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Aschheim zondek reaction in the early diagnosis of pregnancy

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SENIOR THESIS

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GLEN H. JODER

THE ASCHHEIM-ZONDEK REACTION IN THE

EARLY DIAGNOSIS OF PREGNANCY

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TABLE OF CONTENTS

INTRODUCTION-----1

HISTORY-----3

THE ASCHHEIM-ZONDEK REACTION-----10

TECHNIQUE OF THE TEST-----17

CASE REPORTS-----28

SUMMARY AND CONCLUSIONS-----39

ILLUSTRATIONS-----42

BIBLIOGRAPHY-----46

INTRODUCTION

The diagnosis of early pregnancy has long proved to be of the greatest difficulty to the practitioner of medicine. There are many times when a positive diagnosis of pregnancy is of the utmost importance to the patient and to the physician, but until the present time it has been a problem that could not be solved until sufficient time had elapsed to allow a diagnosis by definite clinical findings. The differential diagnosis between early pregnancy, intra-uterine or ectopic, and pathologic and physiologic conditions that simulate pregnancy, frequently taxes the skill of the most expert physician. Even advanced pregnancy may be confused with ovarian cyst and uterine fibroids before the fetal skeleton can be demonstrated by X-ray. Pulmonary tuberculosis, primary and severe secondary anemias, psychoses, endocrine and metabolic disturbances, and numerous other conditions associated with amenorrhea often mimic the gravid state. Likewise, the abnormal uterine bleeding associated with acute salpingitis is frequently attributed to ectopic gestation. The physiologic amenorrhea of gestation and the early menopause often give patients the greatest concern. Thus, a test to determine the presence or absence of pregnancy is

most valuable in these cases.

Even at the present time there is no method or test for determining an early pregnancy that is universally accepted. To meet this requirement such a test, or procedure, must be simple in its technique so that it can be handled without elaborate laboratory equipment and reliable results must be obtained in the hands of competent practitioners.

The Aschheim-Zondek test, although only four years old at the present time, has more universally fulfilled these requirements than any other procedure. Its excellent results have been consistently confirmed by practically all who have worked with it. The test is quite simple, requiring no special training on the part of the practitioner and only the simplest of laboratory equipment.

It will, therefore, be the purpose of this paper to set forth the original technique as presented by Aschheim and Zondek together with various modifications of their test that have been developed and the results that have been obtained by these several methods, including some case reports and the results obtained in my own experiences.

HISTORY

Before going directly to the Aschheim-Zondek test it seems of sufficient interest to review briefly various methods that have been used before in an attempt to diagnose early pregnancy.

It is interesting to note that the problem of determining the state of pregnancy existed even in the old world of civilization, for literature reveals to us the fact that the Egyptians had a procedure for determining the state of pregnancy through the urine 3000 to 4000 years ago. Finkler (13) reports that in an old papyrus there is described the following: "If a woman wished to know whether she was pregnant, she was instructed to bring a vessel filled with earth in which some barley and wheat had been placed. Some of her urine was poured on this at intervals. If the grain sprouted, the patient was considered pregnant." Neuburger also called attention to another custom of this same period and quotes the following from the Brugsch papyrus: "Another direction to see if a woman bears or not, a watermelon pounded is mixed with the milk of a woman who has borne a son, and is given to the patient to drink: if she vomits, she is pregnant; if she has only flatulence, she will never bear again."

Although all the tribes and civilized countries had their superstitions and methods of handling pregnancies there is nothing alluded to in the literature that would show any progress in the early diagnosis of pregnancy, other than the above mentioned, until the time of Hollick, Hodge, and Beach in the period from 1831 to 1864. Hodge (17) called attention to a peculiar deposit of a caseous character (First described by M. Nouche in 1831) which occurred in the urine of pregnant women. This deposit he called kyestine. The urine of pregnant women was allowed to stand in a glass exposed to the air and after a few hours there was formed a deposit of "cloudy flakes" on the sides and bottom of the glass and the urine became more limpid. This was not considered as the characteristic thing, however. The urine again became clouded on the second or third day with increased intensity and soon a more or less perfectly formed pellicle appeared upon the surface of the urine. About the third or fourth day portions of the pellicle began to separate and precipitate out, and by the fifth or sixth day had almost entirely disappeared.

Hodge reports this condition is seldom seen prior to the second month of pregnancy. It is generally most intense, however, between the third and seventh months

of gestation and not often seen during the latter months. He further reports that it is seldom found in other than pregnant women but calls attention to the fact that Kane observed the same thing in certain pathological conditions but in these the pellicle did not usually form until the fifth or sixth day and was thus attributed to the process of putrefaction. The true kyestine was regarded as a gelatino-albuminous substance differing from pure gelatin or albumen and found only in the parturient condition in about 80% of all pregnant women and nearly one-half of lactescent women. Hodge concludes that, "If the observations be carefully made, the finding of kyestine in the urine may be regarded as one of the positive signs of pregnancy, not to be exclusively relied upon, but as assisting us in forming our diagnosis."

Hollick's (18) writings are very much in harmony with those of Hodge only he asserts that kyestine is always to be found in urine of pregnant women after the first month and concludes that on the whole the sign is a very valuable one, and may be much relied upon.

About this same time (1850) Beach (4) also called attention to the appearance of the urine stating that,

"It is generally more abundant, and more pale and limpid." He, however, concludes that before making a positive diagnosis of pregnancy, "Our opinion must not be formed on one, but on the concurrence or the assemblage of reliable symptoms." In this group of symptoms he lists the peculiar changes found in the urine.

Following the kystine test numerous new ideas were brought forward. About the year 1841-2 Lever of London, Simpson of Edinburgh, and Cohen discovered albumin in the urine of many pregnant women, (Hodge). They also found that in many instances there was a coincidence between this state of the urine and various neurotic derangements such as cephalalgia, pain in the eyes, face, chest, back, etc., and frequently it existed with amaurosis, tinnitus, vertigo, coma, and convulsions--conditions which are recognized today as pre-ecampsia and eclampsia or purely nephritic conditions.

M. Blot (quoting Hodge) asserts there is always a trace of sugar in the urine after impregnation occurs; others have noticed the frequent presence of oxalic acid, and occasionally a diminution of the urea; but, the presence or absence of albumin in the urine during pregnancy is the chief observation upon which attention was fixed at this time. In healthy, unimpregnated women, no albumin is found, while in pregnancy this ele-

ment is often found. It was often enough that Cozeaux, a writer of that time, declared that it was the physiological condition of the urine in pregnancy and that-- "If this state be exaggerated, it gradually becomes pathological, and may be accompanied in some instances, by granular nephritis." Blot, however, found it in only 20% of his cases, (Hodge).

