THE ENDOCRINES IN THEORY AND PRACTICE

This article is one of a series on Endocrinology contributed by invitation

LABORATORY TESTS FOR THE EARLY DIAGNOSIS OF PREGNANCY

BY

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The widespread demand for a means of recognizing the existence of pregnancy in its very earliest stages has been successfully met. No fewer than 10,000 pregnancy diagnosis tests are being performed annually in Great Britain alone, and there is every reason to believe that as their usefulness becomes more generally known this number will increase. The routine tests now used are biological tests, but it is not improbable that these will give place later to chemical tests, and it is to this end that much research is being directed.

These biological tests demand the use of some experimental animal whose reactions will make it possible for the enormous increase in the quantities of certain hormones excreted in the urine during pregnancy to be detected and registered. From puberty to menopause oestrin and gonadotropic hormones are at all times being elaborated by the ovaries and anterior pituitary respectively, transported in the blood stream, and excreted by way of the urine and faeces. Oestrin is the general name given to a closely related group of hormones which, when administered to the test animal (usually a mature ovariectomized rat or mouse), provoke certain characteristic reactions normally associated with oestrus—for example, turgescence of the vaginal orifice, cornification of the vaginal mucosa, and distension of the uterus with fluid. The gonadotropic hormones are so called because they control the activities of the gonads, directing their growth, and the induction of those changes which normally are associated with sexual maturity—for example, follicular maturation, ovulation, and corpus luteum formation in the female, spermatogenesis and hypertrophy of the interstitial tissue in the male. The test animal here is either the immature individual (usually mouse or rabbit), in which, by reason of age, the ovary is still inactive, or else the adult animal, in which the gonad is known to be quiescent for the time being.

The amounts of oestrin and of gonadotropic hormones present in the blood, urine, and faeces vary from individual to individual, and from time to time. There is, for example, an increase at the time of the menstrual cycle. But such fluctuations in the non-pregnant woman are negligible when contrasted with the relatively enormous increase in hormone transportation and excretion during pregnancy. During the first few weeks following conception the amount of oestrin (some 300 to 600 mouse units per litre) elaborated by the maternal ovaries (and probably also by the placenta) is not so very much greater than that associated with the menses. It has been estimated that during one complete menstrual cycle in the "standard woman" some 800 to 1,500 mouse units are excreted in the urine. A mouse unit is that amount which is required, under standard conditions, to evoke the typical oestrous reactions in at least ten out of twenty mature ovariectomized mice. But after the eighth week of pregnancy the increase in oestrin excreted is exceedingly great and rapid, until many thousands of mouse units are passed daily. As pregnancy approaches its termination there can be as much as 20,000 mouse

units a litre of urine. Towards the end of the first week of the puerperium this oestrin excretion has again reached its usual non-pregnant level. The increase in the amount of gonadotropic hormones excreted in the urine following conception is equally great, but is much more rapid; it reaches as much as 5,000 to 30,000 mouse units per litre in the first few weeks of pregnancy, and thereafter drops slowly and somewhat irregularly as pregnancy proceeds, to reach its non-pregnant level by about the third day of the puerperium.

Choice of Test

This difference between the times, in relation to conception, at which excretion in the urine of oestrin and of gonadotropic hormones respectively reaches its maximum is the deciding factor in the choice of a pregnancy diagnosis test. In the human female the maximum excretion of gonadotropic hormones in the urine is reached almost immediately following the implantation of the ovum, whereas that of oestrin is reached only after the second month of gestation. For this reason the popular test for human pregnancy is that which detects and reveals this immediate and enormous increase in the amounts of gonadotropic hormones excreted in the urine.

Conception is followed also by a very marked increase in the oestrin and gonadotropic hormone content of the blood, but it is to be noted that the maximum hormone content of the blood does not coincide in time with the maximum rate of excretion by way of the urine, for the amounts in the blood are at a minimum during the early stages of pregnancy, when excretion is at a maximum. This is a sufficient reason in itself for using urine rather than blood in these tests. After parturition, and also immediately subsequent to the death of the embryo *in utero*, the quantity of blood oestrin tumbles down with remarkable rapidity. This fact forms the basis of the blood-oestrin test for foetal death.

