Biochemical Changes in the Reproductive Cycle

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THE PROBLEM of sterility in the "otherwise normal couple" may often be a frustrating one. Although much information has been collected on various aspects of the pathogenesis of infertility in both the male and female partners, much of this is based on observations of relatively obvious factors. When the investigation of the normal couple does not reveal perceptible abnormalities—anatomic, gynecologic, or psychiatric—it may be necessary to look for more subtle aberrations in the activity of the reproductive tract such as derangements in the metabolism of the component tissues. In most instances, however, the significance of these changes is as yet elusive. For example, the metabolic patterns of spermatozoa have been intensively examined, as have the cyclic changes in the metabolic activity of the tissues of the female reproductive tract. It has been suggested that integrated hormonal influences regulate the required sequential biochemical changes. However, it must be assumed that the tissues involved are capable of adequate metabolic responses to hormonal stimulation. The metabolic patterns of a given tissue are inherent in the functional structure of that tissue. Whereas gross deficiencies in functional capacity are easily observed, subclinical reduction in the metabolic performance of an organ system may be extremely difficult to expose. In this presentation, an attempt will be made to correlate the characteristic metabolism of spermatozoa with the metabolic patterns found in the female reproductive tract, not only better to appreciate the teleologic reasons for the existence of these metabolic pat-

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terns, but also to suggest possible biochemical anomalies that cause the enigma of sterility in the "otherwise normal couple."

BIOCHEMICAL FACTORS IN REPRODUCTION

The relatively short interval provided for the establishment of an effective spermatozoon-ovum relationship is a result of the time during which the ovum is available for fertilization. Under the best conditions, the egg lives for about 12 hr. This means that if an embryo is to be produced, fertilization must occur during this period.[48] Insemination, however, may occur at any time. Since spermatozoa may live for several days in the female before ovulation occurs,[95, 104] the metabolic functions of her genital tract are directed toward preservation and extension of sperm vigor for as long as possible, in order that they may be present in an active, functional form during the short period in which a fertilizable egg is available.

These functions are especially obvious coincident with or just before the actual time of ovulation and are the basis for a number of tests presumably indicative of ovulation. These tests evaluate observable changes that derive from several physiologic factors operating in concert and probably reflect the net algebraic sum of positive and negative influences on tissue metabolism.

Most of the changes that occur are considered to be the result of the rising tide of estrogen and the subsequent appearance of progesterone.[22, 26] Estrogen is made available by the gradually developing follicle, from which will emerge the mature ovum, and perhaps also by accessory follicles.[120] The changes observable in the female reproductive tract as a result of the increased estrogen production vary according to the sensitivity to estrogen stimulation of the receptor structures. It would appear that, except in the guinea pig,[1] the vaginal mucosas of the mouse,[79] the rabbit,[108] and the human being[2, 122] are the most sensitive to estrogen. The uterine epithelium is second. The least sensitive is the endocervical canal. The relatively lower sensitivity of the endocervical canal to estrogen stimulation makes this area best suited for characterizing the ovulation phase, since maximal activity of the cervical glands[103] will occur in response to the very high levels of estrogen found ordinarily only in association with ovulation.[26]

Useful inferences can be made from an examination of the cervical mucus. Under the influence of estrogen, the cervical mucus becomes less viscous, clear, and copious.[6, 88, 116] It has a high degree of flow elasticity (spinnbarkeit), in that it can be drawn into threads over 20 cm. long.[29, 32] The cellular content is reduced to few or no leukocytes.[3] The physicochemical characteristics are altered, permitting the sodium chloride present to crystallize in characteristic fernlike or palm leaf patterns.[91] The mucus quality is also most favorable for spermatozoon penetration and survival.[31, 104] The appearance of increasing levels of progesterone following ovulation tends to reverse the estrogen effects. The cervical mucus returns to a scanty, viscous, thick state that exhibits very little spinnbarkeit and no tendency to fernlike crystallization.