About 1914 Abderhalden came forward with a special test for pregnancy which for a time received wide acclaim, but like all other pregnancy tests up to that time, was finally abandoned. The underlying idea is, according to Wells (36), that "The animal body reacts to the presence of foreign proteins by providing specific means of destroying them through proteolysis." In pregnancy, theoretically the chorionic cells of the placenta enter the maternal circulation. Their foreign protein content causes the formation of the protective enzymes--"Abwehrhermente"--which in turn were detected by the ability of serum containing them to digest suitably prepared placental protein in vitro. The whole idea rested on the specificity of the reactions, i. e. each one will reduce only that substance which is introduced into the blood and which causes the development of the protective enzyme. Wells has pointed to the absolutely non-specific and hopelessly paradoxical

results; which were eventually proven as the test gained wider and wider usage. The test is very delicate, its specificity has been disproven and it is therefore of no practical value to obstetrics.

Other tests for pregnancy that came out after the Abderhalden test and deserve merely a passing mention are: the antitrypsin test of Rosenthal, the compliment-fixation test of Fieux and Mauriac, the miastagmin reaction of Ascoli, the epiphamin reaction and those tests which show diminished sugar tolerance--namely--(1) The adrenalin--glycosuria test--during pregnancy a hypodermic injection of adrenalin causes sugar to appear in the urine. (2) Kamnitzer's phloridzin test--the administration of $2\frac{1}{2}$ mg. of phloridzin causes glycosuria. (3) The glucose test--200 gms. of grape sugar per os, causes glycosuria. Fahraen's red blood corpuscle precipitation test--red blood cells settle out of the diluted blood very quickly in pregnancy--i. e., in two hours requiring six hours in the non-pregnant state. Widal's digestive hemoclasia test--200 cc. of milk taken fasting, causes leukopenia (also a test for liver function). De Lee (7) summarizes this entire group by saying they possess merely an academic interest.

Two other more recent tests of current interest are the Mazer-Hoffman (26) test and the Siddall test (26).

The former has the demonstration of the female sex hormone in the whole urine as its basis. The latter test depends upon the effect of the anterior pituitary hormone and to a lesser degree, of the female sex hormone contained in the serum of pregnant women on the genital tract of immature mice. One cc. of blood serum is injected daily and at the end of five days the total mouse weight divided by the weight of the uterus with the ovaries attached gives a ratio of 400 or less, if the woman is pregnant. These two tests have their advocates but neither one has received the universal acceptance nor shown the percentage of correct diagnosis that the Aschheim-Zondek test has shown.

THE ASCHHEIM-ZONDEK REACTION

"The recent discovery of the relationship between the anterior lobe of the hypophysis and the ovary constitutes one of the most important advances within recent years of our knowledge of the physiology of the sex glands," (Mack and Catherwood 23). Froelich, in 1901 (quoted from Mathieu and McKenzie (25) first suggested that there was a relationship between the development of the pituitary gland and sexual maturity. Erdheim and Stumme (Kaplan 19) pointed out that during pregnancy marked changes in the histologic structure of the anterior lobe of the pituitary gland take place. Smith and Engle (33) and Long and Evans (21) demonstrated that the injection of the anterior lobe of the pituitary into mice and rats caused marked enlargement of the ovaries. Aschheim and Zondek began a series of studies in 1925 which showed that the hormone of the anterior lobe of the pituitary was normally excreted in the urine in pregnancy (Parvey 27) and that such urine was capable of producing estrus (bringing the animal to heat). Feeding and implanting infantile mice with anterior lobe of the pituitary gland resulted in advanced morphological and functional changes in the ovaries and menstruation occurred approximately 100 hours

later. They discovered that during pregnancy there was a striking over production of the anterior pituitary hormone leading to its excretion in the urine as previously noted. According to Finkler (13) and Aschheim and Zondek (3) and others this concentration is several thousand times the amount normally found in non-pregnant women. Aschheim and Zondek further discovered that this urine, when injected into infantile mice, brought about the same changes in the ovaries as did the pituitary transplants. This finding is the basis of the test which Aschheim and Zondek proposed for the diagnosis of pregnancy. The test then is an indirect one and according to its originators (3)--"Our reaction does not depend upon a hypothetical unknown body but is dependent upon the demonstration of a body element present in every organismWe demonstrate a hormone whose biological action in the organism is definitely known, and can consequently be identified."

Aschheim and Zondek transplanted various glands into immature animals in order to determine whether the ovarian function could be substituted or stimulated by other endocrine glands than the anterior pituitary lobe of the hypophysis, but they found this to be the only gland that has any effect. Thus they were able to announce one of the most important discoveries

in recent endocrine investigation: "The anterior lobe of the pituitary body, and no other tissue of the body, produces the hormone which sets in action the latent ovarian function, and thereby brings the infantile animal to sexual maturity."

"The anterior pituitary lobe hormone is the "motor" of sexual function. The anterior lobe hormone is the primary, and the ovarian hormone the secondary. The anterior lobe hormone brings the follicular apparatus into action, fires off the follicular ripening, and mobilizes the secondary ovarian hormone in the follicular cells. This then acts in a specific way on the uterus and vagina." (Re-quoted from Mathieu and McKenzie 25).

Aschheim and Zondek first attempted to work out the diagnosis of pregnancy by demonstrating the presence of the ovarian hormone in the urine. They finally gave this up because the hormone could not be demonstrated in small quantities of urine earlier than the eighth to tenth week of pregnancy. Besides they found the hormone was excreted in large quantities at times in non-pregnant women and in those with functional disturbances of the ovarian gland such as may occur at menopause, in certain amenorrheas, hyperthyroidism and myxedema.

The action of the pituitary hormone on the ovaries

is in contrast to the action of folliculin (ovarian hormone). Long and Evans (42) and Allen (1) have shown that the latter produces changes confined to the uterine glands and vagina, the ovarian structures being un-altered. Aschheim and Zondek later distinguished two components of the anterior pituitary hormone, calling them Prolan A. and Prolan B. Prolan A, according to them, is the hormone which causes follicle maturation (Anterior Pituitary Reaction I.--to be described later). Prolan B. is the hormone which causes the formation of corpora lutea and "Blood Points" (Anterior Pituitary Reactions II and III--described later). The "B" hormone is found in the urine only in pregnancy, while the "A" hormone may be found in the urine in such conditions as the menopause, amenorrhea, genital tumors, endocrinopathy and pelvic inflammation. (Finkel 12).

