CLINICAL PATHOLOGY IN GENERAL PRACTICE

THE ASSESSMENT OF GONADAL FUNCTION

BY

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In both sexes the gonads serve two functions: the secretion of the sex hormones and the production of germ cells. These two functions are not entirely independent. Assessment of endocrine function can be made by purely clinical means and by special investigation. The gametogenic function can be studied by special tests alone.

Normal Development

The endocrine activities of the gonads can be considered in four phases: pre-pubertal, at puberty, in the adult, and after the close of the reproductive period.

Before puberty the gonads are physiologically functionless, not apparently through any intrinsic incompetence but because they receive no gonadotrophic stimulation. It is obvious, therefore, that before puberty a diagnosis of hypogonadism is meaningless. At a variable age, usually falling within the range 10–16 years, and for reasons which are quite unknown, the anterior lobe of the pituitary gland begins to secrete effective amounts of gonadotrophic hormone, which stimulates the gonads. Under this influence they secrete their respective sex hormones, which, acting upon their various target organs, bring about the changes of puberty. In the male the interstitial cells of Leydig secrete testosterone; and at the same time the hitherto inert germinal epithelium of the seminiferous tubules begins to proliferate and to differentiate into spermatozoa. Thus, in the male, endocrine and gametogenic functions begin at the same time.

In the female events are rather different. It appears that oogenesis—the production of potential ova—is a wholly foetal phenomenon, so that the newborn female infant possesses preformed all her potential germ cells—perhaps some 400,000. A large proportion of these disappear through atresia before puberty is reached. Thereafter, again the majority are destined to undergo atresia and no more than some 400 are ever likely to become mature ova. At puberty in the female gonadotrophic stimulation leads to the development of Graafian follicles which secrete oestrogen. It is probable that during the first year or two of post-menarchal life no ovulation usually occurs. During this phase menstrual periods are the result simply of cyclically waning oestrogen levels. Eventually, however, one of the maturing follicles becomes predominant, outstrips the rest, and from it the ovum is extruded. Immediately following this event the corpus luteum is formed and secretes progesterone as well as oestrogen. If the ovum is not fertilized and does not become embedded within the endometrium, the corpus luteum begins to degenerate and the levels of ovarian hormones fall. Following this, menstruation takes place and the cyclic changes recommence.

In the male the reproductive phase usually has no clear ending. The testis may continue to produce its hormones and spermatozoa, though on a gradually waning scale, right into old age. For this reason the "male climacteric" does not usually exist or have any outward manifestation. Occasionally, however, testicular function may decrease more abruptly, with the production of climacteric symptoms—such as loss of energy, fatigability, impaired memory and powers of concentration, reduced libido and potency, hot flushes, and so on. These symptoms can be relieved by the administration of testosterone.

In the female the Graafian follicles are used up as described above. When no more remain the ovary ceases to

produce effective amounts of oestrogen. For some time before this final stage is reached the endocrine function of the ovary becomes inadequate to maintain regular menstruation. At the menopause all menstruation ceases. Because the pituitary is no longer subjected to the inhibiting influence of oestrogen, it produces excessive amounts of gonadotrophin, which may be detected in the urine. Other endocrine glands—thyroid and adrenal cortex—tend to become hyperfunctional and so to cause the climacteric symptoms, until a new balance is eventually re-established.

Hypogonadism

Hypogonadism in both sexes may be either *primary* or *secondary*. In the former the gonad itself is the defective organ, failing to respond to gonadotrophic stimulation. In the latter there is failure of gonadotrophin production by the pituitary. It may be possible to differentiate these two types clinically. A clear-cut syndrome—Turner's syndrome—of primary hypogonadism was first recognized in the case of the female, but male examples of the condition are now known to occur. In this there is an absence for all practical purposes of the gonad, and the individual remains stunted in growth, often with a typical squat appearance, and a number of congenital anomalies usually coexist. Outstanding among these are webbing of the neck and cubitus valgus; others include coarctation of the aorta of varying degrees, supernumerary digits, squints, etc. Some elevation of blood pressure is almost invariable. Complete failure of the secondary sex characters occurs.

The key laboratory finding is an increased excretion of gonadotrophic hormone in the urine. This is due to the fact that the sex hormones normally produced by the gonads exert an inhibiting action on the pituitary production of gonadotrophin so that in their absence the pituitary becomes hyperactive. In secondary hypogonadism the characteristic appearance noted above is not found. The individual remains more or less infantile and may be either tall or short. Examination of the urine fails to reveal the presence of pituitary gonadotrophin. In these individuals, therefore, the fault lies in the production of gonadotrophin by the pituitary, the gonads themselves being essentially normal, though remaining unstimulated.

