. 5 and 6. Patient on progesterone 20 mg. daily. A tenth P.O.D. biopsy now shows fullblown eleventh P.O.D. stroma and exhausted glands. Therapy withheld and endometrial inadequacy returned. Therapy with progesterone again begun, normality checked by biopsy, and patient became pregnant. Delivered normal 3640 Gm. male on 2–14–54. Fig. 8. Mrs. H. L., 159124, age 31, 6-year infertility. Biopsy taken on eighth P.O.D. shows throughout glands as shown here under high power, with almost all having the basal vacuoles and nuclear line as noted. Though the glands appear third P.O.D., the stroma appears fifth P.O.D. The surface epithelium is at top of section. Picture completely normal after addition of progesterone as determined by 4 more biopsies, but not pregnant after 4 months of therapy.



11 12

Fig. 11. Mrs. W. M., 131585, para 0, 2-year infertility. History typical of irregular shedding of the endometrium. D. and C. done on 6th day of menses, fifteenth P.O.D., showed exhausted secretion phase endometrial glands. Note thickened vessel in upper left. **Fig. 12.** Same patient as in Fig. 11. Biopsy taken on fourteenth P.O.D. 2 hours after the onset of an 8-day menses. The glands appear as advanced as the ninth P.O.D., but the stroma does not appear to have matured beyond the seventh P.O.D. Patient was placed on progesterone 30 mg. daily beginning at ovulation and became pregnant and delivered normally at term. Subsequently became pregnant without therapy.

control period. There were 3–9 biopsies per patient during control and therapy, with an average of 5 biopsies per patient.

B.B.T. Graphs

All 18 patients had concomitant B.B.T. graphs made, which were analyzed and classified according to Fig. 13. Type I is a normal B.B.T. curve. Type II is the staircase rise. We are inclined to agree with Siegler and Siegler and others[21] that this is a normal curve, since rises of this nature occurred in 4 of the 12 patients who became pregnant. Type III curves, of which Fig. 14 shows many examples, is of interest. It was encountered 36 times in 7 different patients. In no cycle did it occur when coitus did not also occur on the day prior to or the day of ovulation (see Fig. 2). The average duration of secretion phase was 17.6 days, with a range of 15–21 days. Seven of such

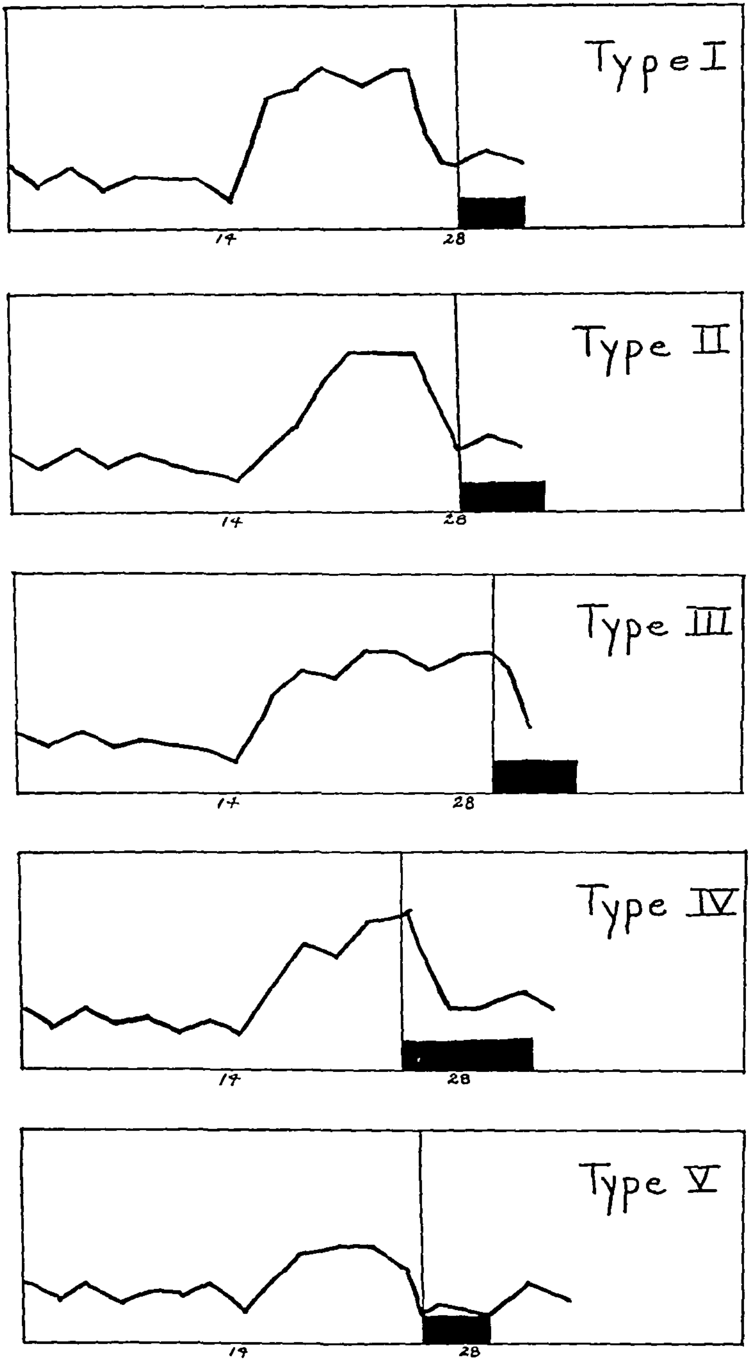


Fig. 13. Types of basal body temperature curves observed in infertility patients.

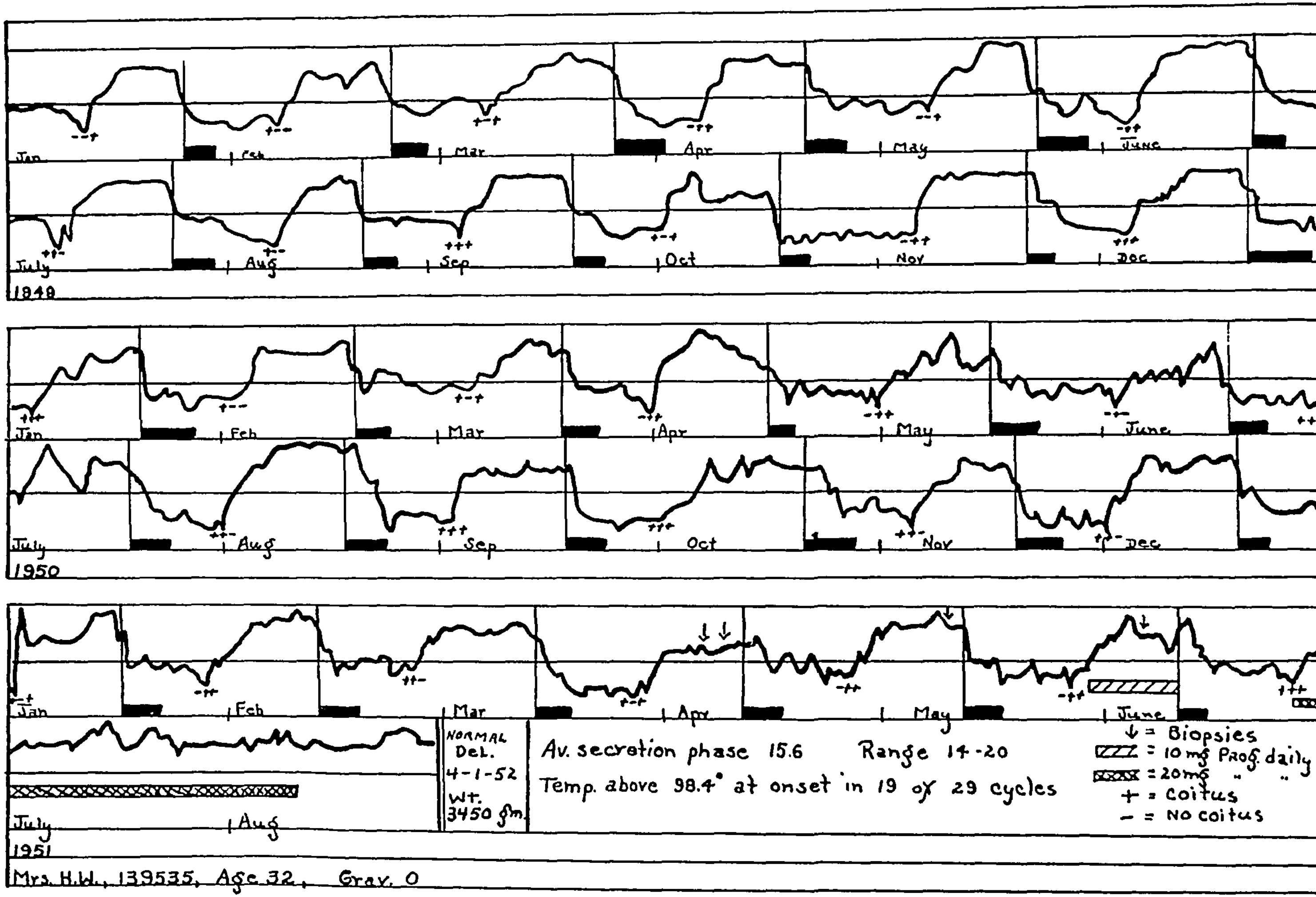


Fig. 14. Basal body temperature curve of 32 months' duration.

cycles were biopsied. The endometrium was deficient only by 2–4 days. These may represent the subclinical abortions due to environment, perhaps different from the germ plasm abnormalities noted by Hertig and Rock.[3] Type IV curve, also seen in irregular shedding of the endometrium, characteristically shows elevation of B.B.T. after the onset of menses and a short secretion phase prior to the onset of menses. This may represent an inadequate endometrial response. Bleeding in these patients usually is heaviest on the third to fifth day of menses which last 7–9 days. Type V curve was seen in 4 patients 17 times. Seibel has suggested that these are probably primarily ovarian inadequacies. Yet 17 of 18 patients had normal Type I and II curves after institution of therapy, perhaps by the "rebound" phenomenon.

From this careful study of the B.B.T. curves in this specific group we have a renewed respect for B.B.T. curves when correlated with biopsies before and after exogenous progesterone. Since the addition of progesterone enhanced the endometrial response in most patients to the proper postovulatory day, it confirms to a certain extent the accuracy of the assumption that the low point of the shift of the B.B.T. is a means of determining ovulation. The number of pregnancies ensuing adds further to this assumption.

Endometrial Biopsies

The second test employed in the study was the endometrial biopsy. If the biopsy is well timed and correlated with B.B.T. and if it is interpreted as to the degree of endometrial maturation as determined by the clinician and pathologist, it is a valuable and informative test. It has the further advantage of being inexpensive and easy to obtain, prepare, and interpret. By correlating B.B.T. and the endometrial biopsies we found that about 40 per cent of the 123 patients showed endometrial deficiencies. This figure is similar to that of Hughes *et al.* and Grant. Because of the exclusions noted, this percentage obviously does not represent the number of patients with inadequate secretion phase endometria seen in the total infertile population. Our method of outpatient diagnosis makes it impossible to arrive at an accurate figure of the incidence of deficient secretion phase endometrium except to say that the minimum frequency in our infertility patients is 10.7 per cent. The actual figure probably approaches 20 per cent, using the criteria of endometrial adequacy as defined.

The 18 Group III C patients, whose case reports are voluminous, are included in this report only as noted under the microphotographs with the exception of Mrs. H. W.

Case Report

Mrs. H. W., age 32, para 0, was a 7-year infertility problem. Her husband, a college professor and statistician, had a perfectly normal semen specimen on 3

15 16

Fig. 15. Mrs. H. W., 139535, age 32, para 0. Biopsy on the seventh P.O.D. appears to have glands no further advanced than third P.O.D. and possibly a fifth P.O.D. stroma. Note the numerous basal vacuoles and the lymphocytic infiltration. **Fig. 16.** The same patient as in Fig. 15. Biopsy was obtained in the same cycle as in Fig. 10, but on the thirteenth P.O.D. 36 hours prior to the onset of menses. Note the glands are still secreting and the stroma is at most eleventh P.O.D. stroma. Also note the peculiar lymphocytic infiltration, the small black stromal cells for the most part being lymphocytes. The deficiency at this stage does not appear as great as that seen earlier.

occasions. All known tests of fertility had been done repeatedly by us and other specialists with negative results. On her first visit, she brought her B.B.T. curve of 26 months' duration. Temperature had been recorded mornings over these 2 years with but 7 exceptions. Days of late rising were noted. The B.B.T. had, fortunately, been recorded vaginally even through menses. Of special interest were the findings of 19 cycles where the B.B.T. was over 98.4° F. at onset of menses (Type III) and in every instance pregnancy would have been possible.

Even though average duration of elevation of B.B.T. in both Type III and Type I were the same, bleeding listed an average of 6.4 days in Type III and 5.2 in Type I or II curves.

It required considerable restraint to defer biopsies for 3 months while evaluating other factors. The results of the first 2 biopsies are shown in Figs. 3 and 4, showing what we considered to be a progesterone-deficient endometrium. An identical biopsy was obtained in the next cycle. She was then placd on 10 mg. of progesterone daily beginning with ovulation. The endometrium appeared better but still lagged by 2 days. Dosage was increased to 20 mg. daily and the patient was scheduled for biopsy on B.B.T. drop or menses. The B.B.T. continued above 98.4° F. until the twentieth week of gestation. A recent biopsy was identical to her first. She has had no therapy and used no contraception since delivery in April, 1952.

DISCUSSION

It must be reemphasized that the study of the endometria of infertile patients should be conducted under ideal circumstances in order to determine the normality or inadequacy of the samples. The pathologist or clinician should not interpret an endometrial biopsy as normal unless he knows its relationship to the patient's cycle. These ideal circumstances are unfortunately often difficult to obtain; but it is much more unfortunate for the patient to assume that an endometrial sample is normal without this criterion. Hughes has shown this previously in his work on endometrial enzymes and glycogen. We have attempted to show this clinically, using the easily available biopsy and B.B.T., and accurately interpreting one in the light of the other.

Statistical analysis of our data superficially attaches significance to the results of the administration of progesterone by both biopsy control and by ensuing pregnancy rate. However, the variable types of abnormalities noted in Table 2, the possibility of error in dating, and the existence of undiscovered variants make it unwise to accept such findings as significant. Following a plan similar to Noyes and Haman, two of us recently had a biopsy-dating correlation of 0.81 on the last 40 biopsies, a figure better than earlier in the study. There remains a group of biopsies that resist classification by any method because of their heterogenous nature. Several patients showed repeated biopsies where the stroma and glands would appear to be developing completely independent of each other and vary considerably from gland to gland or stromal field to stromal field. But the most peculiar abnormality noted in the deficient endometrial biopsies was the increased infiltration by mature lymphocytes. This was not on an infectious basis as shown by the absence of plasma cells and leukocytes. Also this lymphocytic infiltration completely disappeared upon the addition of adequate progesterone.

Furthermore, we hesitate to attach statistical significance to our end

TABLE 2. Luteal Phase Abnormalities

Type	*Frequency*	*Type of B.B.T. curve*	*Form*
I. Morphologic endometrial inadequacy on 6th–8th P.O.D.			
A. Persistence of inadequacy through luteal phase and often into bleeding period	Commonly seen	IV (occasionally I and II)	Occasionally severe —"transitional endometrium"
B. Sudden late shift in secretion phase from inadequate to mature	Seen once	II	..
II. Marked inconsistency between gland maturation (progesterone) and stromal maturation (estrogen and progesterone)	Second most common (approx. 30%)	I (rare), II, III, IV	..
III. Apparent morphologic adequacy but less than 11-day luteal phase on correlation of B.B.T., biopsy, and menses	Quite common (20%)	V	..
IV. Cyclic variations in adequacy	Seen in 2 patients; perhaps more common than suspected	Can almost predict by graph	In reality included normals and all I, II, and III on various occasions

results because the basic suppositions of endometrial adequacy may be unacceptable. This study must, therefore, be considered as preliminary. When employing the defined criteria, at least 10.7 per cent of infertile patients have an endometrial inadequacy. There is no doubt that the broader use of controls, though time-consuming and expensive, would enhance the significance of the findings.

SUMMARY

1. A carefully selected group of patients was segregated from the total

group of private infertility patients to determine whether, by the criteria suggested, an inadequate secretion phase endometrium existed.

2. In this selected group of 123 patients, a tentative diagnosis of deficient secretion phase endometrium was made in 57 patients, 28 of whom were studied further by controlled measures.

3. Ten of the 28 patients had other contributing factors controlled prior to progesterone therapy. These 10 are excluded because the sample would not be considered pure. Five of these 10 became pregnant following progesterone therapy.

4. Thus 18 patients, in whom no recognizable factor other than an inadequate secretion phase endometrium was uncovered, were carefully studied by multiple endometrial biopsies and concomitant basal body temperature graphs before, during, and after progesterone therapy. Twelve became pregnant. Ten of these have delivered and 2 are near term.

5. The criteria for exclusion from the study have been noted, as have the criteria for endometrial adequacy.

6. The types of basal body temperature graphs encountered have been classified; the types of endometrial inadequacies encountered are also classified.

7. For reasons discussed, it can only be said that the successful pregnancies followed study and therapy. It would be unwise to say that the pregnancies were a result of therapy.

8. The correlated use of B.B.T. and endometrial biopsy in infertile patients is inexpensive, easily applicable, and an informative method for uncovering the relatively small number of infertile patients who have an inadequate secretion phase endometrium.

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Annual Conference of the British Society for the Study of Sterility

The 1955 Annual Conference of the British Society for the Study of Fertility will be held in the Large Anatomy Lecture Theatre of the Medical School, Hospitals Centre, Birmingham, England, on Thursday and Friday, June 23 and 24.

Members of the American Society for the Study of Sterility are cordially invited to attend and participate in this meeting. If anyone wishes to present a paper describing some phase of research in our special field, please write Mr. H. H. Fouracre Barns, 31 Weymouth St., Portland Place, W. 1, London, England.

Artificial Insemination with Semen Recovered from the Bladder

Robert S. Hotchkiss, M.D., Asdrubal Baias Pinto, M.D.,
and Sophia Kleegman, M.D.

THE PHYSIOLOGIC CHRONOLOGY of seminal ejaculation in man has been made more clear by the study of partitioned ejaculates.[2] The cellular and chemical constituents in the first and second portions of the divided ejaculate strongly suggest that spermatozoa are mainly stored in the ampullae of the vasa deferentes, at least just prior to ejaculation. It may be inferred that at the initiation of the seminal discharge, the ampullae empty into the prostatic urethra followed by evacuation of the seminal vesicle secretions. The time relationship of the movement of prostatic secretions is more difficult to ascertain, but it has been suggested that some prostatic secretions may precede the emptying of the vasa and the remainder accompany the bulk of the discharge.[3]

MECHANISM OF EJACULATION

The mechanism of coordination of the muscular apparatus surrounding the prostate and urethra has not been completely established. The internal and external urethral sphincters lie at the proximal and distal ends of the prostate gland. The external sphincter is composed of smooth and accessory striated muscles encircling the urethra just distal to the verumontanum at the apex of the prostate.[1] The fibers of the internal sphincter insinuate themselves into the muscular bladder wall at the bladder outlet. Information has been obtained from experiences following transurethral prostatectomy

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which demonstrates that the internal sphincter may be divided or destroyed without consequence to control of urination. Should the external sphincter be injured, incontinence may result. Consequently, the internal sphincter may be relatively unimportant in micturition, but may well be a muscle designed for fulfillment of sexual function. This statement is based on observations that after transurethral resection certain men are unable to ejaculate the semen through the urethra but instead deposit the semen into the bladder.

A hypothesis might be ventured that at the time of ejaculation a normal, intact, internal sphincter closes, or remains closed, thereby preventing the semen from entering the bladder and retaining urine in the bladder when the external sphincter opens to allow the semen to flow into the distal urethra. The propulsion of the semen must be accounted for by the perineal and bulbous muscles surrounding the urethra assisted by certain elements of the pelvic musculature.

RETROGRADE EJACULATION

Retrograde ejaculation may also occur in certain men who never have been subjected to instrumentation of the urethra or prostate and are not aware of a previous injury. Endoscopic observation may occasionally disclose a very large verumontanum, localized inflammation, or a diverticulum, but more often shows no hypertrophy of prostatic tissue or visible abnormalities. The urethrogram may likewise be normal. The inability to accomplish external emission of semen may have prevailed from the onset of sexual activity.

The diagnosis of this malfunction of the male sexual system is easily established. The ejaculatory sensation is always experienced during coitus but semen is not expelled from the urethra. Examination of the voided urine collected directly after coitus will reveal large numbers of spermatozoa, thereby establishing the diagnosis of retrograde ejaculation. Almost invariably the spermatozoa are immotile. The inactivity of the spermatozoa is due to heterotonicity or to acidity of the urine, for the motility may be preserved by the following procedures.

RECOVERY AND PRESERVATION OF SPERMATOZOA FOR INSEMINATION

1. The patient is instructed to avoid fluids for 6 hours prior to collection, thus reducing urinary output.

2. The bladder is emptied by catheterization and washed with 180 cc. of Ringer's-glucose solution.
3. After the irrigation is completed and the bladder emptied, 2 cc. of the same solution is inserted.
4. Manually induced ejaculation is immediately performed.
5. The bladder contents are then removed by voiding or, if necessary, by reintroduction of the catheter.

The fluid thus obtained is a mixture of semen, Ringer's solution, and some urine. Repeated performance of this routine has permitted recovery of excellent specimens for artificial insemination. We present herewith the essential features of 2 cases where this procedure has salvaged spermatozoa for insemination which resulted in conception.

Case 1

(DR. ROBERT S. HOTCHKISS AND DR. ASDRUBAL PINTO)

The patient, a 32-year-old husband, was examined on October 13, 1948. At the age of 26 a transurethral prostatectomy had been performed in Portugal for perineal pain. Prior to this operation ejaculation was normal, but following recovery he realized that semen failed to appear from the urethra, although his sexual ability was otherwise unimpaired. During the past 6 years of married life coitus was practiced twice a week without resulting in impregnation. Spermatozoa had been seen in varying numbers in urinalyses since the prostatic resection.

Physical examination was unremarkable except for some induration of the left epididymis and a palpable left seminal vesicle. The voided urine was negative. Neurologic studies were made but no abnormalities in the reflexes were found, and the patient was regarded as being without neurologic disease.

Many highly motile spermatozoa were recovered by performing the previously described steps in diagnosis and methods for sperm recovery. The total fluid removed from the bladder varied from 4 to 20 cc. and concentration per unit of volume varied proportionately ranging from 10 to 100 active spermatozoa in each high-power microscopic field.

The wife was aged 27. Her menses occurred every 28 days, and detailed examination revealed essentially normal reproduction organs. The couple shortly returned to Portugal where Dr. A. Pinto performed the inseminations. Basal body temperatures were used for timing the procedures with specimens, collected as described on the following days:

April 24, 1949—25 cc. of fluid removed from the bladder had 20 active spermatozoa per high power field. *May 22, 1949*—The specimen was heavily contaminated with urine and showed poor activity. *June 19, 1949*—There were many motile spermatozoa in 7.5 cc. of fluid removed; 1.5 cc. of this was injected by cannula into the cervix and 6 cc. was left in contact with cervix.

Pregnancy resulted and normal boy was born 9 months later. On October 28, 1952, on the fourteenth postmenstrual day, a second insemination was done in the same manner. Dr. Pinto delivered a female baby in July, 1953. In October, 1953, the couple returned requesting a third insemination.

Case 2

(Dr. Robert S. Hotchkiss and Dr. Sophia Kleegman)

The husband, aged 36, was examined on November 15, 1953. There had been no illnesses, but the patient stated that prior to age 27 only a few drops of semen appeared from the urethra at time of ejaculation. For the past 9 years little if any discharge occurred with orgasm, although he retained the full sensation of ejacula-

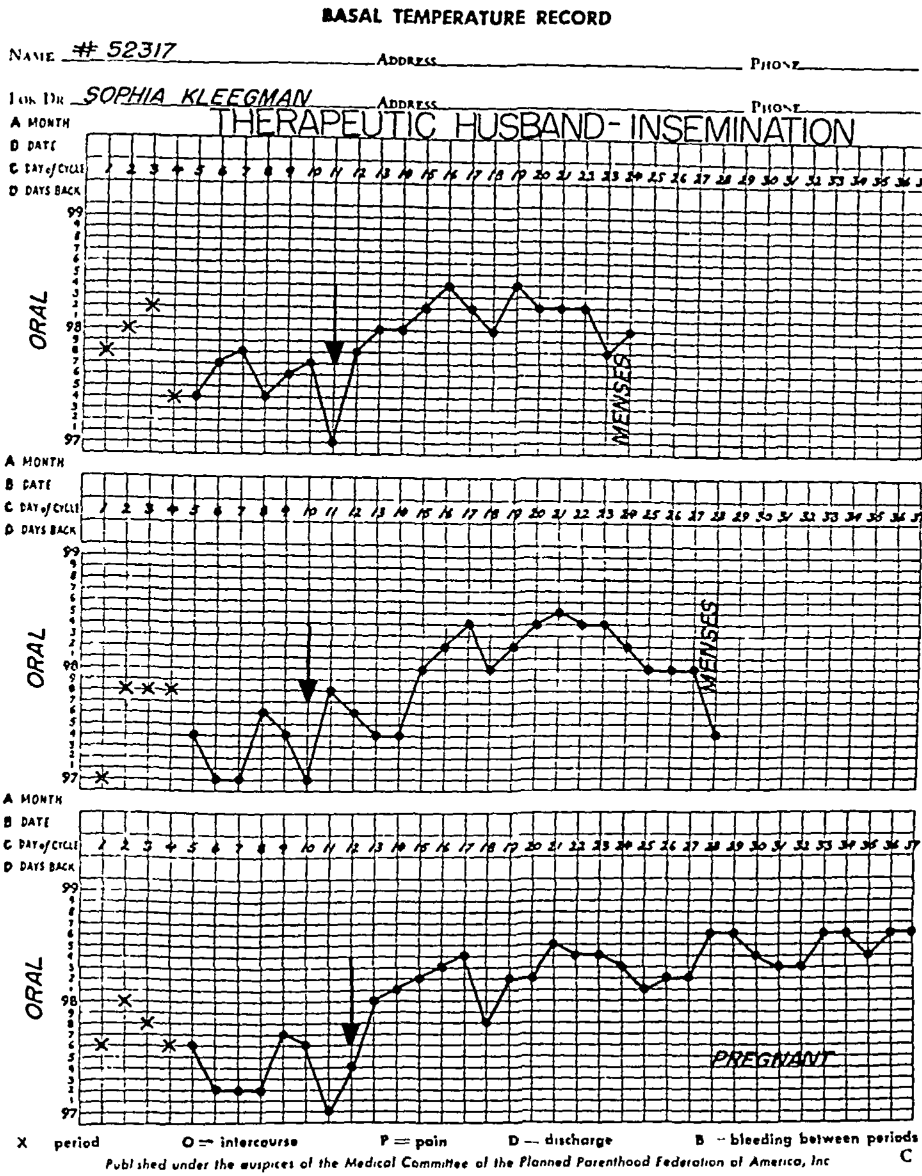

Fig. 1. Basal temperature records of 3 menstrual cycles (patient in Case 2).

tion. During the past 2 years of married life coitus was practiced two to three times a week without contraceptives, yet there were no signs of pregnancy. He had no complaints regarding urination.

Physical examination was essentially normal, although no prostatic secretions were obtained by massage.

He was unable to produce emission of semen. Voided urine collected directly after the attempt showed 200 spermatozoa in each high power field, with occasional cells slightly active.

Endoscopy examination disclosed an intraurethral lateral lobe intrusion of the prostate similar to that often seen in 60-year-old men. A resection of this tissue was suggested but the patient elected to proceed with artificial insemination after it was demonstrated that specimens containing 200–300 highly motile spermatozoa could be secured by the method described above.

The wife, aged 28, had always had excellent general health. At the age of 12 menses began and recurred every 28 days. The only adverse finding in the diagnostic study was a total uterine length of 7.1 cm. and total cervical length of 3.5 cm. Daily vaginal smears demonstrated that ovulation occurred in each cycle during several months' observation. There was some deficiency of the luteal phase. Postcoital aspirations failed to disclose spermatozoa in the vagina, cervix, or endometrial cavity.

Specimens of semen obtained by the described methods were introduced once in each of 4 cycles, determined by vaginal smears. A small part of the specimen was placed by Dr. Kleegman just within the external cervical os and the rest was left in the vault of the vagina. The foot of the table was elevated and the patient was left in the supine position for half an hour following insemination. The inseminations were done on Day 11, Day 10, Day 15, and Day 12, respectively, of each of the 4 menstrual cycles. Excellent motility was observed in each specimen except for that used in the third month. Pregnancy resulted from the last insemination (Fig. 1).

Because the cytology study demonstrated poor production of luteal hormones, 200 mg. daily of oral progesterone was given orally as soon as pregnancy was diagnosed. Eleven weeks after the successful insemination, vaginal bleeding developed, accompanied by cramps. Intramuscular injections of 100 mg. of progesterone were given daily for 4 weeks and bed rest was ordered. The bleeding persisted in very scant amounts for 4 weeks but the patient apparently overcame the threatened abortion. The uterus was enlarged to the period of gestation which, at the time of this report, had progressed to the fourth month.

DISCUSSION

Total retrograde ejaculation occurs only in a small number of men who are affected with infertility. Absolute barrenness is the consequence, and yet the reward of conception may be earned by trial and perseverance by both patient and physician. It is believed that we have reported (A. B. P.) the first instance of 2 normal children conceived in and born to one mother. A third conception by this method is also recorded (S. K.).

Dr. I. Fischer and Dr. E. Coats are likewise submitting reports of 2 conceptions in one woman, 1 ending in abortion and the other in the birth of a normal child now 28 months old. Accordingly there are 5 known conceptions achieved by utilizing spermatozoa recovered from the bladder. Two children, now 3 and 9 months, were born to one mother (Case 1), another mother has 1 child 2½ years of age (Fischer and Coats), one patient is now pregnant (Case 2), and 1 had a miscarriage (Fischer and Coats). It would appear that consideration should be given to the technic when retrograde ejaculation is diagnosed. Furthermore, transurethral resection in young men should be undertaken only with the explanation and warning that retrograde ejaculation may be a sequela to such surgery.

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Testicular Biopsy: Which Infertile Patients Are Benefited?

Sheldon Payne, M.D., and Robert F. Skeels, M.D.

THROUGH THE WIDESPREAD USE of biopsy technics, several of the pioneers in the study of sterility have placed our knowledge of testicular histopathology and problems of male hypogonadism upon a sound basis. Also, in reading the literature, one gains the impression that testicular biopsy occupies an important place in the investigation of oligospermic and azoospermic patients. As we gained experience with the procedure in our practice, we became somewhat discouraged when it appeared that in many instances histologic examination of the testis was not contributing a great deal to the solution of the patients' infertility problems. The present review was undertaken with the hope of establishing some clinical criteria for the selection of patients for whom biopsy would give valuable guidance for specific therapy. It appeared that some of our patients could be spared the apprehension, expense, and occasional discomfort of this procedure, without seriously decreasing their chances for impregnation.

OLIGOSPERMIA

Using Nelson's[6] classification, the following biopsy patterns may be associated with oligospermia:

1. Disorganization, sloughing, or both;
2. Partial maturation arrest;

From the Shelton Clinic, Los Angeles, Calif.

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3. Partial fibrosis;
4. Germinal cell hypoplasia;
5. Normal testis.

None of these are necessarily pathologic entities, and no clues are obtained from the biopsy, as to etiology or specific treatment, which cannot be obtained by other clinical methods.

With the advent of the testosterone suppression-rebound concept, it was hoped that biopsy would afford a valuable method of selecting patients who

TABLE 1. Testosterone Suppression Treatment in 13 Oligospermic Patients Who Had Biopsies

Predominating histology pattern	*Highest pretreatment count* (Million/cc.)	*Highest posttreatment count* (Million/cc.)	*Pregnancy*
Germinal hypoplasia	5	6	No
Germinal hypoplasia	3	6	Yes (A.I.D.)[a]
Complete maturation arrest	0	0	No
Partial maturation arrest	3	Rare	Yes (A.I.D.)
Germinal hypoplasia	4	17	No
Germinal hypoplasia	14	15	No
Disorganization and sloughing	14	3	No
Germinal hypoplasia	15	12	Yes (A.I.D.)
Partial maturation arrest	10	14	No
Partial maturation arrest	3	16	No
Germinal aplasia	Very rare	Very rare	Yes (A.I.D.)
Germinal hypoplasia	5	Rare	Yes (A.I.D.)
Partial maturation arrest	1	13	Yes

[a] A.I.D. = artificial insemination, donor.

would respond to this treatment. Table 1 summarizes our results with testosterone suppression in 13 patients who had biopsies. We reserved the procedure for those patients with fairly severe oligospermia, and our end results have thus far been poor. Only one husband became a father; his count rebounded from 1 million to 13 million, while his sperm motility remained poor. Ordinarily, this amount of improvement would not be considered impressive except that in this case it resulted in conception. No other pregnancies occurred (except those whose wives later conceived by donor insemination).

Results have been similarly discouraging in a larger group of men who did not have the benefit of histopathologic evaluation. An occasional pregnancy has resulted, with or without a significant rebound; some counts improved a little but without conceptions. It is our tentative feeling that testosterone suppression-rebound is like other therapeutic modalities for male infertility; occasional successes are obtained, but its results are not consistent or impressive enough for its inclusion as a very specific or even valuable form of therapy.

We tend to agree with Simmons in finding it difficult to predict accurately the sperm count from the biopsy pattern. In several patients spermatogenesis in the biopsy appeared to be much more active than would be suspected from examination of the ejaculate, and these cases were not always associated with clinically apparent prostatitis or seminal vesiculitis, which might cause partial obstruction of the ducts. Also, occasionally a few spermatozoa were seen in the ejaculate of patients whose biopsy indicated aspermatogenesis. Although opinions vary as to what constitutes a normal semen picture, there are few who would deny that occasional conceptions occur, even with very low sperm counts. It appears, therefore, that it might be safer to base the prognosis for fertility in patients with oligospermia upon repeated semen evaluations.

The treatment of the oligospermic male usually includes the following procedures:

1. General measures—rest, adequate diet, nutritional supplements, and so on.
2. Elimination of tobacco and alcohol, discontinuation of jockey shorts, and so on.
3. Thyroid medication if indicated.
4. Treatment of infections of prostate, seminal vesicles, and so on.
5. Proper timing of intercourse, homologous insemination, and split-ejaculate insemination.
6. Empiric use of drugs and hormones such as testosterone, gonadotrophins, and the like.

Meticulous observation of the above principles will result in a fair number of conceptions. However, knowledge of the histologic pattern of the testis, in our experience, has not been a satisfactory guide to a more intelligent utilization of these measures.

AZOOSPERMIA

Normal-Sized Testes

Men with normal habitus and gonads of normal or only slightly smaller than average size associated with azoospermia may show one of three patterns in testicular biopsies:

1. Normal, indicating bilateral obstruction of the sperm passages.
2. Germinal cell aplasia.
3. Germinal cell arrest.

Formerly it was felt by some that the chief value of testicular biopsy lay in the differentiation of obstructive azoospermia from aspermatogenesis. Now, however, many authorities proceed immediately with the epididymovasostomy, without preliminary biopsy, in the obviously obstructed patient. The biopsy may be taken at the time of the corrective surgery.[2] Although this sequence may be satisfactory in most instances, it should be pointed out that Nelson[5] has reported the biopsies of 21 patients with obstructive azoospermia, and in 6 (28 per cent) spermatogenesis was rated as poor. One of our patients had sufficient clinical evidence to warrant the impression of an obstructive lesion, but biopsy showed also complete maturation arrest. Certainly when there is doubt about the presence of a block, preliminary biopsy should be done.

Reported success rates for epididymovasostomy vary widely, but O'Conor's recent estimates of 20 per cent successful anastomoses and 10 per cent fertility seem to be fairly representative. Roughly 25 per cent of the patients with azoospermia and normal-sized testicles will have obstructive lesions.[6, 8] The remaining 75 per cent will suffer from either germinal aplasia or maturation arrest, both of which are hopeless lesions with our present therapeutic armamentarium. Thus, for a patient with azoospermia and normal-sized gonads, the immediate prognosis can be only about 2.5 per cent favorable to fertility. The patient should be made aware of this gloomy outlook prior to biopsy and other diagnostic procedures, for many will prefer to proceed immediately with donor insemination or adoption plans.

Small Testes, Postpuberal Atrophy

Gross atrophy and fibrosis of the testes occurring after puberty is clinically apparent by the very small size of the testes with otherwise normal maturation of the secondary sex characteristics. The cause of this type of testicular destruction may be inflammatory (mumps orchitis), or more often is not apparent (sclerosing tubular degeneration). The process is irreversible, and the biopsy gives no hint as to the etiology or treatment, although it will give information regarding the extent that the Leydig cells are involved in the process. McCullagh, Gold, and McKendry could find no histologic criteria with which to differentiate primary testicular failure from that secondary to acquired organic pituitary disease.

EUNUCHOIDISM

Patients with a eunuchoidal habitus and other evidences of androgen deficiency may show the following histopathologic patterns:

1. Complete absence of testicular tissue in the scrotum.
2. Eunuchoidal type of Klinefelter's syndrome, with hyalinization and fibrosis of the tubules and Leydig cells. These are instances of primary testicular failure. The testes are very small and assay of urinary gonadotrophins is elevated.
3. Hypogonadotrophic eunuchoidism. Testes in these patients are also very small, and the biopsy pattern is that of an immature, unstimulated, prepuberal testis. Urinary gonadotrophin assay is low or absent. Large doses of chorionic gonadotrophin will produce genital maturation and spermatogenesis in these patients. A therapeutic trial with chorionic gonadotrophin may be employed to verify the diagnosis of hypogonadotrophic eunuchoidism in the absence of urinary gonadotrophin assays or testis biopsy.[1]
4. Eunuchoidism with spermatogenesis. One of our patients was found to be a "fertile eunuch," as recently described by McCullagh, Beck, and Schaffenburg. He had a eunuchoidal habitus and other evidences of androgen deficiency, but spermatozoa were seen (4 million per cc.) in the occasional scanty ejaculate. Gonadotrophin (F.S.H.) assay was normal and 17-ketosteroids were 12 mg. per 24 hours. Testes were of essential normal size. Biopsy revealed the presence of seminiferous tubules and hypospermatogenesis, but interstitial tissue was almost devoid of Leydig cells. This syndrome should also be included in the differential diagnosis of oligospermia, and it can be suspected clinically by the presence of eunuchoidism with essentially normal-sized testes.

CONCLUSIONS AND SUMMARY

Although testicular biopsy will continue to be a valuable scientific tool, from the point of view of the clinician and the welfare of the patient, it appears that the major indication for testicular biopsy is the azoospermic patient with normal-sized testes, in whom there is doubt as to the presence of an obstructive lesion. This is essentially the viewpoint held by some early in the history of testicular biopsy.

The eunuchoidal form of Klinefelter's syndrome can be nicely differentiated from hypogonadotrophic eunuchoidism by testis biopsy, but when facilities are available for urinary gonadotrophin assay, biopsy is not essential.

In other syndromes of testicular dysfunction, especially those associated with oligospermia, biopsy is not an important clinical aid. Either the problem can be elucidated clearly enough by other clinical methods, or, as is often the case, the physician can predict a hopeless situation, no matter what the biopsy might show.

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DISCUSSION

DR. CHARNY: Discussion of this paper will be opened by Dr. William O. Maddock, Detroit, Michigan.

(Discussion of the paper by Doctor Skeels was read by Doctor Maddock.)

DR. CHARNY: The floor is open for discussion.

DR. F. A. SIMMONS, *Boston, Massachusetts:* I think this is a very thought-provoking paper. I am a clinician and I do testicular biopsies. I think that the title question is answered—there is no benefit usually to the patient who is infertile due to small testes. I agree entirely with Doctor Maddock's rebuttal.

I would like to make one point: In 37 men with bilateral gonorrheal epididymitis who have had bilateral testicular biopsies all had normal histologic findings.

DR. L. M. BRODNY, *Boston, Massachusetts:* I would like to make one comment on the question of the philosophy of treatment of patients. I think in all this work one must approach the subject not from the point of view that the patient will benefit, but that a group of patients will benefit. We have had some difficulty in one of the hospitals where I work about introducing certain types of new procedures, and the argument was brought up: "Well, here is a procedure that a patient—the particular patient—will not benefit from." However, I feel that unless the men doing the procedures can develop the technics, unless the pathologists can learn how to read the specimens—and you can take that all down the line, if you will set up a whole group of patients—you will find unless you carry out many of these procedures you are not giving the patient the benefit he deserves.

DR. W. O. NELSON, *Iowa City, Iowa:* I have no quarrel with Dr. Skeels' dissertation. Many of us felt for a good many years that perhaps one of the real values of testicular biopsies was in the screening out of those individuals in whom the prognosis was hopeless and being able to assure them that any form of treatment that we now have available will be quite useless.

When one encounters individuals who, having passed through a whole gamut and whole galaxy of treatment—vitamins, hormones, and various other even less specific procedures—finally come to biopsy, and one finds that the testis, for example, is completely devoid of germ cells, one wonders if that patient would not have been spared far more discomfort and expense if a biopsy had been done originally and the hopeless condition assured immediately.

There is one other comment I should like to make—that is, if one is to attempt to correlate the biopsy picture with the sperm count, it is important to remember that the picture one would see in the testis would be reflected in the sperm count some weeks after the biopsy. Now, ordinarily when people have attempted correlation of the sperm picture in the biopsy, they have made an attempt to do the correlation on the basis of sperm samples that were studied weeks or months before the biopsy was taken. If one attempts a correlation between the biopsy picture and the sperm count taken 5, 6, or 7 weeks after the biopsy, then the correlation is considerably better—not perfect by any means, but definitely better.

DR. CHARNY: It seems to me, assuming the testicular biopsy is accepted as a sound diagnostic procedure, one shouldn't hesitate to do it, any more than he would to do the diagnostic procedures in an individual who had an incurable carcinoma.

DR. SKEELS: I wish to thank the discussants. My main interest in presenting the case actually was to hear some of the controversial discussion. I certainly am not trying to be an iconoclast and disparage the very wonderful scientific work that has gone on ahead and been done through the use of the biopsy tool.

From the philosophic point of view, one certainly can't. I don't want to argue philosophy of medical practice. We try to be scientific in our private practice, but I do feel testicular biopsy isn't something one can order or request as haphazardly as one might request a Wassermann or something like that.

It is my feeling that in Doctor Nelson's example of the biopsy revealing the testis to be devoid of germ cells, usually by using careful clinical criteria one can almost predict the biopsy pattern from repeated examinations of the semen. Again I want to emphasize the fact that certainly the biopsy will be a very important research tool, and that we privately practicing clinicians will continue to look to the research institutions for guidance in our treatment, but in our private practice I find it a little bit difficult to urge patients to go through with a rather drastic diagnostic procedure unless the benefits are more immediately apparent to the patient himself.

Erratum

In the paper by Hendricks and Zuspan, "The Possible Role of Hyperheparinemia in the Infertile Couple," which appeared on page 449 of Volume 5 (September–October 1954), the caption for Fig. 1 is incorrect, and should read as follows:

Protamine-heparin titration in ten Wassermann tubes. Absence of clot formation above 0.12 indicates hyperheparinemia.

Pregnancy after Conservative Surgery for Endometriosis

A Statistical Study

G. E. Norwood, M.D.

An understanding of the relation of endometriosis to fertility has become progressively more important. To add to the rapidly growing significant statistics and impressions available on this subject, an analysis of the histories of 54 married female patients in the childbearing years is herewith presented. Each of these patients has been under the medical care of the author for varying lengths of time during the past 15 years. The discussion and the conclusions derived from this study and presented here relate specifically to the results of conservative surgery for endometriosis, in which the anatomic childbearing function is preserved. Such surgery was done in each of the 54 patients and follow-up studies were carried out for at least 1 year.

MATERIAL

All patients had attempted to become pregnant prior to and following the surgery, and were under routine treatment for infertility. The male fertility factor was checked in each case, both prior to and following surgery, and was found to be within normal limits.

All premature and full-term infants in this series were born alive and are now living.

In 23 cases (Series A) the operation was for endometriosis alone; in 31 cases (Series B) surgery was carried out for endometriosis and for accompanying pathologic conditions.

The patients are classified, also, as to the incidence of pregnancies: Group

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I consists of 21 patients who had been pregnant previous to surgery; Group II, of 30 patients who had not been pregnant previous to surgery; and Group III, of 3 patients who never became pregnant.

TABLE 1. Endometriosis

Grade	Involvement
1	Pelvic peritoneum only
2	Unilateral ovarian
3	Unilateral ovarian and pelvic peritoneum
4	Bilateral ovarian
5	Bilateral ovarian and pelvic peritoneum

The degree of involvement (Table 1) of endometriosis is classified in a manner similar to that outlined by Bacon.[1]

The surgery in the case of each of 51 patients was an abdominal laparotomy. In 3 patients a culdoscopic examination was done and resection of endometriosis was performed through the colpotomy incision. The laparotomies included resection of existing endometriosis and treatment of other pathology as found in the Series B cases and the correction of contributing poor pelvic mechanics. A dilatation and curettage was also done in most cases.

TABLE 2. Repeat Surgery for Endometriosis

Grouping of patients	Year of conservative surgery and age of patient	Age of patient at pregnancy		Year of total surgery and age of patient
		Presurgery	Postsurgery	
A II 3	1938–31	..	Full term 32	1943–36
B II 1	1944–36	..	Abort. 38	1952–44
B I 3	1945–31	Full term 24	Abort. 32, 36	1952–38

In only 3 cases was surgery necessary a second time (Table 2). A hysterectomy was done in each case.

The 3 cases in Group III with no pregnancies previous to or since surgery are shown in Table 3.

Keeping in mind the patients' ages and the fact that the average time between conservative surgery and the first conception following operation was 2.6* years in the other 51 patients, the prospect for conception in the first patient is poor, in the second fair, and in the third good.

* This figure remains very close to the 2.2 years as computed at the time of a previous report[2] of the cases included during the first 10 years of this study.

STATISTICAL STUDY

Since the time factor is considered so important in the etiology of endometriosis, it is interesting to note (Table 4) that in these 54 patients the average interval between the menarche and marriage was 9.9 years, between the menarche and attempted pregnancy was 11.1 years, and between the menarche and surgery was 16.4 years. The average interval between marriage and the first attempt at pregnancy was only 1.2 years, the majority (68.5 per cent) of the patients having attempted pregnancy during the first year of marriage (Fig. 1).

TABLE 3. Summary of 3 Patients Who Had No Pregnancy

Series and grade of patient	*Age at menarche*	*Age at marriage*	*Age attempted preg.*	*Year of surgery and age of patient*	*Patient's present age*
B5	14	23	33	1946–35	42
B2	13	26	27	1947–30	35
A1	11	23	23	1952–28	28

Table 5 presents a comparison of average ages at the events relative to pregnancy in the various groups as subdivided according to the occurrence of pregnancy. Those patients not pregnant either prior to or since surgery (Group III), when compared to either Group I and/or Group II, were, on the average, younger at the menarche, older at marriage, and considerably older when pregnancy was attempted and when surgery was done. Significantly, also, those who became pregnant previous to surgery (Group I) had a later menarche, and an earlier marriage and attempted pregnancy than those who did not become pregnant until after surgery (Group II); due to absence of pregnancy, surgery was performed earlier in Group II.

TABLE 4. Average Ages of 54 Patients at Successive Events Relative to Pregnancy

	Average age	*No. years after preceding event*	*Total years elapsed*
Menarche	13.1	..	..
Marriage	23.1	9.9	..
Attempted pregnancy	24.2	1.2	11.1
Surgery	29.5	5.3	16.4

Table 6 presents further data on the 54 patients according to their age at the time of surgery, and correlates these age groups with the outcome of pregnancy subsequent to surgery. All the 9 patients in the youngest age group became pregnant. In the next two older age groups, there was a drop to approximately 95 per cent, while pregnancies in the 35–39 age group

TABLE 5. Comparison of the Average Ages of the Various Age Groups at Events Relative to Pregnancy

Classification	*Menarche*	*Marriage*	*Attempted pregnancy*	*Conservative surgery*
Group I	13.4	21.3	21.6	29.7
Group II	12.9	24.4	25.2	29.2
Group III	12.6	24.0	27.6	31.0
Groups I and II	13.1+	22.7	23.4	29.4
TOTAL GROUP	13.1	23.0	24.2	29.5

dropped to 87.5 per cent. In regard to wastage, the lowest percentage was in the youngest age group and the highest was in the 30–34 age group. The total number of pregnancies following surgery was 94—32 abortions, 7 premature babies, 54 full-term babies and 1 patient is now pregnant.

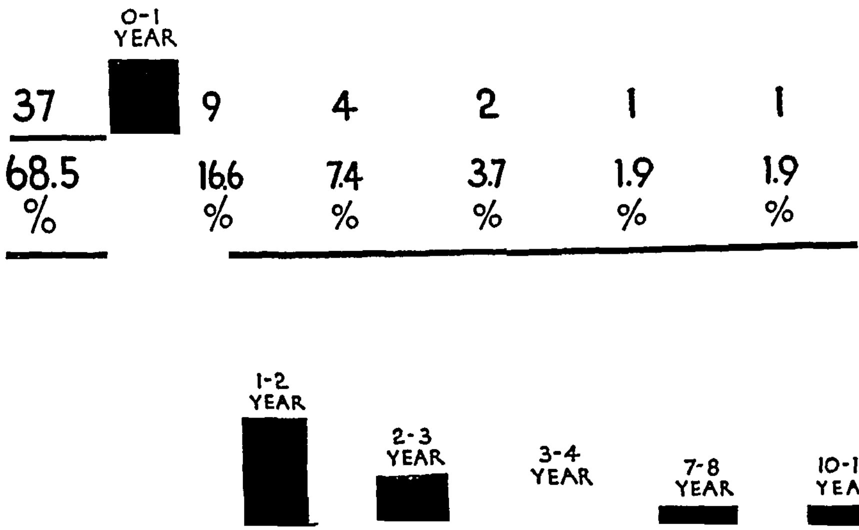

Fig. 1. Number of years after marriage when pregnancy was attempted.

Kelly and Schlademan give 88 per cent as the absolute fertility factor. Figure 2 presents the absolute fertility curves for the total group and also for Series A and B. As a result of conservative surgery, the absolute fertility of the total group of 54 patients was raised from 35.2 per cent to 94.4 per cent.

To determine further the effect of surgery for endometriosis on postoperative fertility, Table 7 presents the postoperative pregnancies of Series A. The group is divided according to the classification of the degree of endometriosis. No statistical evidence is found that is specifically significant.

Table 8 sets forth a comparison of the types of pregnancy before and after

TABLE 6. Postsurgery Pregnancies According to Age Groups

Age (yr.)	*No. of patients*	*Pregnancies (%)*	*Pts. not pregnant*	*Total pregnancies*	*Abortions*	*Premature*	*Full term*	*Now pregnant*	*Fetal wastage (%)*
20–24	9	100.0	0	21	3	4	14	..	14.3
25–29	18	94.5	1	31	12	..	19	..	37.7
30–34	19	94.7	1	31	14	1	16	1	45.2
35–39	8	87.5	1	10	3	2	5	..	30.0
TOTAL	54	94.4	3	93	32	7	54	1	31.8

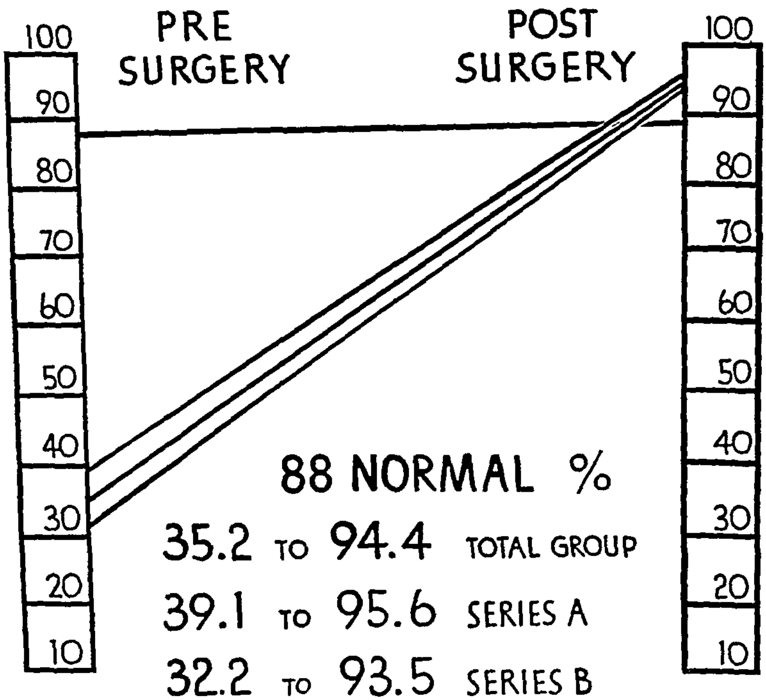

Fig. 2. Absolute fertility curves.

surgery in the patients who were operated on for endometriosis alone (Series A) and of those operated on for endometriosis and associated pathology (Series B). The reduction in the percentage of abortions after surgery was less in Series B than in Series A, these percentages being 2.7

and 16.2 per cent, respectively; and, if the pregnancies producing living infants are added to the present pregnancy, Series A has a 75 per cent potential while the figure for Series B is only 59.3 per cent. There were no ectopic pregnancies postsurgery, in contrast to 2 (14.2 per cent) in Series B previous to surgery.

The types of pregnancies in Group I and II are presented in Table 9. In Group I, after surgery, abortions were reduced by 5.1 per cent; there

TABLE 7. Pregnancies in Series A Patients, with Reference to Degree of Endometriosis

Degree of endometriosis	*Abortions*	*Premature*	*Full term*	*Now pregnant*	*No. patients*	*Fetal wastage (%)*
Peritoneum only	2	..	9	1	9 (1 never preg.)	16.7
Unilateral ovarian	2	1	1	..	..	50.0
Unilateral ovarian and pelvic peritoneum	1	..	10	..	6	9.9
Bilateral ovarian	..	..	..	..	..	..
Bilateral ovarian and pelvic peritoneum	5	..	8	..	6	38.4
TOTALS	10	1	28	1	23	40.0

TABLE 8. Types of Pregnancy in Series A and B Patients before and after Surgery

	Series A (23 patients)				*Series B* (23 patients)			
Type of pregnancy	*Before surgery*		*After surgery*		*Before surgery*		*After surgery*	
	No.	%	*No.*	%	*No.*	%	*No.*	%
Abortions	7	41.2	10	25.0	6	42.9	22	40.7
Ectopic	..	..	..	..	2	14.2	..	..
Premature and full term	10	58.8	29	72.5	6	42.9	32	59.3
Now pregnant	..	..	1	2.5	..	..	..	..
TOTALS	17	100.0	40	100.0	14	100.0	54	100.0

were no ectopic pregnancies in comparison to 6.3 per cent presurgery; and the pregnancies producing living children, including the one now pregnant, were up 11.4 per cent. There were, however, 31 pregnancies in Group I postsurgery against 32 presurgery. In Group II, there were 63 pregnancies postsurgery in comparison to 31 in Group I, and the wastage was 2.2 per cent less.

An appraisal of the percentages of living children and of wastage in the four productive classifications (Table 10) gives the following pre- and postoperative figures based on the number of patients under each classification:

Group II shows the greatest benefit from surgery, with only half as much wastage as productive pregnancies. Group I shows only moderate increase

TABLE 9. Types of Pregnancies in Groups I and II after Surgery

Type of pregnancy	Group I				Group II after surgery	
	Before surgery		After surgery			
	No.	%	No.	%	No.	%
Abortions	13	40.6	11	35.5	21	33.3
Ectopic	2	6.3	..	..	..	..
Premature and full term	17	53.1	19	61.3	42	66.7
Now pregnant	..	..	1	3.2	..	..
Totals	32	100.0	31	100.0	63	100.0

TABLE 10. Percentages of Living Children and Wastage to Number of Patients

Classification	Presurgery				Postsurgery			
	Productive		Wastage		Productive		Wastage	
	No.	%	No.	%	No.	%	No.	%
Group I	16	76.2	15	71.4	19	90.5	11	52.4
Group II	0	0.0	0	0.0	42	140.0	21	70.0
Series A	10	43.5	7	30.4	29	126.1	10	43.5
Series B	6	19.3	8	25.8	32	103.2	22	70.9

in productive pregnancies after surgery, but a very evident drop in wastage. Series A shows a marked increase in productive pregnancies, postsurgery, and a small percentage rise in wastage and in Series B percentage of preoperative wastage, which was higher than the productive, shows a reversal to 103.2 per cent to 70.9 per cent after surgery.

CONCLUSIONS

From a study of 54 patients who have had conservative surgery for endometriosis, the following conclusions (based on statistical evidence) can be drawn:

1. Fertility was lessened by increased age at the time of, and by longer elapsed time between, successive events related to child bearing.

2. Conservative surgery for endometriosis in these 54 patients markedly

increased their absolute fertility, to above the usual average of 88 per cent.

3. No significant difference in postoperative fertility was evidenced by those patients having varying degrees of endometriosis.

4. Surgery gave better results in cases where endometriosis was the only pelvic pathology, as measured by the comparatively greater increase in postoperative fertility of Series A.

5. Accompanying pathology, with its residual effects and the necessity for more extensive surgical procedure, was responsible for the lower postoperative fertility of Series B.

6. An appraisal of the pre- and postoperative percentages of productive pregnancies and wastage to the number of patients shows very favorable operative results.

7. As a result of these significant statistics, impressions, and conclusions, a more logical selection of patients for conservative surgery for endometriosis should be possible.

SUMMARY

In this study of 54 patients, 51 (94.4 per cent) became pregnant one or more times after conservative surgery for endometriosis. Of the 3 who did not, 2 had other associated pathology.

There have been 94 pregnancies since surgery, with the following outcomes: 32 abortions, 7 prematures, 54 full-term infants (61 living children) and 1 patient is now pregnant.

The 61 living children are distributed as follows: One patient has had 5 (4 premature and 1 full term); 3 patients have had 3 each; 14 patients have had 2 each, all full term except that 1 of these patients had 2 premature; 19 patients have had 1 each with only 1 of these being premature. There are, therefore, 37 patients with living children at the end of this 15-year study.

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DISCUSSION

Dr. Carl E. Johnson, *New Haven, Conn.:* I heartily agree with Doctor Norwood's opening statement that an understanding of the relation of endometriosis to fertility has become progressively more important.

Doctor Norwood has referred to Doctor Bacon's study at the Free Hospital for Women in Brookline, Mass. This consisted of 115 cases of intra-abdominal pelvic endometriosis, diagnosed grossly and histologically and treated at laparotomy, with anatomic preservation of the childbearing function.

In Bacon's group 112 patients were married and under the age of 40. Of these 30, or 26.8 per cent, have children after surgery for endometriosis. In Norwood's cases all patients were anxious to become pregnant. In answering Bacon's questionnaire regarding the desire to become pregnant, an appreciable number of answers were in the negative. In this respect the two studies cannot be compared. Doctor Norwood has found that as a result of conservative surgery, the fertility of the total group of 54 patients was raised from 35.2 per cent to 94.4 per cent. This, then, is well above the arbitrary absolute fertility rate of 88 per cent that was selected by Kelly and Schlademan, and is a remarkable achievement.

Eleven of the 41 cases, or about 25 per cent, in Bacon's series that required further operations were found to have adhesions, chronic salpingitis and oophoritis, retention cysts, and fibroids, but no endometriosis was demonstrable. This confirms what many of us have found—that the disease is not always progressive —and is another point in favor of conservatism.

Both Norwood and Bacon agree that no correlation between age and prognosis for symptomatic relief can be shown, but that in general the younger the patient the better is the outlook for subsequent pregnancy.

In his third conclusion Doctor Norwood states, "No significant difference in postoperative fertility was evidenced by those patients having varying degrees of endometriosis." Similarly, Bacon concludes, "The prognosis for symptomatic relief and future pregnancy does not seem to be affected by age, symptomatology, the location and extent of the disease, or the magnitude of the conservative operation performed."

DR. DAVIS: Thank you, Dr. Johnson. This interesting paper is now open for general discussion.

DR. Q. E. FORTIER, *Minneapolis, Minn.:* Since we have been doing a good many culdoscopies, I have some questions. One is that very frequently we find small endometrioses in the pelvis or in the ovaries or in the back of the uterus, and we wonder whether or not those should be removed when very small, and whether or not this will help in the ultimate outcome and handling of the patient.

The other question—in doing a hysteroscopy on a student for dysmenorrhea, we found multiple small nodules that appeared to be beneath the endometrium or in areas where there was little endometrium, and on curettage I found little nodules, and on culdoscopy I found nothing that suggested endometriosis on the pelvis. On sectioning these, which were about 2 or 3 mm. in diameter, they were found to be fibrous nodules containing endometriotic material. Will you discuss this condition?

DR. S. H. STURGIS, *Boston, Mass.:* I would like to ask him the sixty-four dollar question—that is, from his experience can he explain to us or give some explanation why resection of small nodules in endometriosis will increase the fertility rate?

DR. BYRON BUTLER, *Phoenix, Ariz.:* I would like to know, Doctor Norwood, whether you did suspension operations in your cases, and, if so, what type of procedure you did.

DR. J. M. SINGLETON, *Kansas City, Mo.:* I would like to ask Doctor Norwood whether he has any explanation for the etiology of endometriosis and if he saw any relationship between the habitual use of menstrual tampons in this large series of patients.

DR. DAVIS: That is the one hundred and twenty-eight dollar question.

DR. IRVING STEIN, *Chicago, Ill.:* Not to drag out discussion, but I didn't hear the doctor mention whether he did any presacral sympathectomy for relief of pain.

DR. NORWOOD: I first want to thank the discussants for their kindness. I am not certain as to this first question as to these nodules, which were described as fibrous nodules with endometriotic material inside. Were those obtained by curettage?

DR. FORTIER: Yes.

DR. NORWOOD: I have never seen such, so I cannot discuss it in any specific way. We do know, however, that we have an endometrial endometriosis, in which endometriosis occurs not only in the stroma but also in the endometrial glands themselves and in which you get the same type of process that you find in endometriosis in any other area.

On culdoscopy—without doubt I think all of us are using it more—in these three cases I mentioned, where I did a culdoscopy examination and found endometriosis, a specific nodule in the cul-de-sac or even in the peritoneum near the incision, we excised those through that incision and did not do an abdominal laparotomy.

Doctor Sturgis asked why resection of small nodules of endometriosis improves fertility. My own impression is that if there is a nodule of endometriosis close by the cervix with the inflammatory process that goes on at the peak once a week, there is a cervicitis secondary to it, and therefore removing all of it improves the cervical factor. There may be other reasons for that, too.

Doctor Butler asked if we did suspensions. Yes, a few in these cases. My type is a modified Webster-Baldy. But I do it extraperitoneally in most cases and therefore do not have the problem inherent in the old type of Webster-Baldy.

Doctor Singleton asked whether tampons had anything to do with the etiology. I don't know.

Doctor Stein asked if we did presacral neurectomies. Yes, but not in too many cases, because a large percentage of patients in these groups did not have dysmenorrhea. That is one of the fallacies, I think, we are all beginning to recognize. It is not necessary. In fact, in many cases endometriosis does not cause dysmenorrhea.

Suggested Standard for Karyopyknosis

Use in Hormonal Reading of Vaginal Smears

George L. Wied, M.D.

"KARYOPYKNOSIS" AND "PYKNOTIC NUCLEUS" are terms very often used in exfoliative cytology. By derivation from the Greek word "pyknosis," a pyknotic nucleus should be a dense mass. In exfoliative cytology, we speak of a nucleus condensed by degenerative change and shrunk to a dense, structureless mass of chromatin as being pyknotic.

SIGNIFICANCE OF KARYOPYKNOSIS

The presence of pyknotic nuclei in exfoliated, superficial squamous epithelial cells in vaginal smears is generally considered an indication of an estrogenic activity, based on the assumption that cornification of squamous epithelial cells of the vagina is a function of estrogenization.[6] Roth has recommended a "kolpopyknogram" for the control of follicular hormone effects. Of the three cellular indices—(1) the superficial cell index (the relation of superficial squamous epithelial cells to all other squamous epithelial cells); (2) the acidophilic cell index (the relation of superficial acidophilic cells to superficial basophilic cells); and (3) the karyopyknotic index (the relation of superficial cells with pyknotic nuclei to superficial cells with vesicular nuclei)—the karyopyknotic index may be considered the most reliable in cytodiagnosis of an estrogenic activity. Its greatest importance is in follow-up cases where cyclic changes are examined or where effects of administered sex hormones are tested by the vaginal epithelial reaction, one of the most sensitive indicators of the human body.[21]

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This work was supported by the Goldblatt Cancer Research Fund and an Institutional Grant from the American Cancer Society.

CRITERIA FOR KARYOPYKNOSIS

Assuming the cornification of vaginal epithelial cells to be a more or less specific function of estrogenization, we are confronted with the difficulty of a lack of general agreement among the authors; primarily, as to the definite criteria for karyopyknosis, and secondly, as to whether the staining procedure, especially the nuclear staining with Harris hematoxylin, can produce pseudopyknotic nuclei if overstaining takes place. The disagreement of some authors concerning the subjective diagnosis of a pyknotic nucleus seems to be sometimes very broad. de Allende and Orías report a karyopyknotic peak value up to 90 per cent (45 to 90 per cent), whereas Wachtel and Plester could find peak values of only 50 per cent (30 to 50 per cent) at the time of ovulation during the normal menstrual period. We made similar observations in our own laboratory, where different interpreters of the smears computed different percentages of the karyopyknotic index because of subjective differences in their opinions of what exactly constituted a truly pyknotic nucleus.

In order to eliminate as far as possible the subjective factor in the interpretation of smears, the cytologist should use a method which objectively distinguishes pyknotic from translucent or vesicular nuclei. For the purposes of exfoliative cytology, it is not too important to discuss the morphology of pyknosis of nuclei or the question of whether a structureless but still translucent dense nucleus should be called pyknotic or not.

But *it is important to establish an objective standard* by which karyopyknosis can be universally recognized and defined for practical cytologic smear diagnosis. We propose the adoption of an indicator with which we can observe a sudden change from dark red to light red in a nucleus which has progressed to a sufficiently dense (pyknotic) state.

METHOD AND RESULTS

The smears are prepared following the fixation and staining procedure of Papanicolaou. For the hormonal reading we use vaginal secretions from the fornix vaginae only. After fixation, staining, and mounting with balsam with a very thin coverslip, the smears are examined under a phase-contrast microscope. Due to a specific phaseoptical phenomenon, dark, dense bodies shine with a very light red color, whereas the less dark, translucent, and vesicular nuclei retain the original dark color of the stained chromatin.

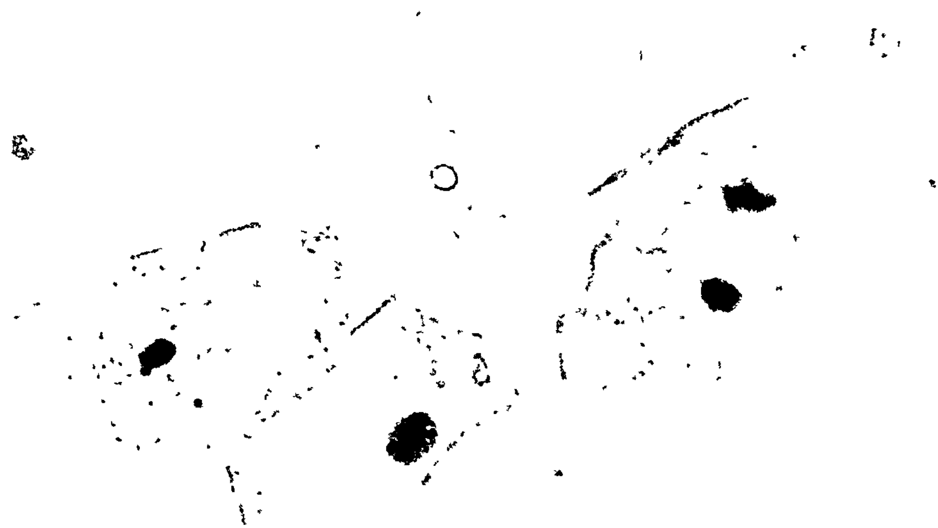

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Fig. 1. Photomicrograph of 5 squamous epithelial cells in a vaginal smear Only 1 of these cells is truly cornified. The pyknotic nucleus shone a bright red due to a specific phaseoptical deviation of the light over dense corpora (Zeiss Phasemicroscope, × 40.)
Fig. 2. Photomicrograph of the same cells as shown in Fig 1, with a microscope without phase-contrast. The pyknotic nucleus of the cornified cell is demonstrated here in its original dark, opaque condition, showing no intranuclear structure. The smear is stained by the procedure of Papanicolaou

In 1951, when we first observed this phenomenon, we complained to the producers of the phase-contrast microscope, because we thought this obvious deviation of the light might decrease the value of the phase-contrast microscope when used for stained material. But then we made a "virtue of this evil," and have used this phaseoptical phenomenon as an indicator since 1952, with the best results.

Figure 1 shows fixed and stained squamous epithelial cells under the phase-contrast microscope with the selective shining light of the darkest and most dense nucleus; Figure 2 shows the staining reaction of the nuclei as seen under the normal microscope. Using this diagnostic aid, even the least experienced in reading smears is sufficient to make accurate counts of the karyopyknotic index. Another advantage of this phaseoptical aid is that the interpreter can use the low dry objective of the microscope (10× or 20×). This makes it possible to examine the 300 cells required for the count in a much shorter time.

SUMMARY

The value of the karyopyknotic index for the cytologic diagnosis of estrogenic activity is discussed. A technic for a more objective interpretation of the smears by means of a phaseoptical standardization of pyknosis is described.

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Fellowship Program

The Population Council, Inc., of New York announces an expanded program of fellowships for advanced training in the study of population. Fellowships will be at the predoctoral and postdoctoral levels. They will be available for study in universities in the United States and elsewhere for the academic year 1955–56.

Preference will be given to qualified applicants from countries other than the United States and Europe. Fellows will normally receive support for full-time work for a period of one year. The basic stipend of $2500.00 per year may be supplemented to provide for travel expenses, maintenance of dependents, and for other exceptional expenses. Somewhat larger stipends may be granted to postdoctoral than to predoctoral Fellows.

Applications for the academic year 1955–56 should be received before March 1, 1955. Requests for further information and for application forms should be addressed to The Population Council, Inc., 230 Park Avenue, New York 17, N. Y.

Conception in a Heifer after Deposition of Semen in the Abdominal Cavity

Odd Skjerven, V.M.D.

THE GENERALLY PRACTICED METHOD of artificial insemination in cattle consists of introduction of semen directly into the uterine cavity. In certain animals with a narrow cervix, uterine insemination is not possible, for which reason the semen must be deposited in the vagina or cervix. Experience has been that the conception rate in these animals is lower than in animals with a passable cervix.

In animals with a nonpassable cervix which have been repeatedly inseminated without becoming pregnant, we have at our institute tried another method of uterine insemination. A sharp cannula attached to the inseminator is introduced through the vaginal wall. The tip of the cannula is then guided by rectal palpation introduced through the uterine wall into the uterine cavity where the semen is deposited. Two of three heifers which had been repeatedly unsuccessfully inseminated by the cervical method became pregnant at our first attempt with the described technic. It was, however, difficult to ascertain the position of the cannula after its entrance of the uterine wall.

At the time of estrus the physiologic movements of the oviducts and their mesenterium tend to transfer small particles placed in the neighborhood of the ovary to the lumen of the fallopian tube. Thus it has been shown that *Ascaris* ova, dyes, or carbon granules placed in different locations in the abdominal cavity may pass into the uterus via the fallopian tubes.[1] Since conception takes place in the tubal infundibulum, it ought to be of no consequence *how* the sperms reach the infundibulum supposing their pres-

From the Department of Sexual Physiology and Sexual Pathology, Norwegian Veterinary College, Oslo, Norway.

ence at the time of ovulation. It was decided to try insemination by depositing the semen into the abdominal cavity.

The first attempt was performed on a nulliparous heifer with normal sexual behavior. By rectal palpation the mature follicle was located in the left ovary. The same equipment as previously described was introduced intraperitoneally through the vaginal fornix near to the posterior os. By rectal manipulation the left ovary was moved towards the tip of the cannula and the semen then deposited. The heifer conceived and the embryo has so far developed normally in the left uterine horn, the animal this day being eight months' pregnant. No reaction was observed from the peritoneum. The semen employed was obtained from an artificial insemination center and was diluted with egg yolk citrate and antibiotics.

It is too early to predict whether or not deposition of semen in the neighborhood of the ovaries will prove to be of practical value in artificial insemination of cattle. So far we regard this single case as a curiosity. Further investigations will be required to elucidate the risk of peritoneal infection by the technic employed. This risk, however, ought to be minimal when semen to which antibiotics have been added is used. Moreover, the bovine peritoneum is very resistant to infection.

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Cervicohysterosalpingography

Preliminary Report on a New Cannula and Technic

Robert G. Hunter, M.D., and George W. Henry, M.D.

As a word grows in length, so usually grows the obscurity of its meaning. The word hysterosalpingography has been in use for many years. There are those who would claim that the addition of "cervico" is unnecessary, the cervix being a part of the uterus. But the cervix has been bypassed and neglected as an object of study until recently. With new instruments and technics, this canal may now be studied, adding to the already extensive work that has been done in investigating the uterine cavity and fallopian tubes. We feel that the term cervicohysterosalpingography is a logical one.

Very few reports of radiographic studies of the cervical canal have appeared in the literature. Asplund published a report in 1951 of observations after examining approximately 1000 patients. Fullenlove reported 300 patients in 1952. No other significant reports have been found.

Until 1951 the methods used to study the cervical canal were: (1) direct vision, (2) biopsy, and (3) cervical secretions. Now, with the new method described below, the cervical canal, an area hitherto virtually unexplored, is open to study and its physiology, its pathologic lesions, and its part in infertility may now be explored with greater certainty.

The primary difficulty has been a simple, completely satisfactory instrument. The cannula in common use is a tube of small caliber about 32.5 cm. long, with ring handles at the proximal end and curved at its distal end. A rubber olive is placed about eight cm. from the distal end and held in

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From the Department of Obstetrics and Gynecology and the Department of Radiology, The Medical Group, Honolulu, Hawaii.

place with a collar and set screw. The cannula is inserted into the cervical canal and advanced until the olive enters the external os where it fits like a cork in a bottle. Countertraction is applied with one or two tenacula about the vaginal portio of the cervix. Radiopaque material is injected with a syringe. The cervix has been completely bypassed and the contrast medium appears first in the uterine cavity.

The instrument used by Asplund is a complicated apparatus. At the distal end of the cannula there is a double cup or cap which is applied to

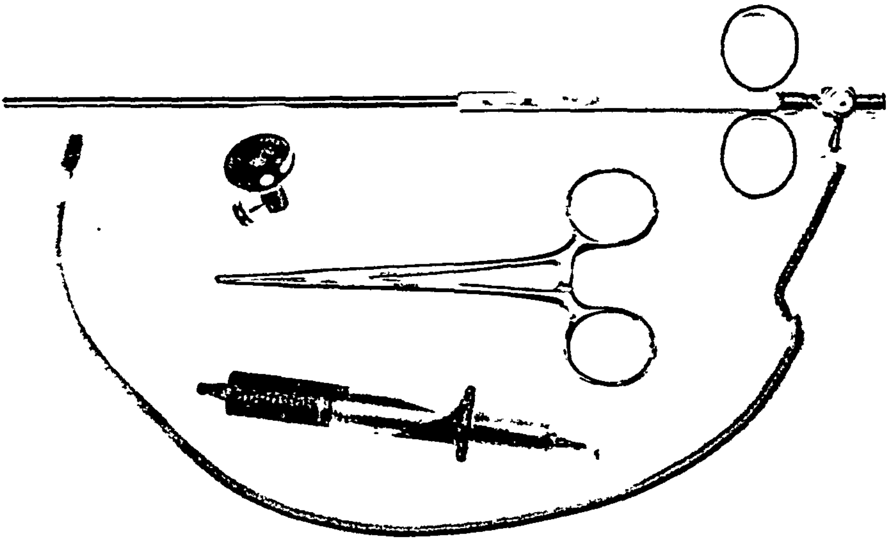

Fig. 1. Parts of new cannula designed for use in cervicohysterosalpingography.

the vaginal portion of the cervix; the air is withdrawn by vacuum pump, thereby applying the cannula to the cervix. Fullenlove's instrument is the conventional cannula with the olive placed near the tip; the external os is dilated as pressure is made on the cannula. Another instrument was presented at the First International Congress on Fertility in Rio de Janeiro in 1951 by a physician from Israel. It was essentially a straight cannula with a metal flange and a cuff of foam rubber.

The instrument used in the following studies was first designed in 1947, following a suggestion from Pendleton Tompkins. Revisions were made during the first year which resulted in the final instrument. This cannula has been in constant use since 1948 and has proved completely satisfactory for both radiagraphic work and for gas insufflations. One hundred cervico-hysterosalpingograms have been done with this instrument from 1948 to 1953. Figure 1 shows the parts of the instrument.

The usual Jarcho cannula was straightened and cut off at 28 cm. A cup was made of sterling silver (because of the ease of fabrication) measuring

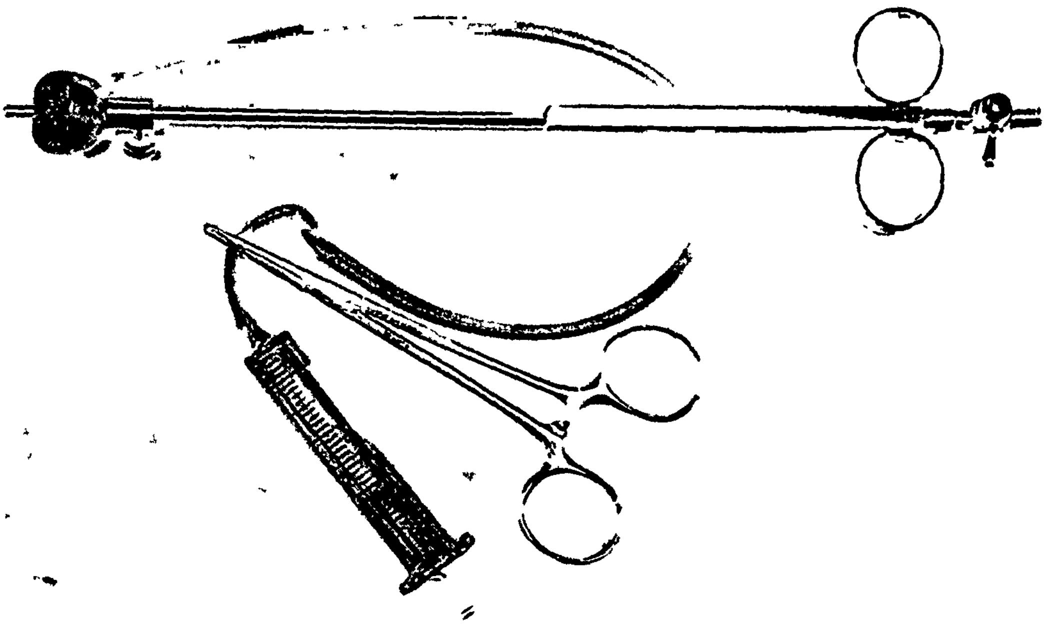

Fig. 2. Cannula assembled.

23 mm. in diameter and 10 mm. deep and a slot was cut, as shown, 4 mm. in width.* The cup was attached to a sleeve with a set screw. A #12 self-retaining catheter with a 5-cc. bulb was cut off distal to the bulb and a notch cut in the catheter itself just proximal to the bulb. A 5-cc. syringe and a hemostat or clamp are needed for inflation of the bulb. Figure 2 shows the instrument assembled.

The cup is placed on the cannula. The distal or bulb portion of the catheter is threaded over the end of the cannula and inflated with 3–4 cc. of air. The cup is pushed against the bulb. Just enough cannula is allowed to extend beyond the bulb to insure its remaining within the canal when

* Manufactured by Ralph E. Woolley, 693 Ala Moana Road, Honolulu, Hawaii.

pressure is applied. One or two tenacula are placed on the lateral portions of the cervix to supply countertraction. For use in gas insufflations no tenacula are necessary. The canal is not dilated with this method and the endocervix is not traumatized.

Various radiopaque media have been used in these studies, but since 1951 only water-soluble media have been employed. At times the edges of the x-ray shadow have been fuzzy without indication that the walls themselves were abnormal. Usually a heavy coating of thick mucus was found.

Fig. 3. Longitudinal fold of cervix and plicae palmitae in a patient with congenital cervical stenosis.

Attempts to remove this mucus by simple irrigation with saline were unsuccessful. A solution of papain was found to be very satisfactory and the resulting radiographs have been much sharper and clearer. Commercial papain powder was made up according to the formula of Rosenthal and Trout.

Early in 1953 the Department of Foods and Soils of the University of Hawaii made available some fresh frozen local papaya latex. This product has a proteolytic activity rated 10 times greater than the usual commercial papain in powdered form. While it has been used in too few cases to draw absolute conclusions, we have been impressed with its prompt action and clearer radiographs. Another product made from pineapple, reported to be much more potent than papain, is to be tried later. The canal is gently irrigated with about 150 cc. of the papain solution under very low pressure, allowing about 15 minutes for its action.

A few cases are presented to show some of the variations seen. All examinations are done with fluoroscopy and spot films of interesting or unusual findings.

Figure 3 shows the longitudinal fold that is usually more prominent on the posterior wall of the canal; the plicae palmitae are clearly shown.

Figure 4 is an oblique view of the same patient. The canal is longer and narrower than average, and observing by fluoroscopy the canal did not

Fig. 4. Oblique view of Fig. 3. The canal is longer and narrower than normal.

change its caliber during the procedure. The back flow was very slow, and the discomfort was the same to her as the usual dysmenorrhea.

The patient, a 30-year-old nullipara, had had a disabling dysmenorrhea of many years' standing. She required narcotics for relief and was absent from work for the first 2 days of each menstrual period. Subsequent dilatation and curettage was done with great difficulty. Dilatation of the internal os and sphincteric portion substantiated the diagnosis of a congenital stenosis. She has since married and is pregnant. There was no discomfort at subsequent menstrual periods.

Figure 5 shows a true hypoplasia and the shadows of a typical infantile uterus. The canal is two thirds of the total cervicofundal length.

A dilatation and curettage was attempted but had to be abandoned because the canal could not be thieaded. The patient was 40 years old, unmarried, and employed as a bookkeeper. Her dysmenorrhea necessitated the use of narcotics, alcohol, and bed rest for 2 days with each flow. Hysterectomy was done at a later date.

Fig. 5. Tiue hypoplasia and shadows of typical infantile uterus.

Fig. 6. Large spiral-shaped canal in a patient with chronic endocervicitis.

Figure 6 shows a large spiral-shaped canal that is shaggy in outline. The isthmus is narrowed. The patient's complaint was that of a profuse mucopurulent discharge. Subsequent dilatation and curettage showed a severe, chronic endocervicitis.

Fig. 7. Results after curettage of patient in Fig. 6.

Fig. 8. A type of cervix frequently seen. Papain removed the mucus causing the shaggy edges; subsequent dilatation and curettage resulted in a normal endocervix.

Figure 7 shows the result of curettage. The walls are now smooth. The patient was relieved of her mucopurulent discharge and subsequently became pregnant.

Figure 8 shows a type frequently seen. The canal gradually dilates toward the isthmus. The edges are shaggy which, according to some authors, indicates a chronic infection. It has been our experience that thick, adherent mucus can produce the same result. Papain removed the mucus as has

Fig. 9. Variations in the cervical lumen found in routine examination.

been previously described. Subsequent dilatation and curettage resulted in a normal endocervix.

Figure 9 shows 4 spot films taken at intervals during a routine examination. Variation in the lumen is shown. According to Asplund, the canal is larger in the secretory phase than it is in the proliferative phase.

Figure 10 is the film of a salpingogram done on a 30-year-old gravida VI, para III. The first pregnancy terminated normally. The second pregnancy was complicated by a placenta previa requiring hospitalization at 35 weeks for 3 weeks when a cesarean section was performed. The third pregnancy terminated at 34 weeks in a very rapid, spontaneous delivery of a normal infant. The next three pregnancies were lost at 20, 18, and 28 weeks, respectively, each one with the same sequence of events. At about 18 weeks the patient was hospitalized because of a 3–4 cm. dilatation of the cervix which occurred without apparent symptoms—the membranes bulged through the os. There was no bleeding and no apparent contractions. The embryos oozed

Fig. 10. Incompetent internal cervical os in a patient with repeated spontaneous abortion.

Fig. 11. Double uterus, as shown by salpingography with old-style cannula.

themselves through this patent os in a period of from a few days to 3 to 4 weeks. A tentative diagnosis of an incompetent internal cervical os was made.

Fig. 12. Same double uterus as in Fig. 11, shown clearly by procedure using the new cannula.

Fig. 13. Sinuses in patient with uterine tuberculosis.

Figures 11 and 12 show the double uterus of a patient who had delivered normally. The usual cannula was employed; it is easy to see how a mistaken diagnosis could have been made. The procedure repeated with the new cannula, showed a double uterus and its cervical communication.

Figure 13 is included because it shows some shadows, the significance of which was verified at subsequent hysterectomy. We are not privileged to see many cases of uterine tuberculosis, and these sinuses were nicely demonstrated on microscopic section. A screw cannula was used on this patient but the essential details are shown.

DISCUSSION

No attempt has been made to classify types of shadows as indicating abnormality except to try to correlate definite radiologic findings with anatomic findings at subsequent surgery or complete examination. As more cases are done, the correlation of specific shadows with actual pathologic conditions can be much more definite.

Asplund has shown in his large series that the measurement of the lumen of the canal is actually greater during the latter half of the menstrual cycle. You have seen some films that have given us the impression that the caliber of the lumen may change during the examination. Perhaps this is segmental and rhythmic, coordinated with the uterine contractions. It is well established that the cervix may contract in response to or associated with fundal contractions during pregnancy and labor. It is not unreasonable to assume that the same situation exists in the nonpregnant uterus but with smaller amplitude of contraction. During the secretory phase, the amplitude of contraction may be greater.

The routine use of the cannula has decreased the amount of discomfort during the examination. Whenever the endometrium is traumatized—as it is with a sound, a biopsy curette, or a cannula—there is conscious pain and often resulting spasm of the tubes. With the usual cannula it is almost impossible to prevent some trauma to the endometrium. Dilatation of the cervical canal usually causes pain, and the resulting uterine contraction may so distort the x-ray picture that the true pathologic condition may be missed entirely.

SUMMARY

One hundred cervicohysterosalpingograms were performed in a 5-year period, with special attention to visualization of the cervix. A new cannula and technic were developed for the purpose. The use of papain as a proteolytic enzyme to dissolve cervical mucus is advocated. The technic

which has been described permits the diagnosis of cervical lesions which otherwise might be overlooked.

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Book Reviews

Proceedings of the Society for the Study of Fertility (No. 5) *Cambridge, England, W. Heffer & Sons Ltd., 1954, 105 pp., 10 shillings.*

Eighteen experts took part in this conference. Their names and the subjects of their papers are: T. N. A. Jeffcoate, *Tubal Patency Tests;* Margaret Hadley Jackson, *Ethiodan (or Pantopaque) as a Contrast Medium for Hysterosalpingography;* E. W. MacMillan, *The Effects of Interruption of the Vasal and Inferior Epididymal Arteries on the Cauda Epididymidis and Testis;* A. S. Parkes, *The Quest for the Ideal Contraceptive;* Donald Young, *The Effect of Mumps on Male Fertility,* J. K. Russell, *Varicocele in Groups of Fertile and Subfertile Males;* F. P. Lisowski. *The Vascularization of the Ovary in the Rat;* D. Pauline Alexander and J. F. D. Frazer, *Some Aspects of Male Fertility in the Rodent;* G. I. M. Swyer, *The Effects of Testosterone Implants in Men with Defective Spermatogenesis;* Bernard Sandler, *The Mechanism of Tubal Spasm;* M. Moore White and Norman Warren, *The Significance of Pus Cells in Human Semen;* E. J. Clegg, *The Arterial Supply of the Seminal Vesicle and Coagulating Gland in the Rat, and the Effects of its Interruption;* M. Brochart, *Origin of Half-stained Spermatozoa Obtained from Live-dead Differentiating Stains;* A. C. Crooke and W. R. Butt, *The Effect of Steroids on the Level of Gonadotrophin in Human Urine;* R. G. Harrison, *The Effect of Ligation of the Vasa Efferentia on the Rat Testis and The Influence of Unilateral Orchidectomy on the Effect of Ischaemia on the Contralateral Testis.*

Jeffcoate reported that at least 43 out of 116 negative insufflation tests, and 13 out of 86 negative salpingograms were erroneous. Even when both tests give a negative result, pregnancy not infrequently follows. The use of complicated kymographic apparatus introduces a possible source of error and the particular pattern of the tracings is of little value in diagnosing tubal development or disease other than tubal occlusion.

Young maintained that mumps orchitis cannot be regarded as a serious cause of male infertility in view of the small number of men affected by it. Mumps at any age without orchitis has no effect on male fertility.

Sandler believes that tubal spasm accounts for 10–15 per cent of all cases of apparent tubal occlusion and it cannot be abolished with certainty by atropine, general anesthesia, Pethidine (Demerol), nitrites, or reassurance. He described the presence of a functional sphincter in the cervix and the possibility of a similar functional sphincter at the uterotubal junction. He discussed effects of sympathetic stimulation and suggested the use of hydrogenated ergot alkaloids as adrenergic-blocking agents. He reported the successful results of such treatment in 6 selected cases.

White and Warren reported on the incidence of leukocytes in human semen and their frequent association with *B. coli* infection of the seminal fluid. They said the presence of coliform bacilli in human semen results in reduced sperm motility and viability. These effects may be due to increased acidity found in association with the *B. coli*. Treatment with specific antibiotics results in marked improvement of semen quality and also cured the sterility in several cases.

This monograph contains a great deal of valuable data and should be read by everyone interested in sterility.

J. P. Greenhill

Mammalian Germ Cells (Ciba Foundation Symposium). *London, J. A. Churchill, 1953. Published in U. S. by Little, Brown & Co., Boston, 1953.*

The Ciba Foundation Symposia have earned a justifiably sound reputation during the few years of their existence, and the recently published *Mammalian Germ Cells* does not fall below the high standard set in the past. This reviewer took part in the symposium and was thoroughly impressed, not only with the quality of the discussions but also with the quiet efficiency which characterizes all the functions of the Ciba Foundation.

The topics discussed, while mostly academic in content, nevertheless carry important clinical implications. The chapters on biochemistry of mammalian semen deal exclusively with species other than the human. Mann describes the discovery of certain unusual substances, inositol and ergothioneine, in boar semen and discusses their possible significance. Gassner, in studies on bull semen, reports on the presence of certain amino acids and demonstrates that their presence in semen, like that of fructose, is dependent partially upon the male sex hormone. Meschaks, in two interesting papers, shows that in warmth-induced aspermia in bulls there is an increased excretion of urinary steroids and a corresponding decrease in excretion when normal spermatogenesis is restored. He demonstrates a similar state of affairs in bulls which had been transported and ascribes the disturbance in spermatogenesis in both instances to hyperactivity of the adrenal cortex with excessive production of neutral steroids.

Several other papers deal with metabolic rates of spermatozoa in relation to fertility without convincing evidence being offered that there is a strong relationship between any one aspect of metabolic activity and potential fertility.

Of great interest is the work of Polge and the group at the National Research Institute (London) on the preservation of semen at very low temperatures. This fundamental work has in the interim proven eminently successful in cattle breeding.

Lord Rothschild presents a most provocative discussion of the movements of spermatozoa in terms of their liberated energy and energy requirements.

The physiology of ovulation is taken up in two stimulating papers by Moricard and his co-workers. Of equal interest is the paper by Chang on the fertilizability of rabbit germ cells and that of Venge.on fertilization of rabbit ova in vitro and subsequent transfer to alien doe.

The matter of embryonic death is dealt with by Carl Hartman and by Casida. In the opossum, Hartman concludes that the majority of pathologic eggs owe their defects not to any deficiency in the uterine environment but to an inherent lack of growth potential in their germ plasms. Casida presents evidence, derived from species other than human, that if fertilization takes place at a time when the female reproductive tract is not hormonally prepared (during the luteal phase, for instance) embryo survival is impaired.

From the purely clinical point of view perhaps the most interesting papers were those presented by Bishop and Donald. These deal with a follow-up of over 1200 cases attending the Infertility Clinic of the Chelsea Hospital for Women (London), particularly in relation to postcoital tests. Bishop analyzes the postcoital test in all its aspects, including the effect of therapy. Certain of his conclusions do not agree with modern teaching. His paper should be read with care by those who insist that, as Bishop puts it, "the physical consistency of the cervical fluid follows a rigid course so that it is almost impossible to determine the exact day of the cycle by . . . the physical consistency of the fluid." Bishop does not agree with this concept.

The value of Donald's paper, which deals with findings in postcoital tests when pregnancy ensued, lies mainly in that he has done a careful analysis of the quality of the postcoital test in relation to the semen quality of the husband, a correlation which has been sadly neglected by students of human sterility. Perhaps his most interesting finding is that a satisfactory postcoital test does not indicate the size of the husband's sperm count, provided the latter is not lower than 20 million/cc. He also agrees with Bishop in his demonstration that satisfactory postcoital tests are obtained over a wide range in the cycle and not, as is taught, only on days coincident with or close to ovulation.

JOHN MACLEOD

Clinical Endocrinology. K. E. PASCHKIS, M.D., A. E. RAKOFF, M.D., and A. CANTAROW, M.D. *New York, Hoeber, 1954, 849 pp., $16.00.*

In something over 800 pages of text, this fine book presents up-to-date knowledge of the ductless glands in a concise and readable form for students and clinicians alike. It is stated in the preface that the proper management of clinical problems can result only from understanding the pathologic physiology involved, and in this particular field an integrated synthesis of physiology, clinical endocrinology, and biochemistry is required. Yet the needs of the practicing physician for definitive therapy, dosage, and rationale of treatment are given paramount importance. It is a challenge to attempt to combine together the complex laboratory background and basic science disciplines relating to the endocrines with a practical outline of office management for the whole range of simple and bizarre endopathies. yet this book admirably achieves its purpose.

Ten sections are devoted in turn to each of the glands, including the placenta, following a consistent form—anatomy and physiology, hyperfunction, and hypofunction. Under these simple headings are discussed all clinical complaints, syndromes, and disorders with endocrine connotations. Such an arrangement gains in simplicity, yet imposes difficulties in deciding where one might expect to find grouped such miscellaneous conditions as sterility, *Mittelschmerz,* and dysmenorrhea (under ovarian hypofunction), or gynecomastia (under testes). However, a very well organized and comprehensive index adequately compensates for such difficulties and provides quick access to the specific information that a busy practitioner requires. The liberal use of cross-references to other pages in the text is another thoughtful and rewarding factor making this information readily available.

Among the 253 illustrations are many charts, diagrams, and photomicrographs which supplement the excellent descriptions of embryology, anatomy, and physiology in each section. All too often in previous texts the placenta has not been accorded its place as one of the endocrine glands. Under this heading will be found a limited discussion of habitual abortions, toxemia, and the complications of the post partum period. At the end of each section a useful list of references is appended.

Section 11 deals with the subject of obesity, even though "obesity in the overwhelming majority of cases is not caused by endocrine disturbance."

Section 12 contains 35 pages on "Procedures and Methods of Study." This is an extremely valuable compendium of laboratory technic, ranging from a description of eosinophil response in the 4-hour ACTH test to the details of an adequate semen examination. Indications, normal values, and interpretation of results are included. Finally, in a table covering 5 pages, representative samples of commercial hormone preparations are listed, stating source, trade names, toleration, and effectiveness of each.

This is not a summary text for the casual student; it is a complete treatise on an important group of associated disorders. This is a good book for the shelves of the general practitioner; it is even more important as source material and a reference text for those specializing in this field.

Somers H. Sturgis, M.D.

Current Therapy 1954: Latest Approved Methods of Treatment for the Practicing Physician. Howard F. Conn, ed. *Philadelphia, W. B. Saunders Company, 1954, 898 pp., $11.00.*

The 1954 issue of *Current Therapy* has, in addition to the editor, 12 consulting editors.

This edition is the sixth of an annual series and has continued the original purpose of bringing to the busy physician authoritative current methods of treatment in a form so devised as to make the desired information readily available. The purpose is to provide a quick adequate reference volume. The diagnosis is assumed to have been made and the authors dwell principally on the treatment or therapy. This volume has 898 pages and there are 192 contributors. It covers the principal fields of medicine, surgery, and the specialties.

The book is divided into 16 sections, in which the principal diseases are classified, such as: 1.—The Infectious Diseases; 2.—Diseases of the Respiratory System; 3.—Diseases of the Cardiovascular System; 4.—Diseases of the Blood and Spleen; 5.—Diseases of the Digestive System; 6.—Disorders of Metabolism and Nutrition; 7.—Diseases of the Endocrine System; 8.—Diseases of the Urogenital Tract; 9.—The Venereal Diseases; 10.—The Allergic Diseases; 11.—Diseases of the Skin; 12.—Diseases of the Nervous System; 13.—Diseases of the Locomotor System; 14.—Obstetric and Gynecologic Conditions; 15.—Diseases Due to Physical and Chemical Agents; 16.—Appendices and Indices.

Many topics are discussed in less than one page, making it possible to review them in a few minutes. The editor does not always give the viewpoint of one physician alone, but in many instances hė gives two or three methods of treatment, all of which are more or less standardized and accepted. The book is well worth having as a reference book in therapy.

Section 14 is of particular interest to the gynecologist and obstetrician. The subjects are antepartum care; abortion (hemorrhage of early pregnancy); ectopic pregnancy; hemorrhage in late pregnancy; pernicious vomiting of pregnancy (hyperemesis gravidarum); toxemia of pregnancy; analgesia and anesthesia, obstetric; postpartum care; normal infant feeding; endometriosis; menstrual abnormalities; abnormal uterine bleeding; amenorrhea; primary dysmenorrhea; sterility, female; menopause; lower genital tract infections; myoma (fibroid) of uterus; tumors of the ovary; cancer of the uterus; cancer of the vulva; pre- and postoperative care in gynecology; phlebothrombosis and thrombophlebitis. For those interested in sterility, the article on page 798 is of particular interest.

Gilbert F. Douglas

ELEVENTH ANNUAL MEETING • JUNE 3, 4, 5, 1955
Atlantic City, New Jersey

THE AMERICAN SOCIETY FOR THE STUDY OF STERILITY

Tentative Program

FRIDAY, JUNE 3–Registration (all day)

Opportunity to attend Endocrine Society Meeting and Banquet

SATURDAY, JUNE 4

9:00–11:00 A.M.	Sperm, Their Migration and Transport
11:00 A.M.–1 P.M.	Psychosomatic Aspects
3:00–5:00 P.M.	Veterinary Problems
5:00 P.M.	Business Meeting

Concurrently with these presentations there will be 12 round table discussions on:

Basal Body Temperature
Artificial Insemination
Endometriosis
Ectopic Pregnancy
Population Problems
Tuboplasty
Semen Assay
Retrodisplacement
Cervical Mucus
Pelvic Pain
Ovulation Failure
Hirsutism and Amenorrhea

Attendance at these round tables will be limited. Members will have the opportunity to sign up in advance. Others will be able to sign up at time of registration for the round tables not previously filled.

SUNDAY, JUNE 5

9:00–11:00 A.M.	The Ovary and the Egg
11:00 A.M.–12 NOON	Guest Lecture
NOON–1:00 P.M.	Endocrine Mechanisms
3:00–5:00 P.M.	Research Tools

Selected movies will be shown on Saturday and Sunday.

THE ENDOCRINE SOCIETY
1955 Annual Meeting

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, N. J., on Thursday, Friday and Saturday, June 2, 3, and 4, 1955.

Use of Serial Vaginal Smears in Detecting Time of Ovulation

Gardner M. Riley, Ph.D., Eugenia Dontas, M.D., and Barbara Gill, M.S.

The development of practical methods for determining the time of maximal fertility in the human female has gained increased interest in recent years. Clinically these methods have proven most useful in dealing with problems of functional sterility. Two methods for approximating the time of ovulation that have been given the most extensive clinical trials are the vaginal smear technic and the basal body temperature record.

Numerous authors[1, 4, 6, 13] have evaluated the vaginal smear method since Papanicolaou[10] published his classic description of variations in human vaginal smears during the menstrual cycle. Rubenstein,[12] utilizing the Papanicolaou technic, noted a correlation of the vaginal-smear variations with those of the basal body temperature curve and later reported the usefulness of combining the two methods.[13] The cyclic cellular pattern has recently been described by De Allende and Orías and by Goldhar and associates.

These numerous publications notwithstanding, the use of the vaginal-smear method has remained largely restricted to those having facilities and opportunities for research. In the present study an attempt has been made to clarify the cytologic criteria for ovulation and to modify the technic for wider application. Observations are made on the relationship of time of ovulation as determined by vaginal smears to the shift of the basal body temperature curve.

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MATERIALS AND METHODS

This investigation is confined to an evaluation of vaginal smears from patients whose chief complaint was infertility. In most instances the smear method was instituted as part of a complete infertility study of the subject and her husband. With few exceptions, the menstrual histories presented no unusual features and the pelvic examinations revealed little or nothing of clinical significance.

The evaluation of the vaginal-smear method as a means of detecting time of ovulation is based upon the examination of a total of 452 series of smears from 300 patients. Basal temperature curves were available for comparison with serial smears in 126 cases.

Collecting Serial Smears

The patient is provided with a supply of microscope slides and instructed to prepare smears in the following manner: Using a commercial, cotton-tipped applicator, a sampling of vaginal cells is obtained and gently *rolled* over the surface of the slide; the slide is dated or numbered and kept in a slide box, without fixation, until the collection is completed. The smears are taken at approximately 24-hour intervals.

It will be noted that these instructions differ from the generally accepted procedures in two important respects. First, a cotton applicator is used rather than a glass suction pipet. In addition to being readily obtainable and convenient in use, the applicator has consistently provided more satisfactory preparations from a technical point of view. The decision to make this change in technic followed several years' experience with the glass pipet in our gynecologic service. The usually stated advantages of the pipet method —insertion to the posterior fornix, noncontamination with cells from the vulva or lower third of the vagina, and forceful expression of fluid over the surface of the slide—are frequently overshadowed by inadequate sampling of mucosal cells. De Allende and Orías emphasize that a "projection" of the extracted material from the pipet will best preserve the relations of the various desquamated cellular elements. We feel that this objective is partly accomplished by insisting that the cotton applicator be *rolled* over the glass rather than haphazardly smeared.

Secondly, the smears are air-dried rather than fixed immediately in alcohol-ether solution. As pointed out by the European workers Isaac and

Wurch, "The great inconvenience of the technique of Papanicolaou and Shorr is that after preparation by the usual means, it is indispensable to fix the slide while still moist by immersion in a solution of alcohol and ether equal parts, and to keep it in this solution until stained." These requirements account in large measure for the fact that such a valuable diagnostic procedure as the serial smear method is not more widely used.

After long adhering to the concept that exfoliated cells must be fixed immediately, we have concluded that smears of satisfactory quality may be obtained without resorting to this initial step. Our experience is in complete agreement with that of Benedek and Rubenstein who state that "the one outstanding difference between smears not dried and those dried is the somewhat increased affinity for eosin in the dried smears." We would further agree that "the mode of fixation and of staining does not alter the decisive criteria of the method." Isaac and Wurch have evaluated the effect of air-drying on smears and conclude that this in itself acts as a type of fixation.

It should be emphasized that avoidance of desiccation is essential in the diagnosis of malignancy by the cytologic method where careful attention to cytoplasmic and nuclear details is imperative. In evaluating phases of the menstrual cycle by the smear method, none of the cytologic criteria for malignancy are used, but rather distinctive cellular patterns are relied upon.

Although a few patients contributed complete series for two or more cycles, the usual routine has been to obtain one or two series preliminary to a day-to-day evaluation. The preliminary series permits the examiner to familiarize himself with the cyclic fluctuations in cellular patterns common to each subject and to estimate the time in the cycle when ovulation is apt to occur.

Staining Procedure

With some minor variations, the smears are stained according to the procedure of Papanicolaou.[11] After the identifying marks on the slides are made permanent with Labink, they are placed in a standard carrier which accommodates 20 slides. Usually a single carrier may be used for one series by omitting a few slides at either end of the series. Exposing the significant slides of each series to all solutions under comparable conditions provides greater uniformity in staining. The steps in the staining procedure are:

1. Place slides in a solution consisting of equal parts of 95% alcohol and

ether for 15 minutes. This secondary fixation, following air-drying, improves subsequent staining.

2. Rinse, successively, in 80, 70, and 50% alcohol, and finally in distilled water.
3. Stain in Harris' hematoxylin for 2–4 minutes.
4. Allow to stand in running tap water until stain changes from red to blue.
5. Rinse, successively, in distilled water, and 50, 70, 80, and 95% alcohol.
6. Stain for 1 minute in Orange G-phosphotungstic acid solution (OG 6, Ortho).
7. Rinse in two changes of 95% alcohol.
8. Stain in polychrome stain (EA 50, Ortho) for 2–3 minutes.
9. Rinse well in two changes of 95% alcohol.
10. Rinse thoroughly in absolute alcohol.
11. Clear in xylol and mount in Canada balsam or other mounting medium.

CRITERIA FOR DETECTING TIME OF OVULATION

Establishing the most fertile time in the cycle—time of ovulation—is of greatest importance to the infertile couple and to the physician who may find it necessary to use artificial insemination. Since ovulation itself thus far evades detection, it is essential to employ technics which will indicate the approximate time of ovulation with a reasonable degree of accuracy. The successful application of the vaginal-smear method depends upon recognition of progressive changes in the cytologic and general smear pattern throughout the follicular phase of the cycle, the existence of a characteristic cytologic pattern coincident with the approximate time of ovulation, and a distinctive alteration in the smear in the early postovulatory phase. The following description will be confined to a definition of the authors' concept of the "ovulatory" and postovulatory smears. Excellent descriptions of the cellular patterns characteristic of other phases of the cycle have recently been reported[4, 6] and need not be repeated here.

The Ovulatory Smear

The term "ovulatory" is used to designate that smear which represents the most advanced stage of the follicular phase of the cycle. The picture presented by this smear is comparable to that described by De Allendē and

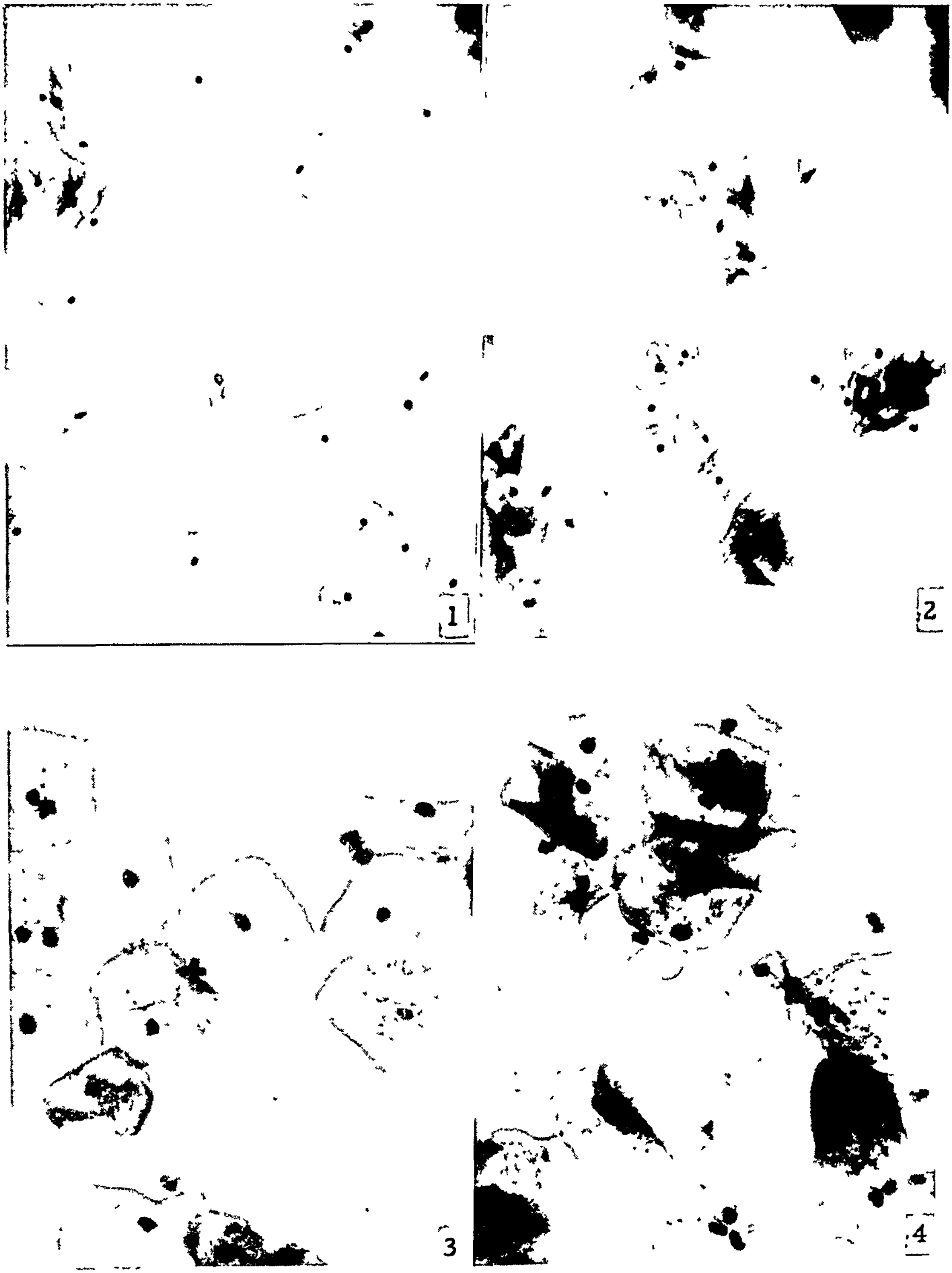

Fig. 1. Ovulatory smear, Day 14 of 28-day cycle (Patient H. Z.). Clear background, large mature cells, well-separated and mostly cornified, only occasional

Orías as the "follicular" smear, so named because "it appears spontaneously at the peak of the proliferative phase of the ovary when the follicle has attained complete maturity." The ovulatory smear also closely resembles the smear described by Papanicolaou[10] as characteristic of the late or "proper" copulative stage of the human menstrual cycle.

The typical ovulatory smear is the best-defined smear of the entire cycle (Figs. 1 and 3). It is characterized by (1) a striking clarity of background; (2) a maximal concentration of cornified cells (eosinophilic cells with pyknotic nuclei) for the cycle; (3) the tendency for individual cells to remain separated; and (4) scarcity or total absence of leukocytes.

All these features must be considered when identification of the ovulatory smear is undertaken. The fact that one or more of these characteristics is not present does not mean that this stage cannot be recognized, though more critical evaluation may be necessary. Not uncommonly a mild leukocytosis *may prevail throughout the cycle, in which case the significance of* leukocytes in the spread must be minimized. The presence of leukocytes, unusual concentrations of bacteria, and excessive mucus may reduce the clarity of the preparation. The percentage of cells showing cornification with pyknotic nuclei at the peak of the follicular phase varies markedly between individuals. Reference to the cellular patterns established for previous cycles is helpful when one attempts to detect the ovulatory smear on the basis of slides provided from day to day.

/The tendency for cells to remain separated is one of the most important features of the ovulatory smear. Characteristically, the cells show little folding or wrinkling at this stage and they may assume a mosaic pattern with little or no overlapping of their adjacent edges. This feature is best appreciated in areas where the concentration of cells is not great. The larger aggregates of cells which are apt to be present in any smear are not significant and should be dismissed as a nonspecific feature of the smear method.

The Postovulatory Smear

The diagnosis of an ovulatory cycle is dependent upon recognition of the

leukocyte. (× 155) Fig. 2. Postovulatory smear, Day 15 of cycle (same patient as Fig. 1). Clumping and folding of cells. (× 155) Fig. 3. Ovulatory smear, Day 12 of 26-day cycle (Patient D. W.). Mainly cornified cells in close approximation but not clumped. (× 309) Fig. 4. Postovulatory smear, Day 13 of cycle (same patient). Characteristic rosette arrangement of cells, folding and curling of edges, leukocytes more numerous. (× 309) early postovulatory smear and the subsequent alteration in cellular pattern characteristic of the progestational phase of the cycle. The postovulatory smear is readily identified 24 hours following the ovulatory smear and the characteristic features of the smear may be recognized 12–14 hours earlier.

The most important single identifying characteristic of the immediate postovulatory smear is the tendency for cells to arrange themselves into small groups or clumps (Figs. 2 and 4). This clumping is to be distinguished from the inadvertent aggregation (piling-up) of cells described above. The arrangement of the cells, together with the tendency to stain intensely with eosin, justifies the use of the term "rosette" as employed by De Allende and Orías to describe the small, compact clumps of cells.

Not infrequently there is a definite reduction in number of cornified cells in the early postovulatory smear, although in many instances this is not a conspicuous feature of the smear. Occasionally an influx of leukocytes may be striking, although this is not a consistent finding. Curling of the cells may be seen but the extent of this change may not be sufficient to aid in detecting the postovulatory smear.

In evaluating a complete series of daily vaginal smears, it is generally helpful to arrange the slides in serial manner against a white background after staining is completed. In many instances there is a striking contrast between the gross appearance of the smears during the follicular phase and that during the postovulative phase. In general, the smears of the postovulative phase appear heavier than those of the earlier phase. The ovulatory smear may be conspicuous as a relatively clear slide appearing to contain little cellular material. It must be emphasized, however, that the absence of clear-cut differences in slide appearance is not to be interpreted as indication of an anovulatory cycle. Extensive exfoliation of mucosal cells throughout the cycle will usually result in serial smears of uniform quality, as will very thin spreads of the vaginal fluid.

CORRELATION OF THE OVULATORY SMEAR WITH BASAL TEMPERATURE RECORDS

Although occasional reports[2] suggest that use of the basal body temperature may not rest on too sound a foundation or that ovulation cannot be related to a particular point on the curve,[9] the bulk of published reports indicate a thermal shift at or about the time of ovulation.

A comparison of the vaginal-smear method with the basal temperature method indicates a relatively close correlation. The ovulatory smear was obtained on the first day of the thermal rise in 28.6 per cent of the 126 cycles studied. In 39.7 per cent of the cycles, the ovulatory smear was recognized the day before the thermal shift; that is, at the low point of the temperature

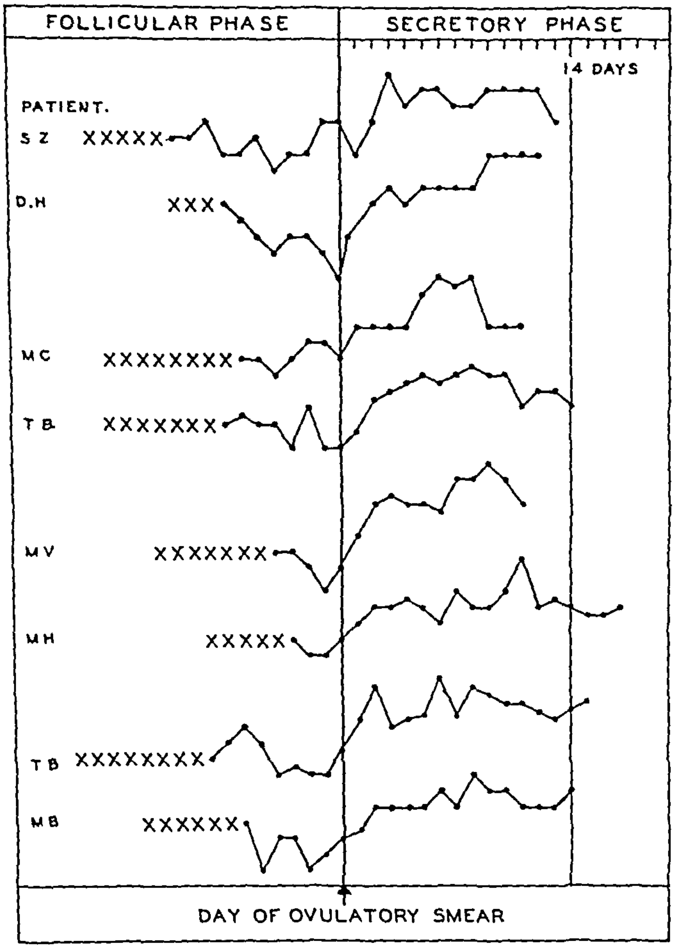

Fig. 5. Selected B.B.T. curves illustrating commonly encountered relationships between time of ovulation as indicated by vaginal smears and B.B.T. curves.

curve. Thus in 68 per cent of the cycles, the ovulatory smear coincided closely with the significant phase of the basal temperature curve. Examples of the most common relationships noted between the day of the ovulatory smear and the time of the thermal shift are illustrated in Fig. 5.

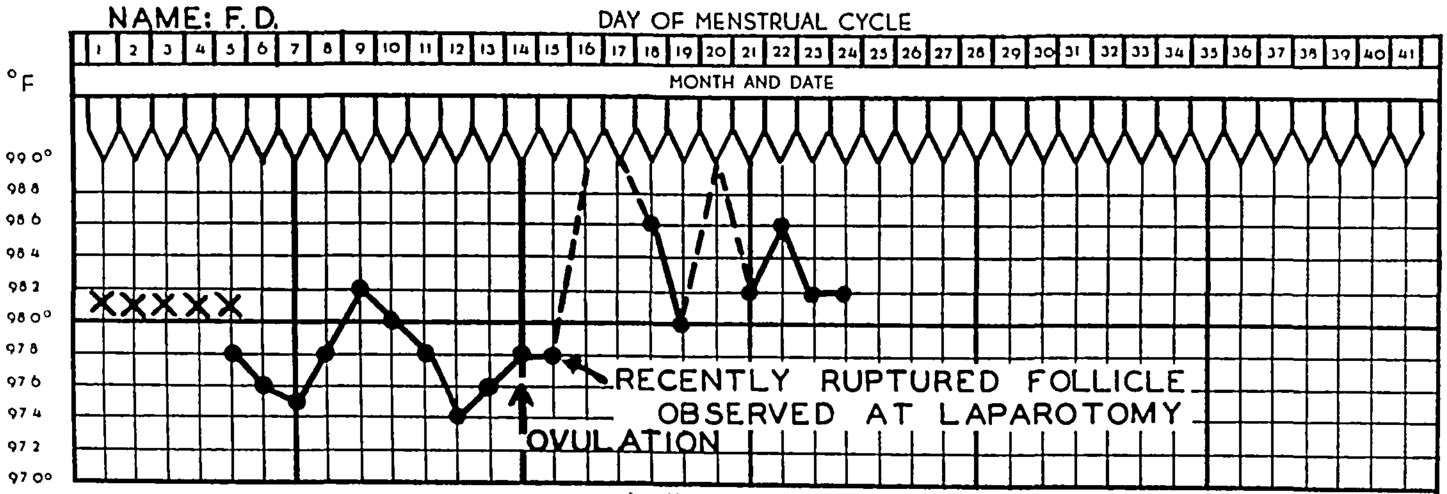

Fig. 6. B.B.T. curve of Patient F. D. The ovulatory smear was recognized on Day 14. A recently ruptured follicle was observed 24 hours later.

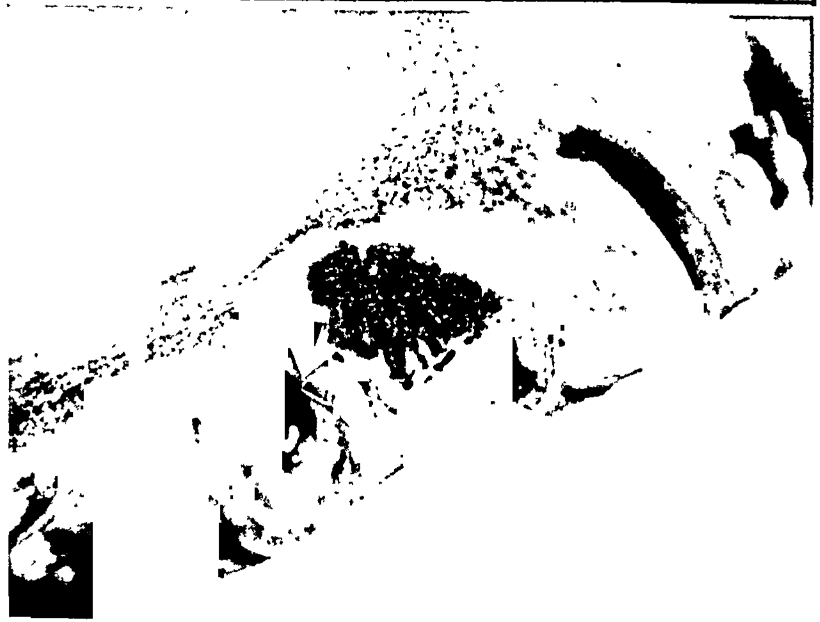

Fig. 7. Appearance of right ovary at laparotomy of Patient F. D. 24 hours after detection of ovulatory smear. A mature follicle protrudes from the surface of the ovary (left of distended tube). Blood was spilling from rupture site at arrow.

In 1 case where the ovulatory smear occurred on Day 14 of the cycle (Fig. 6), it was possible to examine the ovaries at laparotomy 24 hours later. At this time a recently ruptured follicle was observed on the surface of the right ovary (Fig. 7). A trickle of bright, red blood from the rupture site supported the probability that ovulation had occurred within 24 to 48

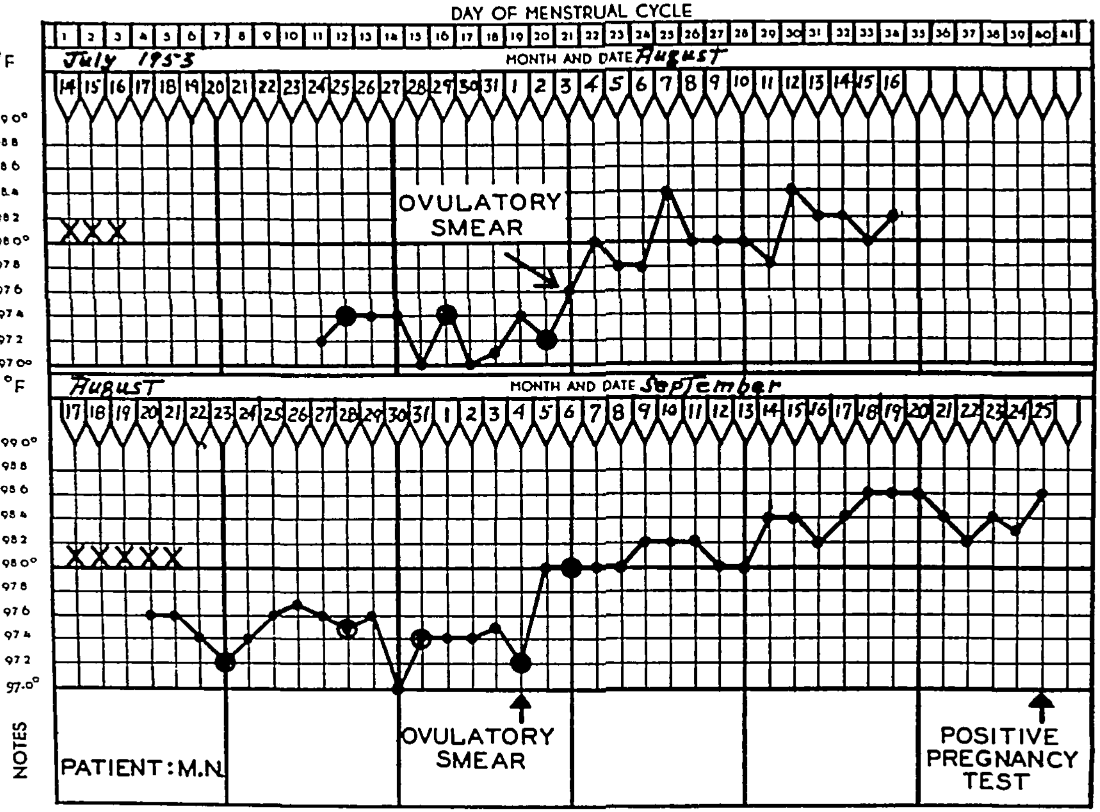

Fig. 8. B.B.T. curves for two successive cycles of Patient M. N. Use of the B.B.T. method was complicated by varying cycle length and daily variations in temperature. Encircled points indicate times of coitus. In the second cycle the ovulatory smear appeared on the Day 19, 1 day prior to the thermal shift. A pregnancy test was positive 21 days later.

hours. Additional evidence that this follicle, observed on Day 15, had only recently ruptured was obtained from histologic examination of endometrial curettings which indicated a late proliferative phase without distinctive secretory change. Noyes and associates believe that subnuclear vacuolation of the cells of the endometrial glands may not be observed until the second postovulatory day. In this particular case it appears that ovulation may have occurred at about the time the ovulatory smear was prepared and approximately 24 hours after the thermal shift.