The blood of pregnant women shows a marked increase in both the pituitary and ovarian hormones (Figure I). With the onset of pregnancy the rise of ovarian hormone is gradual over a period of several weeks. In contrast the pituitary hormone rises rather sharply, a high level being reached a few days after conception. This level is maintained until about the eighth month of pregnancy after which it gradually drops, reaching normal eight days after delivery according to Aschheim and Zondek (3),

Parvey (27), Fanz and Gault (10) and others. Other workers disagree as to the time elapsed after delivery before the hormone is no longer demonstrable in the urine. Stewart (34) finds the reaction disappears from the urine about thirty hours after delivery and other workers were unable to demonstrate the hormone in the urine after the third and fourth days.

Aschheim and Zondek and Finkler (13) have also shown that other conditions, such as carcinoma, especially of the ovaries, early pituitary tumor, lutein cysts, hydatidiform mole and chorionepithelioma cause an increase in the formation of the anterior pituitary hormone. These conditions, however, are rare. Rossler (30) showed that the anterior pituitary hormone is increased in the urine three fold in chorionepithelioma and in hydatidiform mole and in the malignant forms of chorionepitheliomas the increase is from eight to fifteen times the normal amount. These findings have been corroborated by numerous workers including Robert Meyer, Stoeckel, Ehrhardt, Zondek, Mack and Catherwood and others. The incidence of malignant chorionepithelioma following hydatidiform mole (variously quoted at 5-25%) in the eyes of Mack and Catherwood (23) and others necessitates a guarded prognosis with a period of close observation. Thus, the Aschheim-Zondek reaction is a very desirable one in detecting con-

tinued chorionic proliferation, especially in cases which are early and in the operable stage.

Fanz and Gault (10) report that on examining the curve of pituitary secretion, previously referred to in Figure I., they find it to be practically coincident with the development of trophoderm and its derivative, the chorion, and in its latter half of declination is coincident with the disappearance of Langhan's cells. These workers suggest the possibility that Langhan's cells with their trophodermal precursors through their products stimulate the anterior pituitary body to hypersecretion during pregnancy. As previously stated, the presence of the anterior pituitary hormone in the urine can seldom be demonstrated after the eighth day post partum, while after expulsion of a mole it has been observed for months. On the basis of cases which have shown a persistent positive reaction following hysterectomy, some authors recommend the test as a reliable aid in differentiating between benign and malignant types of chorioneplithelioma. Therefore, when the urine examinations give a negative reaction it is possible to assume cessation of chorionic proliferation. But in any case whether it be mole, chorioneplithelioma, ectopic pregnancy, threatened abortion, or normal pregnancy, as long as living fetal elements or their pro-

ducts are in contact with the maternal blood the reaction will be positive.

That the test is entirely reliable in diagnosing early pregnancy and in differentiating pregnancy from other conditions is shown by the results universally obtained with it. Sage (31) reports that a review of all available literature shows over 3000 cases where the test was run with positive results in 95-98% of the cases. In this large number of cases many of them were diagnosed as pregnant within three weeks after the expected date of menstruation and several were in the first few days of pregnancy.

According to Aschheim and Zondek (3), Stone (35), and others reporting on their work these tests included urines from:--well females in various stages of the menstrual cycle; females in the climacteric; females of whom nothing was known except that they were not pregnant; females with a clinical diagnosis of irregular hemorrhage; several males; females with internal disease; endocrine diseases; inflammatory diseases; benign ovarian tumor; fibroid tumors; malignant tumors; amenorrhea; normal undisturbed pregnancies; in puerperium; abortion cases--positive the first four or five days; then negative; and tubal pregnancy.

TECHNIQUE OF THE TEST

The original Aschheim-Zondek (3) reaction, being a biological test, called for the use of five infantile female mice or rats. Since mice were less expensive and rather easily reproduced the originators used them. Infantile mice three to four weeks of age and weighing six to eight grams each were found to be the optimum for the test. Mice normally begin the estrus cycle about the sixth week of life (Parvey 27, Aschheim 2) and if mice older than four weeks are used there is danger of getting false positive reactions because of their maturity. If mice of less than six grams weight or under three weeks of age are used many of them die during the course of the test.

The first morning urine of the patient is collected before she has taken any food or water. This is done because the originators found the anterior pituitary hormone to be more concentrated in this specimen than in any collected through the rest of the day. Mack (22) advised against the use of old urine, unless a preservative has been added, since it is apt to be decomposed and such urine has a toxic effect on the mice and kills them when injected with it. He and Filler (11) also found it desirable to have the patient refrain from taking any in-

ternal medication for twenty-four hours before the urine is collected because such drugs may cause the urine to become toxic to the mice. However, all workers have found the urine can be preserved indefinitely for the test if one drop of cresol is added to each ounce of urine. This fact is especially valuable if the specimen must be sent long distances before being used.

If the urine is not acid it should be acidified and then filtered, if necessary, to get a clear urine for injection. It should be kept cool in an ice box during the course of injections.

Zondek and Aschheim (3) gave each mouse a series of six injections spread over a period of forty-eight hours. All injections are given subcutaneously in the backs of the test animals. Two injections are given the first day at intervals of not less than four hours; three more injections the second day with the same intervals between, and the sixth injection on the forty-eighth hour after the first injection. The first mouse receives 0.2 cc., the second mouse 0.25 cc., the third and fourth mice each 0.3 cc., and the fifth mouse 0.4 cc. at each of the six injections.

All five animals are killed and their ovaries examined macroscopically 96-100 hours after the first injection was given. Aschheim and Zondek (3) have grouped

these changes as Reactions I, II, and III. Since Reaction I is concerned only with changes in the uterus and vagina they do not regard it as positive for the presence of anterior pituitary hormone in the urine. Reaction II is characterized by the presence of "blood points" on the surface of the ovary. These points are a bright red, brownish, or purplish color about the size of a common pinhead. These areas represent gross hemorrhage into the swollen graafian follicles. Robertson (29) believes these blood points are due to the great vascularization which takes place in the ovary, but not being accompanied by an increase in the supporting tissue the hemorrhage into the follicle results.

Reaction III is described by Aschheim and Zondek (3) as characterized by the appearance of corpora lutea on the surface of the ovary. These spots are yellowish, pinhead sized, discrete areas scattered over the surface of the ovary.

The presence of Reaction II or III or both are diagnostic of an increase in the anterior pituitary hormone, which as previously discussed, occurs regularly in pregnancy. If Reactions II or III occur in only one ovary and even if only one blood point or corpora lutea is found the test is regarded as positive.

In cases which are doubtful macroscopically the

ovaries can be fixed in Zenker's solution, imbedded in paraffin and serial sections made and these studied microscopically.

