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A Resume of the tests for pregnancy

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A RESUME' OF THE TESTS FOR PREGNANCY

SENIOR THESIS

SUBMITTED

BY

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I INTRODUCTION

The need of a satisfactory laboratory test for pregnancy has long been recognized by both the physician and the patient. It is very frequently of great interest to both parties to know of a pregnancy at as early a period as possible. In reviewing a large amount of the literature available, it seems that the early diagnosis of pregnancy has been, and still is a difficult problem. At the present time, the so-called "presumptive signs" may lead the physician to suspect the condition, but very frequently he is unable to make a positive diagnosis, until the patient returns in a few weeks for re-examination. This period of waiting is a loss, in some cases, of valuable time, in which pregnancy is contra-indicated and a source of anxiety and uncertainty to women who may have changed their entire plans. But of far more importance is the early differential diagnosis between pregnancy and some form of abdominal tumor. In view of these reasons, many attempts have been made to develop a reliable and easy laboratory test giving the early desired information, at a time when ordinary clinical methods could not furnish proof of pregnancy.

The practical value of any laboratory test, which must furnish reliable evidence of pregnancy before diagnosis is possible by clinical means, is obvious; and the many possible applications for such a method have stimulated numerous investigations designed to produce such a laboratory procedure.

Many of the tests so devised have failed in the hand of other investigators, and have passed by the way-side; others occupy a dubious position as yet, while of others only scattered reports are available. Since Aschheim and Zondek reported a test for pregnancy

in 1928, a large number of investigators have filled the literature with duplications of the original work and modifications thereof. While the work is yet in an experimental stage, and there are some discrepancies as to the results obtained, it is worthy of some summary - which is the purpose of this Resume'.

From the beginning of Mankind the diagnosis of early pregnancy has probably been tried by various means or devices. About 1100 B. C. our earliest known record of such a test is quoted by Aschheim and Zondek (3), they describe an Egyptian papyrus reading: "A woman may determine if she is pregnant by taking some earth and barley in a vessel and adding to it a little of her urine day by day. Should the barley grow, the woman is pregnant, but if the grain does not grow, then she will not bear a child." From this early test up to 1831 the literature on pregnancy tests has apparently gone through the "Dark Ages". Garrison in his book on the History of Medicine makes no mention of the tests for pregnancy.

However, Hodge (31) in 1864 gives us the following method for early diagnosis of pregnancy known as the Keyestine Test: "M. Nauche in 1831 first described a peculiar deposit of a caseous character which he termed Keyestine. If the urine of pregnant women be allowed to stand in a wine glass, well exposed to the atmosphere, in a few hours there will be a deposit of "cloudy flakes" on the side and bottom of the glass, the urine becomes more limpid. This deposit is not characteristic. At the end of the second or third day the urine again becomes cloudy with increased intensity, and soon, more or less perfect, a pellicle forms upon the surface. About the third or fourth day, portions of the pellicle begin to separate and are precipitated so that by the fifth or sixth day the greater part of the pellicle has disappeared. This pellicle is the Keyestine and is seldom seen prior to the second month of utro-gestation". Hollick (33) commenting on this test states that real Keyestine is found only during gestation,

except while the milk is being secreted and not frequently discharged.

It is of interest to note that in 1846 Curtis (12) in an effort to enlighten midwifery on the diagnosis of pregnancy enumerated the following conditions as diagnostic: "Diarrhea, effusions of water, dyspepsia, heart burn, depraved taste, costiveness, prolapsus uteri, gravels, jaundice, difficult breathing and cough, convulsions, toothache, salivation, inflammation of the breasts, daopsy of the ovum and of the extremities".

The author states that Dr. Blundell, after enumerating all the signs that have been found worthy of notice, comes to this sage conclusion, that, "if pregnancy is doubtful, we had better wait 'til the end of the tenth or twelfth month, when unless the gestation be extra uterine, or out of all rule, parturition must sooner or later occur". Undoubtedly this is the surest sign of pregnancy reported in the earlier literature.

There were perhaps many other tests for pregnancy through the ages which have passed one by one into disuse, as there is no mention of them in our more modern available literature.

III LABORATORY TESTS FOR PREGNANCY OTHER THAN THE BIOLOGICAL TESTS

From time to time various laboratory methods have been developed to determine the presence or absence of pregnancy. These are set forth in brief with the reacting principle involved, together with results obtained by other workers upon the test in which information is available.

(1) ABDERHALDEN'S SERO-DIAGNOSIS TEST FOR PREGNANCY

In 1912 Abderhalden (1) described a method for the diagnosis of pregnancy, the principle of reaction involves certain changes occurring in the blood stream of the patient, due to the absorption of foetal elements. It was supposed that a ferment in the maternal blood was developed as a protective mechanism against these foreign proteins, by splitting them down into amino acids.

The method he employed for demonstrating this reaction is given by Holland (32) as follows. "Of fresh clear blood serum from the patient taken fasting, 2 or 3cc are mixed with 1 gm of specially preserved placental tissue in a diffusion tube of parchment, and dialyzed against 20cc of distilled water in a small glass cylinder. Both liquids are covered with toluol, and the operation of digestion is conducted for twelve to sixteen hours in the incubator at 37° C. In that time, if specific protective enzyme exists in the serum, it will have attacked the placental protein, but no other, and the cleavage products will have diffused through the dialyzer, which is not permeable to the normal albumins of the serum.

Of this clear dialyzed fluid, 10cc are mixed with 2cc of a 1% aqueous solution of ninhydrin in a test tube, and boiled for one minute. A bluer color indicates that the placental tissue has been

digested by its proteolytic enzyme contained in the serum, and that the patient must, therefore, be pregnant."

This test in the hands of other investigators showed that men gave positive reaction, as well as pregnant and non-pregnant women. Williams and Pearce, Petri and others (1) contend that the reaction is non-specific. "Bar and Ecallo (1) in 1919 found that the reaction was positive in all the pregnant women tested, but likewise obtained a similar results in 34 percent of all patients in whom pregnancy did not exist. Consequently they concluded that a positive result has no diagnostic significance, while a negative one excludes the possibility of pregnancy."

(2) ANAPHYLATIC REACTIONS

It is reasonable to assume that certain changes occur in the maternal blood stream due to absorption of foreign protein from the foetal elements is capable of sensitization of the pregnant women to these specific proteins, and the possibility of an allergic skin test.

From time to time this test has been tried using placental extracts as allergens. Working with this test William and Kolmer (53), Wintz and Engelhorn (15), Esch (16), Falls and Bartlett (20), found positive reactions in pregnant women as well as women who were not pregnant, but had borne children. The positive reaction occurs late in pregnancy when the so-called presumptive signs are also present, therefore, it is of no value in the diagnosis of early pregnancy.

(3) ROUBITSCHKE'S TEST

In 1922 Roubitschek (45) proposed a test for pregnancy, which consisted of the ingestion of 10 mg of glucose followed by the

injection of 0.5 cc of 1:10,000 epinephrine. Results and reports of other workers upon this technique are not available.

(4) FRANK AND NORTHMANN TEST

Williams (54) quotes, "As the result of the demonstration by Nurnberger and others, that the renal threshold for sugar is definitely lowered during the first months of pregnancy." Welz and VanNest have utilized this phenomenon as a test for the existence of early pregnancy. Frank and Northmann (22) used a technique that is regarded with some favor, while Hirst and Long (30) regard the test as the most reliable and used the following technique.