Figure 8 illustrates the basal body temperature curves of a patient for

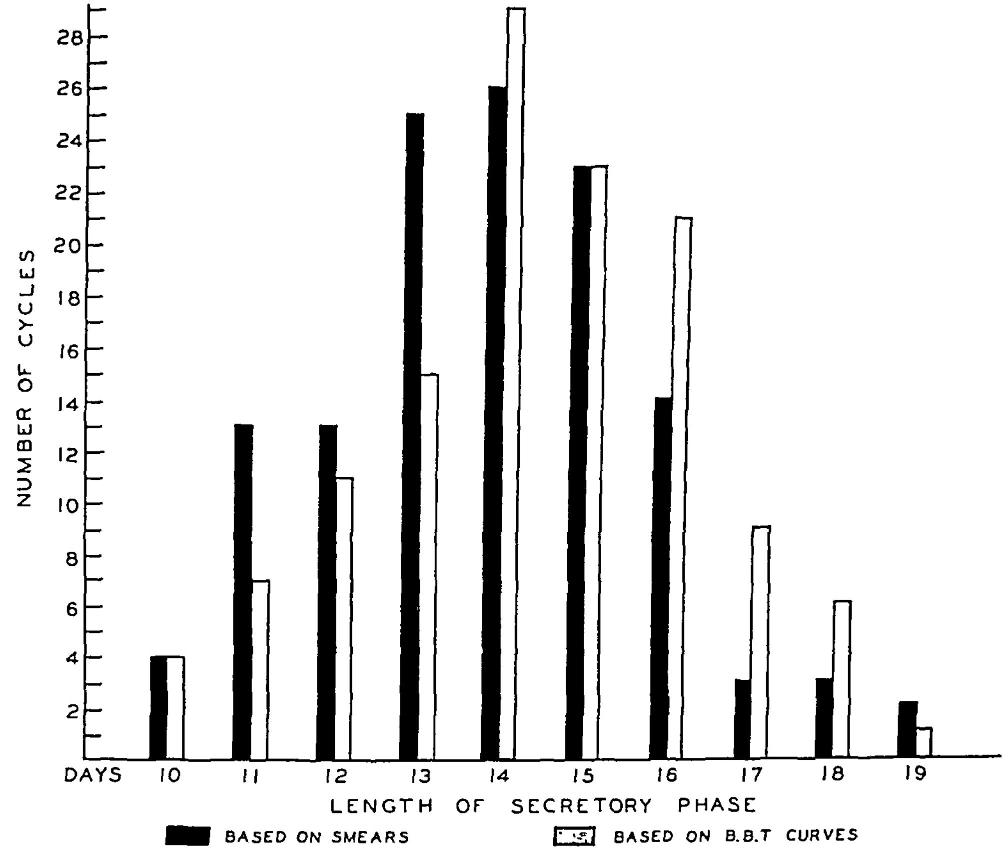

Fig. 9. Frequency of secretory phases of different lengths in a group of 126 cycles. The duration of the secretory phase is calculated from the day of the ovulatory smear or the day preceding the thermal shift.

two consecutive cycles. In the first cycle the ovulatory smear was recognized on Day 21, 24 hours after the last significant low point in the temperature curve. In the succeeding cycle the day of the ovulatory smear coincided with the last low point in the temperature curve. Coitus was recommended on this day, and pregnancy resulted. The multiple, nonsignificant thermal shifts in these B.B.T. curves illustrate the difficulties patients may encounter when relying solely on daily changes in body temperature.

LENGTH OF THE SECRETORY PHASE

Data derived from many sources indicate that the length of the secretory phase is about 14 days. In the present series the length of this phase is calculated from the day of the ovulatory smear to, and including, the last day of the cycle. The interval ranged from 10 to 19 days (Fig. 9). However, 90 per cent of the cycles had secretory phases of from 11 to 16 days. The most common intervals for the secretory phase were 13, 14, and 15 days. While the smear and temperature curve methods did not always point to the same day for time of ovulation, approximately the same spread for the secretory interval was obtained by both methods.

In Fig. 10 a regression line has been plotted to show the relationship between the day of ovulation as detected by vaginal smears and the length of the cycle. As might be expected, as the menstrual cycles increase in length, the day of ovulation occurs progressively later in the cycle. The equation of the regression line indicates that the advancement of the day of ovulation does not quite keep pace with the lengthening of the cycle. Thus for every 1 day that is added to the length of the cycle, the day of ovulation advances only 0.78 of a day. As a result, the length of the secretory phase will vary directly with the length of the menstrual cycle. For example, patients with relatively short cycles of 24 days may be expected to have secretory phases of 13 days, whereas patients with 34-day cycles may tend to have 15-day secretory phases. It is interesting that this relationship also appears to hold for the rhesus monkey, where time of ovulation can be determined with greater precision by rectal palpation. The data of Corner, Hartman, and Bartelmez indicate that the average length of the secretory phase for short cycles (20–22 days) is 10.7 days, and for longer cycles (27 days), 14.7.

While some lengthening of the secretory phase may be expected as menstrual cycles increase in length, it is also apparent that much variation actually exists regardless of the length of the cycle. Thus in 28-day cycles, secretory phases varying from 11 to 16 days were encountered and in 32-day cycles from 11 to 19 days.

In Fig. 10 the dotted lines show the range of the regression line plus or minus one standard error of estimate—plus or minus 1.89 days. Approximately two thirds of the data will be found to lie within this area. For example, two thirds of all patients with 28-day cycles may be expected to ovulate between days 12 and 16 of the cycle. In dealing with problems of infertility it becomes extremely important to recognize the possibility

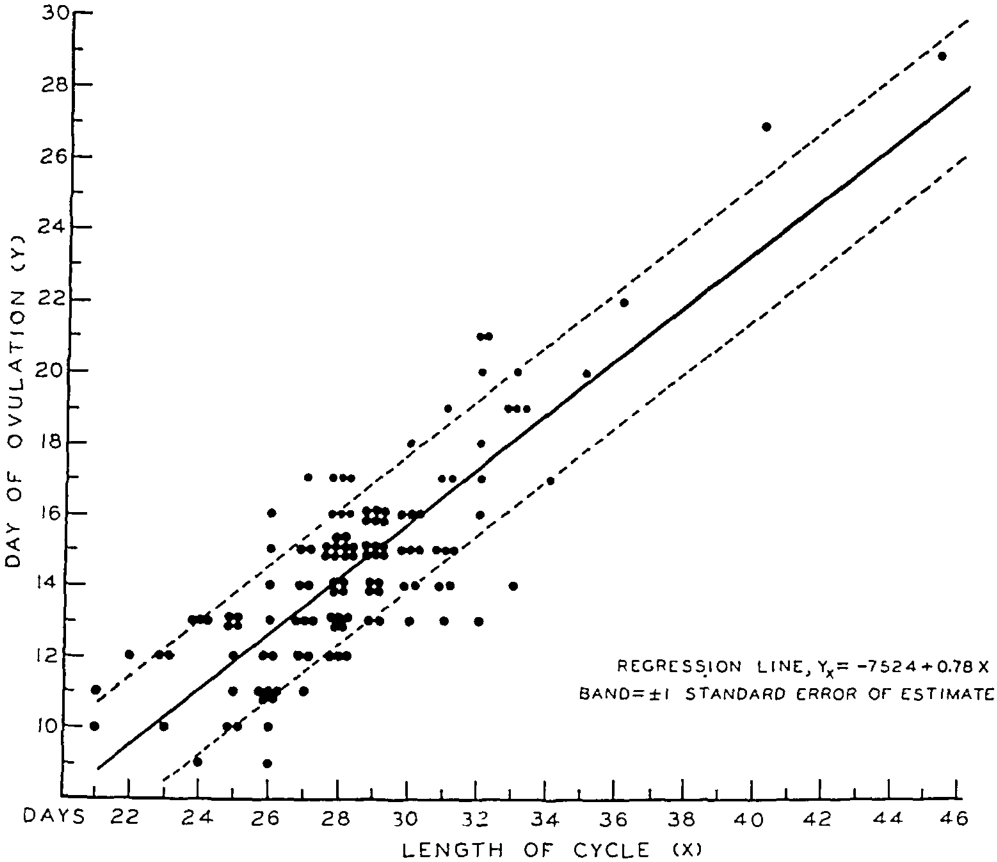

Fig. 10. Length of menstrual cycle and day of the ovulatory smear (day of ovulation) for 126 cycles of women presenting infertility problems. The scatter of points suggests a significant linear relationship between the length of the cycle (X) and the day of ovulation (Y) as indicated by the ovulatory smear. To show this relationship a "regression line" (continuous diagonal line in figure) has been fitted by the method of "Least Squares."

In the equation of the regression line, $Y = a + bX$, the first constant, a, determines the level of the line and the second, b, indicates the units change in Y for each one unit change in X. Thus the line, $Y_x = -7.524 + 0.78X$, indicates that for each day increase in length of cycle there is an increase of 0.78 days in time of ovulation.

One standard error of estimate for the 126 paired items (length of cycle and time of ovulation) is ±1.89 days and is indicated by the dotted lines. Approximately two thirds of the data may be expected to fall within this band.

of such degrees of variability with respect to the time in the cycle when ovulation may occur.

DISCUSSION

The present study indicates that ovulation time may be detected with a useful degree of accuracy by means of the vaginal-smear method. The procedure has been made more practical through simplification of technic, especially in the preparation of the smears, and through a clear definition of the characteristics of the ovulatory and postovulatory smears.

The diagnostic criteria described by Goldhar and associates differ somewhat from those utilized in the present study. Our distinctive "ovulatory" smear more closely resembles the cytologic picture described by these authors for the preovulatory smear. The latter is described as preceding a phase associated with a pronounced leukocytic flush which in turn is followed by the ovulatory smear. The ovulatory smear is apparently distinguished from the immediate preovulatory smear by curling and folding of the edges of the cells, generalized wrinkling of the cytoplasm, and a tendency toward grouping of cells. The presence of leukocytes in the ovulatory smear is considered to be highly variable. This description matches very closely Papanicolaou's[10] "ovulative stage," which is characterized by reappearance of leukocytes in large numbers and the tendency for cells to show folding or curling. It is probable that the "ovulatory phase" of Goldhar *et al.* and the "ovulative stage" of Papanicolaou represent a brief transitional phase between the peak of the follicular phase and the postovulatory phase. Because of the brevity of the phase it may not be seen at all, this being especially true when smears, for practical considerations, are collected at 24-hour intervals. In the present series the so-called "leukocytic flush" was encountered only rarely.

It would appear that the vaginal smear method as a practical means of predicting time of ovulation would be seriously limited if the examiner depended upon the occurrence of the leukocytic flush with only a relative increase in curling and wrinkling as a guide to the ovulatory time of the cycle. On the other hand, it would appear far more practical to use the strikingly distinctive smear which Papanicolaou associated with the "true copulative phase." It is true that a typical smear representing this phase may not always be encountered. However, if all the criteria described above for the ovulatory smear are kept in mind by the examiner, there will be few cycles where a close approximation of ovulation time cannot be made.

The close correlation found in the present study between the ovulatory smear and the thermal shift of the basal temperature curve lends added support to the numerous reports indicating that a temperature change is characteristic of an ovulatory cycle. The chief disadvantage in using only the temperature record as a guide to time of ovulation is the difficulty in recognizing the last significant low point in the curve or the first significant elevation. It is not infrequently with a sense of frustration that the infertile couple attempts repeatedly to utilize the information provided by the ups-and downs of the basal temperature curve hoping that one coital attempt will coincide with the time of ovulation. There remains the possibility that too frequent coitus may actually reduce the chances for successful fertilization. Farris has demonstrated a definite decrease in total count of spermatozoa following repeated emissions and suggests that this depression may further reduce the fertilizing capacity of a "relatively fertile" male. MacLeod and Gold, while noting a depression in the percentage of active cells after continent periods of 6 days or longer, suggest that a reasonable frequency of emission (every 3–4 days) is more conducive to good semen quality in the average individual. In view of these considerations it would appear desirable to employ a method for detecting time of ovulation which would not only localize the fertile interval but also eliminate too frequent coitus preceding this phase. As used in this laboratory, the serial vaginal smear method has proven to be a practical means of attaining these objectives.

SUMMARY AND CONCLUSIONS

Experience with 452 series of daily vaginal smears from 300 patients complaining of infertility indicates that the method may gain wider application through slight modifications in technic and clarification of the cytologic criteria for ovulation.

Satisfactory smears may be prepared by the patient with the use of a cotton-tipped applicator. The unfixed smears are returned to the physician at the completion of the collection period. The Papanicolaou staining procedure may be used following these preliminary steps.

The term "ovulatory" smear is used to designate that smear which represents the most advanced stage of the follicular phase of the cycle and is characterized by striking clarity of background, maximal concentration of cornified cells, tendency for cells to remain separated, and leucopenia.

A comparison of the vaginal-smear and B.B.T. methods indicate a close correlation between the day of the ovulatory smear and the time of thermal shift. The ovulatory smear was obtained on the first day of the thermal rise in 28.6 per cent of 126 cycles and on the day preceding the thermal shift (low point of curve) in 39.7 per cent of the cases.

A regression line has been plotted to show the relationship between the day of ovulation as detected by vaginal smears and the length of the cycle. The equation of this line indicates that for every one day that is added to the length of the cycle, the day of ovulation advances only 0.78 of a day. Thus there is a tendency for the length of the secretory phase to vary directly with the length of the menstrual cycle. In spite of this trend, established on a statistical basis, there remains much variability with respect to the time of the cycle when ovulation occurs. The vaginal-smear method as carried out in this laboratory has proven a practical means of detecting this time of maximal fertility.

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Annual Conference of the British Society for the Study of Fertility

The 1955 Annual Conference of the British Society for the Study of Fertility will be held in the Large Anatomy Lecture Theatre of the Medical School, Hospitals Centre, Birmingham, England, on Thursday and Friday, June 23 and 24.

Members of the American Society for the Study of Sterility are cordially invited to attend and participate in this meeting. If anyone wishes to present a paper describing some phase of research in our special field, please write Mr. H. H. Fouracre Barns, 31 Weymouth St., Portland Place, W. 1, London, England.

What Is Normal Female Fertility?

M. James Whitelaw, M.D.

UNTIL NOW it has been almost universally agreed that failure to conceive during a 24-month period without the use of contraceptives should be used as a time yardstick before institution of a fertility investigation. It is the purpose of this study to demonstrate statistically that this period of time can be curtailed to a maximum of 6 months, and in some cases even to a shorter time.

The evaluation of fertility and fecundity in its various statistical aspects has been left almost entirely to those interested in the field of population trends. Few studies have been made by physicians interested in the evaluation of fertility and fecundity as it relates to the specialty of sterility. In the field of infertility where obvious endocrine, infectious, and anatomic defects are excluded, any study of results by one mode of therapy or another may not be considered of true value without consideration of an adequate statistical index of chance. Such statistical studies have been few, and data for the most part have been collected from records not obtained under controlled conditions; in most instances, the series have been of 100 patients or less. It is obvious that valid results cannot and do not obtain from such haphazard data often gathered by poorly trained workers—data not destined to be used in a reliable statistical evaluation.

Furthermore, it has been stated repeatedly that obesity predisposes to

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infertility, although adequate statistical proof of this fact never has been demonstrated. Based on an analysis of male semen, it has been postulated that after the age of 24, fertility in the American woman decreases.

It is in the hope of shedding light on these and other problems by reliable statistical evidence that this study was undertaken. This report is the first in a series which it is hoped will include 1000 white females, all of whom will have been examined, and the resulting data recorded and classified by a standard method.

MATERIAL

The patients included in this study were all seen in the Department of Obstetrics and Gynecology at the University of California Hospital, within 4 days postpartum. They were selected from both private and clinic groups. All were white, married a minimum of 24 months (during one or more marriages), between the ages of 20 and 34; all were seen consecutively. It was felt that the study should be limited to a single race, and selections and exclusions were made accordingly. The age group–20–34–was chosen since it is presently accepted as being the most fertile period, and because it can be correlated well with statistical population trends and census reports. It was felt also that patients among this 15-year age spread would be less prone to error in reporting events of their past pregnancies, menses, and so on. A 24-month period of marriage was accepted as the minimum for this study.

The method of interview was given careful consideration before the study was undertaken. It was believed that only by the spontaneous type of personal questioning, with questions not previously announced to the patient, could an accurate and truthful statement of fact be obtained. The very rapidity with which the questions were presented precluded meditation or deviation on the part of the patient. Of necessity, the age and experience of the interviewer were considered, and the same questions in the same manner and order were submitted to all patients. At the beginning of the interview it was stipulated that each patient might accept or reject it. Thus, the prerequisites for amassing the statistics presented in this study were: (1) a white woman between the ages of 20 and 34, from 1 to 4 days postpartum; (2) an experienced interviewer of sufficient years for stable judgment and ability; (3) a full acceptance of the interview; and (4) information obtained by a routine series of personal questions, previously unknown to the patient.

Recently we have been bombarded with various kinds of so-called statistical analyses in related fields. In most cases the information included in such analyses has been voluntary, and the individual giving the answers has had time to prepare them previously. With such a method replies are not apt to be as accurate, and in many cases it must be recognized that the truth has been tempered upon consideration. It is obvious that such a method, therefore, cannot give as valid information as can the method used for this study. Nor can statistics gathered by less well-trained workers be honestly compared with that obtained by an individual trained in the study of the particular field being investigated. Such an individual not only asks the questions personally, but codes and records them immediately following the interview throughout this study.

METHOD

All information was recorded on statistical cards devised by the Cancer Research Division of the University of California Hospital. The cards after completion were sent weekly to the statistician, and the author (who was also the interviewer) had no opportunity to review or change any data before the statistical analyses had been formulated. This method of procedure was followed with the sole purpose of avoiding bias in the final statistical tabulation, and to prevent even the slightest change in the method of interrogation.

Of the 258 consecutive patients questioned, only 8 refused to complete the interview on various grounds. This fact was noted on their cards, which were discarded insofar as this study is concerned.

The age of each individual was rechecked by exact birthdate. Weight was estimated from figures obtained immediately preceding pregnancy; height was by actual measurement. A standard table of weights was used to compute ideal weight, and percentages of over- and underweight.

Family History

Questions on family history related only to obesity, diabetes, and cerebrovascular disease.

History of Siblings

This was limited to prematurity, birth weight (normal or over 9 pounds). Weights of siblings were classified as normal, over- or underweight in age groups 0–11, 12–19, and 20 or over. Frequency of diabetes and cerebrovascular disease was considered.

Patient History

This included age and weight at menarche, and average weight from time of menses to first marriage. The menstrual cycle 6 months previous to each conception (24 to 34 days was considered as regular); amenorrhea, menorrhagia, oligomenorrhea, and polymenorrhea were noted. The number of months of first marriage, age, weight, and menses at the time of marriage were recorded. In cases of second and third marriages all pertinent information was obtained. Diabetes, high blood pressure, hypo- and hyperthyroidism, and relative infertility were tabulated. Tests for tubal patency were noted. Endocrine medication, thyroid, and estrogens and length of treatment were listed. Abdominal operations, age performed, and hirsutism and its degree were recorded.

Because all the information obtained was so precise, and was valuable in other studies, absolute infertility and hysterectomy were also noted.

The number of months of exposure, and number without (due either to abstinence, contraceptive technics, or other reasons) before each pregnancy was recorded, as was the total number of months of gestation. Information included the type of contraceptive, its percentage of use, its effectiveness. The records were detailed as to whether contraception was ineffective or not used, whether the resulting pregnancy ended in spontaneous or criminal abortion, miscarriage, premature delivery, dead fetus, or living, full-term infant.

As to the matter of weight, records were made of weight during 6 months previous to each conception; weight was noted as gain, loss, or maintenance. Actual gain during each pregnancy (most figures were obtained from hospital charts); length of labor; and complications of pregnancy (hypertension, toxemia, cesarean section, varicose veins) were recorded.

In the case of one or more marriages, each husband's occupation and status of his health at the time of interview, age, and fertility status (if known) were listed. Inquiry was made as to whether he had been, or was presently, engaged in high-altitude flying, whether he had had arthritis, and frequency of coitus.

RESULTS

Of the 250 patients interviewed, 104 (41.6 per cent) conceived in 2 weeks or less; 23 (9.2 per cent) conceived in 1 month of exposure; 27 (10.8 per cent) conceived in 2 months of exposure; 21 (8.0 per cent) conceived in 3

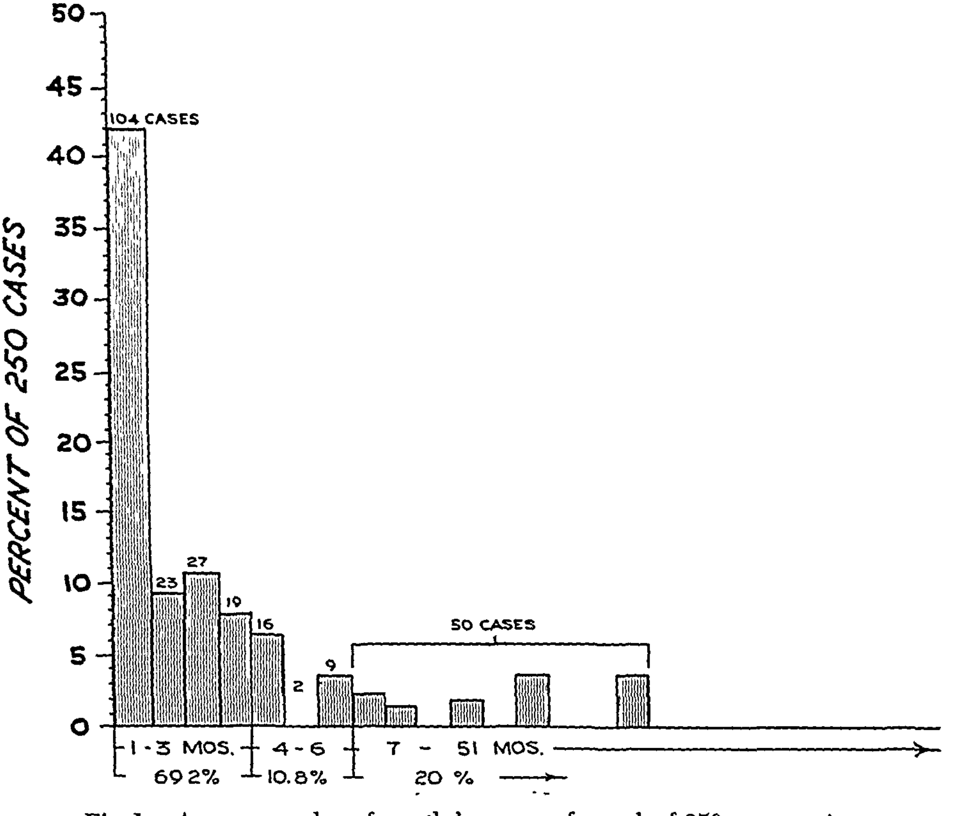

Fig. 1. Average number of months' exposure for each of 250 pregnancies.

months—a total of 69.6 per cent for all groups. These patients were all classified as being of high fertility. In the medium fertility classification there was a total of 26 patients, 15 of whom conceived in 4 months; 2 in 5 months and 9 in 6 months—a total of 10.4 per cent of the whole series. The low fertility group consisted of 50 patients, 20 per cent of the entire series, and these conceived, on the average, in from 7 to 51 months (Fig. 1).

Menses

In the series 15 (6 per cent) patients showed totally irregular menses throughout their histories. Six (40 per cent) of this group were patients in the high index. Of all those interviewed, 94 per cent had regular cycles; in the high fertility bracket 67.2 per cent; in the medium, 8.8 per cent; and in the low, 18 per cent. Of the 6 per cent which comprised the irregular cycle group, 2.4 per cent of the total were in the high; 1.6 per cent in the medium and 2 per cent in the low fertility index. Stated differently, 10 per cent of all those with low fertility had irregular cycles, compared to only 3.4 per cent in the high. In reviewing the relationship of fertility to the age of the patients in this series, there was little or no falling off of fertility until the patient had passed the age of 29.

Contraception

Where contraceptives had been used at some time during the patient's history (and in my series 200 patients fell into this category), 159 (79.5 per cent) of this group were in the high fertility index; 22 (11 per cent) in the medium, and 19 (9.5 per cent) in the low fertility class. Fifty patients denied having used contraceptives at any time, and of these, 14 (28 per cent) were in the high fertility bracket; 5 (10 per cent) were in the medium; and 31 (62 per cent) were in the low fertility group.

Weight

According to the weight table in the high fertility group 16.8 per cent were overweight, 14.0 per cent were normal, and 38.8 per cent were underweight. In the medium group 2.8 per cent were overweight, 0.8 per cent were normal, and 6.8 per cent were underweight. In the low fertility group 6.8 per cent were overweight, 3.6 per cent were normal, and 9.6 per cent were underweight.

Conception Time

In the only two other investigations of this type that appear in the literature[1, 2] to my knowledge, both show a lower average fertility among their patients than that reported here. It is felt that this can be adequately explained–in the first instance, by the type of survey and available records. As has been pointed out, it is not possible to secure an accurate and adequate appraisal of each patient from hospital records, for the reason that the time and experience of the recorder is not known. In the second instance, the series is small, and was obtained partially from hospital records.

Statistically it is of significance that 69.6 per cent of all these postpartum patients conceived in 3 months or less, and this study shows furthermore that 80 per cent conceived within 6 months. These figures are at variance with the accepted precepts that 1 to 2 years after exposure (depending on the authority) should elapse before instituting an infertility investigation. Therefore, it would appear that, in cases where the couple were very desirous of having children, after a 3-month exposure without conception a study of the fertility status justifiably should be done.

Notwithstanding the fact that within 1 month 50.8 per cent of all patients in this series conceived, it may be assumed statistically (law of averages) that among the partners there must have been a definite percentage of males with low fertility. It is interesting to speculate on the statistical chance of these 127 women (50.8 per cent). Can it be assumed that of the 127 males involved, there may have been those of lowered fertility? This problem deserves consideration and investigation, for it is apparent that if the female plays the leading role in conception, our attack should be directed mainly in her direction. It behooves us to try to learn what makes this group of 127 women different from all others.

That irregular menses is a direct contributor is brought forth by the statistics of 15 women who had irregular cycles throughout their histories. Three times as many women who were in the low group complained of this symptom as compared to those in the high fertility index.

Although it has been stated by McLeod,[3] based on a statistical analysis of male semen, that after the age of 24 female fertility decreases, there is no valid evidence to support this in my study. Other dermographers[4] further bear out my findings.

It is highly significant that in our group 80 per cent of the total used contraceptive methods at various times. Of these, 79 per cent of the highly fertile used contraception whereas only 9.5 per cent of those in the low fertility group used it. These figures are at variance with those obtained in the Indianapolis study,[5] where 95 per cent of their "relatively fecund" group used contraceptives, versus 37 per cent of their "relatively sterile" group. This can be adequately explained by the selected type that was used in their investigation.

SUMMARY AND CONCLUSIONS

1. 258 postpartum clinic and private patients between the ages of 20 and 34, white, and married a minimum of 24 months were interrogated consecutively with no forewarning by an experienced interviewer. Eight were excluded because of refusal to answer part of the questions.

2. All answers were tabulated and recorded at the time of interview, and analyzed statistically independently of the author (interviewer).

3. Results of these analyses show that 50.8 per cent of all postpartum private and clinic patients conceived within 1 month or less of exposure; 69.6 per cent within 3 months of exposure; 80 per cent within 6 months; and the remaining 20 per cent averaged from 7 to 51 months before becoming pregnant.

4. Totally irregular menses throughout the history of a patient is shown to decrease her chance of conception.

5. Evidence is given to substantiate the fact that the fertility of the white female does not decrease until after the age of 29.

6. There is no evidence, either positive or negative, that weight plays a role in fertility. In order to determine the part the factor of weight plays, it is necessary to obtain a larger series.

7. The previous use of contraceptives has no bearing on fertility; in this series it was used approximately 9 times more frequently by those of high fertility.

8. It is concluded from these statistics that a maximum time of only 6 months should elapse before instituting a fertility investigation.

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DISCUSSION

DR. FRANCES E. SHIELDS, *Monterey, Calif.*: Dr. Whitelaw's study of these 250 postpartum women has revealed interesting information concerning fertility factors of this group. None of his conclusions seems surprising. The fact that some of his figures do not exactly correspond with the impressions we have gained from our own patients or other published data has little academic significance.

In reviewing my series of donor inseminations, it was found that 40.2 per cent of the patients became pregnant during the first month of inseminations; this is a lower figure than Dr. Whitelaw's. Seventy-two and seven-tenths per cent became pregnant within the first 3 months; this is a higher figure than Dr. Whitelaw's.

Because initial tubal insufflations had been done on many of these patients, it was interesting to find that more patients who had not had a tubal insufflation done became pregnant within the first month than those who had had one insufflation. Of the 10 patients who required repeated insufflations and uterosalpingograms before patency was established, only 1 became pregnant during the first month of inseminations.

It is comforting to have added assurance that contraceptives in general do not alter fertility.

I would like to congratulate Dr. Whitelaw on this paper and his continuing study, and to emphasize his final conclusion that not more than 6 months should elapse before instituting a fertility investigation.

DR. TOMPKINS: Thank you very much, Dr. Shields. Dr. Whitelaw's paper is now open for general discussion.

DR. N. T. WERTHESSEN, *San Antonio, Texas*: Doctor Shields said that there was a difference between the numbers she obtained and those Dr. Whitelaw obtained. I do not think that the difference is large enough to be statistically significant, and I wanted to ask Doctor Shields what is the correlation coefficient between the insufflation and the frequency of pregnancy after a single administration of spermatozoa?

DR. SHIELDS: There were 31 patients in this group. Sixteen of them had not had any tubal insufflation, 14 had had one, and 1 patient had had a good many more than one.

DR. WERTHESSEN: I am wondering whether you believe that the insufflation is a help toward achieving a pregnancy.

DR. SHIELDS: I do not think it is any help. I do not believe it is statistically important. It was not done to help a patient become pregnant. It was done to assure me of the patient's fertility.

DR. A. S. WAINER, *Philadelphia, Pa.*: Dr. Whitelaw, you made a statement that fertility studies should be instituted after 6 months. I wonder if that is statistically significant.

DR. WHITELAW: The statistics on this particular study at the university show that it is valid, that in this group that was studied—of course, this is a selected group, indirectly, since they came to the university—close to 70 per cent of all these patients (69.6 per cent) became pregnant after 3 months, and 80 per cent after 6 months, so it is to be presumed that it will hold in other cases. When I have another 750 I perhaps would have a little more to say.

DR. TOMPKINS: If there are no further questions, Dr. Whitelaw, do you have closing remarks?

DR. WHITELAW: I just want to thank Dr. Shields for her kind remarks and also to say that I think her series more or less corroborates what we found, that roughly 50 per cent of all women by chance will become pregnant within the first month. Thank you very much.

Correlation of the Male and Female Factors in Human Infertility

John MacLeod, Ph.D., Ruth Z. Gold, M.A., and Charles M. McLane, M.D.

In previous studies[15, 19] we have analyzed the factors in the human male which would appear to determine potential fertility. We have restricted our observations to semen quality in relation (1) to a fertile population in which ability to conceive was not a serious problem and (2) to an infertile population in which a conception was difficult or never was achieved. Each population comprises approximately 1600 couples. We have analyzed all aspects of semen quality (ejaculate volume, sperm count, motile activity, and morphology) by the use of simple technics which are available to, and can be used by, doctors in their offices. Until the present study, we have related semen quality to conceptions produced in fertile marriage without considering the gynecologic history. We followed this course deliberately in the hope that we would attain an objective appraisal of the factors in semen quality most conducive to conception as well as those likely to prove detrimental, and with the full knowledge that any conclusions reached might be modified when the history of the female partner was considered.

Several tentative conclusions have been reached by us in regard to semen quality and the possibility, eventual or otherwise, of conception. The first is

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We are deeply indebted to Muriel Lavenda for her valuable technical assistance and for her aid in the preparation of the data and charts.

that above a level of 20 million cells/cc., the sperm count per se is not the most important factor, and, indeed, that the potential fertility of any individual, above a certain sperm count level, cannot be equated with the sperm count. The second general conclusion reached is that there is a far closer relationship between the quality of spermatozoon motility (the rate of forward progression of the cells) and potential or actual fertility. The third conclusion is that, while extremes of abnormal sperm morphology may play an important part in certain cases of human male infertility, the role of sperm morphology in the over-all problem is not as significant as that of motility. However, we have left the possibility open that there may be a close relationship between abnormal morphology and poor motile activity and that, in such cases, impaired fertility may be due to the latter factor. This possibility will be examined in detail in another study.

In the present study we have endeavored to correlate semen quality and known gynecologic disorders in the female partner with conception performance. This has been done on a large population of cases of infertility. The nature of this population is described below.

NATURE OF THE POPULATION

To satisfy the classification of "infertility," only those couples satisfying the following criteria were considered eligible for the study:

1. Those who had been trying unsuccessfully for conception for at least one year at the time of the semen examination.
2. Those who had not produced a conception in the present marriage.
3. All couples were seen during the period between June 1, 1949, and April 15, 1951.

During the above period, 1594 semen examinations were done in individual cases. The period of infertility ranged from under three months (16 couples) to over ten years (38 couples). In 360 of the marriages, previous conceptions were reported. An additional 110 cases lacked information on this point, no history being available. Elimination of the above-mentioned cases left 1124 cases known to be of primary sterility. Of these, 179 couples had been trying for conception for a period less than one year and were excluded as not yet "infertility" cases according to our definition. An additional 29 couples were eliminated because they did not know how long they had been trying. A total of 916 couples remained who were known to have been trying at least one year and who had never produced a conception.

A follow-up on these cases was initiated in April, 1952, so that all cases had a possible follow-up period of at least one year. The procedure was as follows:

1. A questionnaire requesting the gynecologic history of the wife as well as information on pregnancies and their outcome was sent to the clinic or doctor referring the husband. The 916 couples in question yielded the following information:

   132 pregnancies.

   55 patients who had stopped treatment from one to 11 months after the semen examination and who had not produced a conception at that time.

   265 patients still in touch with the referring doctor or clinic one year after the semen examination and who had not achieved a conception.
2. When, in these circumstances, and after a reasonable period of time, no response was obtained from the referring agent, or when the latter reported that he had lost touch with the patients, a printed questionnaire was sent directly to the couple by us. This procedure yielded 34 more pregnancies and the information that 222 couples had not achieved a conception.
3. When the printed questionnaire did not elicit a reply, individual letters explaining the purpose of our study were sent, the response to which revealed 14 pregnancies and 75 additional unsuccessful couples.
4. Lastly, we had eventual personal contact with 14 more couples, 13 of whom were seen at least a year after the initial semen examination.

Thus, of the 916 couples, a full year follow-up was available on:

1. 180 couples who had been successful in achieving a conception within that period of time.
2. 575 couples who had not been successful in that period. (Of these, 105 were successful subsequent to the one-year follow-up, and, while they are not used in this study, the results are included in the 36 per cent eventual success. We obtained a partial follow-up on 56 couples, and no follow-up of any kind was obtained in 105 cases.

Of the 916 couples, more or less complete information was compiled on 755 (82.4 per cent) and partial data on 56 (6 per cent). Each of the latter was carried as one-half an observation (that is, half a failure). The average follow-up on this group was 4½ months, and the "success" (pregnancy) rate computed along the lines indicated corresponds very closely with that arrived at for the end of 12 months by the life table method of analyzing the pregnancies in monthly intervals.

The 105 couples for whom no follow-up of any kind is available cannot be ignored. The possibility of bias is too great. It is essential in a problem of this kind to determine whether the characteristics of the "unknown" group are the same as that of the "known" group or so similar that their contribution to the study would be calculated as essentially the same.

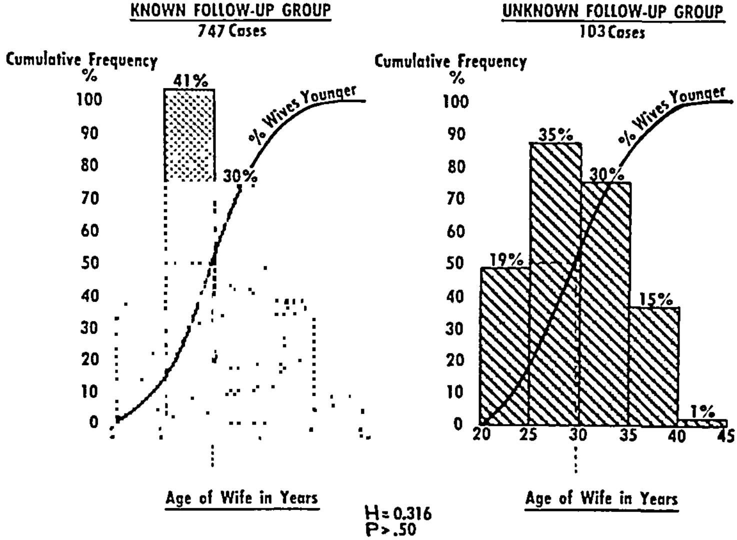

Fig. 1. Relative frequency distributions of age of wives.

The method of testing the differences in distribution between the two groups is that of the H test, a procedure based upon ranks, which tests for differences due to shifts in distribution and in which H is distributed as χ^2 with 1 degree of freedom. The following factors were studied in the two groups:

Age of wife.
Age of husband.
Duration of infertility at the time of semen examination.
Frequency of intercourse.
All factors in semen quality.
Fertility of husband.
Fertility of wife.

In most cases there was a remarkable similarity in distributions in the two groups. A few of the above factors have been selected as examples.

Figure 1 shows the distribution of the age of the wife in both groups. The only difference (not significant) is in the over-30 age group, and, as we shall show later, this difference is, if anything, in the direction of "conservative bias." The similarity in age distributions of the husbands in both groups was found to be even greater.

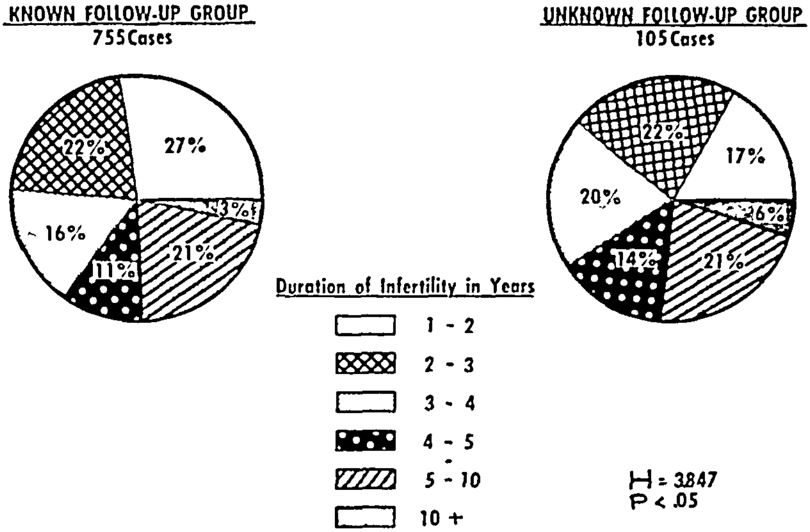

Fig. 2. Distribution of duration of infertility.

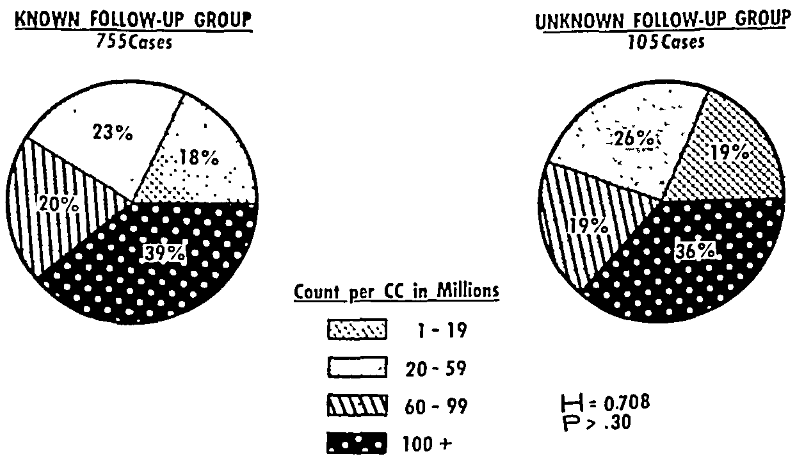

Fig. 3. Comparative distributions of sperm counts.

Figure 2 shows the only significantly differing distribution (the significance is just borderline)—the duration of infertility. But even this factor is not important. If we apply the pregnancy rate specific for the duration of infertility from the "known" group to the "unknown" group, instead of a 23 per cent pregnancy rate we would expect 21.3 per cent. The latter figure would depress the rate for the entire group (916 couples) from 23 per cent to 22.8 per cent.

Figure 3 demonstrates the comparative distributions of the sperm count/cc. and is typical of the similarity shown for all other aspects of semen quality, ejaculate volume, sperm motility, and morphology.

A serious difficulty is encountered when we endeavor to compare the fertility of the wife in both groups. The 105 "unknown" couples are of necessity those for which our gynecologic information is sparse. In this group data were lacking in 50 per cent of the cases as compared with 25 per cent in the "known" group. In the latter group 25 per cent of the wives were classified as of "poor fertility" as contrasted to 35 per cent of the "unknown" group. This difference is not statistically significant.

The following data, therefore, are presented in full awareness that they are based upon incomplete information. But, at the same time, we have not detected any consistent bias which might invalidate any major conclusions reached. The influence of any bias would be diminished by considering rates for specific categories such as women of a given age, semen quality of a certain level of fertility, and so on. Since most of the data which follows is based upon such analyses, we are reasonably confident of our conclusions.

TIME DISTRIBUTION OF 180 CONCEPTIONS

We have chosen the date of the initial semen examination (in our laboratory) as zero time in the follow-up. This does not mean necessarily that the semen examination initiated the first clinical examination of the couple for their infertility. Many couples had sought help elsewhere in the past and either had received conflicting reports on their fertility status or had been told positively that they could or could not have children. But we had to stabilize the time factor in some fashion, hence the choice of our semen examination as zero time and at least one year of infertility as our requirement for the proven infertile state. Of the 180 conceptions occurring within one year of the first semen examination done by us, 48 or nearly 27 per cent of these occurred within one month after the semen examination, and 70 per cent of them in less than six months after this time (Fig. 4). In many of these 48 couples, the conception time was calculated by us from the delivery date and in others by adding 15 days to the last menstrual period, when this date was given. These methods of calculation indicate that in certain cases conception had occurred *before* our semen examination was done; that is, during the menstrual cycle in which the semen was examined. Bender has reported similar instances. Several provocative questions are inherent in the undoubted fact that 48 couples seeking conception for at least one year suddenly attained their objective in the very month they sought clinical help. If all these cases were seeking aid for the first time, objective therapy in itself could not have been the initiating factor. But at least 22 of the 48 couples had been examined elsewhere prior to our semen examination, and in these cases we cannot disregard the possibility that treatment elsewhere of the wife might have produced the desired end at

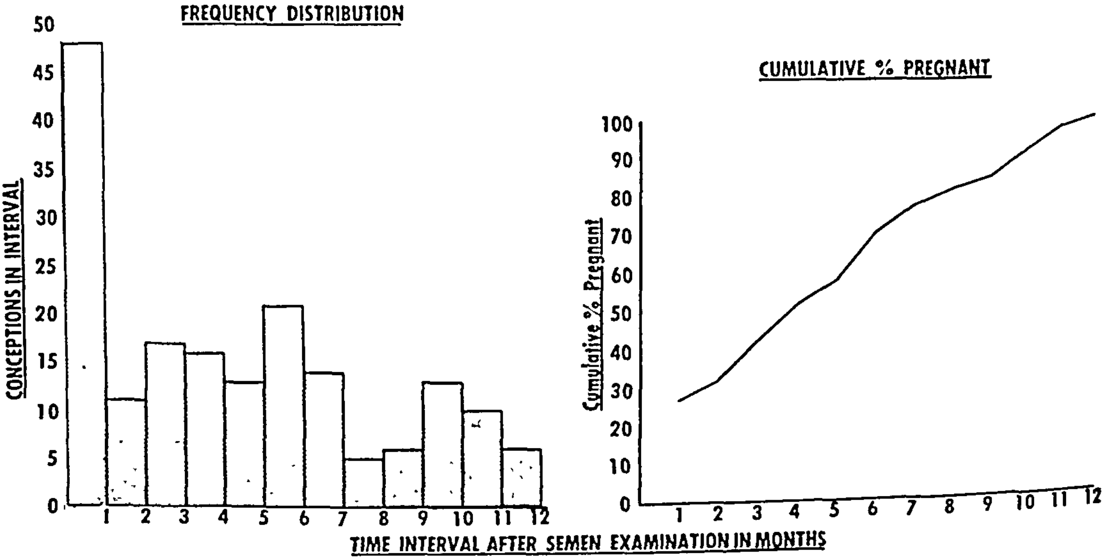

Fig. 4. Distribution of 180 conceptions produced within one year after semen examination.

the particular time a new clinician was sought. Nevertheless, 22 conceptions, or 12 per cent of the total number occurring within one year, took place either in the same month of the initial clinical examination of the couple or immediately prior to their first visit to the doctor.

In four of the 48 couples who conceived in the first month, no information was available as to whether or not a previous semen examination had been done.

One must assume that a psychologic factor aided in these conceptions. This is by no means a new concept in human infertility. Bender and Mazer and Israel, for example, have observed a strong element of the psychosomatic in their studies of infertile couples, and none has expressed the concept more aptly than Bishop when he said: "We have an expression at our Chelsea clinic called 'walking down the ramp.' There used to be a ramp which led from the road outside down to our clinic, and we found when we examined this series of pregnancies (214) that by far the greatest single factor in the patients' achieving pregnancy was walking down the ramp. Much the greatest number of those who became pregnant were pregnant either in the cycle in which they first attended for interview or before we had a possibility to carry out any treatment at all."

Table 1 presents certain pertinent information on all the 180 conceptions which comprise this study and amplifies the data given in Fig. 4. As we shall show presently, the age of the wife and the duration of infertility are

TABLE 1. Time Taken to Produce Conception in Terms of Age of Wife and Duration of Infertility

Time for conception	Number of couples	Age of wife		Duration of infertility		Fertility (%)	
		% < 25	% < 30	1–2 yr.	1–3 yr.	H. Poor	W. Poor
1 month	48	21	75	35%	65%	46	5
2–6 months	78	22	74	35%	67%	34	12½
7–12 months	54	19	65	46%	67%	26	24
TOTAL	180	21	72	38%	66%	35	14
			Not sig.	Not sig.		Not sig.	$\chi^2 = 6.341$ sig.

most important factors in the prognosis for conception. Table 1 demonstrates that while 72 per cent of the 180 women who conceived within one year after our semen examination were under 30 years of age, the age factor was not of importance in determining whether they would conceive in one month or twelve. Nor was the duration of infertility up to three years of any real significance in this regard. We shall refer later to the relation between the potential fertility of the husband and wife and time taken for conception (Table 1).

FACTORS STUDIED IN RELATION TO CONCEPTION

Age of Wife

The cumulative frequency distributions of the "conception" and "no conception" groups and, in addition, the percentage of wives pregnant within one year for each age group are shown in Fig. 5. The age distributions obviously are different. Over 70 per cent of the "conception" group wives were under 30 at the time of our semen examination, whereas only 50 per cent of the "no conception" group are in this category. It is most interesting to note that the conception rate decreases from 37 per cent in the below-25 group to about 14 per cent in the 30-39 age class. None of the wives (14) above 40 conceived.

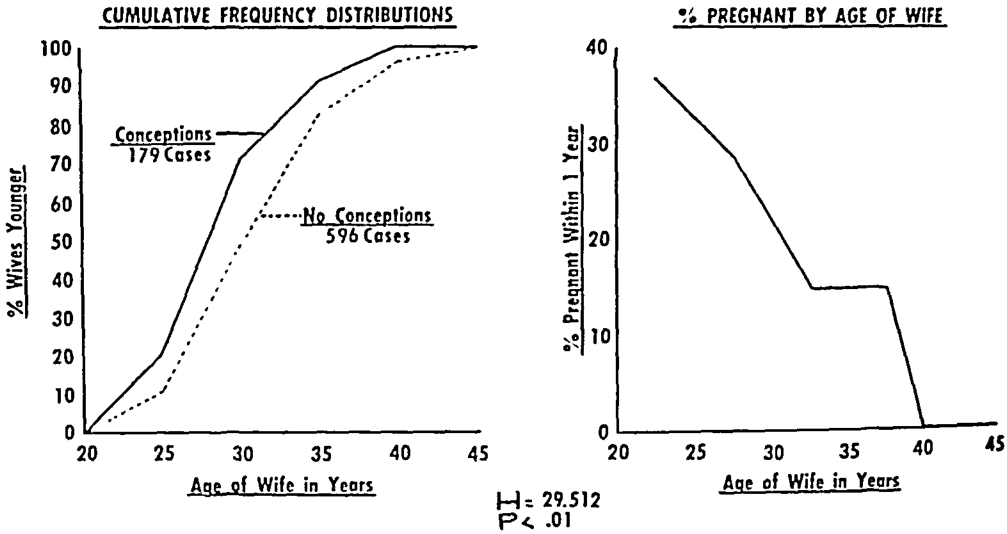

Fig. 5. Cumulative frequency distributions and percentage of wives pregnant within one year by ages of wives.

Age of Husband

The picture so far as husband age is concerned is so similar to that presented for the wife that no figure is shown. However, there is a very strong relationship between the age of the husband and that of the wife (Table 2), 90 per cent of the husbands under 30 had wives in the same age category; with men over 40, only 11 per cent of their wives were under 30. Thus, when we speak of "older" men, say over 35 years, we are speaking of men whose wives are predominantly over 30. In studying the relationship between the ages of husbands and wives and conception, the ideal position would be to

TABLE 2. Age of Wife in Relation to That of Husband

Age of husband	*Proportion of wives < 30 years of age (%)*
< 30	90.5
30–34	56.5
35–39	22.3
40 +	10.8

take wives (or husbands) of a fixed age and study change in pregnancy rates with increasing age of the spouse. But because of the strong association between the ages of husbands and wives, this is difficult—there are too few older wives with young husbands, so that percentages derived from such small numbers would be unreliable. It is, however, quite legitimate and practical to choose suitable divisions in the ages of both partners and study the percentage of conceptions. As seen by Table 3, such comparisons are most illuminating and place considerable onus on the age of the wife as a factor in conception. It is obvious from the table that the age of the wife and not the husband is all-important. We have established[20] that insofar as all criteria of semen quality are concerned, male fertility shows no obvious changes between ages 20 and 40. Also, in a population of known fertility,

TABLE 3. Pregnancy Rate in Relation to Ages of Husband and Wife

	Husband	
Wife	*< 35*	*35 and over*
< 30	31.1%	24.7%
30 and over	15.6%	13.7%

using time taken to produce a conception as the criterion for measuring degree of fertility, we observed a decline in female fertility which appeared around age 25 and followed a steady, increasing course downward in successive years.[19] Figure 5 shows a similar state of affairs in what we must call an "infertile" population, the fertility decline again becoming most apparent around age 25, stabilizing at about age 33, and falling precipitantly at about age 38. We have compared the gynecologic histories in the age groups under and over 30 and do not find a significant difference in the reports submitted to us by the gynecologists. There are no decided increases in the incidence of irregular periods, tubular or cervical pathology, or similar disturbances in the older group that would set them apart from the younger. This age-fertility differential has been observed by others though Barns *et al.* do not consider the age of the wife to be as important in prognosis as the duration of infertility. We shall consider this point in detail.

Duration of Infertility

There is a significant relationship between the duration of infertility and the percentage of patients eventually conceiving (Fig. 6). The pregnancy rate decreases sharply with increasing duration of infertility. In Fig. 6 B, we have included cases with a duration of infertility of less than one year to demonstrate further the significance of these findings. The latter are indicated by a broken line. By our definition, these cases are not bona fide infertility, but it is of interest to see that 50 per cent of the couples conceived within one year as compared to 31 per cent with infertility of 1–3

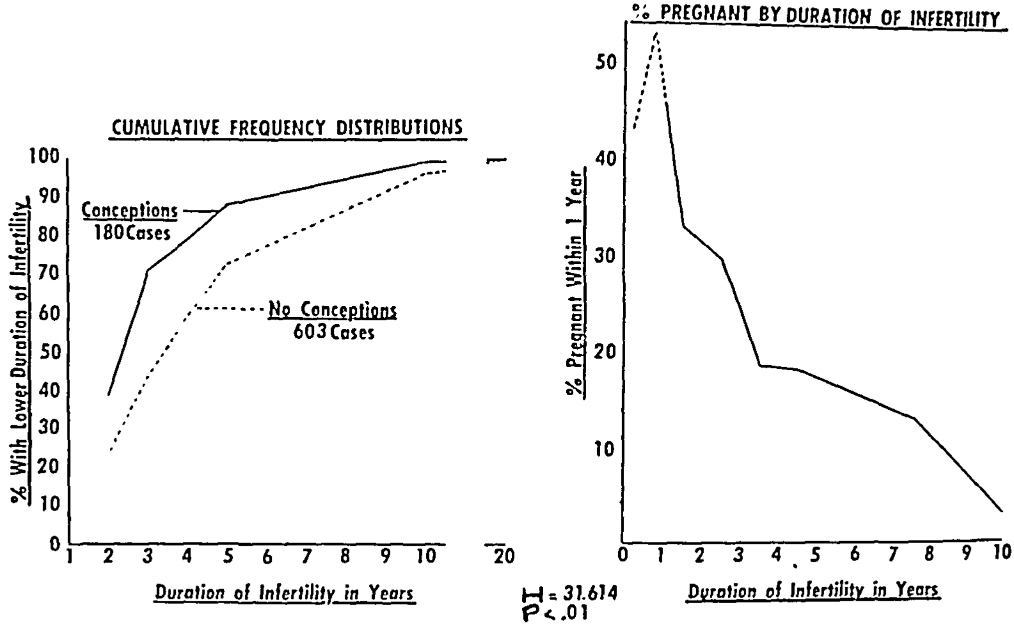

Fig. 6. Relation of duration of infertility to percentage of conceptions.

years, 18 per cent of 3–5 years, 13 per cent of 5–10 years, and only 4 per cent of those trying at least 10 years.

Needless to say, the duration of infertility is related to the age of the wife in that, for example, most of the wives trying 10 years or more were over 30 years of age. Table 4 presents the combined effects of age of wife and duration of infertility on conception.

For those patients trying 1–3 years, there is a significant difference in the eventual pregnancy rate between the women of less than 25 years (43 per cent) and those over 30 (24 per cent). Similarly, in those trying for 5 years

TABLE 4. Pregnancy Rate in Relation to Age of Wife and Duration of Infertility

<table>
<tr><th rowspan="2">Duration of infertility (yr.)</th><th colspan="3">Age of wife</th></tr>
<tr><th>< 25</th><th>25–29</th><th>30 and over</th></tr>
<tr><td>1–3</td><td>43%</td><td>36%</td><td>24%</td></tr>
<tr><td>3–5</td><td>20%</td><td>21%</td><td>16%</td></tr>
<tr><td>5 and over</td><td colspan="2">18%</td><td>7.5%</td></tr>
</table>

or more, the difference in pregnancy rate between these two age groups is highly significant—18 per cent as compared to 7.5 per cent. The differences between the various age groups with an infertility history of 3–5 years is not significant.

It is plain, therefore, that, as Barns *et al.* have observed, the duration of infertility is important in prognosis but we submit that the harshness of

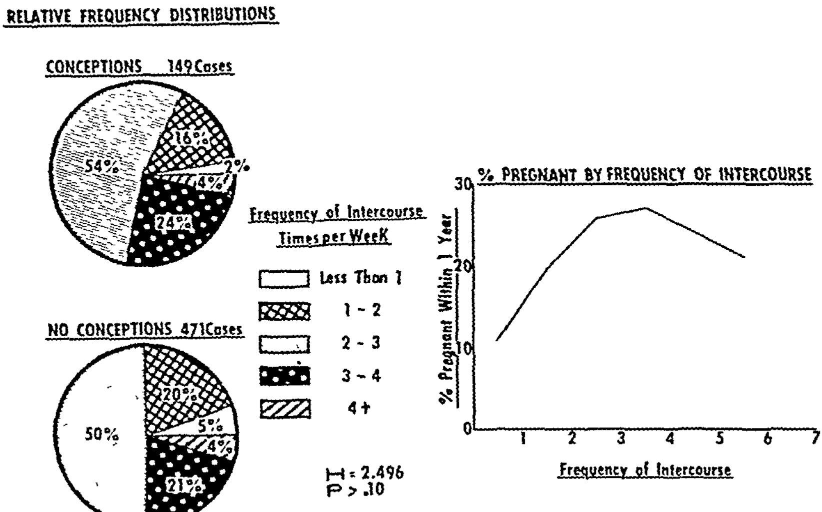

Fig. 7. Relation of frequency of intercourse to pregnancy rate.

the prognosis must be modified if the wife is under 30, and if, as we shall consider later, the husband's fertility is good.

Frequency of Intercourse

In our study of a population of known fertility[19] we found a significant relationship between the frequency of intercourse and the ease with which conception took place (time taken to produce conception). At all age levels of the female partner, we found that the higher the frequency of intercourse, the greater was the ease of conception. We have analyzed this relationship in the "infertile" population (Fig. 7) and failed to find the same degree of significance. However, as can be seen by the graph in Fig. 7, the pregnancy rate for those couples in which the frequency of intercourse normally was less than twice weekly is lower than those whose frequency was twice weekly or more.

SEMEN QUALITY IN RELATION TO PREGNANCY RATE

Ejaculate Volume

There is a significant relationship between the volume of the ejaculate and pregnancy rate (Fig. 8). Only 6 per cent of husbands with an ejaculate volume of less than 1.0 cc. produced conception within one year, as compared to 16 per cent in the 1.0–1.9 cc. class and 27 per cent with over 3 cc. We shall discuss the possible significance of this and the other aspects of semen quality together after the statistics of each have been presented.

Sperm Count Per Cubic Centimeter

There are two levels of sperm counts which have statistical significance. The lower graph in Fig. 9 shows several apparent peaks and depressions, but only at the levels of 20 million and 60 million/cc. can we consider the change to be significant. Above the 60 million/cc. level there is no rise in the pregnancy rate.

Total Sperm Count

There are three levels (Fig. 10) at which the total sperm count appears significant: under 50 million, 50–299 million, and 300 + million. We shall discuss the significance of these later in conjunction with the other aspects of semen quality.

Percentage of Active Cells

The pregnancy rate rises sharply (Fig. 11) and significantly up to the 40 per cent active level and flattens off thereafter.

Quality of Motility

As shown in the graph in Fig. 12, we have used our usual classification of 1, 2, 3, and 4 to indicate rising quality of motile activity; that is, increasing rate of forward progression of the spermatozoa. It is obvious that the pregnancy rate increases as the quality of motility improves, and the increase is statistically significant up to a level of 2+, after which there is no obvious change. It should be noted that 25 per cent of the "no conception" group had a quality under 2+ as compared to only 12 per cent of the "conception" group.

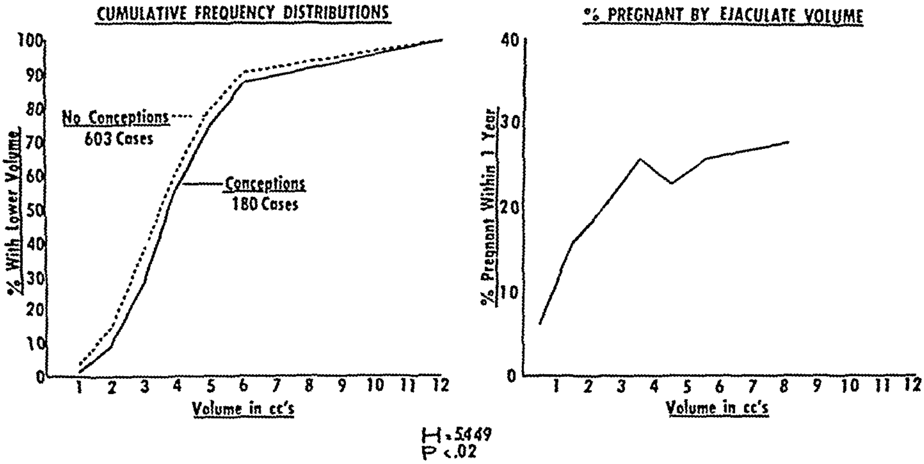

Fig. 8. Relation of ejaculate volume to pregnancy rate.

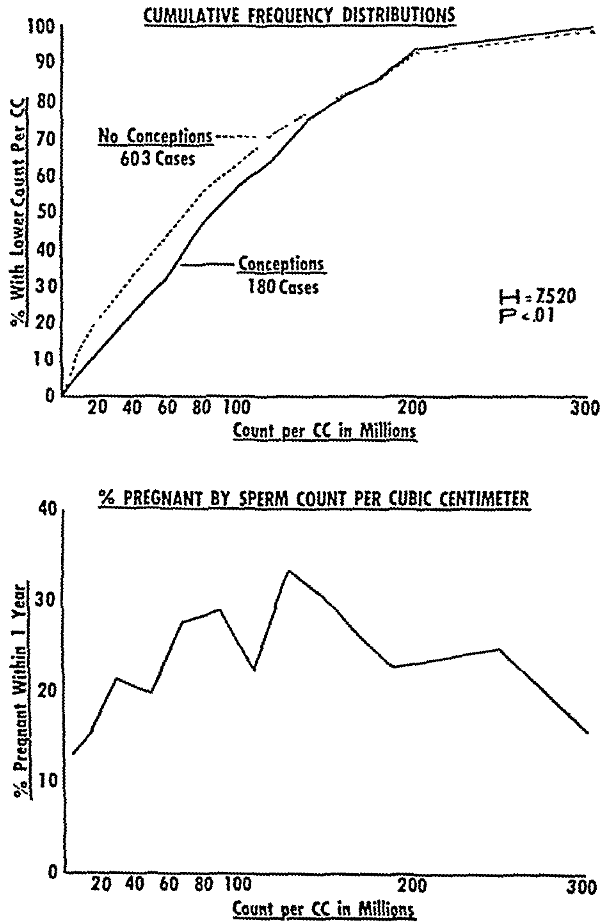

Fig. 9. Sperm count per cubic centimeter.

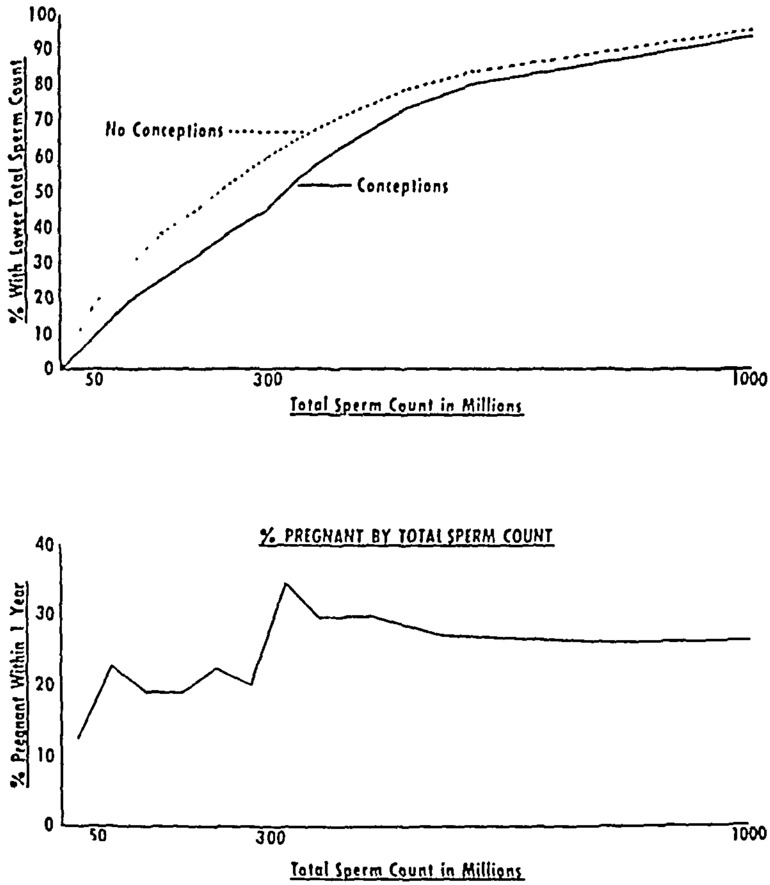

Fig. 10. Total sperm count.

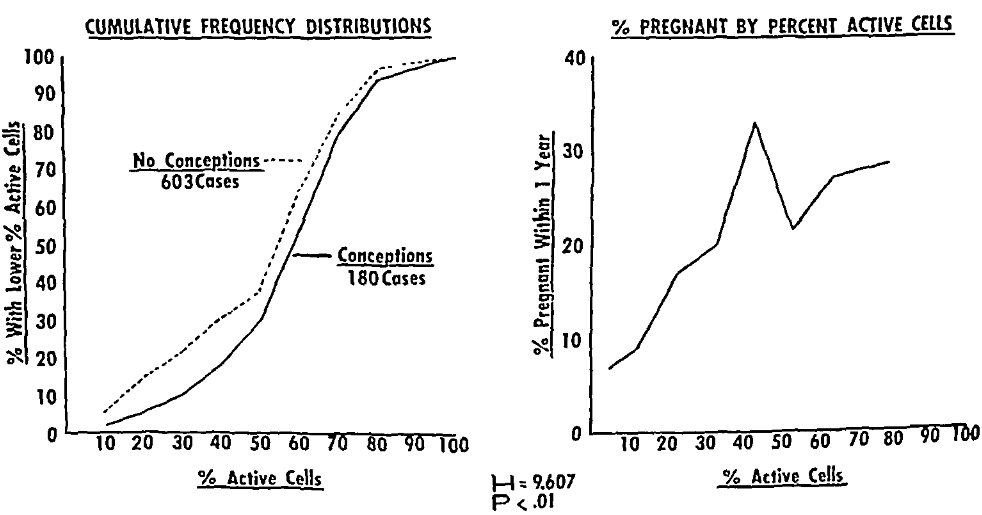

Fig. 11. Percentage of active spermatozoa.

Morphology

Though in Fig. 13 the relation of pregnancy rate to sperm morphology shows a rising trend in the rate with increasingly good morphology, the

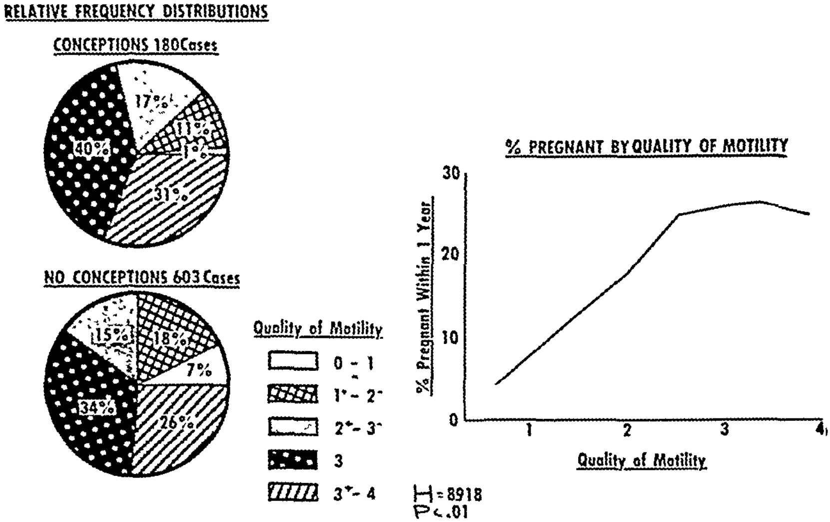

Fig. 12. Quality of motility of spermatozoa.

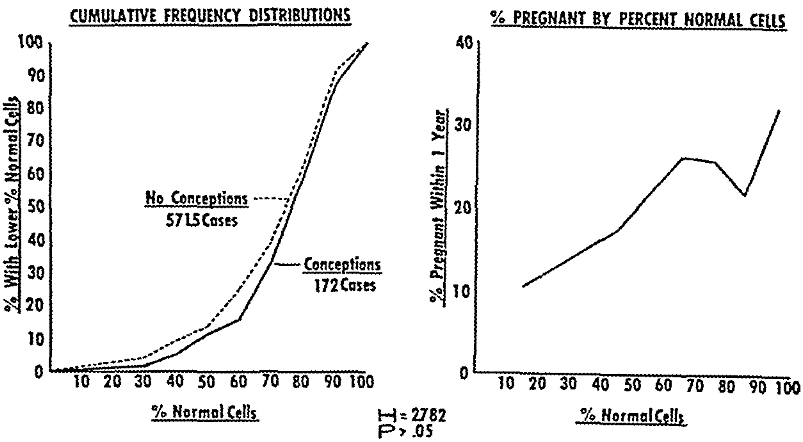

Fig. 13. Relation of normal morphology of spermatozoa to pregnancy.

entire picture is not statistically significant. In previous studies[16] we have found that fewer than 60 per cent normal forms represents a good line of demarcation between a fertile and an infertile population. In the present analysis, the one statistically significant fact is that whereas 16 per cent of those with fewer than 60 per cent normal forms succeeded in producing a conception within one year, 25 per cent of those with 60 per cent normal forms and over were able to do so.

FERTILITY OF HUSBAND AND PREGNANCY RATE

In our previous studies we have considered semen quality to be poor when (1) the ejaculate volume was under 2 cc.; (2) the sperm count/cc. was under 20 million; (3) the percentage of active cells was under 40; (4) the quality of motility was under 2+; and (5) the percentage of morphologically normal cells was under 60 per cent. A semen specimen could be deficient in any one or all of these factors; and a high quality in one factor

TABLE 5. Pregnancy Rate in Relation to Semen Quality

Number of qualities classed as "poor"	*Number of patients*	*Pregnancy (%)*
3	15.0	0
2	96.5[a]	13
1	184.0	17
0	487.5[a]	28

[a] Where only partial data were received, they were carried as one half an observation.

may compensate for deficiencies in another. Table 5 presents an analysis of the pregnancy rate in terms of semen quality when the latter either had no defects or one or more. We have not included sperm morphology in this analysis as one of the defects because, to have done so, the number of cases in two of the categories would have to have been much larger. For example, only the semen of 15 of the 783 men in this study had all three defects (in volume, count, and motility), and conception did not occur in any one of these cases. In 184 cases where only one defect was present in the semen the pregnancy rate was 17 per cent. In regard to this group, it is of interest to examine a breakdown by the defect present in the semen quality and to compare the pregnancy rate in the presence of each defect to that of the men (487) whose semen showed no defect. The over-all pregnancy rate of the 184 cases with one defect is 17 per cent as compared to 28 per cent in the group of individuals with no seminal defect, a statistically significant difference. However, when the 184 cases are broken down according to the defect, the pregnancy rates for the ejaculate volume and count/cc. defects are not significantly different from the rate of those with no defect. Where motility of the cells is the only defect present, the pregnancy rate of 13 per cent is significantly lower ($\chi^2 = 5.582$, $p < .02$).

It is not at all surprising that the pregnancy rate rises with improving semen quality. We have tried to determine the levels of male fertility which are more significant than others. For example, in the past we have classified the husband as poor, fair, or good, according to certain arbitrary criteria of which the following is a summary:

	Poor	*Fair*	*Good*
Count/cc.	< 20 million	20–59 million	60+ million
Percentage of active cells	< 40	40–59	60 and over
Quality of motility	< 2	2–3	3+ and over
Morphology (per cent normal)	< 60	60–79	80 and over

Thus, fertility is "poor" if one or more qualities are poor, "fair" if none are poor but not all are good, and "good" if all are good. Using the above criteria, Fig. 14 shows the pregnancy rate by the fertility of the husband. There is a significant difference between "poor" and "fair," and between "poor" and "good" fertility, but none between "fair" and "good."

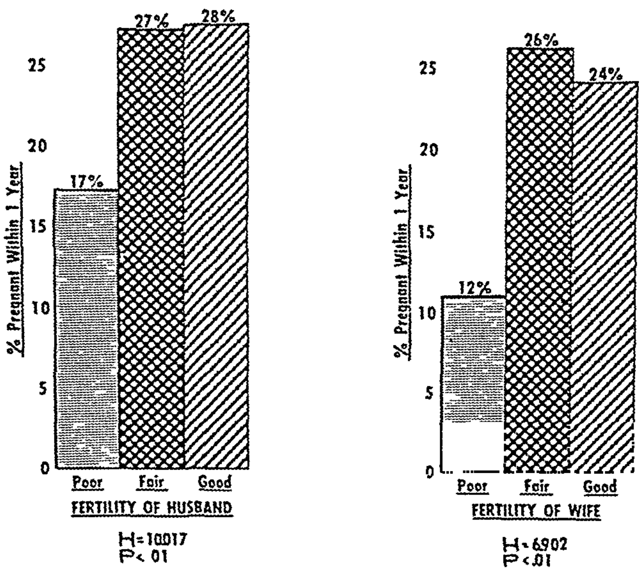

Fig. 14. Pregnancy rate by fertility of husbands and of wives.

FERTILITY OF THE WIFE

Gynecologic data were available for 641 of the 785 cases (82 per cent). Information is lacking for 39 wives in the "conception" group (22 per cent) and for 103 (17 per cent) in the "no conception" group. The source of our information was either from the chart histories of the clinic patients or from the individual doctors who referred the husbands for the semen examination. The ideal study of this kind would be one in which every gynecologic examination was done by the same doctor using standard methods for determination of the fertility status. Unfortunately, this has not been possible, and we admit freely the weakness of the study in this respect. Many doctors (perhaps 50 or 60) sent us their opinions of the fertility status of the wives. and while we have no reason to question their skill and their conclusions, it is obvious, for example, that a diagnosis of poor ovulation from one clinician may not be agreed upon by another. Similarly, we could not always determine whether the diagnosis of closed tubes was based on insufflation or salpingogram, or whether insufflation only was done. However, we had to do our best with the material as it was, and the following are our classifications of the fertility of the wives:

Poor	*Fair*
Poor ovulation	Irregular periods
Closed tubes	Cystic ovaries
Endometriosis	"Slow passage," tubes
	Cervicitis
	Erosion
	Poor postcoital test
	Retroversion
	Hypoplasia

By our criteria, one "poor" condition reported was enough for a "poor fertility" classification. One or two "fair" conditions allowed a "fair fertility" rating, though where more than two in the "fair" category were reported, the wives were placed in the "poor fertility" bracket. Certain doctors replied to our questionnaires by merely giving their impressions of the wives' fertility in general terms as "fair" or "good," and in certain cases the opinion "poor fertility" was not accompanied by a specification of the defects. In each of the above circumstances the verdict of the gynecologist was accepted.

Relating these broad classifications (poor, fair, and good) to the pregnancy rate, we find (Fig. 14), as might be expected, that the conception rate of the "poor" wives was low and significantly different from that of the "fair" and "good" wives. But, as in the husbands, there is no difference in the rate between the "fair" and "good" wives. Table 6 shows the incidence

TABLE 6. Gynecologic Abnormalities

Gynecologic abnormality	Cases with		Cases without		Condition unknown		% of known cases with condition
	No.	% pregnant	No.	% pregnant	No.	% pregnant	
Closed tubes	124.5[a]	18.5	481	23.7	177.5	24.2	20.6
Poor ovulation	76	26.3	529.5	22.1	177.5	24.2	12.6
Irregular periods	72.5	27.6	533	22.0	177.5	24.2	12.0
Endometriosis	17	9.1	596.5	22.8	177.5	24.5	1.8
Hypoplasia	17	11.8	590.5	22.9	175.5	24.5	2.8
Retroversion	58.5	23.9	549	22.4	175.5	24.5	9.6
Erosion	71.5	23.8	536	22.4	175.5	24.5	11.8
Cervicitis	36.0	30.6	571.5	22.0	175.5	24.5	5.9
Poor postcoital test results	120.5	13.3	487	24.8	175.5	24.5	19.8
Fibroids	42	19.0	565.5	22.8	175.5	24.5	6.9
Cervical stenosis	23	17.4	584.5	22.8	175.5	24.5	3.9
Ovarian cysts	25	20.0	582.5	22.7	175.5	24.5	4.1
Low B.M.R.	63	25.4	544.5	22.2	175.5	24.5	10.4
P.I.D.	10	10.0	597.5	22.8	175.5	24.5	1.6
Other	94.5	24.3	514	22.2	174.5	24.6	15.5

[a] See footnote to Table 5.

of the various gynecologic abnormalities reported and the relationship of these to the pregnancy rate. The only gynecologic condition the presence or absence of which relates to a significant difference in pregnancy rate is poor postcoital test result. Of 120 such patients 13 per cent became pregnant within our follow-up period, as compared to 25 per cent of the patients in which no such condition was reported. A logical question is, of course, whether in the poor postcoital reports the husband's semen quality was such as to preclude the possibility of a good postcoital test. We have investigated this point in 90 of the 120 couples. Thirty were eliminated because the wife had at least one other major gynecologic defect. Of the 90 cases in which the postcoital test was reported as poor, the fertility of 42 of the husbands was poor by our criteria. In this group, only 9 per cent (4 couples) eventually conceived as compared to 19 per cent (9) where the husband's fertility was considered by us as good. This difference is not statistically significant, but it is suggestive.

Further, we selected 188 women in which a poor postcoital examination was not reported as a defect, all of whom had a minor gynecologic defect. (This is a population comparable to the 90 cases in which the postcoital test was reported as poor.) In this population, 78 of the husbands were classified by us in the "poor fertility" class. Twenty-five (32 per cent) of their wives conceived within the year. This figure is significantly greater than the 9 per cent who conceived in the face of a poor postcoital test and poor fertility in the husband. We feel that these findings place considerable significance upon the poor postcoital test as a major factor in female infertility. This is true especially when we find that in the other 110 couples of this population where the postcoital test was not reported as poor, and where the husbands' fertility was good, 33 per cent (36 couples) eventually conceived.

We emphasize, however, that only 20 per cent of approximately 600 cases were reported as having poor postcoital examinations. The 487 cases we have listed in Table 6 as not having poor postcoital tests are presented as such because we did not get adverse reports on them. It does not follow that all of these cases were subjected to postcoital examinations, and because of the large number of clinicians involved in the reports we have encountered great difficulty in clarifying this point. We are reasonably certain that many more cases with poor cervical mucus and poor postcoital tests would have been found among the 487 cases had careful examination of the mucus been done in every case.

COMBINED FERTILITY FACTORS OF HUSBAND AND WIFE

We have reached the point where, within the limitations of this study, we can attempt to correlate the fertility findings in both husband and wife with the idea that certain of them may be dominant in the prognosis for conception.

If we combine the outstanding factors—the potential fertility of the husband and wife and the age of the wife—the results are most impressive. Taking the wives under age 30 (Fig. 15), we find that where the fertility of both husband and wife is poor, only 3 per cent of these couples conceived within the allotted period of one year following the semen examination. But in this age group when the fertility of the wife is poor and that of the husband good, 23 per cent conceived. That is to say, a "good" husband can

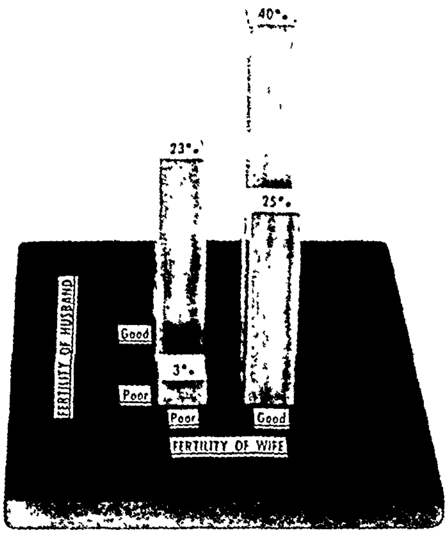

Fig. 15. Percentages of pregnancy by combined fertilities (wives under 30).

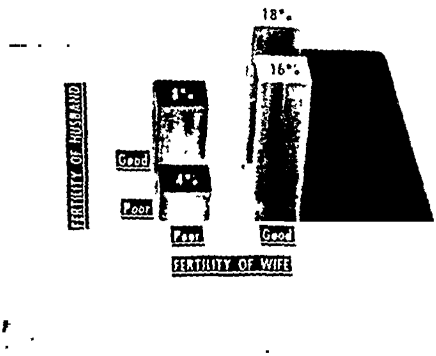

Fig. 16. Percentages of pregnancy by combined fertilities (wives over 30).

compensate for a "poor" wife. Where the husband is "poor" and the wife is "good" in this under-30 age group, again we find that the conception rate is good (25 per cent). Therefore, a "good" wife can compensate for a "poor" husband. Where the fertility of both is "good," we find a further enhancement of the conception rate to 40 per cent, an impressively high figure.

At this point we must not forget that these couples had a real infertility problem inasmuch as they had been unsuccessful in producing conception for at least one year. This fact makes the 40 per cent pregnancy rate for the couples of good fertility more impressive than it appears at first glance.

However, the picture changes when the wife has passed the 30-year mark (Fig. 16). In this age group we find, as would be expected, that where the fertility of both partners is poor, the pregnancy rate is very low. But, in contrast, a "good" husband does not aid a "poor" wife. However, a "good" wife in this age group can help herself up to a certain point, although it is obvious, we already have shown, that the pregnancy rate in the over-30 age group is considerably lower than the younger age group.

DISCUSSION

Throughout these studies our interest has centered on the male factor in human infertility and the relation of semen quality, not only to the fact of conception but also to the ease by which the latter is attained. It is plain that conception in itself does not truly satisfy the definition of fertility. The state of "one-child sterility" can be as frustrating to the residents therein as that of absolute sterility and can pose an equally difficult problem to the clinician. It is also certain that the fact of conception is easy for many couples, but the maintenance of the ensuing pregnancies impossible. Repeated miscarriage without the intervention of a successful pregnancy is probably a condition more fraught with tragedy than one of absolute sterility. Therefore, in considering male fertility one must study the problem in several types of populations with the distinct possibility that different standards will apply. Up to this point we have analyzed the male factor (in terms of semen quality) in three different populations: (1) a population of known fertility in which at least one conception was produced per marriage; (2) a population in which one conception resulted, but difficulty was experienced in attaining a second; and (3) a population in which no conception resulted after years of endeavor.

We have just presented the analysis of a fourth group, one in which the individuals had not achieved a conception within a stated period of time but in which a certain percentage of the cases eventually achieved conception within another arbitrarily designated period. At least one other population displaying a form of infertility must be studied—those in which conception is easy, but maintenance of the ensuing pregnancy impossible. We shall present the results of this analysis in the near future.

Sperm Transfer

In the present study, and as one would expect, there is a relationship between certain aspects of semen quality and the pregnancy rate. The rise in the pregnancy rate with increasing ejaculate volume is significant up to about the 3.5 cc. level, and flattens out thereafter. The volume of seminal fluid cannot be considered seriously except in terms of its content of spermatozoa. The obvious question is whether the rise in pregnancy rate with increasing ejaculate volume is due to the increase in total count of spermatozoa or to some factor involving the volume of seminal fluid deposited at or near the external os at coitus. The latter factor requires a consideration of the fundamental physiologic forces involved in the transference of the spermatozoa from the male to the female reproductive tracts in normal coitus, a process which remains virtually unknown. For example, are the spermatozoa which eventually reach the upper portion of the female reproductive tract forced into the cervical canal at the time of ejaculation or do they migrate from a seminal pool bathing the external os after withdrawal?

These questions are associated intimately (1) with ejaculate volume; (2) with sperm counts either total or per unit volume; (3) with the motile activity of the cells; and, of course, possibly with all these factors together. If the ejaculate volume is important by reason of the formation of a vaginal pool, then the importance of the total sperm count and the motile activity of the cells would be obvious. Motile activity would be necessary for the migration of the cells from the pool into the cervical opening and the *total* count would be essential in supplying an ample number of spermatozoa for the migration. However, we know that immediately after ejaculation (in the test tube, at least) the seminal fluid is a "clotted" mass in the meshes of which the spermatozoa are held immobile for at least several minutes. If this condition holds true in the vaginal deposition of the ejaculate at coitus, then the spermatozoa cannot begin to migrate immediately, and the existence of a vaginal pool in relation to the external os would thus be of value. Though there is no sound evidence that such a pool is physically possible under normal anatomic conditions, one could explain the poor relationship between low ejaculate volume and the pregnancy rate by postulating that a consistently low ejaculate volume would fail to provide continued contact of the vaginal pool with the external os. This would place a premium on volume per se apart from other aspects of semen quality.

If we now consider the possibility that the spermatozoa which eventually reach the fallopian tube are forced into the cervix at the time of ejaculation, then the importance of the sperm count per unit volume becomes primary and the motile activity only secondary so far as penetration of the mucus is concerned.

Sperm Count

It is customary to express the sperm count as the count per cubic centimeter rather than total count, and in earlier studies we found no substantial statistical evidence in favor of changing that principle. However, as we have suggested above, there is no sound physiologic proof in the human that the total sperm count may not play an important role. If we consider first the pregnancy rate in terms of sperm count/cc. (Fig. 9), there are two count levels at which significance can be placed. The pregnancy rate rises at the 20 million/cc. level, beyond which point there is no significant rise until the 60 million/cc. level is reached. Beyond that level there is no further change in the rate. In previous studies of larger and more varied populations we concluded that above the 20 million/cc. level, and provided the other aspects of semen quality—particularly the motile activity—were reasonably good, there was no rise in the fertility potential.

Actually, while Fig. 9 shows two significant count levels, when we examine the aspects of semen quality other than the sperm count, particularly motile activity, we do not have reason to change our earlier conclusion. If, for example, we take the cases with counts between 20 and 59 million/cc. and weigh the motile activity associated with these counts, 117 of them had good motile activity. Twenty-nine (25 per cent) of these wives conceived within the one-year period. If we examine in the same way the cases over 60 million/cc., we find 370 cases with good motile activity, of which 106 (29 per cent) produced conceptions in the year period.

Now, if we examine the same count levels with respect to cases with *poor* motile activity, only 8 conceptions in 63 cases (13 per cent) ensued when the sperm count was beween 20 million/cc. and 59 million/cc. At the 60 million/cc. level and over, where the motile activity was poor, 90 couples produced 17 conceptions (19 per cent).

It is obvious, therefore, that the sperm count is not in itself the important feature in semen quality. The seeming increase in the pregnancy rate above the 60 million/cc. level undoubtedly is due to the fact that only 20 per cent of these cases had poor motile activity as compared to 35 per cent in the 20–59 million/cc. group. The facts that (1) motile activity dominates over sperm count and (2) below the 60 million/cc. level there is a progressive increase in other defects in semen quality already are recorded in other studies.

Turning to the total sperm count (Fig. 10), there are three levels at which the count appears significant: Under 50, 50–299, and 300+ millions. Whether these total count levels have any significance apart from the counts per unit volume is debatable. Though we shall not give the detailed calculations, we have studied the relationship between sperm counts/cc., total counts, and pregnancy rates. We studied these relationships at three count levels in each classification—at less than 20 million, 20–59 million, and 60 million/cc. and over, and at less than 50, 50–299, and 300 million and over in the total count. These relationships are summarized as follows:

	% with total count of	
Count/cc.	*< 50 million*	*300 million +*
< 20 million	79	0
20–59 million	7	6
60 million +	0.4	72

What this summary demonstrates is that where the count/cc. is low, so also is the total count, and where the count/cc. is high, so also is the total count. So that if, as we have done here and in the past, we use the count/cc. instead of the total count, no deception is involved.

Sperm Concentration

These facts leave open the physiologic question of how the spermatozoa reach the cervical mucus at coitus. The question is so important that we must consider other evidence in the literature pertaining to human conception which might throw further light. It is essential, for example, that studies be done on the relationship between semen quality and postcoital examination of the cervical mucus. To our knowledge, only one such study has been done on the human body. Donald, in a similar study to ours, analyzed the factors involved in 193 infertility cases where conception eventually resulted, and the postcoital tests in these cases. He related the postcoital test to the sperm count in the husband and showed that up to the 20 million/cc. level, there is no change in the quality of the test. This evidence is in accord with our thesis that the sperm count per se is not the important factor. It does not, however, tell us how the spermatozoa reach the mucus. Recent evidence in lower animals (rabbit and bull) supports the mounting evidence in the human and raises new important questions as to the advisability of a large number of spermatozoa reaching the vicinity of the ovum. Braden, in a study of the distribution of spermatozoa in the genital tract of the female rabbit after coitus, confirms other work showing that in mammals only a very small proportion of the spermatozoa deposited in the female tract at coitus subsequently reach the vicinity of the eggs. In the rabbit only one out of 10,000–1,000,000 spermatozoa ejaculated attains the ampulla. In the ewe and the rat, the proportion is even smaller. He points out further that the number of cells reaching the ampulla is not related to the number deposited at the external os above a certain minimal number. Chang's figures on the rabbit are in essential agreement. Above a total number of 10 million cells deposited at the external os, there is no increase in the number of cells reaching the tubes. These authors emphasize that the number remains essentially the same in artificial insemination. They agree further that "an important function of the female *genital tract is to limit the number of spermatozoa passing* to the site of fertilization"[6] and that the natural barriers are the cervix and the uterotubal junction.

In the cow, Willett has demonstrated with diluted semen, that above the level of 10 million cells (in a volume of 1 cc.) inseminated artificially into the cervix there is no rise in conception rate. Below this count level and down to about 4 million cells, the conception rate falls off rather slowly. This evidence complements the findings in the rabbit and, indeed, does so in quantitative fashion. It is perhaps more than a coincidence that the maximal sperm count numbers necessary for conception in these species are not far removed from those we have suggested for the human.

Let us examine again our conclusion that above the level of 20 million cells/cc. the potential of fertility does not increase appreciably. If we have an average of 60 per cent of these cells showing good motile activity (a reasonable assumption) and an ejaculate volume of 3 cc., then a total of 36 million spermatozoa would be available potentially for fertilization.

When we remember that in the normal process of ejaculation in the human male, about 75 per cent of the spermatozoa are concentrated in the *first* third of the ejaculate, then 27 million potentially active cells in 1 cc. of ejaculate would be forced against the external os at the time of ejaculation. Dickinson, the acknowledged authority on sex anatomy, states that under normal anatomic conditions, the lips of the meatus must be in contact with the external os in such fashion that the ejaculate is forced against or, as far as is possible, into the mucus of the cervix. Probably only a very small portion of the total ejaculate could be transferred to the cervical canal in this manner, but whatever its volume, the concentration of spermatozoa per unit volume (cc.) and not the total sperm count would be the deciding factor in the number of cells reaching the cervical mucus.

Motile Activity

There is little more to be said about the motile activity in relation to the pregnancy rate. The rate rises sharply with the increase in percentage of active cells up to 40 per cent active, and flattens out thereafter (Fig. 11). This is the exact level which we have found previously to be the point of demarcation between a fertile and an infertile population.

We see a similar state of affairs in the quality of motility—a rising pregnancy rate with increasing quality of motility up to our 2+ level, with a flattening out thereafter (Fig. 12). In a previous study[10] with a fertile population and using "time taken to produce conception" as our criterion, we found a continually rising relationship between quality of motility and ease of conception. The strong fact in both studies is that the quality of motility is more important than the sperm count.

Gynecologic Factors

The fact that so many of the conceptions reported in this study occurred within a brief period after our initial semen examination requires discussion by a psychiatrist and will not be dwelt upon here. Psychosomatic factors undoubtedly are involved, and they would seem to be concerned with the reproductive capacity of the female rather than that of the male. There is no sound evidence that emotional problems inhibit spermatogenesis, and impotence is not a serious factor in male infertility. But the fact that menstruation may be disturbed seriously by emotional crises or tension leaves no doubt that ovulation could be affected equally in similar circumstances.

The age of the wife obviously is most important. The apparent decline in fertility begins about age 25 and becomes precipitate after age 30 or at an age when a woman is usually considered to be in her prime, physiologically and emotionally. It is true that the women in this study were examined only because they had an infertility problem and cannot be thought of as a normal population. But, as we already indicated, we found a decline in fertility in a fertile population at the same age levels when we used "time taken to produce conception" as our criterion of fertility.[10]

In regard to the gynecologic abnormalities and the percentage of cases eventually becoming pregnant with or without the abnormalities, it is obvious (Table 6) that, with certain isolated exceptions, just as many *wives eventually conceived with the abnormality as those without it.* It is difficult to believe, however, in the light of this, that those cases reported with closed tubes actually were in this parlous state. We know, for example, that some "closed tubes" were diagnosed on the basis of only one insufflation, while at least some of the other cases reported to be of "poor" fertility without specifications by the doctor undoubtedly did suffer from closed tubes. For this reason we are confident that the ratios between those "having" and "not having" the various gynecologic infirmities do not represent the true state of affairs. On the other hand, when the *general* fertility state of the wife is considered (that is, "poor" or "good"), especially in conjunction with her own age and her husband's fertility status, the differences in conception performance associated with differences in her gynecologic status are most impressive.

In conclusion, and considering the respective roles of the husband and wife in the problem of infertility, the rather trite fact is that both are important. But while the husband of good fertility does much to help the wife of impaired fertility when she is under 30, he can do little if she is over 30 years of age.

SUMMARY

1. An attempt is made to correlate certain fundamental relationships in the male and female factors in human infertility.

2. The analyses are made on an infertile population, our criteria of infertility being an unsuccessful attempt at conception for at least one year, and lack of a conception in their marriage.

3. Semen quality in the male is correlated with certain gynecologic de-

ficiencies in the female in an effort to determine the relative importance of the male and female in the over-all problem of infertility.

4. The physiology of the transfer of the spermatozoa from the male to the female reproductive tract in normal coitus is discussed and certain theories put forward.

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DISCUSSION

Dr. P. Tompkins, *San Francisco, Calif.:* Dr. MacLeod's paper is now open for general discussion. Some years ago another member of the Society, Dr. Farris of Philadelphia, proposed the idea that the important feature of semen analysis was the number of active cells, rather than the number of cells or the number of motile cells. It seemed to me, in listening to Dr. MacLeod, that he said that the sperm count—the total count—was most important and, next, that the motility was of great importance, and if we combine those two factors it seems to me we are pretty much in line with Dr. Farris' idea. I hope in his closing remarks Dr. MacLeod will comment on the importance of a total motility count, if there be such a thing.

Dr. Irving Fischer, *New York, N. Y.:* I have noticed that Dr. MacLeod referred to the psychosomatic factor. I have noticed, in checking the sperm of donors for artificial insemination, on many occasions the count and the motility varied and, as a matter of fact, on certain occasions the sperm were all dead. I have also been impressed with the fact that, when patients were sent for sperm counts, many times there was a variation in the count. In going over this, I find that the emotional factors are very important in the production of sperm. I would like to ask Dr. MacLeod if he can verify this.

Dr. J. M. Singleton, *Kansas City, Missouri:* I would like to know the significance of the 350,000,000 and the 400,000,000 count and why this seems, in some instances, to cut down on the fertility rate.

Dr. J. W. Goldzieher, *San Antonio, Texas:* Dr. MacLeod, I believe, has made the general statement that the frequency of conception increases with the frequency of sexual intercourse, and I do not believe he made any reservation on that statement at the time. I noticed in the data today that above a certain figure which I do not recall at the moment, the frequency of conception dropped. I would like to know if that is statistically valid.

Dr. J. T. Velardo, *Harvard Medical School, Boston, Mass.:* Both the essayist and the discusser made many interesting points, and I am wondering if they have done anything in the way of studying seminal fructose in infertility in the male.

Dr. Gordon Rosenblum, *Los Angeles, Calif.:* I would like to ask as to how worthy Dr. MacLeod considers the infertile female 40 years of age or more as a candidate for fertility studies.

Dr. Tompkins: Dr. MacLeod, will you undertake to reply?

Dr. MacLeod: I am rather glad, Dr. Tompkins, that you brought up this question of Dr. Farris' theories and those which I have put forward. I have never disagreed at any time with Farris regarding motility of the cells. My contention was that he had stated nothing new and, in fact, had restated original standards. That is to say, as I recall it, the original postulate put forward by Farris was that any man with a count below 83 million active cells in his ejaculate should be considered infertile, if not sterile. What does that mean? It means nothing at all.

Eighty-three million active spermatozoa, using Farris' own figures of an average of 46 per cent actively motile cells in the ejaculate, would mean that there would have to be a total count somewhere in the region of 180,000,000 to 200,000,000 cells. I deny the fact that you have to have that total number of spermatozoa, and I don't think anyone quite saw the fallacy of that reasoning in terms of what I was trying to say, so actually we agree you must have motile activity, but that was a fact I thought evident to anybody.

What I want to emphasize, and what Farris did not, and what I have been continually emphasizing is that motility is not the important factor but the quality of motility. We are not approaching these standards that were put out in the past; we are just as far away as ever.

The second question was related to the emotional factor. I have never at any time, so far as I know, except insofar as impotence was concerned, noticed any effect of emotional factors on the fertility of the male, though we have not analyzed that seriously. But it is my feeling that emotion per se or the psychosomatic factors in the male will not have any important role. There is no question about the effect of the psychosomatic factor in human fertility, but it applies to the female rather than to the male.

One doctor brought up the point that there seemed to be a falling off in fertility above the 350,000,000 or 400,000,000 count. That is quite true. We can't explain it, but if you looked at that postcoital test of Dr. Donald of London you would notice it showed exactly the same thing. He showed the quality of the postcoital test fell off abruptly with a high count. It is hard to explain that. That is why I tried to bring out—I am afraid I garbled the whole issue—the way in which the spermatozoa, under normal conditions of coitus, are transferred from the male to the female reproductive tract. It is a fundamental thing. It is a point that has been neglected, I think, for obvious reasons, because experimentally it is going to be an awfully hard job to observe how they are transferred at the time of ejaculation. There is something to do with high sperm count.

I did not have time to bring up another point: Researchers have shown, in the rabbit and rat, that where you do have a high number of spermatozoa reaching the upper part of the fallopian tubes in these animals you get a condition of polyspermia. In other words, when there is an unduly large number of cells in the fallopian tubes you may get multiple penetration of the ovum, that is to say several spermatozoa or many penetrating the ovum, and they have noticed that in those cases the ova are blighted. I am not suggesting that the husband with a high sperm count is a man to be abhorred, but I think we must, on the other hand, consider this all over again. I think we should re-assess all our concepts and start from scratch, and that is what we are trying to do. I don't say we are right in all we are saying here, but I do hope we will provoke enough discussion and experimental work to throw more light on the subject.

Dr. Goldzieher, I think, brought up the influence of the frequency of intercourse. I think I pointed out at the time that was not statistically significant.

The seminal fructose is a perennial question, so far as I am concerned. It is one with which I am not fully in sympathy. The level will be debated further in this meeting. I am not in complete agreement with those who suggest that the ability of the spermatozoa to burn fructose in order to retain energy is a reliable index of fertilizability. Because, after all, energy is used by the cells for things other than fertilization, and we are not in a position to arbitrarily divide up the energy used by the cell. We are not quite in that position yet.

On the question of women above 40, we showed in this study that none got pregnant, but unfortunately the number was rather low. I think, on the whole, it is quite obvious that female fertility drops rather abruptly after age of 30, and the chances of conception drop off after that age level.

Hypoandrogenic Syndrome with Spermatogenesis

Rodolfo Q. Pasqualini, M.D., and Grato Bur, M.D.

In 1950 our attention was called to a case of eunuchoidism accompanied by normal-sized testes. The patient had neither beard nor mustache; axillary and pubic hair were scanty; the penis was small; libido was depressed; and his psychologic outlook was characteristically eunuchoid. Urinary gonadotrophins were within the normal range. The testes were of normal size. Biopsy revealed normal seminiferous tubules with the complete maturation sequence including spermatozoa, along with a few definitely hypotrophic Leydig cells.

Our report of this case[11, 12, 13, 14] was followed by a report of 4 more cases in 1952,[8] and another in 1953 in which such patients were termed "fertile eunuchs."[9] Since that time 2 more cases have been reported,[7, 16] and the authors have seen one more. These 9 cases have consolidated our original description, which laid the foundation for a new syndrome of testicular insufficiency.

Our 2 cases are presented with special emphasis on the structural and histochemical modifications of the testes and their physiopathologic implications.

MATERIAL AND METHOD

Urinary ketosteroids were determined by a modification of the method of Holtorff and Koch, with which normal values range from 12 to 18 mg. per 24 hours in men between the ages of 18 and 30, with an average of

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15 mg. Urinary gonadotrophins were assayed by the method of Klinefelter, Albright, and Griswold.[6]

The material obtained by testicular biopsy (1) was fixed in 10% formaldehyde, in Bouin's fluid, in 96% alcohol, and in a special fixative for the determination of ascorbic acid. The following stain technics were carried out: hematoxylin-eosin, Mallory's trichromic stain, Rio-Hortega's silver impregnation for the detection of collagenous and reticular tissue, and Weigert's fuchsin-resorcin method for staining elastic fibers. For the study of lipids, Sudan IV was used, while the steroid fraction was studied by optical polarization, acetone solubility, and Schultze's technic. Alkaline phosphatase was determined according to the method of Kabat and Furth.[5] The McManus and Hotchkiss technics were used for detection of polysaccharides and glycoproteins, after enzymatic digestion with ptyalin. The mucopolysaccharide esters were detected by metachromatic technics after digestion with testicular hyaluronidase. Ascorbic acid was determined by the method of Deane and Morse.

CASE REPORTS

Case 1

The patient was a 24-year-old single male. Family history was noncontributory. There was no history of mumps or obesity during infancy or childhood. At 18, medical examination revealed genital hypoplasia and lack of facial, axillary, and pubic hair. A total of 10,000 units of chorionic gonadotrophins was administered at variable intervals during the next 5 months, followed by an unrecorded amount of testosterone propionate for about two years. This course of therapy produced some pubescent changes. From 20 to 23, he received no treatment.

Therapy was then started consisting of administration of 500 units of chorionic gonadotrophins over a period of a few months. This treatment brought about appreciable changes, such as the appearance of axillary and pubic hair, deepening of the voice, slimming of the hips, and awakening of the libido. He acknowledged having had erections, especially nocturnal, at times with emissions, but he had never attempted sexual intercourse. At that time an attempt to make a semen examination failed because of the patient's unwillingness.

Physical and Mental Observations. The patient was in general good health with discrete obesity and eunuchoid appearance (Fig. 1). The voice was relatively deep. He showed marked timidity, emotionality, introversion, and general lack of impulse; intelligence was within normal limits. He weighed 83 Kg. with a height of 172 cm.; the vertex–pubis measurement was 80 cm., as compared to pubis–sole measurement of 92 cm. and arm span of 191 cm. There was slight genu valgum. The skin was soft and dry with a few atrophic striae on the hips. The patient had a eunuchoid facies, with soft pink cheeks and no trace of beard. There was no recession of the temporal hairline and no hair on the breast, abdovessels (Fig. 2). The intratubular compartment revealed full spermatogenesis, with spermatozoa, in 95 per cent of the tubules; in the remaining 5 per cent spermatogenesis had progressed only to formation of primary or secondary spermatocytes. In some sections there was marked cellular desquamation and disorganization of the germinal line with anomaly of spermatogenesis. Sertoli cells appeared normal. The tubular wall reached normal adult maturity in 30 per cent of the tubules; in 40 per cent there was selective development of the reticular fibers; and in the remainder there were variable degrees of sclerohyalinization. The parietal fibroblasts retained their normal morphology.

Elastic fibers were distributed normally except for an increase in the sclerotic

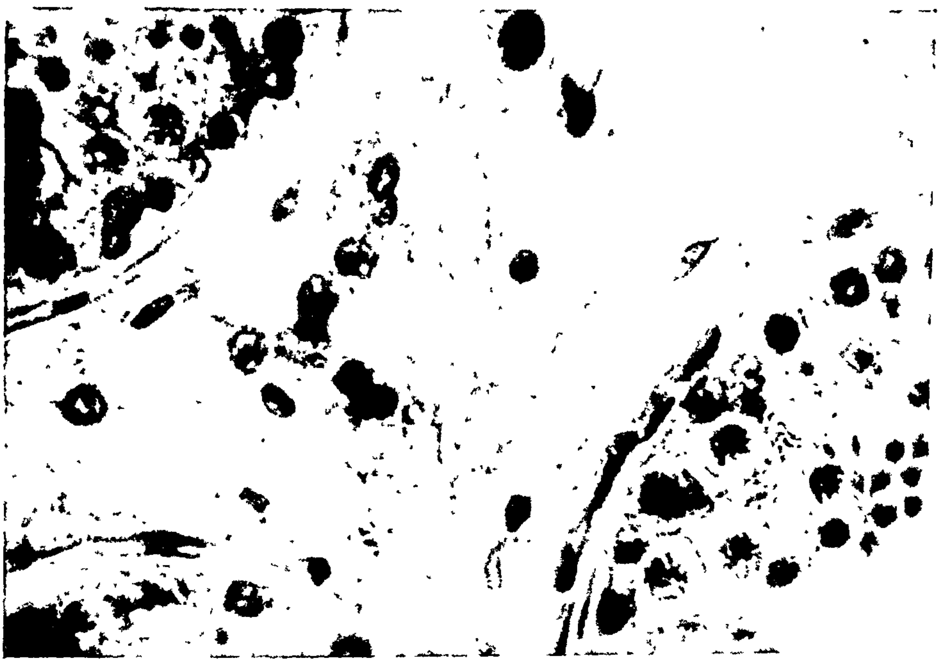

Fig. 3. Case 1. Leydig cells in involution and immature forms. (× 200)

sections. The intertubular connective tissue (Fig. 3) presented a normal distribution of fibroblasts, mast cells, reticular fibers, and fat cells, along with a certain degree of vascular sclerosis. The Leydig cells were decreased in number, appearing as scattered groups of 2 or 3 cells in different states of differentiation. These could be classified as: (a) mature in involution: grossly polygonal cells with abundant lipofuscin in the perinuclear zone; (b) immature: smaller cells, clearly outlined, with compact protoplasm and spherical and nucleolated nuclei morphologically similar to Leydig cells; (c) abortive or fibroblast-like: small groups of cells which were more difficult to recognize as Leydig cells, with fusiform nucleolated nuclei and compact acidophilic protoplasm without vacuoles and without fibrillar differentiation, corresponding to very immature cells. In this biopsy they occurred in a proportion of 60 per cent, 20 per cent, and 20 per cent respectively.

Histochemistry. Lipids were abundant in the spermatogonia, in the primary and secondary spermatocytes, in the Sertoli cells, and moderately so in the fibroblasts of the tubular walls. Leydig cells in involution presented very small lipid drops, while the abortive groups gave an almost negative image. The steroid fraction was vaguely detectable in the involuting interstitial cells and negative in the others. Glycogen was abundant in all intratubular elements, especially in the

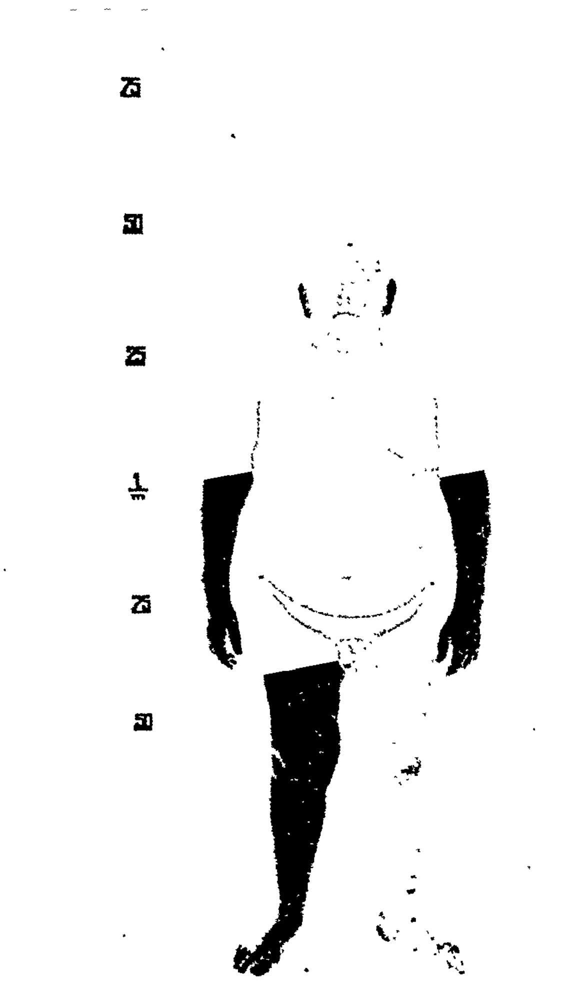

Fig. 4. Case 2. Age 19. Obesity and lipomastia. Very small penis and small testes. Complete absence of body hair.

Sertoli cells and in the spermatogonia, digestion with ptyalin confirming its polysaccharide nature.

Alkaline phosphatase was abundant in the germinal and Sertoli cells and present in the abortive and immature forms of Leydig cells. The reaction for glycoproteins was intensely positive in the basal membrane but less so in the amorphous substance of the interstices. The reaction for ribonucleoproteins was positive for the germinal elements, and in the immature and abortive forms of Leydig cells. Mucopolysaccharides were present in the tunica propria and digestible with hyaluronidase, as in the normal testis.

Case 2

The patient was a 19-year-old unmarried schoolmaster. Family history was noncontributory. He had never had mumps. He was frequently constipated, was very

Fig. 5. Case 2. Testicular biopsy showing small tubules; some without lumina with incomplete spermatogenesis and cellular desquamation. Normal tubular wall. Little interstitial tissue. (× 60)

sensitive to cold weather, and became tired easily, suffering from fatigue, dyspnea, and headaches. Since he was a child he had been aware of his genital underdevelopment. At the age of 15, he was examined by a doctor who prescribed chorionic gonadotrophins; the dosage could not be ascertained but it was probably low, as no appreciable change occurred in the genitals. Since the age of 12 he had had erections without ejaculation. Sexual intercourse had never been attempted and libido was absent.

Physical examination. The patient was short, with generalized obesity (Fig. 4). The voice was moderately low. Intelligence was normal, the patient being an efficient schoolmaster. Height was 153 cm., weight 80 Kg., vertex–pubis measurement 71 cm., pubis–sole 82 cm., and arm span 161 cm. The skin was dry and warm, soft along the trunk and rough along the limbs without myxedematous characteristics. There was bilateral lipomastia, without glandular tissue.

The fingers and toes were short and stubby. There was moderate genu valgum.

The face was round and florid, of infantile appearance, with abundant silky, thin hair, complete lack of beard, and no recession of the temporal hairline. Muscular development was good, with no signs of myo-edema. The thyroid was not hypertrophied; basal metabolism was —20. The cardiovascular apparatus was normal, with a blood pressure of 110/60. X-ray of the sella turcica was normal.

There was excess fat padding the pubic area. The penis was small, measuring 40 mm. in length and 15 mm. in diameter, its base being covered by a skin fold; the prepuce was infantile, the corpus cavernosum spongy and hypotonic. The scrotum was hypoplastic, with little pigmentation. There was an increase in

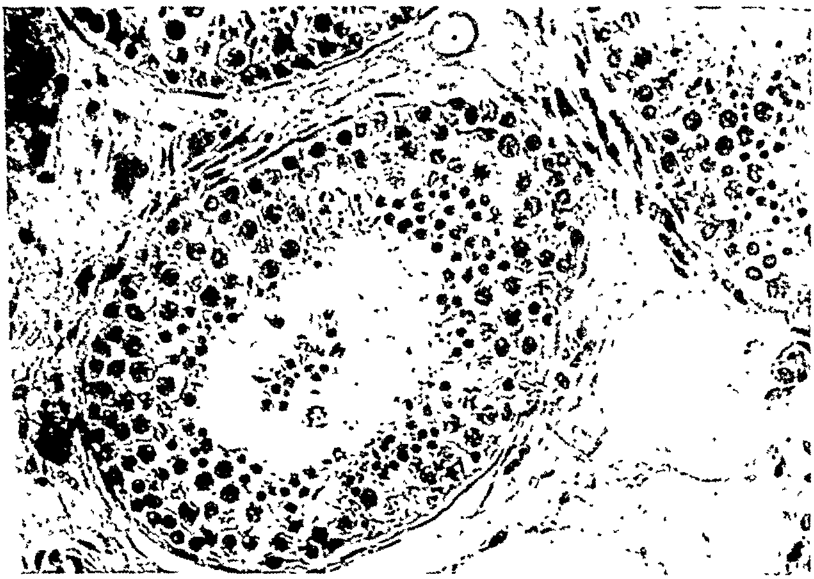

Fig. 6. Case 2. Details of the intratubular compartment. Spermatogenesis up to primary or secondary spermatocytes. Normal Sertoli cells. Normal tunica propria. In the interstices are Leydig cells of the abortive or fibroblast-like type besides the involuting forms. (× 200)

adipose tissue. The testes were small, both measuring 20 × 10 × 10 mm.; the epididymides and vas deferens were frankly hypotrophic. The external inguinal orifices were ample. There was complete lack of pubic and axillary hair. It was impossible to obtain a semen examination because of lack of ejaculation.

Laboratory Studies

Hormonal determination. The excretion of urinary gonadotrophins was more than 6 and less than 48 m.u. per 24 hours. Simultaneous determination of 17-ketosteroids resulted in 3.0 mg./24 hours.

Testicular biopsy. The material obtained (Fig. 5) was massive, and revealed thin seminiferous tubules, approximately 75 μ in diameter, straightlined, with ample lumina and scarce intertubular tissue. In the interstices, the amorphous substance was abundant in some sectors and in others had the homogeneous aspect of the infantile testis. The albuginea and vessels were normal. The intratubular content (Fig. 6) revealed spermatogenesis which resulted in spermatozoa in about 2 per cent of the tubules, and was arrested at primary and secondary spermatocytes in most of the tubules. The Sertoli cells showed the normal adult characteristics in a number of tubules, but in others there was evidence of involution; a few tubules were wholly filled with undifferentiated Sertoli cells and a few large spermatogonia.

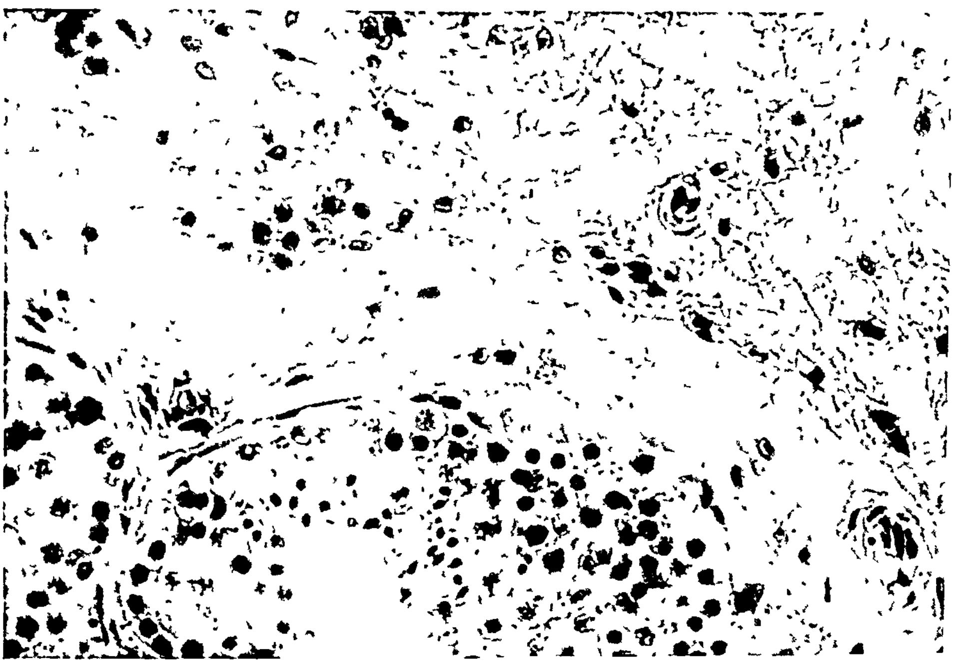

Fig. 7. Case 2. Leydig cells undergoing involution and immature forms. (× 200)

The tubular walls had thick basal membranes which were hyalinized in a few instances, the tunica propria consisting of compact collagenous bundles and thick reticular fibers; the amorphous substance was scarce and the elastic fibers rare. Interstitial tissue (Fig. 7) was more abundant than in Case 1, the fibrillar and cellular structures predominating with little amorphous substance. The fibroblasts were very numerous, and at times it was impossible to differentiate them from abortive Leydig cells (Type C) (see Case 1). Leydig cells were few in number and showed minimal differentiation, the abortive type being the rule (85 per cent) with a few of the involutional type (15 per cent); these contained lipofuscin.

Histochemistry. The lipids formed thick drops in the Sertoli cells but were scarce in the germinal epithelium, the tubular wall, and the interstices; the Leydig cells were almost free of lipids, except for a few of the more differentiated forms. The steroid fraction and ascorbic acid were totally absent. The reactions for glycogen and alkaline phosphatase were weakly positive in the germinal epithelium and in the Sertoli cells, and practically negative in the interstices. The reaction for glycoprotein was strongly positive in the basal membranes and very weak in the amorphous interstitial substance. Ribonucleoproteins were present in the protoplasm of the germinal epithelium and weakly so in the Sertoli and immature Leydig cells; the tunica propria was weakly metachromatic.

DISCUSSION

These 2 patients (Table 1) presented the symptoms which define the hypoandrogenic syndrome with spermatogenesis: (1) clinical characteristics of eunuchoidism; (2) spermatogenesis, and (3) urinary gonadotrophins within normal limits (see Table 1). Case 1 presented the typical

TABLE 1. Observations and Findings

	Case 1	*Case 2*
Age	24	19
External habitus	Eunuchoid	Eunuchoid; obese
External genitals	Normal testes–small penis	Small testes–very small penis
Gynecomastia	No	No
Beard	Absent	Absent
Axillary hair	Very scant	Absent
Pubic hair	Very scant	Absent
X-ray of sella turcica	Normal	Normal
B.M.R.	+5	−20
Libido	Very depressed	Absent
Impotence	Yes	Yes
Urinary gonadotrophins	>12 <24 <48 m.u./24 hr.	>6 <48 m.u./24 hr.
17-Ketosteroids	4.6–11.6 mg./24 hr.	3 mg./24 hr.
After 20,000 r.u. chorionic gonadotrophin	21.9 mg./24 hr.	..
Spermatogenesis	Normal in 95 per cent of tubules	Normal in 2 per cent; up to spermatocytes in 60 per cent of tubules
Sertoli cells	Normal	Normal
Tubular wall	Mature or sclerotic	Sclerotic
Leydig cells	In involution, 60 per cent; immature, 20 per cent; abortive, 20 per cent	Very few; in involution, 15 per cent; abortive, 85 per cent

somatic configuration of the eunuch, which in Case 2 was partly masked by the marked obesity. The vertex–pubis measurements were less than pubis–sole, while the span was greater than the height.

Puberty was delayed and complete sexual maturity never achieved by either patient. In both, libido was markedly diminished and they were practically impotent. Neither had any facial hair and body hair was almost nil; axillary and pubic hair were sparse in Case 1 and absent in Case 2. The picture of eunuchoidism was accompanied by normal-sized testes in Case 1 and small testes in Case 2.

Spermatozoa were produced and discharged by the patient in Case 1. In both cases testicular biopsy revealed the existence of normal spermatogenesis up to and including spermatozoa. The genital characteristics produce the formula: *normal testes–small penis,* as contrasted to the combination of *small testes–small penis* of pituitary hypogonadotrophic eunuchoidism.

Testicular biopsy of these 2 cases revealed: (1) the existence of spermatogenesis, including the elaboration of spermatozoa; (2) a mature tubular wall; and (3) Leydig cells undergoing involution along with the immature and abortive forms.

The urinary gonadotrophins were within the normal limits in both cases—higher in Case 1, corresponding to the higher percentage of tubules with complete spermatogenesis; if the uterine weight of the mice can be taken as index of activity, the output was very low in Case 2. The quantities detected were within the lower limits of normality, but we must take into account that the presumably very low content of gonadotrophin B (I.C.S.H.)[16] in the urine extracts might have affected the results, since it is impossible to separate the action of these two hormones in the determination.

The urinary 17-ketosteroids did not reach the normal limits for our laboratory in either case, and were extremely low in Case 2. In Case 1 administration of chorionic gonadotrophins provoked an increase in the number of ketosteroids in the urine. These determinations acquire more importance when considered along with the clinical symptoms of accentuated eunuchoidism and with the histologic and histochemical features of the Leydig cells. There is no doubt that excretion of urinary 17-ketosteroids was in proportion to the amount of adrenal androgens produced, but, not having performed any fractionation, how much cannot be established.

When we first described this syndrome we attributed it to the absence of gonadotrophin B (I.C.S.H.),[14] based on: (1) the presence in the interstices of Leydig cells, which were few in number and had characteristic signs of immaturity, hypofunction, or involution; (2) the great clinical improvement and the increase in urinary 17-ketosteroids after treatment with chorionic gonadotrophins. Unfortunately, we were not able to obtain a posttreatment testicular biopsy. On the other hand, McCullagh *et al.*[9] confirmed such an insufficiency in gonadotrophin B (I.C.S.H.) in 3 out of their 5 cases. In neither of our patients were there any pathologic reasons for their testicular insufficiency: x-rays of the sella turcica were normal and there were neither metabolic alterations (hypoglycemia, diuresis, and so on) nor diseases in evolution (Wassermann and Kahn reactions negative; normal hemogram; normal erythrosedimentation rate) capable of affecting the secretion of gonadotrophins.

Thus we believe that the principal cause of this hypoandrogenic syndrome with spermatogenesis is a deficiency of gonadotrophin B (I.C.S.H.) of pituitary or possibly hypothalamic origin. As the syndrome develops prepuberally, and the eunuchoid somatic characteristics become evident, we suppose that the production of this hormone, if it ever starts, does so at very low levels, and is insufficient to stimulate the function of the Leydig cells. However, we are inclined to think that a very low production of gonadotrophin B (I.C.S.H.) exists or has existed at some time since there is a minimal stimulation of Leydig cells; on the other hand, a complete absence of testicular androgens would signify a greater degree of immaturity in the germinal epithelium.

From a practical point of view the hypoandrogenic syndrome with spermatogenesis offers more interest than could be presumed from its late introduction into the testicular nosology, which would make it appear exceptional. It is possibly one of the syndromes most easily missed in medical examination, principally because of the practically normal size of the testes. In our 2 patients the characteristics of eunuchoidism were well defined, but they can be very attenuated, low seminal fructose concentration being practically the only sign in the case described by Landau. This leads us to conclude that the surest way to improve the practical diagnosis of testicular insufficiency, as well as our knowledge of the physiopathology of the testes, is to increase the number of testicular biopsies, which should be performed in all cases with symptoms of eunuchoidism, however mild, and even when the testes are of normal size and consistency.

SUMMARY

Two cases are described which embody the syndrome of eunuchoidism, normal spermatogenesis, and normal urinary gonadotrophins which we have named "hypoandrogenic syndrome with spermatogenesis."

The clinical symptoms were: absence or retardation of puberty; somatic eunuchoidism with genu valgum, vertex–pubic measurement greater than pubic–sole, span greater than height, lack of beard growth, and axillary and pubic hair absent or very scarce; small penis, hypoplastic scrotum; libido absent or diminished and partial or total impotence; and lack of physical strength and resistance. Urinary gonadotrophins were normal. Urinary 17-ketosteroids were decreased. There were no signs of a brain lesion.

The testes were normal or small in size. The biopsy revealed normal spermatogenesis with spermatozoa in a variable number of tubules; primary and secondary spermatocytes were found in the remaining tubules; Sertoli cells were normal. Leydig cells were decreased in number, with signs of hypofunction, being either mature in involution, immature, or of the abortive or fibroblast-like type.

It is considered that the origin of the syndrome resides in a primary deficiency of gonadotrophin B (I.C.S.H.) with a consequent lack of trophic and secretory stimulation of Leydig cells. The administration of chorionic gonadotrophins improves the clinical picture and increases the excretion of 17-ketosteroids.

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The American Society for the Study of Sterility

At the annual meeting of the American Society for the Study of Sterility to be held in Atlantic City on June 3, 4, and 5, 1955, it is planned to devote a morning to the projection of 16-mm. films on any topic relating to reproduction.

The Committee requests authors of films to communicate with Dr. Robert S. Hotchkiss, 65 East 66th Street, New York, N. Y., who is arranging this portion of the program. All films will be insured and returned promptly to their owners. The author will be invited to introduce the film at the time of its projection if he is present at the meeting. The Committee would also appreciate any information about films that would be appropriate for the occasion, and the authors' names so that they may be contacted.

