Pregnancy Tests

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Editor's Note: Dr. Mathieu will present some new conceptions in regard to "Sedimentation Distance" in its general application and also in relation to pregnancy tests in the December Sentinel.

THERE has been a constant demand in the minds of the medical profession, and in the lay mind, also, for signs and tests that would diagnose early pregnancy. That space of time between the first missed period of menstruation and the time when it is comparatively easy to clinically diagnose pregnancy, is the time which for the clinician is fraught with a keen desire to be positive in the knowledge as to whether or not there is pregnancy. There are almost as many different clinical signs and laboratory tests for pregnancy as there are theories concerning the etiology of eclampsia. Many of these signs and tests are either valueless or merely fair guesses, while others have as their main strength, a fertile imagination in the mind of the clinician. Nevertheless, some of the laboratory tests have had a foundation, at least of sufficient value to stimulate many minds to work on the problem and the discovery of the female sex hormone and the nature of the hormone of the pituitary gland have grooved the workers toward what now appears to be inevitable success. Though this success has not been attained as yet, it appears certain that the pregnant woman has in her blood, and in her urine, certain constituents that the non-pregnant woman does not harbor. Upon the presence of these constituents are based the various laboratory and biologic tests for pregnancy.

It is well that we take a mountain-top view of the situation and that we be conversant with its present status. With this in mind I am submitting to you pertinent notes recently gathered on the subject during lectures in Vienna and from translations of German literature. I am holding no brief for any tests, but am merely presenting transcribed notes and attempting to describe the most promising tests, many of which I am now using.

It is obvious that pregnancy tests cannot locate the pregnancy; that is, the tests when positive do not tell us that the pregnancy is in the uterus, or in the tube, or in the abdominal cavity, or in the ovary. What is more, if the test is positive, we are sure that the foetus is alive and growing while in cases of hydatidiform mole, ectopic pregnancy and abortion, the tests are of less value. There are six or seven tests, none of which are absolutely reliable, though the Zondek-Ascheim test seems to offer great hopes of eventual perfection.

Tests Dependent Upon Glycosuria of Pregnancy or Lowered Threshold for Sugar

Tests were worked on fifteen years ago by Franke and Nothman on the basis of their knowledge that the pregnant patient is liable to glycosuria; that is, she has lowered threshold for sugar. They are used in various ways as follows:

Give the patient without breakfast, 100 grams of water-free dextrose and 500 cc. of water at 8 A. M. after having examined a specimen of urine to be sure that this patient has not already a

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*Free use has been made of notes from lectures given by Professor W. Schiller of the Kermauner Clinic, Vienna.

glycosuria. Waterfree dextrose is used because one cannot be sure that ordinary dextrose does not contain water, which might cause an error of from twenty-five to fifty per cent. Examine another specimen of urine at 8:30 a. m. and give another 500 cc. of water and then examine the urine again each half hour for three times more. If the test is positive, sugar will be found in the third, fourth and fifth specimens. It is always negative in the first and nearly always in the second. This test is the simplest of the sugar tests for pregnancy, but it is uncomfortable for the patient to take 100 grams of dry sugar. There is apt to be vomiting and the dextrose is expensive.

This test has been modified by the administration of 200 grams of starch, rice and bread, which, however, was found very unreliable. Another modification consists in giving 20 grams of dextrose and 0.50 mg. of adrenalin. Adrenalin allows the liver to throw glycogen into the blood stream, thus temporarily raising the blood sugar level, with at times a temporary glycosuria. The use of adrenalin caused in some instances, palpitation of the heart, and a few patients who were perhaps already in the process of aborting, blamed the adrenalin for the abortion. The test was then simplified by the use of phloridzin given under the trade name of Maturin.

In testing for sugar, the Nylander test is better than Fehling's or Benedict's. The following case report is pertinent:

Mrs. T. (a doctor's wife) age 23, of the Froelich syndrome type, occasionally misses periods or is delayed in the menstrual flow. Her last child was born six months ago. Following the birth of this baby, she had three regular periods. The last one was on July 9th of this year. On August 9th, she had not menstruated. She was in great alarm. The fundus appeared to be slightly enlarged and slightly irregular, though there was no other signs of pregnancy except the amenorrhoea. It was decided to use the Maturin test. She appeared at the office without breakfast. Thereupon exactly 1 cc. of maturin was injected intramuscularly, the urine first having been examined to see that it was free from sugar. The first two specimens after the injection were positive for sugar with Benedict's solution, but were negative with Nylander's. Because the manufacturer of Maturin insists on the use of Nylander's test I presume there must be something about this test that makes it absolutely essential. At any rate, I gave the woman a tentative diagnosis to the effect that she was not pregnant. Within a week following this, she had what appeared to be a normal menstrual period, and there was no sign whatsoever that she had been pregnant.

