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THE CHOICE OF METHODS FOR PREGNANCY DIAGNOSIS*

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MANY modifications of the original methods for the demonstration of the Aschheim-Zondek principle[1, 2] have been developed in its transition from a biologic problem to a practical clinical test for the diagnosis of early pregnancy. In the attempt to adapt it to the requirements of a routine laboratory, immature rats, both male and female, have been used. Rapidity and simplicity have been achieved by the application of the Friedman and Lapham[3] rabbit ovulation test and of the Schneider[4] immature female rabbit test. Among those who have used the rabbit and the rat or mouse tests in parallel, there is but little agreement as to the comparative accuracy and sensitivity.

All of these procedures, in common with other methods involving biologic endpoints, are subject to a variety of errors. These errors may be due to individual and species variations in the test animals. They may represent faulty technic of such nature as to necessitate special adaptations to problem cases as indicated by the case history or by unusual findings in the injected animals. They may be inherent in the actual limitations of the test. Reduction of such technical errors is largely a laboratory problem which can be solved only through vigilance and experience with a great many cases.

In this laboratory the proportion of problem cases in the first 100 tests run was very low. Recently, through the Wisconsin General Hospital, pregnancy diagnosis was offered, as a routine laboratory service, to physicians throughout the state. With the resulting rise in the total number of tests, there has been a relatively greater increase in the number of specimens from cases of abnormal pregnancy and from cases of endocrinopathy simulating early pregnancy. In an attempt to meet the exigencies of such a differential diagnostic service, the laboratory régime

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has passed through several stages. It has been necessary to investigate such matters as the preservation of specimens to be mailed to the laboratory, the injection technic best adapted to the urine received, the establishment of standards on which to base the final report, management of the animal colonies, standardization of the test animals, and other methods of technical improvement and simplification.

The laboratory organization as it is now in operation is based on experience with tests in more than 800 cases, specimens from all of which have been tested by the modification of the Friedman method which will be described. In several instances the test specimens have also been injected into immature rabbits and the same operative technic used as that outlined for the mature rabbit test. Many tests by the rabbit ovulation method have been checked by parallel tests using a female rat modification of the Aschheim-Zondek procedure. The details of this immature female rat modification will be given in this paper. More than 400 tests have been checked by the subsequent clinical course of the case. Those tests found in error have been carefully analyzed and classified. Correlation of the laboratory observations with the clinical data has led to modifications and precautions which tend to reduce the incidence of errors. It is the purpose of this communication to present such technical details as have contributed to accuracy, expediency, and general laboratory economy in the management of this pregnancy diagnosis service. The incidence of errors and their associated clinical conditions will be considered only so far as they have influenced technical developments.

TECHNICAL PROCEDURES

Management of Urine.—A. Preservation: Since many of the mailed specimens may be several days in reaching the laboratory, their adequate preservation is a point of considerable importance. It has been found that, although the hormonal activity may not be altered upon standing, an ammoniacal decomposed urine is invariably toxic to both rabbits and immature rats. Boric acid has proved a most satisfactory preservative which also serves to maintain the desired slightly acid reaction. It is not detrimental to the gonadotropic substance and is less toxic than many other recommended preservatives. Ten grams of boric acid adequately preserve and acidify an 8-ounce specimen. Formalin appears to be definitely inimical to the active principle of pregnancy urine. One incorrect negative of this series can be directly traced to the use of formalin in the urine. The injection of the formalin-preserved specimen produced no detectable effect on the ovaries of either immature rats or those of a mature rabbit. A second specimen, obtained two weeks later and preserved with boric acid, gave a markedly positive reaction. One drop of formalin per ounce of urine was added to the remainder of this second specimen and the preparation was kept in the laboratory overnight before injection. This formalinized urine produced no effect in the mature rabbit or in the immature rat ovaries. This seems to be fairly conclusive evidence that the hormone is readily destroyed by formalin.

B. Toxic Urines: Specimens have been received which, although definitely acid in reaction, were highly toxic to rabbits. No appreciable reduction in toxicity was effected either by the ether washing method or by the ether-glucose detoxification method of Zondek.[5, 6] Wiesner's sulphosalicylic acid precipitation process[7] was equally ineffective in rendering these urines innocuous to injected rabbits. Extracts prepared by the Lloyd reagent-pyridine elution method developed in this laboratory[8] have given correct results with no toxic effects in the injected rabbits. After three washings with an equal quantity of ethyl ether, these urines are usually sufficiently detoxified for injection into immature rats. All trace of the ether must be driven off before injection. Immature rats are extremely sensitive to injection of small amounts of ether.

C. Injection Technic: Both the quantity and number of injections of the test urines have been found to be significant factors in the accuracy and uniformity of result obtained by the rabbit methods. In the extension of the clinical use of the Friedman test, the chief deviations from the technic described by the originators have been with respect to these two points. Friedman and Lapham[3] injected a total of 24 c.c. of urine, in six equal doses over a period of forty-eight hours. Since that time single injection technic, with quantities of urine ranging from 5 to 15 c.c., has been employed by many investigators.[9, 10, 11, 12, 13] Other workers have reported that a higher degree of accuracy is obtained with larger amounts of urine, from 20 c.c. to 30 c.c., administered in two or three injections.[14, 15] Those who advocate a single small injection usually specify that the first morning specimen is required. Obviously the minimum effective dose of pregnancy urine must vary with different specimens. The potency would depend on the hormonal concentration as determined both by the actual concentration of the urine and by the duration of pregnancy.