In the non-pregnant subject the gonadotropic hormones are of hypophyseal origin, but during pregnancy in the human female the pituitary contains no gonadotropic hormones whatsoever, whereas the placenta contains relatively enormous amounts—for example, 1,144 mouse units at the seventh week and 5,800 mouse units at term. For these and other reasons it has come to be generally accepted that the placenta is itself the site of gonadotropic hormone as well as of oestrin manufacture, and does not serve merely as a reservoir. That this is so is also strongly supported by the fact that the presence in the body of placenta-like tissue, such as a hydatidiform mole or a chorion epithelioma, is usually associated with the production and excretion of very large quantities of gonadotropic hormones, larger indeed than those that are found in ordinary pregnancy urine. This difference in the quantity of these hormones in pregnancy urine and in urine from cases of mole and chorion epithelioma respectively furnishes the basis for tests which distinguish between the two conditions, and which recognize the recrudescence of chorion epithelioma.

These gonadotropic substances present in the urine in cases of pregnancy and mole are not completely identical, in respect of the effects they produce in the test animal, with those of anterior pituitary origin. In both, the follicle maturation hormone (prolan A) and the luteinizing

hormone (prolan B) are present, but in the latter the follicle maturation factor predominates, whereas the gonadotropic hormones elaborated by chorionic tissue consist almost entirely of the luteinizing factor. For these reasons anterior pituitary extracts evoke growth and development of follicular granulosa and ova, luteinization of granulosa and theca, ovulation, and hyperaemia of follicles and corpora lutea; whereas pregnancy urine, with its anterior-pituitary-like (A.P.L.) substances, yields but little follicle maturation, luteinization of the theca, atresia of follicles without ovulation, and hyperaemia of follicles and atretic corpora.

Variations in Biological Assays

Out of these facts and speculations the various biological tests for the early diagnosis of human pregnancy have been evolved. They differ among themselves in respect of the test animal, the method of preparation of the urine before injection, and the amount injected, but they are all tests for a significant increase in the amount of gonadotropic hormones excreted. The choice of the test animal is determined by several considerations. It must be such as can be readily obtainable locally at all times; it must be in harmony with the general conditions of the place where it is to be used, in respect of accommodation, food, and husbandry; it must be such as can receive reasonably large quantities of urine (or extract) in as short a time as possible, and be sufficiently delicate in its reactions thereto to give unequivocal results. The methods of preparation of the urine are many and various; some workers inject native filtered early morning urine; by others the urine is rid of its urates, treated with hydrochloric and sulphosalicylic acid and/or washed with ether, in attempts to remove all traces of oestrin, to bring down all surplus proteins, and to render the urine as innocuous as possible. All these methods seem to be equally satisfactory in the hands of different people. The amount injected is determined, among other things, by the size of the animal, the degree of concentration of the urine, the site of the injection, and by the duration of the test. All other things being equal, the more gonadotropic hormones that pass into the circulation within a limited period of time the quicker will the gonads react and the sooner the result will be obtained. But whatever the amount, and whatever the site of the injection, a certain time must necessarily elapse before the ovarian reaction becomes sufficiently obvious and trustworthy.

The Aschheim-Zondek Test

The ordinary Aschheim-Zondek test requires batches of immature female mice, which are injected with small amounts (0.4 c.cm.) of prepared urine six times at regular intervals, and killed after 100 hours. Over a period of many years, and during the course of tens of thousands of instances, its results have been in disagreement with the clinical histories in not more than 1 per cent. of cases. If the concentration of the gonadotropic hormones in the injected urine is sufficiently high, as it is in pregnancy, a positive result is obtained, and in the ovaries white spots and blood spots are discernible to the naked eye. If the concentration of these hormones is not sufficiently high, as is the case if pregnancy does not exist, these ovarian changes will not have been induced and a negative result is recorded. Five days is, of course, a long time to wait, and so several tricks, intended to shorten the time required for the test, have been invented. For example, the urine can be injected into the tail vein of adult mice, which are subjected to red-light irradiation both before and during the test, when the time taken, it is claimed, can be reduced to forty-eight to fifty hours. Rats have been used instead of mice, and as much as 8 to 12 c.cm. of

urine injected in a series of doses over a period of six to eight hours. It is claimed that a positive result, taking the form of intense hyperaemia and hypertrophy of the ovaries, can be obtained after thirty hours.