With this test eighty-five out of one hundred positive results were verified by actual pregnancy. The other fifteen per cent were falsely positive, there being latent glycosuria, corpus luteum cysts or carcinoma of the ovary. On the other hand, in one hundred negative results, there was only one woman who was found pregnant, thus, the test is apparently eighty-five per cent efficient if positive; and ninety-nine per cent efficient if negative except during the premenstrual phase. The test becomes of value at the fifth or sixth week of pregnancy, and is especially reliable after the sixth week. If it is negative, it should be repeated on the following day, giving the phloridzin intravenously after breakfast in the same dose. If any phloridzin gets into the tissues, it is extremely painful. To avoid this, draw up into the needle several drops of saline solution, so that if the vein is missed. the saline solution gets into the tissues and not the phloridzin. Or, if the phloridzin does get into the tissues, put the blood pressure apparatus on and cause a leakage and the formation of an hematoma. This will dilute the phloridzin and relieve the pain. Do not use this test in the premenstrual phase, as it is positive at this time in sixty-five per cent of the cases.

A Test Dependent Upon the Presence of Acetone

Acetone bodies are excreted in the urine of a pregnant woman who is on a diet poor in carbohydrates. They are not found in a non-pregnant woman on an identical diet. This acetonuria appears to be due to a lack of sugar or to a change in the relationship of sugar and fat. In pregnancy, fifteen hours without sugar means acetonuria. To determine this condition, a two day special diet is used in preparation as follows:

8 A. M.—Tea and 20 grams of sugar.
10 A. M.—Two eggs or 40 grams bacon.
12 Noon—Clear soup and 150 grams roast beef.
4 P. M.—Tea and 20 grams of sugar and 2 eggs or 40 grams of bacon.
7 P. M.—Same as at 12 noon.

One or two days of this diet is sufficient. On the following day, examine the urine and if it is free from acetone, start the test, using the same diet but substituting saccharine instead of sugar and omitting white bread.

Examine the urine at one, three, five and seven o'clock. Absence of acetone at the one, three and five o'clock tests will rule out pregnancy with ninety-six per cent accuracy. This test becomes reliable at the same time as the sugar tests (the sixth week) and remains so up to the middle of the seventh month.

Acetone Test in Urine.—Technique of test: Use sodium nitro-prusside only in crystals. Put a few crystals into a small quantity of urine, then one or two drops of diluted acetic acid, and overlay with concentrated ammonia. The positive result is a purplish (not brownish) ring and must turn darker from minute to minute. Brown signifies peptone or proteids.

It is obvious that the presence of acetone in any woman's urine does not of necessity mean pregnancy, but where there is a doubt and no other reason for an acetonuria, this test is most delicate and is a valuable aid in diagnosis.

A Test Dependent Upon the Presence of an Hematoma

There is no specific test for ectopic pregnancy, per se, but there is a good test for the presence of an hematoma which might point to ruptured ectopic pregnancy. It is based on the fact that during the absorption of an hematoma, bilirubin is formed in the tissues surrounding the hematoma; and is absorbed into the circulation. There then occurs an hyperbilirubinemina in the blood serum. Normally, in the blood there is 1/200,000 of bilirubin and if this rises to 1/7,000 jaundice appears, 1/20,000 to 1/30,000 being the usual amount in the blood after a ruptured ectopic pregnancy. After the rupture, either an hematoma forms or there is free blood in the abdomen. The circulation takes up the bilirubin and deposits it in the liver, which gives it off into the bowel where it is changed to urobilinogen; this in turn gets back into the liver and is converted into bilirubin again. This mechanism is called the internal secretion of bilirubin. When this takes place there is an increased elimination of urobilinogen in the urine, and since the test for bilirubin in the blood is difficult and intricate, the test for urobilinogen in the urine has replaced it. The test is called the aldehyde test and is used by mixing the urine with Ehrlich's re-agent when a reddish precipitate forms. Unless the urine be fresh or preserved in a cold dark place, the urobiliiogen may change to urobilin. Urobilinogen may be normal after large meals, so the first urine in the morning is examined. This test is not specific in all hematomata, but can be used in the differential diagnosis between tumor and apoplexy. It is very frequently positive after operations, eight to ten hours after the formation of an hematoma. It is a good strong diagnostic finding where a ruptured ectopic is suspected.

The Urobilinogen test must be quantitive; a normal individual may show small amounts.