1. An "Average Supper" is given the night before.
2. The morning urine is examined and must be negative to Fehling's or Benedict's solution.
3. Breakfast is omitted.
4. Table sugar is given, 7.5 gm for every 10 pounds of body weight, the total not to exceed over 150 gm. This is dissolved into two glasses of water, each containing the juice of half a lemon. All of the sugar must be taken and none lost by vomiting.
5. Voided specimens are collected after one or two hours and tested qualitatively for sugar.

If either specimen shows a definite reaction for sugar, the test is positive. "Slight reductions" being disregarded.

In 1924 Milnor and Fennel (40) used a modified technique and suggest that the assumption of an increased permeability of the renal cells to sugar during pregnancy, upon which these tests depend,

is unwarranted. They believe it more reasonable to assume that there is an unbalance of the internal secretory mechanism in pregnancy, such disturbances of the internal secretory apparatus probably arising in the ovary, liver, thyroid and pancreas.

The value of the test seems to be presumptive as it does not take into consideration patients with a low sugar reserve from other causes.

Williams (54) states that he has had but little experience with the test, but knows of several instances in which it has led to erroneous conclusions.

(5) PHLORIDZIN GLYCOSURIA TEST

Phloridzin is a glucose in the bark of apple and pear trees which is hydrolyzed and split into dextrose and phoretin. When this drug is given it causes a transient glycosuria, with diminution of the normal amount of glucose in the blood. Its action is specific upon the kidney. With this in mind Kamnitzer and Joseph (36) devised a test for pregnancy based upon a sub-threshold dose. If glycosuria occurred within 90 minutes, it was taken to indicate pregnancy. The Maturin test of Kamnitzer and Joseph consists of Phloridizin in 2 mg doses combined with local anesthetic. Considerable work has been done upon this test by various observers. Eastman (14) substituted Fehling's solution for Nylander solution as originally used by Kamnitzer and Joseph (36) and reports 90 percent positive reactions in 136 cases among which were 31 primiparae, and 47 multiparae giving positive reactions, 10 primiparae, and 48 multiparae giving negative reactions. Of these 48 negative reactions, in which pregnancy was a possible diagnosis, 12

were in menopause, 8 had ovarian dysfunction associated with adiposity, 13 were cases of lactation amenorrhea, while 3 were cases of uterine fibroids.

Bronnidoff (6) investigated 350 cases including the male and concluded that the test is non-specific and of no diagnostic significance, while Milnor and Fennel (40) working at the same time obtained positive reactions in most of the cases of proved pregnancy, they also received positive reaction in non-pregnant patients, and concluded, therefore, that the test is of no safe value as a diagnostic procedure.

(6) BLOOD CHEMISTRY IN PREGNANCY

Bunker and Mundel (10) in 1924 reported their studies in the changes in the chemistry of the blood during pregnancy. The studies do not bear out sufficient evidence to elicit information of diagnostic value during the early months of pregnancy.

(7) BILIRUBINEMIA AS A TEST FOR PREGNANCY

In the work of Mandelstamm and Nogeikoff (39) on bilirubinemia, they state it is a frequent sign of pregnancy, but not constant. It would, therefore, have no practical value as a diagnostic procedure.

(8) THE NINHYDRIN FLOCCULATION TEST

Vogel (50) in 1926 developed this test and reported it to be positive in 97.8 percent of 281 cases of pregnancy, and negative in 97.7 percent of 212 non-pregnant women. Investigation of other workers, Grossmann and Meswinkel, (27) report the value of the test unfavorable. In the more recent work of Davis (13) with a modified technique he

speaks very favorably of the test as a diagnostic procedure in both hospital and private practice. He tested 586 cases, 234 pregnant and 352 non-pregnant patients. Of the pregnant cases 100 percent were positive, while in the non-pregnant cases 95.9 percent were negative. It is shown by his report that negative cases, who have the possibility of being more than six weeks pregnant practically excludes pregnancy. The test: (13) "Two reagents are used in carrying this test out. The first reagent is made up of the following ingredients:

Potass. Bicarb.	2.5 grams
Aq. Calcis.	55.0 c. c.
Aq. Dist.	954.0 c. c.

Through this solution carbon dioxide is blown for five minutes. The bottle is then stoppered and shaken for three minutes. The reagent itself can be used for several weeks, but on each occasion that it is used, the carbon dioxide gas must be blown through it.

The second reagent is a one percent solution of ninhydrin (Triketohydrindene hydrate). As this solution keeps for only one or two days, it should be made up fresh each time.

The technique of the test is as follows:

1. To 10 cc of the first reagent, add 0.2 cc of the ninhydrin solution. Then add 1.75 cc to 2.0 cc of the patient's serum.
2. Place the test tube containing the above mixture in boiling water for three minutes.
3. (a) In very early pregnancy, fine blue flocculi are scattered through the solution, which has turned purple.
 - (b) In pregnancy over six weeks, there is a fine emulsion scattered through the purple solution.
 - (c) In non-pregnant women, a heavy cloud of large, coarse

flocculi settles to the bottom of the purple solution".

The author (13) states that the ninhydrin flocculation test can be highly recommended, because of its simplicity and high degree of accuracy.

(9) VENOM HEMOLYSIS TEST

It is well known that the venom of rattle snakes and of the cobra possess the power of hemolysis of red blood corpuscles. Kraus, Graff and Ranzi (35) while working with cobra venom on cells from individuals suffering from various diseases found that erythrocytes from pregnant women during the fourth month had a definite and characteristic resistance. Although this reaction occurs, there is no claim or evidence produced that it is constant in all pregnancies. It is, in all probabilities, of no diagnostic value.

(10) THE MIOSTAGMIN REACTION

This reaction is based upon the observation of Weichardt (52) that solutions of Antigen and Antibody are brought together, diffusion is accelerated, changes in diffusion being associated with changes in surface tension. Using placental tissue as the Antigen, the miostagmin test has been applied to the diagnosis of pregnancy. The results are variable and inconstant.

(11) MANOILOFF'S PREGNANCY REACTION

Rodencourt and Jernokoff (44) give the technique of the test as follows: "One cc of a 2% aqueous diuretin solution is added to 0.3 cc of clear fresh serum. After the mixture is well shaken, 1 drop of a 0.2% alcoholic Nile blue solution is added stirring well. In pregnancy, the serum mixture is discolored, within a few minutes

to one hour, and appears yellowish or yellowish pink. In the absence of pregnancy, it remains blue."

In re-examining the test the authors (44) used serum of pregnant women against serum of non-pregnant women, and found the test unserviceable because of the many color reactions produced. In 75 tests made they never observed the color reaction mentioned by Manoiloff.

(12) ACETONURIA TEST FOR PREGNANCY

The basis of the test is the artificial production of acetone in pregnancy. This being much easier to accomplish than in the non-pregnant. It is supposed that during pregnancy fat oxidation requires larger quantities of carbohydrates for complete combustion. Bulloch (9) tests show that non-pregnant individuals require at least 48 hours to produce acetone, after being deprived of carbohydrates, and the pregnant requires much less time. The Test: The morning urine was voided and saved. Breakfast of cereal with cream and sugar, buttered toast and coffee with cream and sugar, or a glass of milk, at 8:00 a. m. After this no further intake of food until 5:00 p. m. At that time, another urine specimen was voided. The two specimens were then tested for acetone by a modification of the Rothera Test. A positive test for pregnancy is shown when the first specimen is negative for acetone, and the second is positive. A negative test for pregnancy is shown when both specimens show no acetone. If both specimens show acetone, there is an acidosis and the test is void. Bulloch (9) tested 300 cases, 257 were positive, 43 negative. Of the 43 negative, 40 were clinically negative. The earliest positive reaction was 27 days from the last period. In conclusion the author states, "A

positive test proves pregnancy with certainty; a negative does not exclude pregnancy, but renders it highly improbable."