The Friedman Test

But in cases in which urgency is an important factor the Friedman is the better test. Sufficient experience has shown that it is no less trustworthy than is the Aschheim-Zondek. In this test, in which adult rabbits are used, the positive reaction consists in actual ovulation, a phenomenon which occurs normally only after mating or some equivalent excitation. For this reason the rabbits used must have been isolated for three weeks or more, and in order to make absolutely certain that the rabbit itself is not a source of error in a test in which medico-legal interests are involved, it is customary to perform a preliminary laparotomy before the injection is given in order that the ovaries themselves can be inspected.

A trustworthy positive result can be accepted within thirty hours of an injection of, say, 10 c.cm. of native filtered urine into the ear vein. It is because the test animals can differ among themselves in respect of their ability to react to the hormonal stimulus offered that more than one animal is employed in the Aschheim-Zondek and Friedman tests. The use of five animals in the former reduces to a minimum any error due to any individual idiosyncrasy; in the case of the Friedman test it is usual to use two rabbits, each of which is injected. One of them is examined at the end of thirty hours—if the result is positive it can be accepted as being trustworthy; if, on the other hand, there is no evidence of ovulation in its ovaries on inspection the second rabbit receives a further injection of 10 c.cm., and is examined thirty hours later. If this also gives a negative the result may be regarded as reliable. It has been claimed that an even more rapid and delicate reaction is obtained if the rabbits have been operated on previously, and one ovary, tube, and horn removed. It has also been reported that the reaction becomes much more definite if the injection is combined with maceration of the pineal. Further, it has been stated that the pupillary reaction of the rabbit to serum injected into the conjunctival sac immediately after the injection of the urine has given a high degree of accuracy in diagnosis.

The Brouha Test

A third test, the Brouha, takes advantage of the fact that these gonadotropic hormones can cause a definite enlargement of the seminal vesicles of the male mouse. A variant of this test employs the rat, in which the ratio of the weight of the vesicles in milligrams to the body weight in grams is constant. It has been shown that the increase in the size of the vesicles induced by injection of pregnancy urine or blood is proportionate to the concentration of the gonadotropic hormones. But since these tests require seven to ten days to complete, and possess no special advantages, they are not commonly used.

In other countries, and under other conditions, other test animals are to be preferred. Thus in South Africa the female claw-toed frog (*Xenopus laevis*), which is readily obtainable, would seem to be ideal. Two or three cubic centimetres of prepared pregnancy urine are injected intraperitoneally into each of six frogs. If the animals are kept at room temperature (18° C.) a positive reaction is indicated twelve to eighteen hours later by the extrusion of ova through the cloaca or by the presence of eggs in the oviducts. If the animals are kept at 27° C. this result can be obtained as soon as five to six hours after injection. This test has given a very high degree of accuracy, but since it has been found that the frogs to be

tested must not have been kept under laboratory conditions for more than a month their use becomes restricted to those countries where fresh supplies can be quickly secured at regular intervals.

The bitterling (*Rhodeus amarus*) has also been employed as a test animal. This small fish is easily obtainable, but its maintenance under laboratory conditions demands a certain skill. The female (not in breeding condition) has a minute oviducal tube projecting from its ventral surface. If 5 c.cm. of pregnancy urine is added to 2 litres of aquarium water, and if the test is positive, there is a lengthening of this tube by 100 to 300 per cent. within seventy-two to one hundred hours. If a concentrate of urine is used the result can be gained after twelve to fourteen hours.

Accuracy

In these pregnancy diagnosis tests 90 or 95 per cent. accuracy is not sufficient. Errors there must be, for the hormone concentration during early pregnancy is not the same in all women, and it is possible by pure mischance to use in one and the same test a number of animals every one of which is refractory. Nevertheless the aim in these tests must be 100 per cent. accuracy, and no test which yields more than 1 per cent. of error can claim much support. It is for this reason that most of those who are carrying out these tests continue to rely upon the Aschheim-Zondek and Friedman tests, but everyone is waiting for the discovery of a new test animal, and for the elaboration of new techniques which will shorten the time required without affecting the reliability.

