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ASCHHEIM-ZONDEK TEST FOR PREGNANCY—ITS PRESENT STATUS*

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IN 1926 Bernard Zondek and, almost immediately thereafter, our former associate, P. E. Smith, discovered that the implantation of small bits of the anterior lobe of the hypophysis would provoke sexual maturity in immature animals. When the animals are taken on the day of weaning, these remarkable changes can be produced within four days. A simple chain of reasoning led S. Aschheim, an associate of Zondek, to discover that the same chemical substance which in hypophyseal implants so rapidly matures animals is unusually abundant in the body fluids (blood and urine) during pregnancy. It has been known since the classical work of Erdheim and Stumme that the anterior hypophysis hypertrophies in pregnancy. Zondek had just previously shown that the ovarian substance, folliculin, occurs in high concentration in the body fluids of pregnancy. It was therefore natural to inquire whether the new hypophyseal hormone was not also similarly abundant in pregnancy. Their positive findings were quickly extended by the Berlin investigators and now constitute perhaps the most reliable known test for pregnancy and the only reliable early test.

TEST WORTHY OF USE IN GENERAL PRACTICE

It seems remarkable that the medical profession of this country has not made wider use of the Aschheim-Zondek test for pregnancy. This is the justification offered for a survey of the present status of the test. An attempt will be made to discuss briefly both the technique of the test as used by the discoverers and by others, the accuracy or dependability of the test and the sphere in which the test is useful.

TECHNIQUE

As used by Aschheim and Zondek [1-10] the test is performed as outlined below.

The morning urine is sent into the laboratory in clean bottles. They recommend the addition of one drop of tricresol per 25 cubic centimeters of urine if it is necessary for the sample to be sent by mail. A group of five mice, each weighing six to eight grams, is used to test each urine specimen. The total volume of urine injected into each mouse varies from 1.2 to 2.4 cubic centimeters. This total dosage is distributed in six doses during forty-eight hours. It is administered subcutaneously in increasing amounts of 0.2 to 0.4 cubic centimeters each. On the fifth day, *i. e.*, ninety-six to one hundred hours after injections are begun, the animals are autopsied and the genital system is examined, preferably with a hand lens or binocular microscope. The ovaries of untreated animals of this age are always very small smooth glandules, containing, at most, small follicles. In case the ovaries show further development, the type of reaction can be classified as follows, according to Aschheim and Zondek:

Reaction I—Enlarged follicles.

Reaction II — Hemorrhagic follicles — "blood points."

Reaction III—Corpora lutea.

COMMENTS ON REACTIONS AND TECHNIQUE

The presence of Reaction II or III, or both, is an almost certain indication of the presence of the anterior hypophyseal sex hormone in the urine, characteristic of pregnancy in the case of human beings. The reaction is considered positive if only *one* hemorrhagic follicle or *one* corpus luteum is observed in one experimental animal, that is, if either Reaction II or III, or both, are present in any one of the five animals used for the test for pregnancy. However, if Reaction I is given and the animals show evidence of heat (cornification of the vagina and enlarged uterus), the test is repeated with a second sample of urine.

If there is urgent reason to get results in an interval shorter than five days, the Berlin investigators increase the number of experimental animals used in the test and autopsy them in sixty to seventy hours. By this method they cut down the inaccuracy introduced by the variability in reaction of individual mice, and feel they can reach fairly accurate conclusions. The ovaries are studied in serial section if for any reason the macroscopic findings are dubious.

The modifications in technique introduced by other workers have not been numerous; most workers have adhered closely to the conditions of the test as prescribed by the discoverers. Kraus [14] of Prague shortens the test somewhat by autopsying on the fourth day and examining the ovaries in glycerin under the microscope. He believes he can recognize the development of hemorrhagic follicles and corpora lutea at an earlier stage by this method than by macroscopic observation.

Some workers, *e. g.* Mayer [35] and Vogt [22] do not trust macroscopic findings, but always study stained serial sections.