Use six test tubes in a row in a rack. In the first put 2½ cc. tap water and 2½ cc. of the urine; mix, and from this draw 2½ cc. and put in the second tube, and add 2½ cc. of water. Mix contents of the second tube and withdraw 2½ cc. and put in the third tube and mix and so on until the last tube is finished. Discard the 2½ cc. which has been withdrawn from the last tube. The dilution of the 6 tubes is as follows:

1	2	3	4	5	6
1 to 2	1 to 4	1 to 8	1 to 16	1 to 32	1 to 64

Then add ½ cc. of Ehrlich's Aldehyde or Ehrlich's reagent into each tube, shake and mix all tubes and read. A reddish coloration indicates urobiligen and a greenish coloration points to its absence. The use of a white paper as a background will aid in the reading which is done by looking down into the tubes from above. In the normal, it is never positive or reddish in over 1 to 8 dilution or tube number 3. There are several rules applicable to this test:

Rule 1. It takes 8 or 9 hours after the bleeding starts before the test is positive.

Rule 2. It is only when the blood is absorbable that it is positive. It (the blood) might be encapsulated and non-absorbable.

Rule 3. If the bleeding has stopped five or six days before the test, it is of no value and will be negative.

Rule 4. Conversely with rule 3, the test shows positive if there is still bleeding after five or six days.

Rule 5. In a question of ectopic pregnancy: If the sugar test is positive and urobilinogen test is negative, this is a strong indication that the fetus is still alive.

Rule 6. One must rule out apoplexy, passive congestion, and cirrhosis, as these may give a positive result.

Intracutaneous Test

This test is quite new, having been published only a few weeks ago. The method consists in injecting the hormone of the anterior pituitary lobe intracutaneously. Pregnant women give no reaction and the non-pregnant woman gives a marked skin reaction in twenty-four to forty-eight hours. Prolan (hormone of the anterior pituitary) is the substance used and one-tenth cc. is the amount. I have been unable as yet to

obtain prolan and have not tried this test. It appears that the reaction is negative in the pregnant woman since her body fluids contain great quantities of this hormone, and that the positive reaction of the non-pregnant woman is due to the absence of this hormone in her body fluids.

Ninhydrin Flocculation Test

This is a good test and its authors claim an accuracy of 95 to 99 per cent. It is done with serum from the blood of the suspected woman. The serum must be absolutely clear and free from blood stain. There are three fluids involved as follows:

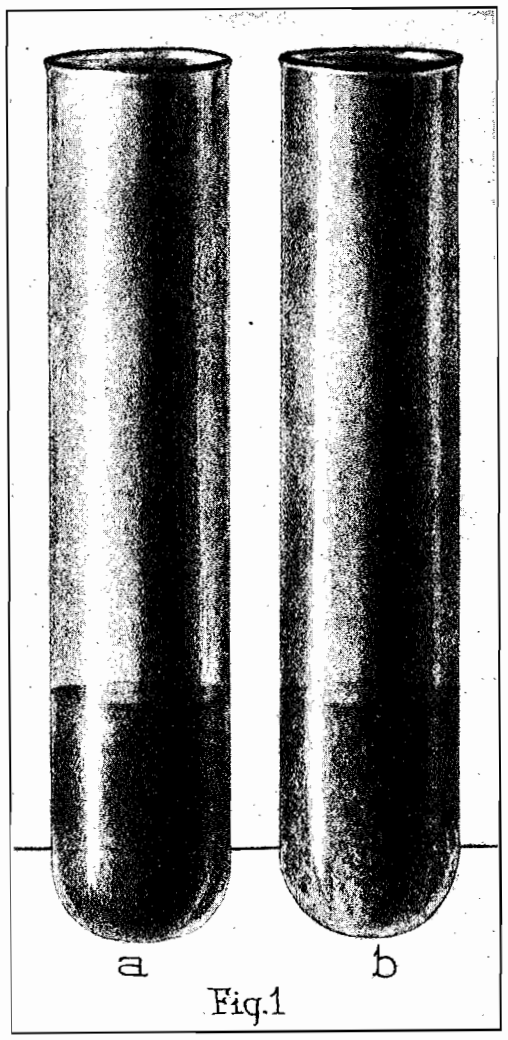

Figure 1

Ninhydrin Test: Tube A. Positive. This is the result in pregnancy. The color is purple. The precipitate is very fine; hardly discernable, and settles very slowly.

Tube B. Negative. This is the result of the test with a known non-pregnant serum. The color is purple, lighter than that of tube A, and contains large coarse flocculi which settle very rapidly.