Chemical Tests

These biological tests are necessarily somewhat expensive. Animals must be bred or bought, fed, housed, and cared for; the work requires a Home Office licence and a degree of surgical skill. It is clear that if there could be devised some chemical test which could conveniently be performed in half an hour or so, and which would remove the necessity of maintaining and slaughtering thousands of animals and yet yield a degree of reliability equal to that of the biological tests, it would surely replace them. Several such chemical tests for the recognition of early pregnancy have been devised, but as yet none of them can compare in respect of trustworthiness and simplicity with the biological tests now in use. But recent work seems to show that it is not improbable that sooner or later a simple, inexpensive, and reliable chemical method of pregnancy diagnosis may be evolved.

Voge, six years ago, first suggested that Knoop's bromine water test for histidine might be used as a pregnancy test, in view of the fact that a connexion between the hypophyseal hormones and the compounds of the β -iminazoyl type had been demonstrated. This suggestion provoked at the time a certain amount of experimental inquiry and a great deal more destructive criticism. However, Kapeller-Adler (1933) rescued this test from the limbo of forgotten things, and by introducing certain modifications, which obviated many of the difficulties and removed certain of the causes of error, succeeded in making the test much more reliable. Renton (1935), in reporting on a series of 100 tests, records that among them there were but seven false negatives. This, of course, though infinitely better than Voge's original figures, is not yet nearly good enough. Another chemical test is that devised by Visscher and Bowman (1934). This was critically discussed in a recent number of the *Journal* by Gladys H. Dodds (1936), who has completed the test on 100 pregnancy cases, obtaining a correct result in 90 per cent., and on eighty non-pregnancy cases with an 88.8 per cent. of accuracy. Again these figures make it impossible for the present to substitute this test for the

biological ones that are now employed, but they are encouraging, and it is to be hoped that they will stimulate further research in this subject.

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CENTENNIAL OF THE ARMY MEDICAL LIBRARY, WASHINGTON*

BY

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It is a very high honour as well as a most pleasant privilege to assist at the Centennial of the conception of the Library of the Surgeon-General's Office, United States Army, now containing more than a million items and the largest medical library in the world. George Adami (1914) described the *Index-Catalogue* and the *Index Medicus* as America's greatest gift to medicine, and in his admirable paper "The Centennial of the World's largest Medical Library: The Army Medical Library of Washington," from which I have freely drawn, Major E. Erskine Hume tells us that Professor William H. Welch not only expressed a similar considered opinion in 1921, but at his last visit to the Library placed it above America's services in connexion with anaesthesia, the insect transmission of disease, and the development of public health laboratories.

The transformation of medicine in America during the last twenty years of the nineteenth century and after was due to several causes: among others to the men of Billings's generation, especially Welch and Osler, to the example set by the Johns Hopkins University and Hospital, to the Library of the Surgeon-General's Office, its *Index-Catalogue*, and the *Index Medicus*. The Johns Hopkins Hospital, the Library, its *Index-Catalogue*, and the *Index Medicus* were largely the offspring of Billings's energetic initiative.

It is surely quite unnecessary to attempt to preach to the converted on the value and inspiring virtue of medical libraries, for this has been so conclusively set forth by Billings, by Oliver Wendell Holmes (1809-94) at the first opening of the Boston Medical Library in 1878, by Osler in "Books and Men," also at the Boston Medical Library when a new building was opened in 1901, and in 1926 at Cleveland in the charming address "The Doctor and his Books" by Professor Harvey Cushing, Osler's pupil and Boswell, who could so convincingly have shown here and now how that tradition survives.

The Army Medical Library

The layman in the street might be puzzled to explain why and how it came about that the Surgeon-General of an army, however great, should have such a library as this attached to his office, which is devoted more to active organization and orders than to medical bibliography ancient and modern. The answer, clear to everyone here, is the personality of John Shaw Billings of the Surgeon-General's Office and the creator of its library. Originally a small collection of reference books for the use of the Surgeon-General (Joseph Lovell) in his office at Washington—according to Billings (1880) some time before 1836—it long remained in a state of suspended animation. In 1840 a manuscript catalogue listed 135 works (228 volumes); at the outbreak of the Civil War (1861) the number of volumes was between three and four hundred.

Besides books, pamphlets, the valuable collections of 450 out of the estimated 600 medical incunabula (books

* Commemorative Oration (abridged) given at Washington, November 16th, 1936.