Androgen Production

In the male the determination of androgen production by the testis would theoretically be the logical method of obtaining an objective assessment of gonadal function. Androgen assay, however, is not a practical procedure for clinical purposes. It can be done by preparing suitable urinary extracts and testing them for their ability to induce growth of the capon's comb when applied direct to it. A more practical method is to estimate the 17-ketosteroid excretion, which can be done by purely chemical means. The 17-ketosteroids—so-called because they possess a ketone group on the 17 carbon atom of the steroid nucleus—are among the metabolites of testosterone. The position is complicated, however, by the fact that the adrenal cortex also produces androgens, some of which are metabolized to form 17-ketosteroids. It is therefore clear that, even in the absence of testicular testosterone, 17-ketosteroids will be present in the urine. Indeed, they may be produced by normal women in amounts not very different from those produced by normal men.

The mean figures for children of both sexes rise from the order of 1 mg. per 24 hours to 5 mg. before puberty. From then on the mean increases more rapidly for males than for females, reaching 14 mg. for adult males and about 9.5 mg. for adult females. The ranges for adults show considerable overlap in the two sexes, being 8–32 mg. for males and 5–20 mg. for females. In old age the mean figure again falls off with increasing years. It is thus quite obvious that the interpretation of the significance of the 17-ketosteroid excretion is none too easy, and the test has only a limited value in the assessment of male gonadal function. Of distinct value in some instances is the study of testicular biopsies. These are not difficult to carry out as a purely out-patient procedure under local analgesia, cause no undue discomfort and no loss of time from work, and enable one to assess the status of the Leydig cells, which are the source of the male hormone.

Oestrogen Production

In the female the simplest and one of the most useful objective tests of gonadal endocrine function is the study of the vaginal smear. This is obtained by inserting a cotton-wool applicator into the vagina, twirling it round a few times, and then rubbing it on a clean microscope slide. To obtain the best results this should be fixed immediately by immersion in equal parts of absolute alcohol and ether, and then stained with haematoxylin and a suitable differentiating stain such as Shorr's. In the absence of oestrogenic stimulation, the vaginal smear consists almost exclusively of small rounded basal cells with relatively large vesicular nuclei, and frequently contains many leucocytes. The fully oestrogenized smear consists of large squames which may have curled-over edges and contain either small densely staining pyknotic nuclei or larger vesicular nuclei (late intermediate cells). Intermediate grades may contain varying proportions of these different types of cell (Fig. 1). One

FIG. 1.—The principal types of epithelial cell found in vaginal smears: (a) basal; (b) primitive; (c) late intermediate; (d) fully cornified, with pyknotic nucleus.

drawback to this method of oestrogen assessment is that the vaginal epithelium is very sensitive to oestrogens, so that a very significantly reduced level must exist before it becomes apparent from the vaginal smear.

Another wholly practical method of oestrogen assessment is by the histological study of the endometrial biopsy specimen. This is easily obtained without an anaesthetic in the adult woman, though in girls and young unmarried women the position is, of course, less simple. An atrophic endometrium denotes either deficient oestrogen production or failure of endometrial response. Which of these alternatives is correct can be decided by studying the response to oestrogenic treatment; if, after an adequate course, the endometrium is still atrophic, a failure of target organ response may be concluded. A hyperplastic proliferative endometrium suggests a high oestrogen level but does not prove it, since the same conditions can result from prolonged though moderate oestrogenic stimulation. Under the influence of progesterone the proliferative oestrogenic endometrium becomes converted into a secretory type. Inadequacy of secretory development in the endometrium obtained during the latter part of the menstrual cycle suggests deficient production of progesterone. Once again, however, this result can arise through failure of endometrial response and can be tested for in the same manner as described above.

The assessment of oestrogen production by the determination of oestrogen excretion or levels in the blood is not yet a procedure of clinical value. In the case of progesterone, however, the estimation of urinary pregnanediol excretion is a not too difficult laboratory procedure and sometimes has practical value. Nevertheless, in normal menstrual cycles the variability of pregnanediol excretion is considerable, so that it is necessary to make several determinations on 24-hour urine collections during the supposed luteal phase in order that useful information may be obtained. It should further be pointed out that accurate methods capable of dealing with small quantities of pregnanediol must be used, since the output may be very small. Less accurate methods, which may have their value during pregnancy, are useless in the non-pregnant state. It is generally believed that the level of pregnanediol excretion reflects the level of production of progesterone by the corpus luteum, but there is no certainty that the parallelism is at all exact.