Fluid No. 1: 954 cc. distilled water; 2.5 grams potassium bicarbonate; 55 cc. lime water.

This fluid keeps indefinitely and before using it must be saturated with carbon dioxide (a 10 litre bottle is filled with carbon dioxide, the above solution is poured into the bottle and then shaken well. This saturates fluid number 1 with carbon dioxide).

Fluid No. 2: 1% solution of Ninhydrin (Triketohydrindene Hydrate).

Fluid No. 3: Blood serum (older than one-half day and not older than three days).

Mix these three solutions as per the following formula:

10 cc. from the first solution.
0.2 cc. of the second solution,
1.75 or 2 cc. of serum.

Put into boiling water bath (but do not boil) for three minutes. (See Fig. No. 1.)

The solution turns purplish and if a control is used it will be found that the solution, which contains the serum of the pregnant woman, will be deeper in color and show a very fine precipitate hard to see, which settles slowly, while in the control or non-pregnant specimen the color will be lighter and the precipitate will consist of a heavy cloud of large, coarse flocculi, which settles to the bottom of the tube very quickly.

I will quote freely from Max Davis, "Although the test has been in use for over two years, the physiological basis for its success has not been definitely determined. It has been suggested that it depends upon the different amounts of serum-albumin and serum-globulin in the bloods of pregnant and non-pregnant women and upon changes in their lability as regards precipitation. But definite proof of this fact as the basis of the test has not been established."

"This test was first developed by W. Vogel, who announced in his first report that it was positive in 97.8 per cent of 281 cases of pregnancy and negative in 97.7 per cent of 212 non-pregnant women. In the first three months of pregnancy, the test proved reliable in only 76 per cent of the cases. The test was checked by various workers, who were not as enthusiastic in its praise as Vogel had been. On the other hand, very good results were reported to me orally by Dr. Schiller, the pathologist at Kermauner Clinic in Vienna. He believed some of the earlier failures to have been due to insufficient saturation of the first reagent with carbon dioxide gas. When this error had been corrected, the results of the test improved. The test first becomes positive at the third week of pregnancy and becomes negative two weeks post partum."

"I now wish to report my own experience with this test in 586 cases, 234 pregnant and 352 non-pregnant patients. The exact condition of the patient was unknown to me before the test in almost all of the cases. The findings were checked, after they had been written down by the laboratory technician. The results are indicated in the table below, which gives a detailed analysis of all the cases."

Cases of Pregnancy	Cases No. of	No. Positive	%	No. Negative	%
(1) 1½-3 months	32	32	100	0	0
(2) 3-9 months	200	200	100	0	0
Total	232	232	100	0	0
Non-Pregnant cases					
(1) Males	120	5	4.1	115	95.9
(2) Pelvic conditions (Pregnancy excluded)					
(a) Fibroids	13	3	23	10	77
(b) Ovarian Cysts	10	0	0	10	100
(c) Carcinoma	1	0	0	1	100
(d) Salpingitis	12	2	16.7	10	83.3
Total	36	5	14	31	86
(3) Functional amenorrhea (over 6 weeks)	16	0	0	16	100
(4) Miscellaneous	780	7	3.9	173	96.1
Total	352	17	4.8	335	95.2

"From this table it is evident that a negative report in any patient who has the possibility of being more than six weeks pregnant practically excludes pregnancy, for there have been no failures in diagnosing pregnancies of a more advanced stage than this. My experience with patients under six weeks pregnant has been limited to two cases, one of which was diagnosed correctly. The second patient also gave a positive reaction when the test was repeated two weeks later. At the present, therefore, the degree of effectiveness of the test in these very early pregnancies cannot be exactly stated by me."

In the non-pregnant patients, there is an error of about five per cent, including five male patients. The type of case in which these errors occurred is as follows:

Females—

(1) Fibroid	3
(2) Salpingitis	2
(3) Appendicitis	4
(4) Cardiac (decompensated)	1
(6) Hypo-ovarianism	1
(5) Epilepsy	1
Total	12

Males—

(1) Syphilis (Wass. neg., primary lesion over 2 years before)	3
(2) Ruptured appendix	1
(3) Ruptured gastric ulcer	1
Total	5

"The gross error is not great, but from the analysis of these cases, it is seen that it occurred for the most part in patients with pelvic or with closely allied (appendix) trouble. The number of this type of case is small, but if this percentage should continue in a large series of cases, it would, of course, detract from the value of a positive report by this test."