Epididymography, Varicocelography, and Testicular Angiography

Their Uses in the Study of the Infertile Male

M. Leopold Brodny, M.D., Samuel A. Robins, M.D.,
Hyman A. Hershman, M.D., and Adolph DeNuccio, M.D.

The inadequacy of available procedures for examining the infertile male prompted a search for pertinent methods of investigation. It was felt that more information about the local anatomy would be valuable. Two major approaches were pursued; first, the study of the continuity and integrity of the genital channels and chambers concerned with transportation of the ejaculate; and second, in vivo visualization of the blood supply of the organs concerned with spermatogenesis. Both problems could be solved by roentgenographic methods. For the former, the luminal procedures of urethrography, seminal vesiculography, and epididymography were adopted; for the latter, the angiographic procedures of testicular arteriography and varicocelography were devised.

LUMINAL PROCEDURES

The luminal pathways through which spermatozoa must pass from their origin in the seminiferous tubules to reach the external urethral meatus can be divided roentgenologically into three sections: (1) the epididymis and vas; (2) the vas and seminal vesicles; (3) the urethra and its appendages.

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Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility at San Francisco, Calif., on June 18, 19, and 20, 1954.

Vaso-epididymography

Thickening, atrophy, beading, or absence of the vas deferens or epididymis on palpation are considered important diagnostic points in the physical examination of the infertile man; but these lesions are often not recognizable by routine diagnostic methods. Even when the genitals are carefully examined by specialists, many defects are not detectable except by surgical exploration. The absence of the vas, especially close to the epididymis, is often difficult to recognize even on very careful palpation. Michelson has stated that congenital anomalies of the vas and epididymis commonly occur in men with impaired fertility and reported 12 such anomalies out of 900 subfertile men (about 1.3 per cent). He also stated that "In the usual routine examination of a patient it is extremely doubtful if the anomalies would have been recognized." The status of the vas proximate to the external ring of the inguinal canal can only be determined by roentgenography or extensive surgical exploration. In view of this fact, it is surprising that radiographic exploration of this area has not received more attention.

Roentgenography of the epididymis was the subject of a detailed study by Lozzi of Italy. He injected the vasa of dogs and found that the procedure was innocuous and produced no abnormal changes in the duct or epididymis. He studied 20 patients with hydrocele, spermatocele, neoplasm, or inflammatory epididymitis and concluded that the procedure was harmless and merited being employed in those instances in which the diagnosis of the nature and site of the pathology was in doubt.

Boreau *et al.* have performed epididymographic examinations for the past 3 years and have emphasized its simplicity and safety. Histologic examination of the testicles and epididymides after epididymography with 50 per cent Diodrast showed no irritation of the tubular linings or any other local tissue changes. They also noted that the catheterized area of the vas healed well without infection or mechanical obstruction. They believed that with ordinary care there was no danger that the contrast medium might rupture the epididymal tube, because of the strength of the walls and because a fine needle was used which did not completely block the lumen of the vas, permitting the excess to flow proximally. No changes of the fertility factors were noted on analyses of the ejaculate after epididymography.

Technic: This procedure can be easily done under intravenous pentothal anesthesia in the pyelographic room with the aid of a small dissecting kit

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and the proper syringes and needles. Under aseptic technic, 2 cm. of the vas deferens is exposed and isolated through a small incision in the upper part of the scrotum. The vas is immobilized by holding it between two towel clips, the points of which are below and at opposite ends of the isolated portion. A small intradermic needle is inserted into the canal of the vas in the direction of the epididymis and about 0.5 cc. of a 30 per cent solution of Diodrast is injected. A suitable high-contrast film is inserted into a sterile rubber bag and placed posterior to the scrotum. The x-ray tube is focused and a radiograph is made (Fig. 1). It is sometimes necessary to inject another 0.5 cc. to fill the epididymal lumen (Fig. 2), and often it is desirable to take oblique or lateral views. This may be done easily by rotating the position of the testicle on the film (Fig. 3).

A frequent inconsistency has been noted between our clinical observations and our surgical and roentgenologic findings. Vasa which feel atrophic appear normal on surgical exposure and on injection have an adequate luminal capacity. Sometimes a thickened vas has perideferentitis which does not affect the diameter of the canal.

Epididymography has proved valuable for the study of tumors, inflammations (Fig. 4), and malpositions of the organs of the scrotum. It should prove to be a useful procedure for investigating the production and transportation of spermatozoa in the infertile male.

Vasoseminal Vesiculography

Seminal vesiculography is easily done at the same time as epididymography. The needle is inserted in the other direction and an additional 2–3 cc. of opaque medium is injected upward and roentgenographic exposure of the bony pelvis is made using the usual technic.

The roentgenologic demonstration of the seminal vesicles and the pathologic lesions concerned with infertility will be the subject of a future [illegible]

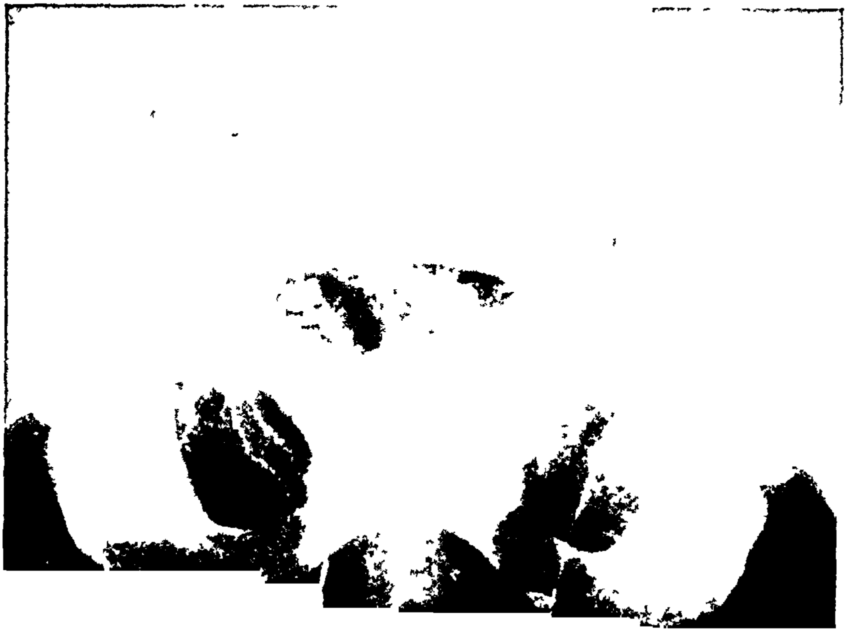

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Fig. 5. Normal vasoseminal vesiculogram. The excess of the opaque medium has flowed into the prostatic urethra. Fig. 6. Vasoseminal vesiculogram in a patient with chronic infection. Note the dilatation of the vesicles.

munication. A normal (Fig. 5) and an abnormal (Fig. 6) vasoseminal vesiculogram are presented to illustrate the results obtainable with this method.

Urethrography

This subject has been recently reported.[2]

ANGIOGRAPHIC PROCEDURES

Testicular Arteriography

That the efficiency of an organ is only as good as its blood supply is especially true of the male gonad. The spermatogenic function is very sensitive to hydrostatic, thermal, and nutritional changes—factors which are locally controlled or influenced by the integrity of the testicular artery and its branches. Previous studies of the vascularity of the testis and epididymis were performed on animals, or on postmortem or surgical human specimens and were inadequate from a clinical viewpoint (Fig. 7). A technic visualizing the vascularization of these organs in the living has been devised and the procedure has been named testicular arteriography. A preliminary report follows.

Technic: The procedure requires anesthesia and is done in the operating room under aseptic precaution. An incision 3–4 cm. long is made in the upper scrotum and the cord is isolated and delivered. The cremaster and fascia are separated by careful dissection until the testicular artery is isolated. Comments on the anatomy of this area will be the subject of a future communication. The vessel is immobilized and its wall pierced by a small hypodermic needle in the direction of the testicle. An x-ray film without intensifying screens is inserted into a sterile rubber bag and placed under the testicle. The x-ray tube is properly positioned over the area and a roentgenogram is taken just as the rapid injection of 5 cc. of 35 per cent Diodrast is terminated (Fig. 8). The entire procedure must be done carefully so that the artery will not be traumatized.

We have also studied the return flow of blood from the testicle and describe the technic in the discussion of varicocelography.

Applications. The conditions for which testicular angiography is diagnostically valuable have not been completely investigated. The development of testicular biopsy in man has provided a means of study of the pathogenesis of cell types in spermatogenesis. Testicular angiograms may provide a means of studying the circulation in the testes and of correlating

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Fig. 7. Testicular angiogram and vasogram of a specimen removed from a 65-year-old male with prostatic carcinoma. The organ was histologically atrophied. The narrow vessels and avascular areas can be noted. Fig. 8. Testicular angiogram of a young male showing normal testicle and semen. The large vessels in the center of the cord are the veins forming the pampiniform plexus. To the right is the testicular artery, and to the left is the branch to the epididymis. Note the architecture of the blood vessels and the marked vascularity of the testicle.

the cell changes with the vascular distribution and with the degree of local ischemia. The following indications are presented on a theoretical basis in the hope that a wider application of this procedure will more rapidly determine its status.

Sequelae of trauma and surgery. Testicular atrophy as a sequela to surgery in the inguinal and genital regions has been attributed to interference with the arterial blood supply, to unrestricted resection of the pampiniform plexus, or to prolonged vasoconstriction. It may be advisable after extensive surgery in the inguinal region to determine by this technic the status of the arterial supply to the genitals. The status of the venous return is discussed at greater length under the heading of varicocelography. The visualization of the adequacy of the blood supply to the testicle after trauma, torsion, or infarction may be the decisive factor for or against orchidectomy.

Inflammation. Vasitis, epididymitis, and orchitis frequently follow mumps, gonorrhea, tuberculosis, or nonspecific infection, and local turbidity is a common complication. The effect of this increased tension and pressure on the blood supply, especially to the sensitive spermatogenic tissues, is worthy of investigation. A study of the testicle by biopsy during inflammation has added much to our knowledge of the natural history and sequelae of various types of orchitis. The visualization of the vascular supply may prove equally illuminating.

Scrotal masses. The vascular pattern has been found to be of value in the differential diagnosis of tumors in other areas of the body and it is possible that neoplasms may show increased or abnormal vascularity. We have not had the opportunity to visualize the blood supply to a testicular tumor, but the angiogram of a hydrocele is quite striking.

Congenital abnormalities. Congenital abnormalities of the vas deferens, the epididymis, and the testicles are quite common, if those with ectopic positions are included. The status of the circulation to and through these organs is very important surgically. It is possible that the high incidence of atrophic testes after surgery for cryptorchidism may be due to an inadequate blood supply and not entirely to any hormonal imbalance.

Infertility. Spermatogenesis is dependent on an adequate blood supply to the gonads.[6] The visualization of the vascularity of the testicle and epididymis may prove valuable for a better understanding of genital pathology, for a clearer interpretation of testicular biopsies, and for the determination of the reversibility of local lesions.

Varicocelography

A varicocele is a mass of tortuous, dilated, and elongated veins in the pampiniform plexus of the spermatic cord and is of two types, primary and secondary. The latter is mechanical in origin and results from interference of the venous return by an intra-abdominal or a retroperitoneal mass. The primary varicocele is of questionable etiology, is commonly seen

Fig. 9. Phlebogram of a varicocele. Note the tortuous pampiniform plexus and the anastomosis of the pathways draining the area.

in the young male from 15 to 40 years old; and is the most important type from the fertility standpoint. It has been estimated that 10 per cent of males have varicoceles, of which 90 per cent are left-sided, 8 per cent bilateral, and 2 per cent limited to the right side. The popular explanation for the etiology of this unilateralism is that the left spermatic vein empties into the left renal vein in an angular manner and not into the vena cava obliquely as does the one on the right side.

Pain accompanying a varicocele is the symptom with which the clinician is primarily concerned and little attention has been paid to the effect of the varicosities on the functions of the epididymis and testicle. There is an apparent lack of exact information on the effects of a varicocele on spermatogenesis.

Sterility as a consequence of varicocele is probably infrequent because of adequate spermatogenesis in the other testicle. The amount of spermatogenic impairment is probably dependent on the degree of venous stasis rather than on the size of the varicocele.

Clinically, slight-to-moderate varicosities in the pampiniform plexus do not change the size or consistency of the testicle, but with extensive vascular changes testicular atrophy has been observed. If the interference with the blood supply is moderate the effect on spermatogenesis is not discernible on biopsy, but if severe, there is a reduction in the amount of sperm formation within the tubules.

Technic. The same precautions that are taken with intravenous pyelography are indicated with varicocelography. There are no additional contraindications or dangers. The procedure is performed by cannulation of a distal vein of the varicocele and the rapid proximal injection of 10 cc. of buffered 70 per cent Urokon sodium (sodium 3-acetylamino-2,4,6-triiodobenzoate) with a large-caliber needle. An x-ray exposure is made of the area as the last 2 cc. is being injected. It is possible to place a small x-ray plate under the scrotal area and obtain a roentgenogram of the local venous drainage, or abdominal films can be taken with the same technic and the venous drainage into the left renal vein visualized. Roentgenograms made in the upright and Trendelenburg positions may prove valuable for the studv of the effects of posture on venous drainage and stasis in the area.

Surgery for the cure of a varicocele is designed primarily to relieve pain and often disregards the effects of the technic on the vascularity of the testicle. Testicular atrophy following the radical cure for varicocele has been so frequent as to make the operation unpopular. The etiology of this complication has been attributed to arterial injury or to too radical resection of the pampiniform plexus. Repeated semen analyses and testicular biopsies over a long period of time are necessary to determine the exact extent to which sterility may be ascribed to varicocele. Angiograms before and after varicocelectomv is indicated to determine the effect of surgery on the blood supplv to the genitals.

This technic will permit the study of the venous return from a varicocele and may lead to important observations as to their cause. It may be possible to visualize retrograde venous flow and diagnose valvular incompetency. We have injected the vein of the pampiniform plexus on the right side and visualized the venous return for comparison with that of the varicocele. The complex anatomic arrangement of the pampiniform plexus must serve other physiologic purposes besides drainage of the organs.

CONCLUSION

The roentgenographic study of the channels and chambers concerned with the transport of the ejaculate and the visualization of the blood supply to the male gonads can provide anatomic information which is valuable for understanding the physiology and pathology of male infertility and many genitourinary syndromes.

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Cervical Aspects in Sterility and Infertility

Werner Steinberg, M.D.

ONLY WITHIN the last twenty years has the role of the cervix in sterility* received the attention which it properly deserves. Until then, references indicating a relationship of the cervix to sterility were scarce indeed. For instance, Donné, to whom we owe the discovery of *Trichomonas vaginalis*, wrote in 1837 that "the reaction of the vaginal liquid is acid and that of the cervical mucus is alkaline, and that pathological changes of acidity or alkalinity can be a cause of sterility." Sims postulated as early as 1868 that one of the questions which must be settled in the investigation of the cause of sterility was "whether the secretions of the cervical canal are favorable or not, and the viability of the spermatozoa." This laid the foundation for the "sperm migration test" which today is named after him. In 1913 Huhner[21] further elaborated this test and also claimed that the cervical mucus was in some way responsible for failure of conception. More extensive research on the subject was initiated by Séguy and Vimeux in France, as well as Miller and Kurzrok in this country. In 1941 Weisman wrote a treatise on the importance of the cervical canal in the task of assisting the spermatozoa in their migration into the uterus.

This paper will present a résumé of studies done during the past 25 years together with an analysis of my own cases.

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Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility, San Francisco, Calif., June 19, 1954.

* Because the word "sterility" is derived from the Greek *stereos*, meaning barren, I only use this term to describe a state where a woman is unable to conceive (*impotentia concipiendi*). The word "infertility," however, stems from the Latin *in* and *fero* which means "I carry" or "I bear" and can be defined as a condition where a woman is unable to carry a conceptus to term (*impotentia gestandi*); thus, I use infertility as synonymous with the state of habitual abortion.

CERVICAL MUCUS

The fact that the lining of the uterine cavity as well as that of the cervical canal have a common embryologic origin would lead one to expect that the cyclic changes which occur in the endometrium can likewise be demonstrated in the endocervix. The earlier findings of Wollner[57, 58, 59] have been refuted by the work of Topkins as well as Bradburn and Webb who were unable to find any definitely cyclic changes in the endocervix. Although the histologic change of the glandular epithelium could not be substantiated, one fact, recently re-demonstrated by Bergman, appeared certain—the cervical secretion undergoes a definite cyclic alteration.

Composition

The results of the various investigations will be summarized in chronologic order. Kurzrok and Miller made the first biochemical studies on the cervical mucus. They thought that there was a "lytic substance" present in semen which had a digesting effect upon cervical mucus resulting in its liquefaction. Séguy and Vimeux, and Séguy and Simmonet were the first to point out that liquefaction was brought about by estrogenic action; they proved that estrogen caused a cyclic change of the cervical mucus at the time of ovulation and thus have to be regarded as the earliest advocates of this theory.

In 1940, Lamar, Shettles, and Delfs devised a method by which they could actually observe the ability of spermatozoa to penetrate the cervical mucus. By aspirating sperm and mucus into a capillary tube they were able to determine the power with which spermatozoa were attracted by cervical mucus and measured the velocity with which they could traverse it. The practical application of this test was developed by Guttmacher and Shettles who contradicted Miller and Kurzrok and claimed that human semen does not possess an enzyme which liquefies cervical mucus. They also confirmed an earlier observation by Moricard that estrogenic hormone, given by mouth or parenterally, could transform a tenacious operculum into a thin, translucent secretion; however, they cautioned that such treatment could inhibit ovulation thus making it a therapy of questionable value in sterility cases.

The year 1944 marks the beginning of studies by Pommerenke and his associates on the physical, chemical, and physiologic aspects of the cervical secretion. They showed that the quantity of the mucus varied greatly with the different days of the cycle[31] increasing in amount as ovulation approached. At ovulation there was a decrease in viscosity[32] as well as maximum penetrability. Administration of estrogens to castrated women also produced marked liquefaction of a thick and tenacious secretion.[33] Later Pommerenke[38] began studies on the chemical composition of the cervical mucus. It was found that the water content was higher in mid-cycle and that certain reducing substances were diminished during ovulation[34] which coincided with a rise of the basal temperature.[39] These reducing substances were quantitatively determined[26] and finally it was pointed out that the decrease of sugar in the cervical mucus was due to an enzyme action, thus making carbohydrates available for sperm nutrition. These chemical studies were continued by Pommerenke and his associates[6, 7, 27] as well as by Shettles.

pH

Studies of the *p*H of the cervical mucus began more than 50 years ago, when Schroeder found it to be about 8.5. Ever since, a number of controversial articles have appeared in the literature, some, such as Meaker and Glaser or Carey and Lardy, claiming it to be always alkaline; others, Kraul and Bodnar, for example, claiming that the *p*H of the endocervical mucus normally ranged between 6.6 and 6.8, thus being slightly acid.

The reason for these discrepancies can be found in the different methods employed. Schockaert and Delrue[41, 42] first used a galvanometric method and showed that the *p*H of the mucus of the lower part of the cervix ranged between 6.3 and 6.8, and they felt that in sterility cases, an explanation could be found in the "hyperacidity" of the cervical mucus. Greenhill, however, noted that: "In women, the vagina is always acid and the cervix always alkaline." Most recently Breckenridge, Pederson, and Pommerenke employed the Beckman glass electrode, compared it to Hydrion indicator paper, and found that the cervical mucus was predominantly alkaline in all phases of the intermenstrual period, with occasional acid samples in 17 per cent. These few references may suffice to prove that there is no unanimity on the subject. Even today we don't know whether the *p*H really changes at the time of the ovulation or not.

Spinnbarkeit

Because of a dislike for the German word *Spinnbarkeit*, first coined by

TABLE 1. Contributing Causes of 100 Selected Sterility Cases

Male Factors:	
Premature ejaculation, impotence, oligo- or astheno-spermia	24
Female Factors:	
General systemic causes:	
Thyroid dysfunction, anemia, obesity, tuberculosis, hyperadrenalism	12
Uterine causes:	
Hypoplasia, malformations, endometrial hyperplasia, endometritis, polyps, myomas, diverticulum, adenomyosis	34
Tubal causes (bilateral only):	
Occlusion, hydrosalpinx, tuberculosis, salpingitis, spasticity, endosalpingiosis	38
Ovarian causes:	
Hypoplasia, oligomenorrhea, amenorrhea, anovulatory cycle, Stein-Leventhal syndrome, cysts over 5 cm., tumors, old abscess, endometriosis	32
Unexplained causes:	
Including psychosomatic factors, premarital repressions, rejection of coitus, guilt feelings, hostility against husband	12
Cervical causes	44

Some cervical abnormality was observed in 44 per cent of these cases. In Table 2, a breakdown is given as to the different types of cervical pathology. These figures bespeak the necessity of devoting to the cervical factor a closer scrutiny and adding the various newer tests to our armamentarium.

The problem then arises as to whether the Sims-Huhner test *per se* is

TABLE 2. Analysis of Pathology in 44 Cervical Cases
Sims-Huhner Test Was Negative or Unsatisfactory

Congenital hyperthrophy	1
Stenosis or angulation of cervical canal	8
Trichomonas cervicitis	10
Monilia cervicitis	6
Pathogenic bacteria	5
Lack of secretion (dry cervix)	
cause unknown	2
postcauterization	2
Tumors	
polyps	3
myomas	1
Hostile mucus (viscous plug, atypical crystallization, low spinability)	12
Mucus hostile to husband's spermatozoa, but not to donor's	4
TOTAL	54

34 patients presented 1 cervical cause for sterility; 10 patients presented 2 causes.

sufficient for the appraisement of sterility cases, or whether other criteria should be added to gain a more complete picture. The time after which the test is to be performed has been a matter of controversy. Huhner[21] originally suggested that the test should be done as soon after coitus as possible. To this technic he has adhered until recently.[22] The *Minimal Procedures* of the American Society for the Study of Sterility[16] recommend a lapse of 2–16 hours before proceeding with the test, whereas Tompkins as well as McLane advise a waiting period of 4–6 hours. Barton and Wiesner allow an even wider discretion: 6–24 hours. If we are to come to conclusions worth while comparing we will have to agree on some more uniform interval. I have always performed the test 2 hours after intercourse.

In addition, the number of spermatozoa which constitutes a positive Sims-Huhner test has been left undecided; Williams' suggestion of adopting 10–20 actively mobile sperms per high power field seems to be an average figure.

Huhner himself[22] claimed that the postcoital test provided all the necessary information for the evaluation of sterility cases. I am in accord with Williams who says "a poor Sims-Huhner test is merely a challenge to better diagnostic methods as concerns the character of the cervical secretions and of the spermatozoa." An unsatisfactory result of a postcoital test may indicate: (1) oligo- or astheno-spermia of the husband; (2) deficient chemical composition of the semen (lack of carbohydrates or hyaluronidase, Niendorf); (3) hostile cervical mucus (possibly serologic in origin).

If no living spermatozoa can be found in the cervical sampling, it is evident that the sperms must have been able to penetrate into the cervical canal and that they have been devitalized there. However, as Rubenstein[11] has shown, immobility does not necessarily mean death of the sperm. He exposed a sample containing immobile sperms to a drop of mucus taken at the time of ovulation, and found that at the moment the inert sperm was brought into contact with the cervical mucus, it recovered motility. This I feel is a very significant observation.

PRACTICAL APPLICATIONS

The determination of the pH of cervical mucus appears to be of questionable value. If the test is to be done, the recommendation of Breckenridge, Pederson, and Pommerenke to use Hydrion paper ought to provide sufficient information for screening use.

The test for spinability can be easily performed in any office; its technic has been adequately described by Cohen, Stein, and Kaye and therefore will not be discussed in more detail. Suffice it to say that the average spinability around ovulation time ranges between 8 and 10 cm.

As far as the fern test is concerned, it is unnecessary, according to a recent publication of Campos Da Paz,[10] to wait for 24 hours in order to allow the slide to dry; one may heat it briefly over an open flame and obtain the same results. Although it is said that there is maximum crystallization at the time of mid-cycle, I have been unable to confirm this. I have seen several cases which showed a good thermal shift of the basal temperature curve and a normal luteal phase of the endometrium, yet the fern test presented an "atypical crystallization." This led me to believe that this phenomenon could not solely be due to estrogen stimulation, since it is assumed that a normally menstruating woman with proper ovulation is in eu-estrogenism.

The most important supplementary test, however, is either the old Miller-Kurzrok test or its modification by Lamar, Shettles, and Delfs. For this one employs capillary tubes, such as are used in hematology, attaching them by means of a polyethylene tubing to a syringe with a needle. After having aspirated some cervical mucus, one leaves a little air bubble and finally sucks up a small amount of semen, after which both ends may be closed up with some cystoscope grease. The capillary tube may be left under the microscope and observed from hour to hour, thus obviating the necessity of keeping the patient in the office for several hours.

In those cases which exhibited death of the spermatozoa, when they got in contact with the cervical mucus, I tried a further experiment. On the basis of the disclosure that polysaccharides provide some form of nutrition for spermatozoa, I tried to add some of these sugars to the cervical sampling to find out whether this may enhance the viability of the sperms. At first I tried adding a minute amount of 5 per cent glucose solution; however, the sperms were killed just the same. Identical results were obtained with a 5 per cent glycogen solution. Finally, I added, according to a suggestion of Grünberger and Holkup, a drop of 5 per cent acacia solution after having titrated the solution with 0.1N solution of KOH until it just became weakly alkaline. Repetition of the capillary test resulted in a survival of the spermatozoa for over 2 hours. Thus, it seems to me that in some cases the reason for the premature death of spermatozoa in hostile cervical mucus is due to a "saccharopenia."

I had originally planned to deal with the details of therapy and also wanted to take up the important chapter on the anatomical changes of the cervical canal which has received much attention (and rightly so) in recent years, but space does not permit this.

In conclusion I would like to propose to the Research Correlating Committee that they include in the next printing of the *Minimal Procedures* the tests which have been outlined in this paper: the determination of the *p*H, the spinability, and the fern tests, as well as a bacteriologic examination of the cervical mucus.

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DISCUSSION

DR. CHARLES R. FREED, *Denver, Colorado:* Because of its anatomic location and the fact that it can be readily palpated and minutely inspected, the cervix often escapes the true portion of blame for sterility that it righfully deserves. This paper endeavors to bring these various cervical factors into focus and to point out the controversy that has existed over them in the past twenty-five years.

At first glance, it appears as though Doctor Steinberg's figure of 44 per cent cervical dysfunction is rather high. I therefore reviewed 32 of my sterility cases at random and discovered to my surprise that the cervix was a contributing factor in some 46 per cent. Although many of these disorders were minor and readily amenable to treatment, this high percentage nonetheless testifies the importance of the cervix in sterility.

There are several good points of the Sims-Huhner test that have been properly emphasized in this paper. The examination of sperm in contact with cervical mucus in capillary tubes is a simple and excellent method of study and affords opportunity for experimentation in an effort to enhance sperm penetration of the cervical mucus of each patient.

I have been using 0.5 mg. diethylstilbestrol, from Day 5 through Day 15 of the cycle with good results in increasing *Spinnbarkeit* without materially altering the temperature graph. Although I prefer not to assume a stodgy attitude, I object to changing the word *Spinnbarkeit* on the basis that it is so well imbedded in our vocabulary that a change would be more confusing than clarifying.

It is regretful that there was insufficient time to include treatment of each disorder and the results obtained. I am grateful for the opportunity of this discussion and wish to commend Dr. Steinberg on a most interesting and provocative paper.

Dr. Steinberg: I have to make a confession, and confessions are good for the soul. Before I had contacted Dr. Greenblatt, I included in the paper all I knew about the cervical factor, and I had it in 36 typewritten pages.

At the same time, I had written to Dr. Greenblatt, asking how much time had been allotted, and it was not until about the first of May that I got an answer back: fifteen minutes. I then took out my stop watch and found that no matter how fast you read, 10½ pages was all I could read in the time and it was up to me to condense it. You saw what happened to me.

I agree with Dr. Freed, to whom I am extremely grateful for discussing the paper, that therapy should have been outlined, but I think if you do cervical cultures on all your patients that exhibit a hostile cervical mucus, you will find different bacteria causing the hostility, and if you have laboratory sensitivity tests done you will find what antibiotics can be used in that particular patient.

The reason why I changed the word *Spinnbarkeit* is that I couldn't understand why a learned professor from Oxford named Clift had to use a German word to describe a simple term in medicine. The English language, I think, is perfectly adaptable and "spinability" is just as good a word, even if people are not used to it.

I am glad that Dr. Freed mentioned that his figures, which he apparently took out after he received my paper, are also 46 per cent. It only points out again the great part the cervix has in the evaluation of sterility cases. Thank you very much.

Abstracts

The effect of incipient vitamin A deficiency on reproduction in the rabbit: I. Decidua, ova and fertilization. Lamming, G. E., Salisbury, G. W., Hays, R. L., and Kendall, K. A. *J. Nutrition* 52:217-225, 1954.

Sixty-four female rabbits were studied. One group received a ration without detectable carotene, the other group received the same ration plus vitamin A. The rabbits were mated after 12 to 14 weeks on the carotene-deficient diet. They were in an incipient state of deficiency, without visible symptoms.

The size and histologic appearance of the decidua produced by a thread sutured in the uterus were normal. At 40 hours and 4 days postcoitum 93.5 per cent of the 154 ovulated eggs, as indicated by the corpora lutea, were recovered from the deficient females, as compared with 78.7 per cent of the 118 eggs of the controls. The percentages of the eggs which had cleaved in the control and deficient animals were 88.3 and 75.4, and at 4 days postcoitum the percentages were 97.0 and 66.7, respectively.

Apparently, incipient vitamin A deficiency causes premature degeneration of the ova of rabbits.

(E. Farris)

The effect of incipient vitamin A deficiency on reproduction in the rabbit. II. Embryonic and fetal development. Lamming, G. E., Salisbury, G. W., Hays, R. L., and Kendall, K. A. *J. Nutrition* 52:227–239, 1954.

Forty-two female rabbits received a ration free of detectable carotene for 12 to 14 weeks before mating; 42 rabbits served as controls. Rabbits were autopsied at 10, 16, or 28 days postcoitum to determine liver and plasma vitamin A levels, number of corpora lutea, and the number and condition of the young or number of implantation sites. The animals were in an incipient state of vitamin A deficiency, without visible symptoms.

In the deficient animals there was a reduction of 14 per cent in number of rabbits mating, and of these 82.4 per cent conceived, as compared to 89.5 per cent of the controls. The number of corpora lutea was the same in both groups. There was a loss of ova before implantation due to ovum abortion in late gestation; only 26.4 per cent of the corpora lutea were represented by normal fetuses at 28 days postcoitum in the deficient animals, as compared to 74.6 per cent in the controls. There was a significant weight reduction of fetuses at both 16 and 28 days postcoitum, even though the dams appeared in good health. Ocular abnormalities occurred in fetuses from deficient rabbits and also changes in the fetal placenta suggestive of decreased vascularity.

(E. Farris)

The oestrogenic activity of some leguminous plants. East, J. *J. Endocrinol.* 10:VIII (March), 1954.

Trifolium subterraneum L. var. dwalganup (subterranean clover) and *Soja hispida* (soy bean) have estrogenic activity which apparently depends on the presence of genistein (5′:7:4′-trihydroxy*iso*flavin) which in itself is a weak estrogen.

In western Australia, where pastures consist almost entirely of subterranean clover, infertility of sheep presents a serious problem. Seeds of *Psoralea corylifolia*, another legume, were powdered and fed for 14 days in a daily dose equal to 0.35 Gm. to 20 female threemonths-old mice, together with normal stock diet. Within 5 days, intensive cornification of vaginal epithelium occurred and persisted. Mating was normal, but to 85 per cent infertile and resulting in pseudopregnancies. Upon restoration of normal diet fertility returned to normal. Ten mice whose ovaries had been removed were similarly treated. Cornified cells appeared within 3 to 6 days and disappeared 2 days after return to normal diet.

(O. J. Pollak)

The effect of a histaminase inhibitor (aminoguanidine) on pregnancy in rats. Roberts, M. *J. Endocrinol.* 10:V (March), 1954.

Much histaminase is present in the placenta and uterus of pregnant rats. Aminoguanidine inhibits in vitro the action of rat placental histaminase, using histamine as a substrate (50 per cent inhibition at a concentration of 2.5×10^{-7}). In vivo, subcutaneous injection of aminoguanidine sulfate to pregnant rats inhibits placental and uterine histaminase.

Various doses were injected subcutaneously twice a day to rats beginning with the 14th day of gestation up to expected parturition. The maximum dose of 500 mg. per day killed 5 out of 8 pregnant rats and reduced maternal placental histaminase to zero. (This dose is also toxic to nonpregnant rats.) A dose of 250 mg. a day or less resulted in 18 per cent, and a dose of 100 mg. a day resulted in 38 per cent of the pregnancies being successful. At 50 mg. a day, all of the 6 treated rats had normal gestation, with placental histaminase equal to 681 placental units (P.U.). This is about one half the normal average of 1470 P.U. (normal range, 408–2238 P.U.). In neither series of the experiment was the offspring affected.

(O. J. Pollak)

Effects of electroconvulsive shock on sexual behavior in male rats. Beach, F. A., Goldstein, A., and Jacoby, G. *J. Comp. & Physiol. Psychol.*

Male rats were observed in a series of 7 standardized mating tests with receptive females and then subjected to 12 daily electroconvulsive shocks. Mating tests were administered after the fourth, eighth, and twelfth shocks and testing continued for 28 days after final convulsion. Sexual activity was not eliminated in any animal, although a few males failed to mate in one or another of the three tests given during the period of shock treatment. Tests conducted after treatment revealed that sexual activity was more intense and frequent than it had been prior to shock. The average delay in initiating coition increased during shock treatment, but decreased subsequently and stabilized at a point significantly below the preshock level. The percentage of the group achieving climax rose significantly and remained well above the pretreatment average. The length of time needed to reach orgasm was shortened by shock treatment. The speed at which individual coital responses followed one another was increased as a result of the convulsions. The length of time needed to recover from the sexually depressing effect of an ejaculation was reduced by the shock. The percentage of the group capable of two ejaculations in the time-limited test was raised significantly by the experimental treatment.

It is tentatively suggested that changes produced by convulsions are mediated by altered hypothalamic and pituitary function. Possible increases in gonadal hormone secretion and in the production of adrenocortical hormones might be involved. There is some reason to believe that hypothalamic reactivity is increased, with consequent changes in autonomic mechanism involved in sexual expression.

(AUTHORS' ABSTRACT)

Contribution of the seminal vesicles toward the composition of whole semen. Mann, T., and Glover, T. *J. Endocrinol.* **10**:IV (March), 1954.

Fructose, citric acid, ergothioneine, and inositol are normal and specific constituents of seminal vesicles in many animal species. All four compounds occur in ejaculates long before spermatozoa appear, in the second to third month of life. Secretory function of seminal vesicles depends upon presence of male sex hormones.

In boars, mean and standard deviation values for ejaculate are: volume, 375 ml. ($\pm$ 24); sperm concentration, 108,000 per μl. ($\pm$ 9,000); fructose, 9 mg./100 ml. ($\pm$ 0.5); citric acid, 173 mg./100 ml. ($\pm$ 9); and ergothioneine, 17 mg./100 ml. ($\pm$ 1.1). Epididymal semen obtained from dead animals contains practically no fructose, citric acid, or ergothioneine, and but 3635 spermatozoa per μl.

Thus, sperm dilution was 33 times greater in the ejaculate than in the epididymal semen. Per 100 ml. of secretion within the seminal vesicles, 43 mg. of fructose, 831 mg. of citric acid, and 81 mg. of ergothioneine were found. The ejaculate contained but 20.9 per cent of the amount of each of the three chemical constituents in the seminal vesicles.

The boar has a prolonged and split ejaculation. Samples collected at 30-second intervals were studied. At first, the ejaculate is free of vesicular secretion but the latter follows closely and persists after the ejaculate is already free of spermatozoa.

(O. J. POLLAK)

Composition of rat seminal vesicles and effect of testosterone propionate on lipid distribution. Cavazos, L. F., Porter, J. C., and Melampy, R. M. *Proc. Soc. Exper. Biol. & Med.* 85:511–515, 1954.

In sexually immature rats (weight, 77 Gm.) the seminal vesicles weighed 2.1 mg. (dry weight) and contained 0.3 mg. total nitrogen and 0.1 mg. lipid. In sexually mature rats (weight, 386 Gm.), the glands weighed 394 mg. and contained 59.5 mg. nitrogen and 4.7 mg. lipid. The average lipid content of seminal vesicles of 3 castrate male rats (approximate weight, 300 Gm.) was 2.9 mg. per gland.

A diffuse cytoplasmic sudanophilia was present in the columnar epithelium of seminal vesicles of sexually mature rats. Following castration there was a marked accumulation of lipid in the basal portion of the cuboidal cells. Administration of testosterone propionate caused the diffuse sudanophilia to reappear.

The accumulation of lipid noted may have resulted from either lipid synthesis and deposition in this region, or the dispersion of the sudanophilic material which may have collected basally during cytoplasmic regression. Phospholipid was observed in the secretory granules. A marked reduction in phospholipid reaction was observed following gonadectomy, due to disappearance of the granules. A marked increase in phospholipid reaction, due to increase in granules, was noted following hormone replacement.

The results suggest that phospholipids are in some way involved in the active metabolism of the secretory epithelium of seminal vesicles, particularly the granules of epithelium cells.

(AUTHORS' ABSTRACT)

Testicular development in the rhesus monkey. Van Wagenen, G., and Simpson, M. E. *Anat. Rec.* 118:231–251, 1954.

This study was undertaken to provide basic data for experimental studies with gonadotrophic hormones in the immature rhesus monkey. Testicular biopsies and autopsy tissues were studied from 40 monkeys of different ages, from fetal life to 11 years.

During fetal life the testicular tubules attain a diameter of 70–80 μ and reach a stage of development not again seen until puberal changes appear. Only spermatogonia and Sertoli's cells are present, but the nuclei and cytoplasm of the latter show differentiation. Interstitial cells are abundant and identifiable as Leydig cells. Definite regression of the testes occurs after birth. The tubules decrease to a diameter of 50–60 μ. Interstitial cells dedifferentiate and are not again distinguishable as Leydig cells until late in the third year. Only a few spermatogonia are seen in the postnatal period, but they increase in size and number by the end of the first year. After a period of slow development for the first three years of life the changes leading to maturity take place rapidly. Leydig cells again differentiate. Sertoli's cells increase in number and differentiate, the nuclei coming to lie basally, the filamentous cytoplasm filling the tubule lumen. Primary spermatocytes appear and the changes leading to formation of sperm follow in rapid succession. The earliest appearance of spermatozoa was observed at 2 years, 11 months, the latest at 3 years, 5 months.

(E. FARRIS)

Congenital malformations produced by injecting azo blue into pregnant rats. Wilson, J. G. *Proc. Soc. Exper. Biol. & Med.* 85:319–322, 1954.

Gillman, Gilbert, and Gillman (1948) demonstrated that a solution of trypan blue injected before and during pregnancy of the rat caused congenital malformations in the offspring. This study is being extended to other azo compounds. One milliliter of a 1 per cent aqueous solution is injected subcutaneously into pregnant rats on the seventh, eighth, and ninth day of gestation. These rats are killed on the twentieth day and the young removed, examined for malformations, and fixed for clearing or dissection.

Of the compounds tested so far, azo blue has proven the most effective in producing developmental anomalies. It caused malformations in 59 per cent of the living fetuses removed on the twentieth day of gestation, whereas trypan blue caused malformations in 49 per cent of young, under the same experimental conditions. The pattern of anomalies was similar after either of these dyes. Hydrocephalus was the most frequent defect, but ocular, vertebral, and cardiovascular malformations were also commonly found after either azo blue or trypan blue. Other visceral and skeletal structures were occasionally affected.

Both azo blue and trypan blue are basically similar in chemical structure, and both differ from other, nonteratogenic azo compounds in that the former possess the diazotized *o*-toluidine grouping. The teratogenic activity appears to be associated with this part of the molecule.

(AUTHOR'S ABSTRACT)

The Vanderbilt cooperative study of maternal and infant nutrition: V. Description and outcome of obstetric sample. VI. Relationship of obstetric performance to nutrition. *McGanity, W. J., Cannon, R. O., Bridgforth, E. B., Martin, M. P.,* Densen, P. M., Newbill, J. A., McClellan, G. S., Christie, A., Peterson, J. C., and Darby, W. J. *Am. J. Obst. & Gynec.* 67:491-500, 501-527, 1954.
(Parts I-IV of this study were published in *J. Nutrition* 51: Dec., 1953.)

A series of two thousand and forty-six white women attending the prenatal clinic at the Vanderbilt University Hospital is reviewed. This group—low to moderate income—represents all patients admitted during four years of study, 1945–1949. Each patient had routine prenatal care, and a nutritional evaluation for evidence of deficiencies, based on physical examination, biochemical assessment, and dietary records. The medical, obstetric, and pediatric outcomes of mothers and offspring are reported.

There was the usual distribution of primiparas and multiparas. The incidence of associated medical and obstetric complications was in line with those reported in textbooks and vital statistics. The incidence of toxemia (preeclampsia and eclampsia) was 5.0 per cent. From the 2046 pregnancies resulted 2068 infants; 92.3 per cent weighed over 2500 Gm., 6.7 per cent between 400 and 2500 Gm., and 1 per cent resulted in abortion. Normal vaginal delivery was accomplished in 91.4 per cent, mid-forceps in 1.3 per cent, breech in 3.9 per cent, version in 0.2 per cent, Cesarean section in 3.2 per cent. Two thirds of primiparas had labor of less than 20 hours' duration; two thirds of multiparas had labor shorter than 10 hours. Postpartum hemorrhage occurred in 1 per cent and puerperal morbidity in 5.4 per cent. The fetal loss of the 2048 infants (excluding 20 abortions) was 3.5 per cent. In the immature group of infants (400–1000 Gm.), the loss was 100 per cent, in the premature group (1000–2500 Gm.), it was 21.6 per cent, and in the mature group (over 2500 Gm.), it was 1.6 per cent. The incidence of congenital malformations was 2.6 per cent. Breast feeding was the sole source for 71.4 per cent of the infants.

As one would anticipate, a given obstetric abnormality was frequently associated with other obstetric difficulties. Similarly, an abnormal fetal issue was associated with resultant obstetric complications.

The main purpose of this paper (V) is to describe the sample of women studied and to show the completely typical nature of the sample. Only the very low incidence of abortions differs from commonly accepted standards.

The relation to obstetric performance of dietary, biochemical, and physical characteristics of 2046 women was explored. All women were studied from first entry into the hospital clinic, and thus nutritional assessments preceded the development and recognition of obstetric and fetal complications. Twenty-five subgroups of women exhibiting abnormal conditions associated with maternal or fetal aspects of gestation were compared nutritionally with the total group as a norm.

The pattern of changes in blood determinations revealed in many instances, as pregnancy advanced, alterations (such as decrease in mean hematologic values, serum protein, and serum vitamin A) that may be explained largely by the recognized hemodilution effect. However, the increase in mean levels of serum carotene and tocopherol, and the urinary excretion of N^1-methylnicotinamide cannot be related to hemodilution. The authors accept these changes as physiologic occurrences of metabolic origin which must be defined before making any attempt to relate deviations from the pattern that may occur with obstetric or fetal complications.

The average nutrient intakes of the women were below the recommended dietary allowances as set forth by the National Research Council, a deficit which was particularly evident in the third trimester of gestation. There was not a single case of frank vitamin deficiency disease. The most common sign of significance was obesity which was recorded in 15 per cent of women examined. The only physical finding and nutrient level which could be correlated was gingivitis and ascorbic acid, and it was evident that the ascorbic acid lack was not the primary determining factor in the development of gingivitis.

The nutritional status of the women with obstetric and fetal complications differed significantly from the total group in 11 of the 25 conditions examined. In all findings, except higher levels of serum carotene of diabetic patients, the occurrence of statistical significance was a reflection of the large groups involved. The actual arithmetical differences were so small as to be of questionable physiologic significance.

When the relationship between obstetric and fetal complications and adequacy of nutrient intake were compared, certain positive correlations were found in groups who had daily dietary intakes during the third trimester of less than 1500 calories and 50 Gm. of protein. These groups exhibited a higher incidence of medical diseases associated with pregnancy, and preeclampsia and eclampsia. In both instances the abnormality appeared responsible for the lowered intake, not vice versa.

(AUTHORS' ABSTRACT)

Physiological adaptation and nutritional status during and after pregnancy. Macy, I. G., Moyer, E. Z., Kelly, H. J., Mack, H. C., DiLoreto, P. C. and Pratt, J. P. *J. Nutrition* 52: Suppl. 1 (Apr.) 1954.

An extensive survey of the nutritional status of 1604 white and Negro women during pregnancy and of their infants was made in Detroit, Michigan. The subjects represented three socio-economic strata and were observed either in a public clinic, in a private clinic, or as private patients. In this report, emphasis is placed upon the observations on 427 women whose pregnancies were uncomplicated and resulted in the birth of a single, healthy infant at term. Clinical, dietary, and biologic methods of assessment were employed. Medical records included appraisal of medical history, prenatal and postnatal course, outcome of pregnancy, and condition of the infants during their first days of life. Food intake records for 7 days and 24-hour "recall" records were used to evaluate dietary methods after testing their reliability with chemical analyses. Biologic observations consisted of microchemical determinations of hemoglobin and, in serum, of total protein, vitamin C, vitamin A, carotenoids, and alkaline phosphatase.

Determination of the six blood components in healthy men and nonpregnant women provided control data. Seriatim values for 100 women from whom blood samples were obtained at successive intervals throughout pregnancy and postpartum permitted comparison of trends shown by the longitudinal data with those indicated by averages for groups at similar stages in the reproductive cycle.

(E. Farris)

Damage to posterity caused by irradiation of the gonads. Muller, H. J. *Am. J. Obst. & Gynec.* 67:467-483, 1954.

The kind and amount of damage to future generations caused by irradiation of the gonads are discussed. Heritable changes (mutations) producing conspicuous abnormalities are the rare, unusual ones. Mutations which produce effects too small or too hidden to be seen under but most carefully controlled experimental conditions are hundreds of times more frequent than the obvious ones. Almost all mutations are detrimental, and those producing small effects are just as damaging as those that are lethal. The difference between the two types lies largely in distribution of the damage among successive generations. The best estimate of genetic damage (from work with flies and mice) is that 85 r. delivered to human gonads would at least double the rate per generation at which mutations arise spontaneously. The example is given that 225 r. delivered to the gonads (whether fractionated in time or not) would produce a detrimental mutant, on the average, in 3 of every 5 children, those affected having some slight handicap which would be manifested generation after generation until the mutation, causing death, would not be longer transmitted.

To avoid adding to the deterimental load of mutations which will take its toll in misery and death in future generations, it is urged that irradiation of the gonads be minimized, that is, supplanted in medicine, whenever possible, by other technics.

(I. H. Herskowitz)

Book Reviews

Regional Block. DANIEL C. MOORE, M.D. *Springfield, Illinois, Thomas, 1953, 374 pp., $11.00.*

Regional Block is directed particularly to the general practitioner and the anesthesiologist in training. Standard and proved local anesthesia nerve blocks are described lucidly with precise details.

The illustrations are numerous and exceedingly clear. The chapter on pudendal nerve block is most informative, with detailed guides, illustrated landmarks, and instructions.

Intra-abdominal anesthesia by peritoneal lavage is suggested as a possible answer to lack of proper peritoneal anesthesia, as in a prolonged operation. An anesthetic solution, 200 cc. of 0.5 per cent Novocaine, is poured into the abdomen and allowed to remain for five to eight minutes.

The technic of spinal anesthesia is presented in detail. This book covers much more than the obstetrician and gynecologist ordinarily uses. It appears to be an accurate guide for one studying and perfecting the art of local block anesthesia.

The format, type, and illustrations are pleasant and in the best of taste.

LAMAN A. GRAY

An Atlas of Pelvic Operations. LANGDON PARSONS and HOWARD ULFELDER, illustrated by MILDRED B. CODDING. *Philadelphia and London, W. B. Saunders Co., 1953, 231 pp., $18.00.*

This atlas was prepared to teach the technical details of pelvic surgical procedures by means of illustrations, but there is sufficient written material on each page to clarify the illustrations which are in reality almost self-explanatory. The authors have included the operations which they themselves perform; hence, they have omitted a few operations which are popular with gynecologists, such as the Sturmdorf trachelorrhaphy, colpotomy for pelvic abscess, and extraperitoneal ligation of hypogastric arteries. However, the authors did not intend to include all gynecologic operations. Likewise, they purposely omitted consideration of the physiologic aspects of preoperative and postoperative management. Incidentally, the Marshall-Marchetti operation should be called the Marshall-Marchetti-Krantz operation.

The book is divided into three sections—abdominal operations, vaginal and perineal operations, and operations for malignant diseases. The authors properly advise that a careful examination be made under anesthesia immediately before the operation, after the bladder has been emptied by catheter. They also insist upon a diagnostic curettage in every case in which pelvic laparotomy is contemplated, in order to avoid unfortunate mistakes. This recommendation is made in spite of the fact that every neoplasm removed is opened and examined before the abdomen is closed.

There are 45 drawings for the Wertheim hysterectomy and bilateral pelvic lymphadenectomy and 57 for the combined bilateral superficial groin dissection and radical vulvectomy, but as the authors themselves emphasize, to understand thoroughly the varied ramifications of the malignant process requires years of specialized study. Such training cannot be a regular part of the experience of every surgeon and gynecologist. Of special interest to readers of FERTILITY AND STERILITY are the 4 plates devoted to plastic surgery on the tubes and 1 plate on wedge resection of the ovary.

With few exceptions the drawings in this atlas are remarkably clear and instructive. The text material is ample and also lucid. The authors also are to be congratulated on their accomplishment and for paying tribute to our own Joe Meigs, their teacher, to whom they dedicate the book. Likewise, the publishers are to be complimented because even though the book is of unusual width, it is easy to handle. Every surgeon and gynecologist can learn a great deal from this atlas.

J. P. GREENHILL

Ion Exchange Resins in Medicine and Biological Research. ROY WALDO MINER (Ed.). *New York, New York Academy of Sciences, 1953, $4.50.*

The twenty papers presented at the New York Academy of Sciences conferences on ion exchange resins have been collected as a monograph covering some of the fundamentals of ion exchange resins, their application to biology, and the therapeutic considerations of these data. The papers are well documented by tables, graphs, and bibliographies, and, as a rule, summaries are added.

This monograph would be of considerable value to the investigator or student of ion exchange, but would be of limited value to the clinician.

WILLIS E. BROWN

The Management of Bilateral Polycystic Ovaries

Irving F. Stein, Sr., M.D.

THE RATIONALE of surgical treatment in correction of bilateral polycystic ovaries is indeed puzzling to many physicians, inasmuch as the cause of the ovarian pathology has never been determined. Endocrine dysfunction most logically explains the ovarian changes; yet endocrine therapy has proved disappointing in the hands of almost everyone who has treated and followed a group of such patients. Roentgenotherapy has also been utilized in the treatment of bilateral polycystic ovaries with but scant success. When no form of treatment is employed, spontaneous readjustment and restoration of ovarian function does not usually occur. In the past 25 years, we have utilized bilateral ovarian wedge resection and recommend it as the routine treatment for bilateral polycystic ovaries, inasmuch as no other form of therapy has given comparable results in the restoration of ovarian physiological function.[10]

In this report, we wish to evaluate our own experience of a quarter of a century and add to it the experience of those members of the American Society for the Study of Sterility who responded to a questionnaire which we sent to the membership.

Bilateral polycystic ovaries occur in only a small proportion of women who present the problem of infertility[8]; it is also found in some single girls whose chief complaints are those of amenorrhea, hirsutism, or both. Other

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The author gratefully acknowledges the assistance of Dr. Bernard M. Kaye and Miss Paula Bennett in the preparation of this manuscript.

symptoms and signs are found less consistently. Menometrorrhagia may be found in the history of a small proportion of these patients with bilateral polycystic ovaries, and unless successfully managed, secondary amenorrhea is likely to follow. Acne and pelvic pain are infrequent; true virilism has not been observed by us. Uterine hypoplasia is found in about three fourths of the patients; breasts are underdeveloped in slightly less than one half of the patients. Other secondary sex characteristics, such as general body development, often show lack of feminizing influence.

DIAGNOSIS

The first cases of bilateral polycystic ovaries which came to our attention (1928) were in women who sought treatment for rather extended periods of amenorrhea.[11] They were childless married women who were chiefly concerned with adjustment of the menstrual cycle. In a few of these, bilateral ovarian enlargement was found upon examination, but in others, either unilateral or no ovarian change was palpable. The latter were tense or obese women in whom bimanual examination was indeterminate.

Having utilized pneumoroentgenography for a differential pelvic diagnosis for several years previously with good results, we applied this method of gynecography[9] to these amenorrheic and infertile women. The films thus obtained were very striking; the bilateral symmetrically enlarged ovaries and relatively small uteri were very clearly defined. Normally the ovaries are no more than one fourth the size of the uterine corpus on the roentgen film; in these women, the ovaries were from one half to three fourths the uterine size. In cases of marked hypoplasia of the uterus, the ovarian shadows were larger than that of the uterus on the x-ray film. Gynecography became a routine means of diagnosis in our hands, since it more consistently and more accurately revealed the bilateral ovarian enlargement than did bimanual examination.

The films were even more striking in the group of single girls (chiefly nurses referred to us because of amenorrhea) in whom only rectal palpation was permitted. Rectoabdominal examination often failed to reveal the enlarged ovaries, which were 4–5 cm. in diameter. Examination under anesthesia was unsatisfactory in these girls.

A definite diagnosis of the bilateral polycystic ovary syndrome depends largely upon proper identification of the enlarged ovaries themselves. We feel that diagnosis on a presumptive basis, relying upon laparotomy for verification is unjustifiable. This has been done by a number of physicians, according to the replies which we received to our questionnaire. The exploratory laparotomy should become unnecessary where there are other methods available for establishing a definite diagnosis. In our experience, bimanual examination has been inconclusive in fully half of our patients with bilateral polycystic ovaries. When palpation of the pelvic organs gives inconclusive information concerning the ovaries, there are several alternative steps:

1. Examination under anesthesia, eliminating voluntary rigidity. However, the ovaries are not always palpable by this method.
2. Culdoscopy, permitting direct vision of a portion of the pelvis. The smooth, pale, cystic ovaries can be observed.
3. Colpotomy, utilized by some physicians. By this means, the ovaries may be palpated, identified, and observed.
4. Gynecography, by which means the pelvic organs can be visualized on the x-ray film, includes pneumoroentgenography and hysterosalpingography.

Vaginal Smears

Other diagnostic aids were utilized from time to time, some informative and others of little help.

Daily vaginal smears, fixed in equal parts of alcohol and ether, and stained by the rapid Shorr stain, have been utilized for rough hormone assay. The pattern of the vaginal smear in bilateral polycystic ovaries resembles that found in the normal female in the second week of the menstrual cycle. For the most part, the large and small cells stain bluish and contain moderate- to large-sized vesicular nuclei. The cytoplasm is either clear or lightly stippled and the cell edges are regular. We do not find complete cornification, nor do we see the characteristic curling of cell edges and clumping which are associated with the postovulative phase of the cycle. Although the vaginal smears show moderate estrogenic effect, they do not reveal either hypo- or hyper-estrinism. These smears are not consistent; hence they offer little aid in the diagnosis of bilateral polycystic ovaries.

Cervical Mucus

In patients with bilateral polycystic ovaries, there is a diminished amount of cervical mucus, which is usually of moderate consistency and has a

Spinnbarkeit of 1–2 cm. The amount and consistency of the mucus does not undergo the usual cyclic variation that one observes in women who are ovulating.

B.M.R.

Determination of basal metabolism rate was regularly and repeatedly made early in our studies, until it became obvious that this determination was of value only in cases of concomitant thyroid disturbances. Only 10–15 per cent of these patients were obese and none was hypothyroid. B.M.R. was usually within the normal range.

Endometrial Biopsy

In the women who presumably ovulate, proof of ovulation can be obtained by performing an endometrial biopsy at a specified time of the cycle. Such timing is not possible in the women who are amenorrheic and seldom, if ever, ovulate. Therefore, the biopsy specimens in women with bilateral polycystic ovaries do not represent a certain definite phase of the cycle and are of questionable value. In our series, we routinely found the predominant type to be *low- to mid-proliferative,* indicating a moderate estrogenic effect. We observed neither atrophy nor secretory activity of the endometrium. Of 2 biopsies in which the endometrium was hyperplastic, 1 was obtained from a patient who had received prolonged hormone therapy previously which, we believe, thus stimulated the endometrium.

17-Ketosteroids

Clinically, many of the patients with polycystic ovaries evidence some masculinization in the form of hirsutism, often accompanied by a masculine escutcheon and body conformation. Thus it would appear that 17-ketosteroid determinations would be informative. However, the results of these determinations in a number of our patients proved to be of little value, as they were normal or very slightly elevated. These results are in accordance with reports of Jones and other investigators. Therefore, we do not consider it essential that a routine 17-ketosteroid determination be made in cases of bilateral polycystic ovaries.

In this condition, we feel that the persistent amenorrhea, hirsutism, and retarded secondary (female) sex characteristics result from defeminization rather than from active androgenic effects. In the differential diagnosis between the Stein-Leventhal and the adrenogenital syndromes, the 17-ketosteroid determination is important inasmuch as the latter is consistently elevated.[4]

Body Temperature

Basal body temperature charts are of value both in direct diagnosis and for comparison following therapy. Before treatment, the monophasic curves which indicate anovulatory cycles are routinely obtained. Following effective therapy, the typical thermal shift indicating ovulation usually appears on the chart.

Pneumoroentgenography

We have utilized all these diagnostic technics and have found that pneumoroentgenography has proved most valuable in demonstrating on film bilateral symmetrically enlarged ovaries, their size and shape, and their relation to the uterine size (Fig. 1). The films also constitute a permanent record available when consultation is desired or a difference of opinion arises. This method of x-ray diagnosis has proved of immense value to us in differentiating among patients with amenorrhea and sterility those with bilateral enlargement of the ovary from those with either unilateral ovarian enlargement or ovaries which were normal or smaller than normal. Obviously the latter would not qualify for the syndrome. A number of women seen in consultation because of amenorrhea and possible bilateral polycystic ovaries were found by gynecography to have hypoplasia of the ovaries and uterus. In such patients there is no indication for laparotomy and bilateral ovarian wedge resection.

In every instance in which the ovaries appeared bilaterally cystic on the x-ray film, and where laparotomy was done, the information obtained from the pneumogram proved to be accurate. Therefore, we feel very strongly that x-ray films after induced pneumoperitoneum should become a routine diagnostic procedure whenever there is a presumptive diagnosis of bilateral polycystic ovaries.

BILATERAL WEDGE RESECTION

Bilateral ovarian wedge resection, which we have described and which we recommend as the treatment of choice in cases of the bilateral polycystic ovary syndrome, evolved from ovarian biopsy. In a few cases of longstanding secondary amenorrhea, we removed a wedge-shaped section of each ovary to ascertain whether the ovarian pathology would explain the persistent amenorrhea. Although the pathologist's examination and diagnosis did nothing to clarify the etiology, we found subsequently that the operative procedure itself resulted in restoration of ovarian function.

A

B

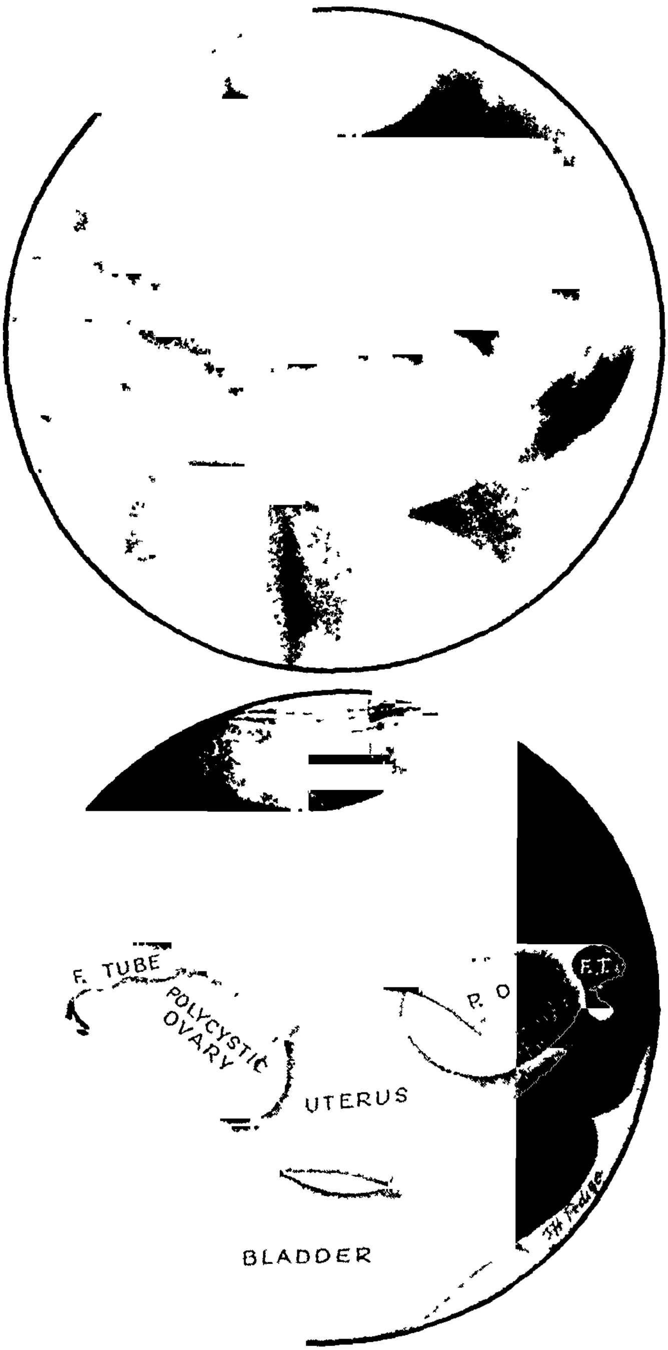

Fig. 1. *A*, Pneumoroentgenogram (Pt. No. 90). Bilateral polycystic ovaries; hypoplastic uterus. *B*, Wash drawing of same.

Our surgical technic is essentially the same as that which we described previously (Fig. 2), and we refrain from performing additional surgical procedures at the time of ovarian resection unless strictly indicated. We do not recommend multiple punctures through the ovarian capsule as we feel that such trauma would favor the formation of adhesions. Ovarian suspension, which is carried out almost routinely by some physicians, is a technic we have performed in only a small number of our patients in whom we

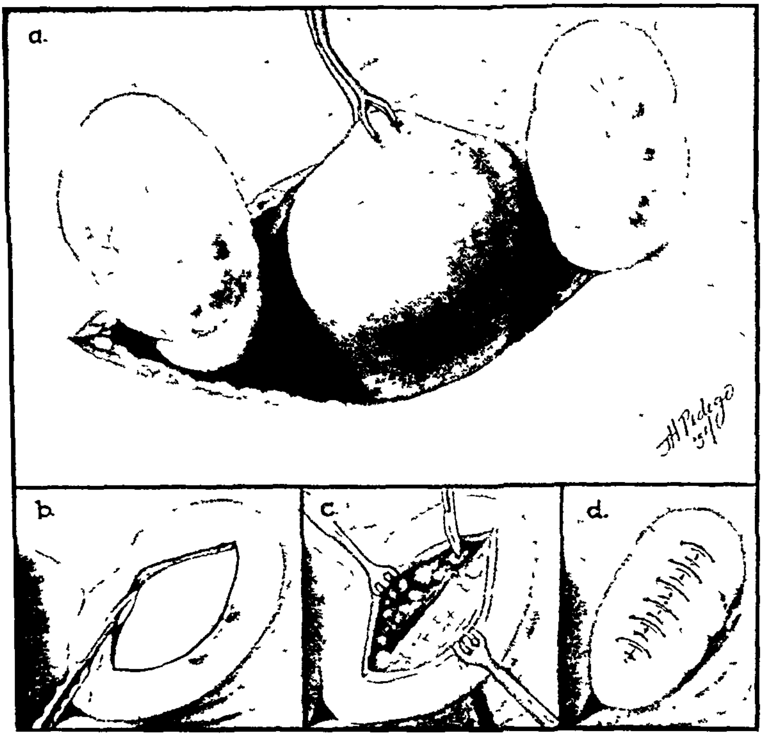

Fig. 2. *A*, Hypoplastic uterus and bilateral polycystic ovaries as seen at laparotomy, Pfannenstiel incision. *B*, incision for wedge resection. *C*, multiple subcortical follicle cysts visible in wedge excision; puncture with knife blade from within incision. *D*, completed repair of ovary.

found the utero-ovarian ligaments abnormally long. In our experience, retrodeviation of the uterus occurred in only 4 patients and in only these 4 was corrective uterine suspension performed. Appendectomy is not advisable at the time of wedge resection inasmuch as the incised ovary is extremely vulnerable to infection. We feel that our best results have been obtained in those patients in whom we limited our surgery to wedge resection alone and have thus narrowed to the utmost the possibility of pelvic adhesions. We feel that extensive ovarian adhesions would definitely defeat the purpose of the operation and might also result in irregular uterine bleeding.

RESULTS OF QUESTIONNAIRE TO A.S.S.S. MEMBERS

Diagnosis

Although we have advocated gynecography as a routine diagnostic method and bilateral wedge resection as the treatment of choice in cases of the Stein-Leventhal syndrome, we recognize that a difference of opinion exists among many physicians. Therefore, we circularized the membership of the American Society for the Study of Sterility in order to establish the validity of other types of diagnosis and treatment for this condition. We asked some pertinent questions requiring specific answers which we shall attempt to summarize.

We received 151 returns, of which 43 were discarded as unsatisfactory for analysis. Some of these 43 respondents were not gynecologists; others stated that they had no experience with the condition and therefore were not qualified to contribute to the subject. In addition, 3 of the 108 physicians who replied categorically denied the existence of a specific syndrome. The other 105 (97 per cent) recognized the bilateral polycystic ovary syndrome. X-ray diagnosis was employed by only 16 of those responding. It was used in 82 per cent of our patients. Forty per cent of the membership utilized no specific diagnostic procedures but relied upon laparotomy to verify a presumptive diagnosis. In addition, 19 members stated that they treated their patients surgically on the basis of history and physical examination alone. Nineteen utilized culdoscopy; 7 stated that they used all methods of diagnosis. Of the remaining 4, 1 used colpotomy, 1 relied upon B.B.T., and 2 utilized endocrine studies for diagnosis (Table 1).

In treatment, 84 of the 105, or 80 per cent, utilized surgical treatment alone, 2 treated medically alone, and 1 radiologically alone. This emphasizes the fact that only 1–2 per cent of our membership has adopted medical or radiologic therapy as the treatment of choice. The remaining 17 per cent of the members combined various methods; some utilized surgical technics only after other measures had failed.

Early in our experience, we found that patients treated medically alone, chiefly by endocrine products—sometimes for periods of many months and even years without benefit—were eventually treated successfully by ovarian resection. We know of no medical treatment which has proved successful in the management of patients presenting this syndrome. Some investigators have used cortisone either primarily or following surgical treatment of bilateral polycystic ovaries. The treatment is based upon the premise that cortisone reduces 17-ketosteroid excretion and depresses ACTH production; thus it may also have a balancing action between growth hormone and adrenal steroids.[4] In the true Stein-Leventhal syndrome, we have found that

TABLE 1. Summary of 108 Responses to Questionnaires Sent to A.S.S.S. Members

Response	Doctors		Patients
	No.	%	(%)
Recognized syndrome	105	97	..
Treated by surgery	84	80	..
Patient menstruated	..	..	75
Patient became pregnant (215 live births)	..	..	37
Treated medically	2	2	..
Treated radiologically	1	1	..
Used combined methods	18	17	..

We sent 151 questionnaires; 43 were discarded as unsuitable for analysis.

17-ketosteroid excretion is almost or wholly normal, in which case cortisone is likely to be ineffective. However, if the 17-ketosteroid excretion shows an increase due to adrenal hyperplasia, cortisone therapy may be expected to be beneficial. Thus far, cortisone has been used in too few cases for proper evaluation.

We have had very little experience with roentgenotherapy, and apparently only about 5 per cent of our members who answered the inquiry utilized this method for treatment of polycystic ovaries. Only 1 who used the method exclusively reported his results as follows: 3 patients were treated; 2 menstruated and 1 became pregnant. Another, who employed both radiological and surgical methods, reported 60 per cent improvement in 22 patients treated by radiology. However, his results in 45 patients treated by bilateral ovarian wedge resection were reported in more detail. He stated that in 32 of the 45, regular menstrual cycles were re-established and 14 pregnancies occurred.

Surgical Treatment

Thirty-six members of the A.S.S.S. who reported using bilateral ovarian wedge resection for management of bilateral polycystic ovaries gave figures which could be summarized. A total of 568 patients were operated on, following which 75 per cent of the women menstruated regularly and 37 per cent became pregnant, with about 215 live births. The percentage of those patients who menstruated regularly and the number of live births are not exact since 2 authors who had followed relatively large series were not specific in these two categories.

RESULTS OF SURGERY IN AUTHOR'S SERIES

In my own series, 88 patients were operated on (Table 2); 80 of these had amenorrhea associated with bilateral polycystic ovaries and 8 gave a

TABLE 2. Author's Results in Fertility Following Surgical Treatment of 88 Patients

	No.	*% of total*
Regular menses established	84	95
Infertile women after elimination of unsuitable patients from series	62	70
Pregnancy achieved	54	88.7 (of 62)
Number of pregnancies	116[a]	..
Live births (4 sets of twins)	95[b]	..
Undelivered	4	..
Recurrence of B.P.O.	0	0
Complications	3	3.4
Carcinoma of uterus	0	0

[a] 118. [b] 96.

history of menometrorrhagia before surgery. In 73 cases, the diagnostic pneumogram was employed. Two of the 88 patients had given birth to 1 child each prior to our first examination; in both instances, bilateral polycystic ovaries were revealed as the cause of their secondary infertility. In order to determine our results in the light of fertility, we eliminated those patients whose husbands were found to be sterile or highly infertile; in our series, there were 12. Four patients were eliminated because in one instance, the husband was killed shortly after the patient's operation; another was divorced; a third committed suicide; and the fourth had a hysterectomy performed because of uncontrolled uterine hemorrhage following an accident. Of the 16 who were thus eliminated, 2 were returned to the series; 1 patient remarried and is now pregnant for the second time, and the other, whose husband was azoospermic, became pregnant after a therapeutic insemination. We also eliminated from our compilation 12 patients who are still single. This left 62 in the married group, of whom 54 became pregnant (88.7 per cent), with a total of 116* pregnancies; there were 95† live births, including 4 sets of twins. There are 4 patients as yet undelivered; 21 patients either miscarried or had stillbirths.

There were some patients who did not menstruate regularly immediately following resection. However, without additional therapy, most of these patients did have fairly regular ovulatory cycles within a few months. There were 3 patients in our series who continued to have irregular cycles of from 4 to 7 weeks, and 1 single girl who had amenorrhea for 1 year following resection, after which she menstruated regularly. There is 1 patient whose surgery is too recent for evaluation. The corrected figure in regard to normal menstruation following surgery is 95 per cent (See Table 2).

DISCUSSION

Recently there have been reports in the literature of studies made of young women 19–40 years of age with endometrial carcinoma; in some of these, bilaterally enlarged ovaries were found to coexist. In 1949, Sommers, Hertig, and Bengloff reported that of 16 women 19–35 years old with adenocarcinoma of the uterus, 4 had typical bilateral polycystic ovaries. Speert, in the same year, had 14 cases of endometrial carcinoma in women under 40 years of age, 11 of whom had cystic sclerotic ovaries. Their cases were characterized by infertility, hyperplasia of the endometrium, and cystic ovaries.

In 1951, Dockerty, Lovelady, and Foust, Jr., reported 36 cases of endometrial carcinoma in women under 40 years of age. The ovaries were polycystic in 14 cases, cystic but of normal size in 7, and fibrotic in 5. Among these patients, 7 had the clinical features of the Stein-Leventhal syndrome.

Another interesting case of the Stein-Leventhal syndrome associated with carcinoma of the endometrium was reported by DeVere and Dempster, of a 23-year-old nullipara who presented menorrhagia and hirsutism, with a

* Two additional pregnancies have been added since completion of this report.
† 96.

previous history of secondary amenorrhea. Dilatation and curettage revealed cystic hyperplasia of the endometrium suggestive of early carcinoma. Laparotomy revealed bilateral polycystic ovaries and a bilateral wedge resection was performed. Six months later, there was a recurrence of cystic ovaries and a diagnosis of adenocarcinoma of the uterus was established.

An unpublished case was reported to us by Dr. R. Glenn Craig of a young woman, aged 28, who had not menstruated for more than 2 years. The ovaries were bilaterally enlarged and a diagnosis of the Stein-Leventhal syndrome was made. Progesterone therapy failed to produce bleeding. Dilatation and curettage and laparotomy were performed. Typical polycystic ovaries were revealed at laparotomy and examination of the endometrial

TABLE 3. Endometrial Carcinoma in Young Women

Author	*Endometrial carcinoma*	*Bilateral polycystic ovaries*	*Hyperplastic endometrium*
S.H.B.[5]	16	4	Not stated
D.L.F.[3]	36	14	Not stated
S.[7]	14	11	11
De V. and D.[2]	1	1	1
C.[1]	1	1	1

specimen showed adenocarcinoma. A previous dilatation and curettage, done when the patient was 21 years of age, showed hyperplasia of the endometrium and metaplasia of many epithelial cells (Table 3).

In our experience, the endometrium—particularly in cases of amenorrhea—is predominantly low- or mid-proliferative in type. Patients who develop adenocarcinoma generally show hyperplasia of the endometrium. Therefore, it is difficult to reconcile these reports of carcinoma associated with bilateral polycystic ovaries with our observations over a 25-year period. We have routinely followed up the patients on whom we have operated; only a few who have moved away have failed to report regularly. We have encountered no instance of endometrial carcinoma in patients who have had bilateral ovarian wedge resection for the management of the syndrome.* From the foregoing reports, an etiologic relationship between bilateral polycystic

* From the record library and the laboratory of pathology, Michael Reese Hospital, 42 cases of endometrial carcinoma were reviewed. The age range was from 33 to 77 years, with only 3 patients under 40. No instance of bilateral polycystic ovaries was found.

ovaries and carcinoma of the endometrium is implied by some investigators, but proof is lacking.

It is our belief that in those cases where hyperplasia or carcinoma of the endometrium, or both, is found, and the ovaries are enlarged, the ovarian pathology is not the same as in the typical Stein-Leventhal syndrome. Dockerty and his associates found no consistency in size or structure of the ovaries in their series of endometrial carcinoma in young women. It is highly probable that the ovarian changes are entirely incidental.

In view of the above, perhaps a diagnostic dilatation and curettage should be performed in all cases where a diagnosis of bilateral polycystic ovaries has been made. Those patients in whom hyperplasia of the endometrium is found should be carefully followed for the earliest recognition of endometrial carcinoma. This offers an interesting field for investigation.

SUMMARY AND CONCLUSIONS

The fact that secondary amenorrhea is so definitely associated with the bilateral polycystic ovary syndrome has led to confusion in the minds of some clinicians. Amenorrhea is only one of the components of a definite syndrome and in itself is not diagnostic; nor is wedge resection the treatment of choice in all cases of amenorrhea, nor indeed in all cases of sterility associated with any and all types of ovarian pathology. In the past 25 years, we have selected only 88 cases for bilateral ovarian wedge resection from a very large group of women who were investigated and treated for infertility. Menstrual function was restored in 95 per cent of these patients, who were chiefly amenorrheic. Fifty-four women became pregnant (88.7 per cent), with a total of 116* pregnancies. There were no recurrences of bilateral polycystic ovaries. We encountered no instances of endometrial carcinoma associated with bilateral polycystic ovaries.

In establishing a diagnosis, gynecography proved to be an important aid and we recommend it for routine use. We recommend surgical treatment in cases where a definite diagnosis of the Stein-Leventhal syndrome has been established.

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DISCUSSION

Dr. Davis: Dr. Stein, thank you very much for a beautiful discussion of a quarter of a century of hard work. What I admire most about your work is your conservatism and your conservative conclusions after that long period of time. The discussion will be opened by Dr. Ingersoll of Boston.

Dr. Francis M. Ingersoll, *Boston, Massachusetts:* Dr. Stein in this excellent paper has brought us up to date on the management of the polycystic ovary syndrome and I am honored to be asked to discuss it. There is little that can be added to his presentation.

Dr. Stein has strongly stressed the fact that the crux of the diagnosis of the Stein syndrome depends upon the demonstration of bilaterally enlarged polycystic ovaries. The symptomatology varies; i.e., most of the patients have infrequent periods, but some complain of menometrorrhagia, all are anovulatory, therefore infertile, some are obese, some hirsute, but some are not. The only finding in common to all is the ovarian pathology. Looking for a patient with bilateral polycystic ovaries among all the patients with amenorrhea, oligomenorrhea, menometrorrhagia, sterility, and hirsutism is like looking for a murderer among 3000 people; the culprit is only apprehended when bilateral ovarian enlargement is found.

Diagnosis of bilateral polycystic ovaries is accomplished by physical examination in a few, but usually by pneumoroentgenography or culdoscopy. We prefer the latter method and feel that diagnosis is usually fairly certain before culdoscopy is done. That is, from the large number of suspected patients careful study by basal body temperature chart and endometrial biopsy will prove them to be anovulatory. Normal FSH-level determinations will eliminate those with precocious menopause. Findings of normal 17-ketosteroids will eliminate those with adrenal hyperplasia or tumor. A normal B.M.R. will rule out those with hypothyroidism. These studies then leave an occasional patient who is anovulatory, hirsute, and sterile who may have polycystic ovaries as an etiological factor. In these cases we do culdoscopy, having had little experience with gynecography. We feel that exploratory laparotomy is rarely indicated as a diagnostic method. The diagnosis of polycystic ovaries should be established before laparotomy for wedge resection is done.

Dr. Stein has demonstrated in his own 88 cases that normal menstruation was restored in 95 per cent of his patients and that of 62 in the married group, 54 became pregnant—88.7 per cent. This is a remarkable series and the obvious proof of the accuracy of his diagnosis and the success of wedge resection.

We have found 37 cases at the Massachusetts General Hospital over a period of 17 years; that is, an average of 2 cases a year. The management of these 37 cases has varied, so that this group is an interesting one to study to compare one type of therapy against another. A standard therapy was not adopted until 6 years ago. Prior to that time each surgeon dealt with the ovaries at operation according to his own plan. Table 1 illustrates the types of therapy and results which are classified as excellent if cycles became regular and ovulatory as proven by basal body temperature curves, endometrial biopsy, or pregnandiol excretion. If postoperative cycles became more regular than preoperative and were sometimes ovulatory and sometimes anovulatory, result was classified as fair. If no improvement resulted therapy was a failure. Two patients had only a small ovarian biopsy taken and neither patient was benefited. These two cases are included to show

TABLE 1. Treatment and Results in 37 Cases of Polycystic Ovaries at Massachusetts General Hospital

		Results		
Type of operation	*No. patients*	*Excellent*	*Fair*	*Failure*
Wedge resection	28	20	6	2
Splitting and eversion	7	6	0	1
Biopsy	2	0	0	2

that the success of the surgery is not due to laparotomy and the alarm reaction, but to adequate resection of ovarian tissue.

Seven patients had the ovaries split open, everted and sewed back to back, a technic advocated by Bailey. Six of these patients had excellent results; 1 patient whose chief complaint was menometrorrhagia failed to be relieved and subsequently had to be reoperated upon and the uterus, tubes, and ovaries removed. Extensive adhesions between ovaries and bowel were found in this case. Twenty-eight patients have had wedge resections of each ovary; 20 patients have had excellent results with restoration of normal ovulatory menstrual cycles, 6 patients have been improved, but were still irregular and ovulated infrequently, and 2 patients had no improvement at all. No explanation is available for one of these failures; the other patient had an elevated 17-ketosteroid level. Following wedge resection the 17-ketosteroids fell to normal levels for a few months, but then rose again. Subsequent therapy with cortisone was unsuccessful and did not succeed in regulating the menstrual cycle. She probably should not be included in this series.

At the present writing from our experience I recommend that wedge resection of at least one third of each ovary is the best method of therapy. That such therapy is empirical is recognized; the rationale escapes me, but it works.

The criterion of success in the group complaining of sterility is pregnancy. Twenty-six of our patients fell into this group, and 14, or 54 per cent, became pregnant. Five patients have each had 2 pregnancies. If we eliminate from this group those with follow-up of less than 6 months (3 patients) and those with infertile husbands, the corrected figure for pregnancy is 66 per cent.

CONCLUSIONS

Dr. Stein, through his years of work, has demonstrated that there is a syndrome associated with polycystic ovaries and that wedge resection is successful in treating this condition. I am glad to have this opportunity to congratulate him on his medical contribution.

Dr. Davis: Thank you, Dr. Ingersoll, for this new information. The discussion is now open to the floor.

Dr. R. B. Greenblatt, *Augusta, Georgia:* I rise to a point of personal privilege, as Senator McCarthy would say. I think I raised the question of whether a Stein-Leventhal syndrome exists, and I want to say I believe it does. But the finding of large, palpable polycystic ovaries does not necessarily mean a Stein syndrome. We have a clinical entity; that is, a pathologic entity. I feel that the 17-ketosteroids may or may not help. We feel many of these patients do not have an elevated 17-ketosteroid urinary excretion. However, we have found several with increased 17-ketosteroids. We feel that they ought to have a cortisone test. If these patients respond to cortisone, we might say it is mild adrenogenital syndrome. If these patients respond to wedge resection and begin to menstruate and ovulate, then that patient had a Stein-Leventhal syndrome. If they don't respond to the cortisone or the wedge resection, we had better look elsewhere.

Dr. Davis: We will take a few minutes if any of you have any pertinent questions or comments. May I ask Dr. Stein if he found any increase in pituitary activity, such as spontaneous increases in growth or any other signs of activity of the anterior pituitary?

Dr. Rita Finkler, *Newark, New Jersey:* I would like to ask Dr. Stein if those few cases of hirsutism were improved after operation.

Unidentified Questioner: Were there any significant histologic findings in the wedge removed from the ovaries?

Unidentified Questioner: I would like to ask Dr. Stein and Dr. Ingersoll, inasmuch as these uteri were all normal, if there were any concomitant biopsies of curettage material.

Unidentified Questioner: I would like to ask Dr. Greenblatt how long he would give for the cortisone test.

Dr. Greenblatt: A period of three months, anyway, with 75 mg. a day.

Dr. Ingersoll: The endometria of these patients are often very atrophic, and sometimes you don't get any at all. Usually there is a small amount of endometrium, proliferative in character, secured by D. and C., prior to exploratory laparotomy.

Dr. Stein: I am indeed deeply grateful to Dr. Ingersoll for the splendid piece of work and the great contribution he has made in trying to clear up the etiology.

Unfortunately, he wasn't able to do so, but the effort should be given an "A." I thank him for his discussion, and also the other discussors.

I don't know enough about the cortisone situation to give a "yes" or "no" as to whether it is a criterion or differentiation or not, but I would accept the work he has done, certainly, and in my paper I have recommended that further observations with cortisone are certainly warranted. He and Dr. Seeger Jones started the ball rolling. Dr. Cohen, my associate, has used it in a few cases. We haven't been enthusiastic.

I want to stress one thing. The fact we had a large percentage of success, I think, is dependent partially upon the long period of time (25 years) we have been able to observe these women since resection. If resection was done within a year, you may only get 10 or 20 per cent success. The percentage over a period of 5 years will go up. I have eliminated any patients operated on in the last year because they have not had time to get pregnant. The exception was one who went six years and then finally this year got pregnant. We had called her a failure in our previous report.

I would say we have had no evidence of pituitary disturbance following resection. A return to feminine type of those who had a rather masculine habitus was observed.

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Optimum Time for Orchiopexy in Cryptorchidism

Frank Hinman, Jr., M.D.

The results of orchiopexy are disappointing. Is it that the surgical procedure is faulty, or can the defect be in the patient or in the testis?

Realization that cryptorchidism is a symptom and not a diagnosis or a disease entity helps us answer these questions. A study of biopsies from undescended testes made during the 8-year period, 1946–1954, was made in order to compare them histologically (as Cooper, and as Robinson & Engle have done) with normal testes from children of similar ages. Our experience has been weighted: our associates in Endocrinology have referred unusual material to us because of their interest in testicular cytologic changes in various endocrine disorders (Table 1).

Extreme variation in direction and degree of development can be seen among sections from cryptorchid patients of the same age or even in the same patient. Our observations made us realize that we cannot talk of the effects of cryptorchidism or of orchiopexy without recognizing that the undescended testis may be just one manifestation of congenital malformation or of endocrine dyscrasia.

Study of this material appears to lead inevitably to two conclusions. First, many testes are constitutionally so abnormal that they will not function no matter what sort of hormonal or operative therapy is applied. Second, if the testes are potentially normal, surgical placement must be done before they are irreversibly damaged by the high intra-abdominal temperature.

From the Department of Surgery-Urology, University of California School of Medicine, San Francisco, California.

Read before the Tenth Annual Meeting of the American Society for the Study of Sterility at San Francisco, California, June 18, 19, and 20, 1954.

CAUSES OF CRYPTORCHIDISM

One of three basic factors prevents descent of the testis:

1. Physical Abnormalities of Gubernaculum and Canal

Physical abnormalities of the gubernaculum and canal may obstruct descent. For example, the patient with unilateral cryptorchidism who is otherwise normal, in whom descent does not occur either with administered hormones or at puberty. Ectopy of the testis would be a variant type. With the testis otherwise normal, early orchiopexy would help this patient.

TABLE 1. Cryptorchidism, with Associated Abnormalities
(University of California Hospital)

Unilateral without other abnormalities		11
Bilateral without other abnormalities		2
Associated abnormalities		16
Obesity	2	
Intersexuality (hypospadias)	5	
Primary testicular hypogonadism	3	
Pituitary hypogonadism	3	
Maldevelopment of the testis	3	
TOTAL		29

In patients with abnormalities of the canal, including most cases with unilateral nondescent, orchiopexy is the only effective treatment, since the patient's own pituitary hormones have proved sufficient to bring down the opposite side. Operation is also necessary if the retained testis is associated with a clinically demonstrable hernia.

2. Abnormalities in Testis

Abnormalities in the testis itself are not at all rare in our particular experience. The classic example is primary testicular hypogonadism. Here, the testis itself is inadequately developed, and in spite of adequate pituitary stimulation (as shown by the high circulating F.S.H. level) never produces enough androgens to promote testicular descent. At the University of California Medical Center, 5 of 17 patients (29 per cent) with primary testicular hypogonadism had undescended testes. Lesser degrees of the same basic testicular abnormality are present in many cases of cryptorchidism in which the only evidence of testicular hypofunction is an increased level of F.S.H., with or without a decreased level of androgen.[3]

The high number of patients seen with seminomas with increased F.S.H. is of interest in this connection. Since cryptorchid testes are also frequently associated with increased F.S.H. and since cryptorchid testes are twenty times more likely to be involved by tumor (seminoma) than the normal testis,[6] we can speculate that there may be some causal relationship between the endocrine and testicular abnormality, and the neoplasm.

The fact that the contralateral, normally placed testis may also be ab-

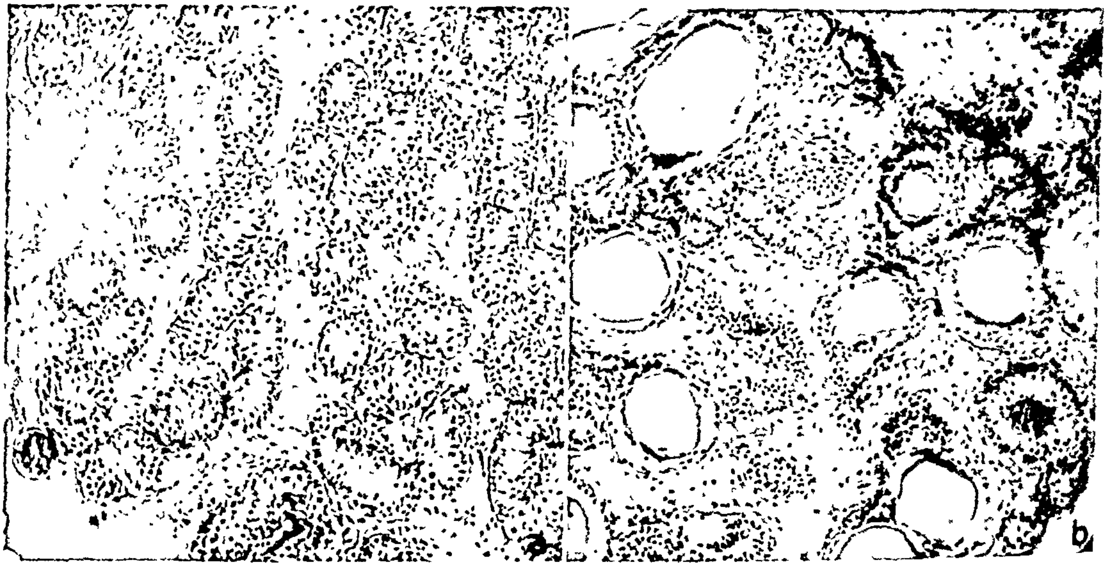

Fig. 1. *A*, Biopsy of "normal" cryptorchid testis in 3-year-old child. *B*, Abnormal testis on opposite side. (× 324)

normal,[7] is additional support for the concept that either there is abnormal testicular formation or that this is a generalized disorder, in contrast to the idea that the testicular changes are necessarily secondary to the higher temperature in the abnormal position.

Cryptorchid testes may be different in the same individual. Figure 1 shows a "normal" cryptorchid testis on one side contrasted with an abnormal testis on the other in a child 3 years of age. An extreme example is the finding of severe developmental defects in one testis, almost to the point of agenesis. In Fig. 2, the hypoplastic testis shows only rudimentary structures with a few Leydig cells.

Orchiopexy, which probably will be attempted for many of these patients with less extreme abnormality, cannot be expected to assure a normally functioning testis. In fact, it is in this group that the greatest number of failures occur. These failures are usually and erroneously assumed to be due to inadequacy in surgical technic.

3. Primary Endocrine Abnormalities

Primary endocrine abnormalities as a cause of cryptorchidism do not need emphasis here. Three out of 15 patients (20 per cent) at the University of California Medical Center with pituitary hypogonadism had cryptorchid

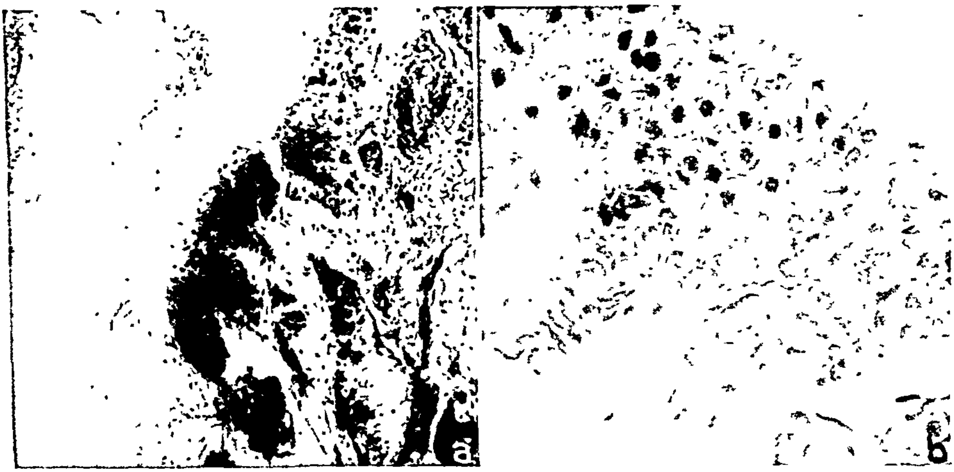

Fig. 2. Low- and high-power views of rudimentary retained testis, composed of Leydig cells in a loose stroma with malformed ducts.

testes. It is conceivable that certain patients with cryptorchidism who respond to hormone administration or in whom the testes come down spontaneously only with the increased pituitary output at puberty, have a subclinical deficiency of pituitary hormonal secretion. Operation on this group is in itself not worthwhile, unless the endocrine abnormalities can be adequately corrected.

EFFECTS OF CRYPTORCHIDISM ON THE TESTIS

The primary effect is delay in maturation. Cooper 25 years ago showed that the retained testis is not necessarily imperfect, but is retarded along the path of normal development—since the earlier the organ is examined, the more normal is its appearance. The secondary changes are increase in fibrous tissue, decrease in the relative number of tubules, the persistence of quiescent nuclei at puberty, and the later disappearance of the more

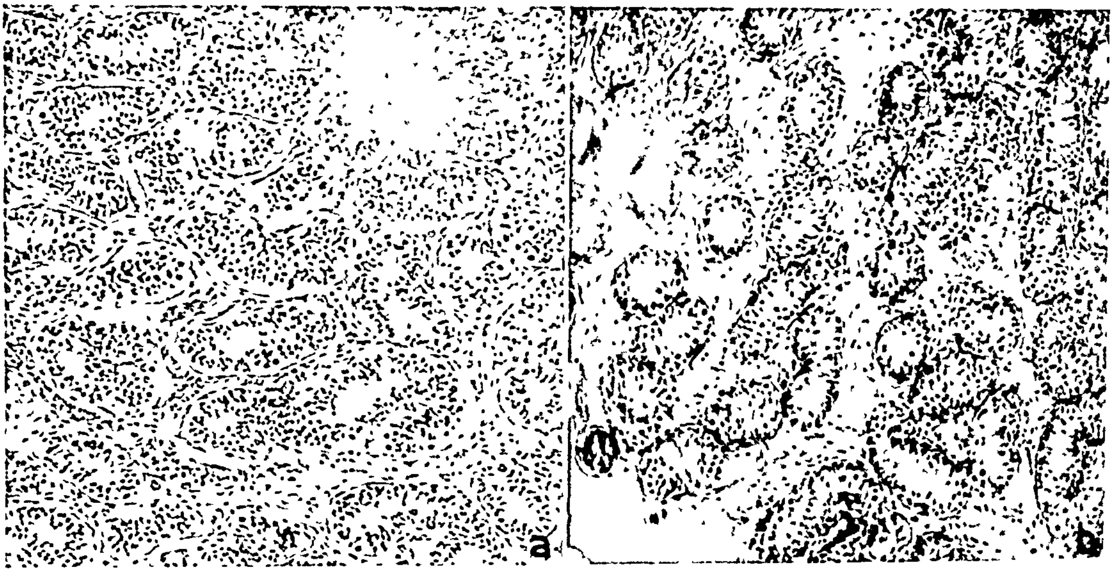

Fig. 3. *A*, Normal testis, 3 years. *B*, Retained testis, 3 years. (× 324)

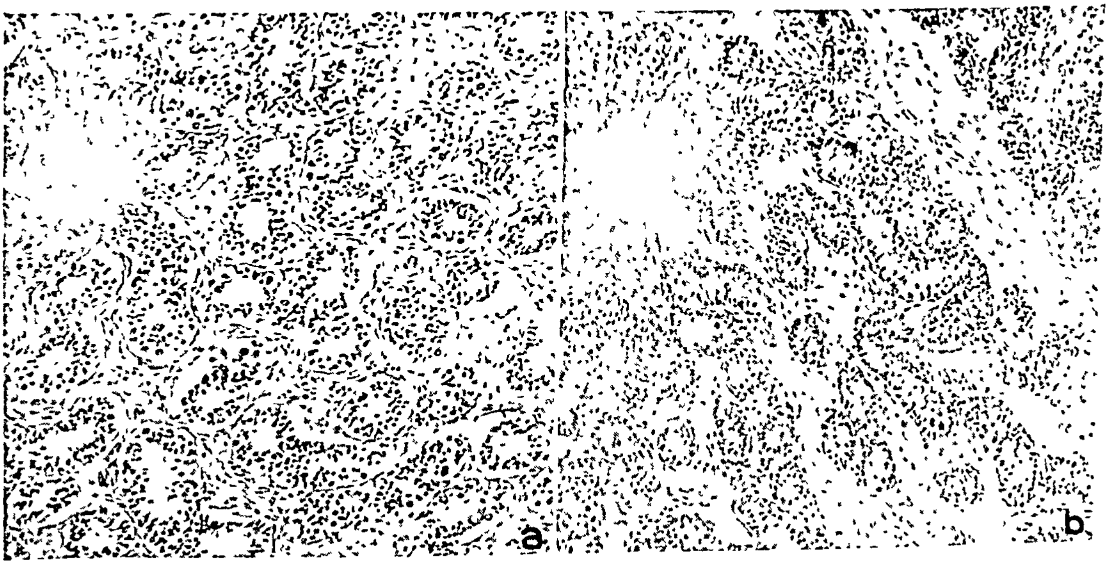

Fig. 4. *A*, Normal testis, 9 years. *B*, Retained testis, 9 years. (× 324)

central rows of tubular cells. We, as well as others,[1, 8] have noted similar delayed sequential changes. Up to the age of 6 years, few changes occur in the testis (Fig. 3). From 6 years to puberty, tubular growth occurs unless the testis is retained. Sections from a boy 9 years old with cryptorchidism (Fig. 4), show small immature tubules in contrast to those from a normal boy of the same age. More striking still is the difference between the normal and cryptorchid testis at 12 years (Fig. 5).

The disorganization and regression at puberty is usually great (Fig. 6). These testes are uniformly incapable of producing normal sperm, and so if both testes have been retained to that time within the body the patient is

Fig. 5. *A*, Normal testis, 12 years. *B*, Retained testis, 12 years. (× 324)

Fig. 6. *A*, Normal testis, 17 years. *B*, Retained testis, 17 years. (× 324)

sterile. Orchiopexy, of course, would not reverse the changes at this age. These changes are caused by increased temperature, which has been repeatedly shown experimentally to produce spermatogenic arrest.[5]

Placement of the testis in the scrotum before puberty perhaps does not significantly improve spermatogenesis (Table 2), although we have no figures on those brought down by the age of 6. It is on the poor results of

TABLE 2. Impairment of Fertility After Orchiopexy

	Impairment by semen analysis		
	Complete	*Severe to moderate*	*Slight to none*
Bilateral			
Untreated	9	0	0
Orchiopexy	14	6	5
Unilateral			
Untreated	1	10	24
Orchiopexy	3	8	25

After Hansen.[4]

orchiopexy that the arguments focus. If the testis is fundamentally normal and if no gross endocrine abnormalities exist, then placement of the testis in the scrotum at an early enough age, before irreversible changes have taken place which prevent proper maturation, should afford fertility if the surgical procedure itself does not damage the blood supply to the testis. These are a lot of "ifs," and they point up the fact that patients with cryptorchidism and especially the figures on the results of orchiopexy, cannot be lumped together for statistical analysis. Rather, each case must be studied for the basic cause of the cryptorchidism to assess properly the value of operation which will be viewed in relation to the age at operation. The argument that because the results of orchiopexy at 6 years of age are not always good, we should operate earlier (at 3 years of age or at birth) is not logical. It does not take into account the fact that the testis and the patient may be fundamentally abnormal.

PRACTICAL CONCLUSIONS

1. Make the decision early, for or against treatment—between the ages of 3 to 6 years, because after 6 years, irreversible damage may occur. For psychologic reasons also, early placement is desirable.

2. Determine if obstruction exists by giving anterior pituitary-like hor-

mone, or more directly, by giving methyl testosterone linguets. This will show what effect puberty will have, without the need for waiting while irreversible changes take place. In unilateral cases, nondescent usually means obstruction, so hormones will seldom be effective. An associated hernia also indicates operation.

3. Operate if hormonal stimulation fails, since puberty also will fail to bring about descent. Hormones have made orchiopexy easier by enlarging the testis and its adjacent structures, and have not been shown to harm the testis.[3]

4. The decision between orchiopexy and orchiectomy will usually be made at the operating table, by consideration of the appearance of the testis and the technical difficulties in bringing it completely within the scrotum. Consideration of four factors will help in the decision:[8]

a. *Cosmetic aspects;* a nylon prosthesis is an excellent substitute.

b. *Hormonal aspects;* testosterone (now as the long-acting cyclopentyl proprionate) adequately compensates for loss of Leydig function.

c. *Fertility aspects;* there is little chance for spermatogenesis unless the testis is reasonably normal in development, has not been too damaged by staying overlong in the abdomen, and can be brought completely within the scrotum.

d. *Neoplastic aspects;* tumor of the testis is twenty times more frequent in patients with cryptorchidism, so we have an additional argument for either bringing the testis to a site where it can be followed by palpation, or removing it if it appears too maldeveloped to produce an effective number of sperm anyway.

In summary, since the retained testis may be irreversibly damaged well before puberty, hormonal stimulation should be tried before the age of 6 years. If it fails, orchiopexy should be done at once. Many failures can be explained by congenitally defective testes or general endocrine abnormality, and are not necessarily the result of the surgical procedure.

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Testicular Biopsy in the Bull

I. Effect on Quality of Semen

H. J. Hill, D.V.M., and F. X. Gassner, D.V.M., M.S.

With the rapid and worldwide adoption of artificial insemination in dairy cattle, more serious consideration is being given to the many factors which may influence the reproductive efficiency of a dairy herd. The beef industry, too, is concerned, as an ever-increasing number of ranch operations are applying this modern technic of artificial breeding, using one or more costly sires to inseminate large numbers of females. This trend has been instrumental in bringing the responsibility of the bull in breeding failure more strikingly into the foreground. Consequently, a greater demand is made on the veterinary profession for a more accurate estimate of the fertilizing capacity of a bull.

While the evaluation of semen has become very important as an aid in the selection of fertile sires, little attention has been paid to the fact that changes in spermatic cytology of the testes cannot always be predicted from semen evaluation alone. Hence, there has been a great need for adoption of a procedure such as testicular biopsy to permit a direct microscopic examination of the epithelial lining of the seminiferous tubules and stroma for the purpose of determining the true state of testicular function. Such information, in combination with semen studies, would permit a more accurate diagnosis of the degree of fertility as well as the effect of therapy.

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Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility, San Francisco, Calif., June 18–20, 1954.

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In the human field testicular biopsy has been used extensively[3, 6, 7, 10, 11, 12, 14] according to the technics described by Charny[6] and by Hotchkiss. None of these authors mentions any damaging effects upon spermatogenesis or any other testis function which could be ascribed to the biopsy technic per se, excepting Bayle, who suggested that small but inevitable hemorrhage caused by biopsy may have led to obliteration of the vasa efferentia in 7 patients.

Review of Previous Studies

Veterinary literature dealing with studies of the effect of biopsy on the bovine testis is scant. Erb *et al.* used an open biopsy method in a study of the influence of vitamin A on semen quality and testis morphology. It is difficult, however, to conclude from the data presented, whether the oligospermia and azoospermia noted after biopsy were the result of surgical insult inflicted by the surgery or due to vitamin A deficiency. This was brought out by Sykes *et al.*, who used a similar biopsy technic during a study of the effect of an energy-restriction diet on semen quality. Their results showed that biopsy produced adverse changes in semen quality and testis morphology which were unrelated to either diet or dietary regimen of the experiment. Moore noted extensive adhesions between the tunica vaginalis and the testes following biopsy, and accompanying progressive deterioration of semen quality. Barker[1, 2] evaluated 3 methods of biopsy in bulls—the "open" method, similar to that used by above-mentioned workers, the Turkel needle puncture, and the Vim-Silverman needle puncture. He concluded that because of the adverse effect on spermatogenesis the exposure ("open") technic should not be used. Of the two instruments used for punctures he favored the Vim-Silverman needle. More recently, Byers reported no ill effects, in general, from testis biopsies on 10 normal dairy bulls aged 8 to 36 months. His technic varied only slightly from that of Erb and Sykes.

During the past 5 years, in studies concerned with testis–accessory sex organ relationships in the bull, the authors have had frequent occasion to examine testis morphology of infertile bulls by various methods of biopsy and to determine the effects of such surgical interference on testicular function and morphology and on semen quality.

MATERIALS AND METHODS

This report comprises the results of studies with 10 bulls of various breeds (5 Holsteins, 1 Jersey, 1 Shorthorn, 1 Brown Swiss, and 2 Herefords), ranging in age from 2 to 7 years. Their history prior to biopsy is summarized in Table 1.

TABLE 1. Histories of Bulls Used in Study

Bull	*Breed*	*Age (yr.)*	*Semen quality*	*History*
C	Holstein	3	Normal	Treated with estrogen; unilateral castration followed by biopsy of remaining testis
D	Holstein	3	Normal	Same as C
18	Holstein	2	Normal	Monorchid by incomplete castration; normal semen; no treatment before biopsy
P	Holstein	2	Normal	Normal bull; slaughtered for venereal disease; no treatment prior to needle biopsy
J-9	Jersey	7	Normal	Normal bull from artificial insemination unit; vasectomy on left side 1 week prior to biopsy on right testis
CSH	Shorthorn	5	Subnormal	Subnormal semen, low breeding efficiency; no treatment prior to right and left biopsies 8 months apart
25	Brown Swiss	2	Subnormal	Same as CSH. Bilateral biopsy
BH	Hereford	3	Subnormal	Breeding failure; oligospermia; treated with FSH, APL, and testosterone propionate 95 days prior to unilateral. biopsy
TH	Hereford	5	Azoospermia	Breeding failure; oligospermia; treated with FSH, APL, and testosterone cyclopentyl-propionate over a period of 152 days and 216 days prior to unilateral biopsy.
24	Holstein	2	Azoospermia	Breeding failure with essentially complete azoospermia; treated with FSH over period of 37 days and 71 days prior to unilateral biopsy.

All bulls were fed and managed at the same level as the dairy bulls used routinely in the artificial insemination unit.

Semen samples were collected weekly, using the standard artificial vagina. Evaluation was made immediately with regard to volume, color, concentration, percentage of motile spermatozoa and rate of motility, percentage of live spermatozoa, and morphology. Initial seminal fructose, number of milligrams of fructose per 100 cc. metabolized in 1–3 hours, pH, and sperm count by the hemocytometer method were also determined.

Technic of Biopsy

The puncture procedure of testis biopsy was attempted in 1 bull. This method was abandoned because of difficulty in securing specimens large enough to be representative of testicular structure. The remaining 9 bulls were subjected to the open surgical approach. They were restrained either by general anesthesia and rope ties, sedation and rope ties, or epidural anesthesia and confinement in stocks. The spermatic cord was infiltrated with 2% procaine hydrochloride, as was the cremaster muscle, to relax this organ which is unusually well developed in the bull. The scrotal skin was made insensitive by local anesthesia. Asepsis was maintained as far as was practical and in no case did any of the bulls show an infective inflammatory orchitis or general septicemia.

The skin over the middle third of the posterior lateral surface of the testis was incised and the incision carried on through the subcutaneous fascia and the parietal layer of the tunica vaginalis until the glistening white surface of the visceral layer of the tunica vaginalis was exposed. The unusually heavy stroma of the bovine testes necessitated an incision into the testicular tissue itself, therefore, a sterile scalpel was inserted through the tunica vaginalis visceralis and tunica albuginea, resulting in an incision of ⅜–¾ inch in length and about ½ inch deep. The biopsy specimen was removed either with a small bone rongeur or a small curved scissors while pressure was applied to the testis. In some cases the tunica albuginea was closed with chromic catgut and an atraumatic needle, while the tunica vaginalis was never sutured. The scrotal skin was closed with Dermalon suture. Hemorrhage was reduced at times by manual compression of the cord until the specimen was removed. Because of extensive hemorrhage encountered in most bulls, Koagmin was administered intravenously and intramuscularly. Each animal received tetanus toxoid and tetanus antitoxin.

RESULTS

Effect on Semen Quality

The pertinent data on sperm count, percentage alive and seminal fructose concentration are summarized in Table 2.

Bulls C and D. Bulls C and D were unilaterally castrated and the re-

TABLE 2. Effect of Biopsy on Semen Quality

				Before biopsy				After biopsy			
Bull	*Breed*	*Age* (yr.)	*No. testes biopsied*	*No. samples*	*Average sperm count* (per cu. mm. × 1000)	*Average % alive*	*Average seminal fructose* (mg./100 cc.)	*Days*	*Sperm count* (per cu. mm. × 1000)	*% alive*	*Seminal fructose* (mg./100 cc.)
C	Holstein	3	1	10	411	65	654	13	70	0	792
								21	0	0	810
D	Holstein	3	1	10	544	50	733	13	40	10	882
								20	22	0	759
18	Holstein	2	1	7	352	60	707	11	110	5	534
								17	30	0	628
								38	30	0	751
J-9	Jersey	7	1	2	1700	85	438	19	715	25	640
								26	180	10	653
								33	23	0	720
								54	14	0	815
25	Brown Swiss	2	2	2	1565	45	632	6	2167	40	630
								15	33	0	393
								67	0	0	748
									RIGHT TESTIS		
CSH	Shorthorn	5	2	10	426	40	598	6	650	50	491
								13	382	10	594
								19	98	5	682
								52	52	5	598
								67	43	0	478
								104	93	40	571
								129	239	45	508
								227	289	45	385
								247	405	60	430
				RECOVERY AFTER BIOPSY OF RIGHT TESTIS					LEFT TESTIS		
				1	405	60	430	1	240	30	518
								21	80	15	403
								59	0	0	455

maining testis biopsied 84 days later. Ten semen examinations made at weekly intervals prior to biopsy showed values considered normal for monorchid animals. Following biopsy there was a drastic reduction in sperm count by the fifteenth day and it was zero a week later. Seminal fructose was not affected (Fig. 1).

Bull 18. Repeated semen evaluation prior to biopsy indicated that Bull

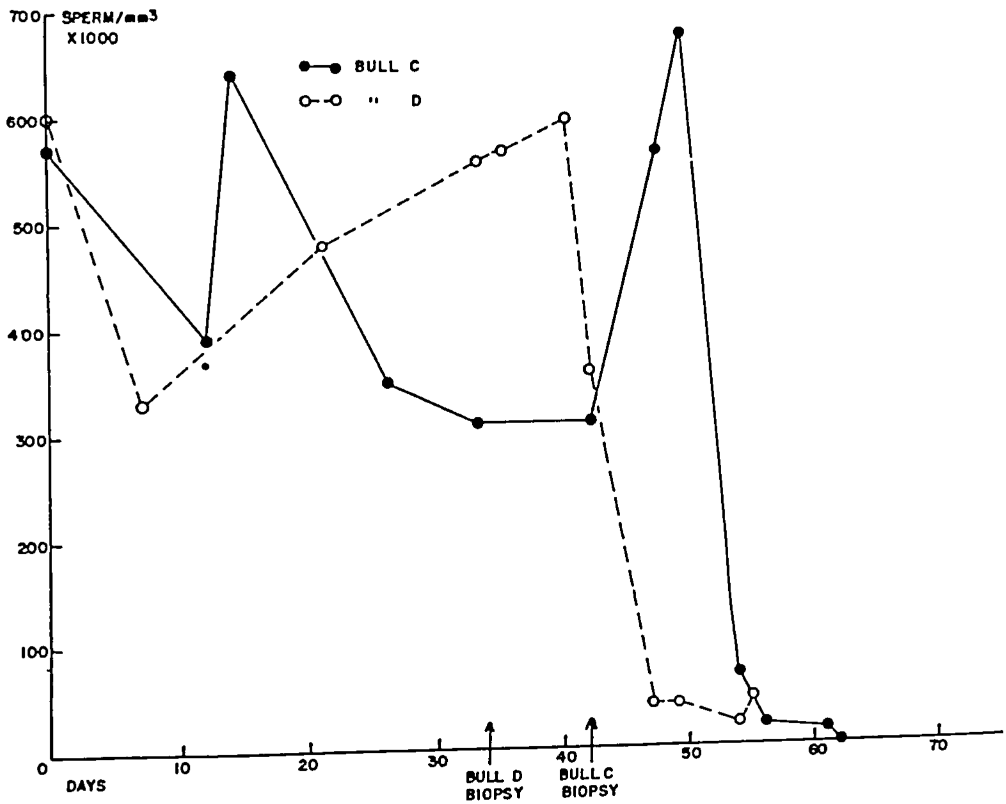


Fig. 1. Semen examinations of Bulls C and D.

18 was normal. However, semen quality deteriorated after biopsy as rapidly as in Bulls C and D with no effect on seminal fructose (Fig. 2).

Bull J-9. A vasectomy was done on the left side of Bull J-9, which was normal, in order to secure sperm from the right testis only. This was biopsied about a week later. It can be seen in Table 2 that by the nineteenth day after biopsy the cell count showed less than half the original value while the percentage of live cells was reduced from 85 to 25. Between 26 and 33 days after surgery these values were essentially zero (Fig. 3).

Bull 25. The effects of biopsy on Bull 25 were similar to those observed

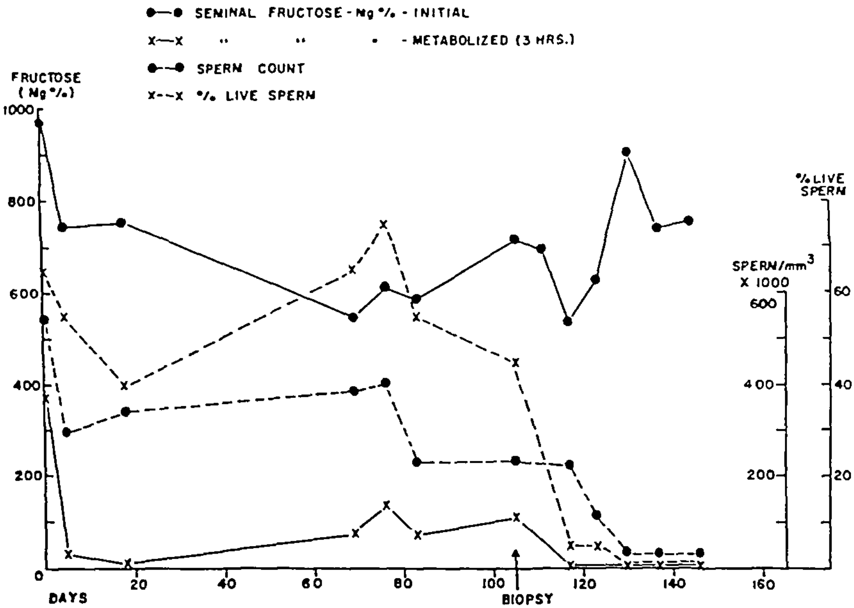


Fig. 2. Semen examinations of Bull 18.

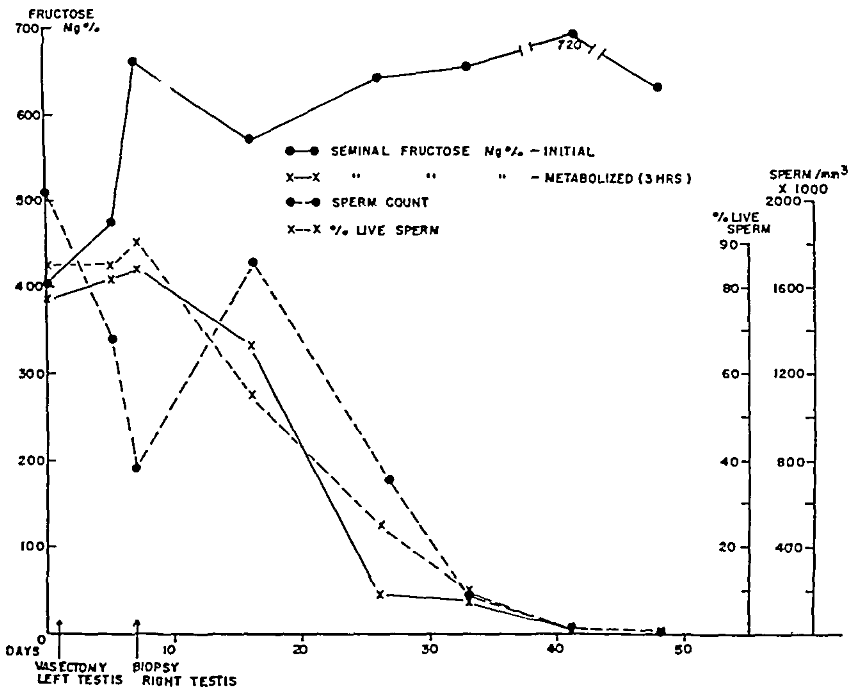


Fig. 3. Semen examinations of Bull J-9.

with Bull J-9. The more rapid deterioration of semen quality is due to the fact that bilateral biopsy was performed (Fig. 4).

Bull CSII. After obtaining a specimen from the right testis, sperm count and percentage of live cells were diminished by more than 80 per cent within about 3 weeks. A distinct recovery of spermatogenesis was noted beginning 4 months after biopsy and continuing to the eighth month

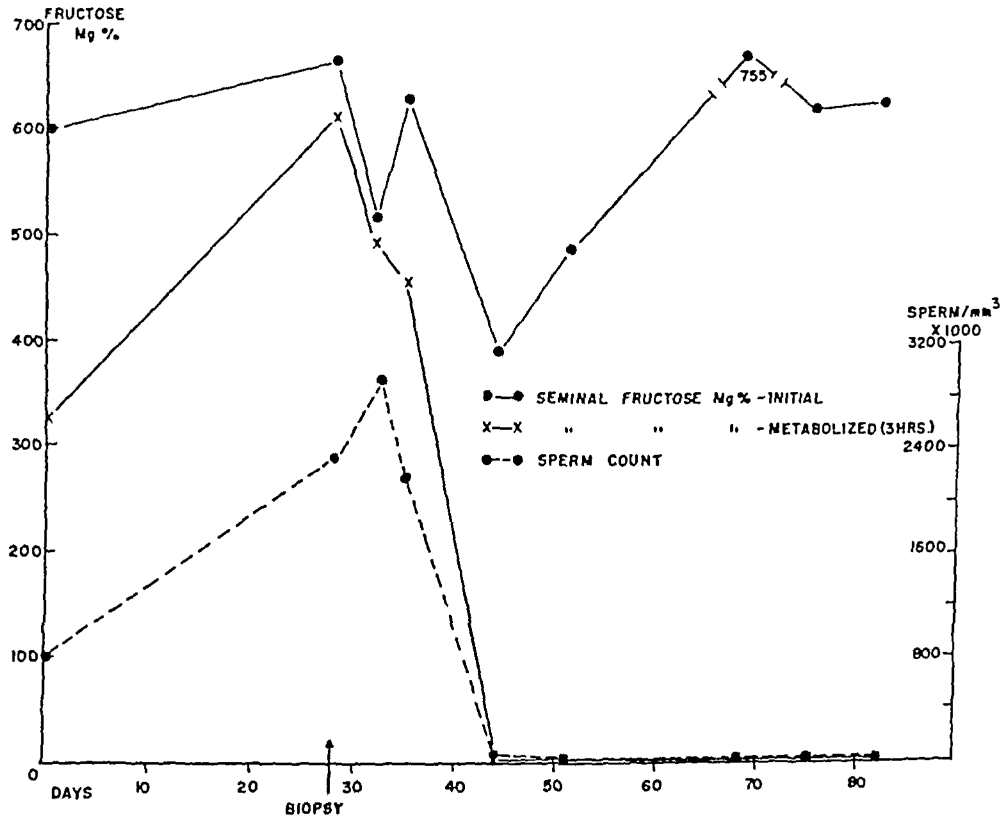


Fig. 4. Semen examinations of Bull 25.

at which time the left testis was biopsied. This resulted in complete azoospermia within a month (Fig. 5). The fact that the cell count was almost zero 21 days after surgery may indicate that the left testis had improved functionally during the 238 days between biopsies and that it was responsible for the bulk of sperm cells produced. Also, since the cell count was not reduced as much after the first biopsy as after the second sampling, one can assume that the right testis had not recovered its function at that time. This was verified at subsequent autopsy.[9]

Bulls BH, TH, and 24. Bulls BH, TH, and 24 were azoospermic at the time testis biopsy was performed. Hence, the only semen value obtainable was the seminal fructose content, which was found to be normal.

Fructose

It was consistently observed that seminal fructose concentration remained within the limits characteristic for the particular individual. Also, since no significant variations were noted in sex drive, it appears that surgical inter-

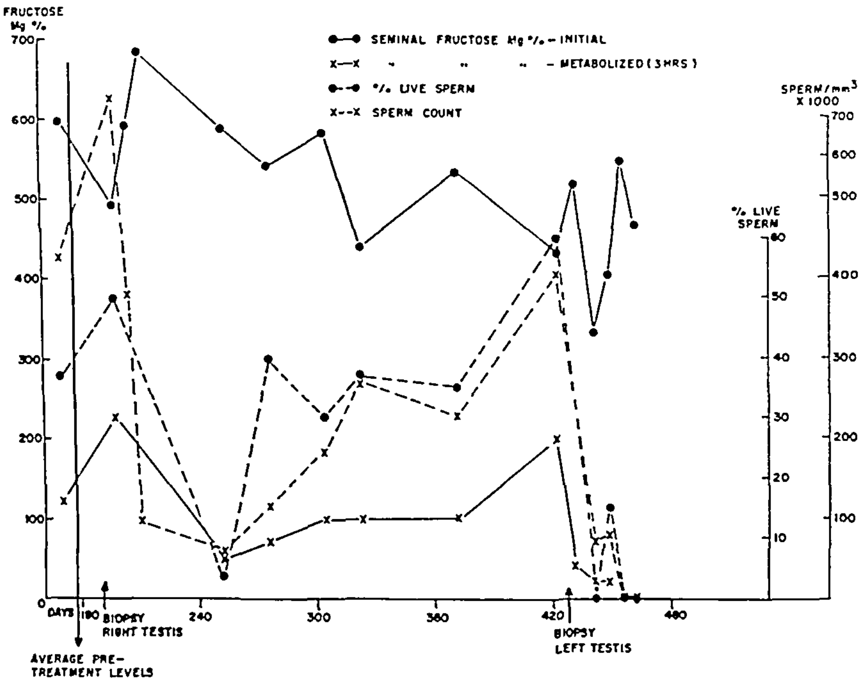


Fig. 5. Semen examinations of Bull CSH (both testes biopsied).

ference with testis structure during biopsy did not adversely affect the Leydig cell system. This is also borne out by the histologic appearance of the interstitial cells. A detailed report on effects of biopsy on histopathology of testes will be presented in another paper.[9]

Testicular Degeneration

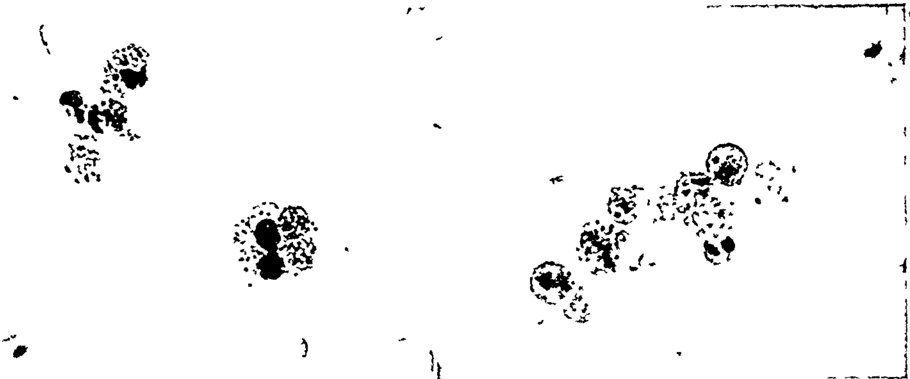
Examination of semen from numerous subfertile bulls showing breeding failure revealed the occurrence of spherical cells of various sizes which were heavily granulated and often multinucleated, resembling giant cells (Fig. 6). In semen of normal bulls such cells are found infrequently and in small numbers. In semen of bulls with low breeding ability, however, they are very numerous and often associated with a high percentage of abnormal sperm cells. They may be just as numerous in semen of subfertile bulls which, aside from low sperm concentration, appears quite normal. A systematic search for these cells revealed their presence in ejaculates of every bull which had been subjected to experimental alterations of testicular function; for example, steroid therapy, thermal insulation of the scrotum, and, particularly, after every testicular biopsy.

These cell forms usually appear in noticeable numbers about one week after surgery, become progressively more numerous as the semen picture becomes poorer, and may remain for several months, or until spermatogenesis returns to normal. Histologic examination of testes specimens shows these cells to be located in the lumina of the seminiferous tubules which are in various stages of degeneration (Fig. 7). The concentration of these cells is not necessarily proportional to an increase in any morphologically abnormal sperm cells, but appears to be related inversely to sperm cell concentration. In our described cases, cessation of spermatogenesis occurred rather abruptly after biopsy; therefore, there was not enough time for morphologic changes to become manifest.

It is evident that the appearance of these cells in large numbers in semen samples is pathognomonic of testes degeneration, involving primarily atrophic changes in the seminiferous tubules. This agrees with Lagerlöf and Blom who observed similar cell forms and postulated on their origin.