(13) AMYLASE TEST OF URINE FOR PREGNANCY

Von Pall (51) states, "The urine of pregnant women has not yet been the object of amylase and diastase tests. Porbet alone mentions that the Amylase content of urine is decreased in toxemia of pregnancy. The author, therefore, tested the urine of 150 healthy pregnant women, making numerous tests, not only quantitative tests of Amylase but also tests of the diastatic effect of Amylase. He found important relations especially between diastase and pregnancy".

The Amylase content of the urine of pregnant women is 3 times as high as that of normal urine. During labor diastase is not markedly changed in regards to pregnancy. The author concludes that, in as much as sources of error were possibly excluded, a marked increase of the Amylase content of the urine is characteristic of pregnancy.

(14) THE ADRENALINE - SOUND TEST

The test is based on the fact that during pregnancy, the head part of the cerebral vascular sympathetic is over-excitabile. This is proved by the vasomotor nasal reflex phenomenon of the white streak in the adrenaline - sound test, which is not demonstrable in non-pregnant females. Muck (41) first used this test in 4 cases of doubtful pregnancy, which gave a positive reaction, and which later were confirmed. He used the test in 5 other cases. Abortion occurred in one, the other 4 were lost sight of. The author states that females affected with epilepsy, lues or hemicrania sympathicotania are out of the question, because the positive reaction may

be due to these conditions in which it is always present.

It appears that the test may have some value in the normal pregnant woman. The limited number of cases used by the author gives it a questionable value as to a reliable diagnostic procedure.

(15) THE WHEAL - REACTION IN EARLY PREGNANCY

The reaction is based on the supposition that when an exactly neutralized isotonic wormosal solution is injected intracutaneously the duration of the wheal is lessened due to a tendency to edema during pregnancy. Obladen (42) states that such wheals as a rule in the non-pregnant last 60 minutes, while in the pregnant it lasted from 30 to 40 minutes. In a series of 78 positive pregnancies in the first to third month, 73 showed a decrease of the wheal-time, while 5 showed no decrease. Thus 93% had a positive reaction. The test on 60 known non-pregnant women there was no shortening of the duration of the wheal-time.

The author believes he has demonstrated by this method a valuable test for pregnancy, but is of questionable value due to the limited number of cases run. The results may prove different in the hands of others. White and Severance report the test to be of little value as a diagnostic aid. (55)

(16) PUPILLARY TEST FOR PREGNANCY

Bercovitz (5) in 1930 reported a pupillary reaction as a diagnostic aid to pregnancy. The test is carried out by drawing blood from the patient, rapidly centrifuging it, then installing the clear serum into her own conjunctival sac giving a change in the size of the pupil (dilatation or contraction) as compared with the

control pupil. The author also found that the reaction did not occur in non-pregnant women, men, rabbits or cats.

A simplified technique given by Bercovitz (5) is as follows: "One drop of 10% sodium citrate is mixed with five or six drops of blood from the finger and installed into one conjunctival sac of the patient from whom it was withdrawn. The entire procedure requires about 2 minutes". The reaction is reported to occur promptly and may last several minutes.

In a series of 150 known pregnancies the author reports 123 positive reactions, 12 questionable reactions, and 15 negative reactions.

Causes of failure are due to a failure to study the pupils carefully before installing the blood, as the pupils of many people are normally unequal.

It appears that the test is not so reliable that it can be utilized as a means of differential diagnosis between pregnancy and other conditions.

IV BIOLOGIC TESTS FOR PREGNANCY

(1) GENERAL DISCUSSION

Since 1925 when Zondek and Aschheim (2) began a series of studies which showed that the hormone of the anterior lobe of the pituitary body was normally present in the urine during pregnancy, and that such urine was capable of producing estrus, a large number of experiments have been carried out on the role of the female sex hormone along the lines of physiology and endocrinology. It is on this fundamental work that the test for pregnancy of Aschheim and Zondek, of Brouha and Friedman depend. Even before this work of Aschheim and Zondek, Evans and Long (17) in 1921, and after Smith (49) 1927 have prepared and demonstrated by oral administration of anterior lobe of the hypophysis has no effect on the date of maturity of rates.

Evans and Long (17) then prepared an extract of bovine hypophyseal substance, which they injected into the peritoneum of adult white rats. They succeeded in producing a very definite change in the reproductive organs.

The results of these injections were as follows:

1. Estrus was absent or inhibited at long intervals.
2. The uterus remained infantile, but the ovaries were twice the size of those of the control animals.
3. Histological examination showed the presence of a very abundant lutein tissues in the ovaries and the formation of this tissue about the egg in unruptured normal follicles and in atretic follicles. Ripe normal Graafian follicles were invariably absent. A powerful specific stimulus to lutein cell transformation was thus present in this hormone.

This appears to be the first definite result obtained from the injection of an extract, which led to our present concept of the various sexual hormones.

At this time a brief consideration of the sexual hormones

that play a role in menstruation and reproduction seems necessary to obtain the proper understanding of the various biological tests for pregnancy.

Frank (21) in a recent article has summarized the role of the female sex hormone as follows:

- (I) "Progestin" (Corner), the special lipid extract of the corpus luteum.
- (II) The Graffian follicle - excretes female sex hormone or "estrin" alone except during pregnancy, at which time the prepituitary hormone also may be found in the follicular fluid.
- (III) The corpus luteum - elaborates not only the female sex hormone but "progestin".
- (IV) The placenta contains female sex hormone, pre-pituitary like substance.

Various Endocrine Influences as Summarized by Frank (21).

- (I) The anterior pituitary lobe hormone stimulates the ovary to produce follicles.
- (II) The Graffian follicle in turn elaborates the female sex hormone, which reaches the tubes, uterus, and vagina by means of the blood stream. This causes early premenstrual changes.
- (III) Following ovulation, the corpus luteum develops. This transitory gland continues to produce:

Female sex hormone as well as a special hormone "progestion," whose presence in the circulation has not yet been demonstrated, enhancing the local uterine and breast changes necessary for the nidation and early nutrition of the fertilized ovum, including sensitization of the uterine mucosa, requisite for successful imbedding.

If the corpus luteum is removed at this early stage, the ovum fails to secure the necessary firm hold in the endometrium, and abortion results.

(IV) The developing placenta, and important gland of internal secretion takes up the production of both female sex hormone and pre-pituitary hormone and as perhaps a safety factor the circulation is flooded with these two internal secretory products to such a degree, that an enormous amount is excreted through the kidneys and bowels throughout pregnancy. The Biologic tests are based upon this excess of hormone, which is secreted at the time or during pregnancy. As this work advanced Evans and Simpson (18) reported that the anterior pituitary body secreted two hormones, of which one was required for normal growth and the other for normal development of the sex glands.