These reactions are in contrast to the immature female mouse ovaries as seen in cases of negative reactions. In these the entire ovaries are about the size of a pinhead, and have a smooth grayish-pink surface; the Y-shaped uterus is thread-like in appearance, and the vaginal orifice is closed. (Finkel 12).

There have been several modifications of the test where mice are the test animals used. This change was largely brought about in an attempt to reduce the mortality among the mice. In Aschheim and Zondek's series of over 1000 cases their mice mortality has averaged around 15%, while other workers have reported such high mortality that they had to abandon the tests. Stewart (34) reports that he overcome this obstacle by treating the urine with ether, i. e., shaking the urine up with ether and then letting it stand, allowing the ether to evaporate off, leaving only the sterile urine. Liese and Auer (20) used only two mice and injected 0.4 cc. of the first morning urine twice daily for three days. Their urines were acidified and collected before 9 a. m. on the morning voided and no chemical preservatives were used and their results were comparable to

those of Aschheim and Zondek. Kaplan (19) injected 0.3 cc. of urine subcutaneously three times a day at three hour intervals or more for three days and examined the ovaries on the third day after the last injection. His results are not unlike those of other workers.

Fluhmann (14) was one of the first to depart from the use of the patients urine. He drew venous blood from his patients, separated the serum and injected a total of 3.0 cc. to 5.0 cc. subcutaneously, twice a day in doses from 0.5 to 1.0 cc, using immature female white mice. In positive results he found the vaginal entroitus of the mouse established by the fourth or fifth day and the animals were then killed, their ovaries fixed in Zenker's solution and examined serially with the microscope, the results being interpreted according to Aschheim and Zondek Reactions I, II, and III.

Evans and Simpson (9) ran their series of tests on sexually immature rats, using six for each test, from 24-26 days old and weighing 40-50 grams each. They neutralized the urine, filtered, and then froze it. A dosage of 1 cc. daily for a total of four injections was given each rat. The ovaries were examined ninety-six hours after the initial injection. These investigators examined the ovaries under the microscope and,

according to them, the positive pregnancy test in the rat is Reaction III. They report that hemorrhagic follicles are not common in the ovaries of precociously matured rats. Though rats are more expensive than mice they find the former much sturdier animals for the test and their mortality was nil. Their results were as accurate as those obtained by other investigators using the original method. Hannan (16) also worked with rats. He used only one albino rat from six to eight weeks old for each test, injecting 3 cc. of the patient's urine subcutaneously in one dose and examining the ovaries after ninety-six hours by the same method used for mice. His percentage of error was considerably higher than all other workers have found. His critics attributed this to the fact that he used too mature rats in his experiments. Ebersson and Silverberg (8) treated the urine with alcohol using $2\frac{1}{2}$ volumes of 95% alcohol. This mixture was centrifuged and the sediment was then suspended in physiologic salt solution. Ether was added to this and the mixture again centrifuged to remove the supernatant ether. This extraction method was repeated two or three times. According to these workers this method left the anterior pituitary hormone in the salt solution, freed from the ovarian hormone, and this was used for injections into rats. They used two female

rats eighteen to twenty-one days old, both from the same litter. One cc. of the material was injected twice a day for one to two days, and the genital tracts examined after thirty-six to forty-eight hours instead of ninety-six to one hundred hours. By this means they eliminated the mortality factor entirely. The ovaries were studied macroscopically, and in doubtful cases, microscopically, and interpreted according to the Aschheim-Zondek method. They claim the preliminary treatment of the urine stabilizes and increases the potency thus minimizing errors in results. Besides this they have cut the time element in half and their results are as good as those obtained by the original method. They further called attention to "Tinctorial and structural changes in the follicular cells seen in histologic serial sections." These changes according to them are definite evidence of a positive reaction and are of great value in cases which are doubtful macroscopically.

Mathieu and McKenzie (25) used only one immature rat between twenty-two and forty days old and weighing from thirty to sixty-five grams. They treated the urine by the original method and injected 0.5 cc. doses twice a day at four hour intervals, subcutaneously for six doses. The ovaries were examined on the fifth day after the initial injection. By this method they obtained

97.3% accuracy in their series of cases.

Friedman (15) was one of the first to experiment with this test on rabbits. He produced corpora hemorrhagica and corpora lutea in rabbits by transplants of rat hypophysis, by intra-peritoneal injection of urine from pregnant women, and by a single intravenous injection of 5 cc. of the same urine. By the intravenous method he was able to demonstrate fresh corpora hemorrhagica in both ovaries, the same as in mice, as early as twenty-four hours after injection. Schneider (32) reports that the rabbit does not copulate or go into heat until the age of five to eight months, depending on the season. The rabbit has no regular cycle and does not ovulate unless it copulates and ovulation occurs in eight to ten hours following copulation. He found rabbits twelve to fourteen weeks old to be the best to use for this method because if older than this there was danger of false reactions from other causes and if under twelve weeks of age the results are inconsistent. Schneider feels that the Friedman test has added great value to the Aschheim-Zondek test because it has greatly decreased the time element, simplified the technique and at the same time has maintained as great a degree of accuracy as the original Aschheim-Zondek test. Beasley (5), Reinhart and Scott (28), and

Magath and Randall (24) have all found the same degree of accuracy with this method, as is reported in their series of cases.

Wilson and Corner (37) report on the possibility of confusion in interpreting the findings in the rabbit ovary. They report there are sometimes large unruptured follicles present which are not diagnostic. These are rounded rather than conical or mamillary in form, show no central depression and are much paler and clearer. They also report the presence at times of hemorrhagic un-ruptured follicles, which are more or less peculiar to the rabbit. These are a deep purple or almost black color and of a smaller size than the ruptured follicles. They suggest the use of a hand lens where differentiation is difficult macroscopically. These same two workers report that they have encountered at times very large, clear follicles, apparently about ready to rupture. Doubling the dosage of urine (10 cc.) caused no change in such follicles and they therefore interpreted them as negative.

It is only recently that any work has been done with patients serum, injecting it directly into the blood stream of rabbits. Brown (6) reports that since absorption of the hormone from the anterior pituitary gland is apparently directly through the blood supply

it seemed logical to expect a rather constant level of this hormone in the blood and that any excess would be excreted by the kidney. Working on this theory he injected separate test rabbits with 5 cc. of urine and 5 cc. of blood serum from pregnant women and he found the reaction from the serum to be about twice as strong as that from the urine. He also found the average effective amount of serum to be 1 cc. per 600-700 grams of body weight and that rabbits weighing between 1500 and 2000 grams each gave the most constant results. He examined his rabbits twenty-four to thirty-six hours after injection and found that in nearly every case the diagnosis could be made on gross inspection.