Gonadotrophin Assay

Mention has already been made of gonadotrophin estimation in both sexes and its value in differentiating primary from secondary hypogonadism. A few words about this estimation may therefore be appropriate. The test is usually referred to as the assay of follicle-stimulating hormone (F.S.H.), since F.S.H. probably is the main gonadotrophin found in non-pregnant urine. There is no doubt, however, that in some circumstances the urinary gonadotrophins include luteinizing hormone (L.H.), so that the test is perhaps better referred to simply as "gonadotrophin assay." The test requires a 24-hour specimen of urine, which should be kept chilled (preferably on ice) during the collection and should be delivered to the laboratory as soon as collection is complete. The urine is subjected to one of a variety of extraction procedures which aims at recovering the total protein content.

The methods which are in use include alcohol precipitation, ultrafiltration, and kaolin adsorption; of these the last is perhaps the most satisfactory technique. The extract is made up into a small volume with saline or distilled water, and groups of immature female mice are injected with fractions at various dilutions. At the end of the injection period (usually four days) the mice are killed, the uteri are removed, dissected free from other tissues, blotted dry, and weighed. Different laboratories have their different "mouse units"; the most commonly used definition is that quantity of gonadotrophin which will cause an increase of 100% in the mean weight of the uterus as compared with that of the controls. It must be emphasized that this assay is a relatively crude affair and that only large differences in gonadotrophin content can be detected. Generally speaking, it is impossible to say that the gonadotrophin excretion is definitely less than normal; at most one can say that the excretion is within the normal range or definitely increased, but this information is usually all that is required.

Semen Analysis and Testicular Biopsy

In the assessment of gametogenic function in the male the key test is the seminal analysis. To be of value the seminal specimen for this purpose should be collected by masturbation after a period of abstinence of three to five days (which should be stated). The specimen jar should be clean, dry, and warmed to something approaching body temperature. The time of production of the seminal specimen should be known. For religious or moral reasons, some men may find themselves unable to comply with these instructions; for them there is no really satisfactory alternative. It cannot be too strongly emphasized that condom specimens are utterly valueless; the presence of various chemicals in the sheath leads to the rapid immobilization of spermatozoa and often to their clumping, so that when examined the spermatozoa are invariably all dead and sperm counts are usually grossly misleading. The production of

seminal fluid by coitus interruptus again usually leads to gross errors, since the ejaculate as produced is not homogeneous and variable losses always occur when this method is attempted. If masturbation cannot be found acceptable, the only reasonable alternative is examination of the wife's cervical mucus some time after intercourse—the test being arranged during the ovular phase of the cycle. Such a test will at least determine whether active spermatozoa are being produced in reasonable numbers or not, though more precise information cannot of course be obtained.

The report on the seminal fluid should note its volume and appearance, the sperm density in millions per millilitre, the proportion of morphologically abnormal spermatozoa, and should give an estimate of the activity and viability of the spermatozoa.

The mean volume of semen is about 3.5 ml.; a volume of less than 1.5 ml. may be of serious consequence, since the interface between semen and cervical mucus after ejaculation within the vagina may be so small as to permit few spermatozoa to penetrate the mucus. On the other hand, if the volume is very large, a normal total number of spermatozoa may be so diluted that again relatively few are able to reach the cervical mucus.

Opinions vary on the lower level of sperm density compatible with normal fertility. Recent accurate studies have tended to lower this figure as compared with former views. It is probable that, provided other features of the seminal fluid are adequate, a density of more than 20,000,000 per ml. is quite satisfactory.

Density aside, it seems that the activity and viability of the spermatozoa are the most important criteria for evaluating fertility. Unfortunately, methods for the accurate determination of these criteria still leave a good deal to be desired. They also vary in different laboratories, so that it is difficult to compare the reports from the various centres. It is fair to conclude that highly fertile seminal fluid contains more than 40% of actively motile spermatozoa which retain their motility for periods in excess of 12 hours *in vitro*.

Although a great deal of attention has been paid to morphological abnormalities, it is doubtful whether most of these studies are really justified. In general, provided the proportion of total abnormal forms does not exceed 40%, the morphology of the spermatozoa is of much less importance than their activity.