Aschheim and Zondek (4) in 1927 announced the urine in pregnant women contained hormones which could produce sexual maturity

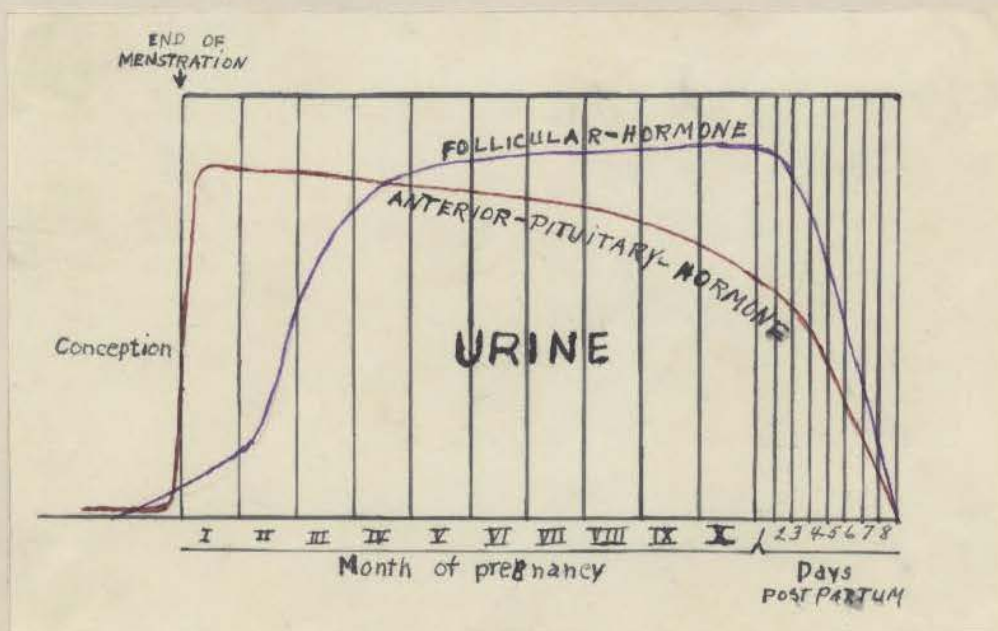


Diagram of amount of follicular hormone and anterior pituitary hormones in the urine of pregnancy. (From Aschheim and Zondek).

in experimental animals. This observation was made the basis for the now famous test for pregnancy, which is so widely used today. They demonstrated that the anterior pituitary hormone appeared in the urine very soon after conception, and that by its presence pregnancy could be diagnosed within seven days after conception. The amount of hormone in the urine of pregnancy increases very rapidly, reaching a peak in the first month after conception, and slowly decreases throughout pregnancy, disappearing within a week after parturition as shown in the graph on the preceding page.

(2) ASCHHEIM - ZONDEK PREGNANCY TEST

This test is concerned chiefly with the ovarian changes produced by the pituitary hormone.

Mathieu and McKenzie (37) states, "The technique as used by Aschheim and Zondek for the pregnancy test requires five immature female mice for each test. They state that the mice used must weigh between 6 to 8 gm., as it has been shown that mice reach maturity about the time that they weigh 12 gm., so that mice approaching this weight cannot be used. The urine used for the test should be the early morning urine because it contains the hormone in the greatest concentration. The reactions of the urine should be faintly acid. Alkaline urine may be corrected by the addition of a few drops of 2% acetic acid, and all urines should be filtered before being used. If the urine cannot be kept at ice box temperature or if it is to be kept for several days before using, it should be treated by the addition of two drops of toluol to 25 cc of urine." The quantities of urine injected are as follows:

Animal No. 1	Six times	0.2 cc
Animal No. 2	Six times	0.25 cc
Animal No. 3	Six times	0.3 cc
Animal No. 4	Six times	0.3 cc
Animal No. 5	Six times	0.4 cc

"They make six sub-cutaneous injections in each animal over a period of forty-eight hours as follows:

1. Monday a.m., 11:00 to 12:00; p.m. about 5:00.
2. Tuesday a.m., 10:00; p. m. 1:00; p.m. 5:00 to 6:00
3. Wednesday a.m., 10:00."

"The animals are killed and opened for examination at the end of a ninety-six hour period from the first injection of urine. Only the findings in the ovary are to be used in the interpretation of

the test. Enlargement of the uterus and a positive vaginal smear cannot be considered as evidence of pregnancy, but only as evidence of ovarian hormone activity, whether it be from the urine injected or the result of stimulation of the ovarian activity by the anterior pituitary hormone."

Parvey (43) states the criterion of a positive test as follows: "A positive test is detected by observing gross changes in the ovaries and these have been grouped by Zondek and Aschheim as reactions II and III. Reaction I in itself is not reliable for diagnosis of pregnancy, as it concerns changes in the uterus and vagina alone. Reaction II concerns the formation of so-called blood-points on the surface of the enlarged ovary. These are discretely distributed, brownish and purplish areas the size of a common pin head, and are the gross representation of hemorrhage into the enlarged graafian follicle. Reaction III concerns the detection of the corpora lutea, which appears discretely on the surface of the ovary as yellowish pin head sized areas. The presence of these changes, blood-points and corpora lutea in an enlarged ovary are diagnostic of an increase in the anterior pituitary hormone, and this occurs regularly in pregnancy. They may be present in but one ovary." A negative test shows the ovaries unchanged.

Since the innovation of this test many investigators have repeated it with the same gratifying results as those obtained by the originators. Mathieu and McKenzie (37) report a series of 150 cases with an accuracy of 97.3%, and error of 2.7%. Jones and Mudge (34) report a series of 255 cases with an accuracy of 97.65%, an error of 2.35%. Parvey (43) reports 191 cases with results in full accord with

claims of Aschheim and Zondek. White reports a series of 163 cases with an error of 3.7%. Crew (11) working with this test reports 907 cases, and of these 479 have been controlled. Of the controlled cases, 288 were positive and 179 negative. There were 9 cases of mis-diagnosis giving an error of 1.88%. The author reports the result of 13 other investigators incorporating 2,368 cases, having been found to give an average error of 1.47%. Sage (46) reports reviewing all available literature which consists of 3,159 cases with positive results from 95% to 98%.

The test in the hands of many investigators, conducting such a large number of tests with results approximating 98% accuracy, is evidence to suffice its usefulness as an early aid to the diagnosis of pregnancy. The test itself seems simple, but offers disadvantages to limit its practicability for general uses for the following reason.

1. Immature female mice must be used, weighing from 6 to 8 gm. This would necessitate a large number of breeding stock being kept on hand at all times.
2. The ovaries must frequently be examined microscopically.
3. Each test requires five animals instead of one.
4. Each test requires six injections into each test animal.
5. The time of the test requires 96 hours as compared with 30 to 48 hours in the Friedman test.
6. In all the expense incurred in conducting the test would seem to render its general usage unpopular.

(3) MODIFICATIONS OF THE ASCHHEIM - ZONDEK PREGNANCY TEST

Since the original work of Aschheim and Zondek, there have been some modifications of the test by various workers.

A Evans and Simpson (19) described a method in which rats were

used instead of mice. They used urine that had been frozen solid prior to the test and injected 1 cc of this for four days. Interpretation of the test was the same.

B Frederick and Silverberg (23) described a modification on the basis of concentrating the active substance in the urine. This was done by using 180 cc of urine together with two and one-half its volume of 95% alcohol. The mixture was kept at a temperature of from 2 to 4 C. for several hours until the precipitate settled out. The precipitate, after centrifugation, was washed with ether and dried at 38° C. A saline solution of the precipitate contained the specific hormone. 1 cc of the extract was injected into each of two rats on three successive days. By this technique they ran 175 cases with results of 100% accuracy and reduced the time of the original test from 96 hours to 36 hours.