DISCUSSION

It is interesting to speculate as to the reasons for this extraordinary lability of the bovine testis to surgical insult, in view of the fact that in the human and in other species repeated biopsies can be taken without serious harm to the organ or its function. First, it should be pointed out that the blood supply to the bovine testis is unusually large and hemorrhage is therefore difficult to control, requiring in some cases intravenous administration of coagulants even after closure of the scrotal incision (Fig. 8). Even then blood often continues to ooze, filling the space between the testis proper and the tunica vaginalis and forming a clot enveloping the lower half or more of the testis. To cite one of many instances, when Bull



A 6B

7

8

9A 9B

Fig. 6. A and B, spherical, multinucleated, granular cells in semen from a subfertile bull. Fig. 7. Same cells in lumen of degenerating seminiferous tubule. Note formation of such cells from germinal epithelium (left lower center of lumen and lower right) Fig. 8. Stream of blood from sutured scrotal sac after testicular biopsy. Fig. 9. A, scrotal sac 19 days after testis biopsy. B, scrotal sac opened to show extent of peritesticular blood clot; this testis was of a yellowish color and soft in texture.

BH was slaughtered 3 weeks after biopsy the scrotum was found to be filled with an organized but unabsorbed blood clot forming a layer about 1 inch thick which enclosed the testicle (Fig. 9*B*). Since neither the visceral nor parietal tunic is very vascular it is reasonable to expect that resorption would be of a low order. This layer undoubtedly had the effect of heat insulation, thus interfering with the thermal control action of the scrotum. In fact, the rapid deterioration of semen quality observed in this study was almost identical to that seen in bulls subjected to thermal insulation of the scrotum. However, in one instance (Bull J-9), hemorrhage as would be indicated by postoperative swelling of the scrotal sac on the biopsied side was at a minimum, yet azoospermia occurred within a month. This suggests that the insulation effect is not the sole cause of degeneration within the seminiferous tubules.

SUMMARY AND CONCLUSIONS

The effect of the open-biopsy procedure on semen quality of 10 bulls, ranging in age from 2 to 7 years, has been reported.

A marked decrease in sperm cell count and even complete azoospermia occurred within 2 to 3 weeks which persisted for as long as 4 months before recovery was noted.

Numerous granular, multinucleated "giant cells" of various sizes were seen in the ejaculates taken after biopsy. The same type of cell has been observed in semen of subfertile bulls and in bulls experimentally subjected to thermal insulation of the scrotum.

There was no change in fructose content of the semen after biopsy.

Improvement in semen quality after biopsy has taken from 4 to 8 months. This fact would preclude the use of such a technic in the bovine sterility clinic for it would be impractical and uneconomical to wait so long after each biopsy for recovery before therapy could be instituted. However, the technic could be of value in determining the status quo of an infertile bull, his fate being thus more accurately evaluated.

The adverse effects of such a technic on testicular histopathology are discussed in detail elsewhere.[9]

Further attempts to develop a satisfactory method of testicular biopsy are justified. Most promising of the methods seems to be that of the Vim-Silverman needle if the diameter of such a needle can be enlarged to provide a specimen large enough for diagnosis.

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Surgical Procedures Affecting Male Fertility

Indications and Contraindications

Isadore Gersh, M.D.

In reporting on the surgical treatment of male infertility Torres in 1940 concisely stated: "The biologic capacity of the male depends on three factors: 1) normal spermatogenesis; 2) patency of the excretory ducts of the genital tract, and 3) the physical ability for sexual union." Review of available literature indicates that surgical procedures have been devised which have a bearing on all three factors. Surgery to bring the undescended testicle into the scrotal position and operations in mumps orchitis have as their ultimate aim the preservation of spermatogenesis. Numerous plastic procedures have been devised to overcome ductal obstructions. For certain cases of sexual impotence due to organic causes an operation has been reported which should be worthwhile in cases of male infertility where this is the etiology.

CONDITIONS AFFECTING STERILITY

Before considering indications for definitive surgical treatment it would be well to note some conditions in which male fertility may be adversely affected by surgery and to mention diseases in which management is important because of the possible effects on fertility. Serious injury of testicular vasculature and even sectioning of the vas has occasionally inadvertently occurred during hernia repair, operations for varicocele and hydrocele, and other scrotal procedures. Orchiopexy should result in a testicle at the lowermost portion of the scrotum, but if the blood supply becomes damaged, the

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already handicapped seminiferous tubules will bear the brunt of the injury. Blind trocar or needle aspiration of the testicle for diagnostic purposes also has real dangers. O'Conor[20] found instances of "marked reaction in the scrotal contents" after the procedure and other patients had appreciable leakage of testicular tissue through the puncture area. Vasostomy, a popular procedure in former years, accounted for 8 of 123 cases of azoospermia reported by O'Conor.

Torsion of the spermatic cord, seen most frequently in adolescents, results in strangulation of the blood supply with hemorrhagic infarction of the testicle. Prompt diagnosis and appropriate treatment are necessary if testicular atrophy is to be avoided. Since the advent of the resectoscope obstructions of the bladder neck in young men have been recognized more frequently and treated by transurethral resection. Although urinary function is improved, the procedure is occasionally followed by retrograde ejaculation of semen. In the older patient this disturbance is annoying, but for the young man who wants a family it creates a serious problem. Treatment of bladder-neck obstructions in young men should involve consideration of this possible complication.

Patients requiring prostatectomy are usually in the older age group and the question of preservation of fertility is rarely a problem here. Transurethral fulguration of the verumontanum area may result in fibrosis with ejaculatory duct obstructions. Growths, such as papillomas on the verumontanum, may interfere with ejaculation and are usually managed endoscopically. Fibrotic changes in the prostate, seminal vesicles, and ejaculatory ducts may be expected to affect the seminal picture qualitatively and quantitatively. Treatment for these conditions requires massages, antibiotics, or endoscopic procedures.

In former years acute epididymitis was generally a result of gonorrhea. Bilateral gonorrheal epididymitis accounted for 19.6 per cent of 102 cases of vasoepididymal obstruction reported by Michelson.[17] Today, in my practice at least, most cases of acute epididymitis are nongonorrheal in origin. Treated promptly and adequately they should not develop tubular obstruction; neglected or considered as minor conditions, the unilateral case may result in a diminished fertility potential while in bilateral cases azoospermia may be the end result. The significance of epididymitis in young and middle-aged men and the importance of the recognition and treatment of the underlying causes must be stressed. Epididymotomy for acute epididymitis was not uncommon in the era prior to antibiotics, and, like vasostomy, is a procedure of the past.

Congenital and acquired deformities of the urethra are factors which often go undetected. The stenotic urethral meatus, recognized by simple inspection, offers obstruction to flow of semen as well as urine, and is simply corrected. Hypospadias and epispadias become of significance when the meatus is in such a posterior position that vaginal deposition of semen cannot be accomplished; plastic surgical procedures in early childhood should correct the deformity. In some patients endoscopic examination of the bladder neck, the prostatic urethra and its contained duct orifices, and the anterior urethra are indicated. One of my patients dramatically illustrated the value of endoscopy. He had azoospermia, but on repeated occasions his urine collected after ejaculation contained many actively motile sperm. Endoscopy revealed a small papillary growth on the colliculus which apparently blocked the ejaculatory ducts during the turgescent state, causing these ducts to empty later in a retrograde manner.

Brodny has shown the value of urethrography in demonstrating interference with flow of ejaculate through the urethra and its accessories. He found that changes in the accessory sexual glands affect the volume of their secretions and that urethral deformities interfere with volume and force of ejaculation.

SURGICAL TREATMENT OF INFERTILITY

Mumps Orchitis

Incidence. Mumps in childhood is a common but usually not serious disease. It becomes significant in male fertility problems because of the not infrequent complication of orchitis in puberty and adulthood.

Michelson[16] in a series of 1083 barren marriages found 13.5 per cent of the men azoospermic. Mumps was the most common inflammatory disease producing degeneration of the spermatogenic tubules, making up 6 per cent of his cases of azoospermia. Werner in 1950 found the reported incidence of orchitis in various epidemics to be from 11.6 to 66 per cent. In questioning 2000 separatees he obtained a history of mumps in 54.3 per cent. Orchitis complicated the disease in 4.9 per cent of all cases and in 19 per cent of those who developed mumps after the age of 13. He found testicular atrophy in 35.8 per cent of all cases with a history of mumps orchitis, in only 1.7 per cent of all the mumps cases, and in 6.8 per cent of those occurring after 13 years of age. In one third the orchitis was bilateral. Of 44 men with testicular atrophy from all causes, mumps orchitis was considered responsible in 43 per cent.

He concluded that mumps orchitis resulted in impaired fertility in 13 per cent of the cases, and that "mumps orchitis cannot be regarded as an important cause of sterility in the male population." However, since testicular atrophy occurs in such a significant percentage (35.8) of cases of mumps orchitis, prevention and treatment of this complication becomes important to those who are interested in fertility problems.

Surgical Treatment. Multiple punctures of the tunica albuginea was advocated by Smith, and later Ballenger advised incisions. In 1946 Nixon and Lewis reported a series of 68 soldiers with mumps orchitis of such severity to warrant surgical intervention. They incised the tunica albuginea in two patients but gave it up as too radical. In the remaining 66 cases they performed open drainage of the hydrocele fluid. Their patients were promptly relieved and temperatures became normal in the average case in 2.1 days.

They felt that early surgical treatment is of value to relieve symptoms, to shorten the period of disability, and to prevent testicular atrophy. More controlled studies with adequate follow-up would be helpful in establishing indications for surgery and results to be expected as regards biologically functioning testes.

The Undescended Testicle

Moore demonstrated that testes of animals require the lower temperatures of the scrotum for spermatogenesis. Clinicians are well aware of this physiologic requirement but basic questions remain unanswered in regard to the actual treatment of the undescended testicle. When should medical measures be used and when should definitive surgery be performed so that the spermatogenic function of the testis can reach its optimum?

In 1952 Charny stated, "The problem of cryptorchidism needs further illumination and precise histologic data. Histologic studies of testes brought down into the scrotum leaves us with little enthusiasm for any surgical technics now employed." In most of their cases biopsies made several months after orchidopexy showed deterioration of seminiferous tubules as compared to the condition at the time of surgery.

Rea reported on 8 married men who had been operated at least 10 years before for bilateral cryptorchidism. The patients were all sexually potent but none of their wives had become pregnant. Their testes were all scrotal but smaller than normal. Biopsies obtained in 4 cases showed atrophic germinal epithelium and no spermatozoa. They found that sperm counts in treated or untreated cryptorchids are lower than normal, while secondary sex characteristics develop normally.

MacCollum, in an earlier report of a follow-up study of 336 cases, concluded that cryptorchidism reduces fertility but that in the operated group fertility is higher. Hansen's studies of 124 cryptorchids indicate that poor fertility results from operations in bilateral cases, and he believes operation is not advisable because of the danger of bilateral atrophy. He found no appreciable difference in the fertility of operated and nonoperated unilateral cryptorchids. So far as fertility is concerned he does not recommend orchidopexy in unilateral cases. Johnson found that spontaneous descent of undescended testicles occurred in 313 of 544 boys, and in about half the cases descent took place during puberty (age 11–13 years).

It would seem that further studies are needed to crystallize the indications for treatment of cryptorchidism as regards spermatogenesis.

Ductal Obstructions

Surgery for ductal obstructions involves mainly the vasoepididymal system, commonly as a result of infections, trauma, and congenital abnormalities. Michelson[17] found 5 per cent of 2100 patients with subnormal semen to have this condition.

Abnormalities of development such as absence of the vasa or failure of fusion of epididymis and testis are not yet amenable to successful repair. The localized obstructions caused by infection and trauma are often successfully repaired. Combinations of pathology, such as contralateral lesions, require variations in technics. Defective spermatogenesis and ductal obstruction may coexist on one side, be bilateral, or contralateral.

The consensus is that ductal obstructions do not result in degeneration of spermatogenic tubules. The establishment of tubal continuity in obstructive azoospermia should therefore yield good results. A primary requisite for successful surgery of vasoepididymal obstruction is functioning seminiferous tubules. Testicular biopsy yields this information.

The accessibility of the scrotal vas has made resection a procedure by which voluntary sterilization is easily accomplished. Too often the operation has been undertaken without adequate consideration, and the patient later has a change of mind and wants it "undone." Successful reanastomosis of the vasa has been reported by a number of workers. O'Conor[20] had 19 successes (50 per cent) in 38 cases; actually the successes were 63 per cent of the cases in which reanastomosis was anatomically possible. Massey reported 4 cases, all successful; Dorsey had 5 successes in 6 cases, while Mauritzen found that vasal continuity could be restored in 35 to 40 per cent of patients in whom vasectomy, trauma, or infection had caused occlusion.

Obstructions of the pelvic portion of the vas are not surgically feasible for repair. Stenosis of the ejaculatory ducts may in some cases be amenable to endoscopic dilatations.

Epididymovasostomy. Epididymovasostomy was described by Martin in 1902. Hagner in 1936 reported on 65 patients with 12 reoperated because of initial failure. Of 35 cases considered surgically feasible there were 21 successes and 16 effected pregnancies. Hagner gave two requisites for a successful operation: "The vas must be patulous above the point of the anastomosis; second, the globus major, or upper portion of the body of the epididymis must contain live sperm."

Reports of the operation and several modifications have been recorded by a number of workers. Michelson[15] recommended use of stainless steel sutures and a temporary splint of similar material. He reported approximately 30 per cent successful operations, based on finding spermatozoa in the semen, and an incidence of 16 per cent ultimate pregnancies. O'Conor[21] recently reported that in his experience about 20 per cent of vasoepididymal anastomoses resulted in exit of spermatozoa but that "actual fertility was restored in not more than 10 per cent." Bayle explored 95 patients. In 70 anastomosis was possible and epididymovasostomy was performed in 67. The results were considered successful in 33 (65 per cent) and 21 produced pregnancies.

A new procedure was recently reported by O'Conor[20] for use where the distal portions of the vasa are occluded. It consists of making an inverted scrotal skin pouch where the free cut ends of the vasa are brought, and the reservoir is used to obtain material for artificial insemination.

SEXUAL IMPOTENCE

Male sexual impotence is occasionally the cause of a barren marriage. Lowsley in 1933 devised an operation for certain cases of impotence, where the condition results from trauma to the perineum, extensive scar formation as a result of inflammatory lesions of the perineum, lack of muscle tone, and advancing age. The operation consists of shortening the ischiocavernosus muscles and plicating the bulbocavernosus muscle with ribbon gut. In addition the two leaves of the suspensory ligament are compressed by a suture to slow the flow of blood out of the penis. In 1953 he reported that about 75 per cent of patients who were followed have been cured or benefited by the procedure.

SUMMARY

Certain surgical procedures which may adversely affect male fertility have been reviewed. Possible hazards of hernia repair and scrotal operations are discussed. Since the advent of antibiotics most cases of epididymitis are nonspecific in origin; they should be adequately treated to prevent vasoepididymal obstruction.

Surgical procedures aimed at preserving and improving spermatogenesis have been used mainly for treatment of the undescended testicle and mumps orchitis. Further studies are necessary to answer questions as to the indications for and results of treatment of these conditions. Does surgical treatment of mumps orchitis preserve spermatogenesis? Will orchidopexy expertly performed at the right time preserve or improve spermatogenesis?

Localized vasal obstructions can be corrected in 40 to 60 per cent of suitable cases while vasoepididymal obstructions have a definitely lower incidence of success. In certain cases of impotence in which this factor is the cause of a barren marriage, the plastic procedure of Lowsley is suggested.

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Serum Gonadotrophins in Normal and Abnormal Pregnancy

I. Quantitative and Qualitative Analysis

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The exact physiologic and pharmacologic properties of chorionic gonadotrophin have not been very clearly established, though a good deal is known of its endocrine pattern. The knowledge of this pattern during normal and abnormal pregnancy is considered to be both fundamental and important, but the reason for this particular pattern needs to be explained.

In the opinion of some the peak in the output of chorionic gonadotrophin that is reached at 60 days is necessary in order to maintain the corpus luteum of pregnancy. Support for this seems to come from histologic studies showing a direct relationship between the number of Langhans cells and the hormone level.[5, 10, 11] It has therefore been deduced that the function of chorionic gonadotrophin (henceforth referred to as CGH) in the first trimester is a luteotrophic one on the newly formed corpus luteum of pregnancy. However, its function, if any, during the last trimester is still conjectural.

The possible role of the kidneys in the establishment of this pattern was first considered by Gastineau *et al.* but they found that the volume of blood cleared of CGH per minute via the urine to be a constant despite the high serum levels during the first trimester. The pattern in a normal pregnancy is

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therefore not due to renal function changes. This, however, does not exclude the possibility that "abnormal" pregnancies, such as preeclamptic toxemia, could not be responsible for increased serum levels where the kidney function is damaged.

Since the renal elimination of hormones does not account for the characteristic pattern, other possibilities such as changes in production, destruction, or extrarenal disposal must be considered. Much has still to be learned about the production and disposal of this hormone and its influence upon the maternal system. This, in essence, is the wide scope of the problem under investigation.

A prerequisite to this study is a reliable quantitative test for CGH—preferably both rapid and easy. In the process of developing this procedure, we found certain interesting data, presented here as a preliminary report encompassing 185 quantitative estimations as well as 137 qualitative analyses in 92 pregnant women.

METHOD AND TECHNIC

From 1 to 8 serial samples were taken during a single pregnancy at intervals varying from 3 days to 4 weeks. Serum from a 10 ml. clotted blood specimen was used in all cases. Sufficient serum can usually be obtained in one half hour; where this does not occur centrifuging may be necessary. Specimens have been kept under refrigeration for as long as 6 weeks without losing any potency. When this material must be kept for longer periods, it can be precipitated with 4 volumes of 95 per cent alcohol, dried with acetone, and stored in the refrigerator. Then it can be redissolved in water, recentrifuged, and the supernatant fluid used.

For quantitative estimations 1 ml. serum is diluted with 9 ml. distilled water. Intraperitoneal injections are made of the entire 1 ml., using a 1 ml. Luer syringe and #23 needle and taking care not to penetrate the bladder or liver of the animal. The animal is returned to the cage and supplied with food and water for the duration of the test.

Chloroform asphyxiation of the rats was used throughout. We found this to be entirely satisfactory as chloroform is not a fire hazard nor does it cause a blanching of the viscera. A killing jar containing paper soaked in chloroform was used, this being both practical and safe. Upon removal from the jar the animal was left on its back till it was established that all respiration had ceased. A "V" incision through skin, muscle, and peritoneum is started over the lower pubic area and extended on either side up to the diaphragm, care being exercised to avoid the bladder, liver, or spleen. The skin flap is everted and the loops of bowel are pulled aside to expose the ovaries and uterus. The ovaries are then examined under incandescent light as daylight or fluorescent light has proved less satisfactory for observing ovarian hyperemia.

The examination was almost always done 4 hours after injection. Both Riley and Albert have found this to be satisfactory. On occasion we have left it for longer periods of time, sometimes for as long as 18 hours. We have found that when levels were less than 10 units/ml. longer periods of time are advisable. An excellent description of the hyperemic response will be found in the original article by Riley.

The International Standard was used as a control. It was found that two International Units (I.U.) were required to elicit a positive response in the rats in 4 hours, the response being uniform and consistent at this level. The test animals were immature female rats of the Yale strain with a weight range between 40 and 90 gm. and an optimum range of 50–70 gm. On the heavier rats the test for vaginal patency was made to determine their maturity. Animals weighing less than 40 gm. may not respond to the hormone because of their immaturity.

During an experiment 1 rat only was used at each dilution to establish an approximate end point. The highest positive and lowest negative were then repeated. This was found to be adequate because of the stated uniformity of the responses in the rats—a prime reason for using this test. Our end points are approximate with a span of 1 I.U. between 0 and 4, 2 I.U. between 4 and 20, 10 I.U. between 20 and 100, and 20 I.U. above 100. We believe that closer spans between dilutions are insignificant and do not warrant the additional rats. Our established values are probably lower than most reports because we have in all cases attempted to establish and report the lowest value.

To avoid error a few practical hints are useful. The animals must be well fed and watered, as lack of either of these may cause a degree of ovarian hyperemia. Room temperature should be kept constant as undue fluctuations may also cause some hyperemia.

For very low titers of CGH, where the dilution factor is small, or large quantities are injected, it is probably well to extend the duration of the test as concentrated material is absorbed slowly. If doubt exists about the response, the ovary should be squeezed gently. If the test is negative the color will disappear; if positive, it will remain or reappear. If further doubt exists the test should be repeated and the time extended. Some rats, especially immature ones, are liable to be refractory to the CGH in which case the

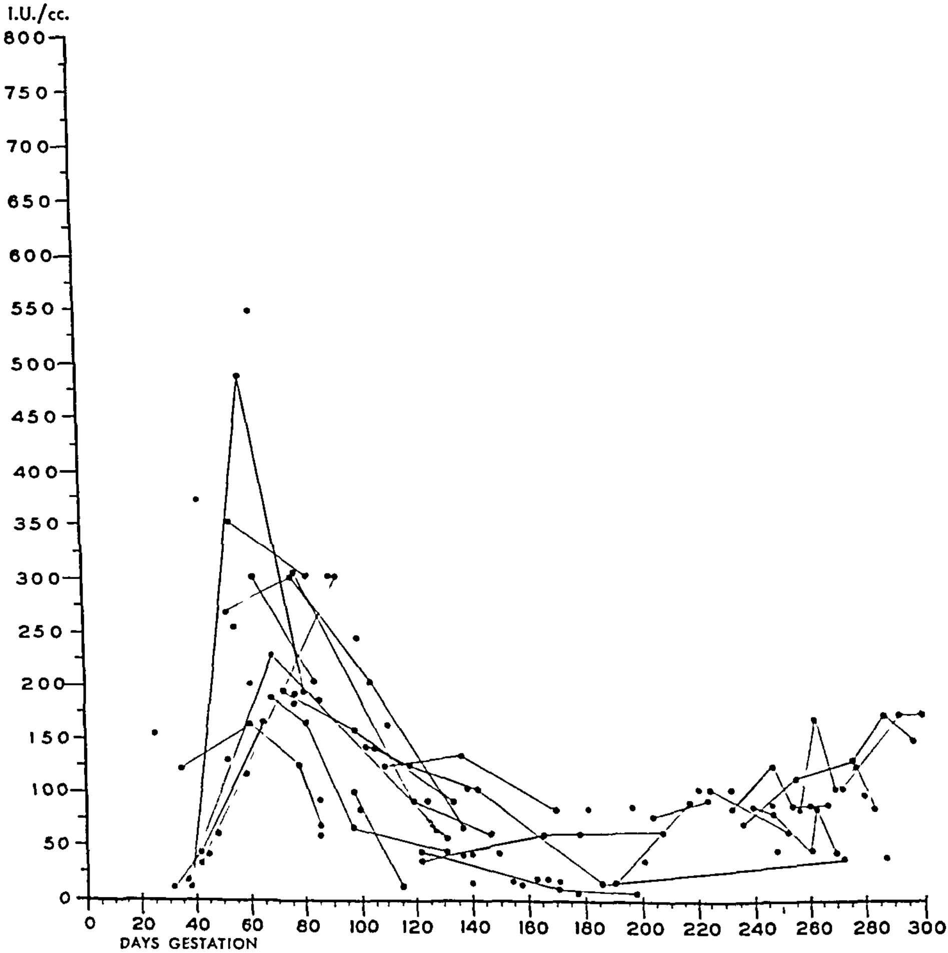

Fig. 1. Endocrine pattern for normal pregnancy.

test should be repeated. The left ovary seemed to give a better response than the right one, but just why this should occur is not known.

Intraperitoneal injection was favored because the material is absorbed over a larger area, resulting in a more uniform response than could be obtained by subcutaneous injection. When using serum as the test medium there is less need for concern about the pituitary gonadotrophins giving a false positive because the small amount in the blood further diluted makes it virtually impossible. We also noted that no toxic manifestations were obtained when using serum as compared to urine.

EXPERIMENTAL FINDINGS

An endocrine pattern for normal pregnancy was established as depicted in Fig. 1. Every case included in this area had a normal antepartum course with the delivery of a normal baby. It is of interest to note that the 6 cases with the highest peak at 70 days had moderate to severe hyperemesis

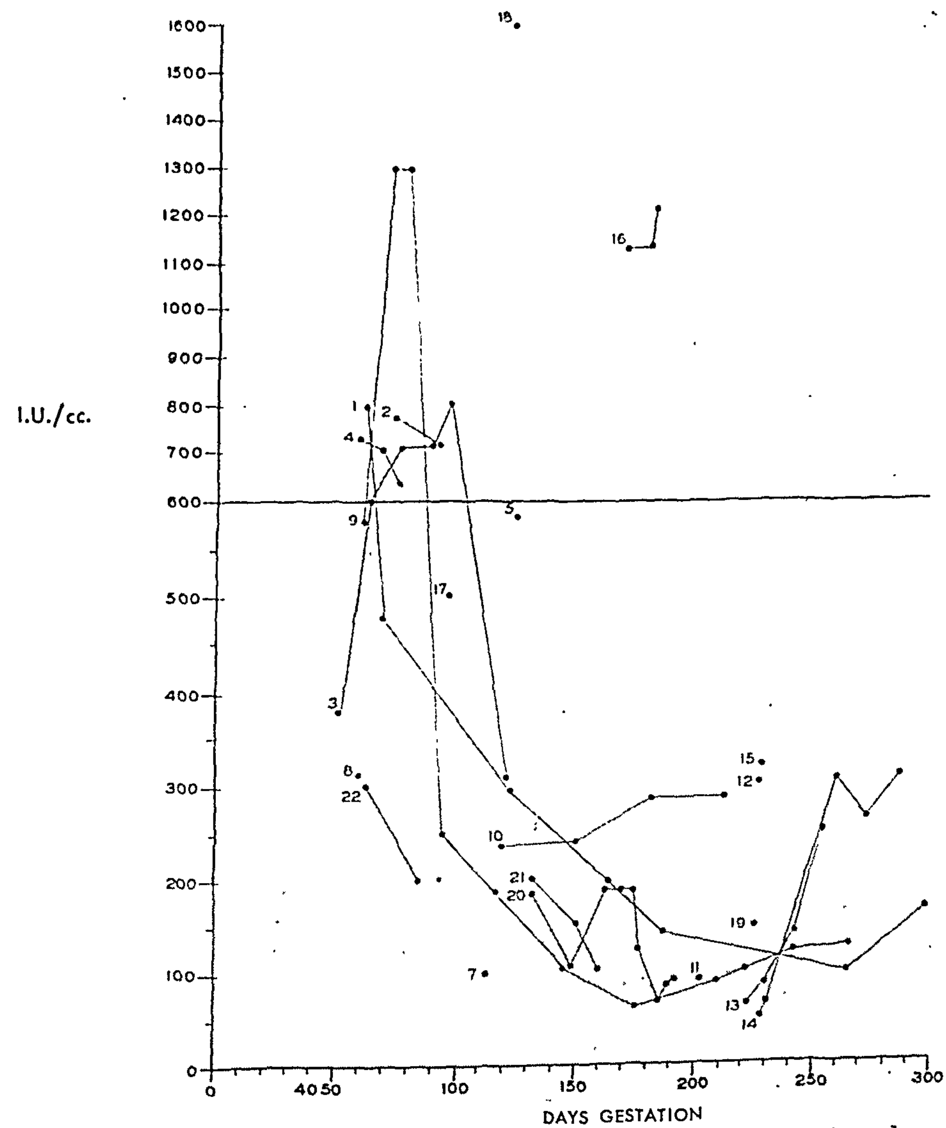

Fig. 2. Normal endocrine pattern during pregnancy (shaded area) and various forms of abnormal patterns.

gravidarum. This endocrine pattern conforms to the findings of others[2,4] showing a maximum peak between 60 and 80 days, after which there is a sharp decline to a low point at approximately 160 days, with a secondary mild rise at 210 days. This curve coincides very well with that obtained when using urine instead of serum, but practically none of the workers doing quantitative analyses with urine mentioned this secondary rise at 210 days, though it has been observed by others using serum. In our work we found this rise to be consistent.

TABLE 1. Abnormal Serum CGH Levels

Chart number	*Diagnosis*
1	Hyperemesis (moderate)
2	Hyperemesis with ovarian cyst
3	Hyperemesis (moderate)
4	Hyperemesis (severe–aborted)
5	Hyperemesis (severe–aborted)
7	Diabetes (N. S. D.)[a]
8	Diabetes (aborted)
9	Twins (N. S. D.)
10	Twins (N. S. D.)
11	Twins (aborted)
12	Twins (premature onset of labor)
22	Twins (N. S. D.)
21	Twins (N. S. D.)
13	Preeclamptic toxemia (induced)
14	Preeclamptic toxemia (induced)
15	Hydrocephalus
19	Anencephalus
16	Hydatidiform mole
17	Hydatidiform mole
18	Hydatidiform mole
20	Placenta previa

[a] *Normal spontaneous delivery.*

In Fig. 2 the normal endocrine pattern is represented by the shaded area while the numbered estimations all represent one or other "abnormal" form of pregnancy. These are summarized in Table 1. The common factor in these cases is the unusually high level of CGH at their particular stage of gestation. In the hydatidiform mole the level of CGH is explained by trophoblastic proliferation. This is probably also the explanation in the cases of spontaneous abortion, for we know that many such cases are distinctly abnormal in their development. It seems logical to assume that in multiple pregnancies there should be more chorionic tissue and therefore higher CGH levels but the explanation in the cases of preeclamptic toxemia or developmental anomalies is still nebulous. Thus, whether this quantitative

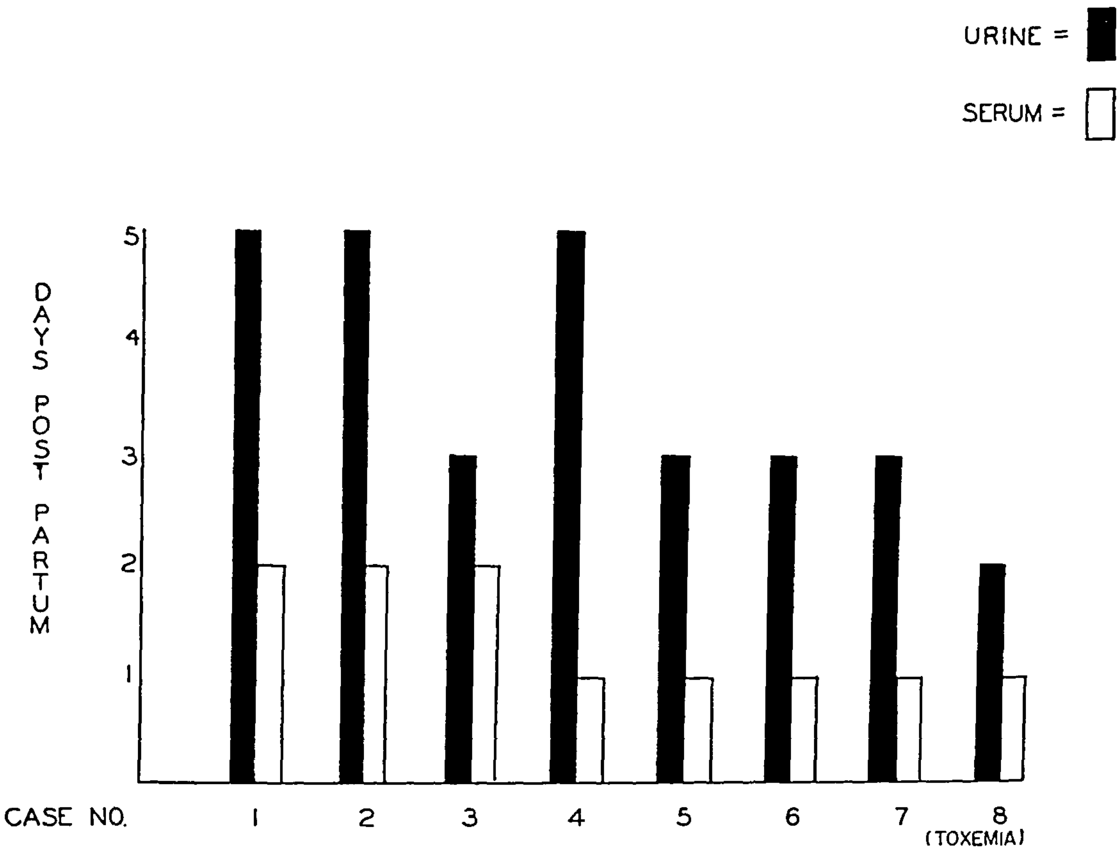

Fig. 3. Disappearance of CGH from urine and serum following term delivery.

estimation of serum CGH permits one to predict twins, abortions, or toxemia if the level should fall outside the established curve, would at this stage only be speculative. However, one factor does clearly emerge. It is possible to do a quantitative analysis in cases of suspected hydatidiform mole with accuracy in 4 hours as compared to the 4 days of the Ascheim-Zondeck test. The saving in time is noteworthy and its usefulness obvious.

ELIMINATION OF SERUM CGH FOLLOWING DELIVERY

Stamler (quoted by Zondek) gave CGH intravenously to dogs and the hormone could be detected in the urine within one minute of injection.

Three hours later only 38 per cent of the original amount was still present in the circulation, but 20 hours later the hormone could still be detected in the urine. Only 11.2 per cent of the total dose was finally recovered from the urine. Gastineau recovered no more than 6 per cent of the total serum CGH from the urine after delivery in 15 cases. It seems that almost 90 per cent of the hormone is disposed of extrarenally, possibly by the liver or pancreas, as a result of "utilization," or via some other unknown route.

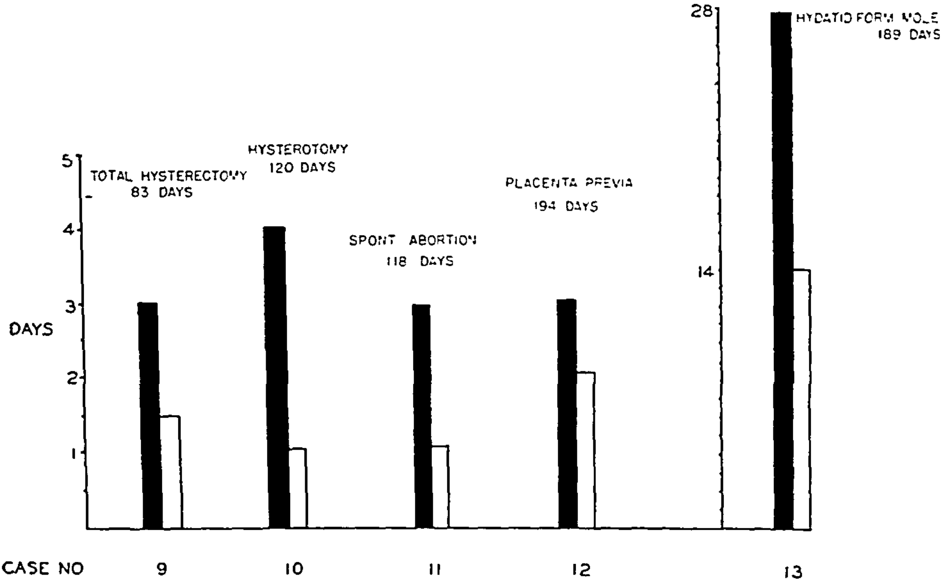

Fig. 4. Disappearance of CGH at various stages of gestation.

We studied the rate of elimination or disappearance of CGH from the serum qualitatively following term deliveries. Eight cases had samples taken from the serum and urine simultaneously every 12 hours postpartum for 5 days. From Fig. 3 it will be noted that CGH disappeared or was not detected in the urine 3–5 days after delivery whereas the serum CGH was eliminated or not detectable after the second day. The significant point is that in each case the CGH was eliminated from the serum 1–4 days before it had been eliminated from the urine, irrespective of the stage of gestation or the method of termination of pregnancy (Fig. 4).

As this finding could have practical value, it seemed necessary to determine whether it was a true one or merely a reflection of the relative insensitivity of the test for serum levels. We therefore took patients who were planning pregnancies or having artificial donor insemination and attempted to make the diagnosis of pregnancy as early as possible using both serum and urine. Twelve successful pregnancies resulted (Fig. 5). The earliest positive results were obtained 16 days after known conception. There were no false positives but 5 cases gave a total of 6 false negatives. In every case where the urine was positive, so was the serum. In no case did we get a positive urine and a negative serum. Where a negative serum was ob-

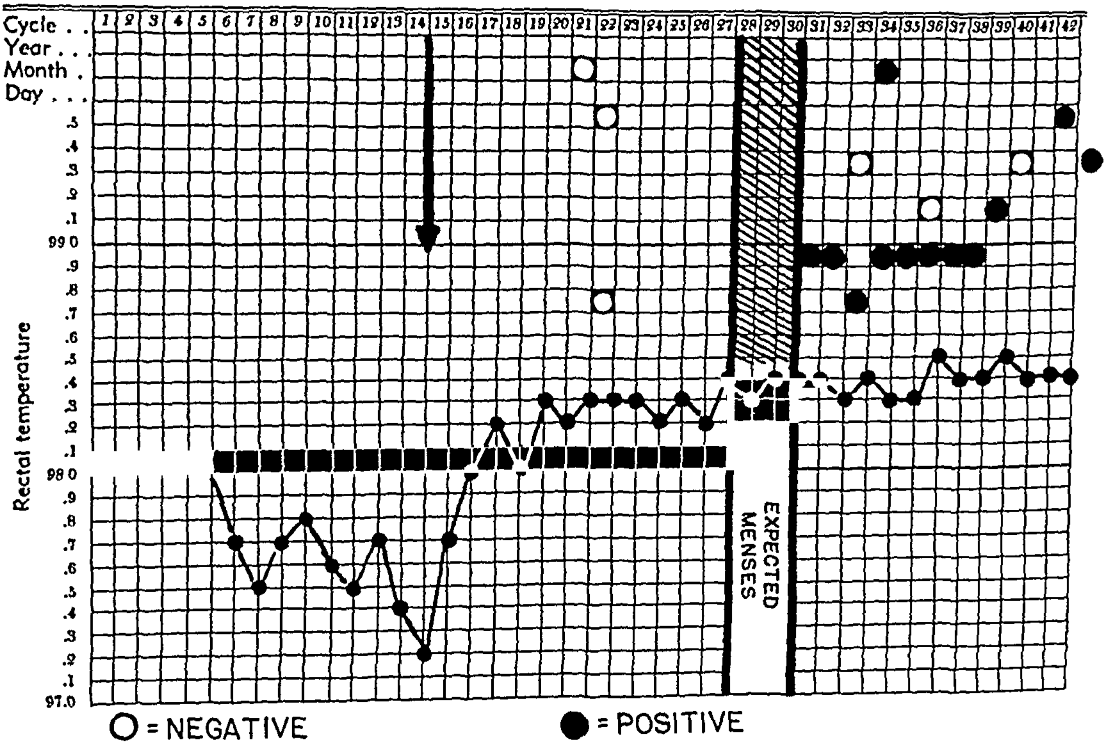

Fig. 5. Early diagnosis of pregnancy using serum CGH.

tained, it was common to both, so that from our experience the test was at least equally sensitive. Smith *et al.*[9] report having obtained a positive serum a day or two before the urine became positive in very early pregnancy, on Day 23. We could not confirm this.

From these results we feel that the more rapid elimination of CGH from the serum is a true one and that it occurs at any stage of gestation following termination of pregnancy by any method. This has practical significance, for normally in the treatment of threatened or inevitable abortions the patient is kept in bed or in the hospital to obviate the possibility of terminating a live pregnancy, till repeated urine pregnancy tests become negative. As CGH is present in the urine for several days after the pregnancy has terminated, this means that the pregnancy may well have been dead in utero for several days. Stated differently a negative test for serum CGH can be obtained from 1 to 4 days sooner than a negative urinary test.

This additional knowledge would permit us to institute definitive treatment that much earlier with corresponding saving of time, money, and inconvenience to the patient as well as bed space for the hospital.

SUMMARY

1. A method for using serum for quantitative and qualitative analysis of chorionic gonadotrophin has been presented.

2. The normal endocrine pattern of serum CGH throughout pregnancy has been given.

3. Of the 22 cases falling outside this established "normal" curve, there were 5 cases of hyperemesis gravidarum, 2 of diabetes, 6 of twin pregnancies, 2 of preeclamptic toxemias, 3 of hydatidiform moles, and 1 each of placenta previa, hydrocephaly, and anencephaly.

4. The earliest positive serum or urine CGH test was obtained on the sixteenth postconceptional day. There were 6 false negatives.

5. Where intrauterine death occurs or pregnancy is terminated by any method or at whatever stage of gestation a negative serum Rapid Rat Test for chorionic gonadotrophin can be obtained from 1 to 4 days before the urinary Rapid Rat Test becomes negative. The practical significance of this has been discussed.

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Seminal Fructose Studies in Infertility

Edward T. Tyler, M.D.

MANN, in 1946, demonstrated that the reducing sugar present in semen is fructose, instead of glucose as had been previously assumed. Mann further observed, as had been similarly demonstrated by MacLeod, that the function of fructose was to provide the spermatozoa with a readily glycolyzable material. Subsequently Mann and Parsons showed that a definite relationship existed between seminal fructose secretion and androgenic function. They demonstrated that withdrawal of male sex hormones in experimental animals through castration resulted in a drop of fructose and citric acid in the semen. They also observed that androgens injected into castrated rats produced a marked response on the part of the accessory sex organs in secreting fructose and citric acid.

These and other preliminary observations have led a number of investigators to initiate studies in order to obtain information on the significance of variations in seminal fructose. Davis and McCune, in 1950, studied 20 specimens of normal and subfertile individuals and found a mean value of 243.2 mg./100 cc., with a range of 59.0–510 mg./100 cc. They noted that semen specimens with low cell concentrations have high initial fructose levels and a low fructolysis rate, whereas specimens with high concentrations have relatively low initial fructose levels and a high rate of fructolysis. They also pointed out that fructose utilization by spermatozoa per unit is slightly

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higher in specimens with low concentration. Subfertile specimens showed lower fructose utilization, for an equivalent number of spermatozoa, than did normal specimens.

Landau and Loughead suggested that seminal fructose values could be utilized as a test for androgenic function and that fructose evaluations might be usable as a means of evaluating the androgenicity of hormonal substances in man. In view of these observations we have been obtaining data for the purpose of determining what practical value seminal fructose levels will have in an infertility practice in which problems of decreased libido, coital difficulties, and the like are also encountered. This report analyzes the data we have obtained thus far.

METHODS

Fructose determinations were done on semen specimens in the following manner: As soon as liquefaction of the specimen was complete deproteinized filtrates were made as described farther on. This was done within 1½ hours after ejaculation, and usually within 1 hour. (Determinations done at longer intervals after ejaculation are not included in the data presented here because variations in rate of fructolysis might make for errors in interpretation.) The filtrates were stored in a refrigerator until the test was completed.

Procedure

1. 0.4 cc. semen is added to 0.2 cc. Reagent 2 in an 0.7-cm. diameter test tube.
2. 0.1 cc. is removed and deproteinized.
3. The remainder is incubated at 37° C.*
4. Further samples are removed at various times.*
5. Deproteinization: Add 0.1 cc. of the product of Step 1 to 1.9 cc. H_2O. Then add 1 cc. Reagent 3 followed by 1 cc. Reagent 4. Heat for 1 minute in boiling water. Blow spermatozoa in and out of pipet 2 or 3 times.
6. Filter or centrifuge.
7. Transfer 2 cc. of the filtrate, which must be completely clear and colorless, to standard test tubes, marked with chalk according to time of removal. (Can be left overnight in the refrigerator.)
8. To the product of Step 7 add 2 cc. of Reagent 5 and 6 cc. of Reagent 6. Heat in water bath for 10 min. at 80–85° C., allow to cool, and measure absorption within 30 min., using a suitable colorimeter.

Preparation of Standards

1. Prepare for standard test tubes A, B, C, and D, add 1.8 cc., 1 cc., 1 cc., and 1 cc. H_2O to A, B, C, and D respectively.

* Steps 3 and 4 are done when rate of fructolysis is being observed.

2. Add 0.2 cc. of Reagent 1 to A. Mix thoroughly and add 1 cc. of A to B. Repeat to C. Repeat to D.
3. Discard 1 cc. from D. A, B, C, and D now contain 0.2, 0.1, 0.05, and 0.025 mg. of fructose solution, respectively.
4. Add 0.5 cc. of Reagent 3 and 0.5 cc. of Reagent 4 to each tube.
5. Repeat Step 8 of *Procedure.*

Reagents

1. Purest possible fructose, 0.2% solution.
2. Phosphate buffer, *p*H 7.4. (200 cc. 0.25N Na_2HPO_4 + 5 cc. 2N HCl; 7.101 g in 200 cc.; 2 cc. HCl + 9 cc. H_2O).
3. Zinc sulphate ($ZnSO_4 \cdot 7H_2O$), 2%.
4. 0.1 N NaOH. Need not be standardized. Commercial solutions are accurate enough.
5. Resorcinol in 95% alcohol, 0.1% (keep in refrigerator).
6. HCl, 30%. 5 parts HCl + 1 part H_2O.
7. Phenol red.

Notes on reagents. On each day when estimations are to be done, add 1 cc. of Reagent 3, 1 cc. of Reagent 4, and 2 drops of Reagent 7 together. The color should be orange-yellow; i.e., *p*H 7–7.2 The pipets used for Reagents 3 and 4 must *never* be used for fresh semen without very thorough washing.

RELATIVE SEMINAL FRUCTOSE VALUES IN FERTILE AND SUBFERTILE PATIENTS

The first series of data concerns initial seminal fructose values in fertile patients compared to those who might be considered subfertile. These fructose values are analyzed from the standpoint of corresponding sperm count and motility, each of the latter being correlated separately.

Table 1 summarizes the findings obtained, utilizing fructose determinations on the initial specimen obtained from 82 individuals. In this summary fructose values are compared to sperm count. It should be emphasized that arbitrary classification into a specific sperm count range is simply for the purpose of a standard for tabulation, for we are all aware of the marked variations in sperm concentration that occur spontaneously in the same individual.

Initial sperm count levels are divided into the following groups: (1) azoospermia; (2) below 10 million/cc.; (3) 10–20 million/cc.; (4) 20–40 million/cc.; (5) 40–60 million/cc.; (6) 60–100 million/cc.; and (7) 100 million/cc. or above.

The results show no variation between seminal fructose levels of patients with marked oligospermia and those with normal sperm counts. If anything, the tendency was for the specimens with higher sperm counts to show, on the average, a lower seminal fructose concentration. One explanation for this might be the fact that in specimens with high sperm concentration the spermatozoa themselves occupy an appreciable volume of the entire semen, and hence the concentration of fructose in the total ejaculate might be lowered. Wide ranges were also found for total fructose content of the

TABLE 1. Initial Seminal Fructose Values at Various Sperm Counts

				Total fructose	
No. patients	*Sperm count* (millions/cc.)	*Range* (mg./100 cc.)	*Average* (mg./100 cc.)	*Range* (mg. ×100)	*Average* (mg. ×100)
6	Azoospermia	308–485	366	485–712	569
(1)	(Azoospermia)	(0)	(0)	(0)	(0)
14	Below 10	40–560	368	12–6413	1429
11	10–20	122–668	425	400–2008	1001
9	20–40	260–560	379	735–3024	1543
7	40–60	111–608	330	344–2128	1175
15	60–100	60–586	313	18–2461	870
(1)	(60–100)	(0)	(0)	(0)	(0)
18	Over 100	180–395	294	377–1975	841

specimens, with no significant deviation in values between normal and subnormal specimens, again with the exception of a tendency toward lowered values with specimens of high fertility.

There were two patients whose specimens failed to show seminal fructose in any appreciable amount on repeated examination. One patient was azoospermic and an attempt to obtain a vesiculogram of the patient was unsuccessful in that no ejaculatory ducts could be located on urethroscopy. This, of course, does not prove that they are not present. However, on exploration, no vas was found on either side. This patient has been cooperative to the extent of providing specimens at intervals for nearly a year, and none of the specimens exhibited volumes of over 0.7 cc. The secretions are presumed to have been almost all prostatic, and this would support the contention that all the seminal fructose is derived from the seminal vesicles.

Semen specimens were also evaluated, as mentioned previously, from the point of view of seminal fructose values compared to the degree of initial sperm motility. Similar numbers of specimens were grouped into arbitrary motility classifications of excellent, good, fair, and poor. While this is not the motility grading we usually use, it is employed here to combine for simplicity such various motility elements as quality and percentage. Table 2 summarizes the findings obtained in a group of 154 specimens, some of which are repeated samples from the same individual.

It may be noted that there is little substantial difference between average seminal fructose values in milligrams per 100 cc. among those specimens showing poor and those specimens showing excellent motility. As a matter of fact, one patient had a consistently good sperm count, usually well over 60 million/cc., with sperm motility that could be generally classified as good, but on no occasion did his specimen demonstrate any significant amounts

TABLE 2. Seminal Fructose Values as Correlated with Grade of Motility in 154 Specimens with Varying Sperm Counts

		Fructose	
No. specimens	*Grade of motility*	*Range* (mg./100 cc.)	*Average* (mg./100 cc.)
39	Excellent	225–380	298
42	Good	228–650	373
38	Fair	176–485	359
35	Poor	140–485	313

of fructose with this type of analysis. Assuming his semen is devoid of any substantial quantity of fructose, this is consistent with the fact that under aerobic conditions, at least, fructose is not essential for good sperm motility and that possibly some other chemical of the semen is metabolized aerobically.

CONSISTENCY OF SEMINAL FRUCTOSE VALUES IN THE SAME INDIVIDUAL

During the course of these studies repeated seminal fructose determinations were done on the same patients in a number of instances where therapy was not a factor and where variations in values obtained could be considered spontaneous. These included, among others, patients on whom two or three determinations for evaluation were done before attempting therapy and a number of presumably fertile husbands who provided repeated specimens during the wife's period of observation and treatment. In almost half, the variation was well over 100 mg. 100 cc. in the same individual.

One individual on whom we have considerable data for over a year showed the marked fluctuation indicated in Fig. 1. These results cause us to have considerable doubt as to the consistency of seminal fructose values in the same individual. They also make us question the suggested usefulness of this diagnostic procedure as an index of androgenic function, since low and high fructose values could be found in the same patients at different times

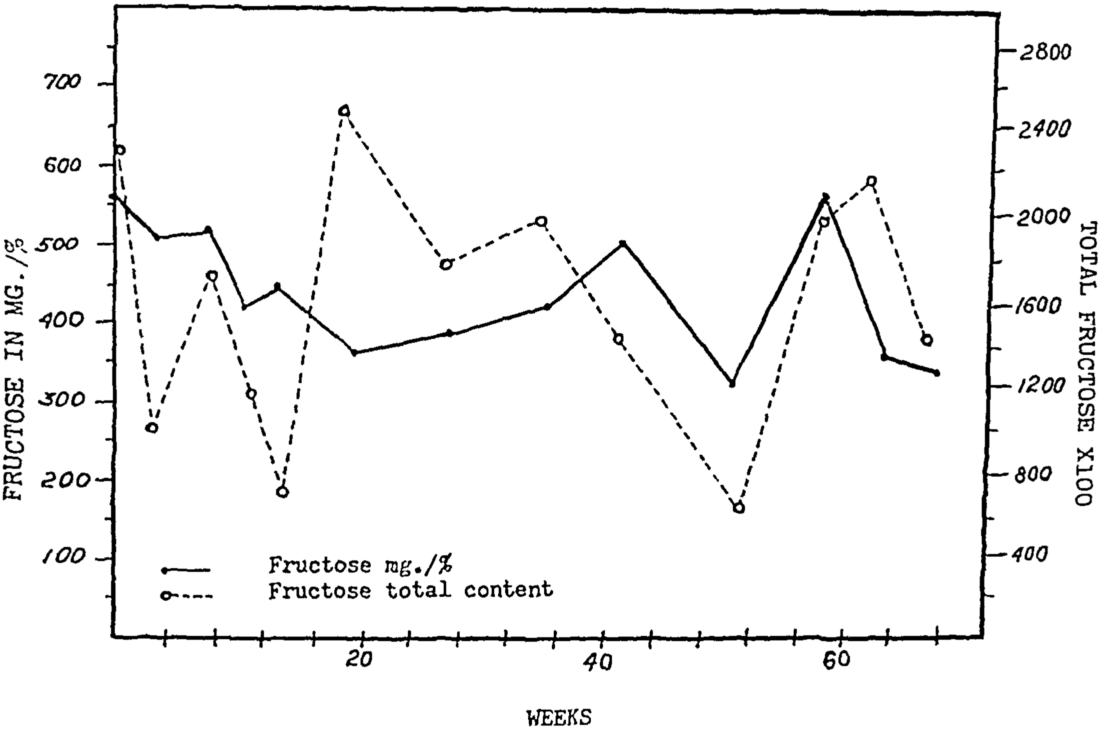

Fig. 1. Seminal fructose values (milligrams/100 cc. and total content) in same individual during a 64-week period. Note wide variations in fructose levels.

without any significant change in any of the obvious signs of good or poor androgenic function.

Testosterone Propionate Therapy

In this connection, among a group of 18 patients who complained of various types of relative impotence, 11 had seminal fructose values which could be considered high, and 7 had values on the low side. This suggested a lack of correlation of fructose values with relative impotence in comparatively normal men. To check upon this latter situation further, 6 patients who complained of decreased libido, infrequent intercourse, premature ejaculation, and so on, and who also had seminal fructose values that were fairly consistent, were given various types of therapy. These included patients with both high and low values. Table 3 charts the variations in seminal fructose obtained in these patients on therapy, indicating in each instance the type and duration of therapy. It was found that testosterone propionate, 50 mg. a day, appeared to result in moderately higher seminal fructose values in 2 of the 6 instances, but none of the other therapy seemed to significantly affect the fructose output. Furthermore, in view of the marked spontaneous

TABLE 3. Seminal Fructose Values (Mg./100 cc.) and Length of Therapy in 6 Patients with Decreased Libido and Coital Difficulties

		During therapy			
			Androgen[a]		
Patient	*Before therapy*	*Gonadotrophin*	*Orally*	*Parenterally*	*Lipotrophics*
J. B.	248	310 (2 wk.)	280 (1 wk.)	486 (1 wk.)	386 (3 wk.)
		205 (4 wk.)	320 (2 wk.)	462 (2 wk.)	
J. K.	272		210 (1 wk.)	305 (2 wk.)	
			345 (2 wk.)	295 (4 wk.)	
S. G.	284	220 (3 wk.)	380 (1 wk.)	512 (1 wk.)	390 (2 wk.)
			274 (2 wk.)		
O. M.	341	300 (1 wk.)	265 (2 wk.)	220 (1 wk.)	260 (2 wk.)
		390 (4 wk.)	325 (4 wk.)	390 (1 wk.)	
J. C.	330		285 (2 wk.)	350 (1 wk.)	260 (2 wk.)
			315 (3 wk.)		
R. C.	414			468 (1 wk.)	508 (3 wk.)
				512 (2 wk.)	
				364 (3 wk.)	

[a] Dosage varied from 25 mg. methyl testosterone (Oreton M) orally daily to as much as 50 mg. testosterone propionate (Oreton) parenterally daily.

variations, it can only be conjectured whether the increases in these 2 cases were actually attributable to the medication.

Relation of Androgens to Seminal Fructose

If seminal fructose values were useful as a test of androgenic activity and a quantitative relationship existed between circulating androgen and fructose levels, one would expect to find a proportionate rise in the latter when substantial doses of testosterone are administered. Since this apparently does not occur consistently, it must be assumed that either relatively small amounts of androgens are adequate for seminal vesicle stimulation and that additional amounts do not alter this function much, or that exogenous androgen in nonhypogonadal individuals does not add appreciably to the level of circulating androgens. This latter possibility could be brought about by some suppression of intrinsic androgens. The majority of patients in this particular series were infertility problems and could not be classified as definitely hypogonadal from endocrine studies.

One patient is of interest, however, as an almost classic example of hypogonadism from both fertility and endocrine production aspects. This patient is a 28-year-old male of typical adiposogenital-dystrophy configuration who consulted me with his wife for donor insemination. This patient's testes measured 1½ × 2 cm. bilaterally. He was smooth-faced, had scant axillary hair, shaved only once weekly, and had difficulty obtaining an erection prior to treatment, which was instituted shortly after marriage. He was given a course of testosterone propionate therapy for approximately 12 weeks which caused substantial growth of pubic but not axillary or other body hair. It also resulted in marked increase in libido and the ability to have and maintain erections and have fairly normal intercourse. The patient's penis measured 2½ × 3 cm.; he had a sperm count of 500,000/cc. 6 months after he had been given the androgen therapy. At this time his libido, ability to have intercourse, and so on, had returned to levels similar to, but not quite as low as, that prior to therapy. Despite his obvious endocrine defects, he was able to produce a semen specimen, which showed a fructose value of 600 mg./100 cc. While one case proves nothing, it does illustrate that in an obviously hypogonadal individual, who responds symptomatically and objectively to androgen therapy, semen fructose values can remain high for some time after withdrawal of androgen.

17-Ketosteroid Excretion

During the course of these studies an attempt was made to correlate 17-ketosteroid excretions with seminal fructose determinations in certain patients. Analysis of these comparative determinations will be presented as part of a subsequent report, but a brief summary is included here. No direct correlation between seminal fructose values and 17-ketosteroid excretion was noted. For example, in Patient J. K. (see Table 3), whose fructose levels were approximately the same on 50 mg. testosterone propionate on alternate days as on no therapy, 17-ketosteroid excretion was almost doubled.

In the same individual 17-ketosteroid excretion appeared to be more consistent than were the seminal fructose determinations. Unfortunately, these latter determinations do not, as is well known, measure androgenic hormonal substances specifically and hence are not as helpful as we would like them to be. However, some patients showed a consistent elevation in 17-ketosteroids on various types of therapy.

One of the substances investigated was betaine,* a lipotrophic agent which was previously reported to have an effect on libido as an incidental finding in certain other investigations.[6] In attempting to evaluate the significance of this finding we employed subjective criteria of the patient as well as repeated 17-ketosteroid assays, although well aware of the limited usefulness of these assays. Utilizing in many instances more than one laboratory for the same determination, we considered a change in 17-ketosteroid excretion of greater than 100 per cent not to be attributable to coincidence. Specimens were obtained under similar circumstances in the same individual, in an attempt to control the physiologic variables in 17-ketosteroid excretion. Of 31 patients who had been given heavy doses of betaine, 18 showed substantial changes in 17-ketosteroids while on the medication. Increased libido on therapy was occasionally reported, but this was difficult to evaluate in this group of patients, as they were in the lower age groups where various complex factors probably were largely involved. Further studies to evaluate the effect of this substance in older men may provide more readily interpretable data on the significance of the variations in 17-ketosteroids noted, and any relation they may have to impotence.

SUMMARY

1. Seminal fructose determinations fail to show consistent levels in the same individual.
2. There is no significant change in seminal fructose values in individuals who are not profoundly endocrinologically hypogonad when they are given androgens or other therapy for stimulating androgenic function.
3. Certain therapeutic substances apparently are effective in altering steroid metabolism as evidenced by 17-ketosteroid excretion, but the clinical significance of this is uncertain, and requires further study.

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DISCUSSION

DR. CHARNY: I must confess that when I received the assignment to discuss this paper of Doctor Tyler's, I rushed to the reference library to either read or reread some of the articles on fructose so that I could get at least some information on the subject that was going to be discussed.

I have had no personal experience with fructose determinations at all so that anything that I may say will not either directly comment on or add to the data which Dr. Tyler has already presented. His report is a negative one, but that should not discourage him in his efforts to continue his work.

There are several facts that are established with regard to fructose. First, that fructose, and not glucose, is the sugar that is present in semen. Second, that it is utilized by the spermatozoa. But why the seminal vesicles go to the trouble of making fructose when there is glucose already available is still a mystery. Why is the nutrient material necessary at all, when the spermatozoa are actually in contact with the seminal fluid for only a short period of time? If the spermatozoa that are going to take part in fertilization of the ovum are transported—as they probably are—up through the cervix and into the uterus and tubes in a relatively short time, either they leave the seminal fluid immediately or they may possibly take along with them, absorbing to them, a certain amount of nutrient material.

There are many questions in this field that remain unanswered because of insufficient data and because of lack of correlation, so that negative reports such as Dr. Tyler's serve a very useful purpose in dispelling at least some erroneous notions that might lead us far afield. I want to congratulate Dr. Tyler on his very fine paper.

Discussion will be continued from the floor.

DR. JAMES LEATHEM, *New Brunswick, N. J.:* I have only one question, Dr. Tyler. Did you have any information—I am perhaps showing my ignorance as far as man is concerned—about whether or not the accessory structures of the normal human being are responsive to a greater degree when operating at the normal level? Certainly in the animal you can elicit function which exceeds normal by stimulation, but it is possible that the accessory organs are operating nearer their maximum in the man, and therefore further stimulation might not induce a response.

DR. MACLEOD: I was very much interested in Doctor Tyler's observations on the individual who had apparently no fructose in the semen, and, as pointed out, we discussed this yesterday. The actual level of fructose in human semen, of course, is inordinately high. That is to say that spermatozoa at 38° in vitro will get along perfectly well in a level of sugar at about 10 to 20 mg./100 cc., so that when the limit of error in these spermatozoa seems to be about plus or minus 30 mg./100 cc., it is obvious that not enough fructose could be present to account for the good fertility.

The whole question of this business of fructose which you raised, Doctor—is there any reason why it should be fructose? I am afraid, as I pointed out before, I am a little bit of a heretic about accepting completely this business of relation of fructolysis to the sperm activity. I have not found any strong relationship between the quality of motility and the rate of so-called glucolysis, rather than fructolysis. That is to say, spermatozoa with rather poor motility may actually metabolize fructose as rapidly as, if not more rapidly than, those with high motility. It is obvious in another species—the bull, for instance, there is a much closer correlation between activity and fructolysis.

I will remain a heretic so far as the human is concerned. I believe he is quite anomalous in many respects.

Dr. Gassner: Dr. MacLeod has pointed out that there are apparently striking species differences on record. We in Colorado have been endeavoring, for the past five years, to show what type of correlation, if any, does exist between activity and viability of bull spermatozoa and their capacity to metabolize fructose.

In our unit at the college, where artificial insemination is practiced, this particular type of test has shown great promise in the selection of fertile breeders, which usually takes many years, relying upon the known return data. That means the amount or the number of calves produced by a particular bull whose specimens have been sent into the field. We have, of course, an opportunity—perhaps less so than other stations in the country, like in New York state—but we have an opportunity to get up to about five thousand actual conception rate data per year. During the past three years in which this work was done we have been able to show consistently a positive correlation between these data and the rate by which spermatozoa will take a sample utilized fructose—let us say removed fructose—whatever happens in the semen. These correlations have been not less than 0.5 per cent to as high as 0.88 per cent. Apparently the amount of fructose in semen—the initial fructose—has very, very little to do with what the spermatozoa will do.

We have pointed out in our publications as early as 1950 that the initial fructose ability has very little to do with what the spermatozoa will do when it has been used in insemination.

There is no question that the amount of fructose produced is definitely under the control of the testicle androgens. We have done a number of experiments with bulls in which we have tried to interfere with testicular functions, selectively tried to damage either the spermatogenic system or the Leydig's cell system, which is difficult to do, but which we have been able to do, and then make restitution by giving various types of androgens—not only testosterone but some of an intermediate nature which are known to be of also an androgenic nature.

So we have been interested in secondary sex gland relationships, and in the course of this basic investigation we have consistently shown that there is a definite control operating via the Leydig's cell system.

I have one question I would like to ask Doctor Tyler. In these patients he has examined has he had any chance to do a particular biopsy to get some kind of idea what the Leydig's cell system looked like? I am referring now to those tests which Heller and Nelson have done, showing that they would establish definite correlation between the sex drive or libido and amount of seminal fluid produced.

I must admit that species differ—the horse differs quite a bit from man. The horse contains very little fructose. At the same time, we also know the horse spermatozoa—Doctor MacLeod has shown that—is very labile. Well, if there is a relationship now between the low amount of substance in the plasma in horses and the concomitant low viability or high fertility or what you may call it of horse spermatozoa, I do not know. The belief is there is a relationship. However, we have not worked with horses.

DR. CHARNY: We are running behind time, and I would like to curtail further discussion on this paper. Doctor Tyler, would you close?

DR. TYLER: Doctor Leathem brought up a very interesting point which I actually have included in the paper but eliminated for purposes of time: there is undoubtedly the question of whether, in individuals with intact gonads, administration of any type of androgenic preparation will increase the circulating amounts over normal levels. We all are aware of the inhibitory effect on intrinsic hormone production when oxygenous materials are given, and this is certainly a consideration that is important and which should definitely be considered.

Doctor MacLeod raised the question of whether there were adequate amounts of fructose in the specimens at levels below 30 mg./100 cc., and in checking Doctor Davis' original figures back in 1940 it would appear that there would not be enough fructose in this particular specimen to last more than about an hour at the rate of fructolysis that was suggested in Doctor Davis' paper. Yet these spermatozoa remained motile for far longer periods than that, and I question whether something else isn't being used.

I believe Doctor Gassner raised two questions: (1) The biopsy specimens on these individuals. I have not attempted to use those because our numbers of biopsies are very limited, and I don't think there would be any purpose in correlating them. We did do relatively large number of biopsies on patients several years back, and I should say that perhaps we should be doing more again, but this is a matter, I think, that will be debated in the next paper.

I would like to point out, in commenting on some of Doctor Gassner's talk, one thing that bothers me is that this test was proposed as an index of androgenic function, and that was the basic question I was trying to solve in this particular report. We know bulls or other animals, when they are castrated, will show no fructose. As far as I am concerned we don't need a laboratory test to prove a man is hypogonadal. I think we need a test which will show certain levels we can call hypogonadal and other levels nonhypogonadal. Thank you very much.

Ovarian Function in Latent Genital Tuberculosis

Masamichi Suzuki, M.D.

THE PROSPECT for cure of latent genital tuberculosis and resultant restoration of ability to conceive, where barrenness is due to the disease, has been enhanced by the advent of antibiotics and chemotherapeutic agents. Favorable early results from streptomycin alone or in combination with other chemotherapeutic drugs have been encouraging.[1, 2, 4, 11, 12] It is too early to predict how many of these patients will continue to be free of the disease, but the indications from our study with streptomycin[17] and those of others[3, 15, 19] are that cure or a remission of the disease may be expected.

Since the majority of these women have associated tubal stenosis, even after successful treatment, salpingostomy or some other type of tubal plastic operation is necessary if conception is to be made possible. Before such a *serious operation is performed, the ability of the husband to procreate and* the woman's ovarian function must be ascertained. Only a few isolated reports on the ovarian function of patients with genital tuberculosis are found in the literature.[5, 8, 10, 11] This paper presents a study of the ovarian function in women with this disease, including a group treated with streptomycin.

MATERIAL

From June, 1949, to April, 1953, in the Atomic Bomb Casualty Commis-

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sion's clinic 41 Japanese women in their reproductive years were found with latent genital tuberculosis. Their ages ranged from 22 to 41 years, with an average of 30 years. The diagnosis was established in the course of sterility examinations in 35 patients and during routine gynecologic examinations in 6. These patients were under the observation of this investigator from a few months to 3½ years. In 30 of the women, endometrial tuberculosis was demonstrated histologically, with bacteriologic confirmation in 26. Three also had associated cervical tuberculosis. In the remaining 11 patients whose endometria repeatedly showed no evidence of tuberculous lesions, the diagnosis was made by the bacteriologic isolation of the organism from cervicovaginal and menstrual discharges, and confirmed by a guinea pig pathogenicity test.

METHODS

B.B.T.

The two most common methods for the clinical investigation of ovarian function—the basal body temperature record and the endometrial biopsy—were used. The basal temperature curves were classified into four types—biphasic, monophasic, bizarre, and short biphasic. The short biphasic curve was distinguished from the usual biphasic curve was a shift of 0.4° F. or higher, with persistent elevation in the latter part of the cycle, but with a biphasic period of less than 9 days.

Basal body temperature records were kept for a total of 177 menstrual cycles. Only 5 were of the bizarre type. There were 64 basal temperature curves with corresponding endometrial tissue taken within the prescribed period; however, 10 specimens could not be dated.

Biopsy

A total of 279 endometrial biopsies were done. Although 148 of these specimens were obtained within 11 days before menstruation, dating was not possible in 15 because of inadequate tissue and in 5 because of extensive tuberculous lesions. Therefore, 128 biopsy specimens representing 128 menstrual cycles were used in this study.

The endometrial biopsy was performed with Meigs' curette[7] in the latter part of the cycle, and repeated biopsies did not cause exacerbation of disease in any patients. The tissues were fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin-eosin. The date of the succeeding menstruation was reported by the patient by letter.

The progressive development of the histologic pattern of the endometrium during the secretory phase of the normal menstrual cycle is sufficiently uniform to permit, with a fair degree of accuracy, the dating of the endometrium during this phase,[9] but it was necessary first to ascertain whether tubercle-studded endometrium could be accurately dated. Obviously, in extensive coalescing miliary or ulcerocaseous forms, the destruction of normal endometrium was so overwhelming as to make dating impossible. Fortunately, the majority of the biopsy specimens were of the miliary type with discrete tubercles and with sufficient areas of normal tissue for accurate dating.

Classification of Endometria

Accordingly, the histologic patterns of the endometrium obtained within 11 days before menstruation were classified into (a) normal secretory (NSP); (b) atypical secretory (AtSP); and (c) absent secretory phase (AbSP). The normal secretory endometria are those revealing secretory changes falling within 3 days of the true dating; the atypical secretory endometria show secretory changes with delayed development beyond 3 days of the true dating; and the absent secretory endometria are those corre-ponding to a proliferative pattern.

Ovarian Function

The ovarian function was determined in 39 of the 41 patients—10 by biopsy, 5 by the basal body temperature, and 24 by both methods. Menstrual cycles were present in 38 patients. One had persistent secondary amenorrhea. Two patients were excluded because of failure to keep basal temperature records and because their biopsies were either inadequate or had not been taken during the prescribed period.

RESULTS

Of the 128 cycles analyzed from the histologic pattern of the endometrium, 111 (87 per cent) were of the normal secretory, 3 (2 per cent) atypical secretory, and 14 (11 per cent) of the absent secretory phase. Of 173 cycles studied by the basal body temperature method, 152 (88 per cent) were biphasic, 4 (2 per cent) short biphasic, and 17 (10 per cent) monophasic curves.

Fifty-one of the 54 cycles studied by both methods were of the NSP-biphasic pattern. The histologic patterns of the endometrium and the type of basal temperature curve conformed except in three instances—one each with the AtSP-biphasic, AbSP-biphasic, and AbSP-short biphasic patterns. Summarizing the results with the two methods, normal ovulatory patterns were observed during 209 cycles (85 per cent) and abnormal patterns in 37 cycles (15 per cent)—6 atypical secretory and 31 anovulatory.

Ovulation

Of the 38 patients studied, an analysis was made of the frequency of the ovarian pattern in 31 on whom records of 3 or more cycles were available (Table 1). In 15 of these 31 patients, the ovulatory pattern persisted. Occasional abnormal cycles were observed in 5 women; and in 7 others, the abnormal secretory patterns occurred more frequently but in less than half of their cycles. Only in 4 patients were more than half of the cycles abnormal. If the 7 patients with more than occasional abnormal cycles are included, however, the incidence would be 11 in 31. A normal ovarian picture was present during one or more cycles of all the women, except one who had persistent anovulatory cycles.*

Menstruation

It was possible to study only one or two menstrual cycles in 7 of the 38 patients. As will be seen in Table 2, 4 manifested ovular, 2 anovular, and 1 both ovular and anovular patterns.

The duration of the menstrual cycles of 38 patients (556 cycles studied) was accurately determined: 9 patients were classified as having irregular menstrual cycles (a quarter or more of their cycles fell beyond the plus or minus 5 days of their mean menstrual cycle). Irregular cycles were observed in 6 of the 11 patients with more than occasional abnormal cycles (including those with more than half their cycles abnormal) and only in 1 of the 20 with more or less persistent ovulatory cycles.

None of the patients studied reported a duration of menstrual flow of more than 7 days; and 5 reported only 1 to 2 days of flow. Thirteen had

* This patient was 41 years of age and had ten pregnancies, the last 3 years before. Her genital tuberculosis probably dates back to 1½ years before when she suffered from tuberculous peritonitis.

scanty flow; two had heavy flow which was not considered abnormally excessive. A few of the patients had an occasional excessive menstrual flow. There was no apparent difference in duration or amount of menstrual flow between those with regular and irregular cycles.

The uterine index[6] was determined on 38 patients: 33 had an index of 0.75 or greater, and 5, an index of 0.7, but none had hypoplastic or infantile uteri.

Two patients with prolonged secondary amenorrhea and one with severe oligomenorrhea had biphasic basal temperature curves but failed to menstruate. A brief clinical résumé of 2 of these cases follows:

Case 1. A 34-year-old woman with primary sterility, who had been in apparently good health, had secondary amenorrhea for 9 years following an illness diagnosed as tuberculous pleurisy. A gynecologic examination disclosed only a slight thickening of the adnexa, without tenderness. Fragments of endometrial tissue obtained by biopsy revealed extensive tuberculous lesions; a few atypical endometrial glandular elements lined by a single layer of cuboidal cells with pleomorphic prominent basal nuclei were also seen among these lesions.

Five mg. of progesterone given intramuscularly daily for 5 days was followed 2 days later by scanty vaginal bleeding. However, two courses of ethinyl estradiol, 0.05 mg. orally for 20 days, failed to induce bleeding. The basal body temperature records from June 27, 1950, through November 20, 1950, were biphasic during 4 cycles but menstruation did not occur. (Figure 1 illustrates 3 of these cycles.) The duration of these cycles was 27–35 days, with the biphasic period ranging from 10 to 14 days. Streptomycin therapy of 40 Gm. was completed on April 21, 1951.

Thirty-eight days later there was a scanty vaginal bleeding for 1 day. Just before the bleeding began, a biphasic basal temperature curve was recorded. Subsequent records kept through 4 cycles revealed typical biphasic curves (Figure 2 illustrates 3 of these cycles), and the patient menstruated with regularity. The patient has been followed for two years since the therapy, and no relapse has occurred.

While repeated endometrial biopsies produced only fragments of tissue, a biopsy obtained 2 days before menstruation in December, 1952, 1½ years after treatment, was adequate to permit the dating of the endometrium and confirmed the true dating. Another biopsy taken 13 days before menstruation in April, 1953, gave a picture of early secretory changes corresponding to the histologic dating of M-16. Although the amount of tissue available by biopsy was still less than normally found, there has been a noticeable increase in her endometrial growth.

Case 2. A 28-year-old woman with primary sterility and secondary amenorrhea of 8 years' duration was first seen in August, 1950. No abnormal findings were recorded except left adnexal thickening. Fragments of tissue obtained by biopsy showed extensive tuberculous lesions. Two courses of ethinyl estradiol,

TABLE 1. Distribution of Endometrial and Basal Temperature Patterns (3 or More Menstrual Cycles Studied)

Patient	Age	Menstrual mean	Cycle type	Menstrual duration (days)	Amount of flow	Endometrium			Basal temperature only			Number of cycles
						NSP	AtSP	AbSP	Biphasic	Short biphasic	Mono-phasic	
NO ABNORMAL CYCLES												
F. N.	22	33.6	Regular	4	Moderate	4	..	..	2	..	..	6
Y. S.	26	33.0	Regular	3	Scanty	3	..	..	1	..	..	4
S. O.	27	24.7	Regular	3–4	Moderate	4	..	..	..	..	..	4
K. M.[a]	27	33.4	Regular	4–7	Moderate	3	..	..	..	..	..	3
T. S.	28	28.0	Regular	5	Moderate	1	..	..	3	..	..	4
Y. T.	28	28.6	Regular	6	Moderate	8	..	..	2	..	..	10
K. T.	28	28.6	Regular	2–3	Scanty	7	..	..	6	..	..	13
C. T.	28	30.9	Regular	3–4	Moderate	5	..	..	1	..	..	6
M. F.[a]	28	30.5	Regular	7	Moderate	7	..	..	5	..	..	12
H. N.[a]	29	29.7	Regular	1–2	Scanty	6	..	..	17	..	..	23
H. H.	30	27.2	Regular	5	Moderate	2	..	..	8	..	..	10
S. K.[a]	31	28.4	Regular	4–5	Scanty	2	..	..	2	..	..	4
R. N.[b]	34	29.9	Regular	1–2	Scanty	2	..	..	2	..	..	4
A. O.[a]	39	25.8	Regular	5–6	Moderate	2	..	..	1	..	..	3
S. I.	23	55.7	Irregular	5	Moderate	4	..	..	..	..	..	4
OCCASIONAL ABNORMAL CYCLES												
S. M.	27	32.2	Regular	3	Scanty	3	..	1	3	..	..	7
C. O.	27	30.8	Regular	5	Heavy	6	..	1	11	..	..	18
M. T.	27	27.5	Regular	7	Heavy	6	..	..	4	1	..	11
T. Y.[a]	32	28.3	Regular	4	Moderate	4	1	..	4	..	..	9
U. I.	35	31.8	Regular	4	Moderate	6	1	..	5	..	..	12

MORE THAN OCCASIONAL ABNORMAL CYCLES												
H. K.	25	28.7	Regular	4–7	Moderate	2	..	1	..	..	..	3
M. K.	28	32.8	Regular	4	Moderate	3	..	1	..	..	..	4
H. D.	29	33.4	Regular	3	Scanty	4	..	1	5	..	4	14
A. H.	25	40.1	Irregular	5	Moderate	5	..	..	9	..	6	20
K. K.[a]	26	35.9	Irregular	4	Moderate	3	1	..	2	1	..	7
F. Y.[b]	28	140.9	Irregular	1–2	Scanty	..	..	..	3	..	1	4
A. H.	39	38.0	Irregular	3	Moderate	2	..	1	..	..	..	3
MORE THAN HALF ABNORMAL CYCLES												
M. K.	30	38.6	Regular	7	Moderate	..	..	..	1	..	2	3
F. Y.	41	26.4	Regular	7	Moderate	..	..	3	..	..	..	3
T. H.	26	54.3	Irregular	3	Scanty	..	..	..	1	1	3	5
S. S.	27	38.9	Irregular	4	Scanty	2	..	3	..	..	..	5

[a] The disease established by bacteriologic methods.
[b] Biphasic basal temperature curves without menstruation not included.

TABLE 2. Distribution of Endometrial and Basal Temperature Patterns (1 or 2 Menstrual Cycles Studied)

						Endometrium			*Basal temperature only*			
Patient	*Age*	*Menstrual mean*	*Cycle type*	*Menstrual duration (days)*	*Amount of flow*	*NSP*	*AtSP*	*AbSP*	*Biphasic*	*Short biphasic*	*Mono-phasic*	*Number of cycles*
R. H.	22	31.3	Regular	2–3	Scanty	..	..	1	..	..	..	1
K. K.	23	26.5	Regular	4	Moderate	2	..	..	..	..	..	2
S. T.	29	28.3	Regular	2	Moderate	1	..	..	..	..	..	1
S. T.	30	27.2	Regular	4	Moderate	..	..	..	..	..	1	1
M. H.	35	30.3	Regular	1	Scanty	1	..	..	1	..	..	2
F. S.	29	29.0	Irregular	3	Scanty	..	..	1	..	..	..	1
K. T.[a]	29	34.2	Irregular	4.7	Moderate	1	..	..	..	..	..	1

[a] The diagnosis of tuberculosis established by bacteriological methods.

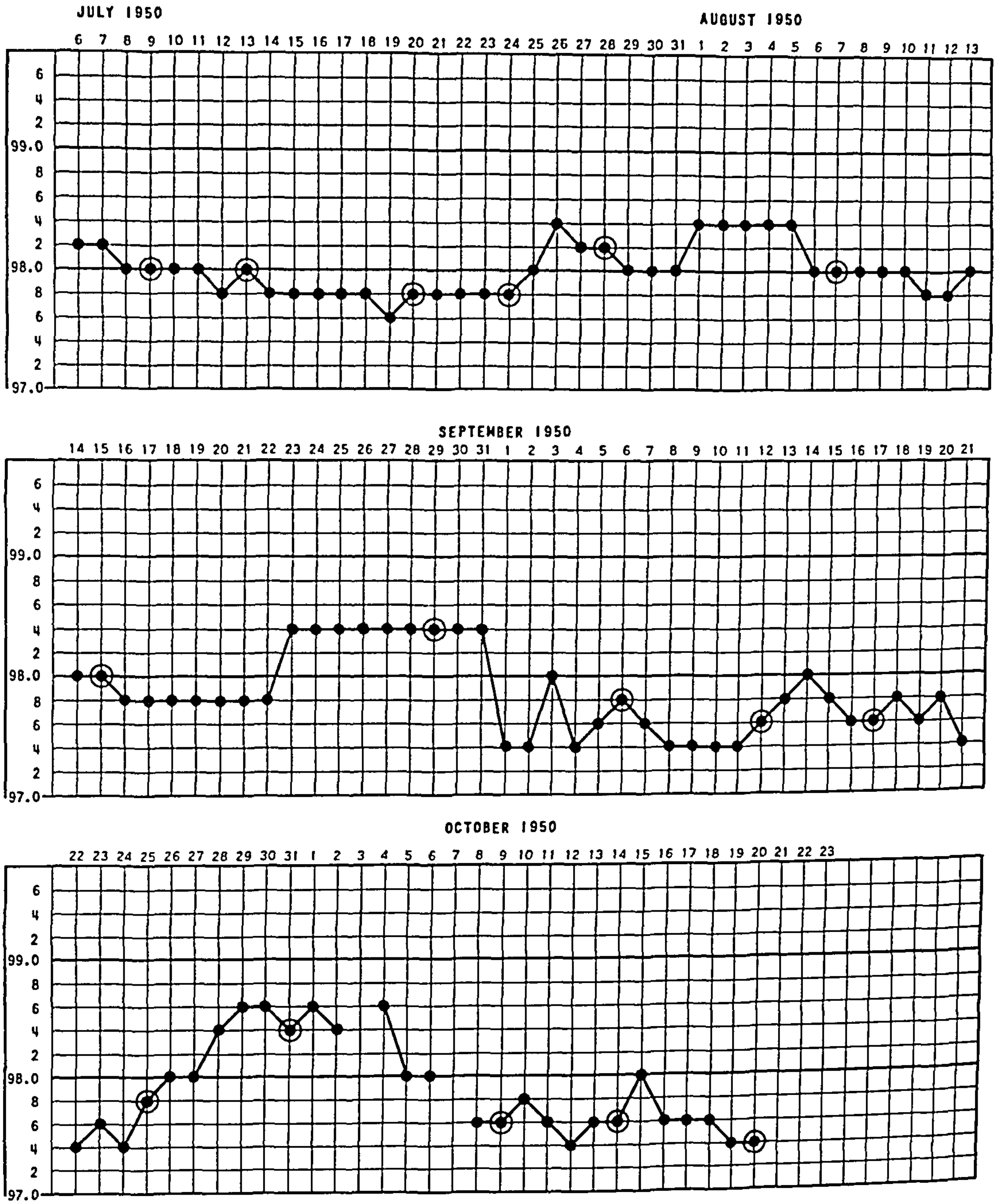

Fig. 1. Basal body temperature chart of R. N., showing cyclic biphasic curves without menstruation.

0.05 mg. daily for 20 days, were given, but no uterine bleeding was reported. Three cyclic biphasic curves, with the intervals between drop in temperature varying from 22 to 33 days, were recorded from August 24, 1950, through December 11, 1950. The elevated biphasic periods were 14, 17, and 18 days' duration.

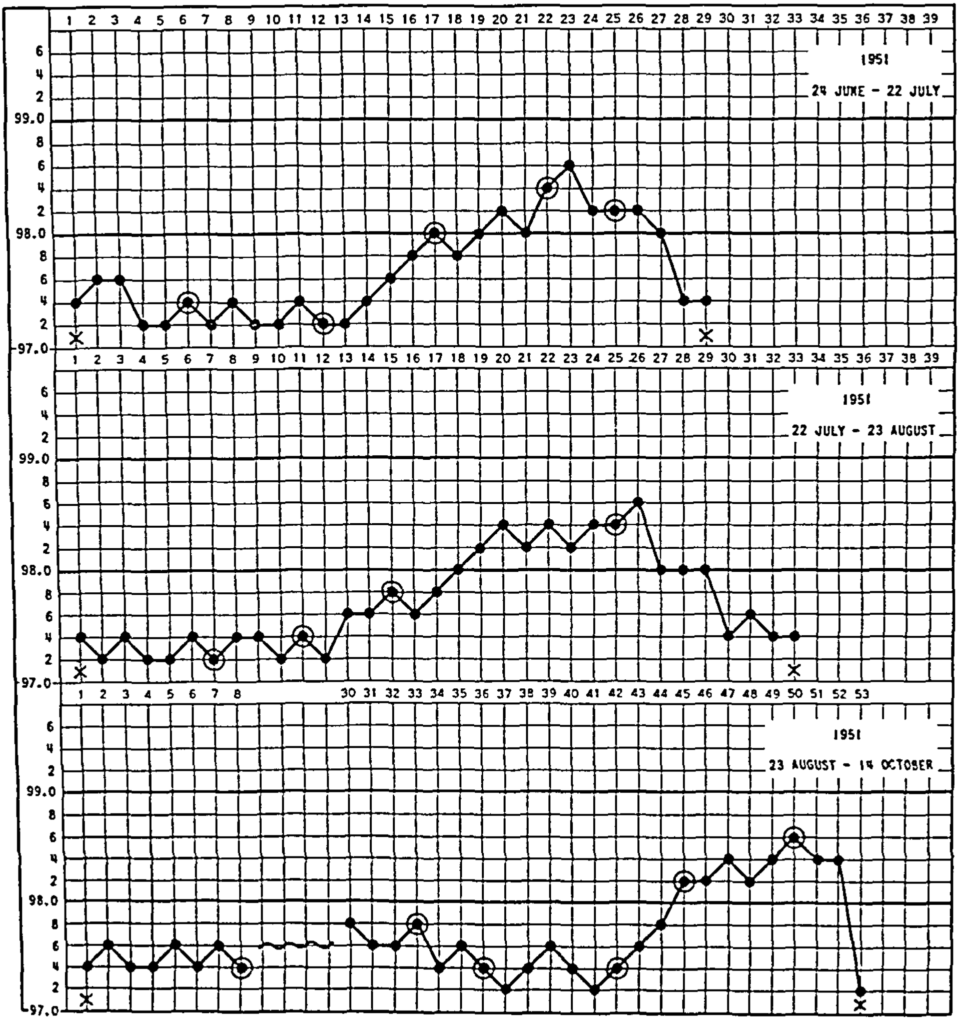

Fig. 2. Temperature chart of same patient as in Fig. 1 after therapy with 40 Gm. of streptomycin, which was completed on April 21, 1951. Cyclic menstruation began May 29, 1951. Typical biphasic curves recorded through 3 cycles.

The patient was treated with 40 Gm. of streptomycin in March and April, 1951. Another complete biphasic curve was recorded between March 15 and April 24. The endometrial biopsy specimens became extremely scanty following the treatment. Although no tubercles were seen in subsequent specimens, relapse was indicated by the finding of tubercle bacilli in her cervicovaginal discharge 1 year later. Her amenorrhea persisted.

The ovarian function of 17 patients who were menstruating regularly was studied before treatment with streptomycin and 6 months to 2 years after treatment. There is no evidence that the treatment influenced ovulation. This has been true both of the patients who relapsed and those who remained free of the disease 6 months to 2 years after treatment.

DISCUSSION

Abnormal ovarian function in women known to be fertile is uncommon except in the first few cycles following pregnancy.[13, 16] Sigler, in his investigation of 953 cases of infertility, reported an incidence of 5.77 per cent of anovulatory menstruation. A majority of his patients had oligomenorrhea or irregular menstrual cycles.

Comparison of Ovarian Function in Tuberculous and Nontuberculous Sterility

In a survey of infertility conducted at this clinic, the ovarian pattern of 107 barren Japanese women—82 with primary and 25 with secondary sterility—who were presumed to be free of tuberculosis, was studied in 3 or more cycles (a total of 600 cycles). No significant difference in the ovarian function and rhythm of menstruation between sterility patients without genital tuberculosis and the patients with the disease in the present study was observed, although there appeared to be a tendency toward a greater number of abnormal ovarian patterns and irregular cycles among the latter group. However, Ryden did not find abnormal ovarian function in his series of genital tuberculosis and was unable to demonstrate the existence of uterine hypoplasia.

Secondary amenorrhea in women with this disease does not appear to be due to ovarian dysfunction, since cyclic biphasic basal temperature curves are observed even when menstruation does not occur. Netter and Netter-Lambert also reported on a genital tuberculosis patient with primary amenorrhea who had cyclic biphasic basal temperature curves.

Cyclic biphasic curves in amenorrheic women, such as reported in this study and by Netter, are identical to those seen in women with normal menstrual cycles except that menstrual bleeding does not occur at the end of the elevated biphasic period. The appearance of menstruation proved by the basal temperature record and by endometrial biopsy to be ovulatory in type in one of the amenorrheic patients following streptomycin therapy supports the conclusion that these curves represent typical ovulatory cycles.

Uterine Physiology

Although on pelvic examination of our patients there were a few uteri of the lower range of normal size, none were smaller than normal. There were no instances of uterine hypoplasia, as determined by the uterine index.

Hormonal Therapy

Hormonal therapy—even large doses—did not induce menstruation.[8, 18] The amenorrhea was probably due to the extensive destruction of the endometrium by the tuberculous lesions, resulting in an interference with the physiologic mechanism of menstruation. One of our patients with prolonged amenorrhea (R.N.) developed scanty vaginal bleeding following a progesterone test but failed to respond to two estrogen tests.

Further evidence that abnormal ovarian function is not the primary cause of the barrenness of these patients is the fact that 3 of our patients became pregnant 1 to 2 years after streptomycin therapy. Unfortunately, two terminated as ectopic pregnancies and one aborted.

It is felt that salpingostomy or another tubal plastic operation can be recommended to patients with tubal stenosis who have been clinically cured for at least 2 years, provided there are no other factors contributing to the patient's sterility and that her husband has normal semen. The risk of ectopic pregnancy, however, must always be considered, even if the operation is successful.

SUMMARY

1. The ovarian function of 39 latent genital tuberculosis patients was studied by the basal body temperature and endometrial biopsy methods.

2. A total of 247 menstrual cycles were studied. Two hundred and ten cycles (85 per cent) were ovulatory in pattern, while 31 (13 per cent) were anovulatory, and 6 (2 per cent) atypical secretory.

3. Three or more cycles in each of 31 patients were studied, and evidence of ovulation was found in all but 1 patient. The cycles of 15 patients were consistently ovulatory in pattern; 5 had an occasional abnormal pattern; and 7 others had frequent abnormal cycles. Only 4 patients had abnormal patterns in more than half of their cycles. The incidence of abnormal ovarian function in patients with this disease is no greater than among sterility patients presumed to be free of genital tuberculosis.

4. Uterine hypoplasia or infantile uteri, as determined by the uterine index, was not encountered in this series.

5. Cyclic biphasic basal temperature curves without menstruation in two patients with prolonged secondary amenorrhea and one with severe oligomenorrhea were interpreted as indicating ovulation, since 1 patient developed ovular menstruation following streptomycin therapy.

6. No definite influence of streptomycin therapy upon ovarian function was noted, but the disappearance of tuberculous lesions resulted in a normal endometrium in the majority of patients studied.

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Evaluation of Sperm Concentration by the Hemacytometer Method

Comparison of Four Counting Fluids

John T. Smith, M.S., and Dennis T. Mayer, Ph.D.

Evaluation of the sperm concentration of semen specimens is of importance both to investigators of sperm physiology and to technicians in the field of artificial insemination. The sperm concentration of an ejaculate may be estimated by visual inspection; but such an estimate would not be satisfactory in the preparation of the higher semen dilutions based on the sperm concentration of an individual ejaculate, and it would certainly be inadequate for research purposes. Even when the more rapid and less tedious turbidity measurements are used, a calibration chart prepared on the basis of hemacytometer counts is a requirement. Therefore, basic measurements of sperm concentration are necessarily made by actual counting with a hemacytometer.

The literature is not specific regarding the type of fluid best suited for hemacytometer determination of sperm concentration. George, in 1952, demonstrated a new fluid for the determination of erythrocyte concentration, which enabled him to count a higher number of erythrocytes. The increased accuracy of count thereby afforded was attributed both to the staining action of eosin B and the fact that the cells lay flat within a single focal plane.

A comparison was made between the number of spermatozoa countable

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with this counting fluid and those countable with other fluids, both eosin-free and eosin-containing, which have been used in the evaluation of sperm concentration. The present paper presents the results obtained from this comparative study.

MATERIALS AND METHODS

Fluid Mixtures Used. The four counting fluids used in this investigation were divided into two groups—eosin-free and eosin-containing fluids. The eosin-free fluids consisted of a 3% saline solution recommended by Day, Anderson, and Herman and Madden, and a sodium bicarbonate counting fluid recommended by Belding. Belding considered his fluid, composed of 100 cc. 5% $NaHCO_3$ and 1 cc. formalin, to be superior to NaCl-containing fluids because of its mucus-dissolving properties. The fluid recommended by George—composed of 100 cc. 3% sodium citrate, 1 cc. commercial formalin 37–40% and 0.6 Gm. eosin B—and a counting fluid developed by Mayer—consisting of 100 cc. 2% NaCl and 1–2 Gm. eosin B—constituted the eosin-containing group.

Collection and Transport of Semen. Semen used in this investigation was collected by use of the artificial vagina from healthy dairy bulls maintained as a part of the Missouri Station dairy herd. Immediately after collection the semen was cooled to 20° C. and transferred to the laboratory.

Dilution and Counting of Spermatozoa. A portion of the well-mixed semen sample was poured into a small vial and drawn into a red blood cell diluting pipet to the 0.005 cc. mark; the tip of the pipet was wiped clean; and the final volume adjusted with a piece of cheese cloth. A small bubble of air was then sucked into the capillary, and the tip of the pipet inserted into a small vial containing a portion of the counting fluid, which was drawn up to the 1.01 cc. mark to give a dilution of 1:200. The dilution technic proposed by Mayer recommends addition of just enough eosin B to the well-mixed semen specimen to give it a pink color and then following the above procedure. This technic makes the original sampling with the diluting pipet much easier, since the pink semen column is easier to see and adjust in a pipet originally designed for deeply colored blood specimens.

However, eosin was not added to the semen prior to sampling in this investigation because the purpose was to compare the spermatozoa countable when using eosin-containing in contrast to eosin-free counting fluids. Filling of all dilution pipets for a single comparison was completed within

5 minutes to avoid any change in the number of countable spermatozoa due to settling. The dilution pipets were shaken for 3 minutes to insure thorough mixing of the semen and counting fluid, and 3 drops of the mixture was blown out to remove any high concentration of spermatozoa remaining in the capillary, before an aliquot was placed on the counting chamber for counting.

Spermatozoa were counted on a Spencer bright line hemacytometer, at magnification of 100, 5 of the 25 large squares being counted in each determination. The same counting chamber and coverglass were used throughout the investigation, but the choice of dilution pipets was left to random selection.

Surface Tension, Refractive Index and pH. Surface tension was determined by use of the capillary height method. The capillary radius was obtained by calculation from the capillary height in double distilled water. Refractive index was determined with an Abbé refractometer and the *p*H with a Standard Beckmann glass electrode *p*H meter.

RESULTS

Data obtained from this investigation have been tabulated in Table 1. The very close agreement between the number of spermatozoa counted with the two eosin-containing counting fluids is striking, the total number of spermatozoa counted being 7099 and 7058, using the counting fluids described by George and Mayer, respectively. Agreement between the number of spermatozoa countable with the eosin-free counting fluids was not as good, since there was a difference of 6.7 per cent (382) in the spermatozoa countable with the 3%NaCl- and 5% $NaHCO_3$-containing counting fluids. Percentages of countable spermatozoa was obtained by dividing the largest number of spermatozoa counted (given the value of 100 per cent) into the number countable with each of the remaining fluids.

The refractive index of each of the two eosin-containing fluids was slightly higher than the indices of the eosin-free fluids. The differences were too slight, however, for this physical property to be considered a factor responsible for the markedly different results obtained with the two groups of fluids.

The surface tension values were different for each of the fluids, and showed no tendency to be influenced by the presence or absence of eosin in the fluids. These results suggest that surface tension of the fluids was not a factor influencing their behavior as counting media (Table 1).

TABLE 1. Surface Tension, Refractive Index, pH, and Sperm Counted with Each Fluid

Fluid	*pH*	$n_{D_{25°}}$ [a]	$r_{25}°$ *(dynes/cm.)*	*Average per 5 sq.*	*Total spermatozoa counted*	*Countable spermatozoa (%)*
George	7.23	1.344	66.49	141.98	7099	100.00
Mayer	5.80	1.345	79.48	141.66	7058	99.42
3% NaCl	6.10	1.342	77.49	107.18	5359	75.49
Belding	8.70	1.343	53.25	114.82	5741	80.87

[a] Refractive index.

The data presented in Table 1 show an interesting relationship between sperm counts and two of the physical properties of the counting fluids. It will be noted that the lowest surface tension and highest *p*H were obtained in the counting fluid of each of the two groups (eosin-containing and eosin-free groups) giving the highest percentage of countable spermatozoa.

Analysis of variance indicated that there was a highly significant difference between the number of spermatozoa countable with eosin-containing in contrast to eosin-free counting fluids. Difference of the between-group five-square averages was 26.84, however; a difference of only 19.5 was required for significance at the 1 per cent level. The difference between the spermatozoa countable with the counting fluids within-groups was not significant.

DISCUSSION

The highly significant difference obtained between the number of countable spermatozoa using eosin-containing in contrast to eosin-free counting fluids was striking. But perhaps more striking was the tendency of the cells to arrange themselves within a single focal plane on the counting chamber when fluids containing eosin were used—the same phenomenon which was described by George as occurring when erythrocytes were counted in eosin-containing counting fluids. However, this tendency cannot be explained on the basis of a difference in any of the physical characteristics determined. The index of refraction was found to be slightly higher in the two eosin-containing diluents, but the *p*H and surface tension values did not show any between-group pattern. It was observed, however, that the lowest surface tension and highest *p*H values were obtained for the counting fluid which gave the highest number of countable spermatozoa. Since a significant difference was not shown for the countable spermatozoa within groups, this effect could not be considered significant.

The phenomenon of layering of the spermatozoa within a single focal plane, though admittedly unexplained, was a desirable one, since the spermatozoa and the hemacytometer lines remained in sharp focus, removing any doubt as to whether a spermatozoon was within a square, in an eosin-containing counting fluid. It has been shown that greater accuracy in evaluating sperm concentration with a hemacytometer may be obtained from using one of the eosin-containing counting fluids described, in preference to the eosin-free counting fluids previously described in the literature.

SUMMARY

Four counting fluids, two eosin-containing and two eosin-free, were compared on the basis of the physical characteristics (*p*H, surface tension, and refractive index) and the number of spermatozoa countable on five squares of a hemacytometer chamber. Results were not explainable on the basis of a different pattern in these physical characteristics. There was, however, a high statistically significant difference in the number of spermatozoa countable between groups (eosin-free and eosin-containing counting fluids), but not within groups. These results suggest the use of eosin-containing counting fluids for hemacytometer determination of sperm concentration.

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ELEVENTH ANNUAL CONFERENCE · JUNE 3, 4, and 5, 1955

Ritz-Carlton Hotel, Atlantic City, N. J.

THE AMERICAN SOCIETY FOR THE STUDY OF STERILITY

Preliminary Program

FRIDAY, JUNE 3

REGISTRATION 9:00 A.M.–5:00 P.M.

Endocrine Society Meeting — Reproductive Tract and Adrenals — Chalfonte-Haddon Hotel.

SATURDAY MORNING, JUNE 4

SECTION I

9:00–11:00 A.M. ***Chairman:*** Walter W. Williams

Sperm and Cervical Aspects

1. J. K. SHERMAN *(by invitation):* Temperature shock in human spermatozoa
2. LEO WILSON: Sperm agglutination due to autoantibodies: A new cause for sterility
3. GEORGE H. ROMBERG: Post-coital cytological studies of endocervical and endometrial fluid
4. C. LEE BUXTON and ANNA SOUTHAM *(by invitation):* A critical evaluation of the post-coital test in problems of infertility

SECTION II

11:15 A.M.–1:00 P.M. ***Chairman:*** Fred A. Simmons

Semi-adoption and Evaluation of Therapy

5. MELVIN R. COHEN: Artificial insemination and *Spinnbarkeit*
6. LOUIS PORTNOY: Experiences with artificial inseminations
7. ABRAHAM STONE and MILDRED E. WARD: Factors responsible for pregnancy in 500 infertility cases
8. CHRISTOPHER TIETZE: Statistical contributions to the study of human fertility

ROUND TABLES

Room A

A. 9:00–10:00 A.M.: Interpretation and value of basal body temperature curves. *Leader:* S. LEON ISRAEL

B. 10:30–11:30 A.M.: Endometriosis. *Leader:* LOUIS M. HELLMAN

C. 12:00 NOON–1:00 P.M.: Pelvic pain. *Leader:* ALAN F. GUTTMACHER

Room B

D. 9:00–10:00 A.M.: Tuboplasty. *Leader:* JOHN ROCK

E. 10:30–11:30 A.M.: Ectopic pregnancy. *Leader:* CARL E. JOHNSON

F. 12:00 NOON–1:00 P.M.: Significance of retrodisplacement in sterility problems. *Leader:* LYMAN W. MASON

MOVING PICTURES *Room C* *Chairman:* Robert S. Hotchkiss

9:00–11:00 A.M.

Physiology of Menstruation. Ovulation and Tubovarian Mechanism. Paracervical Denervation. Ovulation in the Rat. Fertilization of Rabbit Egg. Medical Effects of Atom Bomb.

11:30 A.M.–1:00 P.M.

Male Hormone. Studies in Male Sterility. Development of Testis. Testicular Deficiency.

LUNCHEON RECESS 1:00–2:30 P.M.

SATURDAY AFTERNOON, JUNE 4

SECTION III

2:30–5:00 P.M. *Chairman:* Sheldon Payne

Surgical Aspects

9. HAIG CARAPETYAN *(by invitation):* The surgical treatment of infertility in the female
10. HERBERT H. THOMAS and DAVID DUNN *(by invitation):* Observations on the use of Salpix as a media in hysterosalpingograms
11. PAUL T. TOPKINS, DAVID A. FRENKEL *(by invitation),* and ALVIN M. SIEGLER: A Simplified Method of Using Polyethylene in Tuboplastic operations
12. DAVID J. WEXLER, ANTHONY KOHN, and CHARLES H. BIRNBERG: Conservating surgical management of ectopic pregnancy
13. JOSEPH B. DOYLE: Uterotubal growth and oxygenation after paracervical denervation

ROUND TABLES

Room A

G. 2:30–3:30 P.M.: Artificial insemination. *Leader:* ROBERT N. RUTHERFORD

H. 4:00–5:00 P.M.: Population problems. *Leader:* WILLIAM T. POMMERENKE

Room B

I. 2:30–3:30 P.M.: Semen assay. *Leader:* LEWIS MICHELSON

J. 4:00–5:00 P.M.: Ovulation failure. *Leader:* LEO WILSON

Room C

K. 2:30–3:30 P.M.: Value of "fern" and other cervical mucus tests. *Leader:* ABRAHAM E. RAKOFF

L. 4:00–5:00 P.M.: Problems of hirsutism and amenorrhea. *Leader:* ROBERT E. GREENBLATT

BUSINESS MEETING 5:00–6:00 P.M.

SOCIAL HOUR AND COCKTAILS

6:30 P.M.

SUNDAY MORNING, JUNE 5

SECTION IV

9:00–10:45 A.M. *Chairman:* Joseph W. Goldzieher

Secretory Phase Aspects

14. ROBERT W. NOYES: The uniformity of secretory endometrium as determined by multiple endometrial biopsy dating
15. MAXWELL ROLAND: The use of high oral doses of progesterone to test the responsiveness of the endometrium in anovulatory cycles and chronic aborters
16. EDWARD T. TYLER: Therapeutic dosage of progesterone
17. HERBERT S. KUPPERMAN: Diagnostic and therapeutic use of progesterone in the problem of sterility

SECTION V

11:00 A.M.–12:00 NOON *Chairman:* John O. Haman

Psychiatric Aspects

18. PAUL H. FRIED: Pseudocyesis in relation to the psychiatric factors in infertility
19. WILLIAM BICKERS: Psychic stimuli and their effect upon fallopian tube and uterine contraction patterns
20. LOTHAR GIDRO-FRANK: Some observations on the reproductive performance of women with pelvic pain of long duration

SUNDAY AFTERNOON, JUNE 5

THE AYERST LECTURE

12 NOON–1:00 P.M. *Guest Lecturer:* Edith L. Potter

LUNCHEON RECESS 1:00–3:00 P.M.

SECTION VI

3:00–5:30 P.M. *Chairman:* Carl G. Hartman

Basic Studies

22. NATHAN MILLMAN *(by invitation)* and CARL G. HARTMAN: The oral control of fertility: A contemporary survey
23. JOSEPH T. VELARDO *(by invitation)* and SOMERS H. STURGIS: The effect of steroids on litter size in rats
24. ALFRED M. BONGIOVANI *(by invitation):* Pregnanetriol and adrenal steroids
25. To be announced.
26. J. C. CUMMINS, D. V. M. *(by invitation):* Androgen pellet implantation within the testicle
27. J. G. MOORE *(by invitation):* Tissue culture studies of endometria at varying intervals of the menstrual cycle

GENERAL INFORMATION

Members of the medical and allied scientific professions who are interested in the subject of reproduction and infertility may register. Registration fee for nonmembers is $5.00.

Abstracts

A study of the outcome of pregnancy in women exposed to the atomic bomb blast in Nagasaki. Yamazaki, J. N., Wright, S. W., and Wright, P. M. *J. Cell & Comp. Physiol.* 43: Suppl. 1 (May) 1954.

A study has been made of the outcome of pregnancy in women who were pregnant at the time of the atomic bomb explosion in Nagasaki. Among 30 mothers with one or more "major" signs of radiation (epilation, oropharyngeal lesions, purpura, or petechiae) who were within 2000 meters of the hypocenter, there were 7 fetal deaths (23.4 per cent), 6 neonatal and infant deaths (26 per cent), and 4 instances of mental retardation among 16 surviving children (25 per cent). The over-all morbidity and mortality was about 60 per cent in this group of mothers. This is in sharp contrast to the group of mothers without "major" signs, but within 2000 meters, where the over-all mortality was but 10 per cent, and in the control group where it was about 6 per cent.

The mean height and head circumference of children born to mothers with "major" signs was significantly smaller than in those children born to mothers in the control group.

It is difficult to evaluate radiation effect on this mortality and morbidity since other factors such as trauma, burns, infection, and so on, may have had a deleterious effect on the fetus. The evidence suggests, however, that radiation, either directly to the fetus or indirectly as result of the effect on maternal tissues, was of considerable importance in determining the outcome of these pregnancies.

E. Farris

Fetal losses in hypertension and preeclampsia: Part I. An analysis of 4432 cases. Part II. Prognosis for the fetus according to the week of gestation. Taylor, H. C., Tillman, A. J. B., and Blanchard, J. *Obst. & Gynec.* 3:225-239, 371-384, 1954.

From 1931 to 1950, 4432 instances of toxemia of pregnancy were observed at the Sloane Memorial Hospital. Patients were classified into five groups, depending upon the onset of symptoms (A_1, A_2, B_1, B_2, and C), and each group was further subdivided according to the height of blood pressure and degree of albuminuria on admission to the hospital. These findings were related to the ultimate outcome for the fetus.

A_1. In 306 women with known preexisting hypertension, the total fetal loss was 15.7 per cent, with the percentage of loss for mild, moderate, and severe blood pressure elevation being 8.8, 13.3, and 29.5, respectively.

A_2. In 758 women with established hypertension at first visit to the Clinic and thus with probable preexisting hypertension, the total fetal loss was 13.3 per cent, and the percentages for the three grades of blood pressure elevation were 9.3, 9.0, and 40.0.

B_1. In 2317 patients with preeclampsia, the percentage for fetal loss for the whole group was 6.6, with 4.8, 9.1, and 14.7 for the three degrees of hypertension.

B_2. In 239 patients with the presumptive diagnosis of preeclampsia, the percentage of fetal loss was 13.1 for the entire group, and 11.2, 3.1, and 27.6 for the three blood pressure grades.

C. For 706 patients in whom toxemia developed in the second trimester of gestation, the percentage of total fetal loss was 10.6, with 7.2, 11.2, and 38.0 for the three grades of hypertension.

In patients with *severe hypertension* (180/110) *and severe albuminuria* (3–4+) pregnancy should be terminated in the thirty-third to thirty-fourth week, in the interest of the fetus. In patients with severe hypertension (180/110) and moderate albuminuria (1–2+), as well as in patients with moderate hypertension (160/110–179/110) and severe albuminuria (3–4+), pregnancy should be terminated in the thirty-seventh to thirty-eighth week.

All other patients with less severe hypertension and albuminuria should be continued in pregnancy to spontaneous labor, or at least until the thirty-ninth to fortieth week, before inducing labor.

This formulation is based on the blood pressure and albuminuria on admission to the hospital. Other important factors affect prognosis, such as history of previous fetal loss and evidence of exacerbation or improvement of the condition under treatment after admission.

The probability of loss from prematurity is constantly changing, the contemporary survival rate being higher than the average for the 20-year study here reported.

O. J. POLLAK

Androgenic activity in milligrams of colorimetrically measured ketosteroids in urine: An index of the respective contributions from testicular and extratesticular sources. Hamilton, J. B. *J. Clin. Endocrinol.* 14:452-471, 1954.

Contrary to widespread belief, colorimetric assay of extracts of urinary ketosteroids is not a reliable measure of androgenic activity, which differs with age, sex, and the clinical condition of the subject.

In normal young males, when testicular secretions are not at a peak, the number of international units of colorimetrically measured urinary ketosteroids is high (3.7); in immature children it is lower (0.2), and in adolescents (1.3–2.9), middle-aged men (3.4), old men (2.9), and eunuchs (1.4) the level is also lower. Very high values are found in patients with interstitial tumors. The average number of I.U. per mg. of urinary ketosteroids is 28 per cent higher in young men than in young women (3.7:2.9).

Metabolites from testicular secretions include much androsterone, which has the highest androgenic activity of any urinary ketosteroid. Metabolites from extratesticular sources are present in larger amounts than androsterone but possess less androgenic activity. With such utility index, the proportions of urinary ketosteroids from testicular and from extratesticular sources can be determined, and thus the endocrine function of each can be estimated.

Testicular secretion of precursors of urinary ketosteroids decreases with age. Precursors of extratesticular steroids, apparently chiefly of adrenocortical origin, also decrease with age but to a much lesser level. (72 references)

O. J. POLLAK

Treatment of sterility: Analysis of 400 couples. Gray, L. A. *Obst. & Gynec.* 4:117–183, 1954.

The factors in the treatment of 400 couples complaining of infertility were analyzed. It was concluded that ordinary gynecologic care, correction of abnormalities in the female pelvic organs, efforts to maintain tubal patency by repeated and appropriate measures, and correction of seminal deficiencies seemed to be the most important points.

The overall-all percentage of pregnancy was 31. Organic pathology was found in 283 women. Eleven per cent of the total had major operations. Thirty-two patients had uterine suspension (all but 9 had endometriosis and/or myomas and all had symptomatic indications for surgery) and 15 became pregnant. Five women had salpingostomies and all remained sterile. The uterus was brought forward for retroversion and a Smith-Hodge pessary inserted in 14 patients and 8 became pregnant. Uterine curettage, biopsy, and cauterization of the cervix were performed in 45 women and 15 became pregnant. Tubal insufflation without hysterosalpingography was administered to 189 patients and 36.8 per cent became pregnant. Hysterosalpingograms in 82 women, after insufflation, were followed by pregnancy in 34.3 per cent.

Two thirds of the 228 husbands had a sperm count of less than 60 million/cc., with diminished percentage of pregnancies as compared to those with counts above 60 million. Treatment with general medical care, thyroid, and equine gonadotrophins seemed to be of some value.

(AUTHOR'S ABSTRACT)

Studies on the physiology of follicular fluid. Odeblad, E. *Acta Endocrinol.* 15:313–316, 1954.

In addition to other compounds, the follicular fluid contains a sulfomucopolysaccharide, as an important constituent. The liquor folliculi has a high viscosity, probably depending on the presence of the mucopolysaccharide. The high viscosity (and other physicochemical properties) of the follicular fluid seem to aid in the various stages of the ovulatory mechanism, such as the escape from the follicle, the protection of the egg cell, and the attachment of the egg to the ovarian surface.

(S. J. GLASS)

The significance of cervical bacteria in infertility. Kaye, B. M., Cohen, M. R., and MacLean, H. *Obst. & Gynec.* 3:644–650, 1954.

The theory that certain cervical bacteria, because of their spermicidal qualities, may be an etiologic factor in infertile matings has been advanced. This theory has been studied by the authors in the following manner: Cervical cultures were taken at or near the time of ovulation in 100 consecutive cases of infertility. These were divided into three groups: Patients with clinical cervicitis; patients with clean cervices but with pathogenic bacteria; and a control group with clean cervices, and with about equal numbers harboring pathogens or nonpathogens. Only the first two groups were treated, mainly with vaginal tablets of Terramycin.

This study has failed to confirm the above theory. About equal numbers of women in all three groups conceived, whether or not pathogenic bacteria were found in cervical cultures. The eradication of clinical cervicitis in the infertile patient increases the chances for conception, irrespective of the variety of bacteria present. The enhancement of fertility paralleled the improved results of the Sims-Huhner postcoital test.

(AUTHORS' ABSTRACT)

The gonadal function in female diabetics. Bergquist, N. *Acta Endocrinol., Suppl.* 19, 1954.

The author set out to answer four questions:

1. The onset of menarche of diabetic girls with onset of diabetes before puberty was, at average, at the age of 15. In nondiabetic girls menarche started at age 13.9 years.
2. Of 62 diabetic women treated with insulin (aged 20–39 years) 19 manifested major or minor menstrual irregularities.
3. Active biologic fertility was only slightly impaired in diabetic women and the number of pregnancies among them compared favorably with that of nondiabetic women.
4. The onset of the menopause occurred in diabetic women at a slightly earlier age than among nondiabetic ones.

(S. J. GLASS)

The gonadal function in male diabetics. Bergquist, N. *Acta Endocrinol., Suppl.* 18, 1954.

Male patients with diabetes mellitus, aged between 20 and 45 years, supplied information regarding their sexual function. In 11 out of 64 men there occurred persistent lack of libido and impotence. Ten of these 11 men were in the older age group, 33–45 years of age. Often, there was dissociation between the two symptoms: Some patients complained of lack of libido and others of impotence. Sometimes, the two symptoms were concomitant.

None of the two sexual symptoms was correlated with duration of diabetes. Nor did the diabetes seem more poorly controlled in these patients than in those without symptoms.

There was an uniform tendency to lower 17-ketosteroid excretion in these diabetic males. Poorly adjusted diabetics had significantly lower gonadotrophin values while well-controlled insulin-treated patients excreted normal amounts of gonadotrophin. These observations probably indicate that the decreased 17-ketosteroid excretion in younger men, especially those treated inadequately with insulin, is due to a reduction in pituitary gonadotrophins.

(S. J. GLASS)

Esterilidad masculina (male sterility). Valdes La Vallina, F. *Estudios sobre Esterilidad* 5:1–65, 1954.

This monograph stresses investigation of male sterility in relation to female sterility. Individual chapters and paragraphs are devoted to the concept of the problem, the history, frequency of sterility throughout the world and particularly in Mexico, to rural and urban birthrates, and the relation of infertility to age, religion, and demography. Minimum requirements for the study of sterility are given. A long chapter deals with semen studies, including assay of volume, viscosity, chemical composition, physicochemical, immunologic, and antibacterial properties; morphology, number, and motility of spermatozoa. The various factors in sterility are discussed, such as heredity, age, nutrition, vitamin deficiencies, the influence of climate, light, and temperature, various psychologic factors, intoxications, radiation, drug allergies, and, lastly, medullary lesions. Reference is made to testicular biopsy, especially for the diagnosis of syphilis, tuberculosis, brucellosis, and other bacterial and viral damage to the gonads. Obstructive factors are divided into those of congenital, traumatic, and postinfectious origin. Endocrine factors with the various endocrine syndromes are discussed. Prophylaxis, mainly of venereal diseases, and treatment, mainly surgical repair of obstruction, are discussed.

The author summarizes diagnostic and therapeutic features of 175 cases. [Unfortunately, instead of discussing each patient the various aspects are grouped under separate headings and the number of patients in which a particular symptom was found or a particular drug was used is expressed as percentage of the group. Thus, correlation of findings, therapy, and results is impossible.

The 330 references might seem a lot to those who do not realize that the total number of articles dealing with male sterility is nearly ten times higher.]

(O. J. Pollak)

Characterization of the estrogens in human semen. Diczfalusy, E. *Acta Endocrinol.* 15:317–324, 1954.

The findings reported favor the concept that human testicular tissue is capable of secreting estrogen, the bulk of which is in the "free" form. Estrone, estradiol 17B, and estriol were recovered by solvent partition and countercurrent distribution as well as by fluorimetric analysis of human semen.

(S. J. Glass)

Results in artificial insemination. Haman, J. O. *Tr. West. Sect. Am. Urol. A.* 38–44, 1954.

The author reports on 177 artificial inseminations with donor semen resulting in 134 (75.7 per cent) pregnancies.

In one series, semen was instilled onto the cervix and into the vagina. Positive results were obtained in 56 per cent of cases. In another series, a plastic cup was used to hold the semen against the cervix. The percentage of pregnancies was 83.3. in this group.

The author recommends the cup method since it reduced the number of inseminations as well as the number of cycles necessary for pregnancy, and also reduced the number of complications to zero.

Pregnancy ensued in 24 patients following a single insemination. Thirty women have had multiple pregnancies by artificial insemination. The incidence of miscarriages was the same as in any fertile mating: 17.9 per cent.

In 564 instances, insemination was done on the day of ovulation. Successful insemination occurred most frequently on Days 13, 14, or 15 of the menstrual cycle, never before the Day 10 or Day 24.

Twenty-seven of 63 donors accounted for all 134 pregnancies. One donor impregnated 25 women.

(O. J. Pollak)

Untersuchung des menschlichen Spermas auf 17-Ketosteroide (assay of 17-ketosteroids in human semen.) Huisintveld, L. G. *Acta Endocrinol.* 16:257-262, 1954.

Human semen was treated with 0.1 Vol. HCl at 80° C. under simultaneous extraction with benzene. In this extract, a substance containing the grouping CH_2CO was present. This substance developed a violet color with the Zimmerman reagent. Maximum absorption of the color complex was between 5700 and 5800 Å.

This substance is not identical with any of the known urinary 17-ketosteroids.

(S. J. Glass)

Distribution of succinic dehydrogenase and hyaluronidase in adult rat testis. Greif, R. L. *Proc. Soc. Exper. Biol. & Med.* 85:674-677, 1954.

The vigorous procedures required to extract hyaluronidase from homogenates of rat testis suggested that this enzyme might be tightly bound to some intracellular structure. By means of differential centrifugation in .25M sucrose solution under phase microscopic control, testicular homogenates were separated into "nuclear," "granular," and "soluble" fractions. Succinoxidase activity per milligram of nitrogen was almost three times higher in the granular fraction than in the uncentrifuged homogenate, and the total recovery of succinoxidase in the fractions amounted to over 90 per cent of starting activity.

Due to technical difficulties, the recovery of hyaluronidase activity from the fractions is incomplete. However, in preliminary studies, the granular fraction remains active after repeated washing by resuspension in sucrose. The possibility is raised that hyaluronidase may be associated with the midpiece of the spermatozoon.

(Author's Abstract)

The effects of high epididymal obstruction upon the Leydig cell volume of the rat testis. Harrison, R. G., and MacMillan, E. W. *J. Endocrinol.* 11:89-96, 1954.

MacMillan (Fertility and Sterility *4*:101, 1954) demonstrated that interruption of arteries supplying the caput epididymis results in focal necrosis of the initial segment, obstruction to sperm transport in the vasa efferentia, and eventually fibrosis of the epididymal lesion and the vasa efferentia with obstructive azoospermia.

Quantitative estimation of the total interstitial cell content of testes was made in 9 albino rats killed at periods from 7 to 342 days following experimental interruption of arteries supplying the right caput epididymis. The left gonad served as a control.

Increased interstitial cell volume was noted at 28 and 189 days after operation, by 24 per cent in the former and 45 per cent in the latter. The increase in total volume at 28 days is associated with the disposal of large quantities of shed germinal epithelium. The absolute increase at 189 days is associated with the occurrence of patchy necrosis of the seminiferous tubules.

The results correlate well with experimental evidence of Slotopolsky and Schinz (1925) and support evidence that fails to substantiate Steinach's (1920) claim that the interstitial cells undergo hyperplasia and hypertrophy when the vasa efferentia are ligated.

(AUTHORS' ABSTRACT)

Infertility induced in mice by a single injection of testosterone propionate. Barraclough, C. A., and Leathem, J. H. *Proc. Soc. Exper. Biol. & Med.* 85: 673–674, 1954.

The influence of a single subcutaneous injection of testosterone propionate on fertility was tested in prepuberal mice. Mice, 5, 10, and 20 days old, received a single 1 mg. injection. Fertility was tested when these mice were 3 months' old and compared with littermate controls.

No litters were obtained from 14 mice treated at 5 days of age despite 100 days in breeding whereas all control mice dropped litters 22 to 40 days after being placed with the males. Fertility was only moderately reduced by the androgen when mice were injected at 10 days of age, 8 out of 12 producing litters. However, at 20 days of age the androgen had no effect on fertility.

Ovaries from infertile mice had few follicles, no corpora lutea, an increased follicular atresia, and a marked concentration of sudanophilia in the prominent stroma.

It seems that the age at which androgen was injected was an important factor in the establishment of permanent or temporary sterility.

(AUTHORS' ASTRACT)

Studies in sperm hormones: Demonstration of estrogenic activity. Schaffenburg, C. A., McCullagh, E. P., and Tweed, D. C. *Endocrinology* 54:296–302, 1954.

Present-day concepts of the mechanism by which the testes influence anterior pituitary function are discussed. New evidence is introduced to show that bull spermatozoa contain an estrogenic substance.

Extracts were prepared from frozen centrifuged sperm subjected to acid hydrolysis, brought to 70% concentration of ethyl alcohol, reduced in volume by distillation, and extracted with ethyl ether. Neutralization and *p*H adjustment permitted the separation into two fractions, a phenolic and a neutral one.

Bioassay of the phenolic fraction demonstrated the presence of an estrogenic substance which increases the uterine weight and causes vaginal cornification in the adult spayed rat. The crude phenolic fraction is more active than the purified phenolic material, suggesting the presence of an estrogenic synergist in the crude preparation.

Alpha and beta ketonic and nonketonic preparations from the neutral fraction have no androgenic properties as judged by their failure to increase prostatic alkaline phosphatase in the immature castrate male rat.

(D. C. TWEED)

Klinefelter's syndrome: Report of an autopsy, with particular reference to the histology and histochemistry of the endocrine glands. Burt, A. S., Reiner, L., Cohen, R. B., and Sniffen, R. C. *J. Clin. Endocrinol. & Metabol.* 14:719–728, 1954.

This case report concerns a 63-year-old man with Klinefelter's syndrome, married but childless, moderately eunuchoid, with large breasts, a nodular goitre, small testicles, increased excretion of urinary gonadotrophins, and a normal thyroid function. The testes removed at autopsy were atrophic. On microscopic examination, there were clumps of interstitial cells; rare, completely hyalinized tubules; a few tubules with Sertoli cells; and histochemically the androgenic steroids were reduced. There was a multinodular goitre weighing 160 Gm. The adrenal glands were histologically and histochemically normal. The pituitary gland weighed 0.8 Gm., basophilic and acidophilic cells were normal but the number of hypertrophied nongranulated cells derived from sparsely granulated Schiff-positive cells was increased.

Twenty-one cases of male hypogonadism with a high incidence of thyroid or adrenal hyperplasia are reviewed (34 references) and the observations correlated with the authors' case report.

(O. J. POLLAK)

Infertility in rats and mice following estrogen treatment early in life. Braden, A. W. H. *Endocrinology* 55:112–113, 1954.

A total dose of 20 or 100 μg. of estradiol dipropionate administered over a period of 4 weeks starting at the fourth day of life induced permanent infertility in female rats. A dose of 4 μg. was ineffective. In female mice, sterilizing effect was achieved by a total dose of 5 μg. administered over 4 weeks starting at the second day of life. The sterilizing effect was, mostly, due to failure of ovulation, or, sometimes, due to cystic glandular hyperplasia of the endometrium.

(O. J. POLLAK)

Sex prediction through the use of maternal saliva. Posner, L. B., Livinsay, L. F., and Posner, A. C. *Am. J. Obst. & Gynec.* 67:1082–1084, 1954.

The Rapp-Richardson saliva test has been used in 200 women in their sixth or seventh month of pregnancy. The sex of the offspring was accurately predicted by the result of the test in 62.5 per cent of cases. A boy was predicted correctly in 73 and a girl in 56 per cent.

[The authors are most charitable in their criticism of the method.]

(O. J. POLLAK)

A Morula Stage of Human Ovum Developed in Vitro

Landrum B. Shettles, M.D., Ph.D.