Since many of the urines received for test at this laboratory are mailed from a distance, it is not feasible to obtain the first morning specimen in all cases. A sample from any ordinary voiding is accepted. To guard against inadequate hormone concentration, a larger amount of the test urine must be injected than would be necessary were the more concentrated first morning specimen available. A study of the relative potency of the day and night urine from several cases of early pregnancy at this hospital has been made. Twenty-four-hour urine collections were obtained. The first morning specimen, or the night voidings, were kept in a container separate from the day voidings. It was found that, on a normal fluid intake of 2,000 c.c. or less, the first morning specimens were approximately twice as effective as the day urines. If the fluid intake were excessive, 4,000 c.c., the day and night specimens were practically the same as to volume, specific gravity, and hormone concentration. In one case of very early pregnancy, no missed menstrual period, a single 15-c.c. injection of the first morning specimen invoked a weakly positive reaction. Twenty-five cubic centimeters of the day urine injected in two equal doses produced large hemorrhagic follicles but no rupture points. Upon the injection of 30 c.c. of this specimen, in two 15 c.c. amounts with a ten-hour interval between the first and the second injection, a markedly positive reaction was observed in the ovaries of the mature rabbit. In several other cases of early pregnancy, dual injection has given a positive result when a single injection of the same total amount of urine either produced no effect or only hemorrhagic follicles. This is in accord with Friedman's observation[16] that frequent small injections of urine cause greater stimulation to ovulation in the rabbit than a single injection of equal amount. Friedman[17] has also stated that there is less individual variation in the response of rabbits to a given dose of pregnancy urine when multiple injection technic is used than with single injection of the same amount of

urine. The experience of this laboratory leads to agreement with this statement. Although our rabbits are of standard breed, always obtained from the same breeder, and are housed in individual cages at all times, they do not react with any semblance of uniformity to single injections, whether the interval between injection and ovary observation be sixteen hours, as advised by Wilson and Corner,[11] or forty-eight hours, as recently advocated by Reinhart.[12] Urines from cases of known pregnancy have occasionally failed to elicit an ovarian response when injected as a single dose, while the same amount of the specimen administered in two equal doses with an interval of ten or twelve hours between injections has invariably induced ovulation.

As a result of these observations we have now adopted the following routine for the injection of mature rabbits: The test urines, filtered and preserved with boric acid, are taken from the refrigerator and allowed to reach room temperature before injection. With no aseptic precautions, two 15 c.c. injections, with a ten- to twelve-hour interval between injections, are administered by way of the marginal ear vein. Each injection is followed by 1 c.c. of sterile saline injected through the same needle. The saline injection serves to cleanse irritating urinary substances from the vessel wall and thus prevents the fibrosis and occlusion which may result after a few injections if the vein is not washed out. Another precaution against venous occlusion is the use of a small hypodermic needle, ⅜ inch length and 26 gauge, for injecting the urine. A small needle also insures against too rapid injection. Rarely is a rabbit unable to tolerate two injections of 15 c.c. each, if given slowly. With slow injection it does not appear necessary that the urine be warmer than ordinary room temperature.

The same general procedure has been applied to the injection of immature rabbits for tests. We have found the subadult animals less uniformly responsive to urines of low hormone content in very early or abnormal pregnancies than are the mature animals. Division of the total injection into two or three doses appears to increase the sensitivity of the immature ovaries to small amounts of the active principle. It seems not improbable that the ovaries of many of these young animals may require one or two stimulating doses before they are in condition to produce corpora hemorrhagica. Since rabbits eighteen weeks old usually weigh from 1,500 to 1,800 gm., a total injection of 15 c.c. or 20 c.c. corresponds very well with the 30 c.c. injection used in the older rabbits whose weight is usually from 2,500 to 3,000 gm.

In a series of titration experiments in which female, twenty-four-day-old rats were used to determine the minimal effective dose of pregnancy urine,[8] it was noted that in all cases the maximum ovarian response resulted from the injection of 3 c.c. to 5 c.c. per animal. The Aschheim-Zondek method of injecting graded doses of the test specimen seems to be of no advantage in routine pregnancy diagnosis by means of immature female rats. Division of the total amount of urine into five equal doses, given once in twenty-four hours, proved quite as effective as the injection of the same quantity in ten injections at twelve-hour intervals. Extraction of the follicular hormone is not essential to the accurate diagnosis of pregnancy. However, if theelin-free urines are injected into immature animals, the results of a negative test may serve as an index to hyperhypophyseal function in the nonpregnant. Any uterine or vaginal reaction may then be interpreted as due to follicular development, induced by an excess of follicle-stimulating substance in the injected urine. In this laboratory all specimens to be tested by the immature female rat method are washed three times with ethyl ether, poured into evaporating dishes, and placed in a warm chamber (38° C.) for one hour or more to permit evaporation of traces of the ether before injection. Each test animal is given, by subcutaneous injection, 1 c.c. of the ether-washed urine once a day for five successive days.