C Jones and Murgage (34) modified the test by using two rats instead of five mice. The rats were immature females from 28 to 35 days old. The injections consisted of 1.5 cc of urine three times a day for three days. One hundred and twenty hours after the first injection the animal was killed, and examined in the usual manner. In a series of 255 cases they report an error of 2.35%. In this modification they have conserved experimental animals, but have further increased the time of the test.

D Mathieu and McKenzie (37) further modified the technique by using one rat weighing between 30 and 65 gms. They injected 0.5 cc of urine sub-cutaneously twice a day for three days, and are opened in 96 hours after the first injection and examined. In a series of 150 cases they report the test to be 97.3% accurate.

(4) BROUHA'S PREGNANCY TEST

Brouha (7) and his co-workers note that the anterior pituitary hormone was present in the urine fifteen days following conception, and that folliculin was present after the third month. They also found that the urine of menstruating women also contained folliculin. This led to a 40% error, when the Aschheim-Zondek was applied to such cases. They also found that folliculin did not have a stimulating effect on the male genitalia, while the injection of the hypophyseal hormone caused a marked increase in the size and weight of the seminal vesicles. This formed the basis of their test. In a series of 142 women they report 100% accuracy. Literature of other investigators is not available. However, the technic seems to be too delicate for general use.

(5) SIDDALL'S HORMONE TEST FOR PREGNANCY

The Siddall (48) test depends upon the presence of anterior pituitary and female sex hormone in the blood serum of pregnant women. The test consists of intravenous injections of 1 cc of the patient's serum into an immature virgin white mouse once daily for five days. On the sixth day, a vaginal smear is made, and the animal is killed and weighed. The uterus and ovaries are then dissected out and weighed. The weight of the mouse is divided by the weight of the uterus plus the ovaries and the resultant ratio is the criterion for the reading of the test. If the ratio is below 400 the test is positive for pregnancy, while if it is above 400 the test is negative. In a later article the author (44) reported a series of 139 cases with 2.3% error in the test. More recently Mazer and Hoffman (38) duplicating the test found in the positive cases, marked development of the ovaries due to the anterior pituitary hormone. They found the test positive in 116 of the 150 pregnant women studied with an accuracy of only 76%.

(6) FRIEDMAN'S DIAGNOSTIC TEST FOR EARLY PREGNANCY

The Friedman test for pregnancy has been classed as a modification of the Aschheim-Zondek pregnancy test by many of the experimenters working with the test. The Biologic principle of the two tests are essentially the same, but in view of the fact that the Friedman reaction is a macroscopic instead of a microscopic reading, and that an entirely different species of test animal was used, he should have the distinction and honor of having the test named after him.

Goichi (26) demonstrated in 1920, that mature rabbits possessed mature Graafian follicles at all times, but did not ovulate periodically. Ovulation occurred about eighteen hours after copulation in these animals.

Knowing that an excessive amount of anterior pituitary hormone was present in the urine of pregnant women, and when such urine is injected into mature female rabbits caused them to ovulate, which is the basis of the test.

Heope (29) demonstrated neither artificial insemination or injecting semen or sperms nor stimulating the vulva with electrodes was successful in producing ovulation. Hammond and Marshall (28) showed that ovulation regularly followed coitus with a vasectomized buck, and could also be produced by rubbing the vagina of a female in heat. Friedman (24) holds that following coitus there occurs in the humors of the female rabbit some change capable of provoking ovulation.

With these facts in mind Friedman (24) began using the rabbit in his experimental work on diagnosing early pregnancy.

The author used non-catheterized urine making three daily injections of 4 cc for two days. In 48 hours after the first injection, the rabbit was killed and the ovaries examined. If the ovaries contained either fresh corpora lutea or large bulging corpora hemorrhagica, the reaction was considered positive, but if the ovaries contained neither corpora lutea nor corpora hemorrhagica, but only clear unruptured follicles, regardless of their size, the reaction was considered negative. The rabbits used were sexually mature females that had been isolated for at least 30 to 32 days. In view of the fact, as shown by Hammond and Marshall (28) that it is possible for one doe to "hop" another and cause pseudo pregnancy, each animal was kept in a separate cage. The specimens after collection were stored on ice, then filtered and warmed to body temperature so as not to prevent shock to the animal. The author states that heating the urine over 45° C. for any length of time destroys the potency of the hormones. No aseptic precautions were carried out in this test. In this first series, the authors report 92 cases with 100% accuracy.

(7) MODIFICATION OF THE FRIEDMAN TEST

Brown (8) reported this month a modification of the Aschheim-Zondek test using rabbits as did Friedman (25) with his work. He has made some very interesting observations in his work. For test animals rabbits weighing from 600 to 2,150 grams were used. Those weighing from 1,500 to 2,000 grams gave the best results, while animals smaller proved very unsatisfactory. From 2 to 5 cc of the patient's serum was injected intravenously into the animals ear after it had stood from four hours until over night. They observed that fresh serum injections were often fatal to the animal and suggest the reaction was probably due to some toxic element in the serum at that time, which was not present after four hours. From 24 to 36 hours after the injection the ovaries were examined macroscopically and the observations checked by a microscopic procedure. From the observations made with this technique the earliest positive reaction occurred at 13 hours. The period of gestation in the patients used varied from three weeks to seven months. The results recorded are as follows: "Sera from 5 cases not pregnant were used and gave negative results. On a doubtful case of pregnancy, 3 tests were performed, and were all negative. A case of hydatidiform mole gave a strongly positive reaction and was found to be negative three weeks later. The reactions of the 25 positive specimens examined were: 18 grossly positive; 5 doubtful grossly, but positive microscopically".

The author reports series of 220 tests in all, which have proved correct in almost 100% of the cases.

It is of interest to note that the author found the reaction from injections of serum were much more pronounced than the reactions

produced by the injection of urine. Also more pronounced reactions were reported when the amount of serum injected was increased.

One would conclude from this work that the anterior pituitary hormone is more concentrated in the blood during pregnancy than in the urine; that the reaction of the test depends somewhat upon the amount of hormone induced into the test animal. By the use of this technic the time for conducting the test may be reduced to 13 hours instead of 96 as was employed by Aschheim and Zondek (2).

V EXPERIMENTAL DATA

At the time we started this experiment on the test for pregnancy there could be found no literature on the use of blood serum intravenously in rabbits, followed by observation of the ovaries for any reaction from the anterior pituitary hormone as a diagnostic procedure. However, Brown (8) in St. Louis, was using this technic at the time and through Dr. Harley Anderson we secured the idea with many valuable suggestions in carrying it out, therefore, the test is by no means original.

(1) Technic: Patients used were Dispensary and Hospital patients with one exception. All were known pregnancies or suspected pregnancies—none were known none pregnant. The ages of the patients varied from 17 to 34 years. The rabbits used were non-pregnant females that had been isolated at least four weeks. All animals used weighed from 1,500 to 2,500 grams with one exception, which weighed 1,250 grams and gave us a doubtful reaction considering the physical findings (see Case III).

