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PREGNANCY DIAGNOSIS IN THEORY AND PRACTICE *

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Recent work in sex physiology has shown that two types of hormones are excreted in the urine of the female subject at various stages of the life cycle. They are: (1) hormones produced by the ovaries (and during pregnancy probably by the placenta), exerting their effect upon the secondary sex organs and having no direct action on the gonads themselves; and (2) hormones capable of influencing the morphological and secretory activity of the gonads, and produced by the anterior lobe of the pituitary (and possibly by the placenta during pregnancy). The former group includes a number of substances to which the name of oestrin has been given, while the term "gonadotropic hormones" describes those substances which are not produced in the gonads themselves, but activate these organs. Oestrin is found in the urine for some days after birth, during the whole of the period of sexual activity, and for at least some years following the menopause. The gonadotropic hormones are excreted in the urine for some days after birth, and during the whole life cycle after the onset of puberty.

The excretion of both these types of hormones is very markedly increased during pregnancy, the amounts found in the urine being largely in excess of those present under any other physiological conditions, and the diagnosis of gestation is essentially dependent upon this fact. But whereas conception is almost immediately succeeded by a tremendous increase in the gonadotropic hormone excretion, which reaches its maximum in the early stages of pregnancy, the increase in the elimination of oestrin occurs more slowly, and is gradual and progressive throughout gestation, reaching a maximum at parturition. The gonadotropic hormone content of the urine, rather than its oestrin content, has therefore largely been employed for diagnostic purposes, as it is more especially in the early stages of gestation that such a test is of greatest value to the clinician. In the mare, however, oestrin is the chief hormone excreted in the urine during pregnancy, and the diagnosis depends upon the recognition of certain quantities of the oestrous hormone (Crew, Miller, and Anderson, 1931).

Tests for Gonadotropic Hormones

Now a number of methods have been elaborated for the determination of the gonadotropic hormone content of fluids and tissues. These tests are dependent upon morphological or secretory changes induced in the organs of injected animals by the material that is being tested. The alterations that may be observed in the gonads of experimental animals subject to the action of gonadotropic hormones are numerous, and may be classified as follows.

	Female	Male
Morphological	1. Follicular maturation 2. Ovulation 3. Formation of corpora lutea (a) True corpora lutea (b) Atretic corpora lutea (c) Corpora haemorrhagica 4. Formation of blood spots	Increase in size affecting apparently chiefly the interstitial tissue
Secretory	1. Secretion of oestrin 2. Secretion of corpus luteum hormones	Secretion of male hormone

There has been much controversy as to whether all these effects are due to a single gonadotropic hormone acting in different concentrations and under different conditions, or whether two or more gonadotropic hormones are responsible for the various effects observed. A careful analysis of the results obtained by a number of investigators (Aschheim, 1933; Evans, 1933; Hisaw *et al.*, 1933; Coester, 1932; etc.) strongly suggests that more than one substance is concerned in the control of the various changes produced in the gonads. Moreover, it can also be asserted that extracts containing gonadotropic hormones and obtained from different sources do not all produce the same set of reactions in the gonads. For example, recent work is showing that extracts made from the pituitary gland possess properties which are not shared by those made from pregnancy urine.

Action of Hormones in Urine

But we are more particularly concerned with extracts from urine excreted under different physiological and pathological conditions, and it appears, on the whole, probable that two types of extracts can be obtained—namely: (1) extracts which produce chiefly follicular maturation and oestrin secretion; and (2) extracts which lead to the formation of ovarian haemorrhages and to the production of luteal tissue and of ovulation.