Working with Dr. Harley Anderson and Mr. Feldman on a series of tests using this modified Friedman technique we found 5 cc. injections of serum too large in some cases, the rabbit going into shock and dying soon after being injected. Therefore our dosage was reduced to 2.5 cc. of serum and rabbits averaging between 1500 and 2000 grams were used when it was possible to get them. We lost no rabbits from this cause after reducing the dosage. We were not particular about the age of the rabbits so long as they came within the weight requirements and had been isolated for at least three weeks before being used for the test.

In each of our cases 10 cc. of venous blood was taken from the patient and allowed to stand overnight in the ice chest to separate the serum. We used only one rabbit for each test and in each test 2.5 cc. of the patient's serum was injected into the marginal ear vein of the rabbit. The ovaries were examined forty-eight hours after injection of the serum. The rabbits were anesthetized, an abdominal incision made and the ovaries, tubes and uterus removed in toto. In later tests we merely opened the abdomen and examined the ovaries without detaching them. After examination they were put back in place, the abdominal incision closed and the rabbit returned to isolation for future tests.

As is evident from the above statement, all of our findings were based on the macroscopic examination only and were recorded as Reactions II and III according to the Aschheim-Zondek method. Our cases and results are herewith reported.

CASE REPORTS

Case I.

Mrs. A. This was a case of known pregnancy in a white housewife, aged twenty-one years; Para I, gravida II. Her last period began on June 15, 1931. It was normal and lasted the usual three days. The patient felt life the latter part of October and the forepart of November, 1931.

A physical examination on March 3, 1932, showed a well developed but anemic appearing woman. The abdomen was that of a normal pregnant woman near term. The small fetal parts were palpated on the right side of the abdomen and the fetal heart tones were heard in the lower left quadrant.

On March 4, 1932, 10 cc. of whole blood was taken from a vein in the arm. The serum was separated and on March 5, 1932, 2.5 cc. were injected into the marginal ear vein of the test rabbit. The ovaries of the rabbit were examined forty-eight hours later. Both ovaries were strongly positive, being swollen and each containing several corpora hemorrhagica on its surface which were a bright red color. The uterus was congested and hyperplastic and the tubes were markedly congested. The case was diagnosed clinically as a pre-eclamptic and

treated for the same until she delivered.

Case II.

Mrs. E. M., a white house-wife, age twenty-five years, entered the University Hospital complaining of cessation of her menses, weakness and loss of weight. Her periods began when she was nineteen years of age and have always been regular every twenty-eight days, lasting four to five days each time. There has been no dysmenorrhea or menorrhagia. The last week in December, 1931, she had an attack of influenza which left her quite weak. On January 16, 1932, which was her time to menstruate no flow began. She experienced no pain or discomfort at this time but consulted a physician. Since then she has gradually become weaker, more easily fatigued and had been losing weight. Her last period began on December 19, 1931, at which time she noticed no change in duration, amount, or character of the flow and experienced no pain or discomfort. Vaginal examination revealed a firm perineum and no vaginal discharge, or bluish discoloration in evidence. The cervix was firm and showed a bilateral laceration. The adnexia were not palpable. The uterus was anteverted, anteflexed and in anterior position. The body was about two times normal size and slightly softened.

Increased pulsation of the uterine vessels was present. From these signs a clinical diagnosis of pregnancy was made. A test rabbit was injected with 2.5 cc. of the patients blood serum on March 15, 1932, using the modified Friedman technique. Forty-eight hours later the ovaries were examined and both were strongly positive, showing Reaction II. As this paper was written the case had not yet progressed far enough to establish absolutely a clinical diagnosis of pregnancy, but the test confirmed the diagnosis made on the presumptive signs present.

Case III.

Mrs. J. N., a white housewife, age twenty-four years, came into the University Dispensary on March 19, 1932, complaining of amenorrhea. Her last period was January 2, 1932, and was normal. She had missed her last two periods and prior to that time they had always been regular every month and lasted four to six days each time. She had recently been somewhat nauseated but had not vomited. Otherwise she had no complaints. Patient has had two normal pregnancies. Physical examination revealed a conical, bilaterally lacerated cervix which is somewhat softened and having a slight amount of whitish serous discharge. The uterus

was definitely enlarged and a small nodule about 2 cm. in diameter was palpated on its right side. A clinical diagnosis of pregnancy was made. The rabbit test showed the left ovary to be markedly congested but no corpora hemorrhagica or corpora lutea were found. The right ovary was negative. The rabbit used weighed only 1200 grams and was too young. I feel that the reaction would have been positive had the rabbit been more mature or a greater quantity of serum used. The ovaries were not studied microscopically. Unfortunately we were unable to run a second test on this patient.

Case IV.

Mrs. C. A., a white housewife, age twenty-five years, was admitted to the University Hospital on March 19, 1932, complaining of acute attacks of pain in the lower left quadrant with nausea and vomiting; mass in right inguinal region which causes transient pain. Her menses have appeared every twenty-eight days and lasted five days. The flow is not excessive but there has been some dysmenorrhea and backache. Patient had one miscarriage in October, 1931. Her last menstrual period was three weeks ago. She has had some morning sickness recently. A physical examination showed the mass in the right inguinal region to be a femoral hernia. A

pelvic examination revealed considerable purulent discharge from the vaginal orifice. The vaginal mucous membrane was ingested and had a slight bluish tinge. The cervix was of the nulliparous type but softer than normal. The fundus of the uterus was anteverted and anteflexed, slightly larger than normal, freely movable and not tender to palpation. Palpation also revealed a thickening of the right adnexia with a palpable mass present which was slightly tender and about 3 cm. in diameter. The right adnexia felt normal. No masses were felt by rectal examination. The clinical diagnosis was: right femoral hernia, possible pregnancy, and possible acute salpingitis. On March 22, 1932, a test rabbit was inoculated with 2.5 cc. of the patient's blood serum and the ovaries examined forty-eight hours later. The animal was found to be pregnant so a second rabbit was injected on March 27 and the ovaries examined forty-eight hours later. Both ovaries were swollen, injected and each contained several corpora hemorrhagica. The tubes and uterus were also congested and swollen. The test was interpreted as strongly positive for Reaction II. Operative treatment was deferred on the basis of the results obtained by this test.

Case V.