The serum was drawn from the arm of the patient in a sterile syringe and placed in the ice box in a sterile corked tube. After the clot is broken and allowed to remain there until the next day, 2.5 cc of clear serum was then injected into the marginal vein of a suitable rabbit, using sterile technique. At first we used from 5 to 7 cc of serum for each injection, some of the animals going into shock and dying. We then lowered the dose to 2.5 cc with no fatalities and apparently no bad effects upon the end result of the test. On some of our earlier cases which are not reported here, we also found the use of fresh centrifuged serum would at times cause some of the animals to go into immediate shock and later die. This has not been observed when using serum that has stood in the ice box over night. Brown (8) had made the

same observation.

After 36 to 48 hours we opened the animals under an anaesthesia by means of a midline abdominal incision, and by means of a bottom hook. The ovaries and tubes were brought out of the incision for careful inspection. After the inspection was made the incision was closed by means of No. 1., plain cat gut, suturing peritoneum, abdominal muscles and skin with the same suture. If the test has been negative the animal can be used again at any time. If the test be positive an interval of three weeks should be permitted before reinjection is attempted. By this method the same animal can be used several times for the test.

One case of hydatidiform mole gave a strongly positive reaction and was again positive eight days after the mole passed spontaneously.

In our limited number of cases run, most of them were diagnosed as pregnant by the so-called presumptive signs. The test checked in every case. Of three doubtful cases of pregnancy the test showed two negative, and one doubtful. In the doubtful case, it is quite likely the rabbit was not sexually mature. Microscopic sections were not made to determine whether mature follicles were present, and the test was not re-run. For the other two negative reactions sufficient time has not elapsed to determine the presence or absence of pregnancy by clinical means.

(2) ILLUSTRATIVE CASES

Case 1. - Mrs. A. Ward E., Chart No. 38112. Age 21 years, White. Para I, gravida II. Last period of menstruation was normal on June 15, 1931. Quickening first of November 1931.

General examination revealed a well developed, well nourished woman with no edema of extremities or about the eyes and face. Abdomen: small fetal parts palpated in right side of abdomen. Fetal heart tones heard in L.L.Q. Placental souffle heard best on right side of abdomen. Head was presenting part but not engaged at time of examination. The patient entered the University Hospital March 3, 1932 and was diagnosed as a pre-eclamptic and treated for same.

Test: March 4, 1932. Blood taken from the arm was allowed to stand in the ice box over night. The following day 2.5 cc of serum was injected into the marginal vein of a sexually mature rabbit at 2:30 P.M. Rabbit opened under anaesthesia 48 hours after injection, and there were several in each ovary. Reaction - Positive.

Case 2. - Mrs. E. M. Ward H., Hospital No. 38223. Age 25 years, White. Entered the University Hospital complaining of weakness, loss of weight, and cessation of menses. Patient states was perfectly well up until the last week in December 1931, at which time she had an attack of influenza, which left her rather weak. On January 16, 1931, which was her time to menstruate no period appeared. She experienced no pain or discomfort at that time, but consulted a physician. Since then she has gradually become weaker, more easily fatigued and has lost weight. She has had two periods of nausea in the past two weeks.

Catamenia: Periods started at the age of 19 years and were

regular every 28 days, lasting four or five days.

General examination: Fairly well developed, under nourished, white woman of about 25 years of age. No evidence of pain or discomfort. Thyroid palpable, smooth, and regular in contour (Case II). No tenderness or masses in the abdomen. Rest of General Examination - Negative.

Vaginal: Perineum firm, no vaginal discharge. Cervix firm, with bilateral laceration. Adenexia not palpable. Uterus anterior position. Body about two times the normal size and slightly softened.

Consultants Findings - Outlet: No bluish discoloration or evidence of infection. Cervix: low points towards vulvar outlet. Increased pulsation of uterine vessels present.

Diagnosis: Pregnancy.

Test: March 15, 1932 at 2:30 P. M. mature female rabbit was injected with 2.5 cc of patient's serum and the ovaries were examined in 48 hours. Each ovary showed the presence of several corpora lutea and corpora hemorrhagica. The uterus and tubes were congested and hypertrophied. Reaction - positive.

Case 3. - J. N. (Dispensary No. 46129). Patient age - 24 years. Entered the Dispensary March 19, 1932 stating that she had missed two periods. Has felt some nausea, but has had no vomiting otherwise she has had no complaints. On examination it was noticed that the cervix was conical, and showed no lacerations. The cervix was somewhat softened, and some discharge was present. The uterus was definitely enlarged, and a small nodule about 2 cm in diameter could be felt on the right side of the uterus.

Her last normal period was January 2, 1932.

Diagnosis: Pregnancy.

Test: On March 21st, 2.5 cc of the patient's serum was injected in a virgin female rabbit weighing 1,250 gms. The animal was opened under anaesthesia at the end of 48 hours. On macroscopic examination the left ovary appeared somewhat (Case III) larger than normal, and was somewhat congested. There were no corpora lutea or corpora hemorrhagica seen by the naked eye. From the clinical finding and history we regard this case with suspicion, since the test animal used was not well developed and no microscopic examination of the ovaries were made.

Reaction: Questionable - suggest test be re-run using larger test animal.

Case 4. - Mrs. C. A. Ward H. University Hospital No. 38303. White, age 25 years. Entered the Hospital complaining of acute attacks of pain in L.L. with nausea and vomiting. Mass in right inguinal region, which causes transient pain, and chronic constipation. The inguinal pain was first noticed two years ago, it was sharp at first, then dull. She had had three other attacks since the first, each lasting 3 or 4 days. She also complains of frequent urination but no burning. Menstrues every 28 days lasting 5 days and not excessive, but accompanied by some dysmenorrhea and back ache. Her last menstrual period was three weeks ago, and has had some morning sickness. A miscarriage of a 6 month old fetus occurred in October 1931.

General examination is negative except over the fossa ovalis of femoral vein is a ring admitting the little finger.

Pelvic: Considerable purulent discharge. Hypertrophied lobia minora. Vaginal mucous membrane ingested and slightly bluish. (Case I^V)

Cervix slightly softer than normal, but no enlargement could be ascertained while the fundus being A. V. and A. F. and slightly larger than normal. Adnexia ----- thickening in the right side with a palpable mass which was slightly tender, and about 3 cm in diameter. Right side negative.

Diagnosis: (1) Femoral Hernia. (2) Salpingitis?

(3) Pregnancy ?

Test: On March 23, 1932 a mature female rabbit was injected with 2.5 cc of clear patient serum and upon examining the animal in 48 hours she was found to be pregnant. The test was again repeated on the 27th, using a known non-pregnant animal, which was examined in 42 hours after injecting. Both ovaries contained many corpora lutea and corpora hemorrhagica.

Reaction - Positive.

Case 5. - Mrs. E. W. Ward K. Chart No 38321, White, Age 28 years, entered the University Hospital December 28th complaining of pain in lower back region, pain in pelvic region occasionally. Her last menstrual period was December 29, 1931. She has two children, the youngest four years, the oldest seven years. She states that she had a rather profuse yellow discharge during the last two months, and is troubled by frequent urination and burning. Menses began at the age of 15 years, with a duration of 5 days and is not painful.

Patient feels that pregnancy is impossible as she has been practicing coitus interrptus since birth of youngest child. She states she has not had an orgasim since early marriage. She dreads pregnancy a great deal. Three years ago patient had an abortion, which

was induced by a regular physician. One year ago patient induced her second abortion by taking pills by mouth. She had chills following both abortions.

General examination: The patient was rather thin, white female of about stated age, showed tenderness in epigastrium as well as in both inguinal regions. Pelvic examination revealed a large uterus and Hegar's sign was positive. There was tenderness in the fornices.