METHOD USED AT UNIVERSITY OF CALIFORNIA

In the laboratory at Berkeley, sexually immature rats have been substituted for mice with satisfactory results. The test as performed is as follows: The morning urine is neutralized, fil-

* From the Anatomical Laboratory of the University of California.

tered and, during the test, stored below 0 degrees C. so as to be actually frozen. Six rats, twenty-four to twenty-six days of age, weighing forty to fifty grams, are injected to test each urine. Each rat receives a subcutaneous dose of one cubic centimeter daily for four days (Monday, Tuesday, Wednesday, Thursday). The animals are autopsied ninety-six hours after beginning the injection (Friday). The ovaries are examined under binoculars, eventually dissected free from the oviducts, and weighed. If observations are made by immediately swinging the binoculars over the opened body of the animal with the ovaries *in situ* and only the bursa ovarica opened but the blood supply left intact, then the detection of corpora lutea is much facilitated. The positive test for pregnancy in the rat is the presence of corpora lutea—Zondek's Reaction III. Hemorrhagic follicles, Reaction II, are not common in the ovaries of precociously matured rats. Though the expense of maintenance of a rat colony is greater than that of a mouse colony, and rats would not have been used except that our colony was established, use of the rat has, however, one advantage. Rats are sturdier than mice. Zondek and Aschheim have 15 per cent mortality among their urine-injected test animals. So far we have lost no animals from use in the test.

ACCURACY OF THE TEST

The error involved in the Aschheim-Zondek pregnancy test is small enough to admit the test as a most important diagnostic aid. Twenty-four groups of workers and over three thousand tests are cited in Table 1. In almost no cases did the error exceed two per cent; in fact the majority of workers have found the error to lie between one and two per cent.

TABLE 1.—*Accuracy of the Aschheim-Zondek Pregnancy Test*

Investigator	Number of Cases	Percentage Error
Ascheim and Zondek,[1-10] Berlin	1000	1.2
K. Ehrhardt,[11] Frankfurt	400	2.
E. Solms and E. Klopstock,[12] Berlin	349	1.
F. Wermbter and E. Schultze,[13] Wien	30	
E. J. Kraus,[14] Prag		0
Brühl,[15] Göttingen	192	0 pregnancy cases; 3.75 non-pregnancy cases
Martius,[16] Göttingen	51	1.2 Pregnancy cases; 0 non-pregnancy cases
Praude,[17] Moscow	100	
Brouha and Simmonet,[18] Paris	30	
Odeschati,[19] Italy		
Louria and Rosenzweig,[20] New York	86	1
H. E. Kaplan,[21] Stockton		
Siebke,[24] Kiel	51	0
Füth,[25] Köln	139	1
Hornung,[26] Berlin		2
Kriele,[27] Berlin-Neukölln	12	8
Schmidt,[28] Düsseldorf	171	0
Pankow,[29] Freiburg		0 in normal pregnancy
Gragert and Wittig,[30] Greifswald	46	0
Kehrer,[31] Marburg	97	1
Karg,[32] München	110	1
Esch,[33] Münster Westf.	49	0 except tubal pregnancy
Hellmuth,[34] Würzburg	36	0 except tubal pregnancy
Total number of cases	3088	

All investigators who have applied the test have found it a reliable criterion for normal uncomplicated pregnancy. Many wish to say the test yields 100 per cent results in all normal cases, where applied after one or more missed menstruations. Several workers have noted a positive test seven to eight days, and one only three days after the first missed menstruation. Zondek cites two cases of young women who were tested before a menstruation had been missed. The test was negative for pregnancy, only large follicles were found in the ovaries of the test animals (Reaction I). When retested four to five days after the expected menstruation, the reaction was positive (II and III). Zondek's rationalization is that implantation must occur before the test becomes positive. The test continues to be positive throughout the remainder of pregnancy and persists to the seventh day of the puerperium. Zondek reports the test to be negative on the eighth day.

The errors recorded in the table are attributable to relatively few causes. The test is strongly positive in cases of hydatid mole, and remains so after removal of hydatid mole if only small fragments are left. Chorio-epithelioma also gives a positive reaction.

A few instances of inaccuracy have been reported in cases of tubal implantation. A positive test seems to be almost coincident with the persistence of life of the fetus, a point emphasized by Radtke [36] and others. Instances of a negative test, in cases later proved to be tubal pregnancies, were probably due to death of the fetus before the time of the test. Esch,[33] however, cites a case of tubal rupture in which a positive Aschheim-Zondek test was obtained two days after the fetus was expelled into the peritoneal cavity. In Schmidt's [28] experience the reaction in tubal pregnancy has always been positive except in two cases of old hematoceles.