"An effort was made to determine possible sources of error in the technique of the test and the following were discovered:

1. The introduction of red blood cells with the serum used in the test or the use of hemolyzed blood may give false negatives. (This was tested on known positive cases.)
2. The use of too much ninhydrin or of too little serum may give false positives.
3. The use of tap water instead of distilled water in preparing first re-agent may give false positives.
4. The use of serum over forty-eight hours old, even if kept on ice, may give false positives.
5. Overboiling, up to ten minutes, had no effect upon the correctness of the test."

The Aschheim-Zondek Hormone Test for Pregnancy

During pregnancy as pointed out by Erdheim and Stumme, marked changes take place in the anterior lobe of the pituitary gland. Smith, Engle, Evans and Long demonstrated that marked changes in the ovaries of mice and rats took place after the injection of the anterior lobe of the pituitary gland. In 1927 Aschheim and Zondek reported that in the urine of pregnant women there were present in large quantities two female sex hormones; that of the ovary and that of the anterior lobe of the hypophysis. They attempted to work out a biologic diagnosis of pregnancy by means of the demonstration of the hormone in small quantities of the urine (1 to 2 cc.) and reached the conclusion that the demonstration of the ovarian hormone in the urine was not suitable for a biologic diagnosis of pregnancy because this hormone could not be demonstrated in very small quantities of the urine of pregnant women earlier than the eighth or tenth week and then not with absolute certainty. Moreover, the ovarian hormone might occasionally be found in the urine of non-pregnant women and in functional disturbances and frequently in large quantities during the climacterium. On the other hand they state "The demonstration of the hormone of the anterior lobe of the hypophysis in the urine of humans is particularly adapted to the biologic diagnosis of early pregnancy." By means of their technique they demonstrate a substance which is by no means a specific for pregnancy but rather they show the presence of the hormone of the anterior lobe of the hypophysis. What is characteristic of pregnancy is merely the enormous increase of the hormone

and its marked excretion in the urine of the pregnant woman. By using their test on all varieties of disease, on pregnant and non-pregnant persons, they were able to show a startling accuracy for their test. The sources of error, few as they were, obtained in severe endocrine diseases, such as myxedema and acromegaly and in carcinoma of the female genital tract. The total percentage of error was 4 per cent. Since pregnancy is hardly apt to occur in the myxedematous or in the acromegalic, and since genital carcinoma and pregnancy occur together in a ratio of 1 to 10,000, one can see that these sources of error need scarcely be considered. Some of their positive results were in patients only five days over the missed period. The method appears to have an exactness as great as we can demand of any biologic test.

Knowing that the urine of the pregnant woman contains great quantities of the hormone of the anterior lobe of hypophysis, Aschheim and Zondek made the effect of the hormone of the anterior lobe of the hypophysis upon the ovaries of young mice or young rats the basis of their test. The subcutaneous injection of the urine of pregnant women into immature female mice was followed by remarkable alterations in the ovaries, shown by swelling, congestion and hemorrhage and the premature maturation of the ovarian follicles easily visable to the naked eye.

The specific action of the anterior lobe of the hypophysis manifests itself in the ovaries of infantile mice three to four weeks old and weighing from six to eight grams, or in immature rats weighing about 65 grams. In these animals the hymen is imperforate and there is no evidence of maturation of the follicles, ovulation or appearance of the estrus. In the course of one hundred hours, the time that it takes to make the test, three reactions appear.

Reaction 1. There is maturation of the follicles, ovulation and appearance of the estrus, and the hymen becomes perforate (see figure 2). The appearance of estrus, while not being diagnostic, is a valuable sign which points towards a positive reaction (see figure 3). The follicle grows in size, matures and ruptures, and the ova enter the tube, and corpus luteum forms in the ruptured follicle. From the action of the hormone of anterior lobe, the ovarian hormone is produced in the ripening follicle, which in turn brings on estrus manifested by an enlargement and accumulation of secretion in the uterus. Changes in the vagina and a typical five cycle estral change in the vaginal smears appear according to Allen and Doisy (see figure 3).

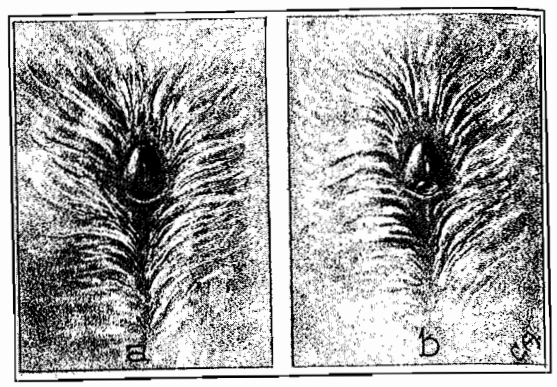

Figure 2
(a) Drawing of imperforate hymen in immature rat **before** the injection of urine containing the hormones, i. e., urine of pregnant woman.
(b) This shows a perforate hymen with the secretion projecting through it, one hundred hours **after** injection of the urine of the pregnant woman.