Diagnosis: 1. Pregnancy. 2. Gastric ulcer. 3. Quiescent pelvic inflammation.

Test: At 2:15 P. M. on March 23rd, a female rabbit was injected with 2.5 cc of the patient's serum. The ovaries were examined in 50 hours after injection, with both ovaries showing both corpora lutea and hemorrhagica.

Reaction: Positive.

Case 6. - Mrs. A. D. Age 22. White. Methodist Hospital. Complaints: 1. Cramp-like pains in lower abdomen. 2. Fever. 3. Intermittent vaginal flow for past two months, one day she would flow considerably, and the next but little. At times the flow would be red, other times it would be brown. She had had no menstrual history since December 1931, and had lost 15 pounds in weight in the last two weeks.

Onset and Development: She states the last menstrual period occurred in December 1921, and felt well until the first part of February 1932, when she vomited eight or nine times daily and continued until she was given morphine. Vomiting and the vaginal flow occurred at the same time. The flow at first was intermittently.

Catamenia: She began menstruating at the age of 11 years. Menses occurred regularly every 28 days lasting 7 or 8 days. There has

been no miscarriage. One normal pregnancy and delivery two years ago.

Diagnosis: Hydatidiform mole. The mole passed spontaneously on March 22, 1932.

Test: Blood was taken from the patient the same day, and a known non-pregnant mature doe was injected by the usual technic the following day. In 40 hours the ovaries were examined and showed numerous corpora lutea and hemorrhagica in both ovaries.

Reading of the Test: Positive.

Due to the fact that the patient's general condition did not improve and her temperature course was up and down, another blood specimen was taken nine days after she passed the mole. The specimen was run in the usual manner and the reaction was positive in both ovaries, but not as strongly so as in the first test.

Case 7. - G. T. Age 34. White. Dispensary Chart No. 54692. On March 3, 1932, patient came to the University Dispensary complaining of bloody show for the past week, which was her regular menstrual time.

General Examination was essentially negative.

Catamenia: Menstruation began at the age of 13 years, it has been regular every 28 days and lasted 3 days except when interrupted by pregnancy; she has four children all living and well, and gives no history of any miscarriage. She eats and sleeps well - gaining in weight. There is no history of nausea or vomiting or quickening. She returned nine days later with the complaint that the flow which began two weeks ago had become worse using four pads the previous day. At this time she had noticed no quickening and the bleeding has been painless, and

there is no history of colds, diarrhea, accidents or hard work. Her last baby was born 7 years ago. No examination had been conducted since that time.

Vaginal Examination: Cervix baggy, drawn to the left and somewhat hardened on the left side. Bloody mucoid material was seen oozing from the os. There is laceration of the os uteri with evidence of endocervicitis.

Abdominal palpation: Fundus not felt, no fetal heart sounds could be heard, while with bimanual palpation, the cervix felt like that of one at four or five months pregnant.

Twelve days later the patient returned with the complaint that she was still flowing in spite of rest in bed and treatment. At this time bimanual examination revealed a soft cystic like mass to the right of the uterus and not attached to it - suggestive of an ovarian or parovarian cyst. Two days later the patient returned feeling well and with no discharge.

Test: Two days before the bleeding stopped a pregnancy test was run with the usual technic.

Reaction: Negative in both ovaries macroscopically

Case 8. - Mrs. C. B. Dispensary No. 54923. Age 17 years. White-housewife, that had been married and now separated. She gave a history of the usual childhood diseases. She has had no operations except removal of her tonsils and adenoids. Menstruation began at the age of 13 years, was regular and lasted 6 days up until pregnancy occurred. There was no history of abortion or miscarriage.

General Examination was negative.

- Pelvic examination revealed the fundus slightly posterior. Hegar's sign was positive and Chadwick's sign was likewise positive at this time of examination.

Test: On March 29th, 1932 2.5 cc of the patient's blood serum was injected into the marginal vein of a sexually mature female rabbit. At 48 hours after the injection the rabbit was opened up under anaesthesia and the ovaries examined. Both ovaries showed, numerous corpora lutea and corpora hemorrhagica.

Reaction: Positive.

Case. 9. - Miss V. M. Dispensary No. 54996. White, single, age 18 years. Entered the University Dispensary April 2, 1932 complaining of no menstruation since February 15, 1932. She has had some abdominal pain, but otherwise has felt well. There has been no morning sickness, and no vomiting. Her Wassermann was negative and urine examination was negative.

General Examination was negative.

Menstrual History: Menses began at the age of 13 years and has been regular every 28 days lasting for 3 or 4 days. There is no history of a previous pregnancy.

Pelvic examination was negative.

Diagnosis of pregnancy not made.

Test: This patient's serum was tested in the usual manner, and both ovaries at the end of 48 hours showed no gross changes.

Reaction - Negative.

(3) PHOTOGRAPHIC SECTION

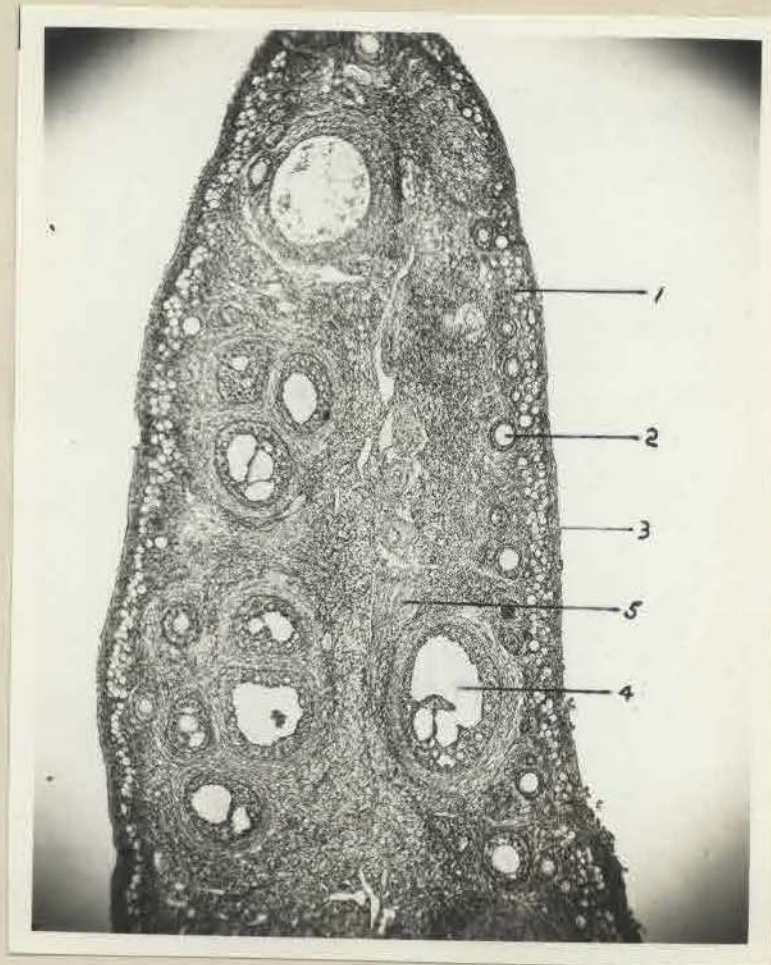


Figure 1. Normal ovary section from a virgin rabbit. x 35.

1. primordia follicles; 2. growing follicle; 3. germinal epithelium;
4. Graafian follicle; 5. corpus luteum atreticum.