Mrs. E. W., a white housewife, age twenty-eight years, entered the University Hospital complaining of having missed her last two menstrual periods. Her last period began December 28, 1931. She complained also of pain in the low back region and in the pelvis. The patient has had an intermittent yellowish vaginal discharge the last two years which has been quite profuse the last two months. Her menses began at fifteen years of age, have always been regular every month and lasted five days each time. They have never been especially painful or discomforting. The patient had one miscarriage three years ago which was induced by a regular physician she says. One year ago patient induced another abortion by taking pills by mouth. The patient feels that pregnancy at this time is impossible because they have been practicing coitus interruptans since the birth of the last child. Physical examination reveals bilateral tenderness over the pelvic region. Pelvic examination reveals a large uterus with a positive Hegar's sign and tenderness in the fornices. There was also increased pulsation of the uterine vessels. On these findings a clinical diagnosis of pregnancy was made. The referring doctor had been giving her medicinal treatment for her ovaries and had told her she had a cystic

ovary. Our rabbit test with serum from the patient's blood confirmed the diagnosis of pregnancy with a strong Reaction II.

Case VI.

Mrs. A. D., at the Methodist Hospital, a housewife, age twenty-two years, entered the hospital complaining of (1) Cramp-like pain in the lower abdomen; (2) Fever; (3) Intermittent vaginal flow for the past two months. The flow was red at times but all other times was a brownish color; (4) No true menstrual history since December, 1931; (5) Loss of fifteen pounds of weight the last two months. Her last menstrual period occurred in December, 1931. Following this the patient felt normal until about February 1, 1932, when she began vomiting as much as eight or nine times daily. This continued until she was given hypodermics to relieve it. She began to flow intermittently at the time vomiting began. Her periods began at eleven years of age, have always been regular every twenty-eight days and lasting seven to eight days. Periods were very painful until the birth of her first and only baby two years ago. She has had no miscarriages. On March 22, 1932, the patient spontaneously expelled an hydatidiform mole. On March 25 a test rabbit was in-

jected with 2.5 cc. of the patient's serum. The rabbits ovaries, after forty-eight hours revealed a strong Reaction II. On April 2, 1932, eleven days after the patient expelled the mole, a second test rabbit was inoculated. Examination of the ovaries forty-eight hours later showed Reaction II but to less degree than the previous test.

Case VII.

G. T., a Dispensary patient, white, house-wife, age thirty-four years. Patient came to the dispensary on March 3, 1932, complaining of irregular vaginal bleeding. Her periods began at thirteen years of age and were always regular every twenty-eight to thirty days and lasted for three days each time. Her last normal period began on September 28, 1932. For one week prior to her dispensary visit she had had considerable bloody show. Patient returned to the dispensary again on March 12, 1932, complaining that the bleeding was constant since her last visit and was more pronounced, saturating four pads daily. The bleeding was painless and the patient has felt no quickening. Vaginal examination revealed a boggy cervix, which was drawn to the left and was somewhat hard on the left side. Considerable bloody mucoid material was oozing from the external os. The

external os was lacerated and there was considerable evidence of endocervicitis. With bimanual examination the uterus felt like a four or five months pregnancy but no fetal heart tones were heard. The possibility of a pregnancy with a threatened abortion was kept in mind and the patient put under treatment in bed. She continued to bleed for the next two weeks in spite of treatment, saturating one to four pads daily but never had any pain. Bimanual examination at this time showed a soft cystic mass to the right of the uterus and not attached to it suggesting an ovarian or par-ovarian cyst. A clinical diagnosis of possible pregnancy associated with uterine fibroid was made. On March 27, 1932, a test rabbit was inoculated with the patients blood serum but gave a negative reaction in both ovaries when examined forty-eight hours later. The case has not been surgically treated yet so we do not know definitely what her condition is, except that pregnancy is ruled out.

Case VIII.

Mrs. C. B., a white housewife, but separated, age seventeen years, came into the dispensary on March 28, 1932, complaining of the possibility of pregnancy. She gave a history of the usual childhood diseases. Her

menses began at thirteen years of age, have always been regular and lasted six days each time. They have caused her no especial discomfort. The patient says she has lost ten pounds in weight since last November. The family history is negative except that one grandmother died of carcinoma of the stomach. The general physical examination was negative. The uterus was not palpable by abdominal examination. Pelvic examination showed a nulliparous cervix slightly retroverted and retroflexed. Hegar's sign and Chadwick's sign were both positive. A clinical diagnosis of pregnancy was made on the basis of these presumptive signs. On March 29, 1932, a test rabbit was inoculated with 2.5 cc. of the patient's serum. After forty-eight hours the ovaries were examined and both showed strong Reactions II and III which are positive for pregnancy and thereby confirmed the clinical diagnosis.

Case IX.

Miss V. M., white, age eighteen years, came to the dispensary complaining of cessation of menses since February 15, 1932. Her menstrual periods began at thirteen years of age and had always been regular every twenty-eight days, lasting three or four days each time. She had had no morning sickness but had experienced

some low abdominal pain. Her last menstrual period began February 15, 1932, and was normal in every way. The clinical diagnosis of pregnancy was questionable. A test rabbit was inoculated on April 2, 1932, with 2.5 cc. of the patient's serum. After forty-eight hours the rabbit ovaries were examined and the reaction interpreted as negative in both ovaries because of the absence of any hemorrhagic graafian follicles or corpora lutea on gross examination. No microscopic studies were made.

SUMMARY AND CONCLUSIONS

A resume of various tests for pregnancy that have preceded the Aschheim-Zondek reaction has been presented. The theory and experimental work on which the Aschheim-Zondek reaction is based has been briefly discussed. There was also presented the original technic of the Aschheim-Zondek reaction together with various modifications of the test that have followed its introduction.

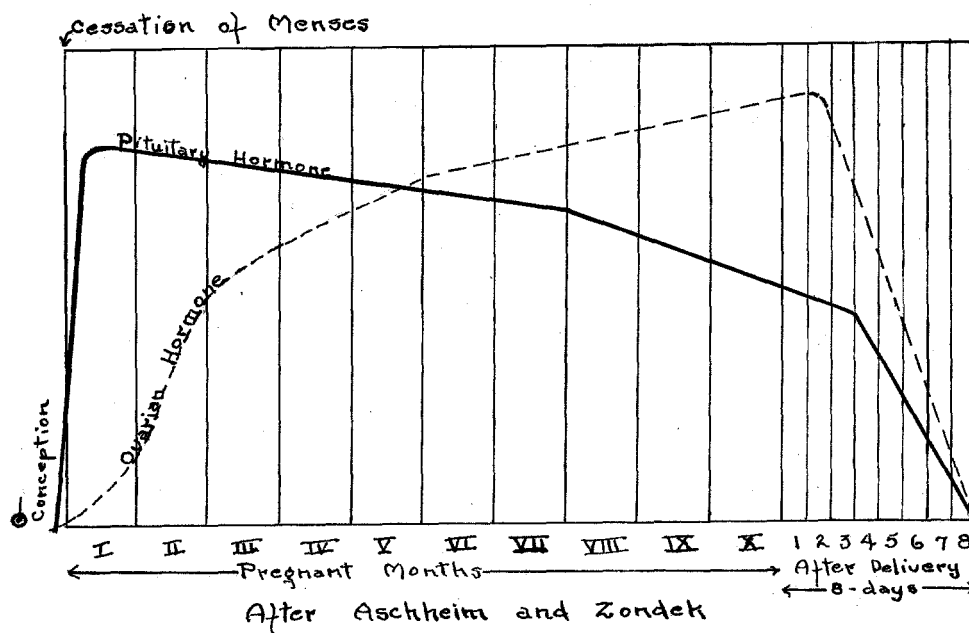
A series of nine cases were tested by the modified Friedman technique and their results included in this paper. For these tests 10 cc. of the patients blood was collected and the serum separated and 2.5 cc. injected into the marginal ear vein of each test rabbit. The rabbits were opened and the ovaries examined macroscopically forty-eight hours later. In the series of nine cases there was only one doubtful result. This we believe was the fault of the technique and not of the test itself since the test rabbit was considerably smaller than the optimum advised by Brown for the test. In the case of hydatidiform mole the reaction appeared to be in direct proportion to the concentration of the hormone in the blood serum, the second reaction eleven days after the mole was expelled being much weaker than the reaction