The orderly sequence of biochemical changes that occur in the female reproductive tract provides a most receptive environment for both sperm and ovum. A number of metabolic components appear to undergo cyclic variations. Among those which seem to be dependent on estrogen stimulation are alkaline phosphatase,[5, 47, 51, 64, 107] diphosphopyridine nucleotide oxidase,[12, 92] adenosinetriphosphatase,[33, 58] beta glucuronidase,[41] sulfatase,[49] serine aldolase,[52] peroxidase,[66] hexokinase,[118] and several dehydrogenases.[13, 93] It would appear that acid phosphatase,[44] succinoxidase,[109] lactic dehydrogenase,[12] and carbonic anhydrase,[67] activity are increased under the influence of progesterone. Nutritive materials, such as glycogen, are gradually stored during the proliferative stages, and both the concentration and availability are maximal during the ovulation phase.[62, 87, 117] The *p*H tends to become more alkaline.[23] The presence of glycogen at peak levels in cervical and vaginal epithelium[81] provides the basis for the Birnberg glucose-oxidase-indicator test, which has been found to be useful for estimation of the ovulation phase.[16, 17, 30] The concentration of demonstrable glycogen appears to be directly proportional to the level of estrogen. The Birnberg test also permits evaluation of cyclic estrogen by following the daily variation in vaginal glucose. The patient may test herself daily and record the results in a "colorgraph" (designed by David J. Wexler) comparable to the BBT curve.[17]

Whereas the mechanisms involved in the storage of glycogen appear to be estrogen-influenced, progesterone, perhaps in combination with estrogen, triggers the secretion of glycogen. Hughes observed that the first indication of glycogen release by the endometrial glands occurs at the end of the second week of the menstrual cycle, coincident with ovulation. The glycogen is hydrolyzed by the increased glycolytic activity evidenced at this time. Augustin found glycogen in endometrial abrasion material only in the presence of ripe ovarian follicles or recent corpus luteum formation. The content of glycogen in the vaginal epithelium follows a similar pattern.[9, 14] Mack inferred that the amount of glycogen in the vaginal smear was directly related to the estrogen level, in that cellular glycogen was absent when estrogen was deficient. The true glycogen content in the vaginal smear is not striking until keratinization has commenced.[9] Lapan and Friedman showed that the values for glycogen and glucose in the vaginal mucus of postmenstrual specimens are consistently lower than those taken during the secretory phase. Higher concentrations are also found during the pregnant state, even though the amount of cervical mucus decreases during this period.

Another aspect of the cyclic biochemical changes occurring within the female reproductive tract may be concerned with providing the special *respiratory* environment required by spermatozoa. Cells, in general, obtain energy for maintenance of their structure and function by the transfer of chemical energy from phosphorylated compounds such as adenosine triphosphate. The purpose of cellular respiratory processes is to regenerate these energy-rich compounds from dephosphorylated precursors such as adenosine diphosphate. Several mechanisms are accessible for the conversion of the prime fuel, carbohydrate, which, by successive oxidation and reduction reactions in the presence of inorganic phosphate, provides suitable phosphorylated intermediates. Two major mechanisms, *aerobic respiration* and *anaerobic* glycolysis, are available to the cell; the pathway utilized depends on the prevailing supply of oxygen and also on the concentration of oxygen-reactive substances present that may divert the oxygen. All carbohydrates used are first converted to glucose phosphate. Under anaerobic conditions, the end product of glycolysis is lactic acid, whereas under aerobic conditions carbon dioxide and water result. Both aerobic and anaerobic mechanisms may be present at the same time in a cell and may in a sense be thought of as competing with each other for the supply of glucose, phosphate, and adenosine diphosphate[53] (Fig. 1). *It is proposed here that anaerobic mechanisms play an important role in both the maintenance of spermatozoon viability and also the fertilization process itself.*

The possible significance of anaerobiosis for each of the components in-

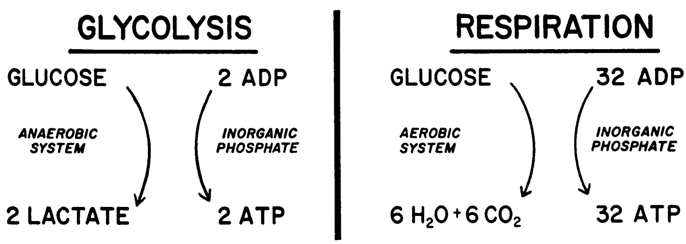

Fig. 1. Regeneration of adenosine triphosphate (ATP). Competition of respiration and glycolysis for glucose, adenosine disphosphate, (ADP), and inorganic phosphate.

volved—the male, the female, and the actual fertilization process—are examined below.