This communication reports the development of a human ovum to a morula stage of approximately 32 cells within 72 hours after exposure to spermatozoa in vitro. Previous efforts have succeeded in bringing human follicular ova to 2-, 3-, 4-, and 8-cell stages after the addition of semen to the medium in vitro.[2-7]

METHOD

The ovum was aspirated, with a 20-gauge needle and syringe, from a mature follicle of a normal ovary in a patient undergoing laparotomy about the middle of her menstrual cycle. The ovum and follicular fluid were discharged into a sterile Petri dish, to which a drop of fresh human semen and some minced fragments of fallopian tubal mucosa were added. The mixture was ringed with sterile petrolatum, covered, and incubated at 37°C. for 72 hours. The tubal mucosa was added because of recent evidence that its fibrinolytic enzyme system is important to the denudation of the ovum and its subsequent penetration by the spermatozoon.[8]

RESULTS

The developing ovum (Figs. 1, 2, and 3) was then studied with the phase contrast microscope under varying conditions of illumination. The zona pellucida, completely denuded of the corona radiata and cumulus cells,

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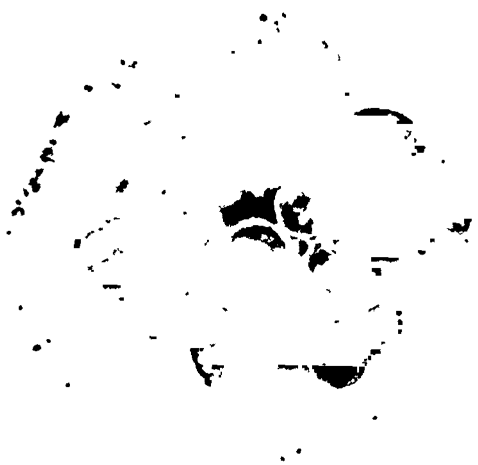
Markle Scholar in Medical Science.

surrounds a morula of approximately 32 cells. The precise number could not be ascertained because of the difficulty in counting the cells at different focal levels of the microscope. The cells, which differ in size partly because



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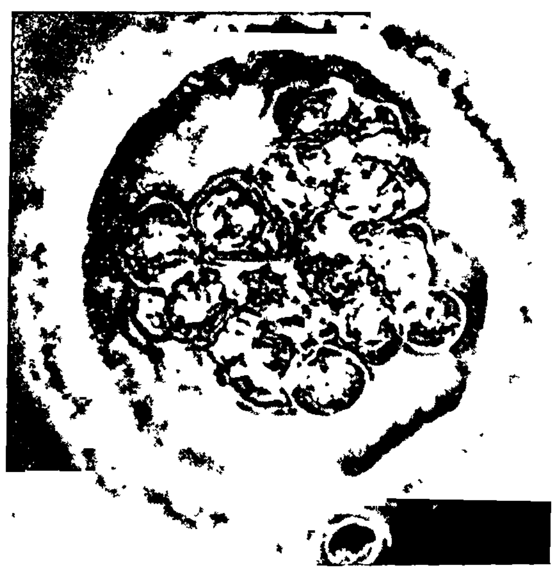

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Fig. 1. Morula stage of human ovum showing early segmentation cavity. Note granules in the zona pellucida. **Fig. 2.** Same as in Fig. 1, with readjustment of light to show third dimension. **Fig. 3.** Same as in Fig. 1, with adjustment of light to show chromosomal configurations.

of asynchronous cell division, surround the early segmentation cavity (see Figs. 1 and 2). Chromosomal configurations in varying phases of mitosis are shown in Fig. 3.

DISCUSSION

Only 4 normal human ova, fertilized in vivo, and recovered at preimplantation stages of development, are available for comparison with the present specimen.[1] On the basis of these segmenting ova, it has been concluded that a 2-cell stage was present in the fallopian tube at 36 hours, a morula of 12 cells was present in the uterus at 72 hours, and blastulae of 58 and 107 cells were in the uterus at 96 and 108 hours, respectively. However, these are probable ages of the respective specimens, in that the time of ovulation and fertilization could not be precisely ascertained from the information available. Fertilized ova undoubtedly vary individually in their rate of development in vivo. The circumstances of the experiment may have affected the rate of segmentation of the present specimen. Accordingly, the discrepancy between the 12-cell morula developed in vivo and the 32-cell morula in vitro at 72 hours may well be more apparent than real.

This specimen represents the most advanced stage to which the human ovum has been seen to develop following insemination in vitro. Continuing efforts are being made to record further developmental stages. Actual chromosomal counts are planned for subsequent specimens.

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Testicular Biopsy in the Bull

II. Effect on Morphology of Testes

F. X. Gassner, D.V.M., M.S., and H. J. Hill, D.V.M.

In a previous paper[7] it has been demonstrated that biopsy of bull testes using an open surgical approach results in rapid deterioration of semen quality within 2 to 3 weeks and that recovery of spermatogenesis may require 4–8 months. These studies with 10 bulls also provided an opportunity to survey testicular structure by histologic examination of specimens obtained by biopsy and at subsequent autopsy, and to correlate the findings with the damage to the function of the testes.

MATERIALS AND METHODS

The history of each of the 10 bulls and the detailed account of the biopsy technic used in this study are reported in a previous paper.[7] Biopsy specimens ranging in size from ¼ to ½ sq. cm. were fixed in Bouin's fluid, serially sectioned at 5 μ and stained with a modified Mallory's triple connective tissue stain. At autopsy performed at intervals of 3 weeks to 10 months after biopsy, sections were obtained from the area of the biopsy site, the center, and the dorsal and ventral poles of each testis. They were treated in the same manner as were the biopsy specimens.

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Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility, San Francisco, Calif., June 18–20, 1954.

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RESULTS

Normal Bulls

Bulls C and D. The relatively normal structure of the testis biopsy specimens (Fig. 1) agrees with the normal semen quality observed before surgery.[7] Fig. 2 shows sections of the biopsy site of the same testicles obtained at slaughter 18 days later. Extensive coagulation necrosis is evident in the tubules near the biopsy site while the remaining tubules show hypoplasia and degeneration of the germinal epithelium. Testis damage, however, extended throughout the organ, as indicated by sections taken from distant parts (Fig. 3) which showed hydropic degeneration, vacuolization, generalized tubular hypoplasia, and fibrosis.

Many lumina contained an accumulation of multinucleated, granular cells resembling giant cells which appear to be associated with, and pathognomonic of, spontaneous degenerative processes of the testes. This is in agreement with observations made by Lagerlöf and Blom, both of whom found similar cell structures in bovine testes and postulated on their origin. Fig. 4 shows a tubule at higher magnification and demonstrates various sizes of these cell forms within the lumen, as well as binucleated, granular cells still attached to the atrophic germinal epithelium. They apparently originate from the germinal epithelium by syncytial confluence of degenerating spermatogonia, spermatocytes, or even spermatids, depending upon the acuteness and severity of the degenerative process.

The possibility is not excluded that these cells may arise from an excessive mitotic activity of degenerating spermatogonia involving only nuclear but little or no cytoplasmic fission.[2, 8] On the other hand, it is unlikely that these giant cells are derived from blood or interstitial tissue elements invading for the purpose of phagocytosis as was suggested by some investigators.[1, 3, 8]

In semen from normal bulls only a few such cells may be found and then infrequently. However, large numbers are often present in semen from subfertile bulls showing oligospermia and breeding failure. The same is true when testicular function is experimentally altered by various means, particularly following thermal insulation of the scrotum.[4] In such cases the sperm cell count rapidly decreases within 2 weeks and these cells appear in increasing numbers, remaining until resumption of normal spermatogenesis 3–5 months later (Fig. 5). Since identical cell forms are found

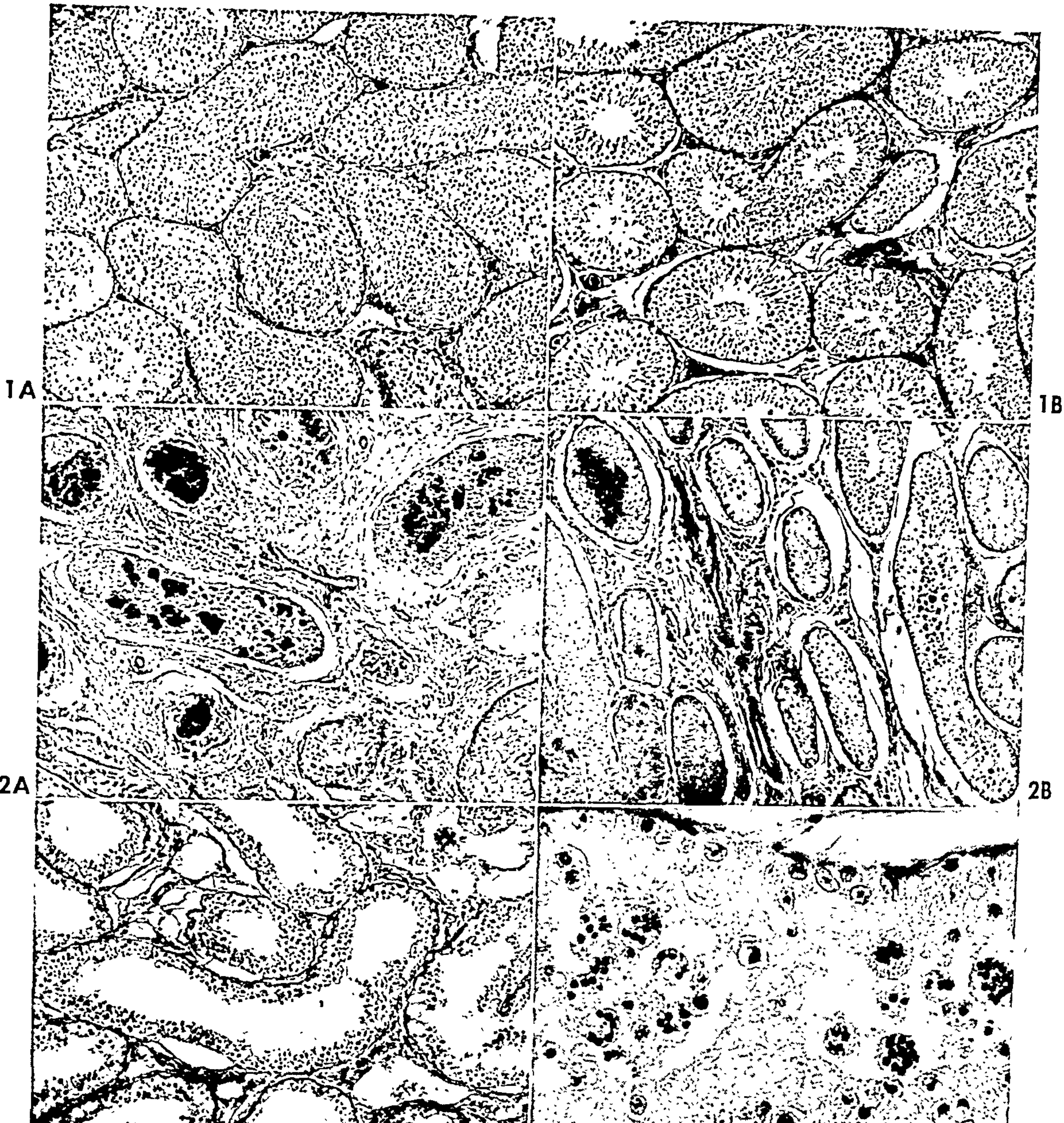


Fig. 1. Testicular biopsy specimens showing essentially normal morphology. A, Bull C. B, Bull D. (× 50)   Fig. 2. A, section of testis of Bull C, taken 18 days later at autopsy. Note necrosis of tubules at biopsy site (upper third) and hypoplasia as well as degeneration of germinal epithelium, elsewhere. B, Bull D, same morphology. (× 50)   Fig. 3. Section of testis of Bull C away from biopsy site (taken at autopsy 18 days after biopsy). Generalized hypoplasia with severe hydropic degeneration and vacuolization of germinal epithelium. (× 50)   Fig. 4. Seminiferous tubule (Bull D) showing degenerative germinal epithelium with many multinucleated, granular cells in lumen. Note formation of such cells from germinal layer (upper right and extreme upper left both binucleated). (× 200)

in semen from bulls with low breeding efficiency the above considerations are of particular interest as an additional tool in the clinical diagnosis of infertility.

Bull 18. The semen quality in Bull 18 was considered normal for a monorchid animal at the time of biopsy. The histologic structure of the biopsy specimen is in agreement with this finding (Fig. 6). Sections of the same testis obtained at slaughter 40 days later show extensive necrosis near the biopsy site (Fig. 7) and widespread atrophy with hydropic degeneration and vacuolization of the germinal epithelium throughout the remainder of the testicle (Fig. 8).

Bull P. Figure 9 shows the structure of a testis biopsy obtained employing the Vim-Silverman needle instead of the exposure method. Three punctures had to be made before a satisfactory specimen was obtained. Due to the unusually heavy stroma of the bovine testis it was not always possible to retain the cut specimen within the cylinder of the needle. While postoperative hemorrhage and swelling were insignificant, considerable damage to the spermatogenic system was evident upon histologic examination of autopsy specimens taken 3 weeks later. Figure 10*A* demonstrates the same degenerative changes described above in an area near the biopsy site. Sections taken from various parts of the testis remote from the biopsy site clearly show severe damage to the spermatogenic system indicated by denudation with vacuolar and hydropic degeneration of the tubular epithelium (Fig. 10*B*). Many lumina contain sloughed immature cells as well as granular giant cells described earlier (Fig. 11). It has been stated[7] that the heat-insulating action of the slowly absorbed, postoperative blood clot incident to the open biopsy method is a contributing factor to the pantesticular degeneration observed. Since the needle biopsy method does not result in intrascrotal blood clot retention but, nevertheless, in testis damage simulating that following the open approach, it is evident that the bovine testis is unusually labile to surgical interference.

Subnormal Bulls

Bull CSH. Bull CSH, a 5-year-old purebred Shorthorn, was a good breeder between the ages of 18 months and 3 years, having a breeding coefficient of 65 per cent. During the fourth year semen quality began to deteriorate and breeding efficiency was reduced to less than 20 per cent. While oligospermia developed and the number of primary abnormal sperm

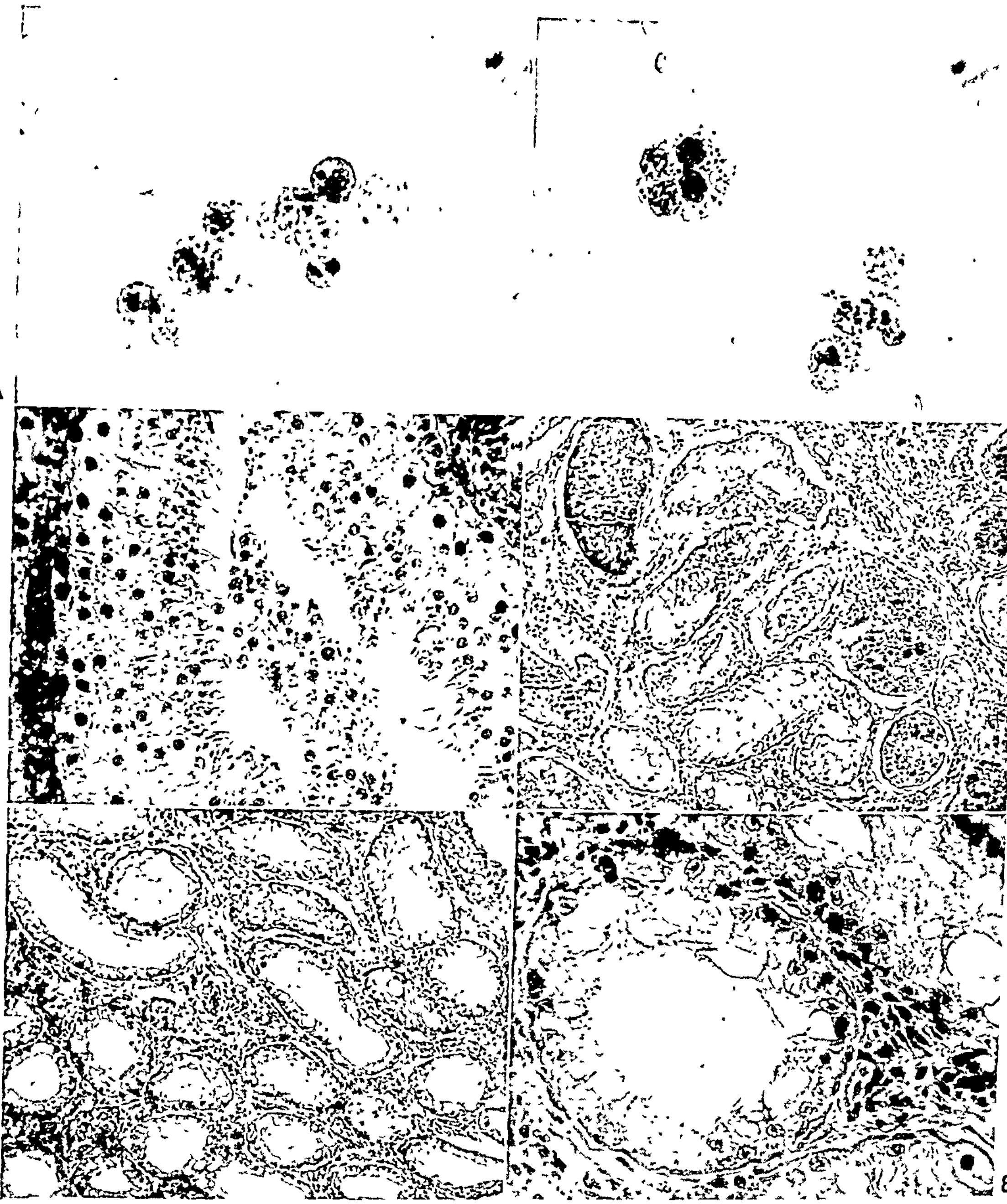


Fig. 5. A, B, multinucleated, granular cells in semen of subfertile bull. (× 430)
Fig. 6. Testicular biopsy specimen from Bull 18 showing normal morphology. (× 200)
Fig. 7. Autopsy specimen taken 40 days later from near biopsy site in same animal. Extensive degeneration with numerous seminiferous tubules showing coagulation necrosis. (× 50) **Fig. 8.** A, appearance of remainder of testis of same animal at autopsy showing widespread hydropic degeneration and vacuolization of germinal epithelium. (× 50) B, same. (× 200)

Fig. 9. A, needle puncture biopsy of Bull P. showing normal morphology (× 50) B, same (× 200) Fig. 10. A, autopsy specimen from near biopsy site in Bull P. taken 21 days after needle biopsy showing fibrosis and degeneration, severe hypoplasia, and vacuolization of germinal epithelium B, section distant from biopsy site showing the generalized degenerative changes described in previous figures (× 50) Fig. 11. A, higher magnification of seminiferous tubules in Bull P. Note heavily granulated, multinucleated spherical cells in lumen. B, sloughed spherical granulated cells in lumen of seminiferous tubule (× 200)

cells increased, sex drive remained normal. The biopsy specimen obtained from the right testis (Fig. 12) shows only a few normally functioning tubules; the others appear to be in process of degeneration. Interstitial connective tissue was increased containing nests of normally appearing Leydig cells. Sperm concentration decreased by 90 per cent during the next 2 months and was near normal again 8 months after biopsy.

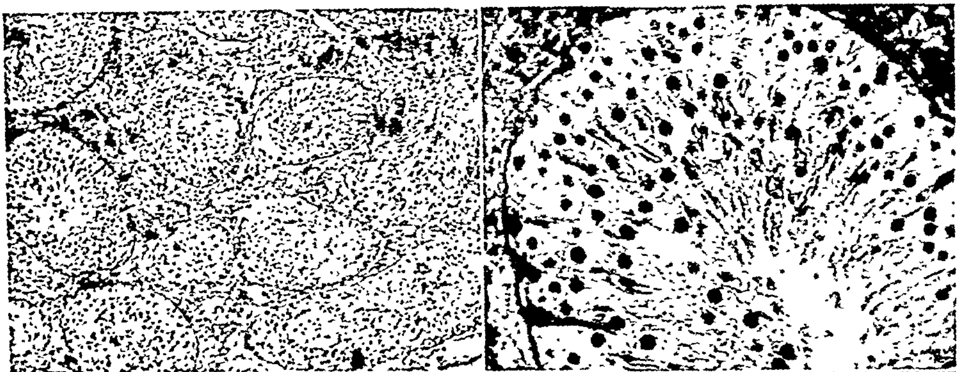
Biopsy of the left testis at this time resulted in a total azoospermia within 30 days. The histologic appearance of the second biopsy specimen was quite similar to that of the section obtained 8 months previously. Since the animal remained aspermic for 2 months it was slaughtered.

Grossly, the testes were small and about one-half the original size. The right testis, biopsied 10 months before slaughter, showed a narrow, fibrotic lesion indicating the biopsy site. The left testis, however, biopsied 2 months previously showed more extensive necrosis at the site of surgery (Fig. 13).

HISTOLOGY. Histologically, there was no sign of spermatogenesis in either testis, whether biopsied 10 months or 2 months before, which indicates that the recovery in sperm production seen 4 months after the right testis was biopsied was due to the activity of the left testis before it was sampled 2 months before slaughter.[7] The fact that sperm concentration in semen was reduced by 90 per cent after the first biopsy indicates that surgical insult to one gonad may well affect the functioning status of the other. This would support observations made by Sykes *et al.*[10]

While the biopsy site in the right testis was indicated only by fibrosis the remaining testicular tissue showed atrophied, sclerotic tubules without spermatogenic cells except Sertoli cells. The tubular basement membrane and tunica propria were thickened. Prominent nests and cords of apparently functional Leydig cells were interspersed among the increased interstitial stroma (Fig. 14). There is no indication that this testis had recovered from the effects of biopsy 10 months previously although it is possible that if repair had occurred it was canceled by the adverse effects of the second biopsy of the opposite testis 2 months before slaughter.

Histologic appearance of the section taken from the biopsy site of the left testis was entirely similar to that described for the other bulls. The remainder of the gonad showed the same changes as noted in the right testis. Of special interest was what appeared to be a marked hypertrophy and, possibly, hyperplasia of the interstitial cells of Leydig. Severe atrophy and loss of tubular tissue are usually responsible for aggregation of Leydig



2A 12B

13A 13B

14A 14B

Fig. 12. A, few normal tubules, others showing various stages of degeneration (Bull CSH). Leydig cell nests with normal cells. (× 50) B, normal seminiferous tubule. (× 200) Fig. 13. A, sectioned right testis of Bull CSH. Note fibrotic lesions remaining 10 months after biopsy. B, left testis. Note the more extensive necrosis of this gonad 2 months after biopsy. Fig. 14. A, right testis of Bull CSH showing tubular atrophy and sclerosis with thickened stroma and accumulation of Leydig cells. (× 50) B, atrophic tubule associated with interstitial cell hyperplasia. (× 200)

cells in large clumps, giving the impression that the units of this system had increased in quantity. In this particular case, however, the Leydig cells were so numerous and enlarged that the hypertrophy and hyperplasia can be considered real and not accounted for merely by atrophy of the tubular parenchyma (Fig. 15*A*). Most of these polygonal or polyhedral cells with good nuclear structure and considerable perinuclear granulation appeared to be functioning normally (Fig. 15*B*). This judgment was verified by the fact that seminal fructose content was within normal range, as was the sex drive. It has been shown that these functions are regulated by testicular steroids and are positively correlated with the secretory status of the Leydig cell system.[3, 6, 9]

Bull BH. Bull BH was severely oligospermic at the time of biopsy, with sperm of poor motility. Histologic appearance of the biopsy specimen was similar to that shown for Bull CSH, except that necrosis and hypoplasia were more pronounced. Upon autopsy 19 days later, the biopsied testis showed the same postoperative damage described earlier, while the unbiopsied testis appeared relatively normal and similar to the other testis at the time of biopsy. There is no definite indication that the remainnig testis was affected by surgical interference with the opposite gland—possibly because too short a time had elapsed between biopsy and slaughter.

Azoospermic Bulls

Bulls TH and 24. Both animals had a record of total breeding failure and were found to be aspermic. Since seminal fructose content and sex drive were normal, both animals were considered to suffer from primary spermatogenic failure. Endocrine therapy consisting of periodic administration of FSH, APL, and testosterone,* separately or in combination, was unsuccessful. In each case the appearance of the biopsy specimen of the right testis verified the original diagnosis of testicular failure (Figs. 16 and 17).

SUMMARY AND CONCLUSIONS

1. Histopathologic studies of testicular tissue obtained by the open biopsy method and at subsequent slaughter of 9 bulls aged 2 to 7 years

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* FSH courtesy Armour Laboratories; APL courtesy Ayers & McKenna and Harrison; testosterone courtesy Ciba Pharmaceutical Products, Inc.

15A 15

16 17

Fig. 15. A, section of left testis of Bull CSH obtained away from the biopsy site showing extensive tubular degeneration with Leydig cell hyperplasia and hypertrophy. (× 50) B, same at higher magnification. Note the prominent Leydig cells. (× 200) Fig. 16. Typical appearance of testicular biopsy specimen showing testis failure (Bull TH). (× 50) Fig. 17. Biopsy specimen indicating azoospermia (Bull 24). (× 200)

have been reported. One bull studied was subjected to biopsy by needle puncture.

2. Within less than 3 weeks after biopsy extensive coagulation necrosis was evident near the biopsy site, while other areas showed a generalized hypoplasia of the seminiferous tubules with hydropic degeneration and vacuolization of the germinal epithelium. This condition was prevalent in the testes of all bulls subjected to the open surgical biopsy technic as well as in the bull biopsied by needle puncture.

3. Many multinucleated granular cells resembling giant cells in various stages of degeneration were observed in the lumina of tubules. Their appearance in semen ejaculates of bulls showing testicular degeneration, either from spontaneous causes or experimentally induced, has been reported in a previous paper.[7] They apparently originate from the germinal epithelium by syncytial confluence of degenerating spermatogonia, spermatocytes, or spermatids, and their presence in semen may be used as another criterion in the diagnosis of infertility.

4. A marked hypertrophy and, possibly, hyperplasia of Leydig cells were noted in sections of testes taken at autopsy of a subnormal bull which had been subjected to biopsy of the right and left testicles at 10 months and 2 months respectively prior to slaughter. Since this response was concurrent with a total loss of spermatogenic elements, it may be considered of a compensatory nature.

5. There is some indication that surgical interference with 1 testis may adversely affect the spermatogenic function of the opposite testis, possibly for a relatively short time only. The unbiopsied gland subsequently may make a compensatory rebound in spermatogenesis, resulting in improvement of semen quality. Further work is required to confirm these indications.

6. No repair of the degenerative germinal epithelium was evident in autopsy sections taken from a monorchid bull as long as 40 days after biopsy. Semen quality data obtained during that time were in line with this finding. Likewise, no recovery was seen in autopsy sections of a testis of another bull which was biopsied 10 months earlier. Since semen quality improved to the presurgical level within 8 months, it is evident that the opposite testis was responsible for this recovery. (Semen quality of 1 normal, unilaterally biopsied bull was beginning to improve 68 days after surgery.)

It appears that recovery from testicular biopsy in the bull does not begin before at least 2 months after surgical interference with testis structure and requires several months more before showing an acceptable degree of improvement in quality of semen.

7. The heat-insulating effect of a peritesticular blood clot often attendant to open biopsy of the bovine testis is suggested as one of the chief reasons that semen quality declines so rapidly and is associated with generalized degeneration of spermatogenic tissue. It has been shown, however, that in the absence of a clot surgical insult alone may play an equally important role in bringing about tubular failure, indicating that the bovine testis is unusually labile to any mechanical interference with its structural integrity.

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Endometrial Aspiration Smears in the Study of Infertility

Further Observations

George H. Romberg, M.D.

In a previous paper[3] published in this journal cyclic cytologic changes in the endometrium, as obtained by the new technic of endometrial aspiration, were described. These investigations were done in cases of infertile women with normal and abnormal menstrual cycles. Because these studies involved basic research which was reported for the first time, it would seem advisable, prior to elaborating further on the endometrial cytology, to review briefly the pertinent findings of that report.

In the original work, daily or frequent samplings in the same patient showed that in the normal menstrual cycle there is a natural variability of endometrial cytologic findings seen at each stage within the same smear, but that the dominant pattern is sufficiently characteristic at all times to permit a recognition of the functional state of the endometrium.

The cytologic approach to the study of the endometrium revealed a wealth of information throughout the cycle, not heretofore tapped by the conventional biopsy, and in two major areas particularly, disclosed endometrial findings of special interest. These concerned (1) perinuclear secretory changes in the glandular cells, and (2) significant hypertrophic changes in the stromal cells, both of these changes occurring in the preovulatory and ovulatory phases of normal menstrual cycles. The explanation for these findings in aspiration smears was felt to be due to the better preservation of

Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility at San Francisco, California, June 18, 19, and 20, 1954.

I wish to express my thanks to Ayerst Laboratories, New York, N. Y., for their assistance with the publishing of the color plates.

cells as obtained by the cytologic technic and the elimination of histologic artifacts which in part are caused by the coarser methods of handling tissue cells in biopsies involving both heating and freezing.

PRESENT STUDY

The present report is based on a total experience of more than 2500 endometrial aspiration smears. The findings to be presented are limited to a detailed account of 5 cases of infertile women covering a period of several months in each instance.

In the first case, studies in a patient with normal menstrual cycles covering a year's study will be described. In the other 4 cases, observations will include endometrial cytologic findings before and after hormonal therapy in infertile women with abnormal cycles. Correlations with endometrial biopsies and basal temperature graphs will be given.

Case 1

The first case concerns a 27-year-old woman whose chief complaint was infertility of 5 years' duration. To date, repeated attempts at relieving her tubal occlusion have been unsuccessful. Her menstrual cycles have been regular, occurring every 27–29 days.

Figure 1 shows partial temperature graphs of six cycles, covering a year's study, indicating a normal thermal shift in each cycle occurring at the time of ovulation. The accompanying illustrations demonstrate normal progressive glandular and stromal cell changes in the preovulatory, ovulatory, and postovulatory phases of two of these cycles, and are typical of the cytologic pattern found in all of them.

On Day 11 of the normal 29-day cycle, 2 days before the low point of the thermal shift (as seen in Fig. 1), mature acidophilic glandular cells with well-developed perinuclear vacuoles, as illustrated in Fig. 2 (Figs. 2–7 in color, p. 309), are found in abundance. The cells in this preovulatory stage are larger and taller than those seen in the early proliferative phase.

The majority of these cells have acidophilic staining cytoplasm. The basophilic forms decrease progressively and are found only in small numbers during the normal luteal phase. The perinuclear vacuoles increase appreciably in size, often occupying about a third of the basilar portion of the cell in the preovulatory phase and retain their limiting vacuole membrane in most instances up to the point of ovulation.

The glandular nuclei are basophilic and granular. They present variable shapes due to the pressure of the secretion within the vacuole. Usually the surface in contact with the vacuole is concave giving a crescent or cuplike appearance to the nucleus. The usual aspiration from the superficial endometrial surface yields predominantly glandular epithelial cells.

The stromal cells (Fig. 3) are also from Day 11 of the menstrual cycle. To obtain adequate sampling of the stromal cells, slightly more pull is exerted in the aspirating process. In the preovulatory phase, the stromal cells are found to be larger than those seen in the early proliferative phase and usually contain two or three prominent chromatin clumps. The stromal cytoplasmic processes take an acidophilic stain.

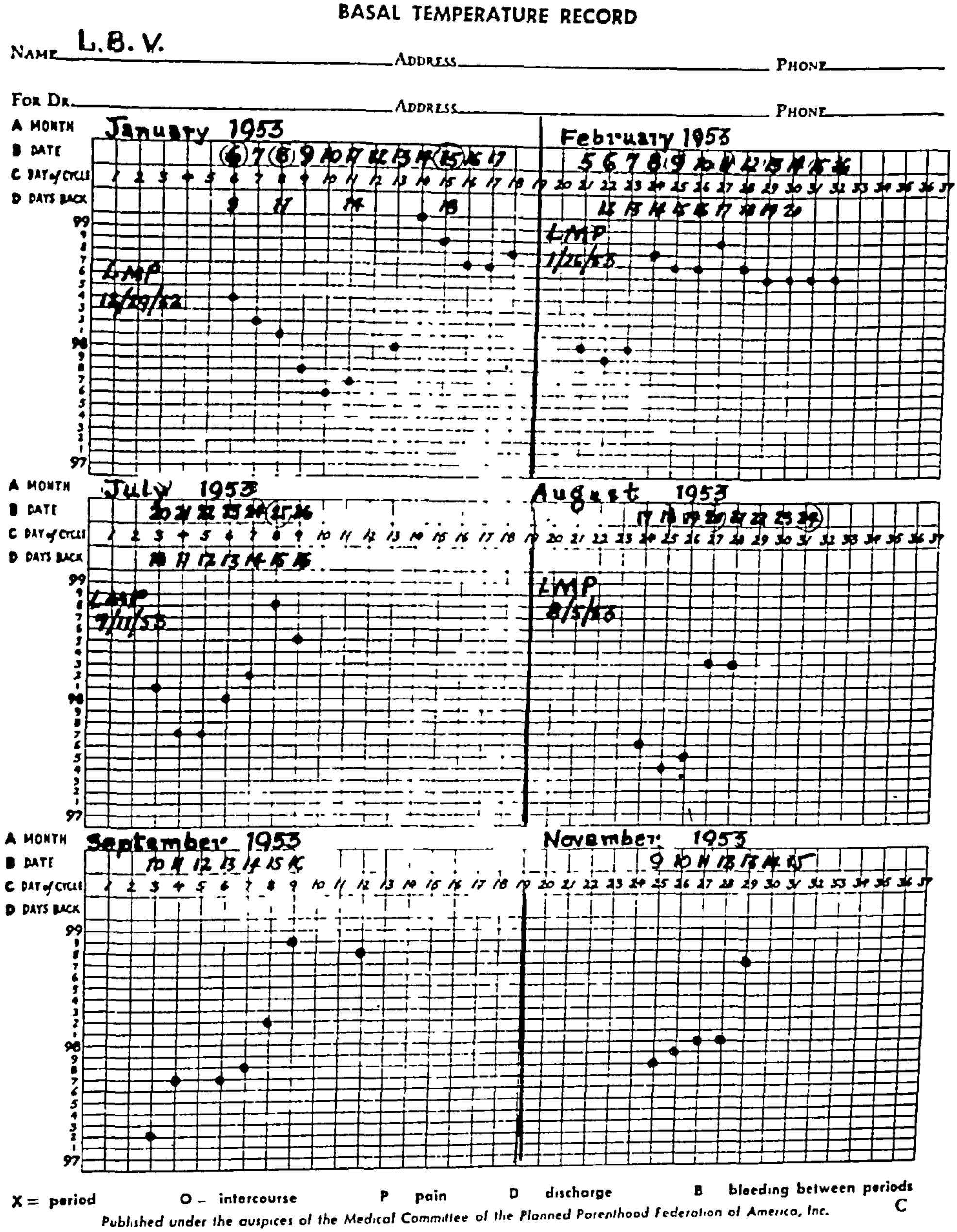

Fig. 1. Case 1. Partial basal temperature graphs of six cycles showing normal thermal shifts at time of ovulation.

The endometrial biopsy (Fig. 4) obtained on the same day from a fragment in the endometrial aspirations, was described by the pathologist as showing normal proliferative endometrium. Note the smaller size of the glandular cells in the biopsy and the absence of any sign of secretory activity which was so clearly seen in the cytologic smears. The hyperchromatic appearance of the stromal and glandular nuclei in the microscopic section is probably due to shrinking action produced by the biopsy technic.

On Day 14, the day of ovulation (Fig. 5) here taken in the second cycle, we see that a progressive increase in the size of the stromal nuclei associated with anisonucleosis has occurred. Note also the increase in the nuclear chromatin which gives the appearance of hyperchromasia. These changes seem to parallel the degree of estrogen stimulation and to correspond to the peak of the thermal shift. A relative increase in stromal edema is often noted at this stage, and is reflected by a paler staining of the stromal cytoplasm and by a greater separation between the nuclei.

Similarly on the day of ovulation, interesting changes are noted in the glandular cells. The perinuclear vacuoles which had developed to the bursting point in the preovulatory phase now are found ruptured in many cells. Characteristically, one sees decreasing numbers of residual perinuclear vacuoles immediately after ovulation with evidence of diffusion of the glandular secretion toward the luminal end of the cell. The majority of nuclei at this stage are located in the basilar position of the glandular cell.

By Day 17 (Fig. 6), which reflects the height of the secretory activity, the glandular cells are larger and broader, pushing the nuclei into a more basilar position, where they appear crescentic in shape. Frequent mitoses are found throughout this phase of the cycle. Special glycogen staining of the acidophilic glandular cells in the immediate postovulatory phase shows increased glycogen content compared to that found in the proliferative phase of the menstrual cycle. Following the peak of secretory activity the glandular cells appear smaller and the cytoplasm shows a less intense acidophilic staining reaction and a decreased glycogen content (Fig. 7). If conception occurs one will still see this type of glandular cell at the time of expected menses instead of the usual regressive glandular changes found in the premenstrual phase.

Case 2

The second case is that of a 27-year-old woman with involuntary infertility of 3 years' duration. Except for occasional hypomenorrhea, her cycles were normal and regular, occurring every 27–30 days. Due to the husband's azoospermia and his unwillingness to consent to donor insemination, this patient, to date, has not conceived.

The temperature graph in Fig. 8 shows a disturbed 47-day cycle with a delayed thermal shift due to a severe virus infection contracted during the menstrual period, and the following menstrual cycle of 29 days. Although the endometrial biopsy, taken at the onset of the next menstrual period, was interpreted as normal secretory endometrium, the cytology of this prolonged cycle shows an abnormal pattern as contrasted with the more normal one found in the following 29-day cycle. Actually it takes a minimum of two to three cycles before the cytologic pattern returns to normal. Two photomicrographs from the disturbed cycle and two from the ensuing cycle are illustrated in Figs. 9–12 (Figs. 9 and 10 in color, p. 309).

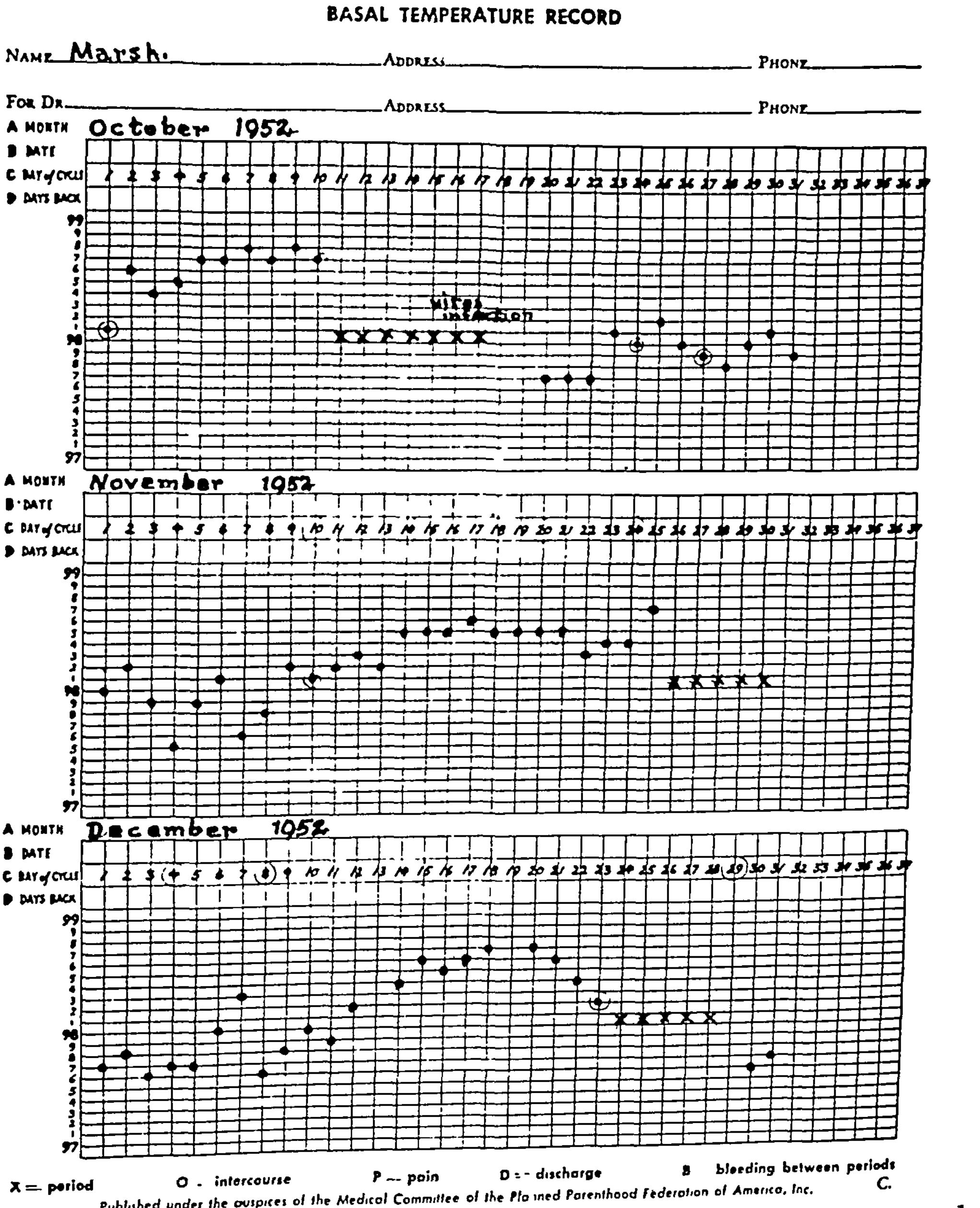

Fig. 8. Case 2. Basal temperature graph of prolonged menstrual cycle with delayed thermal shift.

In Figure 9, from the 47-day cycle and 12 days prior to the onset of menstruation, one sees glandular cells showing a disturbed pattern characterized by their small size and evidence of decreased secretory activity. The nuclei are small and less granular than normal. The cytoplasm shows very faint vacuolization and a mixed basophilic acidophilic staining reaction.

Two days prior to menstruation, in this same 47-day cycle (Fig. 10) the glandular cells are definitely larger and relatively more mature, but they still show a disturbed pattern as evidenced by the basophilic staining reaction. The nuclei are basally located, granular, and show evidence of compression due to the secretion in the perinuclear vacuoles. Most of the cells show a faint acidophilic and a more marked basophilic staining reaction in the cytoplasm. No pregnancy occurred in any patient under study where the basophilic type of cytoplasm predominated in the luteal phase. The finding of numerous basophilic-staining glandular cells in the latter half of the menstrual cycle can be explained by the decreased glycogen content in the glandular cells and endometrial secretions, which was corroborated by special glycogen stains. In oligomenorrhea one sometimes sees the delayed appearance of the perinuclear vacuoles in the glandular cells occurring in basophilic forms. The finding of numerous supranuclear vacuoles at this stage is not clear, but suggestive.

On Day 13 of the ensuing cycle (Fig. 11), corresponding to the low point of the thermal shift in the temperature graph, we see a cluster of normal acidophilic glandular cells with well-defined perinuclear vacuoles and compressed crescent-shaped nuclei usually seen just at the time of ovulation.

The smear taken a week later, 9 days before the onset of menstruation (Fig. 12), shows the normal glandular pattern expected at this time of the cycle as evidenced by the acidophilic cytoplasm, the basally located, cup-shaped granular nuclei, and the increased amount of acidophilic staining secretion in the background.

Case 3

The third woman studied was 31 years of age with 3 years of involuntary infertility. Her cycles were normal, occurring every 28–30 days with occasional episodes of hypomenorrhea and oligomenorrhea which were psychogenic in origin.

Figure 13 demonstrates her basal temperature graphs covering three cycles, including a disturbed one of 44 days' duration. Although within this 44-day cycle there are two apparent biphasic temperature curves 20 and 24 days respectively joined together, there was no intervening menstruation and the pregnancy test done on Day 42 was negative. The preceding menstrual cycle, at the top of the chart, shows a somewhat atypical temperature graph characterized by a very sluggish thermal shift.

Figure 14, taken 4 days before the onset of menstruation, of this first cycle, which was associated with the sluggish thermal shift, shows some of the tall, narrow basophilic glandular cells found in the disturbed cytologic glandular

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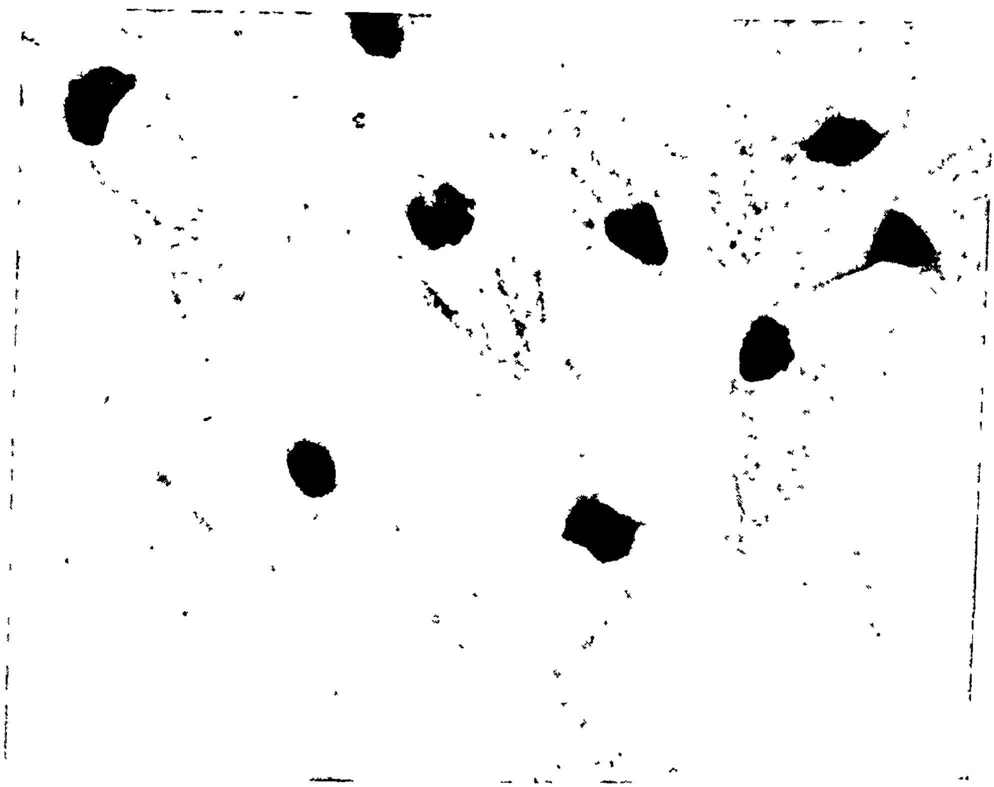

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Fig. 11. Case 2. Day 13. Normal glandular cells at time of ovulation.
Fig. 12. Case 2. Nine days premenstrual. Normal glandular pattern.

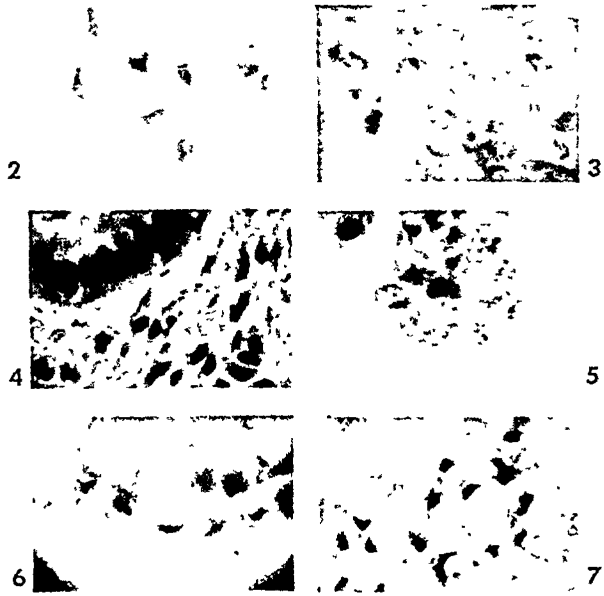

Fig. 2. Case 1. Day 11. Normal glandular pattern. Fig. 3. Case 1. Day 11. Normal stromal cells. Fig. 4. Case 1. Day 11. Biopsy, normal proliferative phase. Fig. 5. Case 1. Day 14. Normal stromal nuclei. Fig. 6. Case 1. Day 17. Normal glandular cells at height of secretory activity. Fig. 7. Case 1. Normal glandular cells in mid-luteal phase of cycle.

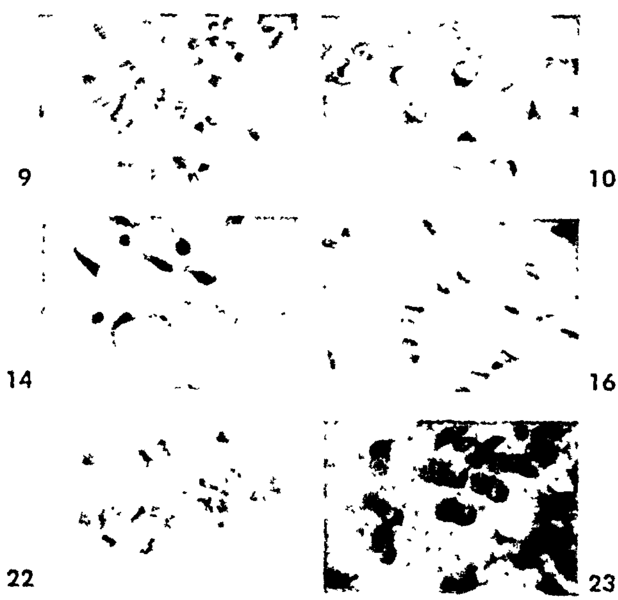

Fig. 9. Case 2. Twelve days premenstrual. Abnormal glandular cells. Fig. 10. Case 2. Two days premenstrual. Abnormal glandular pattern. Fig. 14. Case 3. Four days premenstrual. Abnormal glandular cells. Fig. 16. Case 3. Eight days premenstrual. Slightly atypical nuclei of glandular cells. Fig. 22. Case 5. Eight days premenstrual. Abnormal glandular cells. Fig. 23. Case 5. Day 13. Day of ovulation.

pattern. The nuclei are basally located and granular (Fig. 14 in color, p. 309). The endometrial biopsy taken a few days later, at the onset of menstruation, showed a secretory endometrium with evidence of deficient progesterone stimulation.

In Fig. 15, taken on Day 14 of the prolonged 44-day cycle, and corresponding to the first postovulatory thermal shift, there is a cluster of relatively small but

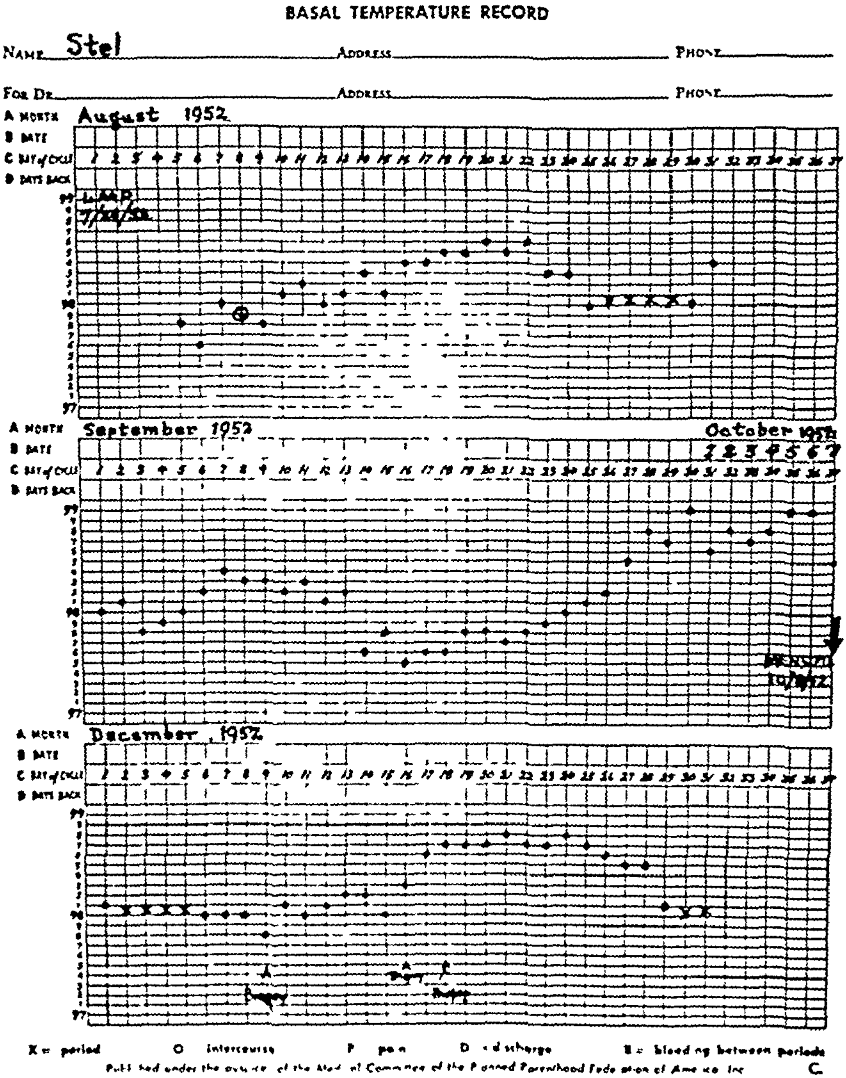

Fig. 13. Case 3 Basal temperature graphs showing disturbed cycle of 44 days' duration which contains two biphasic temperature curves without intervening menstruation.

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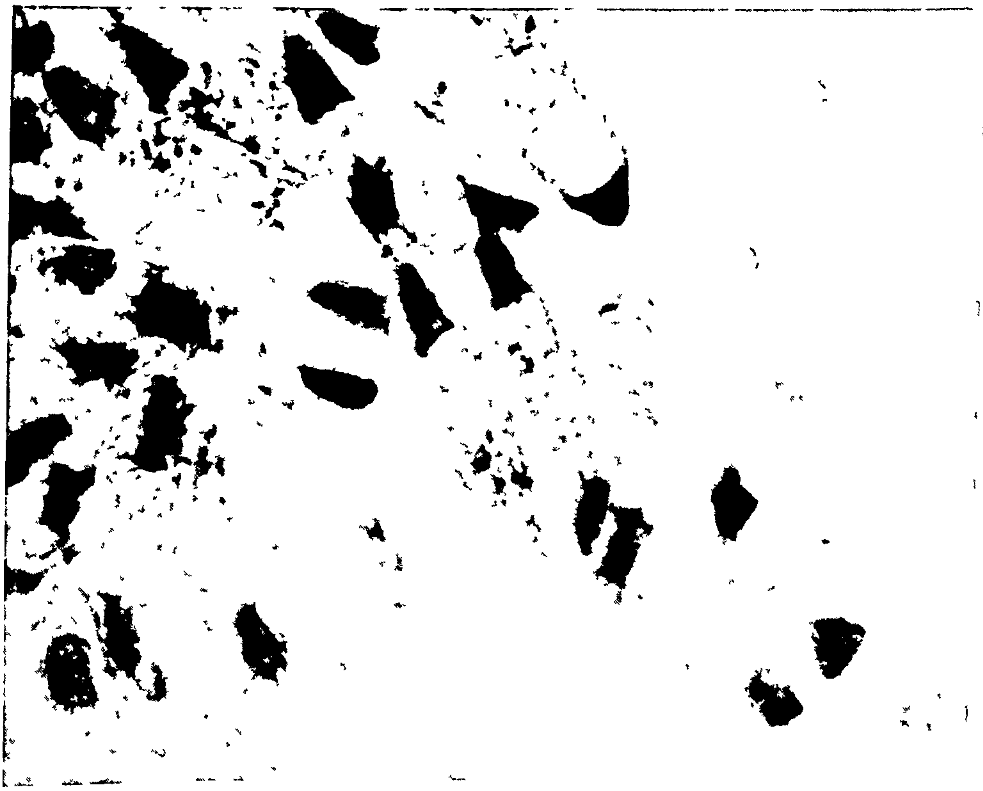

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Fig. 15. Case 3. Day 14. Cluster of acidophilic glandular cells corresponding to the first postovulatory shift in abnormal 44-day cycle. **Fig. 17.** Case 3. Day 15. Glandular cells showing residual perinuclear vacuoles.

mature acidophilic glandular cells showing evidence of secretory activity. These, however, contrasted markedly with the more numerous basophilic forms found in the cytologic pattern of this date.

In the smear taken 8 days before the onset of menstruation in this same 44-day cycle (Fig. 16) and corresponding to the second postovulatory thermal shift, there are glandular cells which at first glance appear to be normal, but on closer inspec-

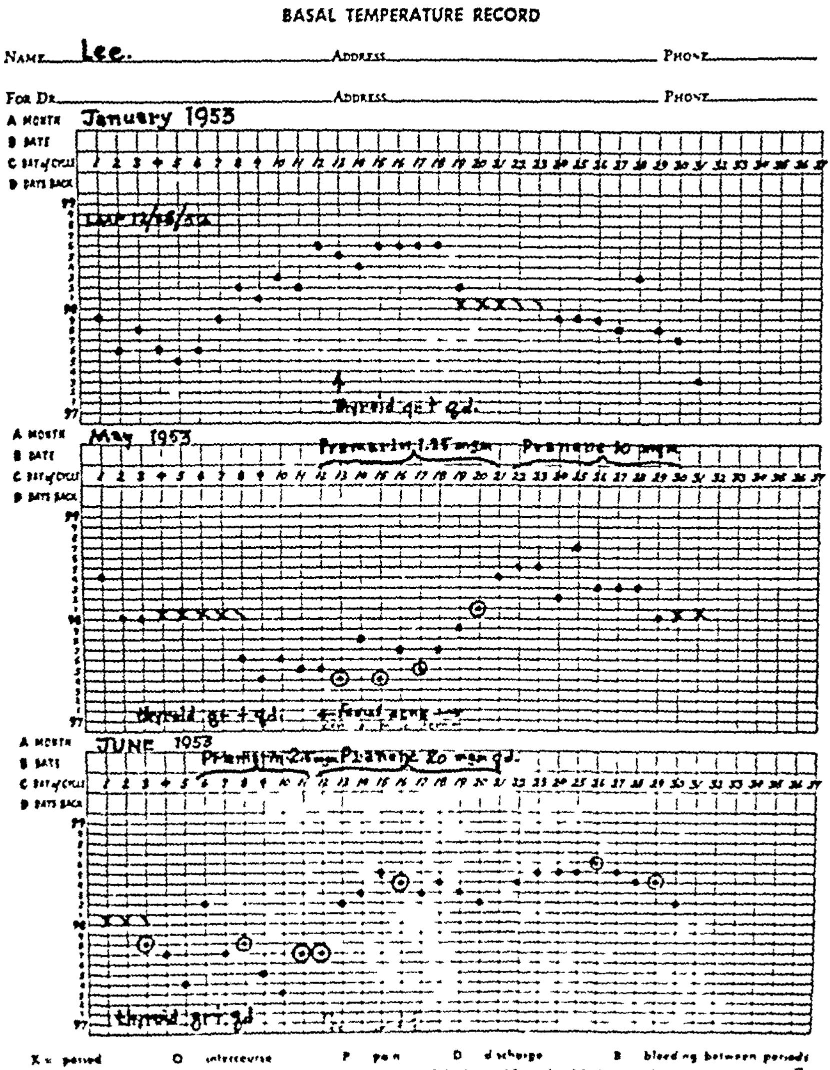


Fig. 18. Case 4. Basal temperature graphs showing progressive improvement following hormonal therapy.

tion show some atypical nuclei and a mixed basophilic-acidophilic–staining cytoplasm. Some of the glandular nuclei are tear-shaped and show clear centers with the chromatin concentrated at the nuclear border. (Fig. 16 in color, p. 309.)

Following sedation and superficial psychotherapy, the ensuing cycle returned to normal. On Day 15, the day of ovulation of this 29-day cycle, the glandular cells (Fig. 17) showed evidence of increased nuclear granularity and of residual perinuclear vacuoles which were not apparent in the endometrial biopsy until 48 hours later.

This patient became pregnant 4 months later and had an uneventful course.

Case 4

The next case concerns a 29-year-old woman with involuntary infertility of 4 years' duration. Previous infertility investigations had disclosed a hormonal imbalance and a low basal metabolism. Subsequent cyclic hormonal therapy had been given without resulting pregnancy.

Figure 18 shows the basal temperature graphs from her case. These are all biphasic in type and show progressive improvement following hormonal therapy. In the second cycle shown, in addition to thyroid therapy, conjugated estrogen* was given from Day 9 through Day 18, and progesterone† was given from Day 19 to Day 27 of the cycle. In the following cycle, the dosage of estrogens was doubled and given one day earlier, on Day 8, and continued through Day 13, and progesterone was given from Day 14 through Day 23. Conception apparently occurred between Days 13 and 14 of this cycle, and a healthy 3630-Gm. baby girl was delivered nine months later.

Figure 19, taken on Day 12 of the second cycle after 4 days of oral estrogen therapy, shows some of the anisocytosis seen in the glandular pattern. Note the variation in size of the cells, nuclei, and perinuclear vacuoles. This mild atypia improved after further hormonal therapy.

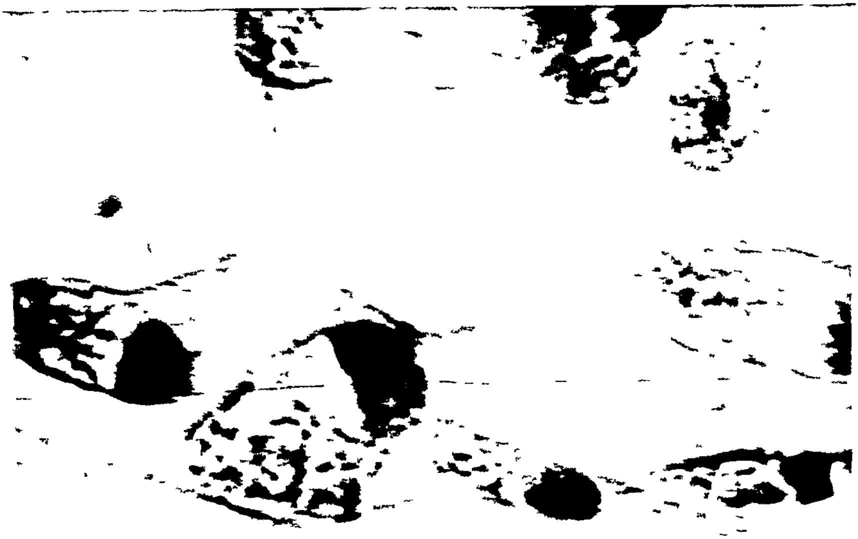
Figure 20 is from the aspiration smear taken on Day 13 of the succeeding cycle. after 6 days of oral estrogen therapy. These show more normal glandular cells as evidenced by the increased acidophilia of the cytoplasm and the more uniform appearance of the nuclear findings. Here again we have another instance of increased acidophilic reaction following estrogen therapy.

Case 5

The final case concerns a 33-year-old woman who had been married for 13 years. She had been referred to me for donor insemination because of the husband's proven azoospermia. Her menstrual cycles showed occasional hypomenorrhea and oligomenorrhea. The basal metabolic rate was low and her blood cholesterol was 400 mg.

All the temperature graphs in her case (Fig. 21) are biphasic in character, showing some irregularity in the thermal shift. They cover a period of 11 months.

* Premarin, 1.25 mg.
† Pranone, 10 mg.



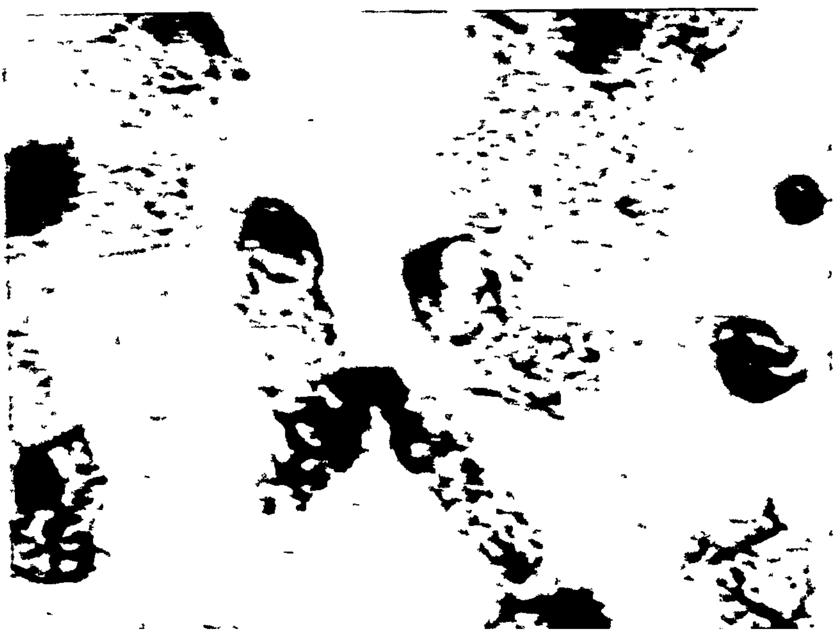


Figure 22 is from the endometrial smear taken 8 days before menstruation and before thyroid therapy was started, and shows atypical bichrome staining reaction in the cytoplasm and atypical vesicular glandular nuclei. Compare these findings with those obtained in a later cycle after thyroid and estrogen therapy had been given. (Figs. 22 and 23 *in color,* p. 309.)

After 6 months of intermittent insemination procedures she became pregnant,

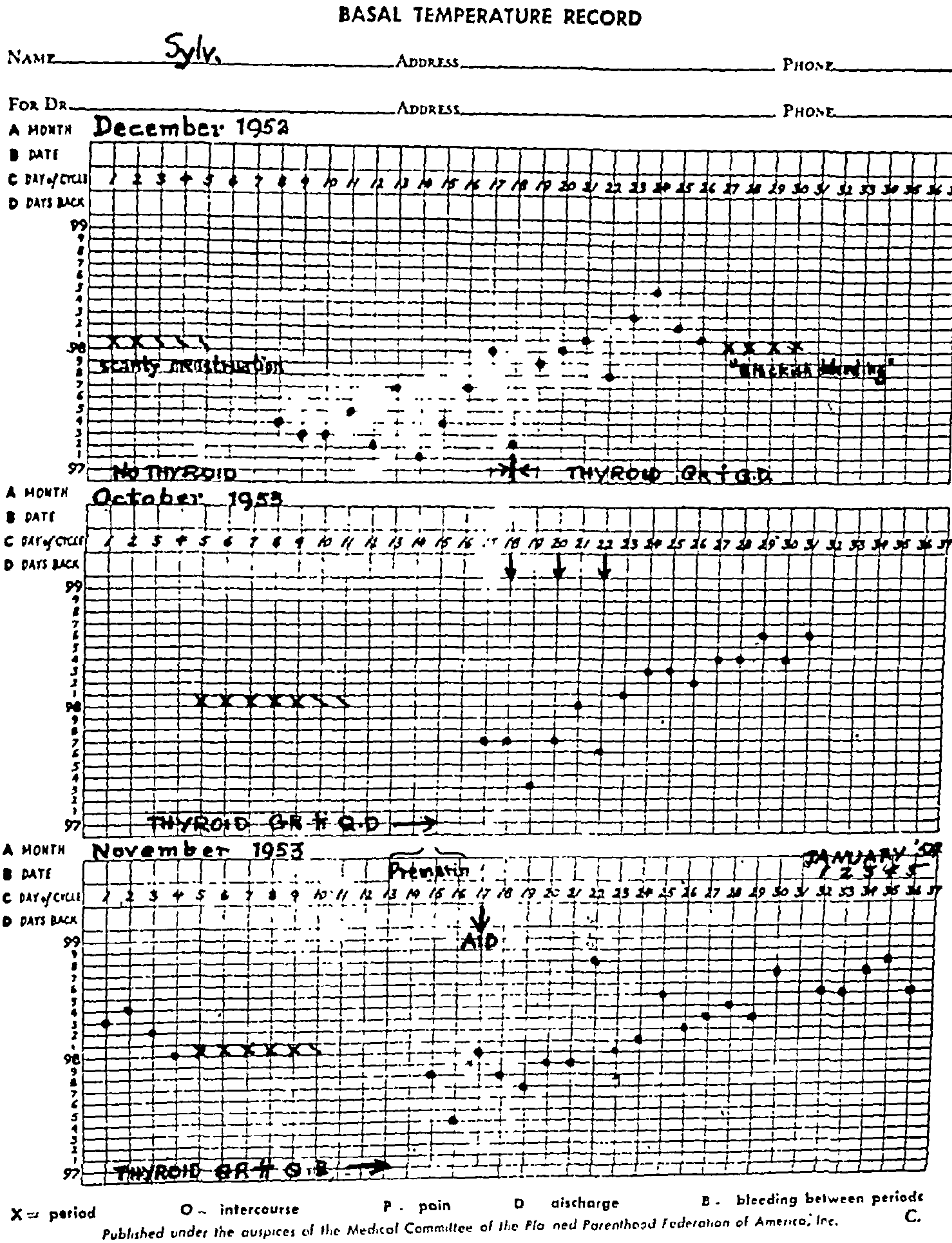


Fig. 21. Case 5. Basal temperature graphs with days of donor insemination indicated by arrows.

but aborted after two months. Four months later she returned for further insemination procedures which were done on Days 14, 16, and 18. These were followed by menstruation on Day 32.

The following month conjugated estrogen* was given daily on Days 9 through 12, and a single insemination was done on Day 13 which resulted in pregnancy. Figure 23 is from the endometrial aspiration smear taken earlier that same day, about 6 hours prior to insemination. Note the acidophilic cytoplasm, the granular,

Fig. 24. Case 5. Day 22. Ten days postinsemination.

eccentrically compressed nuclei and the fully formed perinuclear vacuoles in the glandular cells which are found in large numbers at the time of ovulation.

Figure 24 illustrates the glandular cells from the aspiration smears taken on Day 22 of the cycle, 10 days after the single insemination was done. They show normal nuclei and normal cytoplasmic findings. This patient had an uneventful pregnancy and was delivered of a normal 3175-Gm. baby girl at term.

CONCLUSIONS

This report on the further study of the endometrium as obtained by the new technic of endometrial aspiration smears concerns 5 case histories of

* Premarin, 1.25 mg

infertile women covering a period of several months in each instance. Evidence of characteristic secretory changes in the glandular cells in the preovulatory and ovulatory phases of the normal menstrual cycles is demonstrated. The cytologic response in the endometrium following booster estrogen therapy is reflected in very impressive changes in the glandular epithelial cells. Glycopenia of the glandular cells and endometrial secretions is suggested by the finding of basophilic-staining reactions in aspiration smears, obtained in cycles associated with hormonal imbalance and psychogenic disturbance. Special staining procedures corroborate the decreased glycogen content in the basophilic cells. In some problem cases, where the biopsy and basal temperature graphs are normal, the endometrial smear is useful in disclosing latent hormonal abnormalities. Endometrial aspiration smears continue to show promise of becoming a valuable adjunct in the clinical and research study of infertility.

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DISCUSSION

Dr. W. H. Masters, *St. Louis, Missouri:* Dr. Romberg is taking significant steps to popularize his endometrial aspiration technic, from not only a morphologic but also from a definitive cytologic approach. Certainly the cellular delineation we have just seen and heard described, merits the attention of cytologists in the field of obstetrics and gynecology. If the technic stands the test of widespread evaluation, a valuable addition to our armamentarium is available.

Of particular interest to the endocrinologist is the potential of this technic as a method of evaluating the humoral influences reflected upon the uterine endometrium. The essence of one of our greatest unsolved problems is the complete riddle of endometrial function. Do the ovarian steroids influence the endometrium directly? Do the products of the endometrial glands, acting as catalysts, after this influence in any way? Are there significant humoral entities produced by the endometrial glands themselves?

Our knowledge of endometrial function and physiology is so limited, we can only hail with complete satisfaction any technic that may in any way shed light on this frustrating problem. Certainly it is true that one of our elemental approaches to these important questions must be from a cytochemical point of view. If Dr. Romberg's technic can be successfully used by others to provide convincing evidence of basic cytologic alteration in the endometrium, then he may have made a contribution far beyond the limited scope he presently suggests.

The ultimate widespread clinical use of this weapon is, of course, limited by the availability of basic cytologic training, but the potential of this technic from an investigative point of view is significant.

DR. ROBERT LYON, *San Francisco, California:* I would like to ask Dr. Romberg at what time do the changes of the endometrium occur? Is there a lag from the time of ovulation until you can predict changes in the endometrial cells?

DR. GEORGE E. NORWOOD, *San Marino, California:* What technic is used?

DR. ROMBERG: First, I would like to thank Dr. Masters for discussing this paper and for the very kind remarks he made. Dr. Lyon, I am afraid I am not quite clear about your question. As I understand it, you want to know if there is a lag between ovulation and . . .

DR. LYON: The time glycogen appeared.

DR. ROMBERG: No. I had better explain about that glycogen business. I was naturally very puzzled when I would find this basophilic type of staining, and in looking up the literature I noted that Dr. Zondek, in 1940 or some such time, had alluded to glycopenia. In the paper I thought I made it clear that I have done no glycogen studies because of the time element, and because I understood that in Dr. Papanicolaou's laboratory the smears are allowed to dry before his stain technic is used. You wanted to know about the glycogen. I assume the basophilic reaction was due to decreased glycogen. I have no special stains to prove that, but that is my assumption. Would you like me to answer any more of that question? Have I answered it?

As far as Dr. Norwood's question is concerned, the Papanicolaou technic is used throughout. I found early in the game it was very difficult to do this work under hospital conditions. You follow a patient a day or two and then she is in the operating room. It had to be done under conditions where I could encourage her to come back and not cause her harm or pain. I also found I had to control the situation through the staining and the taking of the smears, right through to the interpretation.

The technic of endometrial aspiration smears for diagnosis of cancer of the fundus is a simple one, and can be rather coarse. In this hormonal work the technic must be very meticulous. I feel, however, that any gynecologist familiar with the procedure would have no difficulty. Thank you again very much.

Failure of Conception in 100 Completely Studied Couples

Analysis of Factors in Infertility

Fred A. Simmons, M.D., and Melvin L. Taymor, M.D.

Textbooks on sterility and journals devoting articles to that specialty within a specialty are replete with reports of successful "cures." The definition of the term "cure" is often obscure. Some authors state that the physician can rarely take credit for the successful solution of an infertility problem, as many of them solve themselves, given enough time. Little has been published on the apparent failure of conception in carefully studied cases.

This report, which must necessarily be labeled preliminary, concerns 100 couples completely studied, in most instances, according to the minimum diagnostic program introduced by the American Society for the Study of Sterility.

It is apparent at once that the significance of the results obtained in this study of 100 unsuccessfully privately treated sterile couples will be of much more value when it can be compared with 100 privately treated couples treated successfully. In this sense this material is strictly preliminary.

MATERIAL

The 100 couples were almost all native white American from the northeastern United States. There were no Negroes, the inclusion of whom un-

From the Sterility Clinic of the Ovarian Dysfunction Clinic, Massachusetts General Hospital, Boston, Mass.

Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility at San Francisco, California, June 18, 19, and 20, 1954.

doubtedly influences figures reported from such large urban populations as Philadelphia, Chicago, New Orleans, and certain clinics in New York. In our institution—the Massachusetts General Hospital sterility and endocrine clinic—the incidence of Negro patients does not amount to more than 2 per cent of the registered patients. These 100 couples are unselected except that the studies were usually complete, the couple was cooperative for as long as requested by the physician, and treatment was unsuccessful. Age and length of marriage are shown in Table 1.

In most instances, the male was normal, but 20 cases with known male factors are included for sake of illustration. All the data available on the

TABLE 1. Age of Wife and Length of Marriage

Age of wife		*Length of marriage*	
No.	*Age*	*No.*	*Years married*
11	20–24	5	1
39	25–29	11	2
41	30–34	21	3
9	35–40	13	4
		16	5
		9	6
		6	7
		3	8
		2	9
		14	more than 10

individual couples were tabulated on graph paper for classification into pertinent categories.

It is the hope of the authors that this analysis of failure in 100 cases may help to define the desirability of the tests recommended and performed, to elaborate on the value of various types of therapy, and to assist the clinician in advising a couple when to abandon the therapeutic measures directed toward relieving their infertility. There is no doubt that one of the responsibilities of the specialist in infertility is appropriately timed advice to the couple to desist from further futile therapeutic work. It will be apparent that there are infertile patients for whom the successful treatment of the diseased state is impossible.

FACTORS IN INFERTILITY

Perhaps nowhere are the physician and the couple more frustrated than in overcoming the factor of *cervical hostility* to the spermatozoa.

For two decades or more the notoriously poor results in relieving *tubal obstruction* have been discussed in the literature.

Ovarian deficiency, fortunately infrequent, has resisted almost all efforts toward correction, with the possible exception of surgery for bilateral polycystic ovaries.

The results of therapy in the male factor—especially for *inadequate sperm count*—are discouraging.

Peritoneal disease, affecting as it does tissue outside the lumina of the

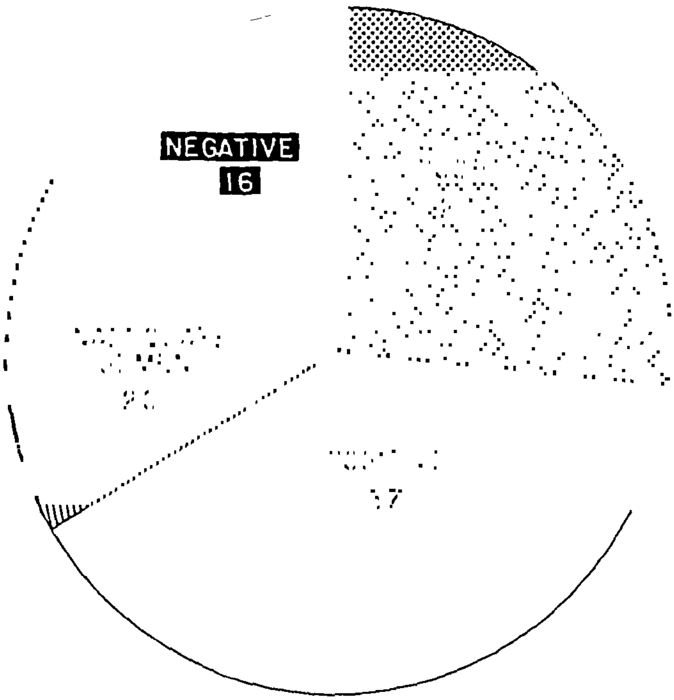

Fig. 1. Cervical factors.

fallopian tubes, is quite unrecognizable without surgery. When diagnosis has been established after laparotomy, correction of the pathologic findings has not met with great success.

The current flurry of assigning all cases of infertility to the *psychogenic factor* in women must be tempered by excluding mechanical factors. In addition, the psychologic factors may not have existed until the appearance of frustration caused by incomplete or unsatisfactory evaluation of the actual fertility index of the couple in question.

Cervical Insemination

It is apparent (Fig. 1 and Table 2) that failure of cervical insemination for whatever reason was of great importance in more than half the couples. In only 37 couples was the postcoital examination of the cervical mucus positive, indicating more than 20 motile spermatozoa per high power field. This test is normally carried out by requesting the couple to have intercourse 2 to 10 hours before the appointment, omitting a douche afterward. The cervix is exposed with a speculum and wiped free of excess mucus with cotton. A Knight nasal forceps is then introduced into the cervical os at the level of the external os and the mucus collected by the forceps placed on a glass slide and examined at once. The examination is ordinarily made without a coverglass which sometimes causes prompt immotility of the contained spermatozoa. The mucus is examined under low power and subsequently under high power, and the number of actively progressive spermatozoa per high power field recorded.

TABLE 2. Cervical Insemination (43 cases)
(Best Pk* recorded)

Finding	*No. couples*	*% of 80*
Negative (0 to rare/h.p.f.)	16	20
Poor (1–20/h.p.f.)	27	34
Inadequate semen	20	..
Positive (more than 20/h.p.f.)	37	46

* Post-coital examination of cervical mucus test.

Disregarding the 20 couples in whom the semen was considered inadequate, we have 80 couples in whom the semen, on more than one examination, showed over 1 cc. of volume with 40 per cent motility, and a 60,000,000 count, of which 70 per cent or more were normal forms. Forty-three (54 per cent) of the 80 patients had an unsatisfactory postcoital test. Of these, 16 (20 per cent) had a negative postcoital test, ranging from no spermatozoa at all to rare spermatozoa, and 27 cases (34 per cent) had less than 20 spermatozoa per high power field.

Eight of the 26 patients who had a dilatation and curettage showed subsequent improvement in the cervical factor, although some required small doses of stilbestrol. Most of the dilatations were done for varying degrees of cervical stenosis—the result of injudicious cauterization before the patient reached us, of congenital failure of development of the cervix, or of unknown etiology.

It may be of some significance that two thirds of these patients were 30–35 years of age. The duration of marriage was 2–12 years. Two patients had had a child. A little over half the patients had used contraception for less than 6 months or not at all.

Almost two thirds of these patients had other reasons besides inadequate cervical insemination to explain infertility.

Negative Postcoital Examination. In the 16 patients where repeated postcoital examination of the cervical mucus revealed no spermatozoa at all or a rare spermatozoa per low power field, the ages varied from 21 to 33 and the duration of marriage from 1 to 7 years at the start of the study. Eight had dilatation and curettage without improvement of the cervical insemination. Fifty-six per cent had other factors contributing to their infertility exclusive of the sperm inadequacy: 5 had tubal defects; 3, peritoneal pathology; and 1, progestational deficiency.

Conclusions. It would seem that when repeated cervical insemination studies indicate absent to rare spermatozoa under ideal circumstances, the possibility of improving that factor with alkaline douches, dilatation and curettage, and stilbestrol in small doses is negligible, unless such response to treatment is relatively prompt. Obviously some of this series started with negative postcoital tests and improved under treatment to "unsatisfactory" or even to "positive." If, however, the postcoital examination reveals up to 20 spermatozoa per high power field, various measures mentioned above will improve the cervical insemination in about 50 per cent of the couples. They may still be resistant to pregnancy because of other factors, including the tubes, the peritoneum, and the ovaries themselves. Despite the excellent contributions of Pommerenke and his group and Buxton and Gepfert of New York, there is a fertile field for evaluation of the cervix as regards its endocrine stimulation (gross and possibly microscopic), development, position, and degree of inflammation—either traumatic or infectious.

Our finding of such a high percentage of significant cervical factors is in contrast to Mazer and Israel, who found inadequate cervical insemination in 26 per cent of 697 couples.

Thirty-three of our 100 unsuccessful couples showed only minor improvement after treatment.

Tubal Patency

It will be noted (Fig. 2 and Table 3) that in almost three quarters of the patients (71 per cent) carbon dioxide gas passed at 120 mm.Hg or less on one or more occasions, with attendant shoulder pain on the patient's resuming the erect position. This indicates presumably normal fallopian tubes. Sixteen cases required higher pressures than 120 mm. twice, or once with an accompanying x-ray confirming the pathology. Another 11 patients had patent tubes in the sense that the gas passed at 120 mm.Hg or over, but only 1 test was done even though the patient had shoulder pain. Early in the study it was not apparent that the tubes might not be normal if the gas passed at pressures under 200 mm. For purposes of this study we arbitrarily selected 120 mm. or above as indicating patency but with possible

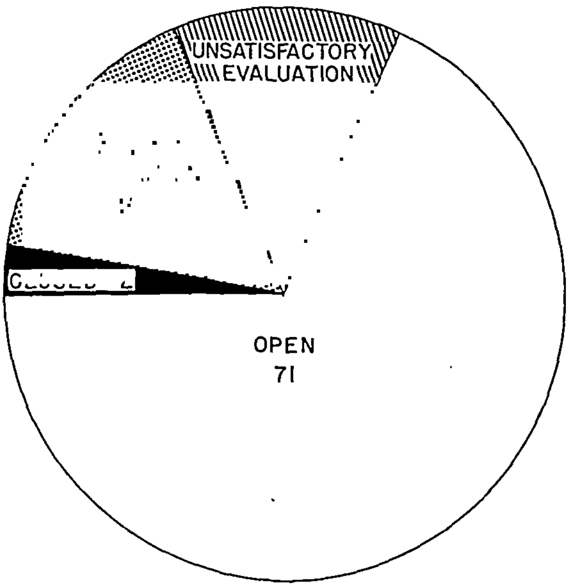

Fig. 2. Tubal patency.

partial obstruction, and passage under 120 as indicating relatively normal tubal physiology.

Etiology of Obstruction. Only 2 patients in the 100 couples had non-patent tubes to gas on at least 2 occasions or to 1 gas test plus x-ray. Two per cent tubal closure is an extremely low figure in 100 infertile marriages, and we were at a loss to explain it except on the basis of all of these patients being private. They were less liable than charity patients to diseases of the tubes, gonorrhea, tuberculosis, postabortal salpingitis and sepsis. One patient had had a criminal abortion before marriage; the other had had profuse menorrhagia and metrorrhagia requiring 3 curettages. In both patients the tubes were finally patent to repeated gas insufflation, but pregnancy has not yet occurred. The last patient had reparative tubal surgery in November, 1954, and became normally pregnant in January, 1955.

We investigated the 16 patients with partially obstructed tubes to see if they were caused by premarital exposure to venereal disease, childbirth or abortion, or appendectomy. Three only had had premarital exposure to venereal disease and 3 had had appendectomy incidental to another exploratory procedure. One patient had had a child and is now awaiting elective exploratory laparotomy for an attack on the peritubal process. It is significant that about 75 per cent with tubal obstruction shown clinically also had a cervical factor. This confirms the observation that, where pathology of the tubes is of an inflammatory or infectious nature, the cervix may also be involved, both working against normal fertility.

There were only 2 instances of endometriosis in the 18 patients with defective tubes. The first was established operatively elsewhere some 8 years before; the second was a clinical diagnosis only on the basis of dysmenorrhea and increased nodularity behind the cervix. Two of these 18 patients submitted to laparotomy—1 managed to achieve 3 pregnancies following the attack on her infertility, but all ended in miscarriage; the second had not conceived 18 months later.

TABLE 3. Tubal Patency (18 cases)

Finding	*No. couples*
Closed (2 or more CO_2 tests or 1 test plus x-ray)	2
Partially obstructed (passage above 120 mm. twice, or once and x-ray with pathology)	16
Unsatisfactory evaluation (passage over 120 mm., but only 1 test done)	11
Patent (passage of gas below 120 mm.)	71

Conclusions. The observations of Grant and Mackey, from Australia, who indicated that repeated tubal insufflation may be more valuable in tubal nonpatency than surgery are confirmed by the 2 patients with closed tubes. Both patients during the course of this study exhibited pain in the shoulder following multiple tubal insufflations but neither has conceived. This implies that while gas may insinuate itself through the fallopian tubes, with attendant shoulder pain, if the necessary pressure is over 120 mm. of mercury, those tubes are probably not functioning properly and constitute a decisive deterrent to fertility.

The high incidence of partial patency in this series suggests that if partial obstruction is found repeatedly, or if peritoneal disease is a possibility, early surgical investigation is indicated. In our experience, even though such patients submit to surgery and do not achieve pregnancy, the couples are satisfied that all possible steps have been taken. They accept the observations at laparotomy as conclusive corroboration of their infertility, and the decision that further investigation is not warranted allows them to accept their sterility positively, even though with disappointment. Thus, much earlier in their marriage, they will cease the futile efforts to produce their

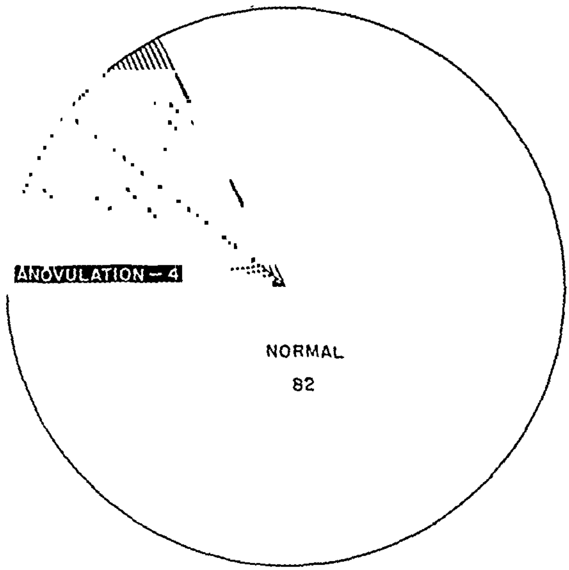

Fig. 3. Ovarian function.

own progeny and may promptly turn their attention to adoption, with a greater possibility of favorable outcome because of their youth.

Ovarian Function

Of these 100 infertile couples, 18 patients gave evidence of ovarian dysfunction: failure to ovulate in 4, infrequent ovulation in 7, and progestational deficiency in 7 (Fig. 3 and Table 4). Ninety-six patients had endometrial biopsies and 90 had properly recorded basal body temperatures long enough for interpretation to be significant. The decision to place these 18 women in the ovarian-dysfunction group was based partly on the biopsy, partly on the B.B.T. determination, and partly on menstrual history.

It seems highly impressive that in 12 there were other conditions contributing to their infertility which were significant in determining the degree of investigation of their infertile state. In only 1 was the male considered unsatisfactory, whereas 8 had a cervical abnormality. One of these patients had polycystic ovaries plus pelvic peritonitis.

Nine patients improved following treatment with surgery, 1 patient achieving 3 unsuccessful pregnancies between the age of 27 and 40, and 1 a full-term, normal pregnancy after a miscarriage.*

We feel it may be significant that in all the patients in this group with ovarian dysfunction who improved, either significant ovarian findings were revealed at laparotomy, or a wedge-shaped resection of the ovaries was

TABLE 4. Ovarian Function (18 cases)

Finding	*No. couples*
Anovulation	4
Infrequent ovulation	7
Progestational deficiency	7
Normal	88

Endometrial biopsies in 96 patients.
B.B.T. kept in 90 patients.

done. The observation that none of these patients responded to cyclic therapy with estrin or progestin does not mean that such therapy or therapy with equine gonadotrophic and chorionic gonadotrophic hormones may not be of value in some patients. Its true worth will not be apparent until 100 couples who have established fertility are analyzed.

One may inquire why these patients were not given x-ray therapy. Nowhere in Boston is there enthusiasm for roentgen-ray therapy of the pituitary gland or ovaries, because of the possibility of deleterious effect from radiation. Admittedly we are overconservative and overcautious. All are offered the opportunity of seeking consultation elsewhere if they request or seem interested in that approach to their anovulatory or ovarian deficiency. We feel that the defeatist attitude toward anovulation, infrequent ovulation, or progestational deficiency should be abandoned in favor of a more vigorous attack of a surgical nature either to help re-establish ovulation or else to demonstrate that the ovaries are irreversibly incapable of producing adequate ova.

* This information reached us too late for exclusion from the study. In February, 1955, we received information of her second normal delivery.

Semen Quality

In 6 of the 20 couples where the male deficiency was pertinent, the husbands had at least one of the following: less than 20,000,000 spermatozoa per cc.; less than 30 per cent motility; or less than 60 per cent normal forms; 2 had less than 1 cc. in volume (Fig. 4 and Table 5). These individuals were originally put into the study as examples of the male factor in infertile

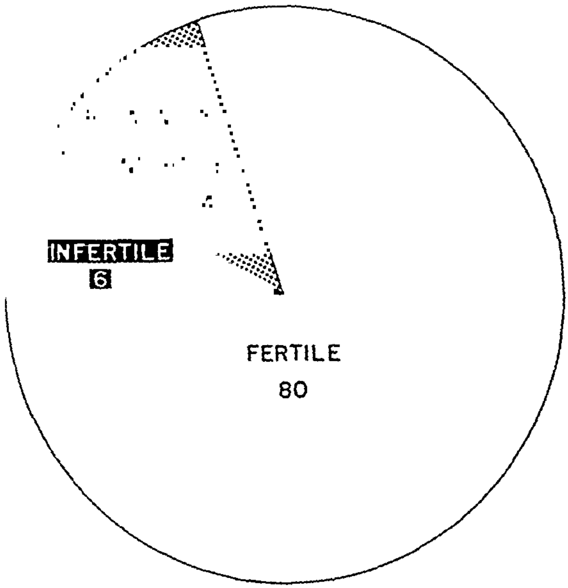

Fig. 4. Male factor.

couples, although 3 of their wives had an insurmountable problem. The first wife had infrequent or absent menstruation due to ovarian deficiency. The second wife had a relatively mild amount of endometriosis, and the third had extensive endometriosis and a psychologic factor. The other 3 wives were normal. Two of these 6 men had testicular biopsies, 1 revealing severe hypospermatogenesis and another, focal atrophy of the tubules. None of these males improved on treatment. Three husbands were still able to produce sperm, shown in postcoital examination of cervical mucus, to the extent of 1–5 active spermatozoa per high power field. In the other 3 wives none was found.

Fourteen husbands had specimens below the lowest clinical limits of normal,* with less than 60,000,000 spermatozoa per cc., less than 4 per cent actively motile, or less than 70 per cent normal forms. Six men had less than 2 cc. of volume. In another 2, there was also a possible cervical reason on the wife's part; one had a congenital deformity of the cervix and another had marked cervical stenosis following one child and extensive cauterization of the cervix. Five of these wives were within normal limits throughout, yet the postcoital mucus test gave always unsatisfactory results, presumably on the basis of the male ejaculate. Seven had other factors.

Prognosis. Three males had testicular biopsies revealing normal testes,

TABLE 5. Semen Quality (20 cases)

Finding	*No. couples*
Less than	
20,000,000/cc. sperm count	
30 per cent sperm motility	
60 per cent normal forms	
1 cc. volume	6
Less than	
60,000,000/cc. sperm count	
40 per cent sperm motility	
70 per cent normal forms	
1 cc. volume	14
Normal	80

hypospermatogenesis, and arrest of maturation. The wife of the male with the normal testes had severe endometriosis, while we considered the wives of the other 2 normal. The "arrest of maturation" patient was treated with testosterone according to the method suggested by Heckel, with no improvement whatsoever. There seemed to be no evidence that those men with less than 60,000,000 spermatozoa per cc. were better from the prognostic point of view than those men with less than 20,000,000. This does not agree with MacLeod's often-quoted statement that the critical level for the male partner is 20,000,000 spermatozoa per cc., provided motility is good. We still feel that, when the husband repeatedly exhibits less than 60,000,000 spermatozoa per cc. with less than 50 per cent motility, a favorable prognosis must be made very guardedly.

Considering the two groups of males as one unit, it is interesting that none showed significant improvement under treatment. The treatment consisted

* According to the standards of the American Society for the Study of Sterility.

of thyroid in adequate dosage, testosterone when indicated, anterior pituitary-like hormone when indicated, and attention to diet and vitamins, diminishing the consumption of cigarettes and alcohol, increasing the amount of exercise, and advice relative to the fertile time of the cycle. In none of the 20 couples did the postcoital examination of the cervical mucus ever show more than 15 spermatozoa per high power field, and the majority had no spermatozoa or rare spermatozoa in the test. Two of the men had previously impregnated their wives and had living children 7 and 8 years old, respectively. Three others have impregnated their wives, but the results were 1–3 consecutive abortions.

Ten of the wives of these men were considered quite normal, and only 3 in fact had extensive mechanical limitations to their fertility. This suggests that in 17 of these 20 marriages the male factor was the decisive reason why the couple did not have children. It also serves to illustrate the desirability of studying in detail the husband of each infertile wife who presents herself for investigation.

Male Factor in Abortions and Stillbirths. While it is very difficult to prove, the male factor is probably significant in the increased incidence of abortion, miscarriage, and stillborn babies. We feel that much more work is needed in this attack on the infertile couple in order to assert that the male germ plasm is not responsible for blighted ova or congenital anomalies in the couple's conceptus.

Volume. In the entire group of 100 couples, 55 males had semen specimens amounting to less than 4 cc. A search of the literature revealed a paucity of statements relative to the normal volume of semen in relation to normal fertility. Perhaps not enough attention has been paid to the gross volume of the ejaculate. In this factor, also, this report is of a preliminary nature. It will be interesting to assay this factor with the subsequent study of the 100 couples fertile after treatment.

There were 8 men who consistently delivered less than 2 cc. of semen. A study of that group reveals that all had better than 50 per cent motility and counts of better than 68,000,000 per cc., all but 2 being over 133,000,000 per cc. Yet only one wife had a satisfactory postcoital cervical test. Again this suggests that the regular volume of the ejaculate may be more important than has previously been mentioned.

Sperm Count. For the sake of argument we should like to point out that in none of the 20 women whose partners had less than 60,000,000 spermatozoa per cc. was the postcoital examination of the cervical mucus satisfactory, although 10 of these women were normal. Therefore, by reference to the cervical factor, only 37 of these 100 couples had satisfactory cervical insemination. This would indicate the desirability of performing postcoital examinations repeatedly in infertile couples and accepting at once the challenge that repeated poor results indicate an unfavorable prognosis relatively early in the couple's study. While artificial insemination with the husband's specimen was carried out rarely in this series, we feel that the semen is grossly or inherent unsatisfactory, and that placing such inadequate semen in the cervical mucus artificially would not increase the rate of conception.

Peritoneal Conditions

If one considers as a peritoneal factor pelvic inflammatory disease and endometriosis interfering with tubo-ovarian function, almost one fifth of these marriages had extratubal or peritubal disease of a significant nature

TABLE 6. Peritoneal Condition (19 cases)

Finding	*No. couples*
Pelvic inflammatory disease	11
Endometriosis interfering with tubo-ovarian function	8

(Table 6). Surgery revealed pelvic inflammatory disease in 11 patients and external endometriosis in 18. In all these patients the tubes were patent to carbon dioxide insufflation. Thus, even though the patient demonstrates satisfactory insufflation tests, there may be disorders adjacent to the tubes which are causing the couple's continuing infertility. Five of the patients with pelvic inflammatory disease had had previous appendectomies. Only 1 had a history of premarital exposure to venereal disease. The duration of marriage before operation varied from 2½ to 7 years, and the time of study before operation from 2 months to 2 years.

While all the husbands were within normal limits of fertility, 75 per cent of the women with pelvic inflammatory disease had unsatisfactory results of the postcoital examinations of the cervical mucus. Of the 3 women with positive postcoital test, 1 had had a baby and 1 a miscarriage. In contrast, all patients who had endometriosis had satisfactory postcoital tests where the husband had normal semen. None of the endometriosis cases had blocked tubes. Murray of Argentina and Westman of Stockholm have previously shown the importance of the peritoneal factor.

Surgery. We agree with Murray and Westman that patients who do not conceive in a reasonable length of time, after all studies have been done, should be offered elective laparotomy earlier in their marriage, and sooner in the study, for two reasons: the incidence of discovery of unsuspected peritoneal disease will undoubtedly be increased by earlier surgery; and meticulous surgical exploration and treatment of peritubal disease, peritoneal disease, and endometriosis may make the wanted pregnancy possible soon afterward. Sometimes the operative findings will be such that the patient can be advised the prognosis is so hopeless that she and her husband may as well abandon treatment and resort to adoption.

All these 19 couples accepted with equanimity the definite fact that if conception did not occur within 1 year of the surgery, the prognosis is essentially zero. On questioning, most patients will state that the finality of the surgeon's unfavorable prognosis, while disappointing, is welcome, bringing as it does a termination of months of hoping for their own children.

We feel that if the patients with endometriosis married over 5 years and over 30 years of age had been offered surgery earlier, the salvage for fertility would have been higher. According to Huffman, conservative surgery in endometriosis may result in successful pregnancies as often as 42 per cent of the time. Certainly if the diagnosis of endometriosis can be established with reasonable accuracy clinically, since the morbidity and mortality of elective exploratory laparotomy for that disease is less than 1 per cent, the couples should be appraised of the potential salvage from surgery. They can then refuse or request it as their zeal for children dictates.

Psychologic Adjustment

In view of the current flurry regarding the psychologic aspect of infertility we studied these 100 patients with a view to analyzing any psychologic factors present. We subdivided the patients considered to have a psychologic factor into those with accompanying organic factors and those without (Table 7). We further subdivided these 19 patients into (1) those cases in which we felt from our interviews there existed a history of emotional or psychologic disturbances antedating attempts to conceive, and (2) those cases in which we felt that the emotional deviation was caused by or markedly aggravated by the failure to conceive.

It is most interesting that there was only 1 woman in the 100 couples who had a psychologic problem for a time before seeking medical advice on her infertility. There were 2 other patients who had no organic causes, but were very tense and upset about their infertility; 1 of these has had 3 consecutive miscarriages. Of the 10 wives who had a psychologic problem accompanied by an organic disturbance unrelated to infertility, 8 had tubal or peritoneal dysfunction, and 1 had ovarian abnormality (Stein-Leventhal ovaries) and reported the birth of a child following the completion of this study. Five had unsatisfactory results of the postcoital test of cervical mucus, including the wife just mentioned who now has a baby. Two husbands had inadequate semen.

An analysis of the organic factors previously reported in this paper would suggest that the psychologic factor in these 10 wives was incidental and

TABLE 7. Psychologic Adjustment (19 cases)

	No. couples
Maladjustment accompanied by organic factor	
Unrelated to infertility	10
Primarily related to infertility	6
Psychologic disturbance only	
Unrelated to infertility	1
Primarily related to infertility	2
Normal	81

merely contributory to their infertility, as all of the organic factors were serious in themselves.

Since only 6 patients had psychologic factors primarily related to their infertility, a brief résumé of their disorders is included.

Case 1. A highly sensitive 26-year-old woman, married for 6½ years, had patent tubes and secretory endometrium, with an adherent, third-degree retroversion on the uterus. The postcoital cervical mucus test always revealed less than 20 spermatozoa per high power field. At times during this study her husband's sperm count fell below 60,000,000 per cc., and he was intermittently somewhat emotionally upset. Early in the study a D. and C. was done for cervical stenosis, with some improvement. Later, in an exploratory laparotomy for the adherent uterus, multiple peritubal and peritoneal adhesions were found. Lysis of adhesions and suspension of the uterus was done over 2 years ago. The couple have subsequently adopted a child. The patient reiterated her feelings of inadequacy and periodic depression.

Case 2. A 29-year-old registered nurse, married 2½ years, repeatedly emphasized that she was gloomy, sensitive about her infertility, discouraged, and depressed. The husband's semen was quite normal; he was extremely obese and, on urging, lost over 40 pounds to regain his normal weight. The wife was also obese and likewise lost enough weight to attain the norm for her age and height. The postcoital examination of the cervical mucus always revealed less than 20 spermatozoa per high power field. Because of abdominal pain and a palpable mass, laparotomy was advised, and large polycystic ovaries of the Stein-Leventhal type found.

Her menstrual cycle varied from 28 to 35 days. One endometrial biopsy revealed 24-day secretory endometrium on the twenty-eighth day and another showed proliferative endometrium. Her basal body temperature records showed poor-to-fair ovulation; a moderate improvement occurred postoperatively. Repeat biopsy has not yet been done. Conception did not occur in the ensuing 12 months.*

Case 3. A 33-year-old schoolteacher, married 5 years, repeatedly referred to her sense of inadequacy. Her husband was definitely psychologically upset, but not wholly about the infertility. He was a conscientious objector during the war and asserted to premarital homosexual tendencies. His semen specimen has never revealed 60,000,000 spermatozoa per cc. The postcoital examination revealed only occasional motile sperm. This couple requested and is undergoing artificial insemination with the husband's semen without success. There were rather erratic, ill-defined basal body temperature curves. They recently adopted a child.

Case 4. A 24-year-old highly educated woman repeatedly commented on her extreme anxiousness about her infertility. She reported frigidity. The postcoital examination of cervical mucus was always satisfactory and the tubes were patent to gas in more than one test. She denied premarital exposure to venereal disease and had had no surgery. Because of bilateral palpable masses and an adherent uterus, laparotomy was carried out after 8 months of study. Multiple intraperitoneal adhesions were found and corrected, following which she conceived and miscarried at 3½ months and has not conceived subsequently. Both the husband and wife have been so anxious about this problem that they have repeatedly sought office consultation for advice as to further maneuvers, and requested a respite from the studies to see if their anxiety would not allay itself. They have applied for adoption.

Case 5. This couple moved away before all the studies could be completed. The wife became pregnant shortly afterwards, and miscarried at 8 weeks. They were greatly upset over what they felt was inadequate supervision of the threatened miscarriage, which became inevitable. The wife was 31 years old, married 6 years, and had a metabolic rate of –21 per cent, for which she was treated with thyroid, as was her husband. The postcoital examination revealed 10–15 motile spermatozoa. The tubes were patent, and endometrial biopsy revealed secretory endometrium. Both partners repeatedly expressed their extreme anxiety about their infertility. The wife reported marked depression and weeping with the onset of menses, which were regular. While the husband repeatedly exhibited

* This patient was reported 4 months pregnant in September, 1954.

less than 60,000,000 spermatozoa per high power field, a pregnancy promptly followed the sterility investigation, resulting, however, in a miscarriage.

Case 6. A 30-year-old woman, married for 4 years, was nervous and somewhat resistant to treatment, but did not become depressed over her infertility until laparotomy revealed multiple ovarian cysts, with a pathology report from Dr. Arthur Hertig that the ovaries were sclerotic and exhibited senile changes. She subsequently exhibited loss of libido and was referred to a psychiatrist for her depression and feeling of inadequacy. She had a history of scant and irregular periods, yet had secretory endometrium on one examination and good-to-poor ovulation shown by the basal body temperature curves. The cervical mucus revealed fewer than 20 spermatozoa per high power field, despite an adequate semen specimen. The couple have adopted two children.

Unknown Causes

Twelve couples individually satisfied all the minimum standards but did not conceive (Table 8). They were cooperative to the utmost in regard to repeated office visits for postcoital examinations at various times of the cycle. Six were subjected to diagnostic dilatation and curettage, 6 had uterosalpingograms despite normally patent tubes, and 1 had exploratory laparotomy for doubtful ovulation and presumed ovarian pathology. The ovaries contained multiple cysts but were not classified as compatible with the Stein-Leventhal syndrome.

TABLE 8. Essentially Unknown Causes (12 cases)

Causes	*No. couples*
Infrequent coitus	3
No organic or psychological reason discernible	12

The 12 husbands were within normal limits of fertility, from history, physical examination, and semen analysis,* albeit at times 2 or 3 were somewhat lethargic in respect to frequency of coitus. One couple had had a successful pregnancy before the study and a miscarriage after the study. The wife's menstrual periods ceased at 41. Five presumably had peritoneal pathology but either declined exploratory surgery or are awaiting the passage of enough time to make them realize the necessity of surgery to satisfactorily diagnose the cause of infertility. Two have had miscarriages and will accept further surgery if and when indicated.

Suffice it to say that we are unable to explain the infertility of these 12 couples on the basis of the causes discussed in this report.

Number of Causative Factors Per Couple

Over 20 years ago, Meaker, in his valuable text, *Human Sterility*, pointed out that there usually were multiple factors in the study of the infertile couple and demonstrated in his cases an average of 2.38 per marriage. We

TABLE 9. Number of Causes Per Couple

Total number of causes	*No. couples*
0	12
1	39
2	35
3	13
4	1
Average	1.5

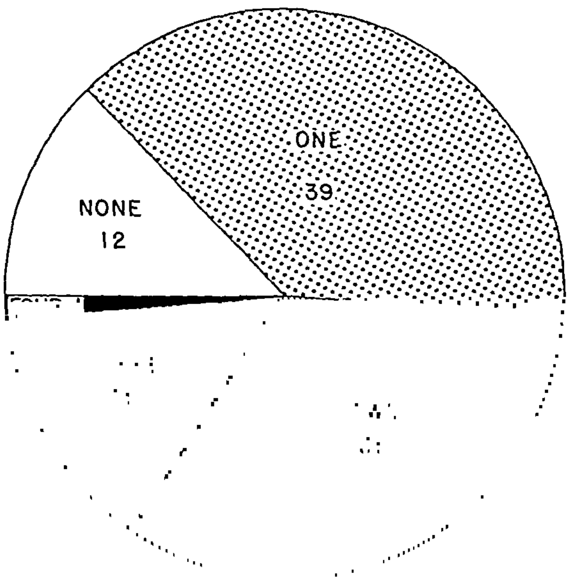

Fig. 5. Number of causes per couple.

have been aware of that fact during this 20-year period and feel that it is significant in these 100 couples that approximately fifty had 2 or more factors (Table 9). Including the 12 couples for whom no explanatory factors appear on our charts, the average for the group is 1.5 per marriage.

There were 39 patients with 1 cause each, 16 of which were limited to failure of cervical insemination, apparently due to the female. There were 2 cases with closed tubes, and 5 with a presumed psychiatric cause paramount.

There were 35 patients with 2 reasons for sterility. We feel this points out the need for a thorough assay of all factors before any treatment is instituted.

Thirteen couples had 3 reasons, while only 1 had 4—this patient had senile or sclerotic ovaries, and usually less than 20 motile spermatozoa per high power field in the postcoital test, despite a diagnostic D. and C., the administration of stilbestrol, and Nutrisol douches. She had also probable tubal pathology, since passage of gas required over 120 mm. of mercury. Only one test was done, however, as she subsequently came to laparotomy, and ovarian dysfunction proved paramount. The fourth reason was a psychologic one primarily related to her infertility and was shown in depression over her infertility and loss of libido.*

Failure to Establish Diagnosis

We attempted to analyze where the diagnostic pattern may have failed in the 12 couples who had no apparent reason for failure of conception. We postulate that there may have been failure of the spermatozoa to ascend through the uterine pathway, for which no appropriate test has as yet been devised. We have had no experience with the intrauterine postcoital test. The possibility that some of these patients might have profited by a uterosalpingogram despite satisfactory gas tests should be considered. The senior author has 7 patients who conceived promptly following the introduction of radiopaque oil into the fallopian tubes, after two or more previous satisfactory carbon dioxide insufflation tests.

Unsuspected tuberculosis of the uterus, tubes, or peritoneum is a distinct possibility. Our over-all diagnostic test usually consists of one endometrial biopsy, if that reveals normal secretory endometrium. We feel that in the future we may do more repeat or follow-up biopsies on patients who fail to conceive when all the other tests are normal and approximately 6 months has elapsed.

The physician may have failed to unearth factors more important than those brought out with a carefully taken history and carefully conducted physical examination and diagnostic maneuvers. There are undoubtedly

* Patient left husband 6 months later and is suing for divorce.

those who will criticize the fact that all patients do not have routine uterosalpingograms. Despite the high incidence of success reported from other clinics using this routine, we defend the adequacy of properly done carbon dioxide insufflations in the majority of patients. This premise may be more adequately borne out by the report of the 100 successful cases to be presented subsequently.

CONCLUSIONS

Since 64 of the couples had unsatisfactory results of postcoital examinations of the cervical mucus, all patients should have this test repeated several times during their study at the optimum time. Twenty-nine cases had unsatisfactory tubal results, suggesting that perhaps all couples should have both repeated gas tests and uterosalpingograms, or that if the combination of tests reveal any unsatisfactory findings, a more prompt surgical attack should be made, all other factors being normal.

Although 82 per cent had normal ovaries, 18 patients had ovarian factors of such severity that a more efficient attack must be directed towards that deficiency. We were not impressed with the cyclic therapy for progesterone deficiency and feel that in all ovarian-deficient cases surgery should be contemplated.

It is hoped that this presentation will encourage the medical profession to insist upon these minimum diagnostic studies at least before offering a prognosis to the patients. If couples fall into the categories described here, and do not respond to treatment within a reasonable length of time, perhaps 18–24 months, they should be appraised of their apparent failure to respond to therapy. Some must even be discouraged from continuing to go from clinic to clinic and doctor to doctor seeking help.

It is hoped that a more vigorous attack may be made on the very resistant cervical factor about which so little is known.

SUMMARY

A review has been presented of our experience with 100 couples completely assayed according to the minimum diagnostic standards of the American Society for the Study of Sterility. Attention to 8 elements in infertility is discussed as follows:

1. Cervical—43 couples.
2. Tubal—18 couples.

3. Ovarian–18 couples.
4. Male–20 couples.
5. Peritoneal–19 couples.
6. Psychiatric–19 couples.
7. Unknown–12 couples.
8. Number of causes per couple.

Postcoital examination of cervical mucus showed inadequate insemination in 73 couples, of which 20 may have been due to deficient or defective semen.

Partially obstructed or closed fallopian tubes were a reason for infertility in 18 couples, and in 11 more the evaluation was considered unsatisfactory because only one gas test was done before x-ray or surgery or both.

Ovarian dysfunction was a factor in 18 wives. Cyclic therapy for progesterone deficiency was more ineffective than surgery.

There was no discernible reason for the infertility in 12 couples.

ADDENDUM

Since this manuscript was submitted for publication, 4 patients have become pregnant. None was in the "normal" group. All were among those operated on for ovarian factor. Thus, 4 per cent have conceived and delivered after having been classed as failures.

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DISCUSSION

DR. TOMPKINS: Thank you, Doctor Simmons. Discussion of the paper will be opened by Dr. Clarence D. Davis.

DR. CLARENCE D. DAVIS, *Columbia, Missouri:* It is, indeed, a pleasure to have the opportunity to discuss this very provocative paper. Doctors Simmons and Taymor are to be congratulated for their forthrightness and willingness to discuss their failures. More emphasis should be placed on this point of view in the future. Everyone is too willing to talk of his successes.

The one major criticism of the material presented here seems to be that the authors did not report consecutive couples who failed to conceive. They state "In most instances, the male was normal but 20 cases with known male factor are included for sake of illustration." This indicates selection of couples. It seems to me that the matter of evaluation of the occurrence of the various factors would have been considerably facilitated had the couples been consecutive and not selected. Furthermore, it would have made the anticipated comparison with 100 successfully handled couples of more value.

The authors emphasized the importance of the cervical factor. This is the most neglected part of the usual sterility survey. They report over 50 per cent of the wives with unsatisfactory postcoition tests. Our incidence is nearly this high. They are somewhat more pessimistic about the correction of the cervical factor than we are. Bacitracin suppositories used on the eighth through the twelfth nights seems to be of definite but limited value in instances of cervical infection. This antibiotic has not caused a monilia vaginitis in our experience.

I am glad to find that the peritoneal factor is discussed. However, this can be suspected by hysterosalpingography in the majority of instances. Doctors Simmons and Taymor perform more laparotomies on the wives than we do. It may be that the described benefits justify this.

Obviously, in the time allotted, only a few points can be touched on. It is surprising to me that they found only 12 couples with entirely negative findings. I am sure that comparable data of ours would show 20 or more.

As the authors point out, a comparison of the data presented here with 100 successfully handled couples will be valuable and informative. We await this subsequent report with interest.

DR. TOMPKINS: Thank you, Doctor Davis. The presentation is now open for general discussion.

DR. SAMUEL J. BEHRMAN, *University of Michigan:* I would like to ask Doctor Simmons, as the cervical factor is so important, whether he was satisfied with the method he used for evaluation as compared to, perhaps, a bacterial count, estimation of *Spinnbarkeit*, or, as used in England, the Clift method.

DR. B. B. WEINSTEIN, *New Orleans, La.:* I should like to comment on the difference in percentage of failures between private and clinic cases. As Dr. Simmons commented, there was a very small series in his data. Some years ago, right here in San Francisco Doctor Guerrero of Mexico analyzed a hundred of his failures. His experience, and ours too, has been that there is a very marked difference in the etiology of the failures in the clinic group and in the private practice group. I should like some comment from Doctor Simmons on whether or not there is a difference in his series which, I take it, were largely from private source, and his experience with the clinic patient—the free patient—in the Boston area. The low incidence of tubal causes is particularly impressive, because we run into a high series of tubal and peritoneal causes.

DR. H. I. KANTOR, *Dallas, Texas:* I would like to ask Doctor Simmons, first, among the patients with ovarian deficiencies, was the incidence of negative-to-poor Hoehner tests unusually high? The second question is, does it seem that there may be a greater therapeutic value to hysterography using the oil in preference to the water-soluble medium?

DR. L. F. HAWKINSON, *Oakland, California:* I was amazed at the number of patients that they considered to have ovulated in this group. I think we will all agree that our evidences of ovulation, even on the bases of body basal temperatures and endometrial biopsies, have been quite inadequate to prove ovulation. It has been my opinion for a considerable period of time that the ABC of fertility is, first, good spermatozoa, at the proper time, an open and developed pathway, and an ovum. In a vast majority of cases we have the first two. If we have ovulation I think it is pretty generally conceded there is enough estrogen to change the fern pattern. The fern pattern is changed by estrogen, and it is maintained that if you get a good fern pattern you have ovulation, and if you have a poor fern pattern you have no ovulation. The number of poor fern patterns we get with good basal body temperature and even fine endometrial biopsy is amazing to me. So I think our criteria for ovulation have been very poor, and I think we assume too many of these women put out an ova with some degree of regularity.

DR. TOMPKINS: I hope Doctor Simmons will redefine satisfactory postcoital tests, and I hope also he will make a remark or two about the incidence of endometriosis in his group of patients. Dr. Simmons, will you close?

DR. SIMMONS: I would like to answer Doctor Tompkins question first, if I may. Regarding a satisfactory postcoital test, I shall read a portion of my talk which I was forced to omit because of the time element.

(Dr. Simmons read the portion of his paper referred to.)

Now, I have no comment to make on Dr. Davis's remarks except to thank him for being so kind.

Doctor Behrman, about the cervical factor. These figures which I presented are the best figures on individual patients after all types of tests and therapy, including such things as the fern test, *Spinnbarkeit* determination, and repeated analysis of cervical mucus, and this is the best that any of these individuals put out in the cervical mucus.

In reply to Doctor Weinstein's question, we are unable to explain why, in this particular 100 patients, we came out with only 2 permanently blocked tubes. There were, I think, only 30 per cent of partially blocked tubes, and I would agree it is probably only due to classification of material. If anyone had asked me what the percentage of tubal factor was, I would have said approximately 30 per cent.

The question about the correlation of ovarian deficiency tests I cannot answer. There were 18 cases with ovarian deficiency. We do have that material at home. I think it is pertinent and shall look it up.

In regard to the relative value of oil versus water, I am one of those who has not yet succumbed to water. If one has a patient with no evidence of inflammatory disease, oil is more successful than any water method I have had experience with.

About Doctor Hawkinson's question about the poor fern tests as indication of failure of ovulation—that test has come in late in our series, some of these cases going back 10 to 15 years. These patients on whom I have reported will be glad to come back and try to correlate the fern test with the ovarian factor. Thank you very much.

Secretory Hypoplasia of the Endometrium

S. J. Glass, M.D., Walter Miller, M.D., and
Gordon Rosenblum, M.D.

FUNCTIONAL INFERTILITY is being subjected to more revealing clinical investigation but its treatment remains largely empirical. Therapy can, however, become less empirical by defining more precisely the clinical observations of the infertile patient in the light of known principles of reproductive physiology. In other words progress can be made not only by the discovery of new ideas but also by more critical evaluation of what we see in the study of each patient with functional infertility.

In the routine investigation of functional infertility much effort is spent in determining whether gametogenesis is adequate, but little importance is attached to the question whether the end organs, such as the uterus, are responding normally to circulating sex hormones. We must bear in mind that sterility can result not only from failure of hormone production or impairment in the metabolism of the sex hormones but also from insensitivity of the accessory sex organs (like the uterus, the prostate, and seminal vesicles) to stimulation by the sex hormones. Therefore in the study of functional infertility it is important to grade, if possible, the responsiveness of these organs.

To this end we have begun a long-term study of the endometrium in patients who remained infertile even though evidence of ovulation was obtained. Premenstrual endometrial biopsies are being analyzed together with estimation of the urinary pregnanediol excretion, cyclic vaginal cytol-

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ogy, and basal body temperature to determine whether the endometrial pattern truly reflects ovarian function.

There is universal agreement that the endometrial biopsy affords the best single index of ovarian function in the study of the infertile patient. Moreover Kurzrok, among others, has also derived valuable prognostic information from the premenstrual endometrial biopsy, being able to predict that deficient progestational changes in the endometrium would either prevent nidation or result in early abortion unless preconceptional and postconceptional therapy was administered. Likewise, Hughes and his associates, as well as Rutherford, were able to correlate infertility and habitual abortion with deficient progestational changes in the endometrium.

We have identified a specific endometrial pattern which we designate as secretory hypoplasia, an all-inclusive term for deficient progestational changes which heretofore have been referred to as early, mixed, or deficient secretory endometrium. We regard the designation of secretory hypoplasia as more expressive of the deficient endometrial response to the ovarian hormones.

One of the major objectives of this pilot study was to determine if pregnanediol excretion does correlate with this endometrial defect.

METHOD

Serial endometrial biopsies were obtained in 15 patients during the premenstrual week. We attempted to carry out the biopsy on Day 26 of the cycle but some were taken at the onset of menstruation. The tissue was divided into two portions, one being fixed in Bouin's fluid for routine histologic preparation with hematoxylin–eosin stain, the other was fixed in 95 per cent ethyl alcohol to be stained for glycogen and alkaline phosphatase.

Pregnanediol excretion was determined in 2 normal fertile women and in 10 patients by the Chaney method on 120 hours of pooled urine collected during 10 nights of the luteal phase.

Further corroboration of the occurrence of ovulation was obtained by cyclic study of vaginal smears taken by the patient during 10 days of the mid-cycle and stained by the Shorr-Papanicolaou method.

All these observations are being repeated after therapy with our combined nutritional-hormonal regimen described elsewhere.[2] The results of

1

2

Fig. 1. Secretory hypoplasia. (× 100) **Fig. 2.** Normal endometrium at same time in menstrual cycle as Fig. 1. (× 100)

treatment as well as the special histochemical studies of the endometrium will be reported subsequently.

RESULTS

Photomicrographs of only a few patients together with suitable normal controls will serve to illustrate the pattern of endometrial hypoplasia (Figs. 1 and 2). The presence of secretory activity in all these biopsies, especially those showing intraluminal glycogen (seen best in color photomicrographs),

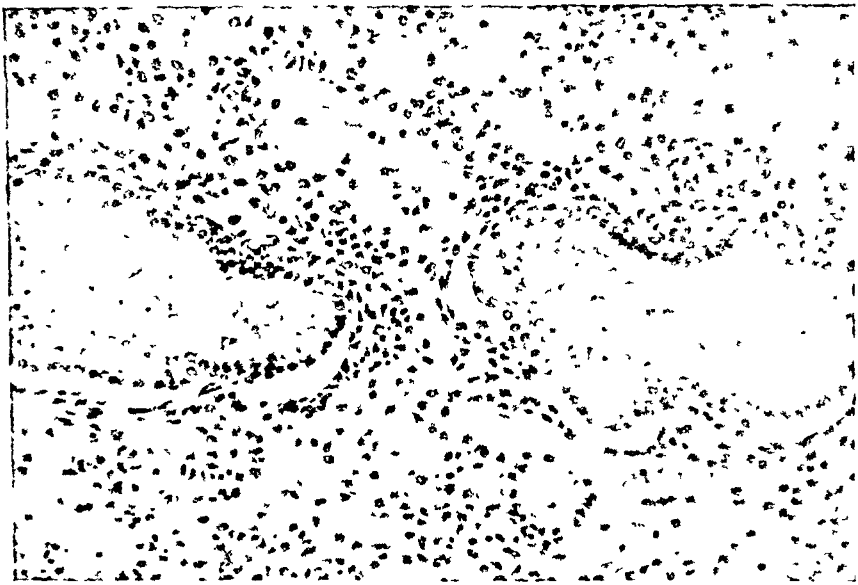


Fig. 3. Secretory hypoplasia of the endometrium. Although the glands show secretory activity as evidenced by periodic acid-Schiff stain the cells are small and the papillations poorly developed. The stroma is scantily cellular and the individual cells do not show predecidual changes.

is ample evidence that ovulation had taken place in the infertile patients. But in those with secretory hypoplasia (Fig. 3) the glandular response is inadequate and occasionally out of harmony with the stromal changes, that is, a dissociated response to the ovarian hormones by the glandular and stromal elements of the endometrium.

The pregnanediol values were low in 7 patients (5–11 mg. excreted in 120 hours of night urine during the luteal phase) and subnormal in 3 patients

(15–16 mg. for the 120 hours) whereas the 2 normal subjects excreted an average of 30 mg. for the same period of the luteal phase (Table 1).

The low values of pregnanediol excretion correlate well with the endometrial secretory hypoplasia.

Even though the results of treatment will be reported later it is a satisfaction to demonstrate an interesting transition in the photomicrographs (Figs. 4 and 5) of one of the patients (E.R.). Here one sees the beneficial result of treatment (Fig. 4) and the tendency to relapse (Fig. 5) when treatment is withdrawn prematurely. Of interest also was the striking increase in the excretion of pregnanediol from a pretreatment value of 5 mg. to 32 mg. after a short course of intensive nutritional-hormonal therapy.