obtained three days after it was passed. On the basis of this limited experience it appears that the test is of value in determining the prognosis in cases of hydatidiform mole and chorionepithelioma. In the other seven cases it was a matter of definitely ruling pregnancy in or out of the picture and although there has not been sufficient time to prove our laboratory results from the clinical progress of each case we feel confident that such will be the ultimate result. The value of the test in differentiating uterine fibroids, and other pelvic conditions from the early gravid state has been shown by all who have worked with the test. That the test is of value in diagnosing early pregnancy is shown by Case IV. This patient's last menstrual period was just three weeks prior to the time when a positive reaction for pregnancy was obtained.

The modified Friedman technique is a great improvement over the original Aschheim-Zondek method in that it cuts the time required to one-half or even less if need be. Only one laboratory animal is needed for each test and these animals can be used repeatedly for subsequent tests provided they are isolated for a period of three weeks or more prior to each test. The mortality of the test rabbits is an unknown factor when 2.5 cc. of serum are used as the dosage.

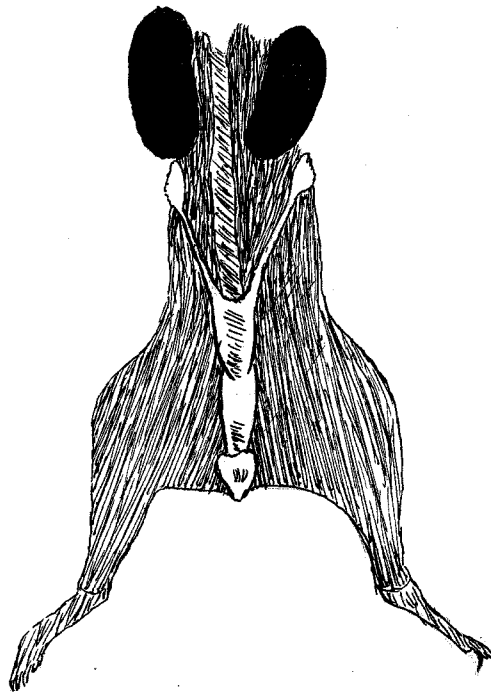
The test is simple, reliable, and can be accurately used by any practitioner whether he be located in a rural community or in the city.

Fig. I.



The ordinate values are based on actual measurements of anterior pituitary and ovarian hormones in urine. The unit for this measurement is the smallest part in cc. of a liter of urine which is capable of producing estrus in an infantile mouse.

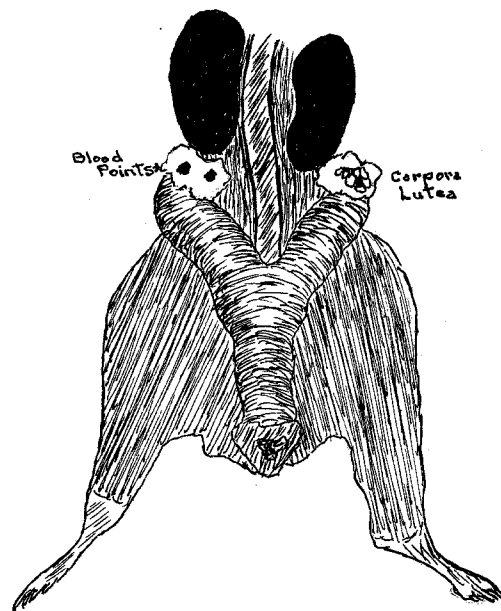
Fig. II.



After Aschheim and Zondek

Genitalia of Infantile Mouse 96-100 Hours After
Injection of Non-pregnant Urine.

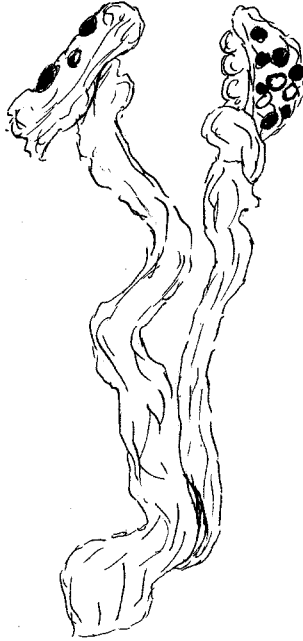
Fig. III.



After Aschheim and Zondek

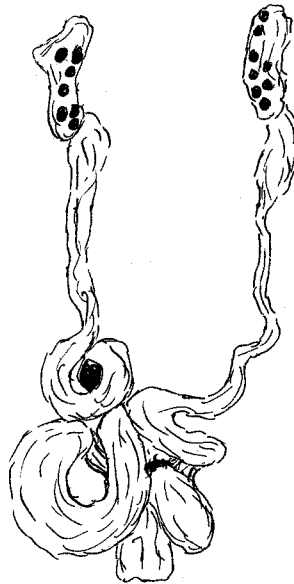
Genitalia of Infantile Mouse 96-100 Hours After
Injection of Pregnant Urine.

Fig. IV.



Ovaries of a Rabbit 48 Hours After Injection With
Serum From a Patient With Hydatidiform Mole.

Fig. V.



Genitalia of a Rabbit 48 Hours After Injection With
Serum From a Pregnant Woman.