The Male Component

It would appear that although the spermatozoon possesses a complete cytochrome system,[37, 76] which permits the direct utilization of oxygen by aerobic respiration, the human spermatozoon acts like an anaerobe.[71, 74, 75] Anaerobiosis favors the uptake of phosphate, indicating that better glycolytic phosphorylation can occur than under aerobic conditions.[20] Spermatozoa produce lactic acid from glucose even under aerobic conditions.[69] This property, which malignant tissues also have, characterizes spermatozoa as metabolically unusual and burdened with an enzymatic fault. However, it is more proper to look upon this phenomenon as a metabolic specialization[74] developed to fulfill the unique requirements of the spermatozoon for intensive motility, survival, and finally its role in fertilization. The most urgent demand on the energy machinery of spermatozoa following ejaculation is that of forward motility, i.e., progression from the entrance of the female reproductive tract upward through the uterus into the fallopian tubes, where actual fertilization occurs. This is indeed a Herculean feat, when one considers the relative dimensions.[106] A spermatozoon that measures 0.05 mm. in length must travel some 100 mm. from the cervical pool. The ratio between length and the distance traveled is thus 1:2000, which would be comparable to an individual of average height swimming a distance of about 2 miles.

There is abundant evidence that spermatozoa thrive best in an anaerobic environment,[18, 54, 71, 78, 98] and that prolonged aerobic metabolism contributes to their senescence.[63, 73, 74] Spermatozoa are extremely sensitive to peroxide,[111, 113] a product of aerobic respiration, and are dependent on catalase for its rapid removal. If oxygen is present, spermatozoa will utilize their intracellular supply of nutrients more rapidly, whereas in an anaerobic environment, the organism tends to use extracellular substrates.[77, 78] A relatively small amount of carbohydrate is necessary to maintain the motility of spermatozoa. It has been shown that as little as 15 mg./100 ml. of glucose is sufficient. If spermatozoa are deprived of nutrients for several hours, motility will gradually cease. However, such spermatozoa can be revived by the simple expedient of adding glucose to the medium. There is one important qualification: If the spermatozoa have been exposed to high oxygen concentrations, the resuscitation rate is completely or severely depressed.[54]

Spermatozoon motility appears to be connected with glycolytic mechanisms,[70, 71, 73] in as much as substances ordinarily inimical to the functioning of aerobic systems, such as cyanide and carbon monoxide, do not block human sperm motility. Davis and McCune have shown that there is better correlation between the rate of glycolysis and the number of spermatozoa than between the net respiratory rate and the number of spermatozoa, indicating that glycolytic potential more aptly characterizes a given sperm population than oxygen consumption. It has been suggested that rate of oxygen consumption per se may be of minor significance in evaluating the actual ability to fertilize an ovum of a semen specimen.[63] Actually, the excessive metabolism that may occur under aerobic conditions seems to be detrimental to the sperm. Greater longevity, in terms of ability to fertilize, appears to be obtainable by restriction of aerobic respiration.[97] Evidence is available from several sources that a number of factors may normally exist whose purpose it is to impede the occurrence of oxidative respiration.[78, 112]

The accessibility of oxygen does not make certain that aerobic metabolism is occurring in a tissue. The consumption of oxygen within a tissue is dependent not only on the availability of oxygen but also on the receptivity of the given metabolic system to the oxygen. If substances are present that can combine with the oxygen at a more rapid rate than the cytochrome system, the oxygen is diverted, the regeneration of cytochrome oxidase (which is necessary if respiration is to proceed) is prevented, and the metabolism of the tissues may continue along glycolytic lines. The existence of oxygen-"binding" substances, such as those possessing an easily accessible sulfhydril group, has been demonstrated in active tissues. Glutathione is such a compound, as is ergothionine, which has been extracted from the semen of some species.[50] Easily oxidizable compounds such as ascorbic acid can also function in this way. The net effect of these substances is to provide a "reductive" environment whose oxidation-reduction potential is negative, indicating a low affinity for electrons and permitting the orientation of the cell metabolism towards anaerobic systems. The addition of thioglycollate to bacteriologic media for the propagation of anaerobic microorganisms is an example of this process. The reductive environment permits the growth of anaerobic bacteria even though the culture tubes are exposed to atmospheric oxygen. A similar situation appears to apply to spermatozoa in seminal fluid.[72] It has been demonstrated that the addition of cystine or glutathione is effective in maintaining spermatozoa in skimmed milk.[57]