TABLE 1. Pregnanediol Excretion During 120 Hours (10 Nights) of Luteal Phase

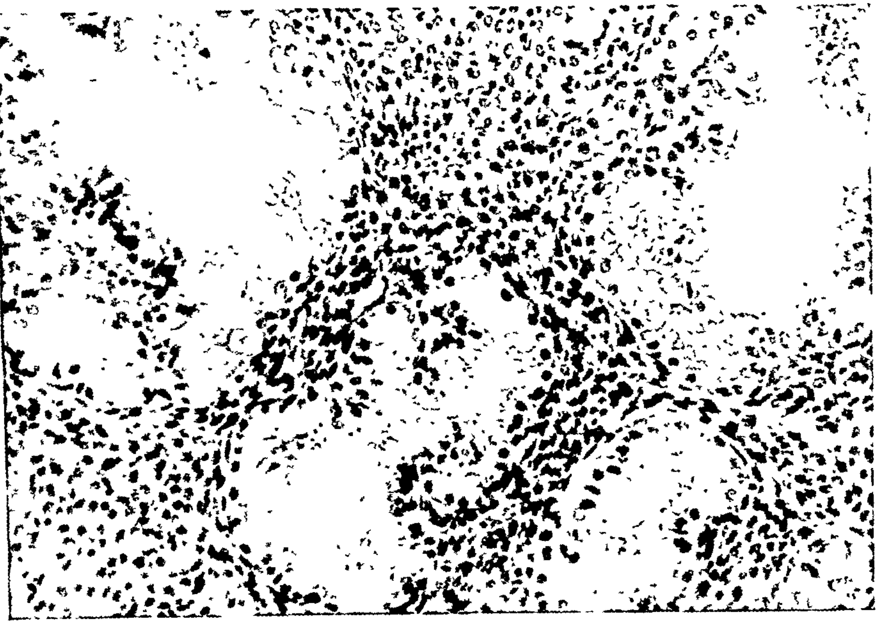
	Excretion (mg.)
2 controls	Average 30.2
7 patients	5.0–11.2
3 patients	15.0–16.5

There are apparent gradations in the endometrial response to ovulation. About one half of the patients under current observation, 15 of whom are serving as a sample for this pilot study, manifest variable degrees of endometrial secretory hypoplasia. The good correlation of the endometrial deficiency with low pregnanediol excretion does suggest that the endometrial defect can be improved with appropriate therapy. The type of therapy employed here—the combined nutritional-hormonal regimen described elsewhere[2]—has been reported by us to yield substantial salvage of fertility. It has long been our belief that intensive nutritional priming of such patients renders the endometrial and other organs of the reproductive system more responsive to endogenous as well as exogenous sex hormones.

SUMMARY

The basic objective of this preliminary study was to seek more precise and quantitative data for the diagnosis of infertility in women who ovulate but who remain infertile unless treated.

We believe that secretory hypoplasia characterizes the response of the



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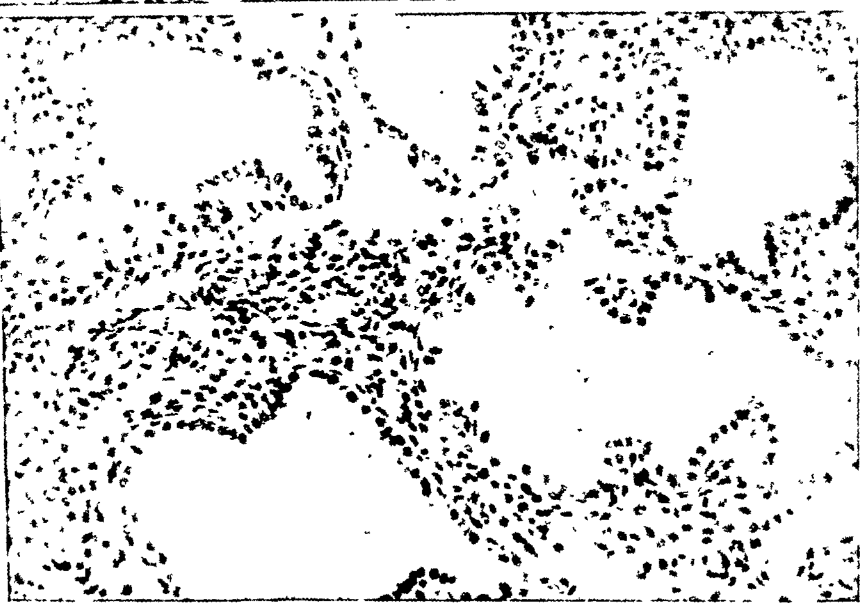

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Fig. 4. Effect of treatment in a patient with secretory hypoplasia. The appearance is that of a normal late secretory endometrium (× 240). Fig. 5. Partial relapse after treatment (same patient; × 240).

endometrium to ovulation in such cases. Likewise the low pregnanediol excretion tends to correlate well with this specific endometrial deficiency.

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DISCUSSION

Dr. Edward C. Reifenstein, *Bloomfield, N. J.*: I should like to express my appreciation of the opportunity to open the discussion of this important problem. Doctor Glass and his associates are to be congratulated for giving us this preliminary report of their studies.

There are several significant aspects of this paper that deserve comment. I shall limit myself to five.

1. It seems to me that we have reached the point in our investigations of functionally infertile women where a correlation and evaluation of the findings obtained from a battery of determinations must be undertaken in order to provide a more accurate picture of the pathologic physiology underlying the disorder in each patient. This is the one way in which we can arrive at a more precise diagnosis for each patient and thus at more rational and individualized therapy. It is obvious that when a sufficiently broad spectrum of tests are applied simultaneously to each patient it will be possible to separate the functionally infertile women into a number of distinct subgroups, each with differences in pathologic physiology. The contributions that such classifications will make to our knowledge of the etiology and the proper therapy of these cases is obvious. The relative roles of the hypothalamus, the anterior pituitary, the ovary, the thyroid, the adrenal cortex, the liver, and the endometrial tissue in each of the various subgroups will become clearer as a result. Doctor Glass and his associates have presented today an integration of the results of only two of the several investigative procedures which they have been using. In addition to those mentioned we can think of others which eventually should be added to the spectrum and subjected to the same scrutiny; for example, the chemical components (glycogen, enzyme, and fructose status of the endometrium) the urinary and the blood levels of the gonadotrophins, of the adrenocortical steroids, of the indices of thyroid gland activity and of the other gonadal hormones (to name a few). Chemical and biologic assay procedures are reaching a stage of development where they should be utilized by the clinical investigators concerned with functional infertility of women.

2. The use of the term "secretory hypoplasia of the endometrium" as proposed by the authors to provide a general descriptive phrase for conditions variously described as "early, mixed, or deficient secretory endometrium" seems to me to be a sound step forward toward the elimination of confusion and to define more clearly the type of case under consideration.

3. Doctor Glass and his group have introduced an ingenious device for circumventing the wide variations in the daily excretion in the urine of the pregnandiol group of metabolites by collecting and pooling the 12-hour nocturnal urinary specimens to give for 120 hours one average 24-hour chemical value during the luteal phase. This minimizes also the errors of collection and probably renders relatively insignificant any variations in the time interval after ovulation at which time the level of metabolite excretion is tested.

4. Doctor Glass and his associates are to be commended also for avoiding undue emphasis on the therapy of these cases until a much larger series of patients has been tested and evaluated and more precise diagnostic groups have been established.

5. I have had the privilege of discussing with Dr. Glass his plans for the future extension of this work; we can see many aspects which will require considerable additional study. We agree that the combined studies of endometrial cytology and pregnandiol excretion in a series of only 9 patients represent too few observations to serve any purpose except that of calling attention to a promising approach for investigating the problem of functional infertility in the female and of encouraging other workers to initiate research in the same direction. Four avenues of future work are immediately apparent:

a. We should like to know how many women with functional infertility show secretory hypoplasia of the endometrium.

b. Similarly, we should like to know how many women with functional infertility show a reduced excretion of pregnandiol in the urine.

c. We should like to know in such patients whether both indices are always abnormal in the same cases, or whether there are patients who exhibit a dichotomy, with one test in what we call the normal range and the other in an abnormal range. It is probably that the endometrium, like other tissues, varies from person to person and from time to time in its sensitivity and responsiveness to hormonal and nutritional factors—some endometria may be resistant and relatively unresponsive and some may be hypersensitive.

d. We should like to know how these indices—measured simultaneously—are affected by the *individual hormones* (such as progestogen, estrogen, androgen, corticoids, the several gonadotrophins, thyroid and so forth) alone and in combination; and by the *nutritional factors* (calories, protein, vitamin A, vitamin B complex, and so forth) alone and in combination with other nutritional factors and with the various hormones. Such information will provide the rationale for sound therapy.

The matter of dosage and of duration of therapy requires study as well. Suitable untreated and placebo-treated control cases are essential to provide a basis for evaluation of the large role that psychogenic factors play in all functional disorders. Such problems as these necessitate the prolonged objective study of a large series of patients before final conclusions can be formulated, and it is most heartening to us all to find Dr. Glass and his associates undertaking these studies with this point of view so thoroughly in mind.

May I say again that it has been a privilege for me to have had the opportunity to make a few comments concerning this interesting investigation at this initial stage.

DR. EDWARD T. TYLER, *Los Angeles, Calif.:* I would just like, in general, to substantiate what Dr. Glass has presented. We have been doing urine determinations on alternate days in the second half of a cycle. One interesting point is the fact that some patients apparently secrete pregnandiol for a very short time and then fail to secrete it late in the cycle. This may be something worth investigating.

DR. GLASS: I want to thank Dr. Reifenstein for his scholarly discussion and cordial remarks. In answer to one of the specific questions he put to me—the incidence of this pattern—in a series of 50 we reported last year 29 showed this pattern of secretory hypoplasia. I did neglect to mention that routine laboratory investigations of the routine things were of course carried out on all these patients. It must be remembered that these patients do present evidence of ovulation but also of imperfect endometrial response and perhaps of some defective ovulatory mechanism per se. The basic objective of this preliminary study was to seek for precise and quantitative data for the diagnosis of infertility. I think we have to make our diagnosis, in many respects, a little bit more precise. I think the data are too few, but the trend is certainly obvious. The correlation is present.

DR. ROBERT GREENBLATT, *Augusta, Ga.:* On what day of the cycle was this taken?

DR. GLASS: Usually on Day 26. Not in all cases—some few on the day of onset of bleeding.

Cyclic Endometrial Changes Without Menstruation

Herman I. Kantor, M.D., and Jack H. Kamholz, M.D.

Menstruation is an integral part of the normal endometrial cycle. As a sign of desquamation, it marks the physiologic end of the secretory phase, and is followed by the onset of a new cycle. Since the endometrium reacts as an end organ of the ovarian hormones, a biopsy taken at the onset of menstruation will indicate the deficiency or absence of any phase of the cycle.[9]

In the presence of traumatic cervical stenosis, the endometrium is usually intact and normal. The most striking difference between this and other types of occlusion is that it may develop slowly. The history often suggests oligomenorrhea proceeding to the amenorrheic stage—best explained by progressive scarring and contracture. When the stenosis becomes sufficiently severe to produce amenorrhea, there are three major possibilities which may account for the fate of the desquamated endometrium and the associated blood:

1. The cyclic variations of the endometrium may cease, thus eliminating both desquamation and bleeding;
2. Retrograde menstruation may occur with retention of the menstrual products;
3. The bleeding may be reduced or absent and the desquamated cells

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We should like to express our appreciation to Dr Henry Ash, D V M., for supervision of the animal experiments, and to Drs. S. W. Cobb, F. I Sebastian, H. R. Levy, W. F. Guerriero, N. Tobolowsky, and J O. Baker for permission to report their cases.

Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility at San Francisco, California, June 18, 19, and 20, 1954.

liquefied by enzymatic action; both being absorbed directly from the uterine cavity.

The first choice would seem to be unlikely. The endometrium, still subject to the influences of the ovarian hormones, should continue in its anticipated responses. Even in an unphysiologic environment such as an endometrial strip transplanted behind the cornea of a rabbit, these estrogen and progesterone effects persist.[11] It would appear that another explanation must be offered.

Retrograde menstruation occasionally occurs and has been reported. Hematometra, hematosalpinx, and hematoperitoneum may result from occlusion of the genital tract.[3, 5, 12] Yet among many patients with cervical stenosis of traumatic origin, few have demonstrated this syndrome.[2] Moreover, disseminated endometriosis—which is found infrequently—should be a common associate of retrograde bleeding.

For the third possibility to be acceptable, it is necessary to show:

1. That retrograde menstruation has not occurred in the patient being studied;

2. That a cyclic endometrium continues even in the absence of visible bleeding; and

3. That absorption may occur directly from the uterine cavity.

REVIEW OF THE LITERATURE

Asherman[2] was one of the first authors to describe cervical occlusion due to trauma. He named the syndrome amenorrhea traumatica. His original report was based on 29 patients but a recent communication[1] states that he has now collected 93 cases. He is greatly impressed by the infrequency of hematometra. In his original report he stated "the endometrium remains in a state of entire quiescence for a long time, sometimes for years on end." However, at the time he did not study the endometria from the patients he treated. For the most part, the symptoms were alleviated by simple sounding and dilation.

When we outlined the present study to him, he wrote[1] "there is no doubt that the endometrium goes through the usual changes" in most cases. In an addendum to his original report, he quoted 24 cases reported by Stamer, only 3 of which had hematometra. Moreover, among 20 cases of Bass it was never present.

In a recent paper by Hunt[7] only 1 of 5 patients with cervical stenosis had associated endometriosis. In another,[8] at the time of treatment the endometrium was reported as in the "late proliferative phase." In a third, the endometrium became available after menstruation had been resumed and showed secretory activity.

Kleegman described cervical stenosis following cauterization, and this was the etiologic factor in most of our patients.

CASE REPORTS

Amenorrhea

Case 1. Mrs. M. B., a 25-year-old gravida I, para I, was seen in 1949 with the presenting complaint of almost complete amenorrhea for one year, preceded by progressive oligomenorrhea for several years. She stated that following the birth of premature twins 6 years ago she was extensively cauterized. The *coup de grâce* to the cervix was administered with a hot cautery by a well-meaning osteopath three years later. At the time of her initial visit to us her menstruation was reduced to an occasional droplet, but there were no symptoms suggesting hematometra.

Examination revealed a solid cervix with a suggestive dimple at the site of the cervical canal. The uterus and adnexa were entirely normal to palpation. Treatment was delayed until the occasional drop of blood made its appearance. At this time the cervical canal was reopened and a curettage was done. A moderate amount of normal endometrium was recovered, and there was a small amount of fresh blood in the uterine cavity. No old blood was encountered.

In order to avoid recurrent stricture, a stem pessary was inserted. Following the procedure, menstruation was resumed, and it has followed a normal pattern since. The total amount of bleeding during each cycle has been slightly less than before the amenorrheic period.

Curettings showed a normal endometrium in a fully developed secretory phase.

Oligomenorrhea

Case 2. Mrs. N. G., a 26-year-old nulligravida, complained of progressive oligomenorrhea following an extensive conization and cauterization of the cervix 7 years ago. At the time we saw her, she said that she could protect herself from the "nuisance of menstruation by using two Band-Aids." There were no symptoms or signs to suggest retrograde menstruation or endometriosis.

Pelvic examination proved the stenosis of the cervical canal to be so extensive that it defied penetration to even the smallest probe. The uterus and adnexa were entirely normal.

In the immediate premenstrual phase, dilation and curettage of the cervix was done. A stem pessary was inserted to maintain the dilatation. There was no blood in the uterine cavity and a moderate amount of normal endometrium was obtained.

phenolsulfophthalein in the urine (Table 1) must then represent direct absorption from the uterine cavity (Fig. 1).

The control experiment for comparative excretion with intravenous injection of dye in the same animal, was carried out two weeks later. During this interval, the dog passed through a period of heat.

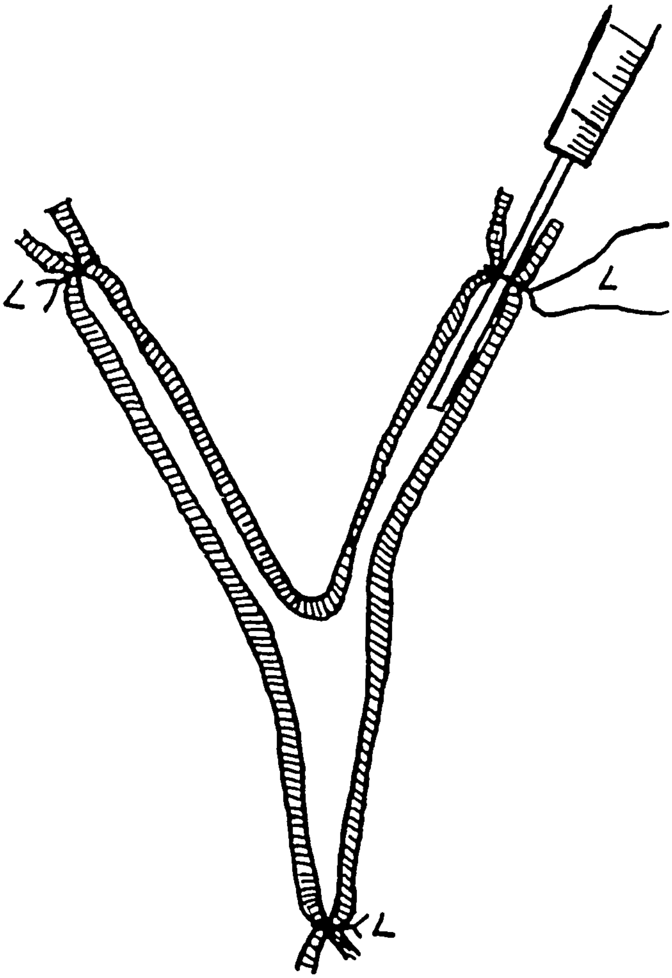

Fig. 1. Method of phenolsulfophthalein injection into dog uterus.

After anesthesia was administered, 1 cc. of phenolsulfophthalein was injected intravenously and laparotomy was again performed. An acute hematopyometra was found. Since the genital occlusion previously accomplished was sudden and complete, this was anticipated. The process was limited to the area isolated by ligature, thus demonstrating the adequacy of the ties. The blood supply was preserved. A complete hysterectomy was done, and a portion of the horn distal to the ligature was included. The

TABLE 1. Phenolsulfophthalein Excretion Studies
Experiment 1

	Intrauterine	*Intravenous (Control)*
	(Percentage of drug recovered)	
1st hour	4	1
2nd hour	3	4
3rd hour	2	4
TOTALS	9	8

Accuracy is only approximate—urine output small.

pathology report for this portion was normal uterine segment and normal endometrium. For the involved portion, the report was hematopyometra with flattening of an otherwise normal appearing endometrium.

Experiment 2

In order to show that such absorption may occur among human beings, a patient was selected in whom both tubes were occluded in their cornual portions. This was seen on hysterographic studies and confirmed by repeated Rubin tests.

The cervix was exposed and the uterus was carefully sounded to insure patency of the canal. A cannula was passed into the uterine cavity and 1 cc. of phenolsulfophthalein was injected. After withdrawal of the cannula, the

TABLE 2. Phenolsulfophthalein Excretion Studies
Experiment 2

	Urine	*Sponges*
	(Percentage of drug recovered)	
1st hour	5.5	
2nd hour	4	
3rd hour	2	
TOTALS	11.5	5.7

cervix was continuously observed and all reflux solution was wiped with gauze sponges before it reached the vagina. The patient remained in the supine position to further encourage intrauterine retention. Table 2 shows the percentage of phenolsulfophthalein recovered.

It seems evident that direct absorption from the uterine cavity may therefore take place.

DISCUSSION

Our experiences as well as those reported in the literature tend to prove generally that stenosis of the cervix does not stop the cyclic variation of the endometrium. All phases may be recovered at the time the initial dilatation and curettage is performed. The postmenopausal patient (Case 3) demonstrated a good endometrial response to administered estrogen. The physiologic hormonal effects remain, although the blood loss may be diminished or perhaps absent. The sequelae of retrograde menstruation and endometriosis are not common. Desquamated cellular structures are probably liquefied by enzymatic action and along with any blood are absorbed directly from the uterine cavity.

Tissue from Cases 1 (amenorrhea) and 2 (oligomenorrhea) were reviewed in order to give further support to this suggestion of in-situ absorption. If, in the presence of cervical stenosis, the uterus may be considered a closed space, the microscopic picture may then be compared with that of endometriosis. In the latter condition, the blood pigments are largely removed by macrophages,[14] and these cells may be filled with hemosiderin particles. Free hemosiderin may also be present in the tissues.

In the first patient, the endometrium contained many macrophages within the compact stroma, most of which were loaded with typical iron pigment. A few were also found within the gland structures. A small amount of free pigment was distributed throughout the tissues.

The sections from the second patient also showed a few macrophages, but none contained iron. However, clumps of free hemosiderin were scattered throughout the stroma.

These findings may provide additional explanation for the absence of hematometra.

The application of this phenomenon to other types of amenorrhea provides fascinating material for speculation. For example, may some amenorrheic women have normal cyclic endometrial sequences with omission of the bleeding phase? May this solve the enigma of the occurrence of pregnancy in the face of amenorrhea? May it explain why an otherwise normal patient may menstruate only once or twice each year?

CONCLUSIONS

Among patients with amenorrhea or oligomenorrhea due to traumatic cervical stenosis, the following observations were noted:

1. The endometrium seems to respond normally to the ovarian hormones.
2. At the time the stricture is dilated, any phase of endometrial activity may be found. The cyclic endometrium continues even in the absence of visible menstruation.
3. The desquamated tissue and blood (if present) may be absorbed directly from the uterine cavity.
4. Retrograde menstruation and endometriosis are infrequent.

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DISCUSSION

Dr. Allan Palmer, *San Francisco, Calif.:* I think this is an excellent report on cyclical ovarian activity and endometrial activity in the absence of bleeding, and would like to substantiate his report by our findings, six or seven years ago, of cyclical ovarian activity demonstrated by the curve in the absence of menstrual bleeding. This finding led us to postulate that there was cyclical activity in the endometrium, and we concluded that the disorder was probably a cervical occlusion of endometrial secretions, both of which were confirmed by biopsies. In one instance there was complete occlusion of the internal os, and in the other case the endometrium was replaced by fibrous tissue.

I might ask, also, if Dr. Kantor has any basal temperature records of these patients, and if so, whether there might not be a slight increase or abnormally high basal body temperature during the time when menstrual flow may have been present if there had not been an obstruction. This might provide one explanation—that there was possibly a low-grade fever due to the absorption of menstrual flow, similar to what we might expect when a toxic protein is absorbed.

DR. KANTOR: I am sorry that Dr. Biskind was not here to discuss the paper because he had received a copy of it some months ago.

In answer to Dr. Palmer's question, unfortunately in this particular group no basal temperatures were taken. However, we do have a basal temperature series in a patient with a complete amenorrhea who had never manifested a problem of infertility. Although a biphasic curve was present, there was no evidence of menstruation, and the patient found no difficulty in conceiving.

Sterility in Central Africa

A. Barlovatz, M.D.

RELATIVELY LITTLE is known as yet about sterility among the colored races. The following report is intended as a contribution to the compilation of standards applicable to the dark-skinned inhabitants of Central Africa, and is based upon 2485 cases of sterility in African women observed in private practice between January 1, 1950, and December 25, 1952, at Stanleyville, the largest center in the forest-covered alluvial basin of the Congo river.

The town lies halfway between the Atlantic and Indian Oceans, 1300 feet above sea level. The climate is uniformly and moderately hot and damp, with an annual rainfall of 65 inches spread over almost the whole year.

The patients described here all belong to tribes living in or within a few hundred miles of Stanleyville. No significant anthropologic or pathologic differences were noticed between these tribes, which all belong to the Bantu race. Europeans, mulattoes, and Sudanese Negroes are excluded from the present series, and no Pygmies are ever seen at Stanleyville.

STERILITY SYNDROME IN THE AFRICAN WOMAN

The Bantu consider sterility as shameful, and the women confess their condition with as much diffidence as a white person acknowledges mental disease. Whereas veneral disease is quite freely spoken of, sterility is hidden before strangers and spoken of only reluctantly. The childless woman considers herself as an inferior being, and the approach of menopause is dreaded like impending death. The desire for children is stronger than in most white people, regardless of economic and social circumstances, and little thought is given to the expense of bringing up the children. We know, however, of

We wish to express our sincerest thanks to Dr. Courtois, Stanleyville, Belgian Congo, and to Dr. Burette of Stanleyville for serologic work and uterograms made and help and counsel given.

no instance where a child was left to starve, after having been weaned; if the parents disappear he is usually adopted and brought up by relatives or even by strangers. A fair proportion, more than 15 per cent, of our patients are unmarried women, often prostitutes without savings or permanent means of support, upon whom a pregnancy must needs bestow tremendous economic hardship. Nevertheless they wish to bear children, though no father will care for them, and when questioned they usually do not even grasp the idea that a baby might be undesirable.

Birth control is rare. We get about 3 inquiries a year on this score, the last one coming from a housewife of 36, who had had 19 children (14 living) and one cesarean section.

Presenting Complaints

Sterility produces in the Bantu woman a number of psychosomatic complaints. We shall mention no detailed statistics, as these complaints are often fleeting, and depend to some extent upon the contact which the physician is able to make. It is also hard to tell in some instances whether one has to deal with a real secondary illness or only with a pretext for consulting the physician.

One has to be on the outlook for minor illnesses which are not as a rule brought to the doctor's notice, say a head cold, or pityriasis, and to inquire about deliveries. Very often the underlying complaint is sterility, and the woman who is sent away with aspirin pills or an ointment reproaches the physician for not having probed further.

The commonest complaint, presenting in nearly a third of the cases, is abdominal pain, either colicky or permanent, localized in the lower abdomen or in the whole belly, more rarely in the left or right side, or in the stomach. We are of course excluding cases where a pathological cause for pain could be ascertained.

Dysmenorrhea is complained of by about 12 per cent of the women, and menstrual irregularity of various types by about as many. Upon questioning, the patients acknowledge that their real reason for consulting the physician is sterility.

The same may be said for watery or creamy discharges, which about 2 per cent of the women bring to the physician's notice.

"Wasting" is complained of by nearly 10 per cent of the women. The subconscious reasoning seems to be: sterility is a serious sickness, therefore I must be wasting.

More frequent still are rheumatoid pains or a feeling of general sickness. The recognition of sterility and the hope of a successful treatment put an end to these pains.

Nervous symptoms and headaches are present less frequently.

Pain, paresthesia, and dysfunction may become localized in the most unexpected places. We have noticed them in the following spots: head, eyes, ears, throat, heart, bowels (constipation), bladder, neck, arms and hands, back, feet, and skin. Imaginary fever existed in 5 patients, and imaginary pregnancy in 4.

Nutrition and Avitaminoses

Although not overly varied, nutrition is usually sufficient to sustain growth and a fair state of health. No major avitaminoses are prevalent among the population. The over-all birth rate seems to be in excess of 25 per 1000 per year, and compensates the losses by death. The population is maintaining its size or even increasing slowly.

Venereal Disease

The common venereal diseases all exist here, the most important being gonorrhea. Only 8 per cent of our husbands claim to have never suffered from this disease, while about 50 per cent have had it two or more times. The incidence of salpingitis among our sterile women is very high, about as high in primary as in secondary sterility, which indicates that postpartum infections do not play a major role as an etiologic factor. It is well known, and we can confirm, that the Bantu mother suffers much less from postpartum infections than the Caucasian. Even before the sulfonamides and antibiotics, pyemia and septicemia were very uncommon indeed, in spite of the frequent lack of hygiene during delivery. It is worthy of note that sterility has been found here in professional truck and cab drivers' families much more often than among other professions. Venereal disease is also especially frequent in drivers and their spouses.

The local prevalence of gonorrhea does not stem from a lack of facilities for treatment, nor from shyness of the sick natives, who often do not mind stating their diagnosis at the door, within easy hearing distance of other patients, while they never speak thus freely of their sterility, but rather from a high drug resistance of the local strains. If the test of treatment is the percentage of immediate cures of fresh male gonorrhea with stated doses of penicillin, streptomycin, and sulfonamides, we get here more failures than, according to the literature, in the United States, England, and France, and are obliged to use higher doses.

Thus it must be borne in mind that gonorrhea has been present in the history of about 9 in every 10 marriages, and should be combated before more elaborate measures are undertaken.

A thorough antibiotic treatment with penicillin, sulfonamides, and streptomycin suffices not infrequently to restore fertility, without any other additional measure. Of course, a lurking syphilis would be cured at the same time with the high doses of penicillin used.

We agree however with the modern authors in temperate climates, who consider syphilis only as a minor and rare cause of sterility. Our opinion stems from the following considerations:

1. We see little clinical syphilis among our patients—half a dozen cases in a year.

2. The percentage of sero-positive reactions (by the Wassermann and Kahn technics) is high among our sterile women (232 positive reactions among 1672 patients, or 13.87 per cent; however it is lower than in the general sick population. In 1951, according to the local laboratory, 17.62 per cent positive sera were found among the patients of the general hospitals and dispensaries.

3. Thus there were fewer positive sera among sterile women than among the general run of native patients whose blood had to be examined.

4. A considerable proportion of positive sera do not denote syphilis; this proportion has been recently evaluated at about 50 per cent in the United States; it should be much higher in Stanleyville owing to the presence of tropical diseases including yaws in childhood.

5. Syphilis causes miscarriages rather than outright sterility.

6. We remember a three months' pregnancy with typical secondary syphilis, who had only two moderately dosed injections, and nevertheless called ten months later with a magnificent and apparently healthy baby.

Inflammation and Tubal Impermeability

On palpation, without taking into account vaginitis and cervicitis noticeable only on inspection through a speculum, more than half of our patients show an abnormal gynecologic status, with a great prevalence of tubal involvement. Table 1 shows the results among the last 1444 women examined.

These figures are not broken down, because in a majority of cases a diagnosis cannot be made without operation.

Lambillon and Drumel working in Leopoldville, Belgian Congo, found among 665 cases of primary and 260 cases of secondary sterility 559 cases of abnormal gynecologic status comparable to our definition (polyps 2, tears and scars 3, fibroids 42, fixed retroversion plus adnexitis 177, adnexitis 325, plastic pelvic peritonitis 10), an over-all percentage of 60 per cent.

The same authors stated that they found on insufflation 50 per cent completely obturated tubes, and 25 per cent partially closed ones. In only 25 per cent of their patients was tubal permeability normal.

TABLE 1. Gynecologic Status of Infertility Patients

	No.	%
Primary sterility	802	
Abnormal gynecologic status on palpation	401	50
Secondary sterility	642	
Abnormal gynecologic status on palpation	348	54
Over-all percentage		52

Technic of Insufflation. We use currently a peculiar insufflation technic, which is at once diagnostic and therapeutic, and has not been preceded by salpingography except in a minority of instances. Most of our patients are indeed quite unable to afford the expense of a salpingogram.

Our insufflations are performed under amyl nitrite inhalation, and with gradually increasing pressure until 30 or 35 cm. Hg are reached, either with the Tubingen insufflator, or with a large syringe connected with a hollow sound inserted into the uterus, the cervix being pinched tightly around the instrument. The result of the procedure is judged by the fall in pressure in the insufflator manometer, or in the syringe, and concurrently by auscultation of the abdomen, alternatively over both ostia. The passage of gas through the tubes produces a blowing sound of sufficient intensity, often also bubbling. It is usually possible to distinguish the sound of each tube, when both are permeable, and, when only one is so, to say whether it is the right or left one. Auscultation during insufflation needs however some practice, if one wishes more information than a simple yes or no.

The routine precautions taken before insufflation include: a preliminary treatment with antibiotics, a minimum of 3,000,000 units of penicillin being used in apparently healthy subjects, and much more when there is any sign of inflammation; after the beginning of such treatment, a minimum of two weeks are allowed to elapse, thus giving the organism a chance to resorb inflammatory exudate if present. The menses must have ceased at least one week before, and be expected in no less than 5 days; if the insufflation succeeds, the patient gets a short prophylactic treatment of 2,000,000 units of penicillin spread over three days, 12 Gm. of sulfonamide, and some streptomycin, so as to prevent infection of the peritoneum by uterine secretions.

During insufflation, an outlook is kept for gas embolism by auscultation of the heart, enabling the operator to stop insufflating before a dangerous quantity of gas has passed into the venous system—an occurrence possible with quite low pressures. It is known that the danger level is in the vicinity of 100 cc. of air.

We have had no fatality in our series, and less than 0.5 per cent peritoneal reactions. These always subside on continuing antibiotics.

A successful insufflation is, according to the literature, heralded by a sharp pain in, or radiating towards the right shoulder. Although we have been on the outlook for this sign, we have failed to elicit it in the great majority of our patients. Our considered opinion is that in the Bantu woman this sign is usually absent and the search for it without value.

The results of our last 444 first insufflations (repeat insufflations in the same patient have been excluded) have been as follows:

TABLE 2. Results of 444 Insufflations

	Positive	*Negative*	
Before November 1951	107	129	
After November 1951	161	47	
	268	176	(60.4% positive)

These figures show clearly that the results are getting better, and even much better; this may be due to the greater number of patients with massive tubal infections who consult us before the women not bothered by pain dare do so, but the skill gradually acquired should also play a part.

Stenosis of the cervical canal we found only 12 times in 444 explorations, without much conviction regarding its etiologic significance.

SEMEN STUDIES

The number of husbands examined is smaller than the number of women. We have excluded from our series the husbands having consulted without their wives, as they are few. A fair proportion of our female patients boast no husband; others live outside Stanleyville and their husbands may not leave their job; still other husbands fail to show up. Lambillon and Drumel and Velghe have published small series of sperm examinations in the Congo native, and report having encountered difficulties getting samples. We have found such difficulties exceptional; it usually needs less than a minute to explain to the patients what is expected of them, and the number of specimens we were able to examine is sufficiently large for statistical treatment.

The classical method is to ask for a sample obtained after a continence of 3, 4, or 5 days. We feel that this method is not appropriate in Central Africa, because it is difficult to make sure whether the patient has followed instructions. It seems also better to study semen as it will be used for fecundation rather than under somewhat artificial conditions of continence, which are likely not to be observed, especially during long periods of time. Our method is to ask for a sample, and more often than not the woman brings it early the next morning, rarely the day after. There has not therefore been in our series below any deliberate continence and our figures should not be compared directly with those published elsewhere after such continence. They may be compared, however, with the figures given by Farris for daily emissions. They form also a useful basis for comparison for investigations performed under similar circumstances.

Frequency of Intercourse

We have interrogated a small proportion of our patients about the frequency of intercourse in young married couples, but do not wish to publish the results of the poll taken, because the answers, usually given rapidly, tend to cluster around two standards which are maybe in the mind of the people as much as in their practice; these two standards are 3 intercourses per week and 3 intercourses per day, the latter being much rarer. We hope to be able to devote more time to this question at some future date.

Methods of Examination

Of the various methods of sperm examination (volume of ejaculate, reaction, viscosity, turbidity, sperm morphology, sperm count, and motility) we have directed our attention to a few only, that may be ascertained without undue loss of time.

The average volume of ejaculate, measured in 150 instances, was 3.12 cc., with a maximum of 7.8 cc. There is no great difference from the average in the white races, especially when taking into account the smaller weight and height of most Bantu men.

It takes however special precautions to make sure that the whole ejaculate is submitted. We therefore do not measure total volume any more.

We have found in our samples the main morphologic variations described in *Fertility and Sterility*, these being more frequent in oligospermia. The following ones have been observed:

Nucleus: too small, too narrow, or inversely too wide, too large, too long;
Acrosome: absent;
Galea capitis: partially or entirely detached;
Collar (protoplasmic extrusion at the basis of the nucleus): frequently seen, sometimes in more than half the sperms;
Centrosome: too small, too long, or too large.

The measure of *p*H we found of little value, as it depends on the age of the sample more than anything else.

Sperm Count. The sperm count we always perform ourselves, and have found the figures shown in Table 3 (first examination of correctly taken sample, without period of abstinence, all re-examinations excluded). These sperm counts refer to sterile marriages.

Thus the husband of nearly every ninth marriage presents azoospermia (11 per cent). Another 4 per cent of husbands show extreme oligospermia (below 1000 sperm/cu. mm.) and altogether 22 per cent husbands show a sperm count below 10,000. If the sperm counts between 10,000 and 19,999 are added, the percentage rises to 36 per cent or more than one third.

Entirely satisfactory sperm counts, viz. over 60,000 per mm., were present in less than one fifth of our series (18 per cent). The obvious conclusion is that a current sperm count of much less than this figure (obtained without imposing a period of abstinence) is sufficient to ensure a satisfactory fertility in the African male. We are prone to put the limit demanding treatment at below 10,000 sperm per cu. mm.

The average frequency of intercourse among our patients appeared to approximate 4 per week, and most of our sperm samples were brought the morning following our request.

According to Farris daily intercourse may diminish the average sperm count by about one half.

It would be unreasonable to consider the male responsible for more than about one third of sterile marriages. If this is so, it follows that, in the Bantu, sperm samples taken without imposing a special period of abstinence and

TABLE 3. Sperm Counts in 1025 Examinations

Spermatozoa per cubic millimeter	*No.*	%
None	110	11
1–99	6	4
100–199	6	
200–499	11	
500–999	21	
1000–1999	31	19
2000–4999	75	
5000–9999	88	
10,000–14,999	70	13
15,000–19,999	66	
20,000–29,999	127	35
30,000–39,999	105	
40,000–59,999	129	
60,000–79,999	73	18
80,000–99,999	36	
100,000–119,999	23	
120,000–159,999	27	
160,000–199,999	17	
Over 200,000	4	
TOTAL	1025	100

numbering only 10,000–20,000 sperm/cu. mm. are compatible with a practically satisfactory fertility.

Motility we found to conform to Caucasian averages, and do not remember a single instance of absence of any movement in sperm samples exceeding 20,000 sperm/cu. mm.

The highest percentage of mobile sperm found in any sample was 94 per cent.

That the sperm count, if it shows a fair proportion of mobile elements, has a rather limited significance, is borne out by the great variations which we observe in the same subjects at an interval never exceeding 2 months, and without any special treatment having been given. Table 4 shows a few instances of such variations.

TABLE 4. Variations in Sperm Count

First count	*Second count*	*First count*	*Second count*
400	0	10,000	160,000
48,000	20,000	18,000	60,000
28,000	8,000	200	2
0	800	1,500	6,000
18,000	40,000	1,400	48,000
32,000	7,000	18,000	42,000
15,000	48,000	12,000	48,000
3,000	14,000	3,500	6,000
6,500	32,000	6,500	24,000
32,000	132,000	10,000	28,000
800	2,000	0	200
24,000	44,000	1,200	19,000
4,500	500	600	200

Leukocyte Counts

Considering the local prevalence of gonorrhea, we find the leukocyte count to be a frequently valuable guide to treatment, and must emphasize that very often a moderate leukocyte count, around 5000/cu. mm., coexists with chronic gonorrhea. The two highest figures found in our series were 29,000 and 25,000 leukocytes/mm.

Causes of Azoospermia

In Europe a large proportion of patients with azoospermia suffer from congenital stenosis of the seminal duct. We have been on the lookout for this condition, but have been unable to diagnose it even in a single instance among our 110 cases of azoospermia. Every time a history of gonorrhea was obtained, and usually also a story of transient and painful swelling of the testicles. On palpation most patients showed a thickening of both epididymides, and all but 7 showed a thickening of at least one epididymis. Usually its lowest part is definitely painful on pressure. Enlargement of the head and of the vas deferens is quite common too; many azoospermic samples come from professional drivers.

Underdevelopment of the testicles is a rather common occurrence, but does not as a rule entail a diminished concentration of spermatozoa, except in extreme instances, when both testicles are no larger than a bean.

TREATMENT

We are well pleased with the success of antibiotics, which suffice in many cases to cure a sterility. They act also well on cervicitis and vaginitis, unless these stem from trichomonas or monilia. Both these types of vaginitis are rarer in the Bantu woman than in the Caucasian.

Tubal insufflation, at once diagnostic and therapeutic, also gives good results, and is now successful in about 3 patients out of 4. It is often repeated several times, when the passage appears to be very narrow.

Uterine malposition does not appear to be a major cause of sterility, and we only rarely advise surgery. Most surgical procedures give, in our experience, disappointing results. Curettage of the uterus however we found successful in a number of cases, although its theoretical indication is rather shaky.

Dilatation of the cervix is useful in some cases.

SUMMARY

The main findings in a private sterility clinic in Central Africa are described. The most prevalent local cause of sterility is gonorrhea with its sequelae: obstruction of the tubae and of the seminal ducts. The sterility syndrome in the Negro woman is described and must be known if one is to recognize sterility masquerading under the most unexpected complaints apparently unrelated to the genital system. A peculiar insufflation technic, at once diagnostic and curative, under high pressure, is used with good results. Standards for sperm counts in the Central African Negro are given. Syphilis is not a prominent local cause of sterility.

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Policies and Practices in Iowa Hospitals Relating to Human Sterilization

Nelle S. Noble, M.D.

REGULATIONS IN Iowa hospitals regarding tubal ligation vary widely. To ascertain the facts questionnaires were sent in March, 1953, to all hospitals in the state. The questionnaire is reproduced in Fig. 1. Of the 176 hospitals queried, 153 replied. These 153 included 130 general hospitals; 6 tuberculosis hospitals; 7 hospitals for mental disease or deficiency; 3 private mental hospitals; 1 hospital primarily for college students; 1 reformatory hospital; and 5 osteopathic hospitals.

Of the replying hospitals, 82 had special requirements applying to tubal ligation and vasectomy which do not apply to other operations; 26 had no such special requirements; the other 45 failed to answer this question.

REQUIREMENTS FOR STERILIZATION

Establishment

The special requirements were established by a variety of groups, most commonly (32 institutions) by the medical staff or a committee of the medical staff; by the medical staff and the administration (12 institutions); or by the medical staff and the board of trustees (10 institutions). The administration itself established them in 6 institutions, the board of trustees in 5, and in 2, the administration and the board of trustees were the authoritative body. The moral law of the Roman Catholic Church, the medical staff, and the governing board established the requirements in 6 institutions. Two institutions reported that the requirements for sterilization were determined by the Board of Eugenics, indicating that they perform sterilizations

I. Are there requirements at your hospital applying to salpingectomy and vasectomy procedures which do not apply to other operations? Yes ☐ No ☐

II. By whom or what body were these requirements established?

III. What officer or body or group is responsible for seeing that these requirements are carried out in your hospital?

IV. Is the approval of more than one physician required? Yes ☐ No ☐
If so, how many? ☐ From what medical specialties?
Are there other requirements? Describe:

V. Are sterilization operations permitted for the following reasons:

1. Diseases of the reproductive organs? Yes ☐ No ☐
2. Too many cesareans? Yes ☐ No ☐
3. To prevent pregnancies which would be dangerous because of diseases of the heart, kidneys or lungs? Yes ☐ No ☐
4. To prevent the transmission of undesirable heredity? Yes ☐ No ☐
5. To prevent an excessive number of children? Yes ☐ No ☐

Date Hospital

REMARKS:

Fig. 1. Questionnaire to Iowa Hospitals.

only by order of that board; 2 listed the Board of Eugenics, Bureau of Hospitals of the American Osteopathic Association, as authority for their requirements, 2 did not answer the question, and 3 obviously misunderstood it.

Enforcement

Enforcement of the requirements for sterilization is usually carried out by the authority or authorities which established them. The medical staff was reported to be responsible in 32 hospitals; in 17, the medical staff and administration; and in 10, the administration alone. In 5 the nurse in charge of the surgery and/or director of nursing service was responsible; 4 hospitals left responsibility to the "owners and operators"; 3 each to medical and nursing staff and to administrator and nursing staff; 2 to administrator and medical and nursing staff; 2 to medical staff and board of trustees; and 1 each to administration and board of trustees and administration, board of trustees, and medical staff; 2 listed the board of trustees alone as authority.

Approval and Consultation

In 79 of the 82 hospitals, the requirements for sterilization had to be approved by more than 1 physician; 39 required approval by 2 physicians, 15 required 3, 7 required 4, 1 required 6, and 1 required 10. Three did not state whether approval of more than 1 physician was required or not.

In many institutions regulations frequently require consultation from specialists in fields other than the one originating the procedure. These vary widely from hospital to hospital depending upon which appropriate specialty is represented on the staff. When available, obstetrical, surgical, gynecological, or medical consultants are often called upon. Additional requirements sometimes imposed are signed consultation reports and signed requests of the husband and wife for the procedure.

Roman Catholic Policy

Procedures which induce sterility are permitted in Roman Catholic hospitals only as an unavoidable incident to the immediate cure or amelioration of a serious pathologic condition for which a less drastic remedy is not available. Thus in ectopic pregnancy the affected tube or part of a fallopian tube may be removed, even though the life of the fetus is indirectly terminated, provided postponement of the operation appreciably increases the mother's danger. The rules are elaborated in a code of morals, "Code of the Catholic Hospital Association," published in *Hospital Progress* [March, 1949], and in the *Moral Code for Catholic Hospitals in the Diocese of Davenport*, published in pamphlet form in April, 1949.

Several Roman Catholic hospitals referred to these codes rather than fill out the questionnaire in detail. Others of the same group indicated that sterilization operations were not permitted simply to prevent an excessive number of children, the transmission of undesirable heredity, or pregnancy which would be dangerous because of organic disease of the heart, kidney, or lung. There were some differences in their opinion as to whether sterilization operations should be permitted for "too many caesareans" or for "diseases of the reproductive organs"—perhaps because of differences in interpretation of the question.

INDICATIONS FOR STERILIZATION

Five of the 100 non-Catholic general hospitals which replied to the questionnaire did not record their policies or views regarding the five indications queried under Question 5. The answers from the 95 hospitals that did reply contained replies varying from one extreme to the other, with all possible shades of opinion between. Thirty-three hospitals permit sterilization operations for all 5 reasons listed, while 6 of these non-Catholic general hospitals do not permit sterilization operations on the female for any reason.

As stated, 5 hospitals had not made up their minds or had no occasion to do so. On the other hand, many had very decided views. Here are a few comments written at the bottom of the questionnaires:

"Not according to our by-laws, but the doctors do them." (Sterilizations for "excessive number of children.")

"Have had very few ligations of tubes. Our doctors are most cooperative regarding sterilization, doing them only when best for patient's welfare."

"If 5 children are alive and patient can prove financial difficulty or poor general health we can sterilize the female . . ."

"Tubal ligation following the second caesarean section does not require consultation."

"What the doctor and patient agree on happens to go here, as they are the parties concerned."

"It must be a diseased organ."

The most frequent comment was that any such operation must be for a good "medical reason."

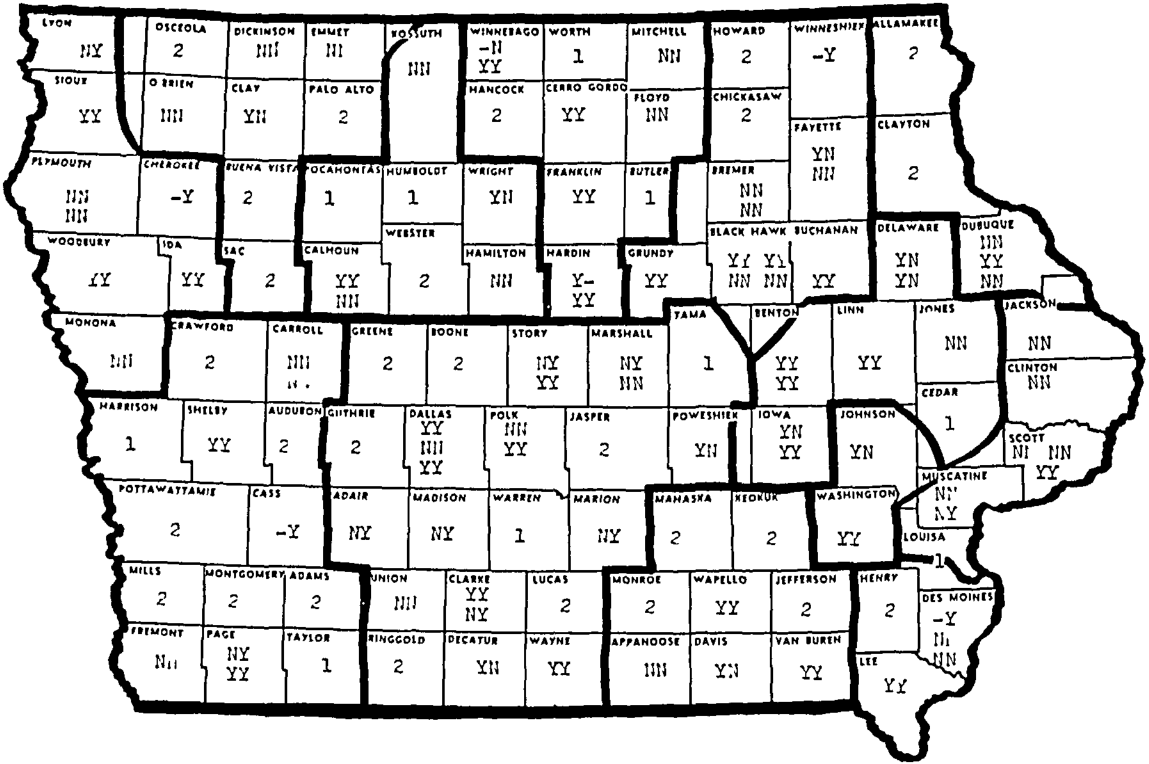

Fig. 2. Regions in the Iowa Hospital Plan in which sterilization operations are permitted for reasons other than pathology.

The multicounty groups are the service regions in the Iowa Hospital plan. Each pair of letters represents a hospital replying to the questionnaire. The first letter (Y = Yes, No = No, - = No answer) indicates whether sterilization operations are permitted to prevent the transmission of undesirable heredity, the second letter indicates whether they are permitted to prevent an excessive number of children. The figure 1 indicates no hospital in that county, 2 indicates a hospital, but no reply to the questionnaire.

One Roman Catholic institution which checked "yes" for diseased reproductive organs but "no" to all other reasons for sterilization reported, "there are no restrictions on any surgery when indicated." A non-Catholic hospital wrote in: "Our physicians are free to act on these matters within the limits which a high standard of medical practice dictates."

Mental Deficiency and Disease

A somewhat different problem is the surgical protection from parenthood of the mentally deficient and the mentally ill. In Iowa this is governed by a state law, administered by the State Board of Eugenics. After approval by the board, sterilization of such persons can be carried out at state expense. In practice, cases are considered by the board only after written consent has been secured from the patient; spouse, if any; and next of kin or guardian. Most of the applications for sterilization thus approved are of patients in state institutions for the mentally deficient or mentally ill; however, the law is also available to persons outside of institutions.

ACCESSIBILITY OF STERILIZATION PROCEDURES

In spite of the limitations for nonmedical reasons imposed on this branch of surgery by many of the hospitals, a geographic analysis indicates that the citizens of Iowa have moderate freedom of access to protective sterilizations. As shown by the map (Fig. 2) each of the multicounty regions into which the state has been divided in the Iowa Hospital Plan contains at least 1 hospital in which sterilization is permitted for eugenic indications. In all but 1 of the regions—that in the northwest, comprising Buena Vista, Clay, Dickinson, Emmet, O'Brien, Osceola, Palo Alto, and Sac counties—there is at least 1 hospital where parents with an excessive number of children may secure surgical sterilization.

The Iowa State Medical Society's *Handbook of Resources Available to Physicians* contains additional information regarding sterilization in the sections relating to the Human Betterment League of Iowa and to the Iowa State Board of Eugenics.

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Abstracts

Sperm agglutination in human semen and blood. Wilson, L. *Proc. Soc. Exper. Biol. & Med.* 85:652–655, 1954.

Ejaculates of 100 men, obtained by masturbation, were normal with regard to volume, sperm count, viscosity, initial motility, morphology, and nonspermatozoal cells. Using a simple microscopic slide method, the author demonstrated sperm agglutinins in the seminal plasma and blood serum of 2 men. Such antibodies have not previously been found under natural conditions and, particularly, not in man.

Agglutination occurred only in the presence of motile spermatozoa and none was seen in the fresh ejaculate until liquefaction began and active sperm movement developed. Temperature influenced the speed and degree of clumping, according to its effect on sperm motility. The motility of the clumped spermatozoa was impaired and subsequently the ability to penetrate cervical mucus was lost. The clumps could be completely dispersed by mechanical agitation after which the spermatozoa reagglutinated, although not quite to the original extent. Complement inactivation did not affect agglutination. The antibody could be removed by absorption. The antibody titers of blood serums were 1:80 and 1:20, respectively. Head, tail, and mixed varieties of sperm agglutination were seen in the early stages but tail agglutination predominated as the clumps increased in size.

The wife of one of the 2 sterile men, after 10 years of barren marriage, conceived following artificial insemination with donor semen.

(AUTHOR'S ABSTRACT)

Effects of ACTH on pregnant monkeys and their offspring. Schmidt, I. G., and Hoffman, R. A. *Endocrinology* 55:125–141, 1954.

Ten pregnant rhesus monkeys were treated with 20 mg. ACTH daily during the last 17 to 67 days of gestation. Two nonpregnant females were similarly treated for 66 and 133 days, respectively. Eleven untreated pregnant monkeys served as controls. This treatment produced marked reduction in circulating eosinophils but had no effect on the number or distribution of other formed elements, on serum sodium and potassium concentration, or on the level of glucose in whole blood.

Three of the pregnant monkeys aborted while 7 pursued an uneventful course and gave birth to apparently healthy offspring. All animals were sacrificed within 24 hours after parturition. Maternal spleens and lymph nodes showed characteristic suppression of activity. In the offspring, these organs were unaffected but there was cortical atrophy of the thymus glands. There were no significant alterations in maternal or fetal pituitaries, thyroids, pancreas, ovaries, oviducts, testes, epididymes, livers, kidneys, myocardium, or bone marrow.

Maternal adrenal glands responded typically to ACTH by hypertrophy of fascicular cells and concentration of lipids in their cytoplasm. Fetal adrenals, in contrast, were very small, had enlarged glomerular zones, atrophic fascicular zones, and redistribution of lipid changes similar to those produced in adult adrenals by cortisone therapy. These findings suggested that excessive amounts of cortical steroids from the enlarged maternal adrenals passed into the fetal circulation and inhibited activity of fetal adrenals.

(AUTHORS' ABSTRACT [I.G.S.])

Color of cervical mucosa as a diagnostic sign of early pregnancy. Farris, E. J. *Obst. & Gynec.* 4:208–212, 1954.

A test for early diagnosis of pregnancy is described. The test depends upon color matching of the normal cervical mucosa with a series of prepared colors (Munsell Color Corp., 10 E. Franklin St., Baltimore, Md.) characteristic of pregnancy. The shades of pink associated with the nonpregnant state are designated N-1 to N-5. The shades of blue associated with the pregnant state are designated as P-1 to P-5, as the intensity of color increases. The cervix of the patient was illuminated with a #1 G.E. photoflood lamp in a Sun-Ray spotlight (Sun-Ray Photographic Co., Inc., 259-309 Lafayette St., New York 12, N. Y.) with a Wratten filter #82-A, giving a reading of 3400° Kelvin. The light was placed 41 inches from the vagina. Specially prepared color chips were matched against the vaginal mucosa of the anterior aspect of the cervix which afforded a smooth surface for comparison.

Fifty-four nonpregnant women were tested at various times during the menstrual cycle. Various shades of pink color were registered. The color was tested 62 times in 50 women who were at least one day overdue. Pregnancy was subsequently proven by the rat hyperemia test and clinically. Practically all depths of pregnancy colors were found within 35 days of the first day of the last menses. One deep shade (P-4) was found as early as the thirteenth day. Seventeen women showed pregnancy color between cycle Days 31 to 35. It was noted that, at first examination, 36 per cent of the women showed pregnancy by Day 35, 66 per cent by Day 40, and 88 per cent by Day 45. By Day 40, cervical color is strongly positive, as a rule. A deep purplish-blue is present from the Day 70 on.

Matching the color of the cervix offers a simple diagnostic test for early pregnancy, eliminating the use of animal assay. Color differences can be distinguished easily and with little experience. Cervical color tests may be diagnostic of pregnancy shortly after the missed period, as early as cycle Day 30.

(AUTHOR'S ABSTRACT)

Evaluation of biologic tests for pregnancy. Benitez, R. E., Phillips, G. W., and Kuhns, D. M. *U. S. Armed Forces M. J.* 5:815–822, 1954.

Data from 6 U. S. Army laboratories were analyzed to develop a uniform pregnancy test. Four methods were investigated: The Friedman test using virgin female rabbits; the Aschheim-Zondek test using virgin white mice; the Hogben test using serum and the *Xenopus laevis* toad; and the test using urine and male green frogs *Rana pipiens.*

The *Xenopus laevis* test using blood serum best met the requirements of economy, simplicity, accuracy, and speed. Sterile serum (5 ml.) is required. Circulating hormone concentration is about 3.3 times greater in blood than that in urine. There are no seasonal variation with the toad (in contrast to the male frog). Each toad can be safely used six or seven times. There are no false positive tests and false negative tests number but 0.8 per cent. The patient must be not less than 21 days nor more than 67 days past expected menstruation.

(O. J. POLLAK)

Book Reviews

Studien am Menschlichen Sperma (2nd ed.). CHARLES A. JOEL, M.D., PH.D. *Basel, Switzerland, Schwabe, 187 pp., 15 plates, 51 figures, and 11 tables.*

In the second edition of his *Studies on Human Sperm*, Joel brings up to date his investigations on seminology and male infertility since the first edition of his work in 1942. Like the first edition, this book consists of three sections. There is first a concise history of the subject of seminology. The second section deals with methods of semen analysis and the evaluation of male fertility. The clinician will find this portion of most interest; particularly helpful are the excellent plates illustrating normal spermatozoa, their physiologic variants, abnormal spermatozoa, and the cellular constituents of semen. The author has added a brief section on testicular biopsy acknowledging its value, but intimating that in most instances of male infertility careful study of the cellular content of the semen will afford adequate information for clinical purposes. With the exception of the Klinefelter syndrome, very little is said of the hormonal findings or etiologic factors in impaired spermatogenesis.

The remainder of the volume is a résumé of the diverse investigations on semen by the author and several co-workers, much of which has been previously published, but which is now brought together in correlated form. Here have been added observations on electronmicroscopy of sperm; the influence of temperature, *p*H, viscosity, light, and other physical and chemical factors on sperm viability; electrophoretic studies; also observations on various enzymes such as diaminoxydase, cholinesterase, and hyaluronidase.

The pertinent literature on the subject of seminology is adequately reviewed and there is an excellent bibliography of 795 references.

A. E. RAKOFF

An Experimental Study of Amniontransplantations to Prevent Intraperitoneal Adhesions. THE TIK LOK. *Gravenhage, Netherlands, Uitgeverij Excelsior, 1953, 83 pp.*

This is a doctoral thesis reporting experimental studies on the prevention of intra-abdominal adhesions. It is written in German with an English summary. The monograph is well-documented with graphs, diagrams, photomicrographs, and an extensive bibliography.

The report deals with a large number of experiments in which the peritoneum of rabbits was insulted by a variety of technics and certain areas were protected with transplants of human amnion.

Three types of experiments are reported: acute trauma, chronic inflammation, and exposure of the uterine cavity.

In each instance the transplanted amnion served as framework for regeneration of peritoneum with the prevention of adhesions.

The author believes that similar results can be expected in homeotransplants into the human peritoneal cavity.

WILLIS E. BROWN

Childbirth Without Fear (Rev. ed.). GRANTLY DICK READ. *New York, Harper & Bros., 1953, 298 pp. $3.00.*

This "revised and enlarged" edition appears, as its predecessor, to be planned primarily for lay consumption. It does, however, give a clear picture of the "principles and practice of natural childbirth."

The controversy surrounding this subject has been wholesome in that it has focused our attention on the emotional aspects of pregnancy and labor, an area all too often ignored by the busy practitioner.

The book is easy reading, even though at times verbose. It is good reading for a physician attending women, for they will surely question him about the book, either for themselves or their daughters. The physician should be informed, whether he approves of the philosophy and teachings or not, and this monograph provides such information from today's most ardent advocate.

WILLIS E. BROWN

Insufflation Utero-Tubaire Kymographique: Méthode pour le Diagnostic et le Traitement de la Sterilité Tubaire. LOUIS BONNET. *Paris, Masson & Cie, 1954, 274 pp., 88 illus., 1600 fr.*

Next to Rubin, who wrote a preface for this book, I know of no one better qualified to write on uterotubal kymographic insufflation than Bonnet. His interest in this subject extends back many years, and in 1934 he made a trip to New York to visit Rubin and learn more about uterotubal insufflation. Bonnet used Rubin's apparatus until 1937 and then devised one of his own. In 1938 he wrote a book entitled *Insufflation Tubaire Kymographique,* for which both Faure and Rubin wrote prefaces.

This book is divided into fifteen chapters, some of which deal with experimental studies, technic of insufflation, kymographic insufflation of permeable tubes, kymographic insufflation of nonpermeable tubes, insufflation of tubes with spasm, insufflation of stenosed tubes, study of hydrosalpinx by kymographic insufflation, diagnostic and therapeutic results, indications for insufflation, and comparison of kymographic insufflation with other methods of insufflation.

Throughout the book the author refers to contributions made by other gynecologists; American references are conspicuous in this field.

The book is well written, in easy-to-read style. The data presented are generally in accord with the ideas of other authorities in this field, notably Rubin. There are abundant hysterograms and kymographs, all of which are clear and instructive. This book will undoubtedly and deservedly prove to be very popular in French-speaking countries. I congratulate Bonnet on his accomplishment.

J. P. Greenhill

Glandular Physiology and Therapy: A Symposium (ed. 5). *Prepared under the Auspices of the Council on Pharmacy and Chemistry of the American Medical Association Philadelphia, J. B. Lippincott Company, 611 pp., $10.00.*

This is the fifth edition of a book which first appeared in 1924 to satisfy an important need at a time when effective hormone therapy was confined to insulin, epinephrine, and extracts of thyroid and posterior pituitary. During the last three decades there has been rapid evolution of endocrine therapy paced by sound methods of experimental approach, by the successful synthesis of the various steroid hormones, and new and accurate technics for in vivo and in vitro study of glandular function.

The thirty-one contributors each expresses his opinions freely. There are twenty-two chapters, each divided into anatomy, physiology, and abnormalities of function, but emphasis is placed upon treatment. In view of the fact that there were so many contributors, the Committee on Publications rendered great service by reconciling the differences of style and nomenclature. A complete list of references follows each chapter.

The contributors are all experts; hence their contributions are authoritative. The data are well presented in a fairly lucid style throughout the book. There is an enormous amount of useful and practical information. The publishers also are to be congratulated on having done their part admirably. This book will undoubtedly become very popular, and deservedly so.

J. P. Greenhill

Laboratory Aids in Endocrine Diagnosis. Roberto F. Escamilla. *Springfield, Ill., Charles C Thomas, 1954, 131 pp., $4.75.*

In the face of much new knowledge in endocrinology, accompanied by a bewildering variety of new diagnostic tests, it is gratifying to find in this monograph a very helpful compilation of the most useful and acceptable clinical laboratory tests. These tests are all based on sound physiologic principles and are simply and briefly described. Their logical grouping makes ready reference to these tests possible for both clinician and technician alike.

The monograph should be particularly useful to the internist who is eager to carry out more comprehensive clinical studies of endocrine disorders.

S. J. Glass

Gynécologie Radiologique et Radiographie Du Sein. JEAN DALACE and J. GARCIA-CALDERON. *Neuchatel, Switzerland, Delachaux & Niestle S. A., 1954, 191 pp., 350 illus.*

This volume on radiologic gynecology is intended as a handy atlas for the gynecologist employing radiologic methods and for the radiologist especially interested in gynecologic diagnosis. The major portion of this atlas is based on the extensive experience of Jean Dalsace, a French pioneer in the x-ray methods useful to the gynecologist. The 350 illustrations comprising the atlas are beautifully reproduced.

The gynecologist specializing particularly in sterility relies upon the x-ray methods developed during the last forty years, and this atlas epitomizes the advances made in this specialty. Concise descriptive legends accompany and clarify each figure if, indeed, there can be further clarification. The authors have purposely omitted lengthy details of technic which they assume are familiar, preferring to present the important films characteristic of the lesions sought by the clinician. Films of atypical findings and films demonstrating shadowgraphs that cause confusion and mistakes in diagnosis are presented for clearer orientation. An admirable candor in acknowledging limits of interpretation is especially to be noted. The vast majority of the films are taken from the authors' personal experience; a few have been borrowed for the sake of completeness from the work of others. These are mostly in the chapters on carcinoma of the uterus and on accidents.

The text consists of 21 chapters; concluding chapter is concerned with the aid of x-rays in the diagnosis of breast lesions. While not yet fully established, this new diagnostic method bids fair, when properly carried out and even in its present stage of development, to be helpful to the surgeon contemplating procedures on the breast.

The present volume renders a valuable service to gynecologists by assembling the typical and atypical x-ray films of gynecologic lesions and their proper interpretation. It serves, moreover, the useful purpose of pointing up accidents and sequelae incidental to and following the use of iodized oils in hysterosalpingography which can be avoided by proper technic and utilization of water-soluble iodide preparations.

In the preface, Professor Funck-Brentano reviews the contributions of French gynecologists and roentgenologists to this special field of medicine and pays tribute to the work of the authors of the atlas. Although the legends are in French, the illustrations are so clear as not to require an intimate knowledge of the language on the part of foreign readers to grasp their significance. For this reason it is unhesitatingly recommended to all gynecologists.

I. C. RUBIN

Actualités Gynécologiques (1st Series). P. FUNCK-BRENTANO (ed.). *Paris, L'Expansion scientifique francaise, 1953, 546 pp., 144 illus., 2500 fr.*

Because gynecology, like medicine and surgery, is in a state of permanent evolution, three years ago Funck-Brentano requested a group of leading gynecologists to contribute articles on up-to-date gynecologic problems. This book is the result. It is a compilation of 42 articles divided into ten sections. All the authors are well-known gynecologists or specialists in related fields. There are two American contributors, Lehfeldt and Rubin.

It is not feasible in a review to mention all forty-two subjects, but suffice it to say that all the important aspects of gynecology are covered in the book. Many articles have excellent reference lists.

The data presented are definitely authoritative and the suggestions for therapy, where they are indicated, are in accord with current leading opinion, not only in France but also in other countries including the United States. One minor point on which I disagree with Funck-Brentano is his chapter on "The Treatment of Vaginismus Due to Persistent Hymen." To relieve this type of vaginismus Funck-Brentano advocates inserting a Champetier de Ribes balloon into the vagina, filling it, making traction on it, and finally excising the hymen over the balloon. Twelve pages and five illustrations are devoted to this operation. Incidentally, two of the illustrations are repeated in another chapter (pp. 498–9). Aside from this and a few minor errors (Sims is spelled Syms), the book is an outstanding contribution. It is easy to read and the illustrations are abundant, clear, and instructive. The intent is to produce a French Year Book of Gynecology and to publish one every two or three years. The editor and his collaborators are to be congratulated on their accomplishment. Likewise, the publishers are to be commended for having done their part well.

J. P. GREENHILL

Textbook of Pediatrics (ed. 6). WALDO E. NELSON (ed.). *Philadelphia, W. B. Saunders Co., 1954, 1581 pp., $15.00.*

This book is a continuation of the series, *Textbooks of Pediatrics*, begun by Griffith, and continued by Griffith and Mitchell and by Mitchell and Nelson. This edition was edited solely by Nelson (and his family). Seventy-two authors made contributions to this book. In spite of the great effort of the editor to keep the size of the book within reasonable limits, it contains 1581 pages.

Nevertheless the editor did succeed admirably in condensing the data presented and particularly in creating uniformity of presentation, which is a real feat. There are 438 illustrations, nearly all of which are clear and instructive. Some illustrations are in color.

The entire field of pediatrics and related fields is covered in the book, so that the volume is really encyclopedic. There are excellent lists of references. Nelson is to be congratulated on having produced an outstanding book which will almost certainly prove to be even more popular than its earlier editions. The publishers also deserve praise for the selection of the type, the clearness of the illustrations, and the sturdiness of the binding.

J. P. GREENHILL

Reproduction and Sex: Survey of Human Biology. G. I. M. SWYER. *London, Routledge & Kegan Paul Ltd., 280 pp., 25 shillings.*

The theme of this book is the application of scientific knowledge to the problems of sexual development, childbearing, and sexual behavior. It is written for the laity because the author believes that many fears and anxieties of adolescence and much marital unhappiness would be reduced if there were wider knowledge of the facts of sexual behavior.

The chapters on the anatomy and physiology of the male and female generative organs are well written and amply illustrated. Parts of these chapters, in particular, are uselessly technical for the layman. There are sections on conception and pregnancy, childbirth, and fertility and infertility, with the causes and treatment of the last. The longest and perhaps the best chapter in the book is "Sexual Behavior and Its Development." Since Dr. Swyer states that the book is "concerned with the normal in reproduction" the chapter on sexual anomalies and disorders might well have been omitted.

There is a glossary, an imposing bibliography and an index. There are abundant references to the studies of other workers in related fields and where there are opposing opinions on a particular subject, both are given. The differences between the British point of view and ours are so slight as to be insignificant.

The book is fundamentally sound and should prove very helpful to individuals seeking information on the many subjects which Dr. Swyer covers so well.

FRANCES E. SHIELDS

Man's Capacity to Reproduce. JOSEPH EATON, M.D. and ALBERT J. MAYER. *Glencoe, Ill., The Free Press, 1954, 59 pp., $2.00.*

Fertility is a complex function conditioned by many factors—biologic, social, and psychologic. Because of the multiplicity of factors which may influence or inhibit fertility, man rarely attains maximum potential fecundity, particularly in civilized populations. A unique exception to such limiting factors is to be found among the Hutterites, a close-knit devoutly religious white sect living in the American Northwest since 1870. Between 1880 and 1950 this sect has increased over 19 times—from 443 to 8542 persons! This extraordinary reproductive record is a result of at least five social-psychological conditioning factors:

1. The culture puts much positive value on having children; any form of birth control is regarded as sinful.
2. The community and its values assure economic support to parents who have as many children as they can biologically conceive.

3. Hutterites can afford and are willing to pay for good medical care.
4. Only very few of the adults fail to get married.
5. There is little migration, traveling, or legal separation or divorce to separate husband from wife during the fertility period. There are few occasions when married women have no opportunity to reproduce.

Whether the increasing social pressures of the surrounding communities will eventually undermine the strict religious tenets of this unique population remains to be seen. Decline in reproductive efficiency will surely follow any acceptance of competing social values.

S. J. Glass

The Mechanism of Labour. Erik Rydberg. *Springfield, Ill., Charles C Thomas, 1954, 180 pp., $4.50.*

In this monograph the author proves his theory that the movements of the fetal head during labor are determined essentially by the shape of the head, and the shape, elasticity, and plasticity of the wall of the birth canal. The evidence presented is based on ingenious experiments which were performed by Rydberg and on roentgen studies of the position of the baby's head before and during descent, and the postural changes in the child during labor. The author also employed moving pictures to study the mechanism of the delivery of the shoulders and the behavior of the infant's head after expulsion from the vagina.

The book is divided into six chapters: 1. "Labour from the Mechanical Point of View"; 2. "On Explanations of the Mechanism of Labour"; 3. "The Shape of the Foetal Head"; 4. "Experiments with Models"; 5. "Application of the Previous Considerations to Actual Labour"; and 6. "Extended Mechanical Analysis of the Experiments with Models." At the end of the book is an extensive summary and a long list of valuable references. Throughout the book the author discusses contributions of obstetricians on the mechanism of labor. This historical review and careful analysis reveals how much time and effort and thinking the author put into his work.

Rydberg is to be congratulated for having presented a simple, lucid explanation of a complicated subject and in a language not native to him. The publishers also are to be commended for their choice of type and paper and because the illustrations are beautiful and instructive, even the roentgenograms, and the book is well bound. This book is a real treat and worthy of very careful study by everyone interested in obstetrics.

J. P. Greenhill

Surgical Treatment of Cancer of the Cervix. Joe V. Meigs (Ed.). *New York, Grune and Stratton, 1954, 462 pp., $12.00.*

This is the first book devoted entirely to the surgical treatment of carcinoma of the cervix. Practically every modern author who has written extensively about this subject has contributed to this outstanding book. The list of illustrious names includes Bastiaanse, Bricker, Brunschwig, Carter, Colby, Graham, Henriksen, Ingelman-Sundberg, Ingersoll, Javert, Kottmeier, McCrea, Meigs, Mitra, Morton, Nathanson, Navratil, Parsons, Quimby, Sederl, Te Linde, Ulfelder, and Werner. The book is edited by Meigs, who wrote an excellent introductory chapter of twenty-four pages. In it he reviews the indications for the surgical approach to cervical cancer, the history of surgical operations, operation versus radiation, classification of cancer of the cervix, carcinoma in situ, choice of treatment, etiology, diagnosis, cervical stump cancer, recurrent cancer of the vagina, lymph nodes, the ureter in radical surgery, and cancer of the cervix and pregnancy, and presents two glossaries of terms used in different parts of the world. At the beginning of each of the thirteen chapters is a short editorial comment by the editor. Every article is illustrated and has a well-selected bibliography at the end.

This book contains all the present knowledge about cancer of the cervix. The information is absolutely authoritative, the text in all instances is clearly and ably written, and the illustrations are numerous, clear, and instructive. The publishers have done their part most ably also.

It is inconceivable to the reviewer that anyone who does gynecologic surgery should fail to own a copy of this book. Since there is only a relatively small number of gynecologic surgeons who can properly carry out the necessary surgical procedures for cancer of the cervix, and since every gynecologist should know how to perform the necessary radical surgery, the vast majority of gynecologists must learn the contents of this book. It is well to quote Meigs' statement, "Now I am no longer worried about the acceptance of the procedure but about the abuse of it, for many surgeons do this operation who have never properly learned its dangers and its actual operative technics."

Meigs not only deserves great credit for having revived interest in the surgical treatment of cancer of the cervix, but also deserves the highest praise and commendation for having conceived and edited this important medical contribution.

J. P. Greenhill

Ovulation in the Living Albino Rat

Richard J. Blandau, M.D.

THIS PAPER deals with some observations on the rupture of the ovarian follicles in the living rat. The morphologic changes which take place in the preovulatory stages and in follicles at the time of ovulation have been described for numerous species in sectioned material. Yet ovulation in the living, intact mammal has been observed only in the rabbit,[5-7, 10, 12] the ewe,[8] the cow,[11] and the human.[1, 2] Since the rat is used so extensively for investigations of ovarian physiology it has seemed important to record the in vivo phenomena in this species. This includes such hitherto unrecorded mammalian data on the length of time required for the individual follicles to complete ovulation and the time interval between rupture of the various follicles in a single ovary.

MATERIALS AND METHODS

All the ovulations to be reported here were induced by injecting gonadotrophin preparations into immature rats according to the technic of Rowlands. Immature female rats of the Wistar or Sprague-Dawley strains, weighing 35–40 Gm., are injected subcutaneously in the nape of the neck with 20 I.U. of a pregnant mare's serum gonadotrophin preparation (Equinex, Ayerst). Fifty-four hours later the animals are injected subcutaneously with 20 I.U. of a chorionic gonadotrophin preparation (A.P.L., Ayerst). The animals are not fed after the last injection but receive water *ad libitum.*

From the Department of Anatomy, School of Medicine, University of Washington, Seattle, Wash.

Part of a paper presented as the Solomon Theron De Lee lecture at the University of Chicago (Ill.), October, 1954.

This investigation was supported by a research grant RS–4241 from The National Institutes of Health.

Procedure

Twelve hours after the last injection the spinal cord of an animal is carefully exposed between the fifth and sixth cervical vertebrae and sectioned. The operative area is packed immediately with Gelfoam (Upjohn) to control bleeding. The ovaries are exposed by making an oblique incision, 5 mm.

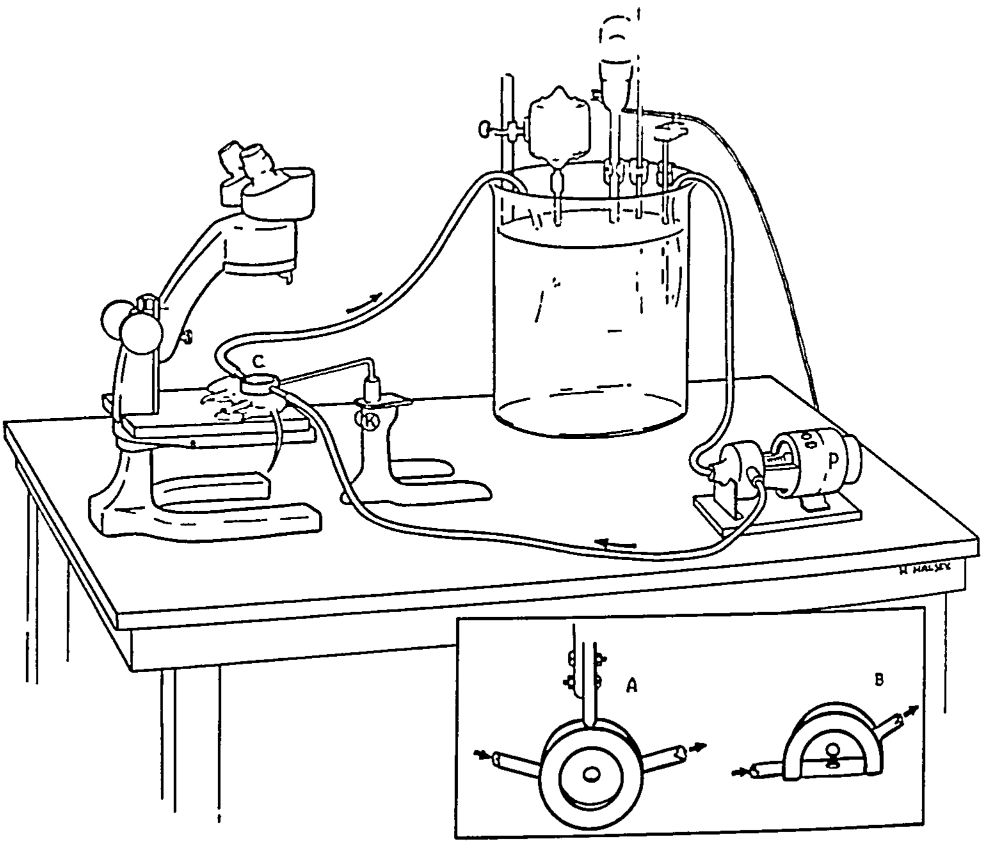

Fig. 1. General arrangement of apparatus used to observe ovulations. The insert shows *A* and *B*, details of the two types of containers described in the text. The pump (*P*) forces warmed water through the peripheral jacket in container (*C*). Container (*C*) is attached to a ratchet so that the height of the cup can be regulated by turning the knob (*K*).

long, in the lateral body wall in the angle between the last rib and the muscles of the back. The skin surrounding the incision is covered with a thin layer of Celvacene* vacuum grease.

The animal is then placed on its side upon the stage of a dissecting microscope (Fig. 1, *C*). A dish (Fig. 1, *A*), which is fastened to a ratchet, is

* Consolidated Vacuum Corporation, Rochester, N. Y.

brought into alignment so that the small circular opening in the bottom of it is directly over the incision. As the container is lowered by turning the knob (K), the periovarial adipose tissue is grasped with a fine forceps and gently drawn into the dish carrying the ovary with it. Great care is taken not to injure the ovary nor to pull excessively on the ovarian blood vessels. The container is lowered until it is pressed against the greased skin. A waterproof seal is assured if the skin is pushed up against the bottom of the dish with a tongue depressor. A fine needle is passed through the mesometrium in the region of the uterotubal junction, thereby anchoring it to the paraffin which covers the bottom of the dish. The periovarial sac is incised close to the oviduct with a finely pointed electrosurgical knife and the large blood vessels coursing over the periovarial sac are carefully cauterized. Ordinarily there is very little bleeding from this procedure. After the periovarial sac has been sufficiently incised it can be peeled back over the surface of the ovary and covered at once with warmed Ringer-Locke's solution.

Observation

In those observations in which various ovulation phenomena were to be timed, a dish as shown in Fig. 1, *A*, was found to be most convenient. It is made of thin copper, is 1½ in. in diameter, and has a peripheral water jacket. Water from a constant temperature bath is forced through the jacket by means of a water pump (P), thus regulating the temperature of the solutions bathing the ovary.

The ovaries of animals prepared in this manner may be observed for as long as 6–8 hours. Ordinarily, however, all ovulations in a single ovary were completed in less than 3 hours so that observations were not carried beyond this period. The length of time required for a single ovulation as well as the interval between ovulations were recorded by means of stop watches.

A number of ovulations were recorded cinemicrographically on 16-mm. Ansco color film. The best results are obtained if a chamber such as illustrated in Fig. 1, *B*, is used. In this case the animal is prepared as described before but is placed on her back and the ovary drawn into the chamber through the opening on the flat side of the dish. The glass-covered opening on the bottom of the dish is brought into position over a substage condenser so that the follicles may be transilluminated from below. Two 100-watt zirconium arc lamps are used—one to illuminate the surface of the ovary and the other for transmitted light. All photographs were made with 16 or

32 mm. MicroTessar lenses attached directly to a motion-picture camera by means of extension tubes.

RESULTS

During the course of this study the process of ovulation was observed in over 300 ovarian follicles. Data were obtained on the time required to complete ovulation in 166 of these. The time interval between ovulation of the various follicles in single ovaries was noted in 11 animals.

The ovaries of immature rats stimulated with gonadotrophins as previously described are greatly enlarged and contain numerous follicles of varying sizes. The ovulatory follicles ordinarily bulge from the ovarian surface and appear very turgid and vascular. Their apices are convex and have a dense meshwork of capillaries. A characteristic feature of the vascular arrangement of the follicles as a whole is the large, flattened venous tributaries which become continuous with the dilated peripheral veins.

Stigma

The first indication of the approach of an ovulation is the formation of the macula pellucida, or stigma, which usually appears at the apex of the follicle. The rapidity with which the stigma forms varies greatly from follicle to follicle. In some follicles it will have completely developed within a few seconds. However, in the majority it proceeds much more slowly, requiring 3 to 5 minutes on the average, before it has fully developed.

As already mentioned the stigma forms at the apex in most of the follicles. However, in a few instances, its formation and rupture occurred near the base of the follicle. One can usually predict where the stigma will form since the flow of blood, in this circumscribed area, is retarded and then ceases. Occasionally very small hematomas appear either within the stigma or at its base. The completely formed stigma is oval, and its border has a somewhat thickened and whitish appearance. Just beyond the periphery of the stigma the dilated blood vessels are frequently arranged in the form of a ring. As rupture of the follicle becomes imminent, one may observe that the superficial germinal epithelial cells which cover the stigma are breaking up into small clumps. The underlying stroma becomes exceedingly thinned out, and a delicate vesicle or pimple forms on the surface. This vesicle is composed of a clear, homogeneous material which appears similar to a structureless basement membrane or to a bleb of viscid follicular fluid (Fig. 8).

The height of the pimple varies greatly in different follicles. In some, it forms an extensive bulge beyond the surface. In others, only a minimal bulging occurs before the stigma ruptures. There is some variation in the diameter of the stigmas and this is one factor which determines the time required to empty the follicle.

Escape of Ovum

The interval between the rupture of the stigma and the escape of the ovum was recorded in 166 ovulations. It was noted that the time required to extrude the egg varied according to the position of the cumulus oophorus within the follicle. If the unattached cumulus and enclosed egg lay in close proximity to the rupturing stigma, it would enter the orifice immediately and act as a plug (Figs. 2 and 5). In 51 ovulations of this type, the mean length of time required to shed the egg was 3 minutes and 36 seconds (ranging from 11 seconds to 12 minutes and 40 seconds). The standard deviation for this group was 2 minutes and 39 seconds. Usually the escape of the cumulus mass and egg was a gradual one, a few cells at a time being extruded from the follicular opening. When most of the cumulus had been pushed through the orifice, the remaining egg mass was extruded very quickly. Great bursts of follicular fluid followed immediately (Figs. 6 and 7).

If, on the other hand, the egg lay more deeply within the follicle at the time the stigma ruptured, ovulation usually was completed much more rapidly. As the stigma ruptured, the follicular fluid would burst from the orifice as if it were under considerable pressure. The cumulus oophorus would escape much more slowly and would tend to elongate as it streamed toward the opening (Figs. 3 and 10). At times as the cumulus mass streamed through the oval opening it would be retarded momentarily by the smaller size of the orifice. Such retardation usually accounted for the prolonged ovulations in this group. In 112 ovulations in which the major portion of the follicular fluid escaped first, the mean length of time required to complete ovulation was 1 minute and 12 seconds (with a range of from 5 to 280 seconds). The standard deviation for this group was 59 seconds.

Table 1 summarizes the intervals between ovulations and the time required to complete ovulations in 3 typical animals. In each case several ovulations had already been completed when the observations were begun. Since all surfaces of the ovary could not be visualized, the ovulations recorded are only those which could be observed on the presenting surface

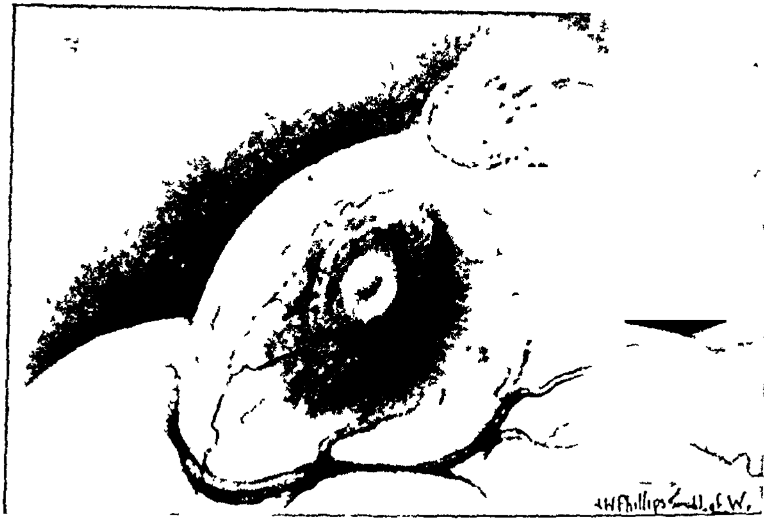

Plate 1. **Fig. 2.** Drawing from a photograph, illustrating ovulation in those follicles in which the cumulus oophorus precedes the follicular fluid through the ruptured stigma. **Fig. 3.** Drawing from a photograph, demonstrating the type of ovulation in those follicles in which the follicular fluid escapes first through the ruptured stigma. Note the elongation of the cumulus oophorus. **Fig. 4.** A follicle in which a venous tributary passes over the center of the developing stigma and greatly prolongs ovulation.

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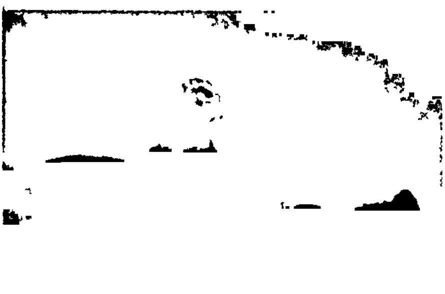

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Color Reproduction
Courtesy Ortho Pharmaceutical Corporation

TABLE 1. The Type and Length of Time of Ovulation of Individual Follicles in the Rat

	Animal no. 262				Animal no. 264				Animal no. 276			
			Time required				Time required				Time required	
Follicle no.	Time of day (A.M.)	Type of ovulation	Min.	Sec.	Time of day (A.M.)	Type of ovulation	Min.	Sec.	Time of day (A.M.)	Type of ovulation	Min.	Sec.
1	8:58	Egg first	8	9	9:06	Follicular fluid first		20	9:01	Follicular fluid first		56
2	9:25	Egg first	2	1	9:13	Follicular fluid first		25	9:11	Follicular fluid first	4	29
3	9:25	Follicular fluid first	1	40	9:13	Follicular fluid first		9	9:12	Follicular fluid first		41
4	9:26	Egg first	6	34	9:14	Follicular fluid first		14	9:12	Follicular fluid first		37
5	9:29	Follicular fluid first		33	9:15	Follicular fluid first		7	9:33	Follicular fluid first		33
6	9:31	Egg first	1	42	9:15	Follicular fluid first		16	9:41	Follicular fluid first	1	59
7	9:32	Follicular fluid first		37	9:20	Follicular fluid first		16	9:45	Egg first	9	40
8	9:36	Follicular fluid first	2	5	9:27	Egg first	4	32	9:54	Follicular fluid first	1	30
9	9:43	Egg first	2	27	9:29	Egg first	2	7	9:59	Egg first	4	47
10	9:44	Follicular fluid first		36	9:42	Follicular fluid first		54	10:07	Egg first	4	58
11	9:53	Follicular fluid first	4	26	9:45	Follicular fluid first	1	30	10:08	Follicular fluid first	1	3
12	10:08	Follicular fluid first	1	5	9:46	Follicular fluid first		32	10:13	Egg first	4	20
13	10:12	Egg first	3	17	10:34	Follicular fluid first		52	10:17	Follicular fluid first	1	32

and in the more peripheral areas. In each case all of the ovulations that could be seen in a particular ovary had been completed in less than 1½ hours. Frequently several follicles ruptured at the same time. This occurs most often during the early phases of ovulation.

Hemorrhage was minimal in the majority of ovulations of either type. If it did occur it was confined usually to some of the smaller vessels at the periphery of the stigma. Gross intrafollicular hemorrhage was not observed in any of the ovulations.

Follicles

Type of Fluid. The follicular fluid escaping from a follicle is of two types: (1) a thin fluid with a viscosity slightly above that of water and which mixes readily with the Ringer-Locke's solution bathing the follicle, and (2) a more viscid fluid in which the cumulus is usually embedded. In a number of ovulations recorded cinemicrographically it was noted that after the stigma had ruptured, the thicker follicular fluid formed an extensive bleb which protruded through the orifice and through which finally the thinner follicular fluid burst (Figs. 9 and 10).

Size. The size of the follicles which will ovulate in the experimental animals varies considerably. The descriptions of ovulations noted above are typical for the larger follicles which bulge on the surface of the ovary and usually are located more peripherally. Ovulatory follicles of intermediate size are more often situated on the lateral sides of the ovary and are tucked in between other follicles of the same or larger size. The stigma which forms on such follicles is often larger in diameter and bulges less on the surface. Such stigmas may rupture very gently and permit the follicular contents to ooze from the follicle. Bleeding at the time of rupture in such follicles is vary rare indeed.

In 3 follicles a relatively large venous tributary coursed over the apex, dividing the developing stigma into two almost equal parts (Fig. 4). Ovulation in these instances was greatly retarded, requiring 15 and 19 minutes respectively in 2 cases and more than 1½ hours in the third case. In 1 instance there was extensive bleeding as the cumulus oophorus squeezed through the small orifice.

Postovulation Change

The collapse of the follicle after ovulation occurs so gradually as to be almost imperceptible. The diameter of the original stigma diminishes until eventually the opening in the apex of the follicle is reduced to pinpoint size or has completely closed. In an occasional follicle there is an outpouching of the granulosa cells through the stigma and in such instances a small tuft of tissue remains after closure and indicates the original site of rupture. During the early postovulatory period when the size of the follicle is gradually being reduced, follicular fluid may continue to drain through the stigma. We have never observed the formation of a fibrin plug in the ovulatory follicles of the rat. As the follicles collapse they lose their translucency and assume a whitish appearance.

DISCUSSION

During the past 20 years ovarian physiology has been the subject of a great many investigations. These have been concerned particularly with the development of the follicles in the normal reproductive cycle and with the pituitary-ovarian interrelationships which culminate in the rupture of the follicle and the release of the egg. It is interesting that even though the morphologic changes in the follicle leading to ovulation have been recorded for many mammals, the actual process of ovulation in the living animal has been described in only a few.

Secondary Cones

Walton and Hammond were the first to observe the formation of the stigma and its secondary cone in the follicles of the anesthetized rabbit. In this animal the secondary cone or nipple forms in the center of the stigma and is the area in which rupture is first noted. In the ewe[8] several secondary cones may appear on a comparatively large stigma. Rupture usually begins on the tip of one of them. Similarly, in the mink,[3] a small papilla pushes out from the avascular stigma as ovulation approaches. Ovulation in the rat differs from these in that obvious secondary cones do not form and the entire stigma is usually involved in the rupture.

Hemorrhage

Bleeding from small blood vessels and the extravasation of blood into the follicle just prior to rupture is the usual occurrence in the rabbit,[7] cow,[11] and the primates.[2, 4] Normally intrafollicular hemorrhage does not occur in the ovulatory follicles of the rat. Small hemorrhages from the edges of the stigma have been described and these appear to be due either to the violent tearing of the stigma during the initial rupture or to the stretching of the oval ring forming the border of the stigma as the cumulus mass is forced through it. As we have shown here, the position of the loosened cumulus oophorus in relation to the stigma is an important factor in the time required to complete ovulation. The loosening of the cumulus in the preovulatory follicles is a well-known phenomenon and has been described in detail in a variety of mammalian species.[4]

Position of Ovum

It is generally agreed that the position of the ovum in the follicle is primarily one of chance. We do not know whether the loosened cumulus oophorus moves toward the stigma as the time of ovulation approaches. Since the cumulus usually is imbedded in the more viscid primary follicular fluid, movement toward the stigma probably could not be expected. It has been shown that in the rat the freely floating cumulus oophorus was located very near the rupturing stigma in approximately one third of the ovulations.

The cells composing the cumulus oophorus form a mass which ordinarily is somewhat greater in diameter than the opening in the follicle. Thus if the cumulus enters the orifice immediately upon rupture of the stigma it forms a plug and is extruded only gradually. As has been pointed out in the majority of ovulations in the rat there is an elongation of the cumulus as it is oriented in the direction of flow of the follicular fluid through the orifice. This would indicate that the cumulus was located at some distance from the stigma at the time of rupture.

Ovulatory Act

From the descriptions of ovulation in the rabbit,[12] cow,[11] and human,[2] one gains the impression that the ovulatory act is not an explosive phenomenon. Even though Walton and Hammond did not actually observe the rabbit ovum leaving the follicle they describe the liquor folliculi as flowing out "steadily and continuously." Markee and Hinsey give a somewhat similar description when they point out that as the tip of the papilla opens the "contents of the follicle flow slowly out of this opening." On the contrary Hill, Allen, and Krammer, describe, also in the rabbit, a very rapid volcano-like explosion of a gelatinous exudate and conclude that "follicular rupture is truly explosive in nature."

Using the surgical technic of exploratory culdotomy, Doyle describes 2 ovulations in the human. In 1 the follicular fluid exuded slowly into the cul-de-sac, in the second there was a gush of follicular fluid at the time of ovulation.

The initial escape of the follicular fluid in the larger follicles of the rat is usually explosive in appearance. Rather large blebs of follicular fluid will suddenly burst from the rupturing stigma. The cumulus oophorus, however, flows out much more slowly. In an occasional follicle even the cumulus may be pushed from it with considerable force. On the other hand, it is interesting that ovulation ordinarily occurs in a very gentle fashion in follicles of intermediate size. In these the stigma becomes exceedingly thin and gradually disappears without an obvious rupture. The follicular contents then flow out slowly and continuously as if there were a gradual oozing-out. Thus in the rat the initial escape of the follicular fluid may be either explosive, as observed in the larger follicles, or slow and continuous in follicles of intermediate size. From the cinemicrographic records there is at least inferential evidence which indicates that an increased intrafollicular pressure in the large bulging follicles effects the initial escape of some of the follicular fluid. There must be very little increase in intrafollicular pressure in those follicles in which rupture is not of the explosive type. At the present time an attempt is being made to measure the progressive changes that occur in the intrafollicular pressures of the ovulatory follicles.

Follicular Cell Differentiation

In observing the orderly manner of formation of the stigma and noting that it may appear elsewhere than at the apex, one gains the impression that its appearance is the result of a progressive differentiation of the cells forming the walls of the follicle. This impression is indeed circumstantial but is enhanced by the fact that two follicles of similar size and turgidity may lie in close proximity—one which may ovulate and one which may become atretic. The reasons for these differences are obscure and probably will remain so until the quantitative relationships between the various hormones favoring ovulation can be determined and until the cytologic modifications of the ovulatory follicles as influenced by these hormones are better understood.

SUMMARY AND CONCLUSIONS

1. A technic is described by which ovulation may be observed in the living immature rat whose ovaries have been stimulated by gonadotrotrophin preparations.

2. The time required to complete ovulation in single follicles varies from 5 seconds to 760 seconds, and depends largely upon the position of the cumulus oophorus in relation to the stigma.

3. In 112 ovulations in which most of the follicular fluid escaped in advance of the ovum the mean time required to complete ovulation was 72 seconds (range 5 to 280 seconds, δ 59 seconds).

4. In 51 ovulations in which the cumulus oophorus preceded the follicular fluid in passing through the ruptured stigma the mean time required was 216 seconds (range 11 to 760 seconds, δ 159 seconds).

5. The time interval between the various ovulations in a single ovary varies considerably and a number of typical examples have been tabulated.

6. Ovulation in the large follicles is usually explosive in nature. In the follicles of intermediate size emptying is characterized by a gentle oozing-out of its contents.

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Psychoanalytic Evaluation of the Problem of "One-Child Sterility"

Marcel Heiman, M.D.

Two recent papers on psychologic factors involved in sterility—"Psychogenic Aspects of Sterility," by *I. C. Fischer*, and "Psychosomatic Aspects of Infertility," by *A. H. Marbach and L. H. Schinfeld*, are indicative of the interest which gynecologists and obstetricians have shown in this approach during recent years. This is especially true for those cases in which physical examination, including all the modern sterility tests, fails adequately to explain the cause of the infertility.

Fischer devotes a paragraph to the entity "one-child sterility"* (secondary sterility)

> The patient conceives without apparent difficulty, then is overwhelmed by the responsibility of motherhood. Or due to a complicated pregnancy, difficult labor or delivery, she suffers enough of a psychic trauma to render her infertile. It is not uncommon in this group to find women whose infertility spontaneously resolves when the first child is ten to fifteen years old. During this time, she matures somewhat, the responsibilities have lessened, she does not believe she can get pregnant anyway, she is under less tension, and becomes pregnant."

Fischer is quite justified in drawing our attention to "one-child sterility" as a special entity, since here the woman has already proved her fertility. In such cases, if one can exclude severe constitutional illnesses or hormonal deficiencies, and if thorough testing of the woman as well as her spouse

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* The only paper on one-child sterility I have found in the literature is by Donald Macomber, published 30 years ago. He assumed as the most important reason "irregularities in the marital habit," and includes in his therapeutic suggestions "attention to diet, exercise and rest."

fails to discover the cause of the sterility, it behooves us to consider the possibility of psychologic factors as either the causative or contributing mechanism.*

STUDY OF PSYCHOLOGIC FACTORS IN INFERTILITY

The physician should not undertake the study of such a couple simply to confirm or rule out a psychologic factor. It is worthwhile to consider psychiatric study of the patient or the family not only in terms of a therapeutic adjuvant, but also as one method employed in understanding the individual who seeks medical help. Much might be gained by using psychiatric interviews as an aid in the study of a case of infertility, rather than expecting the psychiatrists to supply the needed answer.

The present study will present data collected by a psychoanalyst which in small measure may add to our understanding of one-child sterility. This paper will not deal with the mechanism by which psychologic factors result in sterility but will attempt to contribute to the understanding of psychological factors present in some cases of one-child sterility.

The superficial observation of large numbers of patients is inadequate as a basis for understanding the deeper psychologic conflicts. A volume of cases, superficially observed, may be utilized to raise questions and to show trends, but it requires the detailed psychologic study of an individual case to arrive at a clearer insight. And such insight derived from the detailed psychoanalytic study of one patient is relevant only to this particular patient. To draw implications or conclusions or comparisons from the one case studied and to apply these to other cases must be done with singular caution. Only rarely is one single organic factor responsible for sterility, nor is there likely to be one single psychologic factor responsible for it. A combination of organic and psychological factors, and their unique distribution, makes for the specificity of each case.

ATTITUDE TOWARD HUSBAND AND CHILD

In studying families which succeeded in producing only one child, we may ask ourselves the following questions:

1. What does the one child mean to the mother?
2. What meaning might a second child have to the mother?

* The part which the husband plays in such a situation ought not be overlooked. The psychology of the husband, however important it may be, is not being discussed in this paper.

Tentatively the impression was gained that the husband was considered by most women of this group not as a life mate but more as an impregnator. As in some animal species where the male's role consists only in the copulative function, in such families the husband either takes a secondary role in the family or the wife leaves him. Expressions such as "He was cruel," or "He was an alcoholic," or "He didn't support me anyway," or "I had no use for him" are common for such women, indicating the wife's attitude toward her husband. There is a diminished need for a male companion in such women. The man is considered rather as a necessary instrument to obtain the desired child.

NEED FOR CHILD

To answer the question of what is the possible need for the child, we must look at the mother's own family situation to find some answers. I have the impression that underlying the need for motherhood in these women is a more urgent need for a mother. Such women need motherhood because they did not have enough mothering. With the arrival of the child, such a woman has established a mother-child relationship. Unconsciously, through the child, the woman is trying to reestablish a child-mother relationship. By putting herself in the place of the child, the woman may be able to relive the needed child-mother relationship. This would be a plausible reason why the mate is a dispensable third person in this situation. I would like to point out here, and shall elaborate on it later, that in each case, to a varying extent, in these one-child situations, the mother-child relationship has to be looked upon as a defense against unconscious sexual wishes which the woman had as a child toward her own father.

Thus, from what has been said, it may be stated that one explanation for one-child sterility lies in the fact that the first child has unconsciously been used as a means for the reestablishment of a child-mother relationship. An additional child would be undesired, as was the father or a younger sibling at one time, or at the present, the husband. This leads to a tentative answer to the question, "What does the second child of such a patient represent?" Namely, it would bring into the open the "triangular" situation, it would confront the patient with the presence of the third person. I wish to express the idea that sterility in such instances maintains the woman in a fixed infantile mother-child relationship with her first-born.

CASE 1

My own analytic material supplies the unusual situation in which I analyzed a woman during her full course of pregnancy and delivery. Three years later she returned to me for additional treatment because at this time she was unable to conceive despite all efforts. I was able to understand the meaning of the first child and to gain insight into the reasons why she could not conceive a second child.* For the purpose of this paper, only truly relevant material will be presented.

The patient, a 35-year-old white woman, was sent to me because of intolerable anxiety in connection with her forthcoming marriage. I saw the patient a few times before marriage and she returned to resume regular treatment following her wedding trip. She became pregnant immediately and remained in treatment until delivery.

The patient was reared in a midwestern state. She has one full brother, 11½ years younger. She wet the bed until the age of 6. When 18 her parents were divorced and both remarried. Her mother had a second son when the patient was 22. After the divorce she kept house for her father for 1½ years. A year later she became pregnant and a quick marriage was arranged. (There is some reason to believe that she might only have thought she was pregnant. No confirmation regarding the pregnancy and the abortion which soon followed could be obtained.) She divorced her first husband shortly after the abortion.

My medical contact with her began when her second marriage became imminent. The patient's attitude towards me, until shortly before termination of treatment, was one of hostility, hatred, and constant verbal abuse. It became increasingly apparent that the patient had established a sadomasochistic relationship with her mother which she acted out with me during the treatment. Whatever healthy strivings there were to reach out toward her father had been nipped in the bud because he was alternatingly remote and cold, or seductive.

The unborn child was felt by the patient to be something that would destroy her, physically and emotionally, a feeling which was an expression of the patient's own hostile wishes towards her mother. The conception of the baby in the early phase of the treatment was an act of rebellion. It was her immediate reaction to my suggestion not to become pregnant at that particular time. Toward the end of the pregnancy some of the guilt and hostility had abated. There was little doubt in my mind that most of the material in connection with the pregnancy was concerned with being destroyed or destroying.

The situation was quite different when the patient returned for additional

* The full material of this case is to be published shortly in the paper "The Man-Dog Relationship," presented at the Mid-Winter Meeting of the American Psychoanalytic Association in New York City, 1953.

therapy 3 years following the termination of her treatment with me and her delivery. Again the patient was very anxious, this time because of her desire to have another baby and her inability to achieve pregnancy. She came back to me after careful investigation of herself and husband by a competent gynecologist had shown no reason why she could not conceive. Her plan to have another baby dated back about 1 year when she, her husband, and their boy had made a visit to her father. While with her father, the husband and patient decided to have another baby. It was April, the same time of the year that she was married.

As a result of this additional treatment, it was uncovered that for the patient the second child would be analogous to the second child of her mother, a boy born when the patient was between 11 and 12 years of age. The patient revealed that at that time she had ideas of having been made pregnant by her father. While the mother was pregnant, the patient, who had just started to menstruate, had the fantasy of being impregnated while in her bath by semen left by the father. After the child was born she remembers that she played mother to the child—put it to her little breasts and went through the motions of nursing.*

Comment

The material of this case illustrates the statements made earlier. The *patient's difficulty in conceiving the second child was related to her fantasies* that it represented the baby she wished to have by her father. In agreement with this idea was the fact that she first planned the second child while visiting her father. Finally, her attitude towards me was also confirmatory. This attitude was one of warmth and closeness. In contrast to this, her first child—to a large extent—re-enacted her own struggles with her mother. This was shown, too, by her attitude of hostility and aggressiveness toward me during the first treatment.

In still more abridged form, I wish to present material I obtained from another patient in analysis.

CASE 2

This was a 38-year-old married white woman who had a daughter of 9. Some of her most severe feelings of guilt were connected with an incident which had taken place about 5 years ago. At that time she was visiting her home town where her father lived (her mother had died about 5 years before the visit)

* This patient's mother is the prototype of a woman who psychologically represents the mother in a one-child sterility situation, although she had more than one child. Just as her second child was born when the first one, our patient, was 11, so was her third born—the *first* child in her second marriage—when our patient was 22. Later in the paper I shall give some explanation for this behavior.

After coming home drunk from a party, she seduced her husband, although she knew that she was in her fertile period and had no contraceptives available. She became pregnant and had an abortion performed. In this case, too, the awareness that she had seduced her husband for the purpose of getting pregnant came up in analysis in connection with material indicating her fantasies about having a baby by her father. As in Case 1, the desire to become pregnant took place while visiting her father.

Analysis provided material which indicated that the patient's mother had been openly seductive toward her. Her mother would speak in the most derogatory terms about any sexual activity involving men and, on the other hand, would exuberantly praise relationships between women. The patient described her mother as a woman known for her horsemanship who walked around with a horsewhip and once horsewhipped a man who crossed her. She would ripple her muscles like a male athlete. The patient realized in the course of her analysis that she was brought up in a home where doing wrong (i.e., being a homosexual) was rewarded, and where doing right (i.e., developing heterosexually) was punished.

Comment

Helene Deutsch describes "how the woman's own unmastered tie to her mother drives her to compulsive repetition. . . . She, the mother, wants the girl to communicate all her experiences and introduce all her friends to her and sleeps in one bed with her, without regard for the husband. When on occasion I have professionally objected to such behavior, I have several times been assured, 'I myself slept with my mother until my marriage.'"

The case which Deutsch reports, is a 20-year-old patient, the only child of rich parents. Her father had "little interest in family life and was more like a guest in his home than like a parent. . . . The early infantile mother-child relationship had been preserved so splendidly that the eighteen year old girl, at the time of her treatment, was still sleeping with her mother in the same bed and before falling asleep would suck either on the breast or on the finger of her mother. . . . All during treatment the father had only *one* meaning, that of a most unwelcome interloper who every so often tried to wedge himself between the patient and her mother." Helene Deutsch goes on to describe how at first the mother had refused to separate from the patient, and later on, the patient had been unable to separate from the mother. "Long before, the grandmother of this phobic patient inaugurated the process of attaching her daughter (the patient's mother) to herself, and this daughter's unfortunate marriage had intensified the tie.

It is noteworthy that the homosexuality aroused or sanctioned without repugnance by the grandmother—although its sexual component did not become conscious—continued in the mother. In this case (as in almost all cases) the daughter's bed was not only the place of the gratification of the mother-daughter love but also served as an escape from the relationship with the rejecting or rejected husband."

Thus you find the stage set for the one-child sterility. A closeness of mother and child, a twosomeness which excludes any third person.

The degree to which oedipal* fantasies are responsible for a regression which ties the woman to her mother becomes noticeable in another case under analysis with me.

CASE 3

This 24-year-old patient also had 1 child, a girl, who was 2 years old at the time the analysis started. There were long periods during this patient's analysis when the husband was never mentioned. It seemed as though the patient and her little daughter were living alone in their apartment. While consciously she would very much wish that the husband would pay more attention to their little girl, at the same time she gave him very little opportunity to be together with the child. The analysis revealed that she almost identically re-enacted the situation which was present in her parents' home, where she, the patient, an only child, was continuously prevented by her mother from having her father play with her. The mother always said, "Don't hurt her, don't excite her."

Comment

These observations seem to indicate that in certain cases the first child represents the compromise solution of conscious and unconscious strivings. As far as the unconscious motivations are concerned, the child represents a combination of two different needs. To a varying degree the child represents an expression of the fantasy to have a child by the patient's own father. Partly because of guilt for such wishes, and partly because of other reasons, the first child represents, in addition, a re-establishment of a mother-child relationship the way it existed for the patient as a young child prior to the time when her own father played an important role.

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* The term "oedipal" is used to connote the little girl's wishes and fantasies directed at her father (or another person who takes his place). These wishes and fantasies have as their ultimate goal, commonly, the idea to have a baby by the father

DISCUSSION

Among the many meanings the child might have for the mother, I have directed attention to one particular meaning which is important in regard *to the sterility problem—that through the child, the woman is able to relive* the child-mother relationship. Such a relationship satisfies cravings the patient has not adequately satisfied while herself a child, and in addition seems to be a needed defense against the fantasy that the child represents a child by her own father. Such a defense works only as long as no third party enters into the situation. Either the patient's husband or a second child might be considered such a third party. It is for this reason that in a number of cases it was noticed that after having delivered the child the patient would leave her husband.

One-child sterility, therefore, does not always indicate that the patient has adequately fulfilled her motherhood through the first child. It indicates, in certain cases, as I have attempted to describe, that this child is an expression of the woman's inability to fulfill her motherhood. As I pointed out, this inability was based, among other things, upon two factors—the wish to be a little child again and to be back with mother and, as a little girl, the wish to have a baby by father.

Late Second Children

As mentioned earlier, we may find the situation of a one-child sterility in mothers who have more than one child, but the second child is born 10 to 15 years after the first. In cases where the child serves the mother's purpose of a mother-child relationship as outlined in this paper, puberty and adolescence of the child puts an end to the mother's use of the child. It is then that another child is needed to take the place of the first.

Conception Following Adoption

Every so often one-child sterility is mentioned in contrast to the pregnancy of a woman either following the adoption of a child or in connection with the contemplation of adoption. To be sure, the one-child sterility and the pregnancy following adoption need not necessarily be contrasting situations.

Conception After Attempt at Adoption

A case in which conception occurred following frustrated attempts to adopt a child was analyzed and described by Edith Jacobson. We need more such cases in which detailed analysis contributes to our understanding and shows that generalizations are not warranted. Jacobson's patient Sylvia had been amenorrheic. She would not have a child of her own out of a deep sense of guilt over the death of a younger brother. Just as her mother had a deep depression following the death of that boy, which lasted until she could get pregnant again, so Sylvia got depressed after the adopted child was promised and then denied her and remained depressed until she became pregnant during the psychoanalytic treatment.

It is the postscript to Jacobson's article which links in with the ideas presented in this paper. Several months after delivery, despite contraceptive precautions, her patient became pregnant again. "Since she refused to have a second child due to the fear of creating for her first child the same emotional complications from which she had suffered, she had an abortion." Despite all available precautions the patient became pregnant yet another time and again had an abortion performed. She seriously considered sterilization when eventually she again became amenorrheic and thus solved the problem. It becomes quite clear from Jacobson's account of this case that the patient did not wish an additional child because she had identified herself with her own child. I believe nothing shows better the intensity of the raging conflict than the history of this patient who at first, being amenorrheic, could not conceive and later could not keep from conceiving. But the distribution of the inner forces were such that the patient could not permit herself to have a second child.

SUMMARY AND CONCLUSIONS

Observations made on patients in psychoanalysis are the basis for the tentative ideas presented in this study.

1. There is some reason to assume that one-child sterility is often, at least partially, the result of a psychologic conflict.

2. In certain cases the first child is slated to be the only child because unconsciously it represents a re-establishment of the child-mother relationship between the woman and her own mother. At times such re-establishment of a child-mother relationship is sought out of guilt over the woman's unconscious wishes to have a baby by her father (oedipal wish).

3. The twosomeness of this mother-child relationship does not permit the intrusion of a third person. The third person might be either the patient's

husband or the second child. I assume that this is the reason that, in certain cases, women with one child leave their husbands or relegate them to a secondary position.

This contribution to one particular aspect of sterility from a psychoanalytic point of view, the so-called one-child sterility, has been presented with the hope that those obstetricians and gynecologists who are seriously interested in the psychologic aspects of sterility will seek the answers to a most complex problem by detailed psychologic studies of the patient. It is the detailed study of each individual, of each couple, and of each family setting that is necessary in order to arrive at a more comprehensive understanding of the specific problem.

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Serum Gonadotrophins in Normal and Abnormal Pregnancy

II. Function and Action of Chorionic Gonadotrophin Hormones

S. J. Behrman, M.D., M.R.C.O.G., and Philip Niemann, B.A.

THE ENDOCRINE PATTERN during normal and abnormal pregnancy has been investigated by many observers but any interpretation or observation will have only limited value until the significance of these levels is fully understood. This is dependent on knowledge of both the manner of action and the function of CGH in relation to the maternal system. Very little is known about this, or even why the placenta produces this hormone in the first place.

Endocrinologists have stated that the function of CGH in the first trimester is luteotrophic—that is to maintain the corpus luteum of pregnancy. Yet there are reported cases where removal of the corpus luteum during the second and third month has had no effect on the pregnancy. But why is the secretion of CGH maintained throughout the rest of pregnancy with the secondary rise at about 210 days in what has been assumed to be a degenerating organ? Why does one get different levels of CGH in various clinical conditions?* Is this cause or effect? How does CGH act in the maternal system and on what organs?

METHOD AND TECHNIC

For all experiments immature female Yale rats weighing 50–80 Gm.

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Presented at the Tenth Annual Meeting of the American Society for the Study of Sterility, at San Francisco, California, June 18, 19, and 20, 1954.

* See Part I of this report.

were used. The rats were operated upon on Day 1 and used on Day 4. The injection schedules were set up so that the rats receiving aqueous solutions received 5 injections over a period of 3 days and were sacrificed on the fifth day—Day 9. Those receiving oil base solutions received 1 injection per day for 3 days and were also sacrificed on the fifth day. In all 5-day experiments weights were taken and histologic sections made of the uteri, ovaries, and adrenals. Two types of controls were used, one receiving no injections or treatment of any kind, the other receiving injections of Tyrode's solution with the same schedule as above. All rats were fed carrots, oranges, and apples in addition to the standard diet. The interpretation of the hyperemic reaction in the ovaries is detailed in Part I of this report.

EXPERIMENT I

The purpose of this experiment was twofold: First to determine the smallest dose of human chorionic gonadotrophin that would produce a consistent hyperemic reaction in the intact rat, and second to determine whether this reaction could be elicited in the absence of either the pituitary or the adrenal. In other words, we sought to establish whether the reaction was a direct one on the ovary or an indirect one via the pituitary or the adrenal axis. From Table 1 it can be seen that the smallest dose required to give a more or less consistent hyperemic reaction (within the limits of experimental error) was 0.2 mg. CGH.* This dose remained as our standard throughout further experiments.

Of interest was the negative reaction when the pituitary or adrenal was absent. However, when the dose was increased tenfold the hypophysectomized rats responded, but the adrenalectomized rats still gave a predominantly negative response. This is important because we know that the placenta produces ACTH-like and cortisone-like substances, so that when a minimal stimulating dose (0.2 mg. CGH) is used, there is not enough ACTH present to stimulate the adrenal in the intact animal and whatever reaction is obtained is predominantly due to CGH. However, when the 2 mg. dose was used it is possible there was enough ACTH to stimulate the adrenal in the hypophysectomized animals, thus giving a positive reaction. Thus CGH could act on the ovary in the absence of the pituitary provided there was enough adrenocortical action. But even this amount of ACTH would not give a positive reaction in the absence of an adrenal. The con-

* The Upjohn Company, Kalamazoo, Michigan.

TABLE 1. Establishment of Minimum Hyperemic Dose of CGH

	Intact			Hypophysectomized			Adrenalectomized		
	No. of rats		Hyperemic response	No. of rats		Hyperemic response	No. of rats		Hyperemic response
	+	−		+	−		+	−	
CGH									
0.1 mg.	1	7	Negative	2	6	Negative	0	5	Negative
0.15 mg.	0	8	Negative	1	7	Negative	1	4	Negative
0.2 mg. (4 hours)	7	5	Positive	2	10	Negative	0	10	Negative
0.2 mg. (5 days)	5	0	Positive	0	5	Negative	0	5	Negative
2.0 mg.	5	0	Positive	7	0	Positive	3	2	Inconclusive
Human serum (< 8 I.U./cc.)	4	0	Positive	2	2	Inconclusive	1	4	Negative
Controls									
Tyrode's solution (4 hours)	0	5	Negative	0	5	Negative	0	5	Negative
Tyrode's solution (5 days)	0	5	Negative	0	5	Negative	0	5	Negative

trols revealed that neither the actual injection or the presence of the fluid medium in the peritoneal cavity of the rat for 4 hours or even 5 days would give a similar reaction.

EXPERIMENT II

This was planned to verify the finding that the adrenal was the vital organ in the functioning of CGH and not the pituitary, and that the pituitary was needed mainly to provide the ACTH for stimulation of the adrenal cortex. ACTH by itself has no effect on the ovary.

As can be seen from Table 2, FSH* alone gives a hyperemic reaction in the intact animal but not in the hypophysectomized or adrenalectomized animal: This is probably because there is sufficient LH present in this preparation of FSH as well as a functioning adrenal cortex. When ACTH is added to FSH in the hypophysectomized animal a hyperemic response is obtained because there is now again a stimulated adrenal cortex, but no response will be obtained with this combination in the adrenalectomized animal. FSH and CGH in combination will give no reaction in the hypophysectomized or adrenalectomized animal but CGH will give a reaction in the hypophysectomized animal if ACTH is provided to stimulate the adrenal cortex. Again no response is obtained in the adrenalectomized animal.

It therefore becomes clear that the action of CGH is not a direct one on the ovary. Furthermore CGH does not act via the pituitary but needs the pituitary merely to serve the function of providing ACTH to maintain an active adrenal cortex; and the presence of a certain level of adrenocortical extract is mandatory. This is a new concept.

EXPERIMENT III

This was designed to determine whether CGH stimulated the adrenal cortex which in turn caused the hyperemic response in the ovary, or whether the adrenal secreted some substance which had a "catalytic" function in permitting the CGH to act upon the ovary. If the latter was the case it would be interesting to determine which of the cortical extracts was responsible.

Table 3 shows that neither DOCA, compound F, nor cortisone acetate by themselves caused hyperemia in the ovary. A consistent hyperemic reaction was obtained when CGH was added to cortisone acetate but not when it

* Parke, Davis & Company, Detroit, Michigan.

TABLE 2. Hyperemic Response to FSH and ACTH with CGH

	Intact			*Hypophysectomized*			*Adrenalectomized*		
	No. of rats		*Hyperemic response*	*No. of rats*		*Hyperemic response*	*No. of rats*		*Hyperemic response*
	+	−		+	−		+	−	
FSH 1 mg.	9	0	Positive	2	8	Negative	1	9	Negative
FSH 1 mg. + CGH 0.2 mg.	5	0	Positive	0	5	Negative	0	5	Negative
FSH 1 mg. + ACTH 10 U.S.P.				5	0	Positive	0	5	Negative
ACTH 10 U.S.P.				0	5	Negative	0	5	Negative
ACTH 10 U.S.P. + CGH 0.2 mg.				7	2	Positive	0	7	Negative

TABLE 3. Hyperemic Response to Cortical Extracts with CGH on Adrenalectomized Rats

	Adrenalectomized		
	No. of rats		*Hyperemic response*
	+	−	
DOCA 2.5 mg. in oil	0	5	Negative
Compound F 2.5 mg. in water	0	5	Negative
Cortisone acetate 1.25 mg.	0	5	Negative
CGH 0.2 mg., DOCA 2.5 mg.	0	5	Negative
CGH 0.2 mg., cpd. F 2.5 mg.	0	5	Negative
CGH 0.2 mg., cortisone acetate 1.25 mg.	5	0	Positive

was added to DOCA or compound F. This we consider further evidence that the adrenocortical extract has no direct effect on the ovary but merely acts as a catalyst for the CGH, or permits the action of CGH.

CONCLUSION

The chorionic gonadotrophin produced by the placenta will cause a hyperemic response in the ovary of the rat. It does not seem to act through the pituitary or the adrenal glands, nor does it seem to act directly on the ovary unless there is a minimal amount of adrenocortical secretion (cortisone acetate) present, the exact amount of which has still to be determined.

At this stage it is uncertain whether CGH directly stimulates the adrenal gland as well, but it appears that the adrenal cortex must be functioning at a certain level—that is, produce a certain amount of cortisone—for CGH to have its effect on the ovary. This necessity for having an adrenocortical secretion present even though it does not produce any effect by itself has become an increasingly common observation by researchers in the field of adrenal steroids, and has been termed the "permissive action" of the adrenal. Recognition of this fact has been the first contribution to the understanding and interpretation of the action of chorionic gonadotropic hormone secreted by the placenta. Results of further experiments will be forthcoming in a subsequent publication.

DISCUSSION

DR. JOSEPH W. GOLDZIEHER, *San Antonio, Texas:* Dr. Behrman's paper is extremely interesting to us for a variety of reasons. Back in 1947 we were also very much interested in the secretion of CGH, and at that time we were fascinated with the problem of determining how rapidly it was eliminated from the system. However, we decided against the use of the immediately postpartum female because she might have a hormone-producing tissue in the uterus.

On the excretion of CGH from the male, we found injections could still be detected 8 to 9 days later, and if 5 consecutive injections were given to a normal male we could still pick up secreted CGH 20 days later. Unfortunately we did not assay the serum at the same time, and I would be interested to know if Dr. Behrman has done so.

Second, with reference to the relative sensitivity of urine and blood, the critical experiment would be the addition of human CGH to serum and to urine and its application to the animal to see whether it assays of equal sensitivity. I would like to see if Dr. Behrman has done this.

DR. ROBERT N. RUTHERFORD, *Seattle, Washington:* I think we are all hoping for some biologic tests which can be inexpensive. If Dr. Behrman is willing to give us figures on the expense involved or its accuracy, I might leave that question for him to answer.

DR. BORIS RUBENSTEIN, *Chicago, Ill.:* May I ask whether the frog could be used instead of the mouse or rat for gonadotrophin tests?

DR. BEHRMAN: To answer Dr. Rubenstein's question first, yes, you can use the frog, almost any animal, with the same accuracy, if you wish.

I wasn't quite sure about the question, Dr. Goldzieher, but we have not done the assay mentioned in the second part of your question, involving the addition of serum. But one interesting point—we have, as you saw, in one of our cases done urinary and serum CGH assays in a woman who had a hysterectomy, where there was now presumably no tissue left behind, and the same relationship between urinary and serum gonadotrophin as just presented, was demonstrated.

As to the expense, you would of course realize you would have to use anything between three and eight rats for each test if you are going to do quantitative analysis. But for qualitative analysis you only need two, and according to what part of the country you come from, the price is that of the rat—75¢ in our part of the world.

TABLE 3. Hyperemic Response to Cortical Extracts with CGH on Adrenalectomized Rats

	Adrenalectomized		
	No. of rats		Hyperemic response
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Cortisone acetate 1.25 mg.	0	5	Negative
CGH 0.2 mg., DOCA 2.5 mg.	0	5	Negative
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The Formation of a Private-Office Fertility Clinic

Robert N. Rutherford, M.D., and A. Lawrence Banks, M.D.

GREAT FORWARD STEPS have been registered in the evaluation of the infertile human couple. This has been due to the efforts of many individuals once working independently, who less than a decade ago formed the American Society for the Study of Sterility. Since its inception just after the end of World War II, the Society may register as its concrete contributions "Minimum Standards for the Evaluation of the Infertile Couple," its present increased emphasis on the infertile male, and the assembling of a beginning arsenal for attack upon the functional problems influencing human fertility. This is a far cry from the days of incantations, of mystic potions, and of Egyptian snake oil rubbed into the back of the ears in the full of the moon (the time of ovulation for Mother Eve?).

As time has gone on, we all have hoped for massive advances which would resolve the total problem. Such advances have not been forthcoming, despite Rubin's contributions and our better understanding of the basic hormone patterns and the endometrial picture. There has been forced upon us the realization that continued and long-repeated attention to many small details is necessary for a successful outcome in the pair whose chance attempts have not produced a pregnancy within a circumscribed period of time. Heretofore, we felt that a marriage should be allowed 2 years without issue before it merited careful and complete evaluation. Since Whitelaw's report it would seem that we need allow only from 3 to 6 months before we train our diagnostic artillery.