BIBLIOGRAPHY

1. Allen, Edgar: The Oestrus Cycle in the Mouse.
Am. J. Anat. 30: 297-371, May 1922.
2. Aschheim, S.--The Early Diagnosis of Pregnancy,
Chorionepithelioma and Hydatidiform Mole by the
Aschheim-Zondek Test.
Idem: Am. Jr. Obst. & Gynec. 19: 335-342, 1930.
3. Aschheim, S. and Zondek, B.--The Diagnosis of Preg-
nancy From the Urine by Demonstration of the Hor-
mone of the Pituitary Body.
Klinische Wochenschrift, 7: 1404-1411, July 22, 1928,
and 7: 1453, July 29, 1928.
4. Beach, W.--An Improved System of Midwifery, New York.
Baker and Scribner Company, 1850, p. 65.
5. Beasley, B. F.--The Aschheim-Zondek Test as an Aid
in Differentiating Early Pregnancy From Other
Pelvic Conditions.
South. Med. Jr.--24: 821-822, Sept., 1931.
6. Brown, T. K.--A Proposed Modification of the Asch-
heim-Zondek Pregnancy Test.
Am. Jr. Obst. & Gynec. 23: 379-385, March, 1932.
7. De Lee, J. B.--Principles and Practice of Obstetrics,
Ed. 4, Philadelphia.
W. B. Saunders Company, 1924, p. 272-273.

8. Ebersson, F. and Silverberg, M. H.--Anterior Pituitary Hormone in Urine.
J. A. M. A. 96: 2176-2182, 1931.
9. Evans, H. M., and Simpson, M. E.--Aschheim-Zondek Test for Pregnancy--Its Present Status.
Idem: Calif. and West Med. Jr., 32: 145-148, 1930.
10. Fanz, J. I. and Gault, E. S.--Hydatid Mole as a Cause of a Positive Aschheim-Zondek Reaction.
J. Lab. and Clin. Med. 16: 27-36, Oct., 1930.
11. Filler, W.--Value of the Aschheim-Zondek Test to the General Practitioner.
New York State Jr. Med. 31: 891-895, July 15, 1932.
12. Finkel, H. S.--The Aschheim-Zondek Test in the Diagnosis of Pregnancy.
New England Jr. of Med., 204: 203-209, Jan. 29, 1931.
13. Finkler, R. S.--A Successful Test for Pregnancy.
Med. Woman's Jr., 37: 153-157, June 1930.
14. Fluhmann, C. F.--Significance of Anterior Pituitary Hormone in the Blood of Gynecological Patients.
Am. Jr. Obst. & Gynec. 20: 1-15, July, 1930.
15. Friedman, M. H.--Mechanism of Ovulation in the Rabbit.
Am. Jr. of Phys. 90: 617-622, 1929.
16. Hannan, J. H.--The Detection of the Presence of the Hormone of the Anterior Pituitary Body in the Urine As an Aid to the Diagnosis of Pregnancy.

- Proc. Roy. Soc. Med., Pt. I. (Sect. Obst. & Gynec).
23: 14-19, March, 1930.
17. Hodge, H. L.--The Principles and Practice of Obst.,
Philadelphia. Blanchard and Lea Company, 1864,
p. 81, 95, 96.
18. Hollick, F.--Matron's Manual of Midwifery, New York.
F. W. Strong Company, 1848, p. 92, 93.
19. Kaplan, H. E.--The Aschheim-Zondek Hormone Test For
Pregnancy.
Calif. and West. Med. 31: 412-413, 1929.
20. Liese, G. and Auer, E. S.--The Biologic Diagnosis
of Early Pregnancy by the Aschheim-Zondek Test.
Am. Jr. of Obst. & Gynec. 20: 667-670. Nov., 1930.
21. Long, J. A. and Evans, H. M.--On the Rapid Maturation
of the Ovary by Transplants of the Youthful Gonad
to Adults. Anat. Rec. 21: 60, 1921.
22. Mack, H. C.--A New and Reliable Test for Pregnancy.
Am. Jr. Nursing, 31: 802-805, July, 1931.
23. Mack, H. C. and Catherwood, A. E.--The Aschheim-
Zondek Reaction in Hydatidiform Mole and Malignant
Chorionepithelioma.
Am. Jr. Obst. and Gynec. 20: 670-678, Nov., 1930.
24. Magath, T. B. and Randall, L. M.--Friedman's Hormone
Test For Pregnancy.
J. A. M. A. 96: 1933-1935, June 6, 1931.

25. Mathieu, A. and McKenzie, T.--The Aschheim-Zondek Pregnancy Test.
Northwest Medicine. 30: 55-62, February, 1931.
26. Mazer, C., and Hoffman, F.--The Three Hormone Tests for Early Pregnancy.
J. A. M. A. 96: 19-23, 1931.
27. Parvey, B.--The Early Diagnosis of Pregnancy. New England Jr. of Med., 203: 1121-1129, Dec. 4, 1930.
28. Reinhart, H. L. and Scott, E.--A Modification of the Aschheim-Zondek Test For Pregnancy.
J. A. M. A. 96, Pt. II: 1565-1567, May 9, 1931.
29. Robertson, E. M.--The Practicability of the Aschheim-Zondek Test for Pregnancy.
Jr. Edinburgh Obst. Soc. 37: (Pt. II) 124-132, 1930.
In Edinburgh Med. Jr., August, 1930.
30. Rossler, H.--The Diagnostic Significance of Anterior Hypophyseal Lobe Hormone in Urine in Cases of Hydatidiform Mole and Chorionepithelioma.
Ztschr. f. Geburtsh. u. Gynak., Bd. 3--H--96: 516-539, 1929.
31. Sage, E. C.--The Aschheim-Zondek Test for Pregnancy.
Nebr. State Med. Jr. 15: 463-466, December, 1930.
32. Schneider, P. F.--A Hormone Test for the Diagnosis of Early Pregnancy.
Surgery, Gynec. & Obst. 52: 56-60, 1931.

33. Smith, P. E. and Engle, E. T.--Experimental Evidence Regarding the Role of the Anterior Pituitary in Development and Regulation of the Genital System.
Am. J. Anat. 40: 159-214, November, 1927.
34. Stewart, W.--The Zondek-Aschheim Reaction.
Lancet 1: 1347-1349, June 20, 1931.
35. Stone, B.--Clinical Value of the Aschheim-Zondek Test for Pregnancy.
South. Med. Jr. 23: 747-749, August, 1930.
36. Wells, H. G.--Chemical Pathology, Ed. 4, Philadelphia.
W. B. Saunders Company, 1920, p. 205.
37. Wilson, K. M. and Corner, G. W.--The Results of the Rabbit Ovulation Test in the Diagnosis of Pregnancy.
Am. Jr. Obst. and Gynec. 22: 513-519, October, 1931.

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