The tissues involved in the spermatogenic process appear to have a metabolism more similar to that of other proliferative areas. Thus, epididymal spermatozoa appear to have a less unusual metabolic pattern than ejaculated sperm. Lardy has demonstrated that when dormant epididymal sperm are mixed with the secretions of the seminal vesicle and prostatic fluid, there is gradually released a metabolic regulator that produces the change to the metabolic pattern of ejaculated spermatozoa. The effect of this "regulator," he suggests, is to subdue the cytochrome system by keeping it uncoupled from phosphorylating respiratory systems. Weil found an immunologically demonstrable difference between epididymal and seminal spermatozoa, attributable to an antigenic material that is taken up from the seminal plasma before or at the time of ejaculation.

The capacity of spermatozoa to reduce a number of oxidation-reduction indicators such as resazurin,[38] methylene blue,[21, 35] and tetrazolium[82] has been measured. This reductive capacity, in addition to their lactate production[42] and pyruvate metabolism[110]—both of which may reflect the integrity of glycolytic systems—have been proposed as indexes of the relative fertilizing capacity of spermatozoa. This may yet prove to be of major significance. It is possible that even though spermatozoa possess a complete mechanism for direct consumption of oxygen, the cytochrome system, perhaps this aggregate of enzymes and substrates must be kept intact and not dissipated for motility, if sperm longevity in terms of ability to fertilize is to be maintained. Perhaps the machinery associated with oxygen uptake and the oxidative respiratory systems that can provide explosive amounts of energy must be reserved for fertilization, and the subsequent energy-consuming cytokinesis.

The Female Component

If the cyclic changes in metabolism within the female reproductive tract are directed toward providing an environment most propitious for maintenance of spermatozoa, and if the longevity and ability to fertilize are supported by maintenance of anaerobic conditions, it is not surprising to find that anaerobic glycolytic mechanisms may predominate under the influence of estrogen.[39, 40] Glycolytic metabolism gradually increases throughout the proliferative phases of the cycle, reaching a maximum coincident with the ovulation phase, when estrogen is at a peak. Following this, glycolysis decreases, the respiratory mechanisms being more active during the secretory phase. Eschbach and Negelein showed the metabolism of the infantile mouse uterus to be less anaerobic than that of the adult. If estrogen is administered, however, there is a 98 per cent increase in glycolytic mechanisms. The individual studies of Dirscherl, Kerly, Stuermer, Walaas, and their associates have also substantiated the predominance of anaerobic glycolysis in the uterus during the proliferative stages of the sex cycle.

That the cervical canal appears to be more hospitable to anaerobic systems during the ovulation phase is shown by the character of the bacterial population cultured from cervical mucus. After menstruation, both anaerobic and aerobic organisms may be found in the thick cervical mucus.[28] However, during the ovulation phase, there is a striking decrease in the total bacterial count,[3] and usually only anaerobes are cultured from such material. As the mucus gets thicker and menstruation approaches, organisms of both types gradually reappear. The bacteriologic characteristics of the uterus and the vagina are similar in many respects to those of the cervix. Arronet[3] demonstrated a reduction in vaginal bacteria during the ovulation phase, even though there is the highest concentration of glycogen at this time.[62] In this connection, an interesting experiment was performed by Rowson and associates. They inseminated cows with infected semen and slaughtered the animals 2 days later, after which cultures and histologic examinations of the uterus were made. If the cows were in estrus, the uterine cultures were sterile, whereas at any other phase of their cycles, pyometritis developed.

The secretions of the entire reproductive tract, the luminal fluids, and also the follicular fluid, which probably gains entrance into the fallopian tubes, are all reductive in character.[83, 84, 86] That is, their oxidation-potential is negative, and they are capable of providing a reductive atmosphere for spermatozoa. The presence of ascorbic acid, which may also contribute to the anaerobic character of the medium, can be demonstrated[56, 114] in the reproductive tract. Foraker, *et al.* found marked dehydrogenase activity in tubal epithelium. Mastroianni *et al.* obtained lactate as the end product of carbohydrate metabolism in the endosalpinx, even though a good concentration of oxygen is available there, as previously demonstrated by Bishop.[19] This metabolic pattern is analogous to that of spermatozoa; similarly, the mere presence of oxygen need not connote that oxidative respiration is the normal metabolic pathway. The orienting effect of easily oxidizable compounds in this regard has been discussed above.