In cases of abortion, positive results have been reported to occur as late as the sixth to the eighth day after abortion. The test has hence important medico-legal value. It is probable that the results from the test in cases of abortion and partial abortion depend, just as in tubal pregnancy, on the time of death of the ovum or fetus with respect to the time of the test.

Tests have been reported where large follicles (Reaction I) were found after the injection of urine from nonpregnant women in whom carcinoma was present, but such a finding should not be counted among the errors of the method, as a pregnancy diagnosis is never based on Reaction I.

No conditions save pregnancy and its associated phenomena have given Reactions II and III, and among the conditions which have been investigated have been uterine myoma, carcinoma of the body of the uterus, tumor of the adnexa, cystoma, x-ray amenorrhea, unexplained amenorrhea, hypophyseal tumor, and acromegaly.

In summary of the accuracy of the test it can therefore be said: A positive Aschheim-Zondek test is not only given by pregnancy but by hydatid mole and by chorio-epithelioma. A negative test is an almost certain indication of the absence of

pregnancy, at least of pregnancy of longer duration than one month.

IMPORTANCE OF THE TEST—THE APPLICATIONS

Of first importance in the applications of the Aschheim-Zondek test is the early diagnosis of pregnancy—before clinical signs are available or dependable. This is not the only clear indication for the test.

Of next importance is the use of the test in the differential diagnosis of cases in which pregnancy is simulated; for instance, the differential diagnosis between cystic myoma and pregnancy as cited by Wagner.[23] Clinically this case simulated pregnancy. After opening the peritoneal cavity the enlarged uterus was not distinguishable from a normal pregnant uterus. Even when the mass was incised a bag of fluid was encountered similar in appearance to fluid-filled fetal membranes. The uterus was removed, however, because of two clearly negative Aschheim-Zondek tests which had previously been performed. Section of the uterine mass showed a cystic softened myoma.

The test has been suggested as an aid in the detection of the death of the fetus, but the results, at least those reported by Esch,[33] do not confirm this hope. He finds too great a lag after the death of the fetus to make the test valuable. In one case of a seven-month pregnancy reported by him, the heart of the fetus was not audible. For this reason they believed the fetus to be dead. Ten days after this observation the Aschheim-Zondek test was positive. Four days later a macerated fetus was expelled. In another case of incomplete abortion the test was positive eight days after expulsion of the products of gestation. Schmidt[28] describes a rapid disappearance of the test after spontaneous abortion of a fetus dead from syphilitic or renal disease in the mother, but by rapid disappearance he means a negative reaction six days after abortion.

The test promises to be of importance in the diagnosis of hydatid mole and chorio-epithelioma. In cases where the test remains positive too far into the puerperium (*i. e.*, after seven days) or too long after abortion (*i. e.*, after six days), these pathological conditions need to be remembered. Zondek, Aschheim, Wagner,[23] and others emphasize the importance of following patients in whom hydatid moles have been removed by frequent tests as a check on the completeness of removal. Wagner and others find that repeated positive tests after removal of a hydatid mole, though indicating the need of very careful study, do not necessarily indicate the development of a chorio-epithelioma. If, however, the test becomes positive after an interval of negative tests—and pregnancy can be excluded—then the development of chorio-epithelioma is likely.

The fact that the urine from cases having a hydatid mole is so much more potent in anterior hypophyseal sex hormone than urine from cases of normal pregnancy suggests the minimum dose method as of possible aid in distinguishing between normal pregnancy and this condition. Only one-tenth the usual dosage is required to give a positive test in cases of hydatid mole.

Just as the Aschheim-Zondek test is of aid in the differential diagnosis of pregnancy, so also it is of aid in the exclusion of pregnancy in the study of amenorrheas. The test has been found negative in each of one hundred and fifty-one cases of amenorrhea studied in the Charité-Frauenklinik, Berlin. As an instance of such use of the test a case will be cited from Zondek's discussion of hyperhormonal and oligohormonal amenorrheas. In some amenorrheas, hyperhormonal, the urine is characterized by large amounts of *folliculin,* but not of the hypophyseal hormone. Some of these cases have cystic follicles in the ovary. In the latter case the condition can be improved by expression of the cyst. Folliculin is excreted in increased amounts in the amenorrhea of pregnancy as well as in these peculiar amenorrheas of nonpregnant women. In the study of such a case, therefore, one would wish to be entirely sure of the absence of pregnancy by the use of the Aschheim-Zondek test.