Now the substances obtained from the urine of female subjects apart from pregnancy conform, on the whole, to Type 1, while the gonadotropic hormones produced during gestation are, as a rule, capable of bringing about the effects described under Type 2. In practice, in the estimation of gonadotropic hormone content of urine, which constitutes pregnancy diagnosis, certain reactions induced in the gonads of the injected animals have been arbitrarily chosen as end-points. And although some observers have attributed most of these reactions to a definite gonadotropic hormone (the luteinizing hormone, prolan B) it must be emphasized that such an assumption, in so far as it relates to pregnancy diagnosis, is at present chiefly

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of theoretical interest. Of practical importance is the elaboration of a method which will give a positive result with the amount of gonadotropic hormones present in a definite quantity of urine obtained from a pregnant subject, and a negative result when a similar amount of urine from a non-pregnant subject is employed. Such an end has been attained by a variety of techniques, among which the most important are: (1) the Aschheim-Zondek test, (2) the Friedman test, and (3) the Brouha test. This last relies upon the action of the gonadotropic hormones in stimulating (in male animals) the production by the testes of the male hormone which causes growth of the vesiculæ seminales; the test, which is performed on male mice, does not give as reliable results as the other methods. Lately Bellerby (1934) has described a simple test which depends on the induction of ovulation by the injection of gonadotropic substances in *Xenopus*, kept under standard conditions. Results are obtained within nine hours—a much shorter interval than that required for the other tests. Further data are necessary before the value of this method can be assessed.

The Aschheim-Zondek Test

The Aschheim-Zondek test is performed on immature female mice about three weeks old, and depends on the production in the ovaries of certain definite changes. As the urine to be examined may be toxic to the mice, which may interfere with the test, it has to undergo some treatment previous to its injection. Zondek has suggested extracting the toxic substances by shaking the urine with ether, the ethereal fraction then being discarded; and this method has proved very successful. The method employed by the Edinburgh Pregnancy Diagnosis Station has also given very satisfactory results, and is performed as follows:

To each 25 c.cm. of urine one gram of sulphosalicylic acid is added. The urine is allowed to stand for thirty minutes, with occasional shaking. It is then passed through a filter paper, and the filtrate is neutralized with sodium bicarbonate and kept for injection.

Five mice are used for the test, and each animal receives six injections of 0.4 c.cm. of treated urine: the injections are spread over three days—that is, two injections per day, morning and evening. On the morning of the fifth day after the first injection (satisfactory results can also be obtained 100 hours after the first injection) the animals are killed and the ovaries examined macroscopically, and, if necessary, under the binocular microscope. The following changes may be observed in the ovaries: (1) *corpora lutea*—these are usually easily recognizable; (2) *blood spots*—these may be large and easily seen, but on the other hand they may be minute and may have to be carefully looked for (steeping the ovaries in glycerin for a few minutes renders evident any small blood spots which may otherwise not easily be detected); (3) *enlarged follicles*—constituting the so-called “white spots.” The presence in one or more ovaries in the five animals of a corpus luteum or blood spot (or both) constitutes a positive diagnosis. The presence of enlarged follicles is, according to the arbitrary standards, a negative finding, but it has, nevertheless, been found advisable to repeat the test in such cases, as occasionally it does represent a positive diagnosis.

The period of time (four to five days) necessary for the diagnosis does not, in the majority of cases, present any drawback, but it is sometimes desirable to secure a result more rapidly. A number of observers have attempted to develop a rapid test by modifying the Aschheim-Zondek technique (Zondek, 1931; Aberson, 1931; Lassen, 1932), but there is at present no really satisfactory evidence that any of these modifications possess a sufficiently high degree of reliability. On the other hand, the use of the rabbit as a test animal has led to a technique which gives reliable results within much shorter intervals.

The Friedman Test

Friedman (1929) found that the intravenous injection of urine from a pregnant subject into rabbits was followed by ovulation in the ovary in a period of twenty-four hours or less, and immediately realized the importance of this finding in relation to pregnancy diagnosis. The rabbit never (or very rarely) ovulates spontaneously, and hence, if mature animals are segregated, they are suitable for the test. Provided that they are more than 3 months old ovulation can also be induced in immature animals, which are so suitable in many ways for the test that they have been very generally employed.