Presented at the 1954 Meeting of the Western Branch of the American Society for the Study of Sterility, Palm Springs, Calif., November 12–14, 1954.

Our own personal efforts go back some 15 years. Some 7 years ago, we brought up to date our results and found that the large majority of patients came for an office visit or so and then disappeared from our ken. In turn, we saw for a few times many couples who already had seen three or four of our colleagues for an equally few times (usually with no better success than ours). When we had had an opportunity to work with the couple for a period of time (and this period was indefinite at first), our results seemed to approximate those of most published figures. With this in mind, for the next 5-year period we hammered again and again to impress upon these couples—and usually it was the wife only to whom we talked, with the husband remaining in the background—that we must have time to work with them. Time alone, we felt, would offer the best solution for their dilemma as well as for ours. The survey at the end of the next 5 years showed no better results. May I insert here that the figure of relative fertility of the average American couple was accepted as 88 per cent—a figure accepted by this Society several years ago.

A specific plan seemed to be needed. This must include not only our ardent wooing of the continued support and interest of the infertile couple, but possibly other agents as well. We could contribute but one concrete fact—that if we had enough time to work with the couple, they *usually* rang the bell. We undertook to indict our other helpers. The urologist was scrutinized most carefully and was found wanting in our particular community because he was primarily a surgeon and not a family counselor. For exact urologic problems he was unsurpassed. However, the large majority of our infertile males (and the majority was relatively infertile, with counts of from 20 to 50 millions) had many other problems. These had to do not only with their sexual habits and potency, but also with their general attitudes and adjustments toward life. This latter often did not include a conscious desire for offspring, as far as many of these males were concerned. Theirs often was only lip service, repeatedly reaffirmed by the wife but not by the husband's actual conduct. These men were often confused and defeated as far as their wives, marriage, sexual problems, and the like were concerned. The inability to father a child was simply a clinical addition to the symptom complex. Removing this prop from him by finding him relatively or completely infertile often did the marriage most grievous harm. The "barren woman" often was able to turn the tables and indict her husband. While we might gain a scientific answer to the couple's failure, not infrequently it would leave them with neither child nor marriage.

This upsetting of the essential equilibrium in which we all must dwell imposes a dreadful responsibility upon the physician who proposes to explore these subterranean confusions. If that physician were to upset such an equation, was he ready to substitute something better? Often the gynecologist could not. So, into the picture must come the man who understands the interpretation of human behavior patterns and the formation of attitudes. We have used the psychiatrist for the disturbed member of the couple, but we have learned to rely first upon the psychologist to identify the person who should be so referred. In other words, the majority of couples will respond to simple education. Those disturbed by this presentation of simple factual knowledge are selected for more careful analysis by the psychologist. If his study so suggests, the couple goes on for evaluation by the psychiatrist with whom he works. Automatically, this couple excludes itself from further pregnancy efforts until a new equilibrium is established which would allow the addition of a new burden, a 7-pound, squalling infant. To illustrate: a couple has been married for 7 years and their sexual adjustments are inadequate. The girl often endeavors to become pregnant to prove to the outside world that she is at least that much of a woman. She is able to evade her husband's future amatory advances by comment upon her condition, produced by him. In the future, she can avoid the same advances by exhausting herself in the 24-hours-a-day care of his child. He is put in the extremely unhappy position of being a rival and critic of his own child, and no normal father could possibly entertain the homicidal tendencies which he may develop for his own seed!

If we are to investigate the equilibrium, we must be ready to substitute a new one which is better than the original. Hence, in our pattern of care there must be this kind of evaluation which often is beyond the abilities of the sterility specialist–gynecologist.

STRUCTURE OF THE CLINIC

The gynecologist remains as the beginning figure in such a clinic group. His is usually the first contact with the couple and that contact usually is with the wife alone. His role in the mechanical evaluation of the female still is mechanically more complex than the diagnostic scrutiny of the male. We have come to recommend routinely a complete physical examination for the male, just as for the female, since he too is a prospective parent. On several occasions we have had referred to us infertility patients who, on their first complete physical examination, despite several years of "sterility study," were found to have physical defects contraindicating pregnancy. The male may be an equally doubtful risk as a parent, with either physical or emotional defects. In other words, each partner needs a careful physical examination during which time the inquiring physician can derive a working knowledge of the patient's emotional state as well. Often these patients have associated medical problems which account in part for temporary or permanent low fertility.

The medical survey should embody an emotional evaluation as well. Details for such a "psychogram" have been outlined most ably by Ford *et al.* After these two surveys—medical and emotional—are completed, the problem of that couple is subjected to the clinic meeting, where the decision is made whether they should be referred to the consulting psychologist. We have found that it seems better to make the psychologist the arbiter, for we have available one who has had long training in family adjustment problems. He is able to plan and direct psychiatric evaluation, if he feels it is needed, better than the other members of the studying group. The psychologist as a therapist cannot be overstressed.

The executive secretary is the coordinator of the group, with duties which include those of being "mother hen" to the couple. She not only collates the scientific material, as will be mentioned later, but also integrates the studies, counsels as a fellow woman, and endeavors to keep the couple's interest from flagging. Needless to point out, it is often a most severe jolt to have one's reproductive abilities found faulty. She interprets and cushions these shocks well.

The laboratory technician plays a lesser role but should be mentioned as an ancillary specialist. In the earlier days, we found it best to do our own sperm evaluations, endometrial biopsies, and vaginal smears. Much of this is done now by a properly trained laboratory team.

FINANCING

Strangely enough, this aspect of the infertile couple's study became not only a diagnostic tool but the most persuasive element in the continued interest of the couple. As mentioned earlier, in the first 5-year survey of our efforts, we found that our percentage of success was very poor indeed. This was due mainly to the fact that the usual patient came but a few times and then meandered on to the next doctor about whose fertility successes she had heard. Usually, the husband never appeared. During the next 5 years, we endeavored to hammer repeatedly at the need for long-continued supervision after the initial survey was completed. The results were no better.

Several years ago began the concept, which is not new to many of you but which was daring to us, of having a flat fee which covered not only the cost of the medical work-up of the female, but also several evaluations of the male samples, and finally supervision of their reproductive activities for a year's time. The flat fee covered the first 6-month period, and the following 6 months was on a fee-for-service basis. If the male partner needed special medical work-up, this was not covered by the flat fee. If the couple needed special marriage or psychiatric counseling, this was not covered by the flat fee. However, for the large majority of "usual" couples this was a most satisfactory arrangement.

This served to select those who were genuinely interested in their problem. It emphasized the need for an adequate period of time not only for study and evaluation, but also to effect impregnation. It gave us an opportunity to more accurately classify the problem of the patients. Once the plan began functioning, it served to clarify the issues in an amazing fashion for all participating parties. The various phases which this flat fee entitles the couple to embark upon are laid out in the following section.

FUNCTIONING OF THE FERTILITY CLINIC

We found that the original preliminary interview should be conducted by the executive secretary, who would explain the functioning and general program of the clinic. This saved a great deal of time for the physicians involved since much of the preliminary literature could be given and many of the original questions of the patient answered here. If she did not return for her first interview with the gynecologist, that settled that. On the other hand, this gave her time for a discussion of the subject with her husband, which in the large majority of cases would assure us of their mutual support for the year's survey.

First Interview

At the patient's first interview, the physician explains the current progress in the evaluation of the infertile couple, basic physiology demonstrated by simple diagrams, and an outline of the tests to be performed. She then is advised that she will have her physical examination. A similar one is advised for her husband, this at the hands of the internist. This latter examination is insisted upon if the husband has any physical problem known to his wife, any apparent defect in his basic sexual knowledge, or a sperm count of lowered vigor, either in quality or quantity. Her physical evaluation includes the usual physical study, complete blood and urine examinations, blood sedimentation rate, Papanicolaou vaginal smear, occult blood upon the stool specimen, and basal metabolism test. She is given specific timing instructions for:

1. Endometrial biopsy taken on the first or second day of flow.
2. Rubin's test done at the start of the "fertile phase."
3. Basal body temperature chart to be followed for a few cycles to determine ovulation and its position in the "fertile phase." Then the chart is no longer kept so that undue concentration upon the sexual act at a dictated single time may not increase functional problems. The body temperature chart *can* be an unholy device.
4. Planning a "fertile phase." This is the period of 7 or 8 days during which time the exposures to pregnancy are to be timed on a 48-hour cycle, in order to allow time for recreation of an adequate male count. The phase starts a few days before ovulation is to be expected and continues for a few days after it presumably has taken place. This effort to divert attention from a specific day has been markedly successful in keeping a more normal sex act—one prompted by desire rather than by schemed schedule.

Educational Material

Carefully prepared printed material is given to the couple. We have prepared our own simple pamphlets with basic information phrased in our own words. These are inexpensive, inoffensive, and can be brought up to date each year as new information becomes available and routines change. They should be prepared by the doctor himself and written in the language with which he addresses his patients. Routinely, the excellent small booklet by Butterfield entitled *Sexual Harmony in Marriage* is given the wife for study with her husband on general sex physiology and technics.

A two-lecture series is planned to repeat itself every two months. The first lecture includes a sound moving picture entitled *Human Reproduction*,[a] obtainable through the McGraw-Hill Book Company. Factual material is covered by a formal lecture afterward and elaborated in a question-and-answer period later. Statement is made at this time that there is time set aside for either or both members of the couple to have an individual interview with any of the members of the clinic. We find it better to use the general lecture as a fact-dispensing medium. The patients are most loath to discuss personal situations before a group. The solicited interview approach seems superior.

The second lecture is devoted to a discussion of tension mechanisms which can result in relative infertility; to a discussion of sex technics, and to a clinical recital of motives, known or concealed, which may impel the couple to seek pregnancy. Ford *et al.* have given an excellent pattern for psychiatric evaluation. If theirs is a normal positive urge, the couple is little disturbed and seems to wonder somewhat vaguely why the lecturer belabors this point. If the couple is emotionally disturbed and the pregnancy is sought as a cure for some problem existing within their union, they often become most upset and distressed. They usually will re-examine their motives with great care. These are the ones who turn up for the interviews and are routed on to the psychologist for more careful investigation. Until he clears the couple as a mature unity which would be benefited by pregnancy, further studies are withheld. Obviously, for this discussion we are drawing the picture in broad sweeps of the brush.

Records and Data

A punch card system is being elaborated and developed for easy study of the material presented by these couples. Our current system is ready to roll with some 150 items for tabulation. We are endeavoring to use the general system suggested by Simmons at the last annual meeting of the American Society for the Study of Sterility in which he proposed that all couples be graded as to: (1) peritoneal factor; (2) cervical factor; (3) tubal factor; (4) uterine factor; (5) ovarian factor; (6) male factor; (7) psychiatric or emotional factor; and (8) no known reason. His feeling, as I recall it, was that if 3 or more factors were found against the couple, strong advice should be given to them that a successful outcome was most unlikely. Whether this will be of practical value depends upon the long-term figures achieved by many of us.

A tickler system is an excellent device borrowed from the business world. Any couple may be put on a revolving file to be brought up at certain stated periods for restudy and new advice—in other words, it is a device to tickle our memories. This constant resurvey of patients has been most comforting and reassuring to them, for they are in great need of constant reassurance that they "have not been dropped" once their original study and survey period is over.

Other Staff Duties

Bimonthly meetings of the staff are held at which current problems are discussed, new cases are classified after their original study has been completed, and projected plans are reviewed. These have been most helpful to all concerned. The patients delight in the knowledge that their sterility problem is not shelved but is constantly being considered.

The relationship with the other physicians in the community has been very good. The large majority of the men are not particularly interested in large numbers of sterility patients. They handle those couples who yield to simple reassurance or to straightforward medications or to standard tests. Failing success, the physician is more and more frequently referring these problem cases to a clinic of this type for more exact diagnosis or long-term study. Meticulous effort must be made here to see that the patient, once impregnated, returns to the referring physician.

CURRENT WEAKNESS OF THE PLAN

Our two greatest causes for disappointment are explained easily but are difficult of solution. We still have a distressing number of patients who need constant encouragement to continue the study, despite the fact that the flat fee has been paid. This morale problem represents the greatest challenge. Here is where our single greatest chance of success comes. If their interest can be kept alive, the large majority of these patients eventually conceive unless serious organic deficiencies exist.

The other disappointing feature is that these patients have a higher incidence of obstetric loss than the usual chance-impregnated "normal" couple. They have a higher spontaneous abortion rate, a greater frequency of premature labor, and possibly a higher incidence of malformations. This again makes for more of a morale problem than does a similar loss in the couple which has created by rapture and not by careful science. Preliminary warning of such an increased rate of disappointment has been of great value.

Diverticulosis of the Fallopian Tubes

A Clinical and Roentgenologic Study

Alvin M. Siegler, M.D.

In the intramural and isthmic parts of the fallopian tube, nodular thickenings sometimes appear which may cause either a complete occlusion of the lumen or a marked constriction of it. The nodules are located in the muscular wall of the tube and contain nests of gland-like tissue. The lumina of the "glands" are often connected to the central tubal lumen.

There is no unanimity of opinion concerning the proper nomenclature of this entity. It has been termed salpingitis isthmica nodosum, diverticulosis, adenomyosis, and endosalpingiosis. Four current concepts which attempt to explain the etiology and pathogenesis of this condition are as follows:

Inflammation

Chiari, in 1887, ascribed the condition to inflammation and termed it salpingitis isthmica nodosum. Schenken and Burns submitted 329 nodules from fallopian tubes to gross and histologic examinations and noted diverticula alone or in association with an acute or chronic inflammatory lesion in 31 per cent of the specimens. The cells of the mucous membrane of the tube may be caught in the musculature of the salpinx and lie dormant unless they are stimulated by some inflammatory process.

According to Goodall endosalpingiosis is the result of a subacute salpingitis of the productive type. This lesion denotes a transplanting of endosalpingial cellular elements and a reconstruction of these elements into

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The majority of the cases cited in this report were obtained from the records of the late Dr. Samuel L. Siegler.

architecturally glandular forms resembling the parent tissue. It appears to be restricted to those catarrhal infections which involve only the endosalpinx. Plastic tubal closure of the fimbriated end of the tube does not take place but patency, at least of this end of the tube, remains. These glandular elements may invade the tubal wall and the mesosalpinx through the lymphatic tissue or they may occur as the result of contamination by spill. In the latter case the ovary becomes the favorite site of the transplant.

Developmental Defects

Wrork and Broders submitted evidence, after an extensive pathologic study of nodules of the fallopian tubes, which suggested that the disease may not be a result of inflammation. They noted in 41 per cent of 58 patients with adenomyosis of the fallopian tubes some developmental anomaly of the tubes on one or both sides. A cribriform endosalpinx was most common but segmental agenesis and miniature lumina also existed.

Metaplasia of Endosalpinx

Novak and Sampson described endometriosis or endosalpingiosis of tubal stumps arising from the tubal mucosa as the result of metaplastic changes of endosalpinx.

Endometriosis of the Fallopian Tubes

Vehaskari quotes Philipp and Huber who found intramural tubal occlusion most often due to tubal endometriosis. They studied histologically the interstitial portion of the fallopian tubes in 616 patients operated upon for various reasons and found in 277 cases (44.9 per cent), tubal endometriosis.

Bunster denied any knowledge of a specific etiology or localization of tubal diverticulosis. In his opinion the term "tubal adenomyosis" is incorrect because it presupposes that the canals are solely in the thickness of the tubal musculature. Occasionally they are outside in the subserosal web. Furthermore "adeno" implies gland formation and this is inexact. The fallopian tube does not possess glands. In none of the areas of the normal tubes are the secretory cells in glandular form. No secretions have been observed in the lumen or on the human ovum although tubal secretions have been found in lower species. If the human tube does not have glands, glandular function cannot be attributed to tubule formation.

The gross and microscopic pathology of this lesion has been presented in detail by Wrork and Broders and by Schenken and Burns. This aspect is beyond the scope of the following presentation.

MATERIAL AND METHOD

Asymptomatic tubal diverticula are clinically impossible to diagnose. The incidental finding of tubal diverticulosis was made most often during the investigation of infertile women by means of salpingograms. The technic

Fig. 1. The continuity of the right fallopian tube is interrupted for a few centimeters at the isthmus by the presence of many small diverticula. Both fallopian tubes were patent by salpingogram. The lesion was confirmed by laparotomy. (In this photograph and in all subsequent ones, the patient's right side corresponds to the reader's right.)

of salpingography was practically standard throughout the series. The films were taken with the beam in the vertical direction. The gradual or fractional instillation of an oil opaque medium was used during the study.

Each of the hysterosalpingograms was analyzed critically. Special attention was paid to the cornua, to the patency of the tubes or sites of occlusion, and to any roentgenologic alterations from the normal tubal shadows. The majority of hysterosalpingograms were performed in the follicular phase of the menstrual cycle. No correlation was made between the phase of the cycle and the roentgenographic appearance of the fallopian tubes.

After deciding which salpingogram had the appearance of tubal diverticulosis the clinical history was studied and the essential features recorded. These included the patient's age, clinical diagnosis, previous deliveries or

Fig. 2. The ampulla of the left fallopian tube appears to end in multiple diverticula. The ampulla of the right fallopian tube is dilated, but its borders are regular. Bilateral tubal occlusion was found on insufflation. There had been infertility for 16 years.

miscarriages, the presence of dysmenorrhea, previous disease which might influence the roentgenographic appearance of the tubes, and the findings on vaginal examination. The results of microscopic evaluation of any tissue that was removed, which might contribute to the study, were also reviewed.

RESULTS

During 1941–1953, 1160 hysterosalpingograms were performed on women complaining of infertility. A diagnosis of tubal diverticulosis was made in 19 patients, an incidence of 1.6 per cent. Bunster described 12 cases of diverticulosis from 1178 salpingograms. In 284 laparotomies he noted that 4.2 per cent of the patients had tubal diverticulosis. Gori found this lesion in 77 patients among 862 laparotomies, an incidence of 8.9 per cent.

In our series the age range of the 19 patients who had tubal diverticulosis was 24 to 43 years; the median age was 31. Schenken and Burns analyzed

Fig. 3. The multiplicity of diverticula in the interstitial area of the left fallopian tube has obscured the central tubal lumen. Bilateral tubal occlusion was present on insufflation and salpingography. Patient conceived twice following investigation and had 2 normal term children.

the surgically removed fallopian tubes in 208 patients, 187 of whom were Negroes and 21 whites. The ratio of approximately 9 to 1. Fifty-three per cent of the Negroes were 30 to 40 years of age and 43 per cent of the whites were 20 to 30 years of age.

Only 3 patients in our group had had normal term pregnancies prior to investigation for infertility. There had been 11 spontaneous abortions and 2 ectopic tubal pregnancies. Wrork and Broders found tubal adenomyosis in 13 of 100 specimens of tubal pregnancy and 9 in a control series of 116 cases.

Tubal diverticulosis was associated with endometriosis in 2 patients, pelvic inflammatory disease in 2 patients, myoma of the uterus in 8 patients, cervical stenosis in 3 cases, and a retrodisplaced uterus in 2 patients. Dysmenorrhea was present in 8 patients but in only 4 instances was bed rest required at the time of menstruation. Of these 4 patients 1 had pelvic inflammatory disease and the other endometriosis. In Bunster's series pelvic

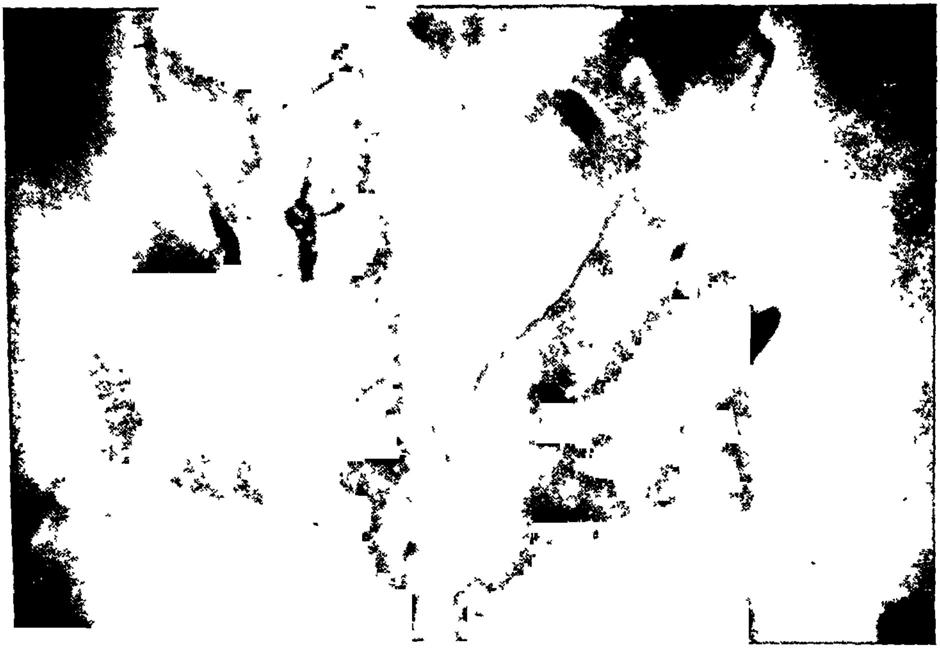

Fig. 4. Bilateral tubal diverticulosis affecting the isthmoampullary region. Patient had had two spontaneous abortions.

adhesions were present in 66 per cent, myoma in 40 per cent, sclerocystic disease of the ovary in 75 per cent, and a retrodisplaced uterus in 58 per cent of the patients.

In our group of 19 cases the right fallopian tube was involved in 6 instances and the left fallopian tube in 13 cases. The lesion occurred predominantly in the isthmus (Fig. 1). In 17 patients tubal diverticula were noted in the isthmus, isthmoampullary or isthmocornual region. In 1 instance the lesion was confined to the ampulla (Fig. 2) and in another to the cornu or interstitial portion of the tube (Fig. 3). It was bilateral in one case (Fig. 4). The affected tube was patent in 8 instances and occluded in 7. In 4 cases the patency could not be evaluated.

DISCUSSION

There have been few descriptions of the roentgenologic appearance of tubal diverticulosis. Hoehne, in 1905, demonstrated by the injection of

Fig. 5. Many outpouchings are seen in the left isthmus and the central tubal lumen is not demonstrable. Bilateral tubal patency was present. Patient conceived 3 months after investigation and delivered at term.

gelatin containing Berlin blue the connection between the lumen of the tube and the branching tubules present in endosalpingiosis. Sampson employed the same principle and published a photograph of the roentgenogram of 1 case. He injected a colored medium into specimens of endosalpingiosis and discerned in microscopic preparations tubules containing masses of injected material. The misplaced mullerian mucosa was everywhere tubal in type and extended almost to the peritoneal surface.

Schenken and Burns instilled Thorotrast into the lumen of the fallopian tubes with diverticulosis previously removed at operation. This confirmed the variations in size, shape, number, and distribution of diverticula seen at the clinical x-ray examination. In some specimens only a few budlike outpouchings of the lumen were observed. In others, the maze of opaque channels in the wall of the tube were numerous and the lumen of the tube was almost completely obscured. Wrork and Broders stated that the study of the ramifications of diverticula by roentgenographic methods following

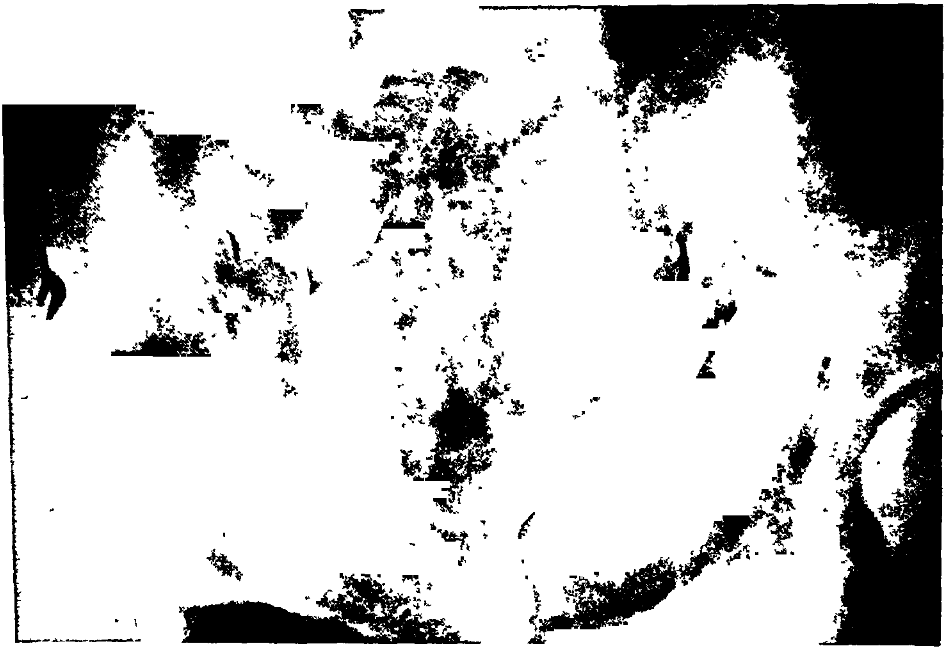

Fig. 6. The uterus is congenitally malformed and markedly dextroverted. Many diverticula are seen arising from the isthmus of the right fallopian tube. There was tubal patency on insufflation.

the injection of radiopaque material was unsatisfactory in most instances due to the minute size of the orifices of such tubules. However, Bunster emphasized the importance of salpingography in demonstrating this lesion. He published a few hysterosalpingograms revealing tubal diverticula.

In our series the diagnosis of tubal diverticulosis was made primarily by salpingography. The characteristic sites were in the isthmic, isthmoampullary and isthmocornual regions of the fallopian tubes. Many diverticula were usually present, resembling the branches of a tree (Figs. 5 and 6). The diameter of the tube which was determined by measuring the most widely separated diverticula conformed to the size of the specimens seen at pathology examination by other investigators. In almost one half of the cases the tube was occluded and there was evidence of occlusion or disease in the opposite fallopian tube.

Diverticula obviously cannot be revealed by tubal insufflation. In fact there was a discrepancy in the results obtained by the two methods with regard to tubal patency. While hysterosalpingography revealed bilateral occlusion in 4 cases, tubal insufflation showed patency in 3 patients and occlusion in 1. Hysterosalpingography disclosed one tube occluded in 5 women but tubal insufflation demonstrated normal patency in 3 and tubal occlusion in 2 of these patients. Although these findings were not confirmed by laparotomy, they do show a significant inconsistency in the results obtained by tubal insufflation and by hysterosalpingography.

Diverticulosis may be suspected in a woman in the fourth decade of life who has had a history of unexplained involuntary sterility of five years or over, no previous pelvic inflammatory disease, no palpable adnexal masses, and no increase in temperature or sedimentation rate. Dysmenorrhea is usually absent. The endometrial biopsy examination should not be suggestive of tuberculosis. When associated with pelvic inflammatory disease or endometriosis the symptoms will be altered. The diagnosis can be made with precision if hysterosalpingography is performed.

The x-ray picture must be differentiated from tuberculosis of the fallopian tubes. However, calcification of pelvic lymph nodes, calcified formations in the tubal lumen or of one or both ovaries, are diagnostic of female genital tuberculosis. Furthermore the fallopian tubes are usually rigid, devoid of peristalsis, and are often occluded at the proximal end of the ampulla. The sactosalpinx is small, its width being less than the size of an ordinary lead pencil. Occasionally fistulous tracks are seen which may be confused with diverticula (Fig. 7). Other roentgenographic signs of tuberculosis may be present and the endometrial biopsy examination may reveal tuberculosis endometritis.

Fifteen of our patients were followed for from two months to seven years. One patient was unmarried; in the remaining 14 married women 4 pregnancies ensued. Three patients delivered normal term children and 1 patient had 2 normal term pregnancies. In 2 of these women bilateral tubal patency was present according to insufflation and salpingographic examination. In 1 patient the presumably unaffected tube was occluded at the cornu. The fertilized ovum therefore successfully migrated through the tube with diverticulosis.

If tubal diverticulosis is discovered as an incidental finding at laparotomy and the tubes are grossly normal they should not be operated upon. The recognition of the disease at laparotomy is greatly aided by considering

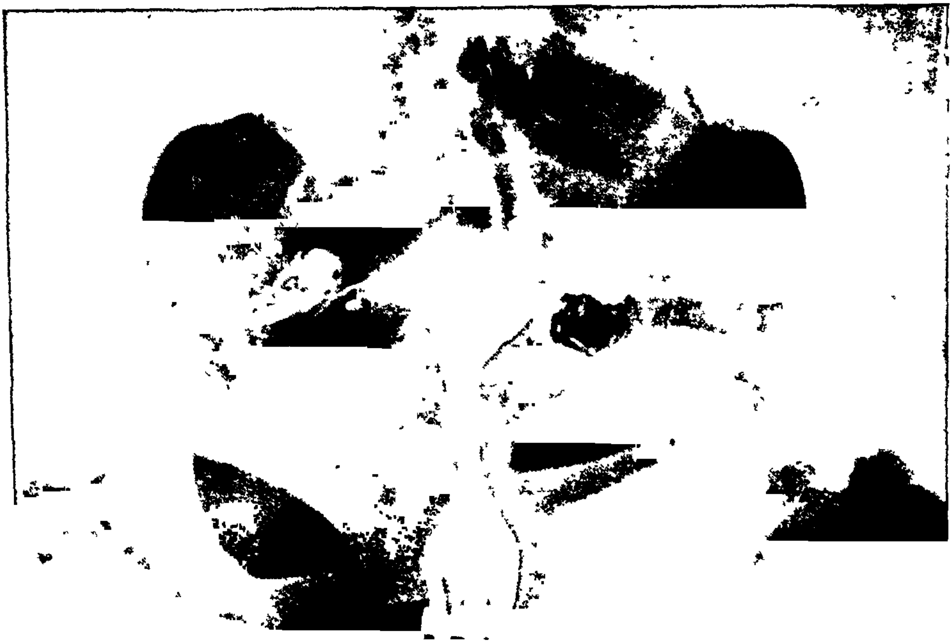

Fig. 7. Bilateral tubal tuberculosis with characteristic irregular small sactosalpinges and many fistulous tracts. Probable lymphatic extravasation is present in the myometrium. The diagnosis was verified histologically.

any irregularity, unusual firmness, or apparent scarring of the isthmus of one of the tubes as very probably being diverticulosis. A woman who has had a long-standing history of infertility with bilateral isthmic occlusion due to diverticulosis might be a candidate for reconstructve surgery. Reimplantation may be attempted. Preoperative culdoscopic examination might be a valuable adjunct in the proper selection of the patient with this lesion who would be suitable for tubal plastic surgery.

SUMMARY

1. During the years 1941 to 1953, 1160 hysterosalpingograms were performed on women complaining of infertility. A diagnosis of tubal diverticulosis was made in 19 patients, an incidence of 1.6 per cent.

2. Tubal diverticulosis is characterized by the presence in a firm, thickened segment of tubal isthmus of multiple small tubular diverticula of the endosalpinx.

3. A characteristic roentgenographic picture may be seen on salpingography.

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Spermatogenesis Following Experimental Testicular Ischemia

Frank Hinman, Jr., M.D., and Gilbert I. Smith, M.D.

Regeneration of the spermatogenic elements of the testis after depression by testosterone and by other agents—such as estrogens, febrile illnesses, and debilitating diseases—has in the last few years been clearly recognized, although the exact stimulating factors for such a rebound have yet to be exposed. The present report concerns the effect of an entirely different noxious agent, temporary ischemia, on spermatogenesis, being a study of both damage and regeneration of the tubular epithelium.

The technic of producing temporary arterial obstruction in the testis of the dog has been described in a previous paper.[2] In brief, after general anesthesia, a tourniquet is placed about the exposed spermatic cord (or in 9 later experiments, about the intact hemiscrotal skin), drawn just tight enough to stop bleeding from the site of the initial control biopsy, and left in place for periods of 1 to 14 hours. Specimens were obtained 30 or 60 days after removal of the tourniquet, fixed in formalin or in Bouin's fluid, and stained with hematoxylin-eosin, and Masson's stain. A total of 42 testes were so studied.

After 2 hours' ischemia, as described in the previous communications, spermatogenesis was depressed, with retention usually of the spermatogonia. Leydig and Sertoli cells resisted damage, and the basement membrane remained intact (Table 1). After 4 hours, however, almost all sperma-

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togenic elements were destroyed, and only a few viable Sertoli cells remained. The Leydig cells formed a solid margin beneath the thick tunica albuginea (Fig. 1). After periods of ischemia longer than 4 hours, all spermatogenic and Sertoli elements were destroyed, and if the ischemia lasted more than 10 hours, the Leydig cells perished also.

DESTRUCTION OF SPERMATOGENIC TISSUE

To obtain more detailed data on the cytologic effects of the shorter periods of ischemia on the spermatogenic cells, and on the possibility of regeneration during the period of repair, a study was made of a second, smaller group of 9 testes, obstructed by mass ligature for periods of 1 (2 testes), 2 (3 testes), 3 (2 testes), and 4 hours (2 testes), and removed 30 days or 60 days later and fixed in formalin and in Bouin's fluid. Individual variation existed

TABLE 1. Effect of Ischemia on Testicular Elements N-33

	Hours of ischemia required to	
	Damage	*Destroy*
Spermatogenesis	2	4
Sertoli cells	2	6
Leydig cells	6	10

between the specimens from any given period, probably on the basis of vascular damage (? spasm) and animal age and nutrition. In general, the specimens obtained in the second series, by obstruction of the entire scrotum, show greater damage than those reported earlier. We have chosen representative sections for illustration.

The destructive changes will be traced in the illustrations:

One-Hour Ischemia

Although the basement membrane is still intact 60 days after 1 hour of ischemia (Fig. 2), only a few spermatogonia remain among the disturbed Sertoli cells. These few germinal cells have vacuolated cytoplasm, with occasional hyperchromatic, shrunken nuclei, or with granular chromatin. In some tubules (Fig. 3) epithelial disruption is more complete, although partial regeneration of the spermatic elements can be seen in certain areas in the 60-day specimens.

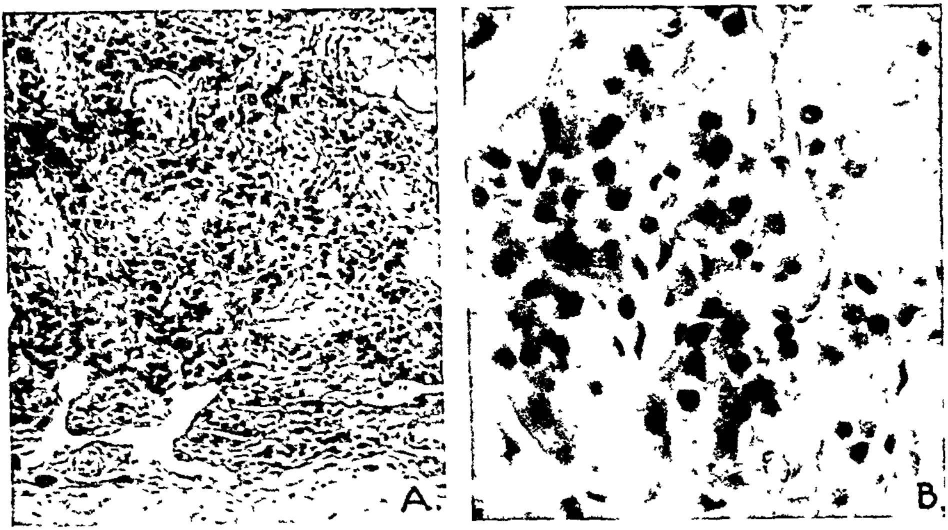

Fig. 1. Leydig cell hyperplasia (from layer beneath tunica albuginea), 4-hour specimen, after 60 days. (*A*, × 80; *B*, × 324)

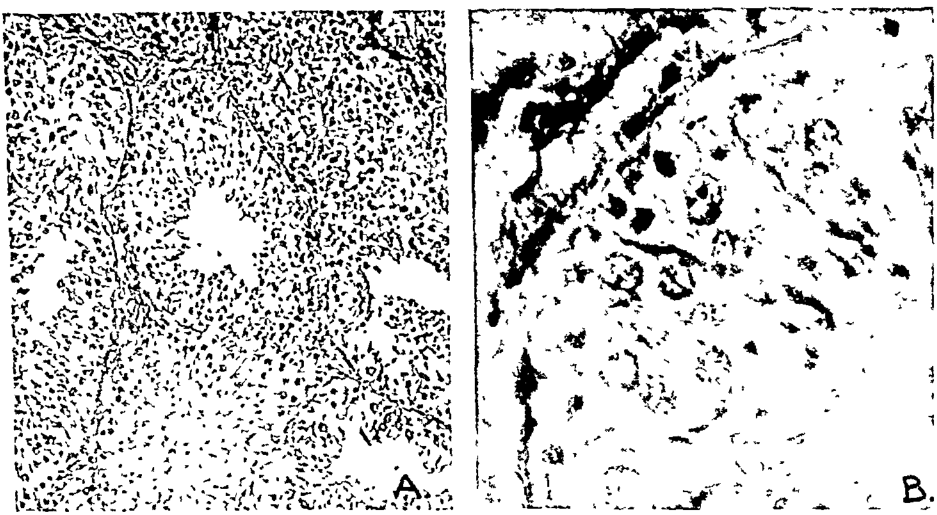

Fig. 2. One hour's ischemia (Bouin's fluid; Masson stain). Few spermatogonia among distorted Sertoli cells. (*A*, × 80; *B*, × 324)

Two-Hour Ischemia

After two hours of ischemia, more extensive changes have taken place (Fig. 4). The basement membrane is now greatly thickened. All spermatogenic elements have vanished. All that remains within the tubules are Sertoli cells with disintegrated cytoplasm and pyknotic, shrunken nuclei. Leydig cells are, however, relatively undamaged at this stage.

3

4

Fig. 3. One hour's ischemia (Bouin's fluid; Masson, × 324). More complete epithelial disruption. No evidence of regeneration.

Fig. 4. Two hours' ischemia (Bouin's fluid; Masson, × 324). Spermatogenic elements destroyed. Sertoli cells disintegrating. Basement membrane greatly thickened.

Fig. 5. Three hours' ischemia (Bouin's fluid; Masson, × 324). Very thick basement membrane surrounds a few unidentifiable cells. Edematous, fibrous connective tissue.

5

Three-Hour Ischemia

Ischemia of 3 hours' duration (Fig. 5) produces extreme damage to the spermatogenic elements. The very thick basement membrane surrounds a few cells which cannot be identified. The interstitial tissue is edematous and fibrous and the Leydig cells (not shown in these sections) are clumped.

Four-Hour Ischemia

At four hours, there is almost complete loss of tubular identity, and the Leydig cells form masses at the periphery (Fig. 1).

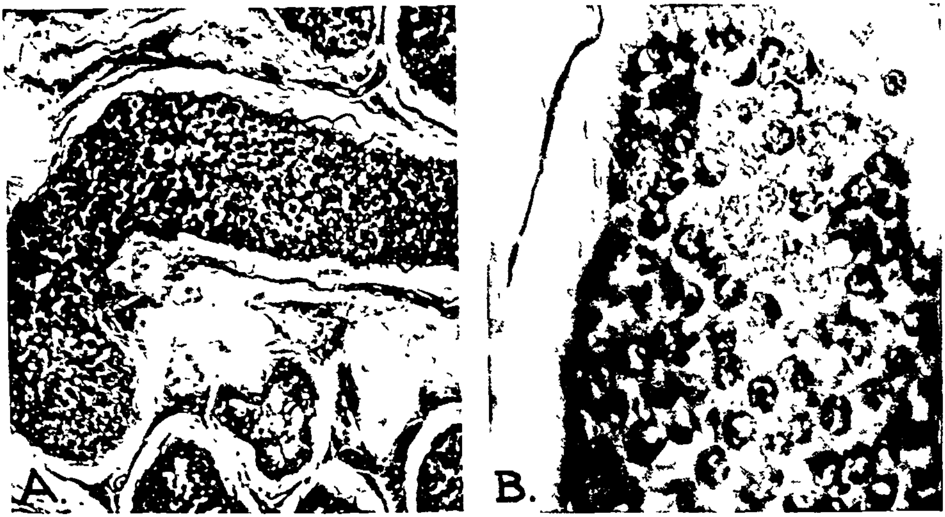

Fig. 6. Attempt at regeneration, two-hour specimen (*A*, × 80; *B*, × 324. Spec. # 160L, hematoxylin-eosin).

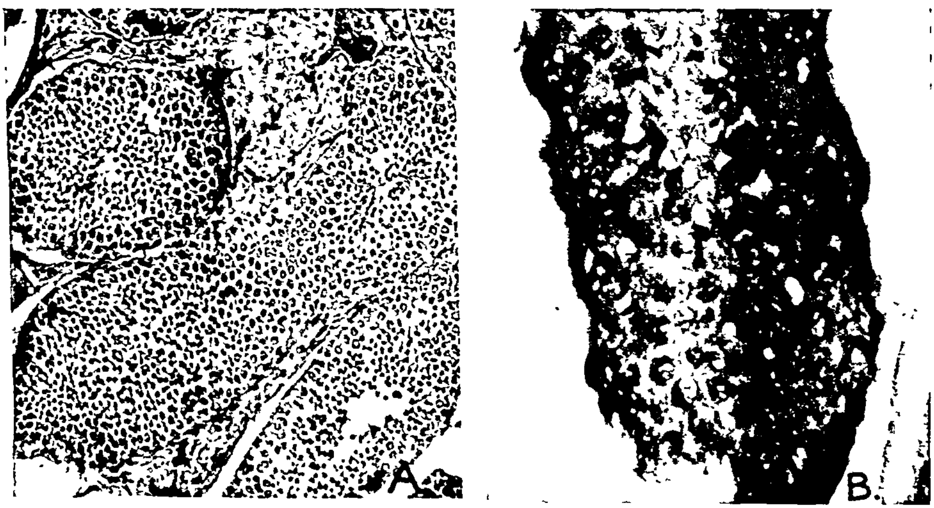

Fig. 7. Same, two-hour specimen (*A*, × 80; *B*, × 324. Spec. # 11R, hematoxylin-eosin).

REGENERATION OF SPERMATOGENIC TISSUE

Regeneration of the germinal epithelium occurred only to an insignificant degree by the end of the experiment (60 days). Only a few specimens showed any regeneration at all. Three testes out of a total of 6 examined 60 days after two hours or less of ischemia (Figs. 6, 7, 8) showed significant regeneration of spermatogonia. Testes examined at 30 days and those sub-

Fig. 8. Same, two-hour specimen (Spec. # 2 R, hematoxylin-eosin).

jected to 3 or more hours of ischemia failed to show regeneration. In the specimens illustrated, nuclei containing clumped chromatin and granular, somewhat vacuolated cytoplasm can be seen. Occasional spermatids are seen among the rather uniform masses of earlier cells, and complete maturation is hard to find. In other words, a rebound phenomenon in a clinical sense did not occur under the conditions of these experiments.

IMPLICATIONS

That vascular obstruction can produce testicular damage is readily demonstrated. Our experiments show the effects of extreme degrees of testicular anoxia, greater than would be expected even in complete torsion of the testis. One might speculate that less complete but recurrent ischemia could occur (? subclinical torsion), causing lesser degrees of spermatogenic damage. The changes noted after shorter periods of complete ischemia resemble those seen in certain patients with infertility (loss of mature forms, thickening of the basement membrane, etc.).

It would appear that complete vascular obstruction of more than 1 or 2 hours causes irreversible changes in the dog testis. The interval in the human being might be slightly longer because of the known greater resistance of human tissue to ischemia. In contrast, we have found a report on testicular ischemia in the rat[1] in which even shorter periods of obstruction causes irreversible damage. The chance for subsequent fertility from the human testis after complete torsion would be expected to be small should the obstruction continue for more than 2 hours.

SUMMARY

1. Complete ischemia was produced in 42 dog testes for periods varying from 1 to 14 hours. Specimens were examined 30 or 60 days later.

2. One or 2 hours' ischemia produces irreversible damage, although some incomplete regeneration occurs. No effective rebound phenomenon was seen under the conditions of the experiments.

3. Although the ischemia in clinical torsion of the testis is seldom as great as in experimental animals, these studies show the most extreme effects which could be expected on the spermatogenic elements.

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Some Factors Modifying Sperm Progression

Roberta Schwartz, B.A., and Hans H. Zinsser, M.D.

THE motility of spermatozoa in semen specimens has been a major subject in every analysis of semen for almost one hundred years. Extensive studies of the number, morphology, live and dead cells, survival time, and motility of spermatozoa have been made.[2, 3, 7-10, 13, 16, 18, 19, 21, 22] Recently Singher and Tyler reported an extremely active factor in the electrophoretic assay of semen, in a further effort to determine the regulators of spermatozoan activity. Prior estimates of motility have been based, for the most part, on percentage of motile spermatozoa or on qualitative calculations subject to the interpretation of the individual probator, and motility has been classified as good, medium, or poor.[4, 5, 12]

A quantitative method for estimating sperm progression has been reported by the authors[17] and is under evaluation in other hands.[14] This has formed the basis for studying the behavior of spermatozoa in Ringer's solution and in secretions of the female genital tract. The ability of local and systemic agents to aid and deter the motility of spermatozoa will be the substance of this report.

METHOD

The capillary tube technic used modifies that described by Lamar, Shettles, and Delfs, and of other investigators, Abarbanel; Gamble; and Haman of Denmark, who have reported the use of capillary tubes in their

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studies of spermatozoan activity. The present technic uses the gravitational gradient to sort the spermatozoa. Semen and agents to be used in the observations are layered into a capillary tube, with no separating air bubble to form an artifact; the sealed tube is kept in a vertical position at body

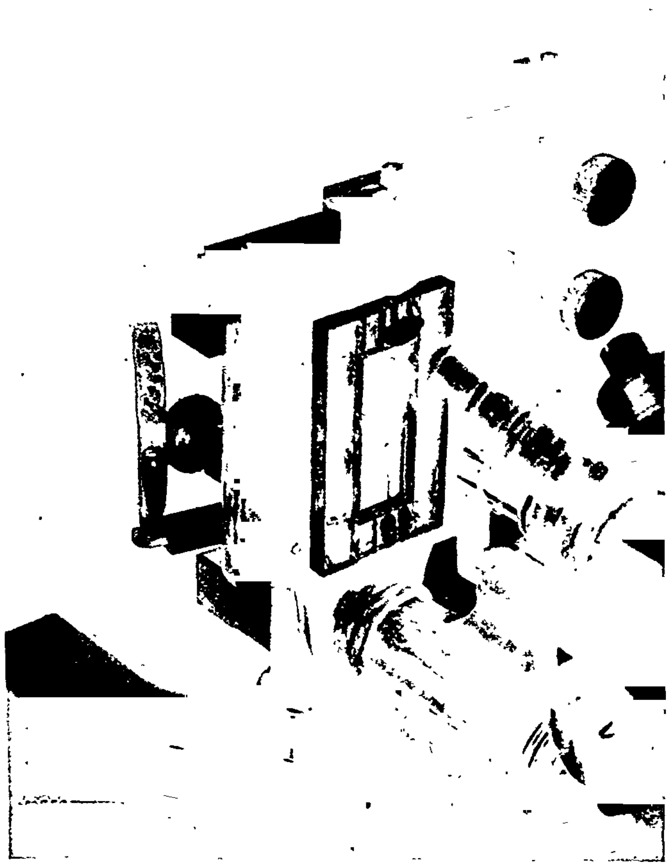

Fig. 1. The arrangement of the microscope in the horizontal position and the sealed capillary tube in the vertical mechanical stage is shown for oil-immersion study.

temperature, examined under oil immersion, and presents a means for long-term quantitative observation under conditions resembling those in the human body (Fig. 1).

The method as reported[17] entails measuring the progression of spermatozoa in Ringer's solution, and has been applied to cervical mucus. Capillary tubes of resistance glass, filled with semen and an equivalent column of

Ringer's solution or mucus are sealed and kept in a vertical position at body temperature. The position of the top field of 5 spermatozoa is recorded at 0 hour and 2 hours, the millimeters of travel of the mechanical stage indicating the distance of progression.

MATERIAL

The 112 semen specimens observed were obtained from volunteers aged 22–33 years in good health, and from patients aged 22–42 years with known

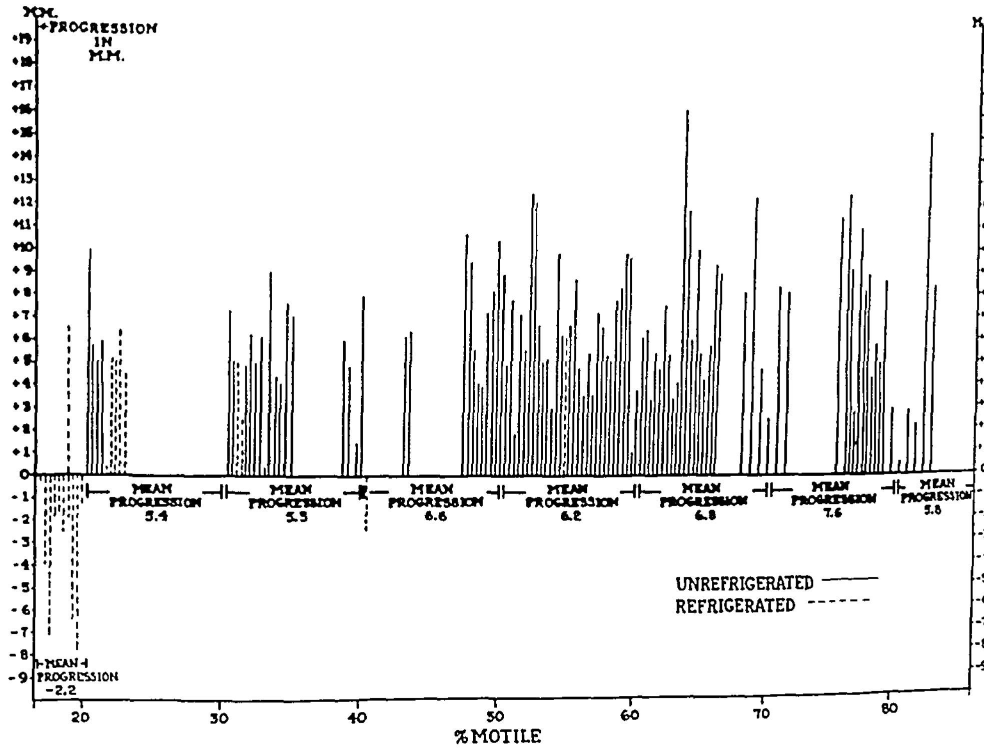

Fig. 2. Correlation of distance of progression into supernatant Ringer's solution with various percentages of sperm motility. (Refrigerated and unrefrigerated specimens.)

problems of infertility. The ratio of specimens from fertile or presumably fertile subjects to those from subjects with problems of infertility was 6:1. Two hundred observations of morning specimens were made under oil immersion within 1–3 hours after ejaculation. Three-day continence was requested; in 14 observations there was 2-day continence, and in 5 observations 5-day continence was reported.

Cervical mucus specimens were freshly aspirated from women of presumed or proven fertility for all observations.

RESULTS

Statistical data are presented in Figs. 2 and 3, Tables 1–3.

Sperm Progression in Ringer's Solution and Cervical Mucus

1. When specimen motility was over 25 per cent, progression was upward in Ringer's in capillary tubes. This statement includes 20 observations on refrigerated specimens. When motility was under 25 per cent, progression was downward. In refrigerated groups there was one exception in each group (Fig. 2).

TABLE 1. Sperm Progression in Different Media

Description	*% motile*	*Count* (millions/cc.)	*No. samples*	*No. observations*	*2-hour progression* (mm.)	*Standard deviation* (mm.)
Cervical mucus and semen, over 25% motility	57	108	17 cerv. mucus 12 semen	25	7.5	5.8
Same specimen, duplicate observ., Ringer's	53	133	28	56	Difference in progression 2.1	1.76
Same specimen, duplicate observ., cervical mucus	76	110	4	8	Difference in progression 0.7	1.40
Same semen, different cervical mucus	51	71	10 cerv. mucus 5 semen	13	Difference in progression 3.3	3.68

2. When there were over 60 per cent of the spermatozoa living, progression was upward in Ringer's; under 30 per cent, progression was downward; between 30 and 60 per cent, there was upward progression in the ratio of 10:8.

3. Duplicate observations of the same specimen in Ringer's solution showed a difference in average progression of 2.1 mm., with a standard deviation of 1.76 mm. (Table1). Duplicate observations of the same specimen in the same cervical mucus showed a difference in progression of 0.7 mm., with a standard deviation of 1.4 mm. However, the standard deviation in different observations was twice as great in cervical mucus as in Ringer's solution.

4. In the small initial sampling to date, penetration and progression of

spermatozoa in cervical mucus was not restricted to any particular period in relation to the time of menstruation (Fig. 3). Distance progressed was not consistent within any one day of the menstrual cycle, nor for any particular day or group of days. In 25 observations of 17 cervical mucus specimens the average 2-hour penetration was 7.5 mm., with a standard deviation of 5.8 mm.; all semen specimens included had motility of over 25 per cent.

5. Spermatozoa penetrated cervical mucus singly, and did not seem to follow the path of the previously observed spermatozoa. Spermatozoa that

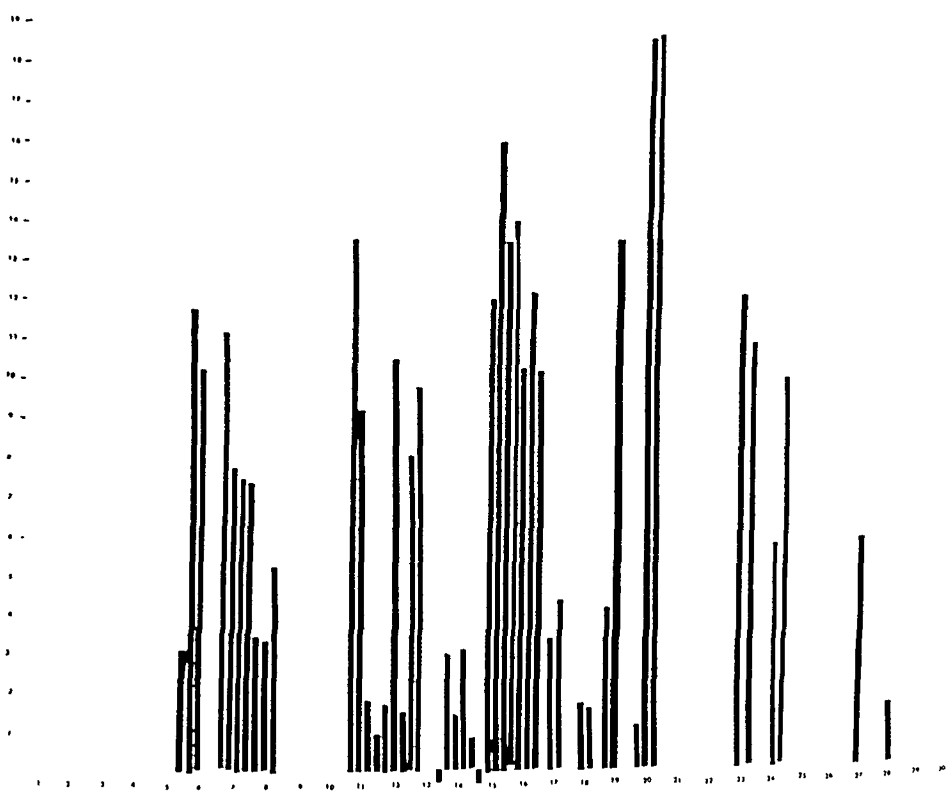

Fig. 3. Progression in cervical mucus taken at various days in the menstrual cycle. No clear-cut mid-cycle increase is seen.

penetrated cervical mucus did not deteriorate as rapidly as those observed in Ringer's solution.

Drugs and Sperm Motility

Systemic Medication

DEXEDRINE. Each subject took 5 mg. Dexedrine 2 hours after dinner the night before a morning ejaculation. There were 24 observations of 11 specimens from 6 donors. Progression in Ringer's solution was 10.1 mm. with Dexedrine, compared to 6.5 mm. for all control specimens at all times of the year (Table 2). However, 52 observations without Dexedrine taken 2

TABLE 2. Effects of Dexedrine on Sperm Progression

	% motile	*Count* (millions/cc.)	*No. samples*	*No. observations*	*2-hour progression* (mm.)	*Standard deviation* (mm.)
Control	56	140	60	104	6.5	2.9
5mg. Dexedrine	62	137	10	24	10.1	1.4

months before, 2 months during, and 2 months after this study yielded an average progression of 7.6 mm. The question of seasonable variation was raised as the Dexedrine had been taken in April and May. This increase

TABLE 3. Sperm Changes After Administration of Radioactive Iodine (I^{131})

	Progression (Av. mm.)		*% abnormal*		I^{131}		*Average count* (millions/cc.)		*Average % motile*	
Subject	*Before therapy*	*After therapy*	*Before therapy* (Av.)	*After therapy*	*µc.*	*Uptake* (%)	*Before therapy*	*After therapy*	*Before therapy*	*After therapy*
G. S.	11	6.3	27	35	3.2	28.9	83	37	76	48
				27	1.8	24.8				
				32						
				30						
G. V.	6.3	6.6	31	39	3.24	41.5				
				27	5.00	39.6	122	120	67	76
W. Mc.	7.8	5.8	31	38	4.6	47.7	163	205	58	74
				53	2.5	66.0				
				37						
W. M.	10.5	9.4	31	26	3.2	17.6	255	138	63	73
				33	1.8					
E. L.	8.5	6.4	36	34	2.6	33.2				
				39	3.2	28.7	205	196	72	66
Average	8.8	6.9	31	34	3.1	36.4	165	139	66	67

either represents a seasonal variation or a residual effect of Dexedrine persisting a week or more after Dexedrine has been administered. The possibility of stimulating sperm motility through drug excitation of the autonomic nervous system will be accorded more extensive investigation.

TRACER DOSES OF I^{131}. The percentage of abnormal forms varied as a result of the administration of radioactive iodine (Table 3). In the dosage range below 3.2 microcuries no significant changes were noted in morphology or progression, although one case at the 3.2 μc. level did show a drop in progression of 54 per cent, and an increase in percentage of abnormal forms in 2 out of 3 specimens. Above the 3.2 μc. level 1 of 2 patients consistently showed an increase in the percentage of abnormal forms. In neither case was progression significantly impaired.

Local Medications

LONG-RANGE EFFECT. 35 observations were made of 7 contraceptive agents layered *below* semen. Capillary tubes were filled first with Ringer's solution, then semen, then the contraceptive agent. In 2 hours the spermatozoa progressed upward an average of 4.6 mm. into the Ringer's solution; *in 3 observations the sperm were immotile and had* regressed an average of 6.3 mm. Controls of Ringer's solution and semen showed an average progression of 5.5 mm. Contraceptive agents had slight inhibitory or no effect on sperm motility at a distance by diffusion upward through semen. Thirty-three observations were made of 5 contraceptive agents layered *above semen. The distance below the boundary of the* agent at which the sperm were immobilized was observed. In 16 observations the spermatozoa were immobilized at an average of 2.9 mm.; all but 5 showed motility beyond that point.

MISCIBILITY AND PENETRATION. That the miscibility of spermatozoa and contraceptive agent is important was evidenced by the fact that some showed mixing of sperm and contraceptive agent in the tube to a distance averaging 1.1 mm. in 2 hours. There was marked disparity of capacity for even dispersion of contraceptive agents in semen-cervical mucus systems.

The following agents were assessed: Preceptin, Servex, Zonitor, Zonitor experimental mass, Ortho-Gynol, Koromex, and Lactikol.

SUMMARY

There was correlation between percentage of motility of spermatozoa and direction of progression: upward, over 25 per cent motile; downward, under 25 per cent motile. There was no correlation between proved fertility and distance of progression.

Progression in Ringer's solution was 6.5 mm. mean average and in cervical

mucus 7.5 mm. mean average. The standard deviation in observations of sperm progression was twice as great in cervical mucus as in Ringer's solution. In other words, the deviation between individual sperm samples is doubled when the factor of differing cervical mucus samples is introduced. However, the difference between observations in duplicate cervical mucus was 0.7 mm., as compared with 2.1 mm. in Ringer's solution, so that erratic observation is not the cause for this variation, although mean progression in mucus was greater.

In the samplings to date distance of spermatozoan penetration of cervical mucus did not correlate with any particular time of the menstrual cycle.

In the cases observed five mg. Dexedrine, taken 10–12 hours before ejaculation, increased the average progression of spermatozoa in Ringer's solution about 50 per cent.

Increase in abnormal morphology of spermatozoa occurred with diagnostic doses of I^{131}; progression markedly decreased in 1 out of 5 cases observed.

Contraceptive materials were estimated quantitatively for their capacity to inhibit penetration and migration through cervical mucus. No agent immobilized spermatozoa at a distance greater than an average of 2.9 mm., and mixing in most cases did not achieve approximation of sperm and spermicide closer than 1.1 mm. Agents evaluated included Preceptin, Zonitor, Zonitor experimental mass, Ortho-Gynol, Servex, Lactikol, and Koromex.

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The Role of Spermatozoa in Habitual Abortion

Charles A. Joël, M.D., Ph.D.

THERE ARE numerous causes of spontaneous abortion, the origins of many of which are as yet unknown. In general, however, the factors in the etiology of spontaneous abortion can be divided into two main groups—exogenous and endogenous. Among the endogenous factors are included the possible existence of lethal factors and of unbalanced changes in the male and female gametes. Among the exogenous factors we can include disturbances in nutrition and some women's diseases (intrauterine adhesions, etc.). Both types of disturbance can cause failure of normal embryonic development.

In the 30-odd years of its existence, the department of embryology of the Carnegie Institute in Washington, which has examined embryos collected from all parts of the United States, has become the center of research in this field. The first director of the Institute, F. P. Mall, held the view that pathologic changes in the embryo were the main cause of disturbances in human implantation. This point of view was generally accepted in gynecologic circles in the 1920's. On the basis of numerous studies, and one can cite in particular the work of Robinson, the germ-plasm theory came into prominence. This theory was supported by George L. Streeter, the successor of Mall. The third and present director of the Institute, George W. Corner, has obtained convincing substantiation of Streeter's conclusions based upon the study of hundreds of ova and uteri of the pig. Corner found numerous moribund and dead young embryos in uteri that showed no pathologic changes when examined under the microscope.

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Special mention should also be made of the work of Hertig and Rock at the Free Hospital for Women, Brookline, Mass., who examined 40 free or just-implanted human eggs, of which one third were pathologically abnormal whereas the endometrium appeared to be perfectly normal. Studies by Hughes at the University Clinic of Syracuse, N. Y., involve the relation of cytochemical changes in the endometrium to human abortion.

The study of these problems in man is complicated by the early implantation of the egg (7½ days) and the rapid union of the egg with the maternal tissue. But in the opossum there is no actual placentation so that this animal is especially suited for the study of eggs and embryos. This study has been mainly undertaken by Carl C. Hartman who with his collaborators has examined 1378 opossum eggs. In these eggs, all of which were free in the uterus, 62 per cent were normal, 28 per cent were unfertilized and 10 per cent were fertilized but abnormal. The ova degenerated in all stages of development beginning with the two-cell stage, and Hartman concluded that some ova degenerate despite fertilization and despite optimum conditions in the maternal uterus.

It is out of the scope of this paper to discuss the extensive literature on the pathogenesis of human ova, and for a description of these changes the reader is referred to the cited literature and to the study of O. Käser, in which I collaborated at the time of my work at the University Women's Clinic in Basel. This study has shown that out of 606 cases of abortion 29.2 per cent were due to maternal causes, 24.8 per cent were criminal, and 35.4 per cent were of ovular origin. The cause of 10.6 per cent of all cases was unknown. In this connection it is of interest that the proportion of molar abortions was 34.1 per cent of all cases, or 52.8 per cent of the abortions with a known cause.

In the last decade there has been a change of emphasis in the causes of abortion to include the endogenous ones, which originate in the *ovum*, the sperm, or the zygote.

MATERIAL AND METHODS

The present study deals with a group of habitual and repeated spontaneous abortions. The cases of habitual abortion showed at least 3 abortions without any intervening normal deliveries, and the cases of repeated abortions showed at least 3 abortions in addition to one or more normal pregnancies.

The group studied consists of 114 cases of habitual and repeated abortions, in which the spermatozoa of the husbands involved was examined. In cases of pathologic findings, the sperm was examined 5 to 7 times at intervals of 4 to 6 weeks. In cases of high degree of oligozoospermia, testis biopsies were taken in order to study the various stages of degeneration.

RESULTS

Out of the 114 cases examined, the ejaculated spermatozoa were normal in 68 men, whereas 46 men showed pathologic spermatozoa. These 46 cases include 26 men with a medium to heavy degree of hypozoospermia, with sperm counts of 30–50 million per cc., a spermiocytogram with about 30–50 per cent abnormal forms, and a motility of 50–60 per cent up to 1 hour. Another 10 patients had a slight-to-heavy degree of oligozoöspermia, with 5–30 million spermatozoa per cc., a high percentage of abnormal forms, and a greater loss of motility. Four patients showed a high degree of oligozoospermia with 3–5 million spermatozoa per cc., with an especially pathologic spermiocytogram, and a great decrease in motility. In 6 patients pathologic changes in the spermatozoa were so great that it was doubtful whether fertilization could be effected. In these last cases there were sperm counts of less than 1,000,000 per cc., and these fluctuated between 250,000 and 900,000. The spermiocytogram showed only solitary normal-looking forms (3–5 per cent) with a rich exfoliation of germinal epithelium (a cell count of up to 40 per cent).

Biopsies taken from 3 of these patients showed from first to third degree atrophy with partially pathologic germinal epithelium, as hyperchromia, atypical mitosis, karyolysis, vesiculation, and degeneration of the cytoplasm.

Of special interest is the group of 6 marriages in which the husbands showed a high degree of oligozoospermia in 7 successive examinations. There were no pathologic findings in the wives of any of these patients. In 4 cases of this group, the wives had 1–3 normal children with a first husband, whereas with the second husband they had 3–4 abortions within 2–3 years and no normal deliveries. With 2 other cases the women had 2–3 abortions. One of the men then died of cancer and his wife had 2 normal children in a second marriage. The second woman divorced her husband and also had a normal child in a second marriage. The patient who was the first husband of the last-mentioned woman married a second time and his second wife had 2 abortions. As this second wife was only 32 years of age the husband agreed to be vasectomized and at the request of both husband and wife, the wife was artificially inseminated. After 3 inseminations the woman became pregnant and gave birth to a normal child.

DISCUSSION AND CONCLUSIONS

An examination of the present data shows that the husbands in 68 cases of the 114 habitual and repeated abortions had normal spermatozoa. In these cases the cause of the abortions does not appear to be due to the male partner in the marriage. In the group of 26 cases with a medium-to-heavy degree of hypozoospermia, it is also impossible to correlate the abortion with the findings in the spermatozoa, since the results of MacLeod and myself show that such spermatozoa are still to be considered normal. The slight-to-heavy degree of oligozoospermia found in 20 cases may be considered responsible for the abortions, but even in these cases this conclusion must be made with considerable caution, as Jackson, MacLeod, and I have seen normal pregnancies and normal deliveries with this type of spermatozoa.

In the last group of 6 cases where the spermatozoa showed marked pathologic changes and where I had doubts that fertilization could take place, the successive abortions can be ascribed with a high degree of probability to the male partner. In support of this is the evidence that 4 women had normal children in first marriages and successive abortions in their second marriages. In addition there were the 2 women who had successive abortions in their first marriages with men with pathologic spermatozoa and normal children in a second marriage. Of particular interest is the man with greatly modified pathologic spermatozoa whose first and second wife produced abortions and whose second wife then bore a normal child after artificial insemination.

A correlation between morphologic abnormalities of the spermatozoa and certain types of abortion is thus within the realm of possibility. But owing to the small size of the sample more definite conclusions on this point have not yet been obtained. The theoretical basis of such a conclusion is also incomplete, since it is so far not known whether a pathologic spermatozoon can fertilize and whether its cytoplasm has any function in the zygote. It is also unknown whether abnormalities of the sperm follow or accompany a change in the cytoplasm or perhaps even a change in the nuclear constitution. But in no case is a "gonad damage" identical with "a hereditary damage."

Although we have as yet not shown an absolute correlation between the morphologic changes of the gametes and the occurrence of abortions, there can be no doubt that the spermatozoa are of special importance, if a certain proportion of these abortions is of endogenous origin. Clinically, our observations are in favor of the spermatozoa as the causative agent in these cases. But when we consider the pathologic changes in the ejaculated spermatozoa it must be realized that its morphologic examination cannot show any nuclear defects, but can at best show changes in the cytoplasm.

Our findings and theories are confirmed by the last article of Hinglais and Hinglais (1954), who make the following differentiation between two functions of the spermatozoon: one leads to fertilization, and another, an ontogenetic function, can be made responsible for faulty ova. Asplund concludes from his material that pathologic spermatozoa may be at the origin of habitual abortion.

The purpose of this paper therefore, has been to draw attention to the importance of the male in cases of abortion.

SUMMARY

Male partners of 114 couples with repeated abortions have been examined. Sixty-eight men had normal spermatozoa and could be excluded, while in 46 the sperm was subnormal. Of these, 26 had a low-to-medium degree of hypozoospermia and then can hardly be blamed for the infertility. Of the 20 remaining males, 6 are of special interest—those with a very high degree of pathologic changes in their spermatozoa. The 6 wives had normal pregnancies and deliveries in marriages contracted with other men. As a proof of the importance of the spermatozoa in abortions, a case is described where in two successive marriages there were only miscarriages and where the second wife had a normal pregnancy and delivery after artificial insemination.

Although the contribution of the male is of obvious importance, no clear-cut relationship between the external morphology of the spermatozoa and abortion has been found.

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Canadian Society for the Study of Fertility

The Canadian Society for the Study of Fertility announces its second annual meeting to be held October 6, 7, and 8, 1955, at the Royal York Hotel, Toronto, Ontario. For further information please write DR. EARL R. PLUNKETT, *Secretary Treasurer*, Canadian Society for the Study of Fertility, 469 Waterloo St., London, Ont., Canada.

Clinical Evaluation of Testicular Biopsy and the Rebound Phenomenon

Paul L. Getzoff, M.D.

THIS REPORT presents a summary and limited interpretation of data acquired from a questionnaire concerning testicular biopsy and the so-called rebound phenomenon as these are applicable to the management of the infertile male. Opinions on this subject were solicited from the members of the American Society for the Study of Sterility and the American Urological Association. One hundred and four replies were received (Table 1).

The limitations and shortcomings of this type of investigation are self-evident. The purpose of this study has been to inquire into the attitudes, personal evaluation, and experience of a cross-section of urologists and other clinicians in this country who manage male infertility problems. We have attempted to collect sufficient information to evaluate the diagnostic and therapeutic values of testicular biopsy and testosterone treatment of infertile men as currently practiced.

In addition to the section of the questionnaire devoted to clinical data, an additional section was provided for information, suggestions, and opinions not covered in the printed outline. The response to this solicitation revealed a keen interest in this project. Many of the physicians took the time and effort to attach detailed reports of their experiences. A few of these

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Presented at the Tenth Annual Meeting of The American Society for the Study of Sterility, June 18–20, 1954, in San Francisco, Calif.

I wish to express my appreciation to the Research Correlating Committee of the American Society for the Study of Sterility for making available the means of production and distribution of the questionnaire upon which this report is based. I desire also to express my gratitude to the 104 clinicians who generously cooperated by recording their experiences and thereby making this report possible.

addenda were several pages in length and reflected the meticulous care proffered their patients as well as the excellence of their professional records.

I. TESTICULAR BIOPSY

TABLE 1. Summary of Replies

104 Replies		
Have never employed testicular biopsies in male fertility studies		31
Have employed testicular biopsies in male fertility studies		73
As part of the routine male fertility examinations	3	
In special cases only (azoospermia and marked oligozoospermia)	65	
Indication unreported	5	
Total number of testicular biopsies performed by the 73 affirmative replies		4850
For purposes of diagnosis	4777	
Histologic results used as a therapeutic guide	3551	
The following numbers of clinicians have discarded the use of testicular biopsies because:		
(a) Have not found this procedure helpful in the management of male infertility		15
(b) Satisfactory histologic interpretation is not available		7
(c) The patients refuse to permit this procedure		40
Have found the testicular biopsy to be a valuable adjunct in the diagnostic work-up		40[a]

[a] This number includes some of the clinicians who have discarded the testicular biopsy for reasons noted in (b) and (c) above.

Additional Remarks from Questionnaire

"The average sterile couple expects resultant pregnancy in 8–10 examinations and treatments. If no results, they usually discontinue the study by not meeting appointments."

"I obtain testicular biopsies only when there is a question of doing a plastic procedure."

"In the vast majority of cases, semen analysis, combined with the history and physical findings, is sufficient to establish the status of the problem and to act as a guide to treatment, progress, and prognosis."

"A high percentage of males are willing to give a specimen but are not willing to have a biopsy."

"I have discarded testicular biopsies in azoospermia."

"Testicular biopsies are expensive, occasionally painful, and not always helpful."

"In patients with a history of bilateral epididymitis, a biopsy is done. If sperm are found, a secondary epididymovastomy is done."

"Of great academic interest, but practical use is limited to azoospermia."

"Histologic reports are ambiguous."

"The chief reason for not employing testicular biopsy is that it would not alter in any way my treatment of the condition."

"I am concerned with the possibility of prolonged injury resulting from testicular biopsy."

"Not proved helpful as far as therapy is concerned."

"One might consider that schizophrenia and latent schizophrenia are absolute psychiatric contraindications to testicular biopsy, and severe anxiety states, particularly in those males manifesting hypochondriacal concern about themselves and their bodies (even if they do not mention their genitals), constitute relative psychiatric contraindications to the procedure."

Discussion

Thirty-one of the 104 clinicians queried in this study who manage male infertility problems have never employed testicular biopsies for either diagnostic purposes or as a therapeutic guide. When this figure is readjusted to allow for those who have discarded this procedure, this ratio more than doubles itself to the significant number of 67 out of the 104 clinicians not currently utilizing the histologic method as an adjunct to their investigation of infertile males.

It is noted that although 98 per cent of the testicular biopsies were performed for diagnostic purposes, only 73 per cent were used as a means of determining the course of treatment. The difference in these numbers could be an important consideration if the former represents a lack of acceptance of testicular biopsy findings as a therapeutic guide in the treatment of male sterility. The indication, stipulated by the group who favored the use of testicular biopsy, was limited to cases of severe oligozoospermia and azoospermia. Only 3 clinicians in the entire group advocated the use of testicular biopsies in the routine study of male infertility. Except in instances of azoospermia secondary to epididymal obstruction, there was no comment expressed relevant to specific applicability of the histologic findings.

It seemed significant that fully half the clinicians who have performed testicular biopsies have discarded this operation. Forty per cent of this group expressed the opinion that testicular biopsy studies had little more to contribute than the information gleaned from a meticulous clinical appraisal of the patient and a similarly thorough semen analysis. An almost equal number stated that their patients would not submit to a testicular biopsy. The remaining 20 per cent have discarded testicular biopsies because a satisfactory histologic interpretation is not available to them.

II. REBOUND PHENOMENON

Forty of the 104 replies indicated no experience with the use of testosterone therapy in the manner described as the "rebound phenomenon." The remaining 64 clinicians had employed this form of treatment in 840 males who had been rated as infertile.

The method of selection of cases for the testosterone therapy was based on testicular biopsy (44 cases), hormone-excretion determination (39 cases), and in every instance of oligozoospermia. Under this heading of oligozoospermia, there were 21 different definitions which ranged from "azoospermia" and "very low" to 70,000,000 spermatozoa/cc. In 184 instances, either no criterion was offered for regarding the patient as infertile, or the rationale for the therapy was not given.

Of the 840 treated cases, no response to treatment was evident in 508 cases. There was a variable increase in sperm count in 276 cases (Table 2). This increased count was maintained for an average of 1.7 months (134 cases). However, improvement was temporary and the count returned to pretreatment levels in all the cases.

There were only 63 instances in which the sperm count was impaired by the testosterone therapy. No permanent depletion of the sperm population after treatment was described.

Pregnancy eventually occurred in the wives of 23 treated males whose count had not improved, 4 men who had undergone slight improvement, 3 cases that had revealed moderate improvement, 4 males with appreciable improvement, 21 cases described as markedly improved, and 1 case which was labeled as "100%–1,000%" improved.

Details of Replies Reporting Successful Experience

Only 14 clinicians reported the successful use of testosterone in the treatment of infertile males. The number of pregnancies achieved is shown in Table 3. It was thought that the interest and completeness of this report would be enhanced by presenting the individual replies and analyzing the experience therein.

Reply No. 1. Treated 3 patients in whom the sperm count was below

TABLE 2. Summary of Replies Reporting Successful Experience with Rebound Phenomenon

			Sperm count after testosterone										Pregnancy eventually occurred in wives of treated husbands whose sperm count had:					
				Improvement														
Reply No.	*Total cases treated*	*Method of selection* (Sperm count—1,000,000 per c.c.)	*No improvement*	*Slight* (Less than 10%)	*Moderate* (10%—25%)	*Appreciable* (26%—50%)	*Marked* (51%—100%)	*"100—1000%"*	*Increase maintained* (mo.)	*Increased count returned to pretherapy level* (No. of cases)	*Poor count further impaired*	*Permanent impairment*	*Not improved*	*Slightly improved*	*Moderately improved*	*Appreciably improved*	*Markedly improved*	*Improved "100—1000%"*
1	3	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..
5	8	40	8	..	..	..	..	..	..	..	..	..	8	..	..	..	..	..
8	3	20	3	..	..	..	..	..	..	3	3	0	2	..	..	..	..	..
9	100+	40	60	..	20	10	5	..	24	30	0	0	..	..	..	1	2	..
16	8	60	2	2	3	..	..	..	6	1	0	0	..	..	2	..	..	..
18	3	20	3	..	..	..	..	..	..	0	3	0	3	..	..	..	..	..
32	6	70	4	2	..	..	..	..	3	..	0	0	..	2	..	..	..	..
57	12	40	1	..	..	..	10	..	36	..	0	0	..	..	..	..	10	..
58[a]	4	40	3	2	1	..	1	..	12	3	0	0	..	..	..	..	1	..
64	30	20–60	15	6	3	3	3	..	..	30	0	0	1	1	1	3	4	..
69	22	40	14	..	..	2	3	3	12	4	0	0	2	..	..	..	3	1
80	3	60	2	1	..	..	..	..	..	..	..	0	..	1	..	..	..	..
92	29	40	21	..	..	1	7	..	13–14	0	6	0	..	..	..	..	1	..
95	6	40	6	..	..	..	..	..	..	..	..	..	..	6	..	..	..	..

[a] Statistical error is reported exactly as recorded on the reply.

Reply No. 80. Treated 3 patients in whom the sperm count was below 60,000,000/cc. No improvement was evident in 2 cases and slight (less than 10 per cent) improvement in 1 case. The latter patient reported that his wife became pregnant.

Report No. 92. Treated 29 patients whose sperm count was below 40,-000,000/cc. Twelve cases showed no improvement. There was 1 pregnancy in the wife of a man who manifested marked improvement following treatment.

Reply No. 95. Treated 6 patients whose sperm count was below 40,000,-000/cc. Although no improvement following treatment was manifested in any of these cases, each man reported that his wife became pregnant.

Discussion

Of the 104 clinicians queried in this study, 64 reported the use of testosterone in 840 male patients whom they had rated as infertile. Successful experience with this mode of treatment was reported by 14 clinicians who treated a total of 237 patients; pregnancy eventually occurred in the wives of 56 treated males.

The variable criteria of oligozoospermia provides considerable room for question as to whether or not a significant portion of the "success stories" were obtained from males who were fertile before treatment. Furthermore, allowances must be made for those men whose infertile state was associated with subnormal steroid activity and in whom testosterone constituted a specific requirement for restoring germinal epithelial physiologic performance. This latter group is not contained in the category defined as "rebound phenomenon," (i.e., healthy, endocrinologically normal males with substandard semen findings).

The effects of testosterone therapy in these cases indicated that there were almost twice as many men who did not experience any improvement in their sperm counts as men in whom some improvement was noted. Even in this latter group, the majority of the counts ultimately returned to pretreatment levels. Although a small number of cases showed continued low sperm counts following testosterone therapy, all of these cases eventually returned to pretreatment levels after treatment was discontinued.

Closer study of the 56 pregnancies which eventually occurred in wives of treated males reveals the apparently significant fact that no improvement had been reported in the sperm counts of fully 40 per cent of this group.

SUMMARY AND CONCLUSIONS

The scope of this presentation does not permit any justifiable conclusions relevant to the worth of either testicular-biopsy studies or testosterone treatment in the management of male infertility. These were not our goals because the margin of error would be indefinable in such a large study in the absence of both control groups and standardized laboratory data and a host of other variables.

Nonetheless, several significant points have come to the foreground whose validity is perhaps more certain.

I. Testicular Biopsy

The majority of the clinicians canvassed in this investigation do not subscribe to the thesis that histologic information about a sterile male has sufficient worth to justify a testicular biopsy.

I do not believe that this is an instance of sheer nihilism on the part of the profession. It seems more probable that the bulk of clinicians are being conservative in the face of indecision. This indecision is seemingly based on the paucity of published evidence describing a rational specific therapeutic regimen for the anxious infertile male patient. The emphasis in the work-up of the sterile male has been placed on clinical study and a complete and accurate semen analysis.

Yet it must be agreed that this phase of medical investigation constitutes a genuine challenge in the medical investigation of male sterility. Only 3 physicians report the adoption of testicular biopsies as routine procedures in the handling of male sterility. One might justifiably pose the question of whether the information obtained by these clinicians resulted in their obtaining both a more accurate prognosis and a higher ratio of conceptions than those individuals who rely exclusively on clinical examinations and semen analyses. The details of affirmative replies would contribute much to clarify some of the basic issues identified with this subject.

II. Rebound Phenomenon

This phase of the analysis has provided some revealing information about the trend in the current treatment of male infertility.

Sixty-four clinicians furnished at least 20 different definitions of oligozoospermia. These concepts of oligozoospermia varied from 1,000,000 spermatozoa/cc. to 70,000,000/cc. Additional modifying factors which received insufficient consideration are the morphologic characteristics and the degree of motility of the spermatozoa. Therefore, regarding some men as being infertile because they have relatively low sperm counts (20,000,000–40,000,000/cc.) ignores the fact that such males have been demonstrated to be in reality fertile. Furthermore, excellent cytologic and motility characteristics of spermatozoa have proved to be efficient compensatory factors in rendering men with relatively low sperm concentrations more fertile than men with higher or normal sperm counts but poorer spermic detail.

The conception rate with testosterone therapy was less than 4 per cent. Of the 840 cases which had been so treated there were 56 conceptions reported. In the latter group, no improvement was noted in the semen picture of 23 men whose wives became pregnant. If further allowances are made for other factors (substandard hormonal pattern, the treatment of men who were in reality fertile prior to treatment, etc.), the conception rate which could be attributed to the rebound phenomenon would even be further reduced.

It would seem reasonable that the successful experience with any type of recommended therapy must of necessity remain in question until there is some reasonable semblance of unanimity as to what seminal characteristics identify male infertility. The criteria for the evaluation of the infertile male, published by the American Society for the Study of Sterility in 1951, has already been vitiated by the more recent reports of such brilliant investigators as Williams, MacLeod, Hotchkiss, and several others. Modification of these criteria is essential.

Current Reviews

WILLIS E. BROWN, *editor-in-charge*

Foreword

With this issue, FERTILITY AND STERILITY is embarking on a new venture in our abstract service. We believe it will prove a valuable addition to the Journal and provide assistance and perspective for the reader. Our appreciation to Doctor Pollak who, for five years almost single-handed, has carried the abstract section of the Journal. We look forward to his continued participation and assistance.

The rapid progress of the sciences and medicine has made it impossible for the busy practitioner to keep in touch with new developments in any special field. It was the opinion of the editorial board who discussed this subject over the past two years that our Journal would serve its readers best by departing from the publication of selected abstracts. The desirable features of an abstract department should be the careful selection of the most informative articles which appear in the current literature, to have them abstracted intelligently, and to interpret their significance to the readers.

It is our present plan to review the literature in our special and related fields, to provide an expanded bibliography, to abstract the choice articles, and to select an editorial consultant thoroughly conversant with the subject who will review these abstracts and add his editorial comments from his personal experiences. Such editorial comments will be practical as well as scientific so that they can be of value to all our readers.

Dr. Willis Brown, our associate editor, who is in charge of this new department, has written this first section. In it he reviews the recent literature on the role of the ovary (excluding the ovum) in infertility. He discusses these various contributions in the light of their clinical significance.

It is hoped that each issue will carry a review of one phase of the problem of infertility written by a member of our editorial board or an invited guest. We hope that he will interpret the literature liberally in the light of his own extensive experiences so that the casual worker in this field may add to his own knowledge and enlarge upon his experiences.

The editorial board will welcome any criticisms and suggestions which you may have in this formative stage of this new department so that it may best serve the physician in his daily practice and the research worker in his special field.

M. EDWARD DAVIS

The Ovary

Perspective

The ovary is the fundamental organ of reproduction in women, providing the primary germ cell, the preparation of the implantation site, and the endocrine support of the young embryo. A critical review of the role of the ovary in infertility seems to divide itself into three sections—that dealing with ovary as an endocrine organ, that treating of ovulation, and the early ovum and conceptus. This review of the role of the ovary in infertility will employ the outline below.

Outline

I. Certification and timing of ovulation

A. Objective evidence of ovulation
Culdoscopy; laparotomy

B. Presumptive (indirect) signs
Mittelschmerz; B.B.T.; pregnanediol; endometrial biopsy; cervical mucus; vaginal smears

II. Factors bearing on ovulation

A. New theories of ovulation

B. Gonadotrophins and their modification
Chorionic; pituitary; rebound and augmentation

C. Systemic factors
Nutrition; thyroid; general health

D. Neurogenic and emotional factors

E. Radiation
Gonad; pituitary; whole body

F. Ovarian surgery
Endometriosis; resection of ovary (Stein-Leventhal syndrome)

III. Effectiveness of luteal function

A. Physiology
B.B.T.; pregnanediol

B. Endometrium
   Serial biopsy
C. Cervix
   Mucus: Penetrability; viscosity
D. Tube
   Motility; epithelium

IV. Clinical applications

I. CERTIFICATION AND TIMING OF OVULATION

During the past few years there have been numerous publications dealing with this aspect of ovarian function. In general the data presented falls into two categories—objective and presumptive (indirect). From the clinical standpoint we must rely primarily on indirect evidence—such as *Mittelschmerz*, basal body temperature curves, chemical tests for pregnanediol, changes in vaginal smears, endometrium, and cervical mucus.

Objective Evidence

Doyle,[1] with an ingenious technic of *culdoscopic observation*, has noted and described the clinical picture of ovulation in humans. The time for such observations was determined by *basal body temperature*. While not a clinical procedure, it is objective evidence of ovulation in women.

The occasional observation of ovulation at laparotomy and the recovery of ova from the genital tract also provide objective evidence of ovulation and are useful for certification in research programs, but are not suited to clinical use.

Presumptive Evidence

There has been intensive study of the presumptive or indirect evidences of ovulation for clinical use. The following publications bear on this problem.

Noyes and Haman[2] have compared the reliability of *basal body temperature* and *endometrial samplings* with menstrual dates and found them to be 80 per cent accurate within 2 days. These authors describe the interpretative data employed in their studies.

Similar studies are reported by Sturgis[3] and Jackson,[4] both of whom reviewed critically the technic of determining ovulation, indicating their *post hoc* quality. These authors independently concluded that *basal body temperature* is probably the cheapest and easiest clinical test, but that the *serial biopsy* is the most reliable index and provides in addition information regarding the development of the corpus luteum and endometrial response.

Pommerenke[5] reviews the many *collateral cycles* that accompany the ovarian rhythm and indicates those of most clinical importance.

Corner, Farris, and Corner[6] have studied the *age of the corpus luteum and the endometrium* in conjunction with *the Farris hyperemia test* and found correlation within 1 day in 80 per cent; there was also agreement in each of 6 instances that ovulation had not occurred.

Vollman,[7] in a statistical study, correlated the *cervical mucus, Mittelschmerz, and basal body temperature* with fruitful coitus and established a fertility index on the basis of subsequent pregnancies. His data suggest a broader zone of fertility than is usually reported.

Tumonova[8] made similar studies, including *pH of cervical mucus* in his study of indices of ovulation.

Siegler and Siegler[9] studied the *basal body temperature* in several cycles of 202 women and describe *variations of the curves* and their significance in terms of ovulation and fertility. They indicate a broader spread of ovulation throughout the cycle and call attention to the many individual variations. Typical curves were found in 82 per cent of cycles studied.

Goldhar, Grady, and Masters[10] studied daily *vaginal smears* and found about 70 per cent correlation with ovulatory cycles.

Roland[11] studied the *cervical mucus arborization* pattern in ovulatory and anovulatory cycles and in menopausal women treated with estrogens. The persistence of fern pattern in the late cycle is presumptive evidence of failure of ovulation.

Roth and Burger[12] compared *vaginal smears, basal body temperature, and pregnanediol excretions* in a study of ovulation and found close agreement.

Critique

Most of us would agree with Shorr[13] that no single clinical evidence of ovulation should be employed; each technic has certain advantages while lacking either accuracy or clinical adaptability.

It would appear that the best clinical *pre*ovulatory indices are the serial vaginal smear and the Farris hyperemia test, and that of the *post*ovulatory indications, the endometrial biopsy is the most reliable, with the basal body temperature and pregnanediol tests offering certain useful clinical information.

Thus, instead of simplifying the problem of determining ovulation, it now appears necessary to accumulate serial data, over several cycles, by several technics. While time-consuming, this offers the best criteria for interpreting the factor of ovulation in the study of female infertility. Each investigator will come to employ those technics available to him and will develop special skills in their interpretation. In my own office I employ *Mittelschmerz* (when present), basal body temperature, and serial endometrial biopsies over 3 cycles for determining ovulation and estimating its timing.

II. FACTORS BEARING ON OVULATION

In the past few years there has been a large number of publications bearing on those factors affecting ovulation. Many of them are vague or only clinical reports, others represent careful experimental studies. Representative references and abstracts are reported here on each of five areas—gonadotrophins, systemic factors, neuro- and psychogenic factors, radiation, and ovarian surgery.

New Theories of Ovulation

There are two interesting reports offering slightly new concepts of the mechanism of ovulation. Roland[14] suggests that a lack of an inhibitor factor, which normally represses the "non-chosen" follicle, may permit many follicles to reach partial maturity, with an attendant estrogen suppression of the pituitary resulting in anovulation. Barjaktarovitch[15] suggests that a reflex stimulus from the distended follicle causes a release of both luteinizing hormone and oxytocin of the posterior pituitary, with resultant stimulation of the theca to contraction and expulsion of the ovum.

Gonadotrophins

The status of pituitary-derived gonadotrophins has not changed in recent years. The prompt development of antihormones has sharply limited their clinical usefulness.

Runner and Gates[16] and Austin[17] have demonstrated quite clearly by transplantation technics that ovulation of a normal fertilizable ovum can be obtained in prepuberal mice and rats with chorionic gonadotrophins.

Despite ample evidence of the lack of adaptability of this biologic response in mice to women, chorionic gonadotrophin continues to find some advocates in clinical medicine. Gianaroli[18] reports its employment but utilizes the somewhat unreliable vaginal smears for evidence of ovulation. Ishizuka[19] administered chorionic gonadotrophins in the midfollicular phase to ovulating women and reports advancing the time of ovulation. This he interprets as "induced ovulation." The evidence of chorionic-gonadotrophin-induced ovulation in anovulatory women is still questionable.

Weir[20] has described the employment of estrogen early in the cycle to enhance pituitary function, thus clinically confirming work of Brown and Bradbury[21] and others that estrogen will cause the release of pituitary gonadotrophin.

The employment of the rebound phenomenon in obtaining enhanced pituitary-gonadal function has not been adequately studied in women. It is partially discussed in an excellent review by Siegler.[22] It is undoubtedly the explanation of the frequent occurrence of pregnancies following artificial cycles.

Systemic Factors

In the field of general or systemic influences on ovulation, several interesting reports have appeared. Excellent general reviews presented by Davis,[23] Sturgis,[24] and others provide perspective and specific suggestions for diagnosis and therapy. In addition there are two reports dealing with the importance of nutrition and pregnancy in women by Glass and Lazarus[25] and Seymour.[26] Studies on animals by Runner and Gates[27] and Lee, King, and Visscher[28] indicate loss of fertility in both forms of malnutrition, obesity and underweight.

The relation of cortisone to ovulation in rabbits is reported by DeCosta and Abelman,[29] and apparently it has no effect on ovulation in this species. However in women suffering from androgenic stimulation, Wilhelm and Marks[30] report successful suppression of 17-ketosteroids, following the use of cortisone with ovulation and pregnancy. This is one of the more intriguing aspects of therapeutic hormonal balance in sterility. Similiar effect on ovarian function is reported by Greenblatt.[31]

Thyroid has held a unique place in gynecology and infertility. Typical of many reports are those of Jarvis,[32] and Johnson and Bradbury[33] who noted enhanced basal body temperature curves and pregnancy following its use. Others deplore its promiscuous use and Tyler[34] points out its inefficacy in a series of patients.

Neuro-Emotional Factors

Recently there have been a number of studies related to neurologic or psychologic interference with ovulation. Pirard,[35] and Netter and Lambert,[36] in a large series of superior cervical ganglion blocks, found pregnancies resulting in women with anovulatory cycles. They attribute the success of this procedure to a reflex vasodilation in the hypothalamic region. While the total series is large, the number of patients with anovulatory sterility that conceived is quite small (13 out of 23). These intriguing results must await further confirmation before general adoption of the procedure.

Much has been written about the emotional or psychologic aspects of infertility. Most authors, however, relate this to tubouterine spasm rather than to suppression of ovulation. Pseudocyesis and other emotional amenorrhea is a fruitful area for study.

Radiation

The past several years have seen a great host of publications on the effect of gonadal and/or pituitary radiation in infertility. As in most case where objectivity is lacking, emotionalism and adapted collateral evidence so beclouds the issue that it becomes almost impossible to formulate an unbiased opinion.

Writers on this subject fall into three groups. There are those gynecologists who use such radiation and report series of cases of such size and weight as to command serious consideration. Second are those scientists, usually biologists or geneticists, who condemn it unqualifiedly. Their opinions are based on animal experimentation and theoretical grounds. Some of the animal experimentation bears only the most remote relationship to human reproduction. And last are those clinicians who are attempting to appraise the facts concerned—chiefly by more critical analysis of statistical data available.

Payne,[37] Rakoff,[38] Israel,[39] Bonar and Garber,[40] Asherman,[41] and many others describe respectable series of cases, carefully selected, and fairly well controlled. In essence they recommend that when anovulation is the sole cause of infertility —and all other factors have been corrected—in women between twenty and thirty-five the employment of radiation is indicated. In their opinion the results, as measured by the number of apparently normal children, warrant their continuing this therapy despite the many dire warnings of genetic deviation.

Those opposing this therapy do so on highly logical interpretation of animal data. Rugh,[42] Russell,[43] and Muller,[44] all distinguished scientists, have warned of dire consequences and plead with us to desist.

And finally there are the reports of Kaplan[45] and Rubin[46] which provide the largest series of cases. It would appear that the third-generation children were presented as a trump card. It is unfortunate that Kaplan did not follow up the male children of irradiated grandmothers. It might be feasible (and it may have been done) to compute the incidence of mutations, monsters, abortions, and so on, in the general population and compare this with the combined figures for second- and third-generation pregnancies of irradiated patients.

It is indeed unfortunate that the term "stimulating x-rays" was employed, for it is now known that such is not the case. It has been suggested that perhaps persistent follicles were responsible for this endocrine stalemate of anovulation or amenorrhea, and that perhaps wedge resection and irradiation accomplish the same elimination of such follicles although by separate methods. If such is the case, surgical resection of follicles would relieve some patients and satisfy the geneticist; we can reserve irradiation for surgical failures.

So there you have it. An increasing number of clinicians are impressed by the accumulating statistics of success and apparent safety, and an increasing number of geneticists are becoming alarmed.

Taking average figures, it would appear that an extensive medical work-up, including a four-exposure series for pyelography, a three-exposure series for lumbosacral spine derangements, and a barium enema and fluoroscopy, might well deliver an equal number of roentgens to the ovary as those deliberately delivered in specific therapy of anovulatory infertility. Stanford and Vance[47] have recently reported an extensive study of the theoretical gonadal radiation received in standard radiographic studies and suggest certain factors bearing on this item.

For the benefit of those of us on the sidelines, we could hope for an early resolution of this argument. A physician attempting to advise his patient whether to accept a childless home, to take a chance on an adopted child of uncertain parentage, or to accept a risk of irradiation is certainly in a difficult position. The cold light of science is much needed in today's life, but it does not warm the aching heart of a barren woman.

This problem seems to have much in common with artificial insemination. Our decision will have to be made primarily on personal basis.

Surgery

Last to be discussed are those derangements within the ovary itself which affect ovulation, such as sclerocystic disease, endometriosis, and infections and their residues. McGoogan[48] has reviewed the frequency of the several ovarian lesions in this regard. The literature has shown an increasing awareness of the *Stein-Leventhal syndrome*. Buxton and Van de Wiele,[49] Andrews,[50] Leventhal and Cohan,[51] Bergman,[52] and others[53] have given excellent descriptions of this disorder. It is rapidly becoming apparent that there are many variations or degrees of clinical deviation in this syndrome. It further appears that critical preoperative study is necessary for success. Buxton[49] and others suggest a pre-surgery test with cortisone, and this may have merit.

The etiology of the disorder and the rationale of therapy still elude us. Further study is in order. In the meantime, critical study of patients with these clinical manifestations will further our knowledge.

Endometriosis has not significantly occupied the attention of gynecologists interested in sterility during the past few years. The presumed role of delayed fertility and the frequency of endometriosis is still unsettled. Both Robertson[54] and Simmons[55] have reviewed the role of medical and conservative surgical care of the endometriotic infertile woman.

Critique

The variety of factors bearing on ovulation are slowly being delineated. It is no longer adequate to certify anovulation—one must also determine the factor or factors responsible for such ovarian failure.

The absence of effective pituitary gonadotrophins continues to delay studies and therapy of anovulation. Chorionic gonadotrophins continue to intrigue us but have not been able to induce ovulation in the anovulatory woman. The rebound phenomenon is proving increasingly useful in anovulation.

General systemic influences are emerging as more important than specific endocrine disorders. The study and care of infertile couples encompasses increasingly broader responsibilities in the realm of nutrition and general health.

While much is being proposed regarding the theoretical relationship of neuro-emotional factors in reproductive failure, there is scant and very questionable support for the effect of such influences on ovulation.

Radiation is still a controversial issue. While I do not endorse its indiscriminate use, the evidence for interdicting its occasional judicial employment is not adequate to allow barrenness to continue without every possible treatment. Mutations are a definite hazard, yet not all mutations are bad.

Enhanced ovulation and fertility have followed the disruption of the ovary by both radiation and surgery, perhaps by the elimination of the polycystic state. Where feasible, surgery would seem safer.

It is gratifying to see an increasingly conservative approach to anatomic derangements of the ovary such as endometriosis, polycystic and sclerocystic disease, and benign neoplasia. Fertility is often preserved or enhanced by these efforts.

III. EFFECTIVENESS OF LUTEAL FUNCTION

Finally, the ovary is responsible for certain essential functional changes in the tube, endometrium, and cervix. Defective luteal function may result in failure of pregnancy due to abnormal cervical mucus and sperm ascent, to abnormal transport of the ovum, or to the unsuspected early abortion of non- or mal-implanted conceptus.

Progesterone is truly a progestational hormone; luteal deficiencies, while admittedly uncommon, have not received the attention warranted, primarily because of lack of scientific information and cost of therapy.

Steinberg[56] has postulated three types of ovarian cycles with luteal deficiency—anovulatory-aluteal, anovulatory-luteal, and ovulatory-hypoluteal.

Palmer's[57] excellent review of hypoluteal infertility outlines the criteria for this syndrome: endometrial samplings with hypoglycogen content or inadequate predecidua, abnormal or hypoperistalsis of tube, deficient vaginal smears, and depressed pregnanediol excretion.

Two forms of therapy are available—stimulation with chorionic gonadotrophin as previously described by Brown and Bradbury[58] and replacement with progesterone.

The earlier reports of McLennan and McKelvey on abnormal corpus luteum function and irregular development and shedding of the endometrium have been confirmed.[59] Hughes[60] has also demonstrated a hypoglycogen state due to hypoluteum function correctable with chorionic gonadotrophin. Buxton[61] and others[62] have reported similar findings. In all probability the successful pregnancies reported by those employing chorionic gonadotrophin are of the hypoluteal variety.

Abnormal tubal and uterine peristalsis in hypoluteal states has been described by Rubin, Palmer,[57] Bickers,[63] and others. The role of this function in infertility is not clear.

Changes in cervical mucus favorable to the ascent of sperm were once thought to be associated with ovulation and presumably luteal function. Many recent investigations suggest this to be primarily an estrogen function.

Recent studies have indicated a larger requirement of progesterone and the advantages of combined estrogen-progesterone therapy.

Recent developments of long-acting progesterone by several of the pharmaceutical houses should enhance our effectiveness in this regard.

Critique

Hypoluteal states are one of the most fertile fields for study in the infertile and habitually aborting woman. The identification of this syndrome is still difficult and lacks somewhat in clinical practicality. Unusually flat or short elevations of the second phase in the basal body temperature and low pregnanediol excretion curves are suggestive, but subject to inherent errors.

Serial endometrial biopsies offer objective evidence of a specific deficient response of that organ and indicate a local need for augmentation regardless of the body temperature response or pregnanediol excretion values.

Hypoluteal states, while not common, are most readily amenable to clinical therapy, responding to both the stimulation of chorionic gonadotrophins or to direct supplementation with progesterone.

The development of relatively inexpensive and long-acting progesterone for clinical use opens a new area for extensive exploration and clinical trial in this area of infertility.

IV. SUMMARY OF CLINICAL APPLICATIONS

Thus has our knowledge of the role of the ovary in infertility advanced during the past few years. Better understanding of the methods of certifying ovulation and its timing have been developed. Of the factors bearing on ovulation, the area of general health and nutrition has shown increasing importance. The limitations of gonadotrophic therapy remain. Slight improvement in clinical management of anovulation is available through the rebound and augmentation technics. Radiation and partial ovarian resection are gaining some acceptance with partial clarification of the issues involved. Hypoluteal states are now clinically recognizable and remain an important area for study in infertility and frequent early abortion.

Serial endometrial biopsies have emerged as the most important single diagnostic procedure in infertility of ovarian origin. They can be done easily in the office without anesthesia or great discomfort to the patient. The simultaneous study of a series of endometrial samples taken in the latter half of the menstrual cycle provides more useful clinical information regarding ovarian function than attempted correlations and interpretations from a single specimen.

Not only can ovulation be certified, but also the effectiveness of luteinization and endometrial response can all be interpreted with reasonable accuracy. On the basis of such objective data sound therapy can be programmed.

When anovulation is present, a schedule of superb general health and nutrition is essential. The employment of the rebound phenomenon or pituitary augmentation are often effective in inducing ovulation. Of the many technics proposed for the rebound phenomenon, probably the easiest is the artificial cycle induced with steplike increases in estrogen over 21 days; the continuous suppression of ovarian function with large doses of estrogen or testosterone is also effective. Moderate single doses of estrogen given on Day 10, 11, or 12 often enhance the release of pituitary gonadotrophin and may be followed by ovulation.

If the endometrial biopsies indicate a hypoluteinism or a deficient response of the endometrium, augmentation is feasible by stimulation of the corpus luteum with chorionic gonadotrophins or direct supplementation with progesterone. The doses of these, as all biological therapeutics, must be determined by the clinical response of the individual patient.

In patients with enlarged and/or cystic ovaries, cortisone may be of therapeutic and prognostic value. In carefully selected cases surgical resection of the ovary seems of value. It would seem wise to defer radiation to that group of carefully selected anovulatory women where all else has failed to induce ovulation.

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Book Reviews

Reproductive System. Frank H. Netter, M.D. *Summit, N. J., Ciba Pharmaceutical Products, Inc., 286 pp., Vol. 2, The Ciba Collection of Medical Illustrations. $13.00.*

The foreword of this outstanding contribution was written by John Rock and the preface by the editor, E. Oppenheimer. The latter points out that this book is intended to supplement, rather than replace, standard reference works. Of the 233 plates dealing with the reproductive system 89 appear in print for the first time in this book. The other 144 plates were previously published in the Ciba *Clinical Symposia* or in special folders or brochures. In the preparation of the book Netter had the cooperation of the following authorities: N. S. Assali, Albert Decker, Joseph A. Gaines, Charles R. Geschickter, Robert A. Hingson, George W. Mitchell, Jr., Josef Novak, I. C. Rubin, Somers H. Sturgis, Samuel A. Vest, and Pearl M. Zeek. In his interesting introduction Netter relates the history of modern scientific anatomy and names the artists who had a fascination for drawing the human body during the Renaissance.

The book is divided into 14 sections, as follows: (1) "Development of the Genital Tracts and Functional Relationships of the Gonads"; (2) "Normal Anatomy of the Male Genital Tract"; (3) "Diseases of the Penis and Urethra"; (4) "Diseases of the Prostate and Seminal Tract"; (5) "Diseases of the Scrotum and Testes"; (6) "Normal Anatomy of the Female Genital Tract and Its Functional Relationships"; (7) "Diseases of the Vulva"; (8) "Diseases of the Vagina"; (9) "Diseases of the Uterus"; (10) "Diseases of the Fallopian Tubes"; (11) "Diseases of the Ovary"; (12) "Pregnancy and Its Diseases"; (13) "Anatomy and Pathology of the Mammary Gland"; and (14) "Intersexes." At the end of the book is an extensive complete cross-reference index containing more than 3000 items.

It is difficult to conceive of any physician who is not acquainted with Netter's beautiful drawings, because during the last decade the Ciba company has widely distributed booklets containing Netter's illustrations. In the reviewer's opinion no one surpasses Netter in his ability to graphically portray the human body. Not only are the structures meticulously correct in size, shape, location, and relation to other tissues, but also the coloring is magnificent and accurate. Every physician should have a copy of this outstanding contribution. Certainly everyone interested in sterility should possess one, particularly because it is sold without profit; hence the price is low. Netter, the artist, Oppenheimer, the editor, the collaborators, and the Ciba company are to be highly commended for having produced this masterpiece.

J. P. G.

Urology. MEREDITH CAMPBELL (Ed.). *Philadelphia, W. B. Saunders Co., 1954, 3 vol., 2356 pp., 1148 illus., $60.00.*

In the preparation of this colossal contribution Campbell had 51 collaborators. In spite of the large number of contributors, the book presents a smoothness which is gratifying to the reader. Of special interest to the readers of FERTILITY AND STERILITY is the 33-page chapter "Infertility in the Male" by Robert S. Hotchkiss, and the small sections on sterility due to radiation, cryptorchism, epididymitis, orchitis, epispadias, and hypospadias. Of course, many of the chapters are of importance to everyone interested in sterility, including "Anatomy and Surgical Approach to the Urogenital Tract in the Male" by Austin I. Dodson; "Physiology of the Prostate and Seminal Vesicles" by Charles Huggins; "Physiology of the Testis and Scrotum" by Carl R. Moore; "The Urologic Examination" by Gershon J. Thompson; "Embryology and Anomalies of the Urogenital Tract" by Meredith Campbell; "Urology in the Female" by Lawrence R. Wharton; and "Endocrinology in Urology" by Earl T. Engle and Joseph W. Jailer. There is no chapter on infertility in the female.

Every chapter is beautifully and profusely illustrated and has an extensive, useful list of references at the end. It is impossible to discuss the individual chapters in this review. Suffice it to say that the three volumes contain practically all the useful knowledge in the fields of male and female urology presented by leading authorities. This book will prove helpful not only to urologists but also to gynecologists, pediatricians, endocrinologists, internists, and others. The editor, his collaborators, and also the publishers are to be congratulated on having produced this monumental contribution.

J. P. G.

Contributions to Embryology. Vol. 35; Nos. 231-241. *Washington, D. C., Carnegie Institution of Washington (Publication #603), 1954, 237 pp., 54 plates, 53 illus., $12.00 paper bound, $13.00 cloth bound.*

There are eleven contributions in this volume:

1. Early abnormal embryos of the rhesus monkey (George W. Corner and George W. Bartelmez);
2. Studies in the development of the baboon (*papio ursinus*): A description of two presomite and two late somite stage embryos (Christine Gilbert and Chester H. Heuser);
3. The formation of neural crest from the primary optic vesicle in man (G. W. Bartelmex, with the collaboration of Mary P. Blount);

4. Observations on regional circulation times in the lamb under fetal and neonatal conditions (S. R. M. Reynolds, G. M. Ardran, and M. M. L. Prichard);
5. Androgen-induced female pseudohermaphroiditism in the monkey (*Macaca mulatta*): Anatomy of the reproductive organs (L. J. Wells and G. van Wagenen);
6. Development of the human diaphragm and pleural sacs (L. J. Wells);
7. Architecture of distended and nondistended human umbilical cord tissues, with special reference to the arteries and veins (Anna W. Chacko and S. R. M. Reynolds);
8. Venous drainage of the placenta of the rhesus monkey (*Macaca mulatta*) (Elizabeth Mapelsden Ramsey)
9. The early development of the human nephros (Theodore W. Torrey)
10. On the preimplantation stages of the human ovum: A description of four normal and four abnormal specimens ranging from the second to the fifth day of development (Arthur T. Hertig, John Rock, Eleanor C. Adams, and William J. Mulligan)
11. Localization of the erythrocyte-forming areas in the early chick blastoderm cultivated in vitro (George W. Settle)

In 4 or 5 of the 9 young rhesus monkey embryos which were studied by Corner and Bartelmez, the embryonic abnormality was thought to have resulted from constitutional defects of the embryo itself, and in the other cases the possibility exists that the abnormality of the embryo resulted from primary failure of the corpus luteum. According to Hertig, Rock, Adams, and Mulligan, the cause of abnormalities in segmenting human ova appears to lie in the intrinsic quality of the zygote, rather than in its environment.

Cineangiographic studies made by Reynolds, Ardran, and Prichard on newborn lambs and a goat, showed that after ventilation of the lungs, there is not only a more rapid circulation through the pulmonary vessels, but an approximately fourfold increase in volume of this blood flow. In female monkeys transformed into intersexes (pseudohermaphrodites) by Wells and van Wagenen, by introducing testosterone propionate into the maternal circulation during pregnancy, various changes in the reproductive tract were produced. The induced modifications persisted for several years after birth. The treatment failed to cause the gonads to differentiate into testis-like structures. Chacko and Reynolds' studies on the architecture of the umbilical cord reveal several new facts. Ramsey presents evidence that the maternal sinus is a constant feature of the macaque placenta.

This volume, like its predecessors, is an excellent presentation of authoritative studies made by outstanding contributors. The text is easy to undersand, the drawings and photographs are as nearly perfect as is possible to attain, the typography is clear, and the paper is sturdy. This is an important volume for everyone interested in embryology and obstetrics.

J. P. G.

The Effects of Cortisone on the Infertile Male

Lewis Michelson, M.D., Samuel Roland, M.D., and Peter Koets, Ph.D.

THE REPORTED EFFECTS of cortisone on the sperm count and other related physiologic phenomena have been conflicting. Trabucco described marked improvement in the sperm counts of oligospermic and azoospermic patients who were given cortisone.[9] Studies of the effects of large doses of cortisone on a group of rheumatoid men showed a definite decrease of the number of spermatozoa in the semen. No significant change in motility or morphology was observed.[4] In a group of 7 boys with virilism due to adrenal hyperplasia, rapid growth of the testes with active spermatogenesis occurred following the administration of cortisone, and Leydig's cells appeared where formerly neither germ-cell differentiation nor Leydig's cells were present. In the studies of Wilkins and Cara the testicular maturation was thought not to be a direct effect of cortisone, but due rather to the release of pituitary gonadotrophins, which occurred when the excess secretion of adrenal androgens and estrogens was suppressed.[10]

The increased excretion of urinary gonadotrophins due to cortisone has been reported.[4, 5, 8] Sohval and Soffer reported excessive urinary gonado-

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We express our thanks to Dr. Warren O. Nelson and Dr. David Wood for their study and interpretation of the testicular biopsy specimens.

We thank Dr. William C. Kuzell for his advice on the medical procedures.

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trophic activity in 9 of 22 patients who received daily doses of 150 mg. cortisone or 100 mg. corticotrophin for from 1 week to many months. In their cases the increased urinary gonadotrophin titers appeared within 1 week of treatment.

Maddock, Chase, and Nelson describe urinary gonadotrophin increases in each of their patients following the administration of cortisone in daily doses of 500 mg. for 26 to 32 days, with no consistent changes in testicular morphology or estrogen excretion. They concluded that cortisone caused an increased pituitary excretion of follicle-stimulating hormone (FSH), with relatively little change in interstitial cell-stimulating hormone (ICSH) secretion.[4]

With this background of information it was thought that perhaps cortisone given to a group of infertile males (who had no obstructive pathology) might improve spermatogenesis. While Maddock, Chase, and Nelson administered 500 mg. daily with resulting decrease in the sperm number, our purpose was to determine the effect of small dosages of cortisone–25 to 50 mg. daily.

MATERIAL AND METHODS

A group of 10 infertile males (7 oligospermic and 3 azoospermic) was selected. From their history, physical examination, testis biopsies, and semen studies it was concluded that none of these men had any obstruction in his efferent sperm-carrying duct system. No contraindication was found to cortisone administration. Testis biopsies were done in all instances prior to cortisone medication. Before, during, and after therapy repeated sperm counts were made. In most patients 17-ketosteroid and urinary gonadotrophin excretion were determined.

At weekly or biweekly intervals during therapy 24-hour urine specimens were taken for 17-ketosteroid assays and at other intervals for urinary gonadotrophin assays. On several occasions consecutive daily creatinine determinations were made on 24-hour urine specimens, and it was felt that the close agreement in creatinine excretion indicated that full 24-hour urine excretion was being obtained.

Thorn tests were performed twice on each patient at 1-week intervals, using a stimulating dose of 25 mg. of corticotrophin and a normal decrease in circulating eosinophils was observed in all.

Table 1 lists the dosage and total amount of cortisone given to each patient.

Seven of the men were given 50 mg. of cortisone taken orally daily for 5–9 weeks, and 3 were given 25 mg. daily for 8–8½ weeks. The drug was given in divided doses over the entire day. In all but 3 patients the drug was gradually tapered off during the last 8 or 9 days. The total quantity given each patient varied from 1500 to 3375 mg. No untoward effects were observed.

TABLE 1. Cortisone Dosages

Patient	No. days			Total (mg.)
	50 mg. daily	*25 mg. daily*	*Tapering dose daily*	
L. R.	..	61	8	1600
V. A.	63	..	9	3375
C. K.	61	..	9	3275
J. W.	..	57	8	1500
G. L.	..	56[a]	8	1475
B. A.	61	..	9	3275
A. A.	35	..	..	1750
W. C.	62	..	9	3325
D. D.	48	..	..	2400
J. P.	49	..	..	2450

[a] Represents total of 2 periods.

Biopsy

The testicular tissue obtained by biopsy from the different oligospermic patients (Table 2) varied greatly in its histopathologic appearance—from complete germinal aplasia of practically all tubules to normal appearance of a high percentage of tubules, with active spermatogenesis, the remaining tubules showing varying degrees of impaired spermatogenesis and sperm production. Peritubular sclerosis of varying extent and severity was present in 6 instances. Leydig's cells appeared normal in 6 of the patients. In the seventh (A.A.) biopsy specimen the Leydig's cells tended to be large and vacuolated. In this patient the seminiferous tubules showed mild pathologic changes.

The biopsy specimens from J.P., the only patient who showed marked and sustained improvement in the characteristics of the sperm population,

TABLE 2. Summary of Seven Oligospermic and Three Azoospermic Patients

Patient	*Age*	*History*	*Physical examination*	*Testis biopsy*
			OLIGOSPERMIA	
J. P.	29	Allergies to wool, milk. Hay fever, asthma. Precortisone therapy: thyroid gr. 2 (BMR —23), Vit. B comp., extract liver, amino acids, folic acid, 6 mo.—no demonstrable effect on sperm ct.	Negative	(L) Similar to right but very much milder (R) Partial-to-complete arrest in the two spermatocyte stages in almost all tubules. Moderate sloughing. Moderate to complete sclerosis of the basement membrane and tunica propria 15% tubules Leydig's cells normal
B. A.	28	Bilateral acute orchitis age 7. Duration a few days. Etiology unknown.	Testes small	(L) Tubules small; germinal cell aplasia; few sperm (R) Only Sertoli's cells in tubules Leydig's cells normal
W. C.	25	Always underweight	Height 6′4″, eunuchoidal habitus; both testes smaller and softer than normal; epididymides small and hard	(L) Tubules varying greatly in size and contents; insignificant sclerosis; 30–40 per cent only Sertoli cells, remainder impaired spermatogenesis with tendency to arrest of spermatogenesis at spermatocyte stage; marked sloughing (R) Similar, but less extensive Leydig's cells normal
A. A.	28	Pregnant-mare serum for 1 yr., 3 yr. previous—no demonstrable effect on semen	Left testis smaller than right	(L) Normal-size tubules; mild hypoplasia and spermatocytic arrest; mild sloughing in 20% of tubules (R) Same Leydig's cells tending to be large and vacuolated
D. D.	27	*Pre-cortisone* testicular biopsies with left epididymitis; testosterone 25 mg. weekly, vitamin B complex, extract of liver, amino acids, folic acid, 4 mo.	Prostatic secretion 50–200 pus cells per h.d.f.	(L) Most tubules large with active spermatogenesis; mild generalized fibrosis; widespread sloughing (R) Same Leydig's cells normal

TABLE 2—(*Continued*)

Patient	*Age*	*History*	*Physical examination*	*Testis biopsy*
J. W.	33	Chinese; left testis always small	Left testis small; left epididymis small and hard; right spermatocele	(L) 70% tubules moderate fibrosis, good spermatogenesis; remainder many small, fibrosed, poorly active, with poor spermatogenesis. (R) 45% tubules fairly normal, good spermatogenesis; remainder small, completely fibrosed or only Sertoli cells or poor spermatogenesis present Leydig's cells normal
G. L.	36	Virus hepatitis at 19 yr.	Prematurely gray; left testis small, epididymis small, hard; right testis and epididymis normal	(L) 80% tubules severe sloughing and sclerosis, poor spermatogenesis (R) Similar but more extensive and destructive Leydig's cells normal
			AZOOSPERMIA	
C. K.	30	Negative	Testes small; left epididymis thickened; right globus major cyst	(L) Tubules averaging less than normal size; 15% moderate sclerosis; spermatogenic arrest at primary spermatocyte stage (R) Same Leydig's cells normal
V. A.	29	Negative	Negative	(L) Tubules normal size; spermatogenic arrest at primary spermatocyte stage; occasional spermatid and spermatozoon (R) Same Leydig's cells normal
L. R.	22	Negative	Right spermatocele	(L) Few tubules, moderate sclerosis; spermatogenic arrest at spermatocyte-spermatid stage (R) Same Leydig's cells normal

are of special import and are given here in detail. The pathologic process in the testes involved the germinal cell epithelium as well as the basement membrane and tunica propria of the seminiferous tubules. In the right testis the sclerosis (moderate to complete) included about 15 per cent of the tubules. The remainder showed insignificant sclerosis. Spermatogenesis was greatly impaired by the tendency to arrest at the two spermatocyte stages in almost all tubules. Moderate sloughing was present. Sperm production was greatly lessened.

In the left testis the extent of the sclerosis was similar to the right, but in a milder and more moderate degree. There was little tendency toward spermatogenic arrest and the occurrence of polynucleated cells. There was some slight sloughing. Sperm production was fairly good. Dr. Warren O. Nelson, to whom we are indebted for making the histologic studies on this biopsy specimen, concluded that "most of the normal sperm in the ejaculate are from this testis, while most of the abnormal forms are from the right testis."

In the 3 azoospermic patients the main defect was arrested spermatogenesis at the primary spermatocyte stage. A few spermatozoa were seen in one (V.A.) but none in the other two (L.R. and C.K.). In C.K. there was a considerable reduction in both the numbers and layers of germinal epithelium. In L.R. and V.A. only the layers of germinal epithelium appeared reduced. A moderate, not extensive, sclerosis was present in C.K., and absent in L.R. and V.A. Leydig's cells appeared normal in all 3.

RESULTS

Sperm Count

The administration of cortisone caused a temporary depression in the number of spermatozoa in all the oligospermic patients (Fig. 1 and Table 3). A possible exception was B.A., in whom so few spermatozoa were seen before and after cortisone treatment that it was impossible to determine whether the drug had had any effect on the sperm count. The drop in the other 6 varied from approximately 50 per cent to 97 per cent during periods of from 2 to 8½ weeks. In no instance did the semen become azoospermic. In all patients the sperm counts returned to essentially precortisone levels. In G.L., who discontinued cortisone after 5 weeks, a depressed sperm count promptly returned in 1 week to precortisone levels. At this point cortisone was started again and 2 weeks later the count was again depressed. Cessation of medication again resulted in an increase in sperm counts to essentially the premedication level, followed by a drop. We have been unable to obtain a later specimen than that shown in Fig. 1, 25 weeks after starting cortisone or about 16 weeks after it was discontinued.

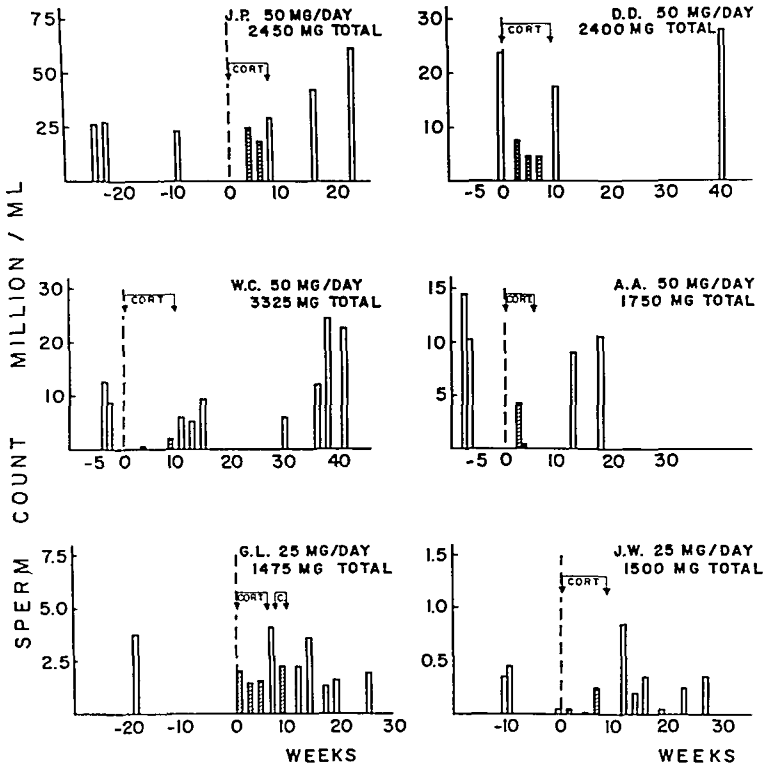

Fig. 1. Effect of cortisone on sperm count (shaded bars indicate counts made during cortisone administration).

In only 1 patient (J.P., Table 4) did the number of spermatozoa show a sustained increase in the postcortisone level over the precortisone count (see Fig. 1 and Table 3). When first seen, 6 months prior to cortisone therapy, the number varied from 26 to 27.5 million per cc., with poor motility and morphology. Sixteen weeks after cortisone was started (or 10 weeks after it was discontinued), the semen showed 42,500,000 spermatozoa per cc. and 7 weeks later the count was 61,500,000.

TABLE 3. Sperm Count and Urinary Ketosteroids

Patient	*Sperm count* (mill./cc.)			*Urinary 17-ketosteroids* (mg./24 hr.)[a]	
	Before therapy	*Cortisone*		*Before therapy*	*During cortisone*
		During	*After*		
		OLIGOSPERMIA			
J. P.	26.2	24.7	29.5	19.0	25.9
	27.5	18.2	42.5		16.9
	23.0		61.5		15.7
					12.0
B. A.	2–4 sperm/ 25 h.d.f.	2–3 sperm/ 25 h.d.f.	2–8 sperm/ 25 h.d.f.	18.2	21.1
					14.2
					13.2
W. C.	12.5	0.3	6.0	. . .	. . .
	8.75	2.0	°°°		
			24.5		
A. A.	14.5	4.2	9.0	9.8	18.9
	10.25	0.45	10.5		15.9
					14.9
D. D.	23.75	7.5	17.3	. . .	. . .
		4.6	26.7		
		4.5			
J. W.	0.3	0.05	0.85	12.1	24.5
	0.35	1 sperm/ 50 h.d.f.	°°°		14.2
	0.45		.05		
	0.05		°°°		
		0.25	.035		9.6
G. L.	3.75	2.0		15.1	33.4
		1.5	4.1		16.7
		—	—		
		2.2	1.3		
			1.9		
		AZOOSPERMIA			
C. K.	0	0	0	. . .	. . .
V. A.	0	0	rare	. . .	. . .
		0			
		rare			
L. R.	0	0	0	. . .	24.9
					15.1
					18.1

[a] Normal range 7–27 mg./24 hr.; average 15 mg.