Testicular biopsy has already been mentioned. Its value in the assessment of gametogenic function is twofold. In the first place, in cases of azoospermia testicular biopsy enables one to decide whether this is the result of failure of sperm production or failure of sperm delivery through obstruction of the efferent ducts. In the former circumstance the biopsy would reveal absence of spermatogenesis and in the latter a normal testicular structure. In the second place, the biopsy is of prognostic value where the seminal fluid is grossly inadequate, since a study of the histology of the testis may enable one to decide whether treatment has a chance of effecting improvement or could not possibly be of value. It is a fact that in most cases of oligospermia the biopsy reveals severe disorganization of the germinal epithelium in most tubules, while the other tubules are unaffected; clearly in these cases the stimulus to the testis must be normal and its modification by treatment cannot hope to produce any tangible results.

Tests for Ovulation

The direct study of ova is unfortunately not a practical procedure; we are, for example, quite unable to say whether the ova are morphologically normal or not, even though ovulation appears to be taking place. Our assessment of the occurrence of ovulation or its failure has to be made on deductive grounds. The principal methods in use for the determination of the occurrence of ovulation are: basal body temperature records, endometrial biopsy studies, and vaginal smear examinations.

Basal Body Temperatures.—If the temperature is carefully taken every morning as soon as the patient wakes up, and before any activities are undertaken, characteristic changes are found in ovular cycles. It is of little consequence whether the temperature is taken orally or rectally; provided the thermometer is left in place for three to five minutes and the mouth is kept closed, oral temperatures are normally quite satisfactory. Generally speaking, the temperature runs at a mean value of about 97.4° F. (36.3° C.) during the first half of the cycle, then it rises and thereafter runs at a level of approximately 98.2° F. (36.8° C.) until shortly before the period begins. It then falls to its previous level and the changes are repeated in the following cycle. In this way a biphasic pattern is produced, and this pattern is characteristic of ovulation (Fig. 2). The temperature rise during the latter half of the cycle has been shown to be due to the action of progesterone. It follows therefore that such a pattern could occur even in the absence of ovulation, provided a corpus luteum were formed. Indeed, this happens occasionally,

FIG. 2.—Basal temperature records. The black shapes represent menstrual bleeding. (a) The biphasic record of regular ovular cycles. (b) An anovular cycle, with no rise of temperature (monophasic) and relatively scanty period, between two ovular cycles. (c) Irregular ovular cycles; the intervals from the start of the periods to the arrows marking the occurrences of ovulation are inconstant, but the post-ovular intervals are of more uniform duration. (d) The temperature rises slowly in the post-ovular phase in this record. (e) The temperature falls slowly from the post-ovular plateau. (f) A combination of (d) and (e), so that little more than a mid-cycle depression remains to represent the biphasic pattern. (g) Here conception has occurred and the post-ovular plateau persists. (h) In this case delayed ovulation, not conception, accounts for the non-appearance of the period.

the ovum being trapped within the follicle instead of being extruded. Nevertheless, trapped ovum is probably so rare a phenomenon that it is safe to say that regular biphasic basal temperatures reflect regular ovulation. It cannot be too strongly emphasized that the temperature record merely indicates that ovulation *has* occurred. It does not indicate with any certainty *when* ovulation occurs and it certainly cannot predict when ovulation is *going* to occur. Consequently, the advice sometimes given to women wishing to conceive—to time intercourse in accordance with their temperature record—is likely merely to perpetuate their infertility, since, once ovulation has occurred, intercourse can no longer lead to the fertilization of that particular ovum. If ovulation is not taking place the biphasic pattern described above does not appear, the temperature running a more or less uniform course. When the cycles are irregular but nevertheless ovular, the temperature pattern reveals that it is the pre-ovular rather than the post-ovular phase of the cycle which is variable. If the period is overdue the basal temperature record may indicate whether the delay is due to conception or to delay in ovulation. In the former circumstance a normal pre-ovular phase followed by a prolonged post-ovular plateau will be found; if the plateau exceeds three weeks the existence of pregnancy is almost certain. In the latter case either the post-ovular rise will be missing or it will have followed a prolonged pre-ovular interval.

Endometrial Biopsy.—An endometrial biopsy taken shortly before a period is expected will reveal a secretory pattern in an ovular cycle. Such a result indicates the existence of a functional corpus luteum, from which the conclusion that ovulation has occurred is usually justifiable. If the periods are irregular it is best to take the biopsy on the first day of bleeding; if the bleeding is taking place from a proliferative type of endometrium an anovular cycle may be inferred.