Three types of technique have been used—namely, (1) a single injection of urine, (2) multiple injections of urine, and (3) the injection of a concentrated preparation of the active gonadotropic substances made from the urine to be tested.

The first two of these methods have apparently yielded very good results in the hands of several observers, although it is to be noted that in many cases accurate results cannot be secured in less than forty-eight hours (for example, Ehrhardt, 1932). These methods were at first tried at the station in Edinburgh, but were not found sufficiently satisfactory. This may be due to the interval elapsing between the collection of the urine and its receipt for examination being longer than has been the case with other investigators. We therefore decided to elaborate a method which would give results possessing a high degree of reliability within twenty-four hours, if possible. And the method I am now using as a routine in Edinburgh is actually capable of doing so.

The active gonadotropic hormones are obtained from the urine in the following manner.

To 50 c.cm. of the urine is added 50 c.cm. of rectified spirit, and the mixture is allowed to stand for some minutes. The precipitate, which consists of inactive material, is centrifuged off and discarded: 150 c.cm. of rectified spirit is then added to the supernatant fluid. This brings down the active factors; the precipitate is again centrifuged off, and the supernatant fluid is now discarded. The precipitate is extracted with ether to remove any remaining toxic substances, and the fraction not removed by the ether is dissolved in 5 c.cm. of saline and neutralized; 3 c.cm. of this solution is injected intravenously, and the animal is operated on twenty-four hours later. If for any reason the reaction is doubtful (in about 10 to 20 per cent. of cases), then the remaining 2 c.cm. is injected, and the animal is again operated on after a further interval of twenty-four hours.

Unless a definite positive test is given by one ovary both ovaries are always inspected. A positive diagnosis is returned if (1) one or more ovulated follicles, or (2) one or more haemorrhagic follicles, are observed.

Both ovulated and haemorrhagic follicles may, of course, be present in the same animal. In the great majority of cases (about 80 to 90 per cent.) the result is definite within twenty-four hours. Uncertainty at this stage may arise for these reasons: sometimes the ovaries of normal rabbits contain small blood spots; occasionally, too, after the injection of gonadotropic hormone, the only effect after twenty-four hours is the production of a blood spot. Hence if such a condition is found in the ovary on inspection it may be difficult to decide whether the blood spot has arisen as a result of the injection or independently of it. Twenty-four hours later, however, the question can be decided, as, with the method employed, a more marked effect is seen in the ovary if the test is positive. Rarely, too, follicular maturation only is seen twenty-four hours after the injection, and in such cases a further examination at forty-eight hours is undertaken.

Fortunately the above difficulties do not often arise, and it is only in some 10 to 20 per cent. of the cases that it is not possible to give a diagnosis within twenty-four hours.

Reliability of Tests

It is important to determine what degree of reliability can be achieved with the Friedman test, and how this compares with the Aschheim-Zondek test. Mack and Agnew (1934) have recently reviewed the literature on the subject and classified the results in several thousands of cases, including a large number of their own. The Aschheim-Zondek test gave a mean accuracy of 96.6 per cent. in 8,685 cases described by various investigators, while an accuracy of 98.5 per cent. was obtained by means of the Friedman test in 1,899 cases. In their own cases they were able to get slightly better results with the Friedman than with the Aschheim-Zondek test. At the Edinburgh station 3,151 A.Z. tests, controlled by clinical findings, were performed during the period 1930 to 1933, and the actual error was 1.75 per cent.—an accuracy of 98.25 per cent.