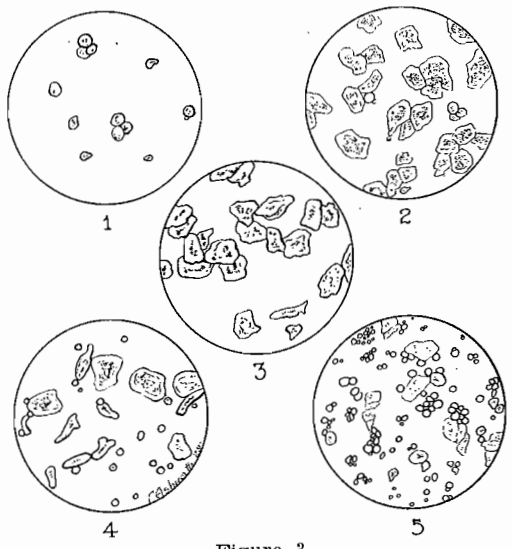

Figure 3
Microscopic Diagnosis of the Estrus Cycle in the Albino Rat*

Stage 1. Pre-estrus: Before heat begins—no sexual excitement. Vaginal smear shows small epithelial cells with nuclei—no leucocytes.
Stage 2. Estrus: The heat period—copulation accepted. Vaginal smear shows large squamous epithelial cells without nuclei—no leucocytes.
Stage 3. Late estrus: The heat period is over—no sexual excitement. Vaginal smears are thick and cheesy, containing clumps of large squamous cells without nuclei. Late in this stage there may be some large epithelial cells with nuclei—there are no leucocytes.
Stage 4. Metestrus or postestrus: The stage of degeneration and leucotysis. Vaginal smears show moderate numbers of polymorphonuclear leucocytes, squamous cells and large epithelial cells.
Stage 5. Anestrus or diestrus: Pause—the resting stage. The vaginal smear shows polymorphonuclear leucocytes as the predominating cells and moderate numbers of large epithelial cells and strings of muccus.

*Method described by Long and Evans—Memoirs of the University of California, Vol. 6, p. 1, 1922.

Reaction 2. Massive hemorrhage into the enlarged follicle. The whole ovary is hyperemic and the vessels markedly dilated. Macroscopically, the hemorrhage can be recognized as a sharply circumscribed point, brown to bluish red, the size of the head of a pin projecting above the head of the ovary, which they have designated Petchia.

Reaction 3. Formation of corpora lutea atretica with luteinization of the theca cells. Aschheim and Zondek have shown