The germinal epithelium is seen lining the free surface of the ovary, between this and the outer epithelium is a basement membrane. The section shows a vast number of primary follicles which have migrated inward as they developed.

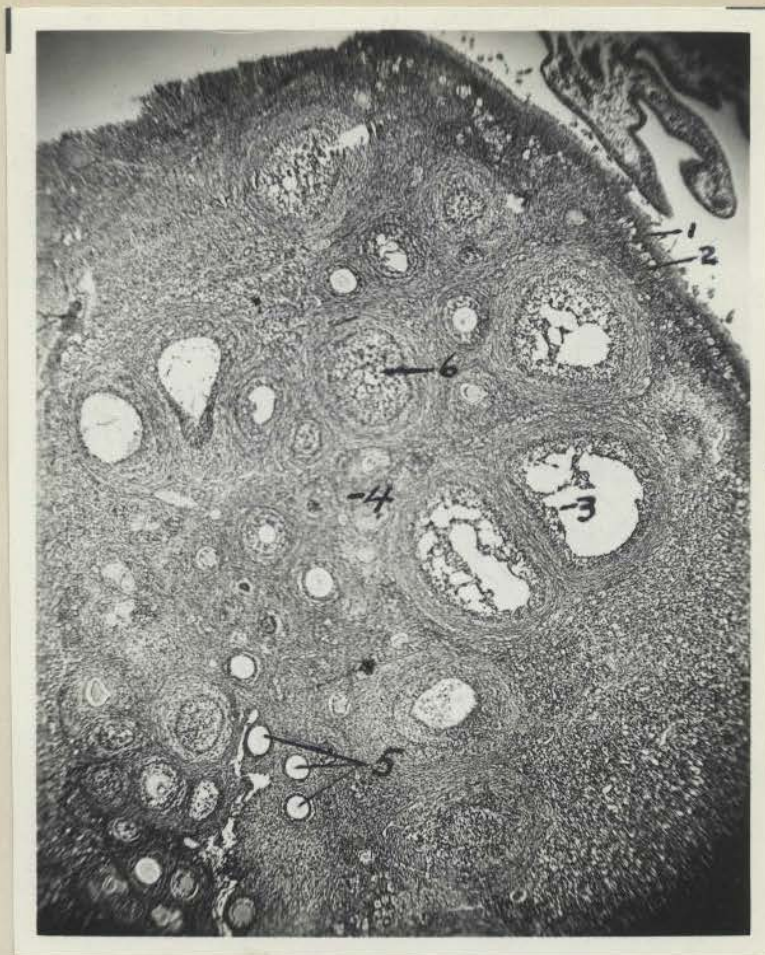


Figure 2. Cross section of a normally mature rabbit ovary. x 35.

1. germinal epithelium; 2. growing follicles; 3. Graafian follicle;
4. corpus luteum atreticum; 5. blood vessels; 6. peripheral section of
vesicular follicles.



Figure 3. Section of right ovary from mature rabbit 24 hours after injection of 2.5 cc of patient's serum, x 35. 1. primary follicle; 2. surface of ovary with germinal epithelium; 3. follicular liquid; 4. granular precipitate; 5. membrana granulosa; 6. theca externa and interna.

It will be noted that the Graafian follicle in this section has been stimulated to size several times larger than the ones seen in the normal ovary (Fig. 1 and 2) also that the follicle has migrated to the periphery of the organ, the outer part of the wall is very thin and has ruptured allowing part of the follicular liquid to escape, while the remaining liquid has in part formed a fine granular appearing precipitate. This is a typical section of a positive reaction to the test.



Figure 4. Section taken from the ovary as Figure 3 shown a Graafian follicle that has ruptured and discharged the follicular liquid. The base of the follicle has filled in with blood, while the surface has contracted forming a typical corpus hemorrhagicum, and is easily seen macroscopically on ovaries giving a positive reaction to the test.



Figure 5. Gross appearance of right and left ovaries of a sexually mature virgin female rabbit 48 hours after injection of 2.5 cc of patient's serum. This is a typical reaction showing both corpora lutea and corpora hemorrhagica in each ovary.



Figure 7. Gross appearance of rabbits ovaries showing difference in size and appearance. A and B, following injection of serum from pregnant patient; C, no change in ovary following injection of serum from non-pregnant patient.

(4) COMMENT

Although the number of cases run by this technic has been quite limited, the results compare favorably with those of similar tests. The use of blood serum gives a higher concentration of anterior pituitary hormone, than is found in urine of pregnant women as was shown by Brown (8). The method is rapid and the technic is simple. By using the same animal several times brings the cost of the test down to a practical value even to the general practitioner. The test is as reliable as are the physiological functions of the body. Errors may occur in technic and interpretation as in any other test. The test is of great value in determining the presence of hydrotidiform moles.

VI OMISSIONS

In this Resume' all of the more modern tests for pregnancy have not been discussed. The following tests have been omitted in view of the fact that they all occur in foreign literature and the articles were not available.

1. Sero-electric Test.
2. Prolan Test.
3. Bromine Water Test.
4. Porges - Pollaczek's Test.
5. Luttge - Von Mertz Test.

VII CONCLUSIONS

1. Tests for pregnancies have been tried for at least 3000 years, but none have proven of practical value and reliable until the biological test was introduced by Aschheim and Zondek in 1928.
2. In reviewing the technique and results of the various laboratory tests for pregnancy, it would appear that, and especially those concerned with the experimental production of glycosuria, possess only a presumptive value. The pupillary test may prove of value if properly executed. None of these tests have proven very popular either for a reason of technic or reliability.
3. The biological tests have gained great popularity since their advent. Most of them are as accurate as any test we have for medical diagnosis of today.
4. The biological tests are based on an increased presence of anterior pituitary hormone in blood of pregnant women or an elimination thereof in the urine, which when injected into a virgin experimental animal causes the formation of mature follicles, hemorrhagic spots and atretic corpora lutea in their ovaries.
5. The Aschheim - Zondek test in the hands of various experimenters has proven 98% accurate. The disadvantages of this test are, that immature mice, the age of 20 to 21 days old are required, and the ovaries must frequently be examined microscopically.
6. These same objections are obviated with the Brouha test, in which, the animal must be nearly mature, and the reaction is read macroscopically. However, the time required for this test is a distinct disadvantage.
7. The advantages of the Friedman test over the Aschheim - Zondek

tests are that only one test animal is required, and the results may be determined after twenty-four to forty-eight hours by macroscopic examination. The rabbit is much more sensitive to the reaction than the mouse or the rat.

8. The anterior pituitary hormone is more concentrated in the blood serum per unit of volume than in the urine, therefore, using blood serum instead of urine has given nearly 100% accuracy by this technic as shown by Brown.
9. The modified technic of the Friedman test is practical, accurate, and economical.
10. All of the hormonal pregnancy tests are of great value in distinguishing either early or late pregnancy from other conditions. The greatest value is in detecting early pregnancy in cases in which continuation of the pregnant state would be dangerous to the physical well-being of the patient.
11. These tests are of no less value in the differential diagnosis of early pregnancy, fibroids and other uterine tumors, ectopic pregnancy, hydatidiform mole, chorioepithelioma, abortion, toxemia of pregnancy, functional amenorrhea, and the menopause which have not been discussed in this Resume'.
12. A negative hormonal test does not exclude the possibility of pregnancy; repeated tests should be applied.

VIII

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