Birnberg and I have reported the impression that follicular fluid entering the fallopian tubes may contribute to the maintenance of an anaerobic environment.[15] Evidence was obtained by testing follicular fluid with the use of the glucose oxidase-orthotolidine system for the detection of this sugar. A prompt reaction occurred, probably from the glucose present in the fluid. However, within 15 min. the color reaction had completely faded, even though the color system is usually relatively stable. This reversal of the orthotolidine reaction by follicular fluid is suggestive of the presence of a powerful reductase system in follicular fluid capable of reducing the blue form of orthotolidine back to the leuco form. This calls to mind the changes seen in the oxidation-reduction dyes used in bacteriology, such as methylene blue, and also of the color reactions used to measure the reductive ability of spermatozoa mentioned above. It is known that follicular fluid has a beneficial influence on spermatozoa.[8, 61] Perhaps follicular fluid not only furnishes some of the nutrients for spermatozoa in the maternal environment, but also helps supply the necessary oxidation-reduction environment for fertilization. Recently, the presence of large quantities of sperm antiagglutinin, which functions only in the reduced form, has been reported in follicular fluid.[65] It is interesting to recall in this connection the observation of Redenz that the sperm stored in the female bat in the autumn are activated by follicular fluid secreted at the springtime ovulation.[89]

Fertilization

There is a strong possibility that the female reproductive tract maintains an environment that permits anaerobic metabolic activity not only for maintenance of spermatozoa but also for fertilization of the ovum itself. Brooks showed that both the activation and fertilization of ova require an appropriate oxidation-reduction potential.[24, 25] Since fertilization occurs in primitive forms outside of the body, where the concentration of oxygen may be appreciable, the maintenance of an environment that permits anaerobic metabolic activity requires that substances be present that can prevent the union of oxygen with the cytochrome system. Barron *et al.* have documented the importance of sulfhydril compounds in relation to the fertilization of arbacia eggs.[10, 11] If materials are added that combine with the soluble sulfhydril groups, there is an increase in the rate of respiration of the eggs, and development is retarded.[98] Addition of larger concentrations of the sulfhydril inhibitory agents reduces the metabolic rate. Parachlormercuribenzoate, an active sulfhydril inhibitor, causes a contraction of the cortical layer of the arbacia egg, and this interferes with the fertilization process.[60]

If a substance is introduced that raises the effective oxidation-reduction potential—e.g., by the addition of an oxidative dye—fertilization can be impeded. Runnstrom demonstrated that porphyrindin or porphyrexide, at a concentration of 5×10^{-6} M, can block the fertilization of sea urchin ova. The addition of sulfhydril-containing compounds, like glutathione, reverses the inhibition by the oxidative dye. Gothié showed that in order that *Ascaris* ovocytes mature, their environment must be anaerobic during a period in which fertilization and the first stages of division take place.[45, 46]

In-vitro insemination of human and rabbit eggs has been described by Shettles and by Moricard.[83, 84, 99–101] In each instance the insemination was accomplished under relatively anaerobic conditions in a medium that contained follicular fluid and fragments of fallopian-tube tissues. It is possible that the follicular fluid and the fallopian-tube tissue fragments may be necessary to contribute the oxidation-reduction systems that permit the glycolytic type of metabolism required for sperm penetration and fertilization.

DISCUSSION

Why anaerobic glycolytic mechanisms may have such an important role in supplying the energy for certain elements of the reproductive process must be a matter for speculation. Tissues in general use this form of energy production whenever rapid proliferation is required.[53] Although a yield of energy-rich phosphate bonds that is as much as 16 times greater is generated by aerobic mechanisms (Fig. 1), anaerobic energy systems prevail whenever delicate differentiation of cellular activity is needed. This may be related to the more intricate energy requirements of the elegantly balanced processes associated with proliferation and differentiation. Control of the required cellular energetics is probably more easily achieved by anaerobic systems than by aerobic systems, with their explosive release of energy that results from the direct utilization of oxygen. An analogy may be seen in the atomic bomb, which produces huge amounts of uncontrollable energy. To apply this energy, one must devise a number of impediments that harness the energy, but at the same time reduce the total energy obtainable. This is also presumably the reason that the intricate mechanism of patterned muscle contraction, with all its nuances of movement, which require a multitude of energy gradations utilizes anaerobic metabolism as its immediate energy source.