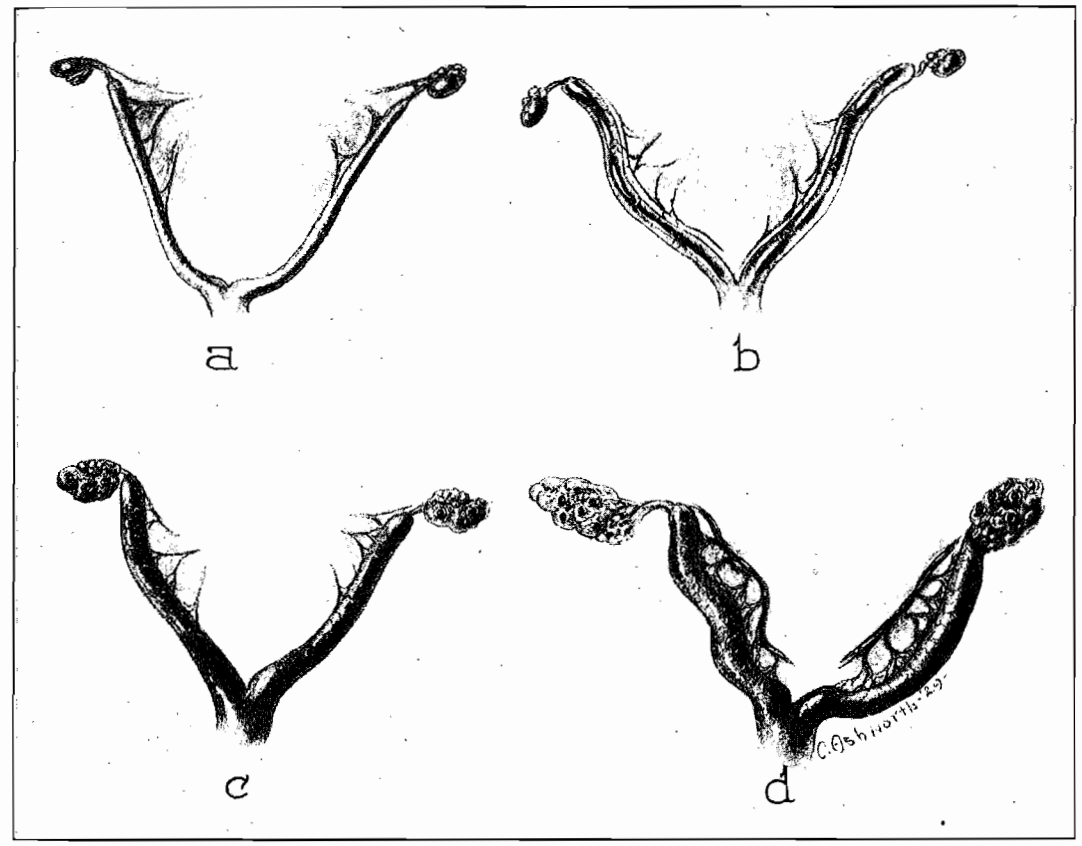

Figure 4*
(a) The uterus, tubes, and ovaries of the normal immature rat.
(b) Uterus, tubes and ovaries of the immature rat following the injection of known non-pregnant urine. Note the somewhat cystic enlargement of the uterus and the absence of ovarian change. This change in the uterus is probably due to the small amount of ovarian hormone in the urine of the known non-pregnant woman. This urine was collected in the post menstrual phase of the non-pregnant woman.
(c)(d) The uterus, tubes and ovaries of immature rats which were injected with the urine of pregnant women according to the technique of Ascheim and Zondek. Note the engorgement of uterus and tubes and the great enlargement of uterus, tubes and ovaries. The dark spots in the ovaries represent the hemorrhagic areas. (See figures 6, A, B and C.) The hymens in (a) and (b) were imperforate while the hymens in (c) and (d) were perforate and the vaginal smears showed estrus. See figures 5, 6 and 7. These specimens were from rats of the same litter and all of approximately the same weight.

*I wish to acknowledge my indebtedness to Dr.Manville of the University of Oregon for assistance in rat experiments, and to Dr. Menne andthe Department of Pathology for preparation of microscopic material.

that there is an explosive over-production of the hormone of the anterior lobe of the hypophysis almost immediately after the implantation of the fertilized ovum in the uterus. This over-production continues throughout pregnancy but ceases a few days after delivery. There is also an over-production of the ovarian hormone, but it does not become very marked until in the second or third month and is not suitable for diagnosis. The reactions through the hormone of the anterior lobe of the hypophysis, which are applicable to the diagnosis of pregnancy, are reactions 2 and 3. Reaction 1 is not diagnostic in itself but warrants further tests with the urine. To repeat, the positive reactions which show pregnancy are seen in (a) hemorrhage into one dilated, often partially luteinized, follicle (b) a corpus luteum atreticum.(See figure 4.)

Technique

The technique of the method is as follows: The examination is carried on by means of infantile mice, three to four weeks old, weighing from six to eight grams, or immature animals so that the results are due to the injected urine and not to spontaneous maturity. For each urine examined use five animals. The action of the urine must be tested on several animals for the following reasons: (1) One animal must be used as a control. (2) One animal might die as a result of the injection. (3) All animals do not react equally strong. (4) The pregnancy action is to be considered positive, even though it appears in only one animal. The morning urine is used because in this urine the hormone is in the greatest concentration. If the urine cannot be tested immediately a disinfectant must be added. For this, one drop of pure tricresol is added to every twenty-five cc. of urine, and the urine is well shaken. Before the test is performed the reaction of the urine is tested. If it is alkaline or neutral, acetic acid is added until it becomes weakly acid to litmus. The urine is filtered and the clear filtrate is used. Aschheim and Zondek describe their technique of the injections as follows: The urine is injected in 6 portions, which are spread over 48 hours. We like to begin the examination on a Monday or a Tuesday, because thus the examination can be finished on a Friday or Saturday. The injections of the 6 portions of the urines are done as follows:

1. Monday A. M., 11 to 12 o'clock;
   Monday P. M., about 5 o'clock.
2. Tuesday A. M., 10 o'clock;
   Tuesday P. M., 1 o'clock;
   Tuesday P. M., 5 to 6 o'clock.
3. Wednesday A. M., about 10 o'clock.