This case also was the only one to show any significant change in the morphology and motility of the spermatozoa. The motility has risen from 30–40 per cent (half actively progressive) to 50–60 per cent (the majority actively progressive), and the morphology from 56 per cent normals to 68 per cent normals. Prior to cortisone therapy this patient had many allergic manifestations: asthma, and hypersensitivity to poison oak, house dust, and egg. He had been given a high-protein diet, vitamin B complex, amino acids, folic acid, and crude liver extract for several months. He was also better satisfied with his new job and had gained 12 pounds.

TABLE 4. Patient J. P.—Sustained Improvement of All Sperm Characteristics

		Sperm		
Date	*Period in therapy*	*Number per cc.*	*Motility (%)*	*Ovals (%)*
10/13/53	Precortisone	26,250,000	30	..
10/22/53	Precortisone	27,500,000	40	56
1/21/54	Precortisone	23,000,000	40	43
4/2/54	Cortisone started, 50 mg. daily	..	..	..
4/29/54	On cortisone	24,750,000	40	50
5/13/54	On cortisone	18,250,000	30	50
5/21/54	Cortisone discontinued	..	..	..
5/27/54	After cortisone	29,500,000	50	60
7/29/54	After cortisone	42,500,000	50	67
9/16/54	After cortisone	61,500,000	60	68

In one other case (W.C.) an increase in the number over the precortisone period was first noted 29 weeks following discontinuance of cortisone. The count before therapy ran from 8,750,000 to 12,500,000, posttherapy 24,500,-000 to 22,500,000. As the improved count has been observed in only 2 specimens in a period of nearly 2 months we do not feel justified in terming it as yet a sustained increase. The patient received only cortisone therapy.

Reproductive Ability

Pregnancy occurred in 1 case (D.D.). No sustained improvement in the sperm count could be demonstrated. (Table 5; see also Fig. 1 and Table 3.) No improvement was noted up to the time cortisone was started or 10 days after cortisone was discontinued, which was 2–4 days before conception occurred. However, 31 weeks postcortisone the count exceeded the pre-cortisone level. He had been on thyroid and a combination of vitamin B complex, amino acids, folic acid, and crude extract of liver orally for about 6 months previous to cortisone therapy. He also received 25 mg. testosterone intramuscularly weekly for 3 months up to about a month before cortisone was started.

TABLE 5. Patient D. D.—Impregnation of Wife, No Sustained Improvement in Sperm Count

Date	Period in therapy	Sperm Number per cc.	Sperm Motility (%)	Sperm Ovals (%)
4/1/54	Precortisone	23,750,000	30[a]	76
4/1/54	Cortisone started, 50 mg. daily	..	..	..
4/22/54	On cortisone	7,500,000	40	64
5/6/54	On cortisone	4,650,000	50	74
5/20/54	On cortisone	4,550,000	30	57
6/1/54	Cortisone discontinued	..	..	..
6/10/54	After cortisone	17,250,000	50	52
Between 6/12 and 6/15/54	After cortisone; conception occurred	..	..	..
1/4/55	After cortisone	26,750,000	65	65

[a] Motility varied from 30 to 70% over a period of several months previous to cortisone.

Azoospermia

In 2 of the azoospermic patients no change could be demonstrated in the ejaculated semen. In the third (V.A.) a rare spermatozoon was seen in 2 of several semen specimens examined during and postcortisone therapy. Such a minimum finding does not demonstrate any effect of cortisone. We have retained the original classification of azoospermia as several specimens were examined previous to cortisone and no spermatozoa were seen.

Semen Volume

No sustained alteration before, during, or after cortisone administration was observed.

Hormone Excretion

The urinary 17-ketosteroids excretion in the 5 oligospermia cases from whom we were able to obtain pre-cortisone assays showed an initial rise

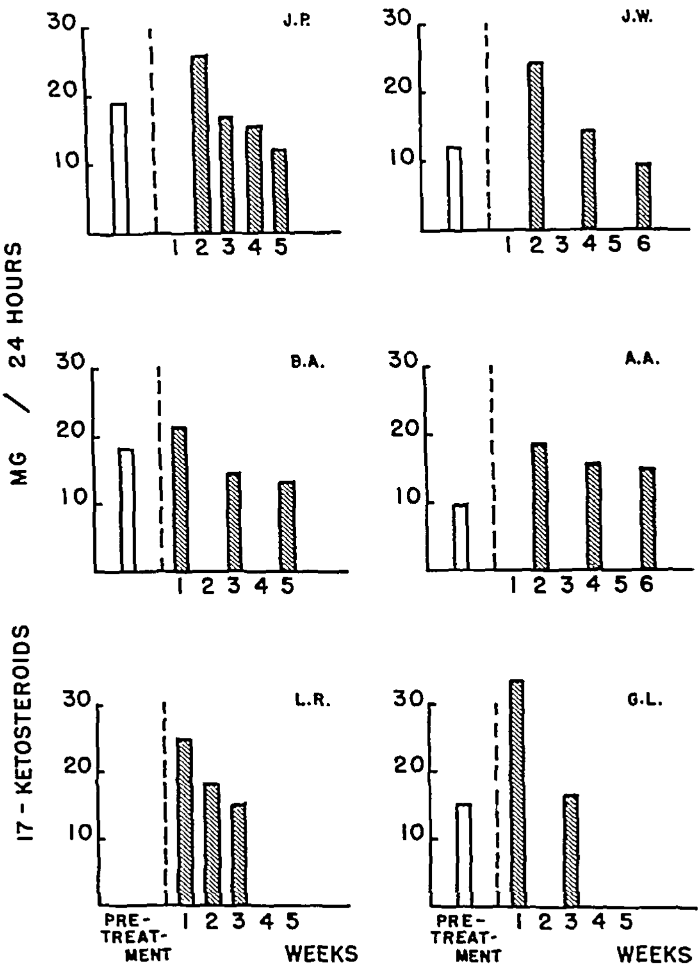

Fig. 2. Effect of cortisone on urinary excretion of 17-ketosteroids (shaded bars indicate determinations made during cortisone administration).

(Fig. 2; see also Table 3). In 3 patients this was followed by a drop to below pretreatment levels in 3 to 6 weeks. In 2 patients 1 had not reached the pretreatment level in 3 weeks and the other in 6 weeks. We were unable to obtain satisfactory assays in the other 2 cases. In 1 azoospermic case (L.R., arrested spermatogenesis), although we had no pretreatment assay of 17-ketosteroids, the pattern appeared to follow the course of a fall to below a pretreatment level. In the other azoospermic cases we were not able to obtain the urine for an assay.

Urinary gonadotrophin excretion showed an increase in 2 cases of oligospermia and 1 of azoospermia. The changes in the levels of these hormones for the remaining cases were varied, not sustained, and inconclusive.

DISCUSSION

The depression of sperm counts and the rebound to former levels after cortisone was discontinued resembles a similar phenomenon observed with the administration of testosterone.[2] The mechanism by which cortisone depressed the sperm counts of these patients is unknown. A suppression of cortical androgen and estrogen is thought to occur. Cortisone given to rats in large doses has caused a reduction in adrenal weight, but gonadal weight and gonadal histology were unchanged and decrease in prostate weights and increase in seminal vesicle weights took place.[6]

Increased excretion of urinary gonadotrophins has been explained by some to be due to the inhibition of antigonadotrophin formation.[1] Smith has suggested increased renal clearance to account for the elevation in urinary gonadotrophins. Although no regressive changes in testicular morphology were found by investigators who gave cortisone to humans,[4] rats,[6] and birds,[3] the increased urinary gonadotrophin excretion observed during cortisone administration could be explained by depressed gonadal function.

SUMMARY

1. The number of spermatozoa in the semen of 6 of the 7 oligospermic patients was greatly depressed, temporarily, by the daily administration of 25–50 mg. of cortisone for 5–9 weeks. All of these rebounded to essentially precortisone levels. One case showed a sustained increase above this level to a normal count. One other showed an increase above the precortisone level but the count is still subnormal. Only 1 case showed a subsequent fall to below precortisone level, which has persisted. In the seventh case there were so few spermatozoa that no conclusion could be drawn as to any alteration in their characteristics.

2. The motility and morphology of the spermatozoa in 5 oligospermic

cases was not significantly altered. These characteristics improved to normal in the 1 case in which the count became normal. In the seventh case the findings were inconclusive.

3. A pregnancy occurred in 1 case. No improvement could be demonstrated in the precortisone spermatozoa characteristics up to and including the tenth day postcortisone and within 2 to 4 days of conception. However, 31 weeks postcortisone the count had exceeded the precortisone level.

4. No demonstrable change was noted in the azoospermic cases.

5. The semen volume was not significantly altered in any patient.

6. The urinary 17-ketosteroids in the 5 oligospermic patients in whom we had a premedication assay showed an initial rise. This was followed in 3 to 6 weeks by a drop in 3 cases to below the precortisone level. In 1 case the precortisone level was not reached in 6 weeks. The remaining patient was followed only 3 weeks but a drop to nearly this level had occurred. In the 1 azoospermic case, no precortisone assay was obtained but the pattern appeared to follow that of the oligospermic cases.

7. The urinary gonadotrophin level showed a rise in 3 patients. In the rest of the group the findings were inconclusive.

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In Vitro Speeds of Bovine Spermatozoa

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THE RATE OF progressive movement of the spermatozoa has been used as one criterion in physiologic studies for evaluation of semen both initially upon ejaculation and after storage or treatment. In most instances the rate of spermatozoan travel is determined by visual estimation with the aid of a microscope, and a rating is then assigned according to some arbitrary scale. Little information has been reported concerning actual individual spermatozoan speeds *in vitro.*

Adolphi reported the average speed of spermatozoa as 4.02 mm. per minute for bull spermatozoa, 3.00 mm. per minute for ram, and 2.58 mm. per minute for dog spermatozoa. Walton used a micrometer eyepiece and a stopwatch in determining the rates of travel by sea-urchin spermatozoa. However, the semen was under the influence of an electrical field and thus the 18μ per seconds reported was not a measure for free-swimming spermatozoa. Grave and Downing reported on some observations of sea-urchin spermatozoa traveling along a capillary tube, but no specific rates of travel were given. A more recent study was made by Rothschild and Schwann,[7] who used a time-exposure photographic method to determine that the rate of travel for sea-urchin spermatozoa was approximately 190μ per second. Lamar, Shettles, and Delfs reported a rate of travel of 3 mm. per minute for human spermatozoa in a capillary tube containing cervical mucus. Yamane and Ito also used a capillary-tube method in their study and reported rates of travel for stallion spermatozoa ranging from 87 to 200μ per second. Phillips and Andrews reported an average rate of travel of 4.83 mm.

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per minute for ram spermatozoa, with rates up to 15 mm. per minute in the first portion of the capillary tube. Rothschild,[5] in a preliminary report, indicated that the mean speed of bull spermatozoa was 117μ per second. Using a photographic method and employing the probability-after-effect principle, the same author reported a rate of travel for bull spermatozoa at 111μ per second.[6] He compared[6] this method to a photograph matching method giving a rate of 123μ per second. The fastest spermatozoon observed traveled at a rate of 250μ per second.

Thus the reports on actual sperm speeds to date are based on a very limited number of spermatozoa or ejaculates, and in general the methods reported are too time-consuming for routine use in either the experimental laboratory or the practical field situation. This paper reports the results of an attempt to rapidly assess spermatozoan speeds *in vitro* by clocking their travel over a measured distance under the microscope.

MATERIAL AND METHOD

In an attempt to obtain a quick estimate of the rate of travel by an individual spermatozoon and a mean rate for all active spermatozoa in an ejaculate, the time required for each of several spermatozoa to travel a given distance was determined. The time interval was recorded to the nearest 1/10 of a second with a stopwatch. A 10-power micrometer eyepiece, with a 100-unit scale, was calibrated by means of a hemacytometer field to obtain the distance traveled. The calibration factor was 6.95μ per unit for the 10-power objective and 3.16μ per unit with the 20-power objective.

Preparation of Semen

The ejaculates used in this study were obtained from the regular herd bulls maintained at the University of Illinois. All observations were completed within 30 minutes of the time the semen was ejaculated. The semen was prepared for examination by placing a small drop on a warmed microscope slide and dispersing the spermatozoa in a drop of 0.9% saline. A coverslip was placed over the drop and gently pressed down. Thus a thin layer of semen was created which restricted the sperm movement largely to one plane. The slide was held at 38° C. for observation.

Speed Determination

The time required for each of 10 individual sperm cells to travel the length of the 100-unit scale was recorded. The first 10 sperm cells that could be aligned with the long axis of the scale constituted the group that was timed. Four similar groups of 10 spermatozoa were clocked within consecutive 45-second periods for each of 24 ejaculates. All observations within each group of 10 spermatozoa were made with the same objective power. However, the sequence of powers within each series of 4 groups was changed so that every possible sequence was used in observing the 24 ejaculates. The mean speed of the 10 spermatozoa in each group was used as the estimate of the mean speed of all active spermatozoa in the sample during that 45-second period of observation.

Another investigator, using a visual estimation method, assigned to each semen preparation an initial rating for "per cent motile" spermatozoa and rate of travel of the spermatozoa. The percentage ratings were based on the commonly used scale of 0–100 per cent motile spermatozoa with 10 percentage unit intervals. The speed of travel ratings were based on a scale ranging from 0, indicating no progressive movement, to 4.0, indicating excellent progressive movement, with 0.5 unit intervals for the intermediate estimated speeds of travel.

RESULTS

The measured speed of the 960 individually observed motile spermatozoa in the 24 ejaculates under the conditions of this study ranged from 10 to 352μ per second, with an average rate of 114μ per second. The mean speed of all 40 spermatozoa in each ejaculate ranged from 32 to 226μ per second. The mean speed of each group of 10 spermatozoa varied from 27 to 254μ per second.

The mean speeds in microns per second for each group of 10 spermatozoa observed under the 10- or 20-power microscope objectives and during the consecutive 45-second periods are presented in Table 1. The mean speeds of all spermatozoa observed at the $\times$ 100 and the $\times$ 200 magnifications were 117.6 and 111.0μ per second.

Differences in Speed

An analysis of variance revealed that the differences in the mean speeds of the groups of 10 spermatozoa were significantly greater (0.01 level) for the spermatozoa from different ejaculates than for the spermatozoa from the same ejaculate (Table 2). Also there was as much variation in mean speeds between ejaculates from the same bull as there was between ejaculates from different bulls. The differences in mean speeds between those spermatozoa observed under a 10- or 20-power objective were significant

TABLE 1. Mean Speed of Sperm Travel (μ/sec.)

	Interval from start of observation (sec.)				*All*
	0–45	*45–90*	*90–135*	*135–180*	
× 100	125.3 ± 43.5[a]	131.0 ± 42.8	108.3 ± 57.2	105.9 ± 35.9	117.6 ± 45.4
× 200	122.3 ± 66.4	109.6 ± 51.0	118.1 ± 42.4	94.3 ± 40.1	111.0 ± 48.4
All	123.8 ± 50.8	120.3 ± 47.3	113.2 ± 49.1	100.1 ± 37.7	114.3 ± 45.1

[a] Standard deviation from the mean.

(0.01 level). The sperm speeds became significantly lower (0.01 level) with an increase in the length of time from slide preparation to observation. The mean speeds in microns per second were 123.8, 120.3, 113.2, and 100.1 for the consecutive periods of 0–45, 45–90, 90–135, and 135–180 seconds, respectively. Orthogonal comparisons show that there was no significant difference between the first and second period. However, the mean speeds were significantly lower (0.01 level) in periods 3 and 4 than in the preceding periods. Table 2 summarizes the analysis of variance.

TABLE 2. Summary of Analysis of Variance

Source	*Degrees of freedom*	*Mean square*
Total	95	
Between ejaculate[a]	23	8251[d]
Between bulls[b]	9	8131
Within bulls	14	8329
Within ejaculates	72	235
Powers[c]	1	1023[d]
Periods[c]	3	2626[d]
P_1 vs. P_2[c]	1	145
$P_1 + P_2$ vs. P_3[c]	1	1249[d]
$P_1 + P_2 + P_3$ vs. P_4[c]	1	6485[d]
Error	68	118

[a] Mean square tested by the within ejaculate mean square.
[b] Mean square tested by the within bulls mean square.
[c] Mean squares tested by the error mean square.
[d] Significant at the 0.01 level.

There was no significant correlation between the mean speed as determined by the clocking speed method and the estimated percentage of motility of the spermatozoa or the original sperm concentration, as the correlation coefficients were 0.06 and 0.04, respectively. Likewise there was no significant correlation between the estimated ratings and the estimated percentage of motility or the original concentration. The correlation coefficients were 0.04 and 0.18, respectively.

DISCUSSION AND CONCLUSIONS

In comparing the two methods of differentiating between ejaculates on the basis of speed of spermatozoan travel *in vitro*, the correlation coefficient of 0.05 was not significant. In Table 3 it is seen that the upper half of the mean speeds were all grouped in only the top estimated rating assigned. Also there was as great a range in microns per second within the 2.0-through-3.5 assigned ratings as between them. There is an indication that the number of spermatozoa present may tend to influence the estimated ratings as the correlation coefficient between estimated rating and original sperm concentration was 0.18 as compared to 0.04 between the clocking speed and original concentration.

TABLE 3. Comparative Rates of Sperm Travel

Number of ejaculates	*Estimated rating*	*Clocked speed in μ per second*		
		Mean[a]	*Range*[b]	*Fastest sperm*
11	4.0	150.7	98.5–226.4	352.2
4	3.5	90.6	76.0–104.4	187.6
7	3.0	85.0	62.9–108.1	150.8
1	2.0	96.6	..	111.8
1	1.5	32.2	..	119.7

[a] Mean speeds of all 40 spermatozoa in each ejaculate.
[b] The range in mean speeds of all 40 spermatozoa in each ejaculate.

The data show a wide variation in speeds between spermatozoa within the same ejaculate. Since these great differences exist, it may be of value to determine the individual sperm speeds in physiologic or metabolic studies of semen. Also since the mean speeds for the ejaculates were significantly different, it appears feasible to differentiate between ejaculates or experimental samples of semen on a basis of sperm speed.

Clocking Speed Method

The clocking speed method of determining sperm travel has its limitations. The major limitation is the human bias present in the selection of spermatozoa to be measured, reaction times in starting and stopping the watch, and in maneuvering the micrometer eyepiece. Also the spermatozoa may not travel continuously in one plane; thus the speeds might be variable even for an individual spermatozoon. However, in all probability, some of these limitations can be minimized by refinement in technic.

On the other hand, the clocking speed method does have some distinct advantageous features. It is simple, quick, and inexpensive. Speeds of individual spermatozoa are easily determinable as well as a mean speed with a standard deviation for an ejaculate. The clocking speed method can be used on a wide range of sample conditions, being limited only to the extent of being able to distinguish individual spermatozoon action. The results obtained are in terms of an objective measure of units of distance during a period of time. The results are not as dependent upon the skill and judgment of an investigator as in estimation methods. In view of these facts the results reported by different workers should prove to be on a much more comparable basis. Thus, the clocking speed method of determining spermatozoon speeds *in vitro* may be a useful tool especially in semen investigations in the laboratory.

SUMMARY

With the aid of a micrometer eyepiece and a stopwatch, a quick measurement of the speeds of spermatozoon travel was made. Groups of 10 spermatozoa were measured under $\times$ 100 and $\times$ 200 magnification during consecutive 45-second periods for each of 24 ejaculates. The mean speed of the 960 spermatozoa observed was 114μ per second, with a range from 10 to 352μ per second. The mean spermatozoan speeds for ejaculates varied from 32 to 226μ per second.

A statistical analysis revealed that the differences between the speeds of spermatozoa from different ejaculates were significantly greater (0.01 level) than the differences between the speeds of sperm cells from the same ejaculate. There was as much variation between ejaculates from the same bull as between ejaculates from different bulls. There was a significant (0.01 level) difference in speeds between those spermatozoa observed at $\times$ 100 and

× 200 magnification. The mean speed declined significantly (0.01 level) with an increase in the length of time between preparation of a slide and observation.

There was no significant correlation between the measured speed and an estimated rate of travel, the estimated "per cent motile" of spermatozoa, or the concentration of spermatozoa in the ejaculate. The clocking speed method to determine either a mean or individual spermatozoan speed may be a more useful tool in semen studies than methods using visual estimation only.

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Clinical Significance of Pregnanediol Excretion

Joseph Rogers, M.D.

PREGNANEDIOL is the urinary excretion product of the corpus luteum hormone, progesterone. The development of relatively simple methods for the measurement of pregnanediol has led to numerous studies of this steroid in relation to the metabolism of progesterone. It is the purpose of this paper briefly to review current concepts of the significance of pregnanediol excretion.

HISTORICAL BACKGROUND

Marrian in 1929 isolated an inactive steroid alcohol from the urine of pregnant women and determined its molecular formula. This compound was named pregnanediol by Butenandt. Subsequent to the isolation and purification of progesterone in 1934 it was postulated on the basis of the similarity of the chemical structures of the two compounds that pregnanediol might be a metabolic product of progesterone.

In 1936 Venning and Browne[37] isolated pregnanediol from pregnancy urine as a water-soluble complex salt, sodium pregnanediol glucuronidate, and in 1937 Venning and co-workers[41] isolated the compound during the luteal phase of the menstrual cycle and reported recovery of pregnanediol in the urine after intramuscular injection of progesterone. These observations served to establish pregnanediol as the urinary metabolite of progesterone. Subsequently, Venning[34] published a method for the determination

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of sodium pregnanediol glucuronidate and there developed a large volume of literature on the excretion of pregnanediol, using this method of determination.

MEASUREMENT

In 1941 Astwood and Jones introduced a method by which pregnanediol is recovered in the free state rather than as the complex sodium pregnanediol glucuronidate (NaPG). In the last few years this method or varieties of it for measuring free pregnanediol have proved increasingly popular.[7, 10, 14, 18, 21, 30, 33] The method in use in this laboratory is a modification developed by Astwood, Grady, and Wermer of the method reported earlier by Astwood and Jones.

Procedure

A 24-hour urine is obtained. If the volume is more than 1500 cc. an aliquot is used. The volume of urine is measured and poured into a round-bottom flask, 100 cc. of toluene is added, and then 1 cc. of 18N sulfuric acid for each 10 cc. of urine. The flask is attached to a reflux condenser and the contents boiled for 15 minutes. The flame is removed and the flask allowed to cool with gentle shaking until boiling ceases. The ingredients are poured into a separating funnel, shaken, and the toluene extract allowed to separate before discarding the urine. The toluene extract is washed twice with 2N sodium hydroxide and twice with hot distilled water (50 cc. each time). The extract is poured into an Erlenmeyer flask and excess water is boiled off on a hot plate. A column is made using aluminum oxide (6½ Gm. water/100 Gm.) about ¾ inch deep in a 2-cm. diameter sintered glass funnel. Twenty cc. of toluene is added, the aluminum is suspended by vigorous mixing with a spatula and then allowed to settle. The aluminum oxide is covered with a layer of sand, care being taken not to disturb the column. The toluene extract is drawn through the column by gentle suction, and the column is then washed with 10 cc. of toluene. Blue and green fractions will come through with the toluene. The column is washed with 0.5% absolute alcohol in toluene until all the pink color is washed through. These fractions are discarded. Three per cent absolute alcohol in toluene is then used to remove the next fraction containing the pregnanediol, the color of which ranges from yellow to brown.

The column is washed with approximately 100 cc. alcohol to be sure that all the pregnanediol is removed. (A slight brown residue remains in the column but does not contain pregnanediol.) The extract is evaporated to dryness and transferred by means of a medicine dropper to a test tube with 95% alcohol. This is evaporated to a measured volume of 1–4 cc., depending upon the amount of pregnanediol expected, and exactly 3 volumes of water are added slowly while heating. This solution is boiled gently and allowed to cool slowly. When dealing with small quantities of pregnanediol, as in menstrual cycles, the solution is allowed to stand at room temperature for 24 hours in order to obtain maximum precipitation. The precipitate is collected on a sintered glass funnel with gentle suction. If the precipitate is not white, it is redissolved in 95% alcohol and reprecipitated and refiltered. When pure, the precipitate is dissolved in alcohol, transferred to a small previously weighed bottle placed in the oven to dry, and then weighed.

PREGNANEDIOL EXCRETION IN THE MENSTRUAL CYCLE

Pregnanediol has been shown to be excreted during the menstrual cycle following ovulation and until 1–3 days prior to the onset of the menses.[5, 9, 20, 32, 38, 43] For a time it was felt that the endometrium was necessary for the reduction of progesterone to pregnanediol. The evidence for this was the inability to isolate NaPG from the urine after the administration of progesterone in a woman who had had a hysterectomy,[39] and the observation by Hamblen, Ashley, and Baptist that curettage during the luteal phase of the menstrual cycle was followed by an abrupt fall in NaPG excretion. However, Buxton and Westphal later recovered pregnanediol from the urine of men receiving progesterone and Venning and Browne,[40] using larger doses of progesterone, succeeded in recovering pregnanediol from the urine of hysterectomized women and concluded that endometrium was not necessary for the conversion of progesterone to pregnanediol.

OVULATION

Using the method described, pregnanediol appears to be excreted within 24 hours after ovulation and continues to be excreted till the onset of menstruation. Figures 1 and 2 show pregnanediol excretion by normal subjects. In our experience[28] pregnanediol excretion correlates poorly with temperature elevation, pregnanediol having appeared in the urine before, during, and after the temperature rise. In clinical usage daily collections of 24-hour urines are impractical so that it is the usual practice to rely on one or two measurements of urinary pregnanediol as an indication of ovulation. For this purpose specimens are best collected on Days 20 to 24 of the usual 28–30 day cycle.

The advantages of pregnanediol excretion as a test of ovulation are its relative convenience, inexpensiveness to the patient, and the elimination of inadvertent interruption by endometrial biopsy of a much wanted pregnancy. Studies of endometrial biopsy material when correlated with pregnanediol excretion indicate a close correlation, so that the finding of pregnanediol in the urine may be taken as evidence of secretory endometrium and active corpus luteum. The studies by Brown and Bradbury in

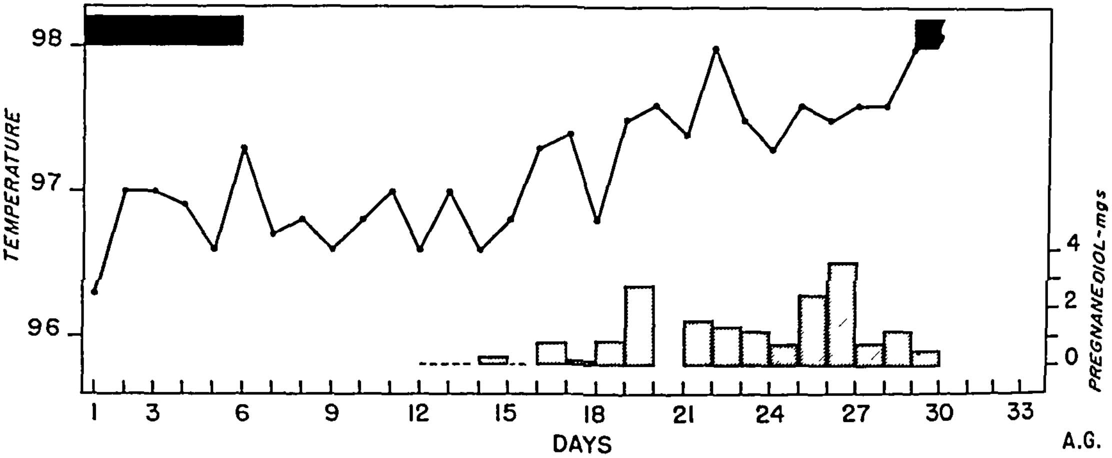

Fig. 1. Pregnanediol excretion in normal menstrual cycle. (Reprinted from Rogers, J., and Sturgis, S. H., *J. Clin. Endocrinol.* *10*:89, 1950, by permission of Charles C Thomas, publisher.)

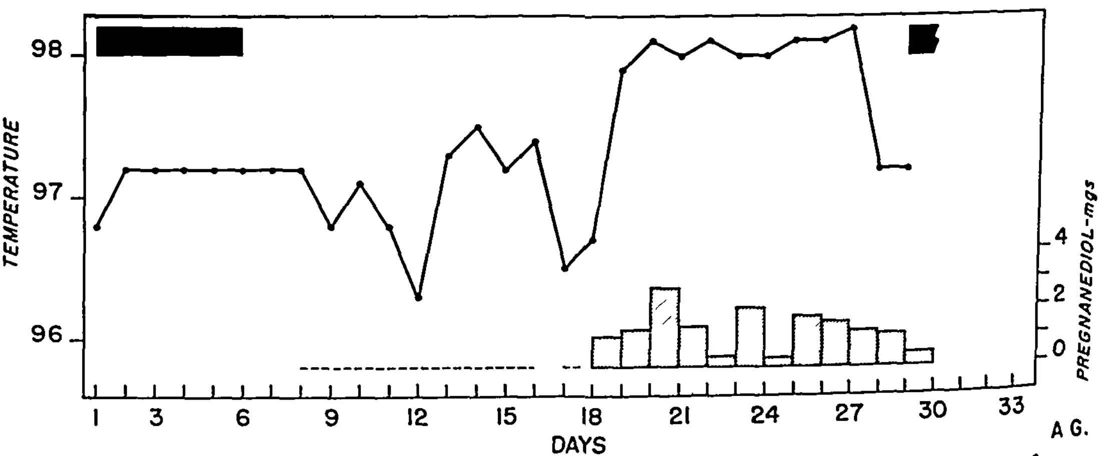

Fig. 2. Pregnanediol recovery in normal menstrual cycle. (Reprinted from Rogers, J., and Sturgis, S. H., *J. Clin. Endocrinol.* *10*:89, 1950, by permission of Charles C Thomas, publisher.)

prolonging corpus luteum activity by the use of chorionic gonadotrophin further indicate the correlation between corpus luteum activity, secretory endometrium, and pregnanediol excretion. Because of the necessity of serial collections of urine, pregnanediol excretion does not lend itself to the clinical study of the exact timing of ovulation except perhaps on a research basis.

The problem of inadequate corpus luteum function has been mentioned by several authors. Whether such an entity actually exists remains unsolved. There are some women (Fig. 3) whose pregnanediol excretion is lower than that normally encountered, suggesting the possibility that the corpus luteum of that particular cycle is deficient in the production of progesterone.

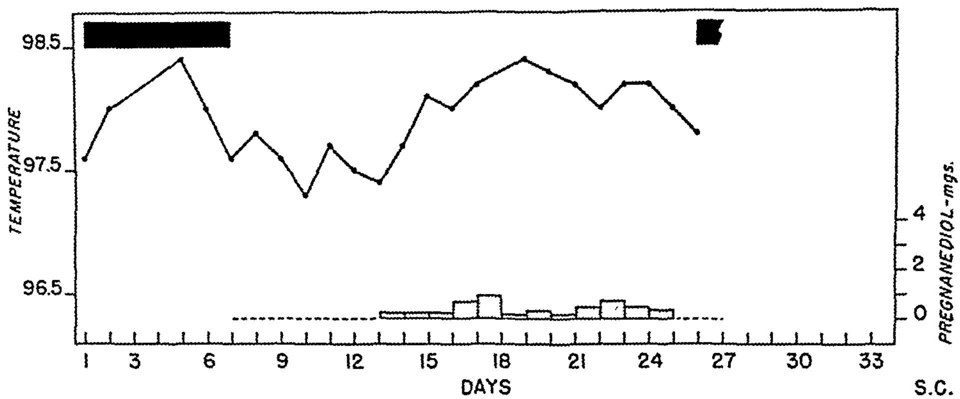

Fig. 3. Diminished pregnanediol excretion. (Reprinted from Rogers, J., and Sturgis, S. H., *J. Clin. Endocrinol.* *10*:89, 1950, by permission of Charles C Thomas, publisher.)

PREGNANEDIOL EXCRETION IN PREGNANCY

The demonstration by Venning[35, 36] of the pattern of excretion of sodium pregnanediol glucuronidate in pregnancy (Fig. 4) led to further observation by other workers and the hope was raised that pregnanediol excretion could be used as a reliable chemical test for pregnancy. However, a major drawback to its use as a pregnancy test is that the amount of pregnanediol recovered during the early weeks of pregnancy may not be sufficiently increased over that found in the luteal phase to be diagnostic.[43] However, good results have been reported in the measurement of pregnanediol excretion as a diagnostic aid in pregnancy.[15–18] In a series of 248 cases Guterman reported 92 per cent accuracy in diagnosing pregnancy.[16]

THREATENED ABORTION

Although the measurement of pregnanediol has not been widely accepted as a pregnancy test, it has proved of considerable value in the prognosis of threatened abortion. Cope[8] was one of the first to point out that a falling level of pregnanediol in the urine might be a reliable indication of intra-uterine death. Hamblen[16] later reported on falling titers prior to abortion, and Guterman[17] was able to prophesy the prognosis of threatened abortion in 93 per cent of 73 cases on the basis of a falling titer of pregnanediol when it should have been rising.

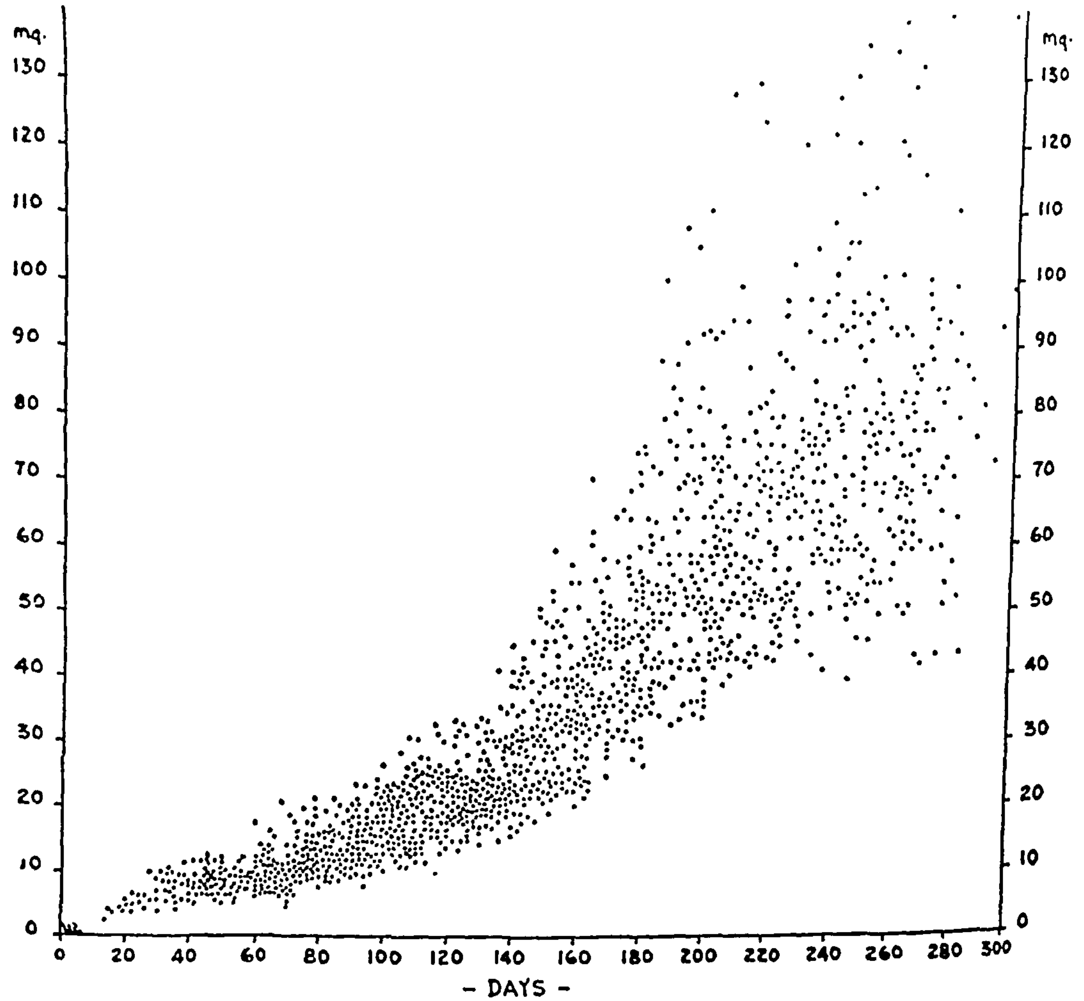

Fig. 4. (Courtesy E. H. Venning.) Excretion of various hormone metabolites in normal pregnancy. From Venning, E. H., *Obst. & Gynec. Survey* 3:661, 1948. (Reprinted courtesy of the author and the Williams & Wilkins Company, publishers.)

An interesting chapter in the development of our concepts of pregnanediol excretion started when the Smiths reported that diethylstilbestrol administered to pregnant women resulted in an increased excretion of pregnanediol as measured by the Venning method, and concluded that the estrogen administered stimulated progesterone secretion. This observation led to the popular use of stilbestrol in threatened abortion, habitual abortion, and pregnant diabetics. Subsequently, Davis and Fugo,[11] using a method measuring pregnanediol in the free state, failed to confirm the observation of the Smiths that stilbestrol increased pregnanediol excretion. They pointed out that in the Venning method all the glucuronides are included in the final determination and suggested that diethylstilbestrol glucuronide as well as pregnanediol glucuronide had been included in the determinations reported by the Smiths. Sommerville and co-workers[30] came to similar conclusions and further demonstrated that in pregnancy the administration of diethylstilbestrol appears to decrease the excretion of pregnanediol. Such observations have led to doubt concerning the value of stilbestrol during pregnancy.[12]

Measurement of pregnanediol excretion has been reported to be helpful in conditions associated with high gonadotrophic excretion other than pregnancy, such as moles and chorionepitheliomas, where pregnanediol may not be found in the urine, therefore serving to differentiate these conditions from pregnancy.[15] However, de Watteville reported 3 cases of hydatid mole in which pregnanediol was present, rendering this differential point less secure.

METABOLISM AND THEORETIC CONSIDERATIONS

The liver appears to be the site of conversion of progesterone to pregnanediol. This has been indicated by experiments in which progesterone pellets implanted into the spleen with rapid absorption through the liver were less effective than similar pellets placed in subcutaneous tissue. Masson and Hoffman demonstrated in rabbits that administration by gavage required 200 mg. of progesterone to obtain a progestational effect comparable to that elicited by 0.25 to 0.5 mg. administered subcutaneously. In the rabbit who had had a partial hepatectomy, however, comparable progestational changes were obtained by the administration of 25 mg. of progesterone by gavage.

Pearlman and Pincus administered massive oral doses of Δ^5-pregnen-3β ol-20-one to a postmenopausal woman and recovered minute amounts of pregnane-3(α),20(α)-diol, from the bile. Pearlman and Cerceo isolated pregnan-3(α)-ol-20-one and pregnane-3(α), 20(β)-diol from the gallbladder bile of pregnant cows. It has also been shown that after the oral administration of progesterone small amounts of pregnanediol could be isolated from the bile of human subjects in whom T-tube biliary drainage was established for the relief of common duct obstruction.[27] Such observations serve again to implicate the liver as the site of conversion of progesterone to pregnanediol.

Measurements of pregnanediol excretion after a large oral dose of progesterone have been made in patients with various forms of parenchymal liver disease and in extrahepatic biliary obstruction.[26] The results are difficult to interpret. In some patients with hepatic disease pregnanediol excretion is markedly diminished, in a few it is increased, and in others it is normal. The results suggest that in some individuals with hepatic disease the metabolism of pregnanediol itself is interfered with but that in others the metabolic difficulty is in the conversion of progesterone to pregnanediol.

In patients with obstructive disorders of the biliary tract significant increases in urinary pregnanediol may occur, suggesting that in biliary obstruction and interference with this pathway of excretion more pregnanediol is excreted correspondingly in the urine.

SUMMARY AND CONCLUSIONS

1. Pregnanediol, the urinary metabolite of progesterone, can be measured by relatively simple methods.
2. The finding of pregnanediol in the urine of women in the luteal phase can be accepted as evidence of ovulation.
3. The measurement of pregnanediol as a pregnancy test is of value in some instances, but is helpful chiefly in aiding in the prognosis of threatened abortion.
4. Some aspects of the relation of progesterone metabolism to the liver have been briefly discussed.

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Placental Insufficiency ✓

Cervical Mucus Arborization as Aid in Diagnosis

Bernhard Zondek, M.D., Isador Forman, M.D.,
and Kenneth L. Cooper, M.D.

Many tests have been devised to determine the adequacy of ovarian function during the menstrual cycle. Those commonly employed are basal body temperature curves, vaginal smears, endometrial biopsy, and bioassay of hormone excretion in the urine. Several authors[2, 3; 11-14] have reported on the cyclic change in cervical mucus as a simple test for estrogen and progesterone activity. The principle of this test is based on observations by Papanicolaou that when cervical mucus obtained during the estrogen phase is allowed to dry, it "crystallizes" and forms a typical arborization pattern. Under the microscope this smear shows a fascinating picture of flowers and leaves resembling fern or palm leaves. This phenomenon has been described as fern- or palmlike (PL) formation. The mechanism of aborization has been investigated by Zondek.[13]

Papanicolaou suggested that the pattern of crystallization might depend on the activity of the estrogenic hormone. Dried smears of mucus obtained during the post- and premenstrual phases show abundant vaginal and cervical epithelial cells, leukocytes, and mucus in patches or threads (Fig. 1). From Day 8 to Day 22 of the cycle a striking arborization pattern de-

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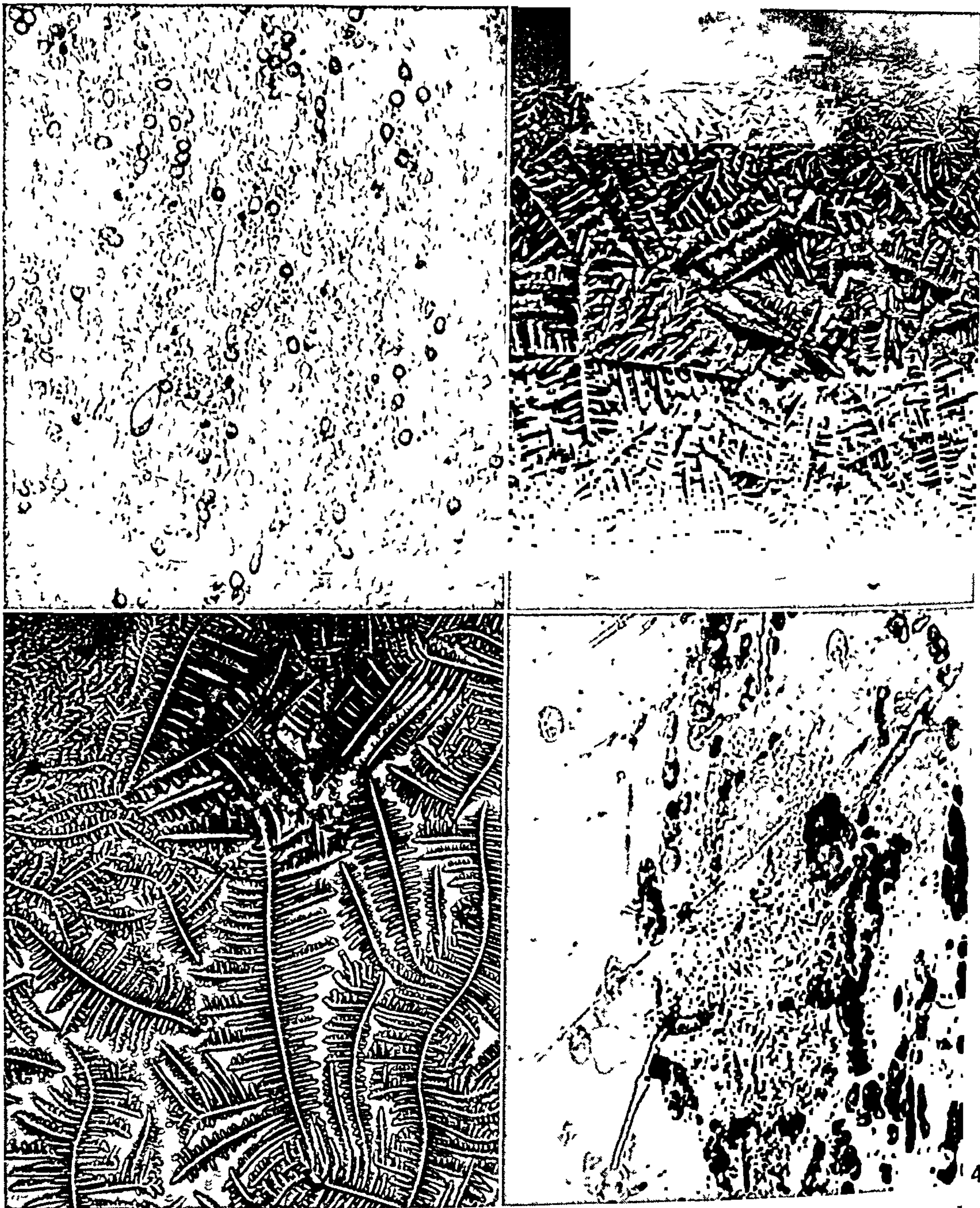

1 2 3 4

Fig. 1. Negative arborization reaction. Cellular pattern: leukocytes, vaginal and cervical epithelial cells, and mucin threads. **Figs. 2 and 3.** Arborization, strongly positive reaction (+++); 2, thick smear; 3, thin smear. **Fig. 4.** Arborization, slightly positive reaction (+).

velops: Under high magnification, main stems are seen among the leaves, with side branches showing toothlike projections. Only a few cellular elements are found. Occasionally, the cells may line up in rows and may resemble the fern formation but, if examined under high magnification, the side branching and toothlike formations are absent. This was called "pseudo-arborization."[13, 14]

The PL reaction may appear with variable intensity. If most of the dried mucus is in a state of arborization, the reaction is designated strongly positive (3 plus) (Figs. 2 and 3); if arborization is present in several places, it is positive (2 plus); and, if present only in isolated areas on the slide, it is graded slightly positive (1 plus) (Fig. 4). Campos da Paz[4] found that PL formation was most abundant at the peak of estrogen activity and has used this fact as a guide in the study and treatment of the cervical factor in infertility.

ARBORIZATION IN PREGNANCY

In the premenstrual phase and in early pregnancy there is a marked increase in the number of cervical glands, with hyperplasia of the glandular epithelium.[5, 7] These glands produce considerable quantities of mucus but this secretion, when dried, does not show the "crystallization" phenomenon. Also, estrogen levels are fairly high in these situations and yet the PL reaction is inhibited. One can assume, therefore, that arborization is possible only when the cervical mucus is under the influence of estrogen which is unopposed by progesterone activity.*

Effect of Progesterone

The arborization inhibiting effect of progesterone can be overcome during the menstrual phase by the administration of estrogens (10 mg. estrone) but this cannot be accomplished during pregnancy.[13] We administered 10–20 mg. estradiol benzoate to patients in early pregnancy without any effect. A group of women in the early weeks of pregnancy were given 10 mg. estrone by injection and in addition 15–30 mg. stilbesterol orally (1 patient received 90 mg. estrone by injection and 270 mg. stilbestrol over a period of 9 days), without change in the cervical mucus (Pl remained negative). In patients receiving very large doses of stilbestrol during the sixteenth–

*The ratio of progesterone to estrogenic hormone was ascertained to be 15:1, that is, 15 mgm. of progesterone will inhibit the action of 1 mgm. of estrone in the production of cervical mucus arborization.[13]

thirty-sixth weeks of pregnancy (100–150 mg. daily to 13 Gm. in several months) the cervical mucus remained refractory throughout.

Is the refractoriness of the cervical mucus in pregnancy due to the inhibitory action of progesterone? Our clinical experience would seem to indicate that this is not the case. During the first days and weeks of pregnancy the amount of progesterone produced in the body is not appreciably greater than in the premenstruum, judging from pregnandiol excretion in

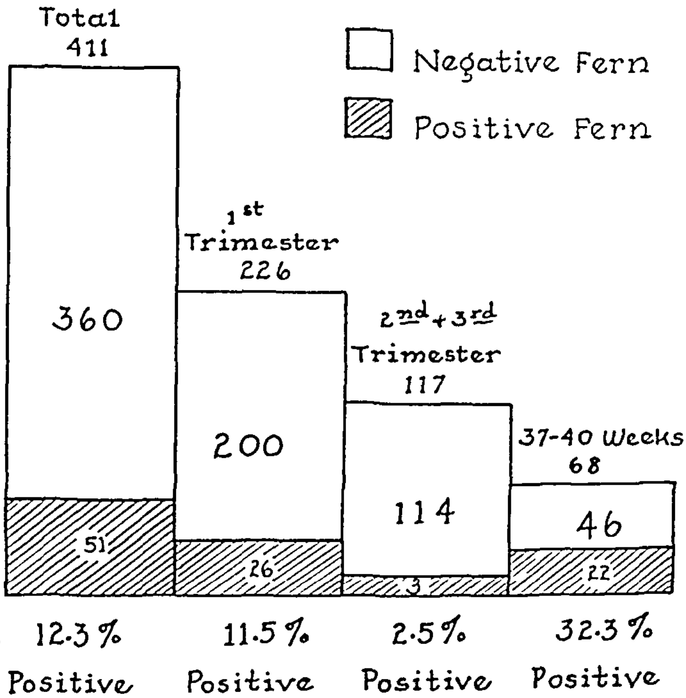

Fig. 5. Arborization of cervical mucus in 411 pregnancies.

the urine. But even if the progesterone production is much greater in early pregnancy than heretofore supposed, very large amounts of estrogens should overcome the inhibitory action of progesterone. Since we were unable to accomplish this, we may assume that a special mechanism exists in pregnancy which inhibits the excretion of mucus containing electrolytes essential for the aborization mechanism.[13]

INDICATION OF ABNORMAL PLACENTATION

Study

During the past year, we have studied the cervical mucus of a group of 411 pregnant women. In this series, 226 women were in the first trimester and 185 were in the second and third trimester. Of this latter group, 68 were studied at weekly intervals during the last 4 weeks of pregnancy. Of the 226 women in the first trimester of pregnancy, 26, or 11.5 per cent, showed a 1 plus reaction (Fig. 5). In the group studied in the second and third trimesters (between fourteen and thirty-six weeks), only 2.5 per cent showed positive PL reaction; of the 68 patients in the last month of pregnancy, 22, or 32.3 per cent, showed a positive reaction (1 plus).

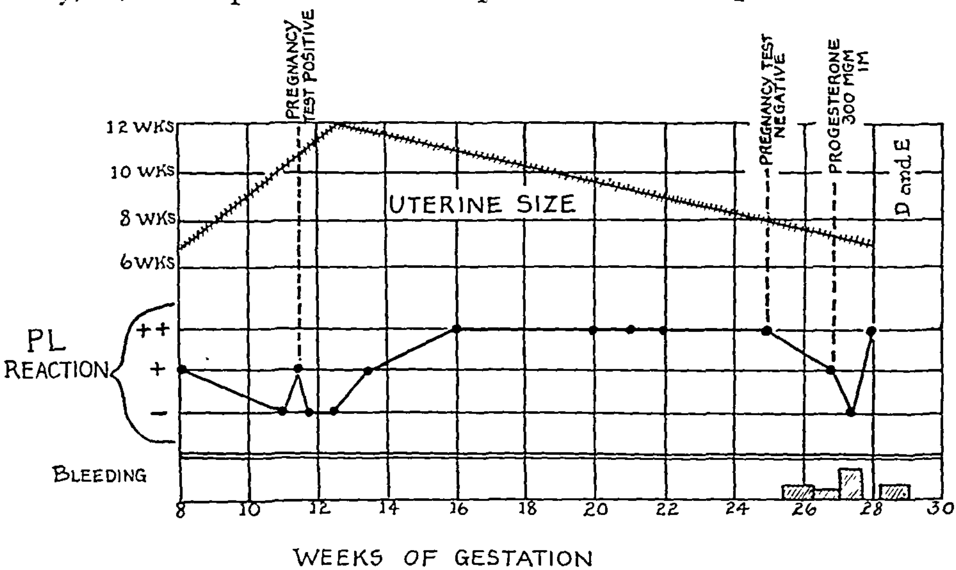

Fig. 6. Patient V. T., age 20. Missed abortion.

Roland, in suggesting the cellular pattern without aborization in the cervical mucus as a test for pregnancy, gave no statistical data, but implied that the test had a high degree of accuracy in early pregnancy. Therefore, early in our study, we were somewhat disappointed by our relatively high percentage of false-positive reactions. However, as the study progressed, we found that a number of these patients had abnormal placentation which eventuated either in tubal pregnancies, abortions, or intrauterine fetal death (missed abortion) (Fig. 6).

Results

Of the 226 patients studied during the first trimester, 200 showed negative reactions and 26 slightly positive to positive reactions (Table 1). In this latter group, 15 pregnancies terminated unfavorably. Two were tubal

TABLE 1. Outcome of Patients with Positive PL Reaction in Pregnancy

Name	Para	Gravida	Weeks gestation 6–8	10	12	14	16	18–20	22–28	32–36	Clinical diagnosis
V. T.[a]	1	3	+	−	+	++	++	++	++	..	Missed abortion
L. A.	3	4	++	++	++	..	..	..	..	..	Failed to return
S. S.[b]	0	1	−	+	..	..	..	..	..	..	Tubal pregnancy
D. J.	1	3	..	−	+	+	..	..	..	..	Abortion
D. W.	1	2	++	++	++	+	++	−	+	..	Still pregnant
G. E.	2	3	..	..	++	++	..	..	..	..	Abortion
V. S.	1	2	..	..	+	..	..	..	..	..	Abortion
F. G.	5	6	++	..	..	..	..	..	..	..	Tubal pregnancy
C. W.	1	2	..	..	+	..	..	..	..	..	Abortion
E. S.[c]	4	5	++	+	..	..	..	..	..	..	Abortion
L. S.	2	4	+	+	−	−	−	..	..	..	Still pregnant
M. B.	2	4	++	++	..	..	..	..	..	..	Failed to return
J. N.	0	1	..	..	+	+	+	..	..	..	Failed to return
R. L.	2	3	..	..	..	+	+	+	−	..	Disturbed pregnancy
L. B.	1	4	+	+	−	−	..	..	..	..	Disturbed pregnancy
C. W.	1	2	..	..	..	..	+	+	++	..	Abortion
A. H.	0	2	++	++	++	++	..	..	..	..	Abortion
E. G.	1	2	+	+	+	..	..	..	..	..	Abortion
J. W.	3	4	..	+	..	..	..	..	..	..	Abortion
F. C.	0	1	++	+	−	−	−	..	..	..	Still pregnant
J. V.	0	1	..	+	++	..	..	..	..	..	Abortion
C. H.	0	1	++	++	..	..	..	..	..	..	Abortion
M. M.	3	5	..	−	−	++	..	..	..	..	Still pregnant
V. W.	1	3	..	++	+	..	..	..	..	..	Still pregnant
E. S.	1	2	..	++	..	..	..	..	..	..	Abortion
								..	..	..	
A. H.	1	2	..	++	+	+	+	+	−+	..	Still pregnant

[a] See Fig. 6.
[b] See Table 2.
[c] See Table 3.

pregnancies (Table 2) and 13 ended in abortion (Table 3). Three patients did not return to the clinic and 8 are still pregnant. Of these 8, 2 have had intermittent vaginal bleeding and have been classed as "disturbed pregnancies." One patient (Table 4), who is at present in her twentieth week,

TABLE 2. Ectopic Pregnancy (Mrs. S. S., Age 35)

Date	*Period of gestation*	*Bleeding*	*Cervical mucus*	*Diagnosis*
11/11/53	8 weeks	Spotting Sept. 29 Nov. 4	Negative	Intrauterine preg.
11/16/53	8 weeks	Spotting Nov. 14	+	Disturbed preg.
11/18/53	9 weeks	..	+	Disturbed preg.
11/21/53	9 weeks	Bleeding	..	Inevitable abortion versus ectopic pregnancy
11/22/53	..	..	..	Left tubal pregnancy (colpoceliotomy; laparotomy)

TABLE 3. Abortion (Mrs. E. S., Age 33)

Date	*Period of gestation*	*Bleeding*	*Cervical mucus*	*Diagnosis*
1/22/54	? Preg.	None	++	Cervical polyp Questionable preg.
2/ 5/54	5 weeks	Staining, Feb. 3	+	Threatened abortion
2/12/54	6 weeks	Staining, Feb. 6, Feb. 7	+	Threatened abortion
2/21/54	8 weeks	Painful bleeding	..	Aborted D and E

TABLE 4. Disturbed Pregnancy (Mrs. L. S., Age 29)

Date	*Period of gestation*	*Bleeding*	*Cervical mucus*	*Diagnosis*
2/ 9/54	6	None	+	Disturbed pregnancy
2/23/54	9	None	+	Disturbed pregnancy
3/10/54	11	None	+	Disturbed pregnancy
4/ 2/54	14	None	–	..
4/ 9/54	15	None	–	..
5/11/54	19	None	–	Still Pregnant

has had 3 previous pregnancies. The first was normal and full term, the second ended in an intrauterine fetal death at 35 weeks, and the third aborted at 6 weeks. In this pregnancy, the PL reaction, which was positive (1 plus) at 6, 9, and 11 weeks, became negative at 14 weeks and has remained negative to date (nineteenth week).

DISCUSSION

Functional Placental Insufficiency

On the basis of these observations, we are inclined to believe that a slightly positive PL reaction during pregnancy may be due to a functional insufficiency of the placenta. It is not at all clear at the present time why the cervical mucus changes its physical properties and the cervical cells allow the permeation of electrolytes as soon as the placental function is disturbed. Further studies are necessary to clarify this mechanism. In 13 of our patients with positive PL reaction the pregnancy terminated in abortion, suggesting that the placental insufficiency was irreversible in these cases. However, a positive reaction does not prove that the damage to the placenta is irreversible since the placental disturbance may subside, leading to normal placental function and continuation of the pregnancy. In any case, a positive PL reaction in early pregnancy probably indicates a disturbed function of the placenta.

Prodromes of Abortion

In recent years, many investigators have studied this problem. Farris observed that a "weak" reaction in the rat hyperemia test for pregnancy was an aid in predicting abortion. In 1950, Randall *et al.* reported on a study of cervical cytologic smears in 340 women during early pregnancy. In the same year, Benson and Traut employed a vaginal smear method and concluded that a "trend toward abortion can be determined by vaginal smears"; and in 1953 Koff and Tulsky studied pregnandiol levels of 335 patients with threatened abortion and found that 97 per cent of them aborted when hormone levels were low (less than 5 mg./24 hr.).

Undoubtedly all of these methods are of value in the management of the abortion problem. However, they are all either technically difficult to perform or to interpret and, therefore, unavailable to most physicians. The examination of dried cervical mucus, on the other hand, is a very simple procedure. The smear can be read within 10 to 20 minutes and the findings are so clear-cut and simple to interpret that the test can be made a routine procedure in office and clinic.

CONCLUSIONS

Between Day 7 and Day 22 dried cervical mucus shows arborization (PL positive). In the premenstruum arborization fails to appear, the mucus containing a cellular pattern only (PL negative). The same picture is seen during pregnancy until the last month.

A slightly positive PL test was found in 26 out of 226 women in the first trimester of pregnancy. Fifteen of these 26 pregnancies proved to be disturbed pregnancies terminating in uterine or tubal abortion.

The change in the cervical mucus during pregnancy, manifesting itself in arborization, is probably caused by placental insufficiency, the nature of which is to be investigated by further studies. The functional insufficiency may be irreversible, leading to abortion, or reversible, restoring the full function of the placenta. The exact mechanism of arborization (PL positive) during pregnancy is not clearly understood. A change in the estrogen-progesterone balance would reasonably explain this phenomenon. However, our failure to produce the PL phenomenon in early pregnancy with massive doses of estrogens leads us to question the validity of this hypothesis. From the clinical point of view, it would seem that arborization of the cervical mucus early in pregnancy may serve as a prognostic sign of impending abortion.

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DISCUSSION

Dr. Edward T. Tyler, *Los Angeles, Calif.:* I want to congratulate Doctor Forman on his very fine presentation. A new simple method for obtaining prognostic data in instances of threatened abortion should provide considerable help in a field where information of this type is so important. If continued investigation confirms these original observations physicians will be able to take advantage of a very simple laboratory procedure for following the course of certain pregnancies.

We routinely examine cervical smears in infertility patients and can correlate in a general way the variations in cervical crystallization with various stages of the menstrual cycle. But, unfortunately there are many instances where one finds crystallization when it should not occur and conversely it fails to appear when it should logically be present. These instances occur when hormonal studies, biopsies, etc., fail to indicate an abnormal cycle, and it would therefore be reasonable to conclude that the crystallization test is not as reliable as we would like, at least in evaluating nonpregnant cycles. It should also be noted that anyone experienced in examining cervical mucus can predict fairly readily whether it will crystallize, by observing the characteristics grossly. One suggestion which may prove helpful to those aspirating cervical mucus is the use of the long 3.0 cc. syringe, without the Luer-lock. I have found that aspirating secretions with this type of syringe provides, in general, a more practical way of obtaining cervical mucus, and gives more suction than one would be able to to obtain with the long 1 cc. syringe.

DR. SOMERS STURGIS, *Boston, Mass.:* I would like to ask Dr. Forman a question. In the normal menstrual cycle, if by Day 22 or 24 you have a negative test, does that necessarily mean ovulation or could it mean low estrogen or deficient ovarian function instead?

The discussion is now open to the floor.

DR. LYMAN W. MASON, *Denver, Colorado:* I should like to ask Dr. Forman a question. I thought I was listening carefully, but I may have lapsed several times, and he may have said so, but I should like to ask if he knows or if any work has been done to determine just what is the chemical substance which crystallizes to form this pattern.

DR. ROBERT B. GREENBLATT, *Augusta, Ga.:* I have been very much interested in the relationship of progesterone to the inhibition of this fern pattern. It takes a lot more than 7 or 8 days to inhibit that pattern with large doses of progesterone. It can be modified considerably in patients who have a 3 plus reaction after considerable dosage with progesterone. The fern pattern may not disappear within 10 days. Maybe reduced to 1 plus reaction. I wonder whether you have made similar observations.

DR. R. NOYES, *Stanford University Hospital, San Francisco, Calif.:* Do you happen to have the figure on how many patients who had a negative fern test aborted or had a disturbed pregnancy?

DR. ABRAHAM STONE, *New York, N. Y.:* May I ask Dr. Forman whether he has correlated the fern test with temperature chart to see whether there is a drop in temperature at the time when the fern appears?

DR. FORMAN: I would like to thank Dr. Tyler for his most interesting discussion of our paper. In answer to Dr. Sturgis' question as to whether a negative reaction on Days 22–24 necessarily indicates ovulation, I would say that it does not, unless one has checked the cervical mucus earlier in the cycle. However, in the regularly menstruating women, one may assume that there has been estrogen priming. Therefore, one may use this test as a fairly accurate index of ovulation—especially if samples of mucus are examined on, say, Day 10 or 15 and again on Day 22 or 24.

We have correlated the basal body temperature curve with examination of cervical mucus and have found considerable agreement in our results. We feel that the presence of arborization phenomena in the follicular phase and its disappearance in the luteal phase is, if anything, a more accurate indication of ovulation than the basal body temperature curve. We have not used this test to time ovulation, but I am sure that this can be done.

Dr. Mason asked if any work has been done to determine just what is the chemical substance which crystallizes to form this pattern and I would say that Dr. Zondek and his co-workers have done a great deal of work along this line. I believe that it is their feeling that the sodium chloride in the mucus is responsible for this phenomenon. Other body fluids exhibit this tendency, but only in cervical mucus is it dependent on the action of estrogen, unopposed by progesterone.

Dr. Greenblatt raised the question of inhibiting the fern phenomenon by the administration of progesterone. He is perfectly right—it does take considerable quantities of progesterone to achieve this. Dr. Zondek has established that 15 mg. of progesterone is necessary to overcome the effect of 1 mg. of estrone. This ratio is based on experimental work which he carried out on postmenopausal patients. Dr. Cooper and I carried out similar experiments on younger women in whom supracervical hysterectomy with bilateral salpingo-oophorectomies had been performed and found the ratio to be more nearly 10:1.

As to the question how many of our patients with negative ferns aborted, I must confess that we did not follow that group closely enough to answer this. As far as we could determine, only 4 of the 200 patients aborted, but there may have been more. We were particularly interested in the positive group and they were followed more closely. However, we intend to continue this study to determine the outcome in both groups of patients.

I might say that this is a preliminary report and, of necessity, no definite conclusion can be drawn, but we believe the evidence is intriguing enough to warrant further investigation.

Pelvic Tuberculosis

Its Relationship to Sterility, Present and Future

Donald W. deCarle, M.D.

Of all the disease entities that affect the organs of the pelvis, especially those of reproduction, tuberculosis is probably the most serious. For a clear understanding of the depredations of this disease and its profound effect on fertility, it is necessary only to review any one of the standard textbooks on gynecology. In discussing its pathology in his most recent edition of *Operative Gynecology,* Te Linde gives a detailed description of the terrific havoc caused by pelvic tuberculosis.

It is the intent of this report to consider the effects of this disease upon fertility and pregnancy up to the present time. The results of various forms of treatment during this time will be evaluated, along with those of 4 patients of the author. With this as a basis, an attempt will then be made to prognosticate the most advantageous methods of the future handling of patients with pelvic tuberculosis, in the future, especially in its relation to fertility.

GYNECOLOGIC EFFECTS

It is indeed unfortunate that the very process which activates healing in tuberculosis has certain untoward sequelae. In tuberculosis of the pelvis, these processes have a very detrimental effect upon future pregnancies. Healing of the tubes in this disease is through a process of fibrous infiltration into the walls of these structures, according to Te Linde and others. This primarily interferes with a normal function of these organs—tubal peristalsis. It further results in multiple strictures, both inside and outside the tubes. These, in turn, may cause either distention and hydrosalpinx or

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further shrinkage with distortion and obliteration of the lumen. It should also be noted that this same healing process can seriously involve the ovary. Thus, there may be enough disruption of normal ovarian function to prevent satisfactory ovulation. As a result, up to the present time, subsequent pregnancies are comparatively rare in the presence of pelvic tuberculosis.

PREGNANCY

In his discussion of the association of pregnancy with this disease, Donaldson has found that the incidence is difficult to assess with any degree of accuracy. He believes that the literature is misleading. This, according to him, leaves the impression that pelvic tuberculosis is more common than is usually assumed. Further, it has been his experience that inability on the part of the physician to recognize unsuspected pelvic tuberculosis in an otherwise healthy woman has been another reason for this difficulty. As a result, he has found that pregnancy has rarely been reported in the presence of a proved case of this disease. In his experience, this is true of ectopic as well as intrauterine gestation. Of the former, he cites but 9 such cases from the literature.

Bland, however, in reporting a case of ectopic pregnancy in which pelvic tuberculosis was found at surgery, included 32 other such cases collected from the literature up to 1940. Since then, as reported by Donaldson, cases of a similar nature have been described by Hicks; Shannon and Heller; Mann and Meranze; and by Geisendorf in 1951. Pink, in 1944, commented in detail on the case of a patient in whom 2 such pregnancies were found in the presence of associated genital tuberculosis within a period of 2 years.[3]

From a further review of the literature, the occurrence of a normal intrauterine pregnancy would seem equally rare in the presence of pelvic tuberculosis. The fact, however, that it does occur was discussed and actually shown at autopsy by Cooper; Vineburg; and Thom, Schmorl, and Kockel during the last century and more recently by Wiseman and Retan, and Puxedder.[3]

As opposed to this group, however, case histories of patients in whom intrauterine pregnancies occurred following conservative surgery in the presence of pelvic tuberculosis have appeared from time to time in the literature. Fruhinsholz and Feuillade,[3] as early as 1924, described one of the first of such patients. This was followed by similar case reports by other authors such as Roulland[3] and Donaldson.

THERAPY

However, in spite of the marked advances made by this form of treatment, pelvic surgery in the presence of tuberculosis of this area has in the past carried a 20 per cent mortality, according to Brown, Gilbert and Te Linde. It was not until the introduction of streptomycin that our modern treatment of tuberculosis in general and pelvic tuberculosis in particular began. Here, for the first time, medical treatment with a specific agent aimed at the acid-fast tubercle bacilli was introduced. Although bed rest and sanitarium care still form the bases of treatment for this disease, nevertheless the introduction of this antibiotic markedly changed our attitude toward tuberculosis in general. As an example, interruption of a pregnancy associated with this disease, even though it happens to be an active pulmonary lesion, is no longer considered necessary to save a patient's life. Along with para-amino salicylic acid, which delays the development of resistance to streptomycin on the part of the tubercle bacillus,[4] this antibiotic is widely used in the treatment of pelvic tuberculosis. Although this combination of drugs has not replaced surgery in the treatment of pelvic involvement, it has made such treatment safer and far more effective. It has markedly lowered the mortality of pelvic surgery in its presence. Sired, Falls, and Zummo used these drugs preoperatively and in the postoperative period in some 16 such patients with the loss of only 1. Brown, Gilbert, and Te Linde had similarly good results. Greenhill quotes Ryden, who found 9 out of 13 patients cured at surgery following the use of these drugs preoperatively.

In 1951, the first reported case of a patient with pelvic tuberculosis treated exclusively with streptomycin and PAS was referred to by Ewart Williams. In the author's opinion, this marks the basis for future treatment of pelvic tuberculosis. Along with some of the newer drugs (such as Rimifon, isonicotinic acid, and isoniazid of the so-called I.N.H. group) which have been found to increase still further the tissue permeability and effectiveness of streptomycin, streptomycin and PAS could conceivably replace surgery to an increasingly marked degree.

PERSONAL EXPERIENCE

All forms of active treatment of patients with tuberculosis notwithstanding, it should never be forgotten that preventive medicine is and will continue to be the chief agent in combating this disease and any other of its kind. Thanks to the various national and local agencies, gynecologists see comparatively few patients with tuberculosis of the pelvic organs. It is for this reason primarily that there is a paucity of material. Conclusions must therefore be made from comparatively small, isolated groups of such case reports.

Since 1950, there have been but 11 patients who have come to my attention in private practice in whom a diagnosis of pelvic tuberculosis was established. Of these, 2 had positive endometrial biopsies, 1 showed pathognomonic tubercles among the curettings and 1 was diagnosed by the process of elimination. In the remaining 7, tubercles of the pelvic organs were found at surgery. Only 4 of these 11 patients sought medical help because of inability to conceive.

The case reports are presented because they are typical examples of the sterility problems associated with pelvic tuberculosis at the present time. Unfortunately, the last case may not be included as that of a patient who was successfully treated medically. Pelvic tuberculosis was never positively established as the underlying cause of her sterility.

Case 1

This patient was treated intermittently for sterility over a period of some 15 years before repeated endometrial biopsies showed tubercles. A previous biopsy taken elsewhere had been diagnosed as a possible corpus carcinoma. This patient was clinically cured following almost 1 year's treatment with bed rest, streptomycin, and PAS. She is now approaching the menopause, and it is therefore doubtful that she will ever conceive.

Case 2

Because of this patient's inability to conceive, a myomectomy was advised and a large intramural fibroma was removed from the anterior uterine wall, at which time tubercles covering the pelvic organs were discovered. Following 9 months of sanitarium treatment along with streptomycin and PAS, the patient was clinically cured. Repeated hysterosalpingography, however, showed both tubes to be dilated, typical of hydrosalpinx resulting from the healing process of this disease. Because of the apparent loss of all tubal peristalsis, surgery seemed inadvisable because of the poor prognosis, although it was considered.

Case 3

The patient was a 21-year-old nullipara who had been previously confined to a sanitarium with pulmonary tuberculosis. Because of lower abdominal pain, irregular menses, and progressive dysmenorrhea, she was seen by a gynecologist.

Pelvic surgery was advised for an adnexal mass. Because of her desire to have children, a second opinion in regard to her pelvic condition was requested, at which time I saw her. In spite of medical treatment and conservative surgery—unilateral salpingo-oophorectomy—this patient has failed to conceive.

Case 4

This patient reported for investigation within a year after marriage because of her inability to become pregnant. Because her pelvis gave the impression of having some inflammatory process present and because she at one time had had a questionable pulmonary infection, a diagnosis of probable pelvic tuberculosis was made by the process of elimination. After a course of streptomycin and PAS, the patient was entirely free from any subjective or local symptoms. This treatment was given in a sanitarium under the supervision of a specialist in the field of tuberculosis. Unfortunately, this patient was compelled shortly thereafter to move elsewhere. A report has been received, but unfortunately not substantiated, that she did eventually conceive but subsequently miscarried.

CONCLUSIONS

If one may be allowed the privilege of prognosticating on the basis of information gleaned from the past, it would seem that some rules in the handling of pelvic tuberculosis could be made. This not only refers to this entity in general but particularly to its relationship to infertility. First, it must be a foregone conclusion that with continued improvement in public health methods in the field of preventive medicine, there will be a diminishing number of patients seen with pelvic tuberculosis, especially in this country.

Secondly, although there are many gynecologists who are not ready to accede to the concept of medical treatment alone in cases of this kind, the trend, in my opinion, will be definitely away from surgery. With the drugs already established, especially streptomycin in its various forms in combination with PAS, the trend should be toward medical treatment exclusively. This belief is founded on the continued introduction of newer drugs. The so-called I.N.H. group, of which isoniazid is an example, have already shown a definite advantage—increased effectiveness of streptomycin. Their one advantage is a greater ability to penetrate the caseous masses, with less distortion in the healing. This is a quality absolutely necessary in tubal and ovarian tuberculosis, if future pregnancies are to occur.

Again, if more pregnancies are to be obtained, some method other than that of endometrial biopsy, dilatation and curettage, and/or the process of elimination must be found for earlier and more accurate diagnosis. We hope that as time goes on this will result in the establishment of treatment sufficiently early to forestall the destructive effects following the healing processes of pelvic tuberculosis.

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Grants-in-Aid

The Ortho Pharmaceutical Corporation is providing two $500.00 grants-in-aid for 1956. Applications for these grants should be sent to Dr. Robert Greenblatt, Chairman of the Research Correlating Committee of the American Society for the Study of Sterility, at the Medical College of Georgia, Augusta, Ga. A brief outline of the research project for which the grant will be used should accompany the application.

Ambivalence and Conception

Alfred Stern, M.D.

THE TREATMENT of sterility has been advanced during the past decades by newly acquired knowledge and skills. However, in spite of systematic evaluation of irregular findings and logical application of therapeutic procedures, too many cases remain still unimproved, including some which, anatomically and biochemically, are least abnormal. Attention is directed toward some seemingly trivial observations that in some instances may well lead to the core of this problem.

VAGINAL DOUCHE

The physician who habitually asks the sterility patient whether she takes vaginal douches will be impressed by the discovery that many of these women apply the douche regularly once or even more frequently during 24 hours. He will be more surprised to learn on further questioning that these douches are taken "naturally—after intercourse." It is evident that such procedure is hardly conducive to the promotion of conception. It should therefore be discouraged.

But there is more to it. The douching habit is more widely spread in our civilization than one would assume. Often it has been taught to the young girl by her mother or by nonprofessional and professional advisers who want to impress her with the virtue of cleanliness. According to my experience, the girls who have been most deeply impressed with the idea of cleanliness become the women who douche most orthodoxly. Compulsive reactions lead them to avoid any "contaminations," or if this is not feasible at least to undo them. Truly, they want a child; but they would like to avoid

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becoming or being pregnant. They believe in the emotional, moral, and social values of marital and family life; but they are in some way incapable of giving up their chastity. They are not fully conscious of their rejection of contact and they do not clearly appreciate the meaning and effect of their douching. Thus a considerable number of women who would like to have a child, but who subconsciously or consciously entertain an emotional barrier against contact, are applying primitive contraceptive methods.

Some patients can easily be made to recognize their ambivalent feelings concerning conception. They will realize and admit them when pointed out. They will make up their minds and either discontinue prevention or stop asking for sterility treatment. Patients whose resentment of conception is more covered up and of a more neurotic nature will have greater difficulties in changing their attitudes—if they ever succeed at all. Some may make the apparent concession of giving up douching altogether or at least douching after intercourse. But they may show other mechanisms of defense.

VAGINAL OVER- AND UNDERSECRETION

Dryness and tightness of the vagina may interfere with intercourse. The incidence and meaning of undersecretion by Bartholin's and cervical glands, with the resulting lack of lubrication of the pathways, need no explanation to the expert therapist.

But the opposite phenomenon of excessive excretion and "reflux" after intercourse deserves some scrutiny. Lack of sphincter activity with the resulting spilling and disposal of the sperm deposit is often the mechanical result of anatomic relaxation of the pelvic floor, correctible by surgical repair or merely by elevating the lower end of the bed. But many women who after numerous childbirths have descending parts and missing sphincter tonus do *not* fail to conceive. "Reflux" of sperm after intercourse is not necessarily the result of anatomic change. It may often be interpreted more correctly as the result of rejection rather than of relaxation, one of the "trivial" means by which the repelling female system rids itself of invasion.

READINESS FOR CONCEPTION

The majority of women who see the physician for treatment of sterility are desirous of success and therefore prepared to overcome somatic as well as psychologic obstacles. This very preparedness is of great help in the treatment, sometimes of more help than the therapeutic procedures aiming at the correction of coincidental anatomic or endocrine deviations. The impressive roster of successful conceptions after operative and hormonal treatments contains probably many cases of women who become pregnant, not because of the technic of their treatment, but because of the very fact that they were ready for consultation and cooperation. Several decades ago, before the brilliant methods of tubal insufflation and endocrinology were advanced, it was printed and taught that gynecologic examination alone (not counting instances when the displaced uterus was lifted) could occasionally overcome sterility. This assumption may have been the result of experiences with cases where readiness and consultation coincided. In our times of better therapeutic armament such coincidence still remains important.

Our understanding of the individual sterility problem—much advanced by inspection, palpation, x-ray visualization, basal temperature curve, hormone evaluation, and so on—can be still improved by probing into whether the patient really wants (or wants enough) to conceive. Some little emphasized and often withheld patterns of behavior habits, such as postcoital douching, may point toward an essential emotional handicap in the patient's striving for conception.

It is not proposed in this paper to detract from the tremendous merits of the accepted treatment of sterility. Instead, an attempt has been made to stress the relationship between the patient's ambivalence and conception. Overemphasis on the idea of being "clean" (if understood too narrowly) is incompatible with insemination; overemphasis on the idea of "integrity" (if felt too strictly) is incompatible with the sharing of one's body, be it with husband or fetus. A woman who in adult life does not advance and adjust her sense of personal values is likely to set in motion mechanisms which interfere with the success of marital life. These mechanisms may easily remain unknown to both patient and doctor.

SUMMARY AND CONCLUSIONS

Postcoital douching can be instrumental to sterility. Seminal reflux can be the result of relaxing (or antiperistaltic?) dysfunction of the female pars copulandi. Connections are sought between these mechanical interferences with conception and an underlying psychologic aversion to conception.

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Report of a Birth Regulating Program

Nils P. Larsen, M.D.

The problem of population control has engaged the attention of man since prehistoric times. Originally the perils and difficulties of day-to-day life kept the size of any group within the limits that the land could support. In later civilizations, endemic and epidemic disease—aided by man's inhumanity to man—acted as limiting factors upon the density of the population.

Even with the operation of natural bounds upon overpopulation, methods for the reduction of human fertility—from birth control to legal infanticide—have been advocated or practiced by nearly all nations. "A Yale University study of 190 cultures ranging from primitive to modern found evidence of the practice of birth control in every one of them. Extremely few women in the world produce their biological capacity which averages around 25 children in a lifetime."[4] In pre-European Hawaii, abortion sticks were used. Undesired babies were frequently given to friends, while the children of chief stock crossed with the lowliest group were killed by law.

This balance between mortality and birth rates has been upset by the progress of medical science. If it is to be regained the instinctive and untutored attempts to limit human fertility will have to be reinforced by other medical contributions. Unless this happens, the ill-effects of overcrowding and inadequate food will nullify many of the advantages of today's increased longevity.

Puerto Rico excellently exemplified how modern health work, without concomitant reduction in birth rate, produces crippling overpopulation. "The death rate has been cut in half and the birth rate has climbed in the past 48 years. The result is a rate of natural increase which has almost tripled. . . .

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The number of people increased 87 per cent between 1910 and 1946, for example, but the number of gainfully employed rose only 50 per cent."[5] An Indian economist recently summed up the situation: "Death control without 'birth control' will multiply miseries."[4]

In Hawaii in 1923, the infant mortality rate was 140. In Hilo, the second largest city, it was 269. Among the Filipinos the rate was 366 and among Hawaiians it was 305.[3] Today, some thirty years later, the rate is 21.

In 1938 the average Hawaiian worker earned $2.67 per day on the sugar plantations. This wage could feed, house, and clothe a maximum family unit of husband, wife, and four children. Food alone consumed 50 per cent of his total earnings. The addition of one more child destroyed the fragile balance of income and expenses.[6] It was painfully evident if the workers were to remain solvent their family size must not exceed six. In our self-contained, relatively isolated units, health, self-respect, and well-being were challenged by too many children.

The task for doctors seemed clear. The emotional facets of this problem which to some made conception control unethical could well be weighed against the suffering that could be prevented by a rational policy of family regulation. A little over 100 years ago the medical profession was severely condemned from most English pulpits because doctors were interfering—by giving smallpox vaccine and preventing epidemics—with God's method of deserved punishment for humans who had sinned. Some of the religious opposition to birth regulation as one means of preventing human suffering is quite similar.

The plantation population of Hawaii seemed ideal for study of the means of dealing with this problem. A good health system with a corps of well-trained doctors and excellent hospital facilities was available to all plantation workers and their families. Separate groups of plantations on each of four islands gave the opportunity to establish control and experimental groups. The plantation doctors became interested in developing a program of proper birth spacing on their plantations and a succession of methods were tried.[2]

The Graffenburg ring was used, but some infections resulted. Properly used it was efficient, for a number of patients became pregnant the month after removing it; however, sterility occurred in most cases in which infection had resulted.

Diaphragms were tried, but the intellectual level of the people was such that the women used them irregularly and incorrectly. One worker's wife hung her diaphragm over a nail on the wall; after the nail had punctured the rubber, she kept on using it and wondered why she had become pregnant.

Condoms were available at the plantation stores but suffered from the same handicaps as diaphragms. The rhythm method was advocated by some, but proved too uncertain, requiring more understanding than even the other contraceptive technics.

The only sure way seemed to be sterilization, which was made available without charge for any laborer or his wife who desired it for a legitimate reason and who qualified medically. Any laborer's wife with a fourth or later pregnancy was offered postpartum sterilization. If there were medical indications, sterilization was done on patients of lesser parity. The hospital stay was not prolonged by a postpartum sterilization, and the complications were negligible.

Ninety-eight per cent of all deliveries are in a hospital and most plantation wives realize the economic problems created by too many children.[1]

To indicate the attitude of the plantation doctors on this problem, I quote a letter of December 27, 1940, from Dr. Marvin Brennecke of Kauai who initiated postpartum sterilizations on the plantations.

> There were 36 done at Koloa Hospital and 35 done at Eleele Hospital since June, 1937, when I started doing them. This makes a total of 71. . . . I still feel that this procedure is of great value to the mother and her family, but we must pause to consider the problem from a sociological and economic point of view since the operation has gained an impetus in the Islands. The operation has many merits but let us also consider any possible disadvantages. Will the procedure have any social or economic ill-effects in the Islands? I do not think so, but let us consider a few of the points. . . . The sociological problem of population trends is not in itself important. In our present democracy the immediate status and welfare of the individual is still the primary objective in our work . . . not the loss of potential mothers in this particular community through postpartum sterilizations. . . . In two years each would have had another pregnancy. Since we sterilized 71 women, in ten years there would have been 350 more babies in this district. To be conservative, let us subtract 50 per cent for those who may have used effective contraceptives or for those who would have remained sterile. This still leaves 175 additional people in the Koloa District. Adding similar figures from all districts of Hawaii the sum will be impressive . . . over a period of fifty years the number increases in geometric proportions. Figures in themselves are not important, but they may help us in gaining a clear perspective of the advantages and disadvantages of such an effective and easy method of birth control.

Careful records of sterilization operations and other health procedures have been kept.[1] In Table 1 a 12-year report of sterilization frequency is recorded for the sugar plantation hospitals of four of the islands.

The technic employed in male sterilization is the excision of an inch from each vas and a folding over and ligation of the distal end. It is usually done under local anesthesia. The operations are scheduled for the beginning of a week-end, so there is minimum loss of work time. Patients are instructed to take precautions for three months after operation to avoid possible impregnation. There are no data available on the failure rate; however, one case of spontaneous resumption of fertility fourteen years after sterilization is known.

TABLE 1. Sterilization Operations

	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	Totals
							POSTPARTUM						
Hawaii	51	35	45	26	18	19	33	33	27	36	31	18	372
Maui	32	22	7	6	4	10	18	9	14	28	26	13	189
Oahu	23	37	22	10	4	4	4	2	1	2	9	7	125
Kauai	24	16	18	8	8	18	10	16	20	15	16	11	180
TOTAL	130	110	92	50	34	51	65	60	62	81	82	49	866
							SALPINGECTOMIES (NONPREGNANT)						
Hawaii	4	17	11	2	6	7	12	6	6	4	14	6	95
Maui	32	5	18	10	3	1	12	16	11	5	2	1	116
Oahu	2	0	2	0	0	1	2	1	0	1	3	6	18
Kauai	3	4	0	2	3	1	14	12	1	0	0	0	40
TOTAL	41	26	31	14	12	10	40	35	18	10	19	13	269
							VASECTOMIES						
Hawaii	12	11	5	3	7	8	7	10	9	8	3	4	87
Maui	11	11	2	1	1	4	3	7	5	2	0	3	50
Oahu	7	5	7	3	10	5	3	3	1	3	2	3	52
Kauai	5	2	2	0	5	8	4	4	1	9	3	1	44
TOTAL	35	29	16	7	23	25	17	24	16	22	8	11	233

Women are sterilized by the excision of a segment of tube and insertion of the proximal end of the tube into the broad ligament. It is usually done under spinal anesthesia; puerperal cases on the third postpartum day. Failures, if any, are not known, but we must grant there has been no meticulous follow-up.

During the 12 years covered by the statistical summary the population on the sugar plantations (due to increased mechanization) declined from 83,000 to 53,000. The amount of sugar produced actually increased. The number of women of childbearing age varied from 17,300 to 11,800 and the number of infants born per year varied from 2000 to 1100. The number of sterilizations performed is shown in Table 1. The birth rate dropped until

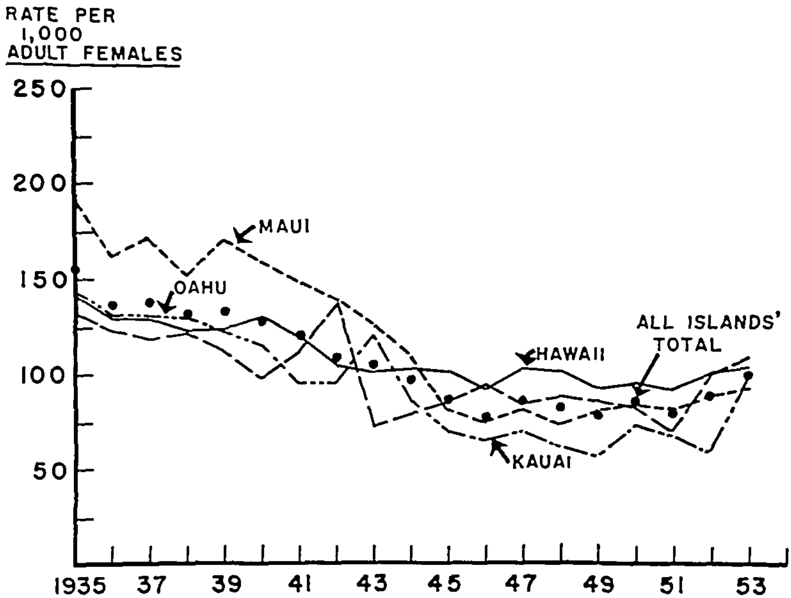

Fig. 1. Birth rates on four island groups of sugar plantations, 1935–1953. Note the steady drop to 72 in 1949 and the subsequent rise to 100 in 1953.

1949, when it was 72 per 1000 adult females (Fig. 1). It has risen each year since and in 1953 the rate was 100. Figure 2 shows the drop in infant mortality. The maternal mortality declined from 1.5 per 1000 births to 0.3.

How much of the improvement in infant mortality was due to the free use of proper birth spacing methods is hard to estimate. Since these methods were more widely used on the plantations than elsewhere in the Territory and since the infant birth rate fell first on the plantations, it would seem the program did help to lower that death rate. The birth spacing program was more vigorously promoted on the plantations than elsewhere. Other factors to improve health, such as better milk, had been applied to the whole territory. At least with four instead of twelve children, families were less crowded, had more food, and the mothers were less harried.

Neither venereal disease nor sex crimes increased, as was predicted if birth control information became widespread (Fig. 3). In view of all these

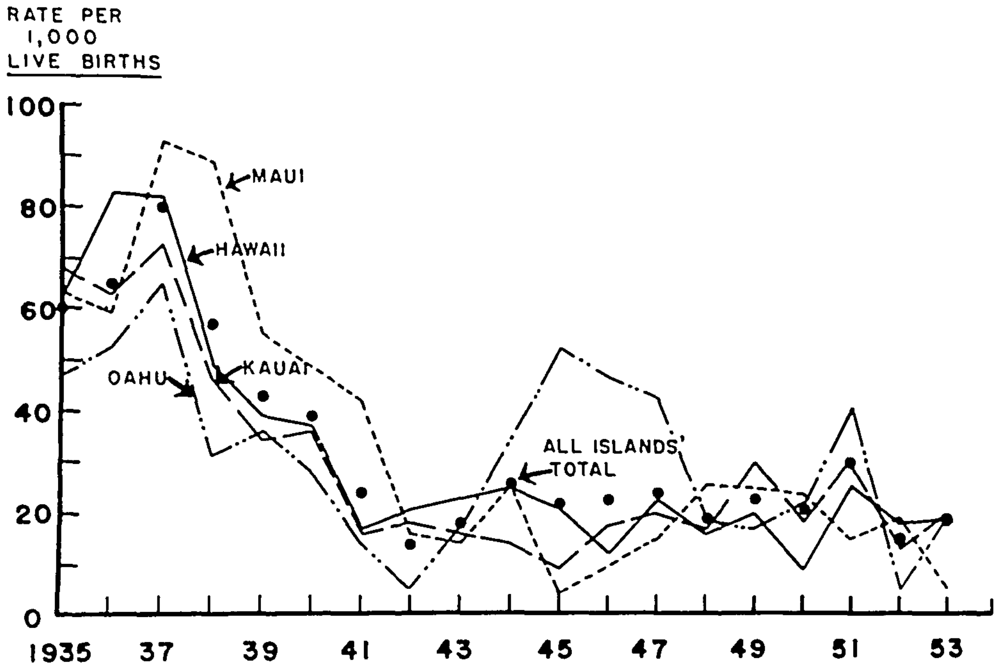

Fig. 2. Annual infant mortality rates on sugar plantations by islands. After a precipitous drop from 1937 to 1942 it has remained fairly constant in the low 20's.

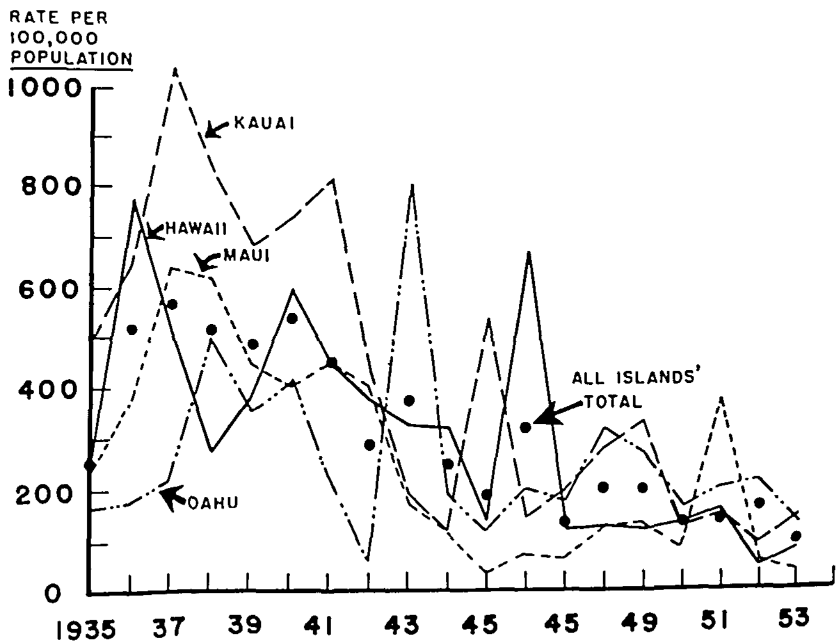

Fig. 3. Annual new cases of venereal disease on sugar plantations by island, 1935–1953. Much credit for the drop must be given to antibiotics.

facts, most people of our four islands view our program of family limitation as a great boon; at least, there is no vocal objection.

COMMENT

A series of tables and charts are presented to indicate that in an area where the teaching of proper birth spacing is considered an essential part of the public health program there has been a precipitous drop in infant mortality. This drop occurred on the sugar plantations before it occurred in the Territory as a whole. The health of the mothers (harder to measure) was also improved. The statistics suggest that methods for proper birth spacing should be part of a coordinated program for improving infant and maternal health. The figures indicate that immediate postpartum sterilization is feasible and safe, the complications minor and few.

CONCLUSION

The current study reinforces our opinion expressed ten years ago in a preliminary report[2]: "A definite and carefully watched birth spacing and birth regulating program associated with all the other care that mothers should be given, can improve the health of mothers and infants and still not reduce the birth rate too much . . . a program of birth spacing, including post-partum sterilization, is necessary for the attainment of the highest infant, maternal, and community health." The methods used have not resulted in any known detrimental effects and have resulted in beneficial ones. They have been accepted by a community whose members are largely of the Catholic or Buddhist faith.

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Hydrotubation

Separate Examination of the Patency of Each Tube with Isotonic Saline Solution

Hideo Yagi, M.D.

HYDROTUBATION is a term which I introduced in 1929 to describe a new technic for diagnosing patency of the fallopian tubes by means of isotonic saline solution instead of air or carbon dioxide.[2] The idea came from the experience of performing hysterosalpingographies in the diagnosis of sterility. With hysterosalpingography, I was struck with the following observation: If more than 10 cc. of iodized oil can be injected easily under low manometric pressure, it is almost a positive indication that one or both of the tubes are patent, as may be verified by roentgenography. On the other hand, if there is tubal closure or stenosis, resistance is encountered, and when added pressure is applied, oil will leak from the uterus into the vagina. This led me to conclude that tubal patency could be tested with the use of a nonradiopaque medium such as isotonic salt solution, without the aid of x-ray pictures. Isotonic salt solution, at body temperature, is an ideal nonirritating medium. Its use is free from the potentially dangerous after effects, notably embolism, that are sometimes associated with the use of radiopaque oils.

METHOD

In applying the test, salt solution at a pressure of 2 m. is used instead of air or carbon dioxide. By noting the quantity and speed of flow, we can

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estimate the degree of tubal patency. The resulting data can be plotted as a hysterosalpingogram and may be kept as a permanent record.

My original paper describing this method was published in 1930[3] and simplified in 1936.[8] The English translation[4] brought forth immediate response in other countries[5, 6, 7] as exemplified by Slámová's citation of my technic in 1937. In 1944, I worked out a new technic for the separate hydrotubation of the individual tubes.[9, 10] The principle of the new method lies in

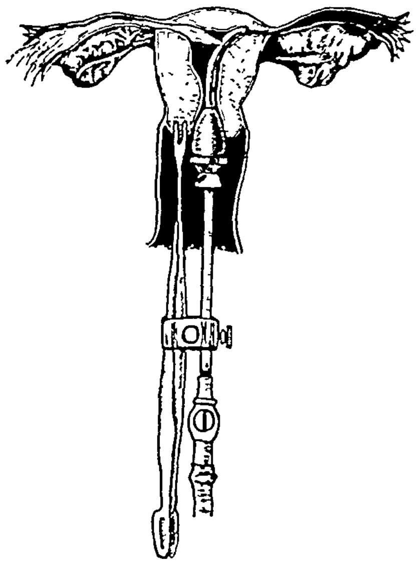

Fig. 1. The principle of separate hydiotubation lies in blocking one of the tubes with the tip of the cannula while injecting the salt solution into the other.

blocking one of the fallopian tubes at its uterine cornu with the tip of the special cannula, which resembles the end of a uterine sound, and then injecting the salt solution into the other tube through openings below the tip of the cannula (Fig. 1).

The cannula is 22 cm. long. The tip, measuring 7 cm., is made of malleable silver and resembles the common uterine sound. Two openings are situated 1 and 2 cm. respectively from the tip, on the side of greater curvature of the cannula. An adjustable rubber cone or olive surrounds the cannula. After measuring the uterine depth—the distance between the external cervical os and the tubal ostium—the cone is properly set with a metal screw and applied closely against the cervix with a Museux forceps which is attached to the cannula. After testing one tube the cannula is partially withdrawn, rotated through an arc of 180 degrees, and then directed against the other side. By this method, separate hysterosalpingograms can be prepared for each tube.

The test is performed with patients having either primary or secondary sterility. Prior to the test, pelvic examination is performed to exclude uterine

Fig. 2. Apparatus for hydrotubation. Note openings near end of cannula tip.

bleeding and acute and subacute infectious conditions which are contra-indications for the test. Additional tests should include bacteriologic examination of cervical secretions and the usual tubal insufflation and x-ray salpingography.

Hydrotubation may be performed at any time except during the pre-menstrual and menstrual periods. However, the optimal period is between one and two weeks following the cessation of menstruation.

Equipment

The special cannula devised for the purpose of separate hydrotubation is used. A graduated glass cylinder of 50 cc. capacity is employed as a reservoir for the sterile salt solution and is connected with the cannula with a sterilized narrow rubber or nylon tube. The upper surface of the salt solution is initially raised to a level of 2 m. above the uterus. This gives a

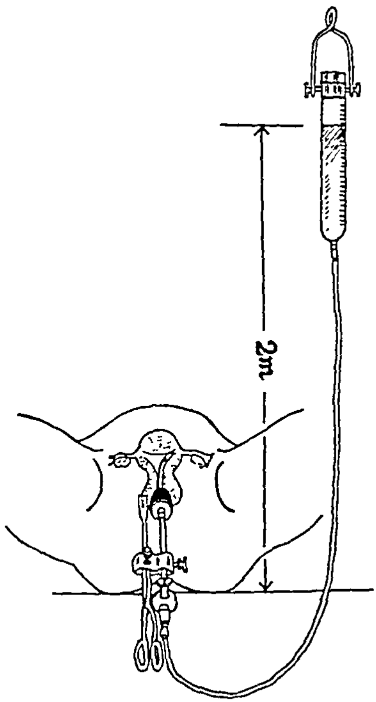

Fig. 3. Schematic representation of apparatus in use.

pressure approximately equal to that of 150 mm. of mercury. The solution is permitted to flow by gravity (Figs. 2 and 3).

TECHNIC

The patient is placed in the lithotomy position. By pelvic examination, the position and approximate size of the uterus is determined. A graduated uterine sound is introduced to ascertain the distance between the cervical os and the uterine cornu. The upper, malleable portion of the cannula is bent to fit the internal uterine contour. The cannula is then gently inserted into the uterine cavity, the tip being pressed so as to occlude the right tube at the uterine cornu. The rubber cone is moved into position to close, by pressure, the cervical os, and is held by a screw. A Museux forceps is applied to the cervix and is then clamped to the cannula.

While the operator watches for leakage of solution at the cervix, an assistant regulates the height of the cylinder and notes the saline flow, minute by minute. If there is no leakage from the os and the amount of solution escaping is more than 10 cc., the tube tested is presumed to be patent. In such case, the hysterosalpingogram reveals an ascending curve.

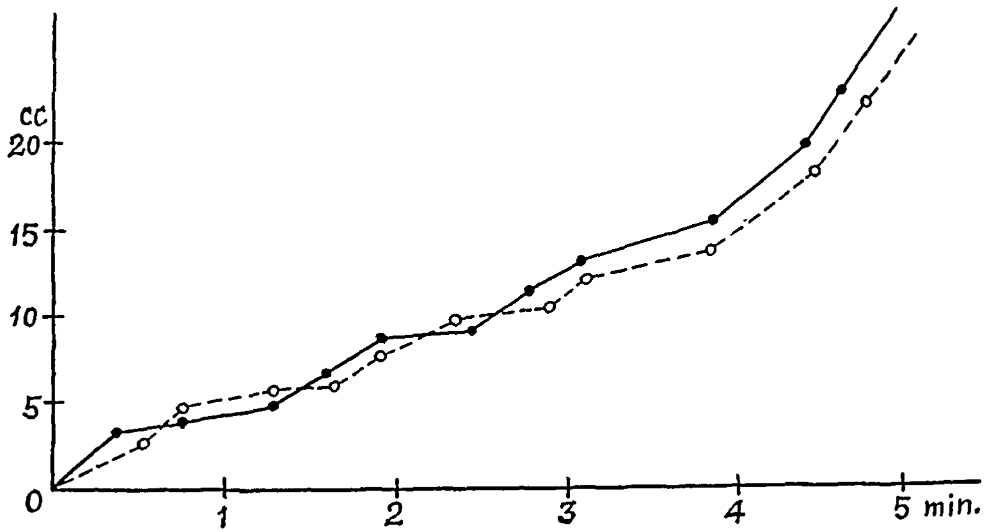

Fig. 4. Flow of the salt solution is plotted against time. Note that the curves are of the ascending type denoting that both tubes are open.

The cannula is now freed and turned 180 degrees so that its tip fits the opposite uterine cornu and the second test is carried out. A second curve is then made for the right tube (Fig. 4).

ANALYSIS

The curve varies according to the tonicity and patency of the tubes. If tonicity is weak or low, the curve may be very steep (Fig. 5). If, on the other hand the tonicity is strong, a slowly ascending curve is obtained. Thus, when the tube is patent, the hysterosalpingogram is always of the ascending type. When tubal occlusion exists, the curve becomes horizontal and runs parallel to the base line. When the curve is horizontal and very close to the base line, this indicates that the inflow of solution is very small and that the occlusion is at the cornu itself (Fig. 6). An ascending curve which is followed by a horizontal curve extending further from the base

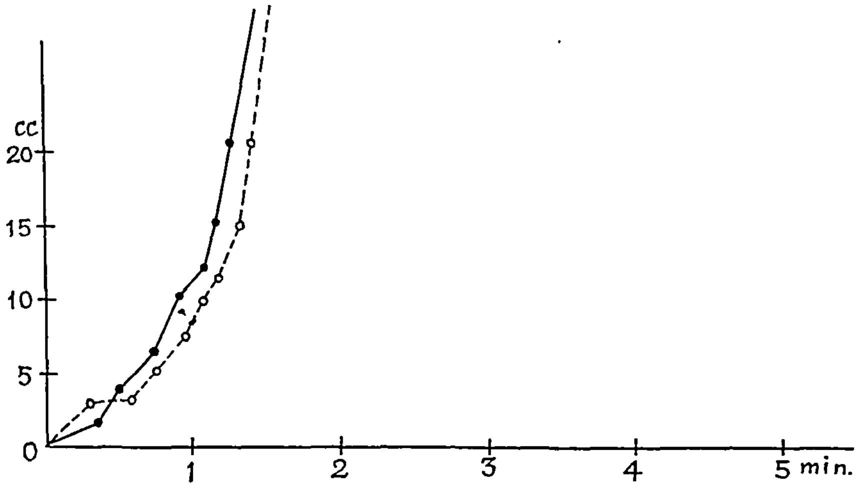

Fig. 5. The steeply ascending curves denote low tonicity of the patent tubes.

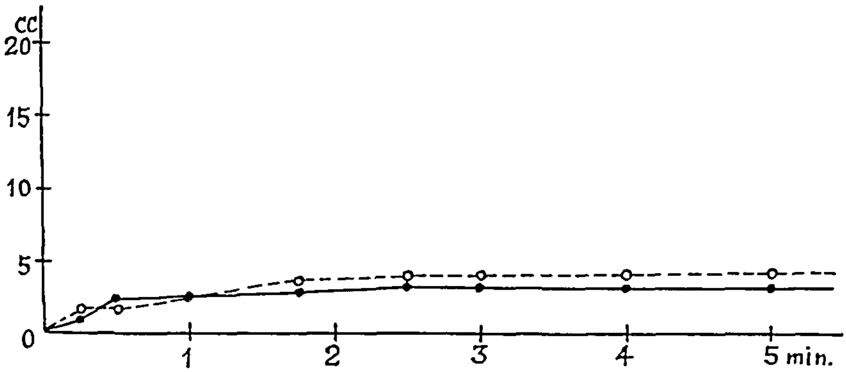

Fig. 6. Hysterosalpingograms of two tubes occluded at or near the uterine cornua. Note flat or horizontal character of the curves denoting retention of the fluid.

line indicates occlusion nearer the abdominal end of the tube (Fig. 7). When the fall of the column of water stops, as it may in some cases, we "hold" the pressure for a maximum of five minutes to rule out obstruction due to temporary spasm in the uterine cornu or in the tube.

By this method, then, it is possible to diagnose the side and site of occlusion without recourse to x-ray pictures. The principle may also be applied to the use of air or gas. The equipment is cheap and can be assembled by practitioners. The test can be repeated, as necessary, without danger of irritation of the pelvic peritoneum. Therapeutically, this procedure may be employed to open some occluded tubes so that pregnancy may ensue.

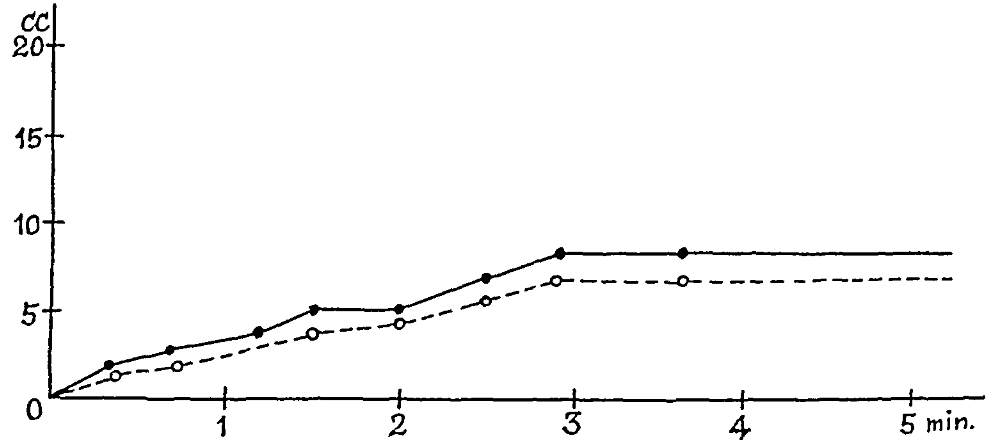

Fig. 7. The initially ascending character of the curve shows that fluid is entering the tubes. The curves on becoming "horizontal" indicate that the tubes on filling take on no more fluid and are therefore presumed to be closed at their distal portions.

During the past 20 years, I have performed 3000 hydrotubations with accurate diagnoses in 95 per cent of the cases as determined by follow-up with laparotomy or by hysterosalpingograms.

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Current Reviews

WILLIS E. BROWN, *editor-in-charge*

Sterility Investigation

BARRENNESS is a major concern of women. The importance of this biologic failure is manifest in the special social customs and laws evolved to provide for this contingency in all cultures.

Advances in medicine have followed a typical pattern of exploration and analysis of the gross or symptom complexes, the establishment of a syndrome, and its fractionation into several specific entities with individual etiologies, pathologies, and therapies. Such is the case with infertility; as research and experience increased, our focus changed from the "sterile woman" to infertile marriage—to a study of the partners.

The old terms, "sterile" or "sterility," were early modified by the use of such qualifying words as primary, relative, absolute, and so on, attempting to identify those women who *could not* conceive from those who had not but *might* conceive. Primary and secondary sterility have been introduced to indicate the never-gravid from those who could not conceive again. The terms "infertile" or "subfertile" have received more attention and connote a clinical state where permanent sterility has not been certified.

Following the widespread use of the Sims, Huhner, and other tests to measure the role of the male in this syndrome, our concepts shifted to an infertile mating and our terminology reflected this by popularizing "infertile marriage" or "infertile couple."

Finally, our studies have come to include those women and marriages where conception occurs but blighted embryos result in habitual abortion or stillbirth. While this is not strictly within the province of infertility or sterility, nevertheless the marriage is barren and the home childless.

The most startling advances in medicine have come as the result of special interest in some aspect of biology or its allied sciences. Thus the students of fertility have come from diverse fields bringing skills, knowledge, interest, and disciplines as they serve the common goal—reproduction. From this special interest has emerged a body of knowledge and a group of interested clinicians all dedicated to the common end, a newborn at every hearthstone.

THE IMPORTANCE OF STERILITY

Barrenness is an important clinical and social syndrome. The emotional turmoil imposed on such a couple is not only of great concern to the individuals but may adversely affect diverse physiologic and emotional processes and manifests itself in apparently unrelated areas of both medicine and social behavior. An example is the psychoneurotic woman who, impelled by her desire for a child, exceeds the bounds of social acceptability and filches a newborn from another's cradle.

Infertility or barrenness is a distressingly common state. Sutterlin[1] indicates that there are approximately 3,000,000 (12 per cent) infertile couples in this country. Llewellyn-Jones[2] and Jeffcoate[3] indicate that from 8.5 to 15 per cent of marriages are infertile in England. Barlovatz,[4] while giving no figures, implies it is an important entity in Central Africa. Sargent[27] suggests that there are 500,000 husbands in the United States needing and desiring treatment for infertility. It would appear that infertility is increasing. Whether this represents a true increase in infertility or an increase in registration of the problem is unknown.

It is perhaps pertinent to compare the 3 million infertile marriages with the 1–2 million diabetics, or 1.5 per cent; a daily average of 500,000 hospitalized patients with psychoses, and approximately 2 million psychotics in the population; or a 1.5 per cent incidence of cancer.

The clinical experience of Turner[5] and others indicates that approximately 65 per cent of infertile couples suffer permanent sterility and that 35 per cent exhibit various correctable defects. It is our duty as physicians to categorize these unfortunate couples and to extend to them the full benefit of our experience and knowledge through adoption, therapeutic insemination (both homologous and donor), or the establishment of fertility—fecundity and fruitfulness.

Infertility (or sterility) is not a scientific or clinical diagnosis but is a description or statement of a frustration syndrome. It is no longer acceptable to treat sterility; one must first assay the various causes. Thus infertility as a syndrome becomes qualified as "infertility due to oligospermia," "infertility due to cervical hostility," "infertility due to anovulation," and so on.

The present "Current Reviews" will attempt to survey the recent literature on the diagnostic aspects of clinical infertility and to offer perspective and interpretation of those procedures necessary to effective clinical diagnosis and therapy.

CLINICAL INVESTIGATION OF STERILITY

While custom usually dictates that the woman is the primary patient, Wall,[9] Murphy,[13] Stein,[14] Hamblen,[15] and others emphasize the importance of the proper orientation of both husband and wife in this effort, preferably at the preliminary survey. The importance of the biologic or reproductive unit has been emphasized by many authors and is finding its way into the titles of papers, such as "The Dual Responsibility in the Infertile Marriage"[6]; "Principles of Investigation of the Infertile Marriage"[7]; "Treatment of Sterility, Analysis of 400 Couples"[8]; "Analysis of Clinical Data on 500 Childless Couples"[5]; "The Management of Marital Sterility"[9]; and many others.

Despite the increasing emphasis on the conjugal unit as a basis of study, the employment of the Sims or Huhner test is infrequently stressed. Only a few (Kleegman,[10] Finkler,[11] and Sandler[12]) of the long list of papers studied in this review emphasized this fundamental test.

The importance of a careful anatomic survey of both husband and wife is pointed out by all authors. The various authors report male infertility as being important in from 10 to 40 per cent. The role of the male both in infertility and habitual abortion is described by McBurney,[16] Nuñez,[17] Nicoll,[18] and others. The role of constitutional or functional factors is stressed by Turner[5] and Finkler.[19]

An analysis of these reports and others provides evidence of interest. The number of women or couples coming to these authors who have had partial or incomplete studies is of interest. Overstreet[20] emphasizes the number of infertile couples who have sought medical help and received only inadequate or incomplete diagnostic studies. Gray[8] notes the frequency with which patients do not complete diagnostic studies for one reason or another. This problem is found in other countries, as reported by Ferreira[21] from Brazil and by Jeffcoate[22] from England. It is important, therefore, that a comprehensive plan of study be outlined to the infertile couple and their cooperation for a complete study obtained.

The incidence of multiple factors has been repeatedly emphasized and the reports of Gray,[8] Turner,[5] Kleegman,[10] Simard,[23] and others show multiple factors varying from 40 to 80 per cent. It is therefore incumbent upon the physician to make a complete diagnostic survey to evaluate all potential impediments to fertility before undertaking therapy based on the first or obvious finding.

While subsequent "Current Reviews" will consider in detail the various problems of infertility and their investigation, it seems wise to outline a simple plan of office procedure. (Details of infertility investigation have been outlined by the Research Correlating Committee of the American Society for the Study of Sterility.[24])

A PLAN

1. Since the wife usually presents herself, it is well to proceed with a preliminary survey of the woman including history and examination.

2. A survey conference with both husband and wife should be scheduled at the next mid-interval, to be preceded by planned coitus for a Huhner test (for details see Kleegman,[10] Finkler,[11] and others). At this conference proper orientation of the husband in the infertility complex is possible and his understanding usually enhances cooperation. The Huhner test not only gives valuable information but serves to bring the husband directly into the investigation of the reproductive failure. It provides a valuable clinical assay of male performance or fertility index through evidence of:

   a. Patency of male apparatus
   b. Relative appraisal of semen quality (number of spermatozoa and incidence of abnormal forms)
   c. Sperm survival
   d. Cervical and vaginal hostility

A tubal patency test* should be carried out at this visit. Good results are obtained with all such explorations, and any one of them may be used for preliminary survey. I personally employ a modified insufflation test, reserving other technics for detailed study as indicated.

3. During the next two weeks special studies and laboratory tests (including BMR, blood count, etc.) on both partners can be completed.

* Details of the special studies will be presented in subsequent "Current Reviews."

4. The wife is asked to return for endometrial biopsy on Day 25 ±2 of the cycle.
5. A summary conference is scheduled for both husband and wife soon thereafter for presentation of data with perspective, prognosis, further studies* needed, or a therapeutic regimen recommended.

This infertility survey should not consume more than a few weeks. From such a survey the majority of factors will be discovered, and the couple will embark on a definite program of further detailed study or therapy.

RESULTS

Complete investigations of infertile couples have been reported on sufficient series of marriages to permit an appraisal of the results.

Ferreira[21] reports discouraging over-all results of 2.5 per cent, while others, notably Gray,[8] Turner,[5] and Finkler[11] indicate successful pregnancies in from 30 to 40 per cent of infertile couples.

Of the many factors involved, age of the couple offers a significant correlation. The younger the woman the better the prognosis. It is also *emphasized that the longer the duration of infertility (after two years)* the poorer the prognosis. Bender[25] reports that primary sterility, in which 47 per cent achieved pregnancy, offers a better prognosis than secondary, where only 34 per cent became pregnant. Similar results are reported by Rutherford.[26]

Simard[23] and others emphasize the multiplicity of anatomic factors and indicate their approximate distribution as follows: Uterine—12 per cent; tubal—36 per cent, ovarian—19 per cent; cervical—13 per cent; and male—7 per cent (usually 20 per cent). This is in essential agreement with others.

Finally an appraisal of the profitable duration of therapy has been made by several authors. Turner[5] indicates that 83 per cent of the successful pregnancies occur within the first year. This is generally confirmed by Gray,[8] Bender,[27] and others. Thus prolongation of therapy beyond this interval, unless new factors are identified, is usually fruitless and should be discouraged.

SUMMARY

Recent literature suggests that infertility is a syndrome of dual responsibility requiring complete and critical appraisal and analysis of both husband and wife.

* Details of the special studies will be presented in subsequent "Current Reviews."

The etiologies of infertility are multiple, and complete study of both husband and wife is essential.

A simple, logical plan followed completely will provide most of the needed data for proper diagnosis, prognosis, and therapy. The essential steps and data are:

1. Anatomical and functional examination of husband and wife.
2. Conjugal examination (Sims, Huhner tests)
3. Tubal patency
4. Ovulation (endometrial biopsy)

The fertility index can be enhanced and conceptions obtained in approximately 40 per cent of infertile marriages by meticulous attention to details of diagnosis and treatment.

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Book Reviews

The Energetics of Development: A Study of Metabolism in the Frog Egg. L. G. BARTH and LUCENA J. BARTH. *Columbia University Press, New York, 1954, 117 pp., $3.00.*

This small and commendable monograph on the energetics of development by this research team of Columbia University is a further contribution to the developing field of chemical embryology. It assembles quantities of data obtained from studies on the frog *Rana pipiens* which as yet have not been woven into an acceptable group of unifying concepts. The earlier developmental concepts of the relation of morphologic patterns to development, such as relations of the first cleavage to the gray crescent, bilaterality, and other variables are here replaced by the physiologic and biochemical aspects of the problem. The ultimate goal of studies on energetics is to explain aspects of development in terms of chemical reactions. It is important to understand how energy is transferred during development from energy-rich to energy-poor compounds in order to produce cellular growth and cellular differentiation, and how the different enzymes regulate a developmental chain reaction leading to the proper development of embryonic parts.

Individual data are arranged and discussed under 5 headings: (1) introduction to the various aspects of energetics; (2) the storage of energy in the egg; (3) the release of energy as development continues after fertilization; (4) localization of the release of energy; and (5) the metabolism of protein and its distribution during development. Many problems are presented that cannot be solved satisfactorily at the present time but the treatise is highly stimulating for those interested in the more penetrating analysis of development from the chemical aspects.

CARL R. MOORE

La Prophylaxie en Gynécologie et Obstetrique. *International Congress of Obstetrics and Gynecology. Geneva, Switzerland, Librairie de l'Université Georg, 1954.*

This huge book contains more than 200 papers which were read in Geneva at the International Congress of Gynecology and Obstetrics. The papers were presented in the 5 official languages of the Congress; English, French, Spanish, Italian, and German. At the end of each article are summaries in languages other than that of the paper itself. The chief subject of the Congress was prophylaxis and every aspect was covered by the participants. The discussions dealt with maternal, fetal, and neonatal morbidity and mortality, neoplasms in women, and the teaching and organization of prophylactic obstetrics and gynecology.

It is manifestly impossible to discuss individual papers. De Watteville and his associates are to be highly complimented not only for compiling this enormous book, but also and especially for having made arrangements for more than 1800 obstetricians and gynecologists to attend the Congress. Those who were not in Geneva will find this book packed full of valuable information not only about prophylaxis in obstetrics and gynecology but also about many other subjects. The book is highly recommended.

J. P. Greenhill

The Management of Endocrine Disorders of Menstruation and Fertility. Georgeanna S. Jones, M.D., *Springfield, Ill., Thomas, 1954, 198 pp., 37 illus., $5.75*

This volume was intended as a primer in gynecological endocrinology for the medical student and the busy practitioner, and within certain limitations it fulfills its object admirably. The style is simple, readable, and lucid, and the organization of material excellent. The highly simplified presentation of the complex neuro-endocrine interrelationships is probably necessary for didactic purposes. Nevertheless, a number of important omissions exist. In the chapter on pituitary hormones, no mention is made of the Gorbman ultrafiltration technic, a most practical procedure, and in a discussion of the *Rana pipiens* test, the reduction of urine toxicity by the kaolin adsorption technic is omitted, although it is described for the *Xenopus* test. In summarizing the pituitary hormones, it is somewhat naively stated that three gonadotrophins have been isolated in chemically pure form; to the reviewer's knowledge chemical purity has been achieved only for the posterior pituitary hormones, oxytocin and vasopressin, by du Vigneaud and his group. No mention is made of the necessary presence of pituitary hormones for chorionic gonadotrophin to be active, although this fundamental observation is the basis for several modern bioassay procedures.

The next chapters on the ovarian steroid hormones, although summarizing most of the important data, contain a number of fundamental errors: the androstane ring is incorrectly called an estrane ring on p. 36, and is again described, instead of the pregnane nucleus, in connection with progestational steroids. In illustrating pregnaneolone (i.e., pregnanolone), the structural formula of Δ^5-pregnenolone is pictured instead. The double bonds in the estrogen ring A are assigned fixed positions. It is hoped that future editions will bring steroid nomenclature and biochemistry up to date.

No mention is made of the Shorr or the iodine stain for determination of estrogen effect, nor the Kober or fluorescence reactions for the chemical estrogen methods. The described pregnandiol methods are outdated, and for some reason, gravimetry is held to have some advantage over colorimetry contrary to general opinion among steroid chemists. More detail, including illustrations if possible, seems highly desirable regarding the luteal changes in the human endometrium, in view of the importance of endometrial biopsies.

In the clinical sections, the ovarian hilus-cell tumors go unmentioned, as does the use of presacral air insufflation as a differential diagnostic tool in amenorrhea with hirsutism. Dysmenorrhea is not discussed at all, although there are known to be endocrine aspects to this problem. Therapeutic advice given is limited to that advocated by the author; this is probably very wise in view of the intended audience. Thyroid is advocated enthusiastically; in other respects there is ultra-conservatism as in the hesitancy to consider ovarian irradiation despite the recent work of Rakoff and others. If the reason given for not using this treatment—ignorance of its mode of action—were applied in other fields, one might also hesitate to prescribe salicylates, colchicine, cortisone, or chlorpromazine, to name just a few effective agents whose mode of action is also unknown. The book ends with a thoughtful chapter on tumors of the chorion. There is a bibliography of 344 references which it is hoped in future editions will give more weight to the excellent work which has been done in Britain and France since the war. Twenty misspellings, largely of proper nouns, were noted; the typography and illustrations are excellent.

JOSEPH W. GOLDZIEHER

Die Sexualität des Menchen: Handbuch der Medizinischen Sexualforschung. HANS GIESE (Ed.). *Stuttgart, Germany, Enke, 1955, 648 pp., 132 illus.*

This book consists of contributions by 21 authorities on the subject of sex in humans. The editor is Hans Giese, Director of the Institute for Sex Research at Frankfurt A. M.

In the preface the editor states that while the Kinsey reports took the United States by storm, they did not arouse the same response in Germany, most likely because the methods used by Kinsey and his associates had been employed in Germany for many years, especially by Hirschfeld, and this type of research had ended. This book has two main parts: "The Regular Symptom Complex of Sexuality," including biological basis and courses of sexual relations; and "Clinical Problems of Sexuality," including functional disturbances and abnormal sexual relations. There are 19 chapters covering every phase of sexuality in humans. Among the subjects are anthropology of sexual life, anatomy and physiology of the sexual organs, the biological basis of sexuality, chemical basis of sexuality and fertilization, hereditary basis of the sex of humans, sexuality in homosexuals, the social forms of sexual relations, childhood, puberty, maturity and old age, sexual intercourse, anatomy and physiology of sex disturbances, functional sex abnormalities, disturbances of the endocrine function of men and women, precocious puberty, intersexuality, psychopathology of sexuality, sociology of prostitution and morals.

There are numerous references throughout the book. That they are predominantly German is understandable in the field of sexuality. The book is authoritative and evenly written, though there are many contributors. The type and illustrations are clear and the book is well bound. The book is highly recommended as a storehouse of valuable information about all phases of sexuality.

J. P. Greenhill

Beiträge Zur Sexualforschung. Organ der Deutschen Gesellschaft für Sexualforschung. H. Bürger-Prinz and H. Giese (Eds.). *Stuttgart, Germany, Enke, 1954.*

This contribution consists of five separate monographs. The first is "Concerning the Pattern of Sexuality" and the second is "Methods of Treating Sexual Disturbances." Both of these subjects were presented at the Second Congress of the German Society for Sexual Research in Königstein in 1952. There were eight contributors to the first monograph and ten to the second. The third monograph in the series is "The Phenomena of Transvestitism in Men" by H. Bürger-Prinz, H. Albrecht, and H. Giese. The fourth monograph, "The Inquiry of the Intimacy Sphere," was prepared by Ludwig von Friedeburg. The fifth monograph entitled, "Studies on Human Homosexuality" was written by G. M. Kempe and Reinhard Redhart.

Each contribution has a well-selected list of references, mainly German.

Those interested in the subject of sexuality will find these monographs as well as the accompanying book on "The Sexuality of Humans" (see above) very instructive and interesting.

J. P. Greenhill

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