Table Showing the Results Obtained with the Aschheim-Zondek and Friedman Tests on a number of Cases Tested in Edinburgh

Cases	Aschheim-Zondek Test		Friedman Test	
	Positive	Negative	Positive	Negative
Definitely pregnant	74	2	75	1
Definitely non-pregnant	1	66	1	66
Abortion	1	5	5	1
"Delayed menstruation"	0	2	2	0

We have also examined 151 cases with both the rabbit and the mouse test; the data are shown in the table. In 138 cases (seventy-three pregnant and sixty-five non-pregnant) the results of both tests were in agreement. Six cases of early abortion urine were also examined, and in five of these the Friedman method gave a positive result while the Aschheim-Zondek was negative. In one case the Friedman test was negative and the Aschheim-Zondek positive. In two cases in which menstruation was delayed for some time the Aschheim-Zondek test was negative while the Friedman yielded a positive result. The possibility that these apparently false positives were due to undetected early abortions cannot be excluded. Lastly, there were two cases in which the Friedman test was definitely wrong (one false positive and one false negative), and three cases in which the Aschheim-Zondek test was definitely wrong (two false negatives and one false positive). These results are substantially similar to those previously reported by a number of observers, and agree in showing that there is little difference between the reliability of the Aschheim-Zondek and the Friedman tests.

Validity of the Tests

The various diagnoses described represent tests, not for pregnancy, but for the presence in the fluid examined of gonadotropic hormones capable, in the definite amounts injected, of causing certain alterations. A positive result, therefore, means no more than that those amounts of hormone are present in the quantities of fluid examined, and it is then necessary to make a differential diagnosis between the various conditions in which these hormones may be excreted. It must be emphasized, however, that in the great majority of cases a positive test does actually indicate gestation.

The increased excretion of gonadotropic hormones occurs not only during a normal pregnancy but also during ectopic gestations. It commences very shortly after the embedding of the fertilized ovum, and continues as long as the living products of conception or chorionic elements are in biological contact with the maternal tissues. Positive effects have been obtained with urine collected

even before the missed menstruation. If pregnancy is interrupted the excretion of gonadotropic hormones rapidly falls, and the test becomes negative within a few days.

The pregnancy test is also positive in cases of hydatidiform mole and chorion epithelioma: indeed, in these cases the excretion of gonadotropic hormones is usually at a much higher level than during pregnancy, the amount excreted attaining, in some instances, several 100,000 units per day (Ehrhardt, 1930). When either of these conditions is suspected a quantitative estimation of the gonadotropic content of the urine may be carried out, and a finding of 100,000 or more units per litre strongly suggests that a normal gestation is not present. A few cases of malignant disease have also been recorded in which the urine gave a positive pregnancy diagnosis test.

After the menopause the excretion of gonadotropic hormones is markedly increased, but extracts usually only cause follicular maturation effects. At times such urines give positive Aschheim-Zondek reactions, and this also applies to the urine excreted by patients after operative or radiological castration (Brandstrup and Lassen, 1933).

Finally, it is of interest to note that in normal pregnancy the excretion of gonadotropic hormones may occasionally fall to such a low level that the pregnancy test becomes negative (Runge, Hartman, and Sievers, 1932). A negative test in the presence of decidual tissue may also be observed in rare cases in which the tissue between the embryonic and maternal circulation is fibrosed (Kleine, 1933). A similar fibrosis has been described by Phillip (1931) in two cases of hydatidiform mole in which the Aschheim-Zondek test was negative.

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The second (1934) edition of the *List of International Fellowships for Research*, issued by the International Federation of University Women (Crosby Hall, Cheyne Walk, S.W.3; price 2s.), contains a useful index which enables quick reference to be made to those which relate to specific subjects. The list is classified under three headings: fellowships open to graduates of all countries, irrespective of nationality; those for study abroad which are restricted to students from two or more specified countries; and those which are limited to students of one specified country. Most of the fellowships are without sex discrimination, but the exceptions to this rule are duly specified. No attempt has been made to give an exhaustive description of the exchanges which can be arranged for students and members of faculties of the European universities, since this has been already made available with much additional information in the handbook published by the League of Nations Institute of Intellectual Co-operation. The sole intention of the present list is to place at the disposal of students in a readily accessible form such knowledge as will enable them to pursue post-graduate work in foreign countries. Most of the fellowships and scholarships are intended for advanced post-graduate or research work, and scholarships for undergraduate study are not included, except when the opportunities offered are equally available for both graduates and undergraduates.