Vaginal Smear.—If daily vaginal smears are followed characteristic changes occur at and after the time of ovulation, from which it is possible to infer that this event has occurred. It must be emphasized, however, that *daily* smears are necessary for such a study. The method is thus of limited value and can be applied only to patients in hospital, or to those who can be instructed to take and prepare their own vaginal smears.

The detection of pregnanediol in the urine during the latter half of the cycle has sometimes been advocated as a test for the occurrence of ovulation. Clearly, it is in general of less practical value than temperature records or endometrial biopsies.

Observations on changes in the cervical mucus have also been advocated for the detection of the occurrence of ovulation. Their limited usefulness does not warrant further elaboration.

Next article on Clinical Pathology.—"Liver-function Tests," by Professor N. F. MacLagan.

The Wellcome Foundation, Limited, has increased its authorized capital from £1m. to £3m. The issued capital has been increased to the same amount. At the time of his death in 1936 Sir Henry Wellcome owned all the shares in the Wellcome Foundation, Limited. By the terms of his will he established the Wellcome Trust, a public charity administered by five trustees to whom all the shares were bequeathed. The objects of the Trust are the furtherance of research work bearing upon medicine, surgery, and allied sciences, and the entire declared dividends of the company are at the disposal of the trustees. The Wellcome Foundation, Limited, trades under the name of Burroughs Wellcome and Co., and the capital increase will bring the issued capital more into line with the capital actually employed in the business. It foreshadows a large programme of expansion starting with a new £350,000 pharmaceutical manufacturing unit at the Wellcome Chemical Works, Dartford, on which work has already started.

Reports of Societies

THE CLINICAL ASPECTS OF IMMUNITY

A symposium on the "Clinical Aspects of Immunity" was held on March 23 at the Royal Society of Medicine, under the chairmanship of the president of the Section of Medicine, Sir HENRY COHEN. The opening speaker, Professor A. A. MILES (Lister Institute), emphasized that specific immunity usually meant antibody immunity. The two factors to be considered were the capacity of the host to produce protective antibody, and the ability of the bacterial antigens to induce antibody formation. Bacteria contained many antigens, only a few of which were capable of inducing protective antibodies. The body did not discriminate, and was as likely to make an irrelevant response to the antigens of an invading organism as it was to produce a protective antibody.

It was difficult to separate relevant from irrelevant antigens for the production of vaccines, and to assess the results of immunization. The ideal test of a vaccine was a controlled clinical trial in a susceptible community, but this had rarely been made. Vaccine had therefore to be devised largely on the basis of laboratory evidence, which was valuable but required careful interpretation.

In diseases like diphtheria, immunity was very largely antitoxic, but in invasive diseases such as cholera, enteric fever, and whooping-cough the role of toxins was less certain. Resistance to endotoxins did not always confer protection against infection by the living organism. Endotoxins were often also surface antigens; in cases where anti-endotoxic antibodies had been shown to be protective the reason was probably that they were acting as opsonins, not as antitoxins.

The search for relevant immunizing antigens had been outstandingly successful in the pneumococci. When virulent pneumococci lost their capsule they lost virulence, became useless as vaccines and susceptible to phagocytosis. Anti-capsular antibody was protective against infection and was clearly an opsonin. Another example of a non-toxic surface antigen largely determining protective immunity was the M-protein of *Str. pyogenes*. Although *H. pertussis* possessed several surface antigens, none had been conclusively proved by laboratory methods to be protective. The effective antigen had not yet been demonstrated by *in vitro* reactions.

In addition to toxins and surface antigens determining resistance to the host's defences, bacteria might possess antigenic substances that appear to be auxiliary pathogenic factors. These should not be accepted as important for artificial immunization unless there was evidence, not only that they were associated with the virulent form of the organism and had an observable effect on the host in conditions resembling those of natural infection, but that they were capable of inducing protective antibodies. For example, streptococcal hyaluronidase and fibrinolysin could not be implicated, because they did not immunize against streptococcal infection.

Antibody Formation in Virus Diseases

Professor A. W. DOWNIE (Liverpool University) spoke on the relation of antibody formation to the course of virus diseases. Not all viruses were good immunizing agents. Acute generalized diseases with viraemia such as measles and smallpox often gave lifelong immunity with high titres of circulating antibody. Localized surface infections such as influenza and the common cold gave poorly maintained immunity. Lymphogranuloma and psittacosis viruses were poor immunizing agents. Relapse was common, and the complement-fixing antibodies produced had little relation to protection.

The relation of antibody formation to the course of the virus infection could best be considered in the acute