SUMMARY*

1. The Aschheim-Zondek test is remarkably dependable in cases of normal pregnancy.

2. It is positive a few days after the first missed period.

3. The test is useful in differential diagnosis of pregnancy from other amenorrheas, tumors of the uterus, etc.

4. There is also a distinct province for the test in the diagnosis of hydatid mole and chorio-epithelioma.

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* It is to be hoped that the present summary will encourage routine use of the new test and that the technique of its performance is described with sufficient detail to encourage any diagnostic laboratory to include the test in the service it renders physicians. The equipment is very simple, but unfortunately involves a continuous supply of litters of the test animals (rats or mice) known with certainty to be not younger than twenty-one nor older than twenty-six days of age when the test is begun. Pending equipment of the usual laboratories for the test, as a convenience for the physicians of the state, and for a fixed charge, the anatomical laboratory of the University of California will continue to carry out the test in all cases requested. Merely 100 cubic centimeters of morning urine are required. If a postal journey of a day or longer is necessary, four drops of tricresol should be added to this quantity of urine. Otherwise any preservative is preferably omitted.

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THE FUTURE OF MEDICAL PRACTICE—MEDICAL SERVICE ORGANIZATIONS

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TODAY the individual practitioner of medicine works under many disadvantages. People are taken sick at night and suffer equally during holidays and over the week-end, as on other days. He is thus called upon to give a service which only the most enduring can satisfactorily render. When he is sick and during his vacations his expenses continue and his income ceases. Often he has little time to do the essential laboratory work and, to get the reports he requires, he has to send his patients from pillar to post. At times, much overworked and frequently poorly recompensed, he is also troubled in spirit, for he sees many patients who can ill afford to follow the advice he should give them and, however much he financially sacrifices himself, he can do little to prevent the high costs of serious sickness from crippling many of the families he attends. If successful, he, from small beginnings and after many years of practice, acquires a considerable following. If he then becomes incapacitated or dies, his office closes, no successor reaps the benefit of his labors, and those who have put their faith in him have as a body, no one to whom to turn. Physicians and patients thus jointly suffer and equally desire relief from such a situation.

To quite a number state medicine appeals as a remedy. This, if it comes, would mean that the sick would be taken care of by the state and that physicians, surgeons, and nurses would become employees of the state.

If it were possible to evolve a plan in which politics and patronage played no part and which would continue to attract the same degree of intellectuality as the profession now attracts, there would be much that could be said in its favor. Past experience, however, with bureaucratic management of shipping, railroad, and public utilities business does not speak well for its likely success in this new field. Nevertheless there is a distinct trend toward it, and a social trend, like a secular trade trend, gains ever increasing impetus and wins ever widening support, unless the causes that lead to its inception be removed. Finally, it is apt to attain such a force that it is able to bring about a trial adoption of its tenets in spite of its disadvantages.

If the medical profession is to avoid such a contingency, it must take the lead in remedying the existing conditions. As the writer sees it a satisfactory plan must provide:

1. That all sick people can obtain competent medical and surgical service without undue delay.
2. That this must not cost more than the recipient can afford to pay.
3. That those who give the service would receive a just return for their work.
4. That the rendering of the service would not involve undue or excessive strain upon the members of the profession.
5. That it should remain possible for physicians and surgeons who achieve unusual success to earn incomes comparable with those of the leading lawyers and business executives in the same community.

With the above in mind, the writer visualizes a time:

1. When it will be the recognized duty of the state and municipality to provide such a service for all the indigent sick, and to pay those who give the service.
2. When the great mass of the people will pay a health rate just as they now pay a water or gas or electric rate, and that this rate will insure them competent ever available medical and surgical service. He hopes that private corporations, who in their standing and efficiency will compare with the great public utility companies, will arise to organize and develop and give this service. He questions whether the members of the medical profession alone are fitted by training, experience or bent to successfully develop or manage such corporations but believes that this could be successfully done if representatives of the profession associated with themselves men of the same order of training and intelligence as those who have developed the big life insurance companies. With such a system in existence, he would expect to see: (*a*) hospitals and service stations built on inexpensive sites; (*b*) corps of medical men of all types, on duty at, let us say, eight-hour intervals, giving their whole time to this work, they pre-