In the gravid uterus, glycolytic systems predominate during the proliferative stages, in which the framework and cellular structures of the endometrium must be organized. However, during the ensuing secretory phases, when the function of the tissues is to manufacture rapidly utilizable materials for the maintenance of fertilized ovum and the developing embryo, the tissues utilize the richer energy sources associated with oxidative respiratory systems.

Merely increasing the metabolism of the cell by providing greater amounts of energy does not necessarily lead to acceleration of the proliferative process. The increased energy must be channeled in a given direction in order to facilitate the processes that are necessary for the construction of the tissue. The addition of substances that disrupt the usual pattern of energy transference can disorganize this channelling.[98]

Future investigation into the validity of the views presented in this article may help explain the infertility of the "otherwise normal couple." There are several possible foci that may be aberrant. The oxidation-reduction potential within the female reproductive tract may not be conducive to maintenance of spermatozoa or to proper fertilization. The metabolic patterns within the spermatozoa may not be organized to adjust to the vicissitudes of the changes in the oxidation-reduction potential in patients with inherent metabolic errors or with borderline aberrations in their endocrine systems. The clinical estimates of viability of spermatozoa based on observations of motility must be looked upon as an index of the ability of the spermatozoa to survive, and as such, are extremely useful. However, it should be kept clearly in mind that the in vitro conditions under which these observations are made are not comparable to the in vivo environment to which the sperm is exposed, especially with regard to the difference in oxygen tensions, and also the chemical substrates found in the luminal fluids. If anaerobiosis plays an important role, as has been suggested in this article, then perhaps the appraisal of viability of spermatozoa should be made under anaerobic conditions similar to those that may be expected to be present in the female reproductive tract. This factor may be extremely important in understanding the reasons for the infertility of the otherwise "normal couple." It is quite possible that spermatozoa motility as observed in the usual manner does not reflect the actual conditions in the female partner. The oxidation-reduction potential in her reproductive tract may be so altered that an otherwise normal spermatozoa cannot maintain its viability long enough. Sillo, after discussing factors that restore or enhance spermatozoa motility states: ". . . . the adding or invoking of motility of spermatozoa by any of the above solutions does not necessarily mean that their fertilizing ability has been improved." Evaluation of the integrity of spermatozoa by measurement of motility alone may not be a real index of their ability to fertilize. Excessive motility may represent a wasteful dissipation, by disorganized metabolic patterns, of energy that should have been directed toward maintenance of the ability to fertilize. An analogy may be seen in the tachycardia and fibrillation that are manifested by a failing heart.

The maintenance of an environment conductive to anaerobic metabolism—which may involve the maintenance of an adequate supply of substances that permit anaerobiosis, even though oxygen is available—seems to depend primarily upon the action of estrogen. Pretreatment with estrogen is necessary for fertilization of normal ova placed in an ovariectomized rabbit.[85] The effect of the progestational steroids may be such as to interfere with the biochemical pattern required for support of this anaerobic environment. Chang has called attention to the importance of "capacitation" of spermatozoa—the physiologic changes they must undergo in the female before acquiring the capacity to fertilize ova. Estrogen is required for this process. Progesterone may inhibit the capacitation of sperm by inducing an unfavorable uterine environment.[27]

Implications of the influence of other steroids may be seen in the study of Roberts *et al.*, who demonstrated that within 4 hr., estradiol-17-beta injected intravenously into ovariectomized rats produced a marked elevation in glycolysis of the uterine tissues, although there was no concomitant enhancement of respiration. The administration of cortisol acetate could prevent the accentuation of glycolysis induced by the estradiol. It would appear, then, that the maintenance of the anaerobic metabolic environment necessary to sustain spermatozoa in the female reproductive tract and also the conditions required for normal fertilization requires a multiplicity of endocrine factors operating in concert, including estrogen, its metabolites, and other steroid elements.[113]

SUMMARY

Subtle aberrations in the biochemistry of the reproductive tract may be the cause of elusive sterility problems in the "otherwise normal couple." Integrated hormonal influences regulate the cyclic metabolic changes necessary to provide the proper chemical environment for both the maintenance of sperm viability, and also the fertilization and subsequent development of the ovum. The evidence for the important role anaerobic mechanisms may play in these phenomena is discussed.

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International Fertility Association

The North American Conference on Infertility, sponsored by the International Fertility Association, will be held at the Barbizon-Plaza Hotel, New York, June 23–25, 1961.

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