The urine is injected into the young mice subcutaneously in the following quantities:

Animal 1 six times, 0.2 cc.
Animal 2 six times, 0.25 cc.
Animal 3 six times, 0.3 cc.
Animal 4 six times, 0.3 cc.
Animal 5 six times, 0.4 cc.

"As we have already stated, only the findings in the ovaries of the young mice are to be considered in interpreting the pregnancy reaction. Enlargement of the

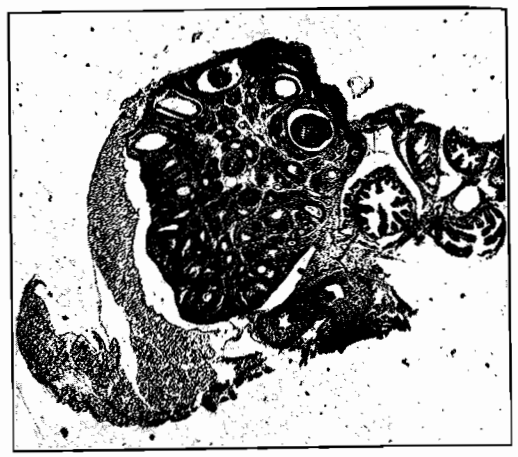

Fig. 5A. Shows section of entire ovary of normal immature Albino rat weighing approximately 65 grams. Magnified.

uterus and a positive vaginal smear merely mean to us evidence of homonal activity which can be caused by the ovarian hormone as well as the hormone of the anterior lobe. The pregnancy test may therefore be carried out without vaginal smears. We advise, however,

Fig. 5B. Microscopic section of a portion of (5A) ovary of normal immature Albino rat weighing approximately 65 grams. Note the immature follicle in various stages of development with intrafollicular stroma. Compare this with sections from injected rats (6A, B and C).

Fig. 6A. Section of the entire ovary of immature Albino rat weighing approximately 65 grams 100 hours after the injection of the urine of a known pregnant woman according to the technique of Ascheim and Zondek. Note the hemorrhagic areas, the Graffian follicle with thecal cell formation and the corpora lutea.

Figs. 6B and 6C. Higher magnification of portions of 6A. Note hemorrhagic areas and almost completely developed corpus luteum with lutein cells growing inwards; and stimulated follicle with beginning thecal accumulation.

that a vaginal smear be made because in many cases our attention is sharpened. When we begin the test on Monday we make a smear on Monday and then not again until Wednesday evening. Then smears are made Thursday morning and evening and Friday morning (see figure 3). The young animals are all killed on Friday morning when the test is begun on Monday, and on Saturday morning when the test is begun on Tuesday. Death is caused by the use of ether. The sex organs are examined most carefully, especially the ovaries. In the majority of cases the diagnosis can be made by macroscopic inspection of the ovaries. If this is decisive, if the corpus luteum cannot be recognized with definiteness, the ovaries are serially sectioned. The ovary must be fixed in Zenker's fluid. Formalin fixation is not sufficient, because the shrinkage of the cells may lead to erroneous interpretation. The test is moreover repeated with the same urine.

There have been many modifications suggested but the authors insist that the above technique is the most accurate. The authors summarize their results in the following three statements:

(1) "The hormones of the ovary and the anterior lobe of the hypophysis, which are over-produced during pregnancy and not utilized in the metabolism of the pregnant individual, are excreted in large quantities in the urine throughout the entire duration of the pregnancy."

(2) "The demonstration of the presence of the ovarian hormone in small quantities of urine is not suitable for the biologic diagnosis of pregnancy because large quantities of the hormone may be excreted in the urine in other conditions than pregnancy, as for instance functional ovarian disturbance (menapause, hyperhormonal amenorrhea, etc.)"

(3) "The demonstration of the presence of the hormone of the anterior lobe of the hypophysis in small quantities of urine 1.2 to 2.4 cc. of urine, is admirably adapted for the early biologic diagnosis of pregnancy when carried out according to our technique."

They also tried this test on many control cases, which showed that (1) the various stages of the menstrual cycle in mature, healthy women, had no influence on the excretion of the hormone of the anterior lobe of the pituitary in the urine; (2) women who had passed through the menapause were investigated with absolutely negative results; (3) cases with a clinical diagnosis of irregular bleeding (pregnancy excluded) were all negative; (4) the urine of men gave negative results; (5) many cases with internal diseases, including cardio-vascular disease, tuberculosis, pneumonia, hypertension, pyemia and other diseases were all negative; (6) all examined endocrine cases gave a negative pregnancy reaction; (7) in 42 cases of amenorrhea, in which pregnancy could be excluded, all urines were negative for pregnancy reaction.

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