Pregnancy Diagnosis by Rabbit Tests.—A. Selection and Maintenance of Animals: All of our rabbits are obtained from a breeder whose records of age, weight, number of pregnancies, and period of isolation have been found dependable. The young female rabbits are separated from the males at weaning and are kept with other females of the same age until they are eighteen or twenty weeks old. From such a group, animals weighing not less than 1,500 gm. are selected, at seventeen or eighteen weeks of age, for use in tests by the immature rabbit method. For tests with mature rabbits, only such animals are accepted as are known to be six months or more of age, to have had at least one litter, and to have been kept in individual cages three weeks or longer. It is practically impossible to obtain immediately postpartum rabbits from any breeder. However, knowledge of the breeding record tends to rule out individual variations. Fewer refractory animals are encountered, hence greater confidence can be placed in the accuracy of negative results.

Different breeds of rabbits exhibit marked variation as to follicular development. Among the rabbits which we have used, it has been noted that the Australian reds, Australian whites, Flemish whites, and mixed breeds less constantly present ovaries with large follicles than do the standard chinchilla grey rabbits kept under the same environmental conditions.

The environment of the rabbit exerts considerable influence on follicular development. Although kept in separate cages, rabbits brought to the laboratory from ordinary outdoor hutches are not in heat when received. They must be kept in warm quarters for several days before they are physiologically in condition to react to the injection of pregnancy urine. Rubaschkin[18] and others have reported that guinea pigs in captivity breed less frequently in winter than in summer though they may become pregnant at any time if kept warm. This nonbreeding of guinea pigs submitted to low temperature may well be assumed to be due to a lack of follicular development. It seems logical to infer that housing in cold quarters may likewise be responsible for the absence of follicles in the ovaries of rabbits so kept. Stockard and Papanicalaou[19] observed that domesticated animals, particularly rabbits, kept under uniform conditions of temperature and feeding frequently lose the seasonal variations of sexual behavior characteristic of such animals in their native wild. This, no doubt, explains the fact that rabbits kept in isolation in a warm laboratory are, as a rule, constantly in condition to react positively to the injection of pregnancy urine.

Infection in an animal precludes its use in successive tests. Immature and young adult rabbits seldom survive more than one operation if they have coccidiosis. Anesthetization is usually fatal to animals with respiratory infections. Incorrect negatives may result from the use of rabbits bearing staphylococcal infection. In our laboratory, ovaries of animals with stitch abscesses and those of rabbits with suppurating eye ulcers have been found to possess no large follicles. No reaction could be induced in these ovaries by the injection of known positive urines. When the lesions were completely healed, the ovaries were again responsive to gonad-stimulating substance. Animals in which the ovaries have become adherent, encapsulated with scar tissue, or have the blood supply interfered with are discarded from the test colony.

B. Repeated Use of Test Rabbits: Examination of rabbit ovaries by operation has many advantages over that by autopsy. The ultimate cost of the test is lowered by the reduction in turnover in the colony. By repetition of tests in a single animal, standardization of the colony is automatically effected. The first positive result obtained in a rabbit brands that animal as one capable of responding to gonadotropic

material. Reference to carefully recorded descriptions of the appearance of the ovaries of an animal at a previous test is frequently helpful in the interpretation of results which might otherwise be doubtful.

It has been observed that two weeks after the injection of pregnancy urine, the ovaries of immature rabbits have assumed the appearance of those of normal uninjected control animals of the same age. The period of pseudopregnancy in the mature rabbit is of longer duration. The corpora lutea, resulting from stimulation by pregnancy urine, persist for three weeks or longer. Some of the ovaries of these mature animals are so extremely hypertrophied that it is doubtful if they ever resume normal size and appearance. This arouses the suspicion that repeated stimulation of the ovaries to such excessive development may eventually produce a refractory state akin to that described by Hisaw and associates.[20] As a precaution against incorrect negatives, all such results obtained in rabbits that have been used for more than three tests are checked by immediately injecting a test dose of pregnancy urine extract of known gonadotropic potency. A positive reaction, twenty-four hours after the injection, demonstrates the sensitivity of the rabbit and establishes the accuracy of the negative test with respect to that specimen.

Pregnancy diagnosis was still in the status of an experimental procedure in this laboratory when it became apparent that operative technic involving approach to the ovaries by way of midline ventral incision would not permit more than two or three successive tests per rabbit. In spite of extreme care as to asepsis and surgical manipulation, the animal's usefulness was soon terminated by extensive peritoneal infections, herniation with fatal strangulation, intestinal and ovarian adhesions, or bad healing of the external wound. An operative procedure was, therefore, devised for demonstrating the ovaries by way of a dorsal approach. Application of this method has proved highly successful. The difficulties attendant on the transabdominal operation are largely avoided. Many of the animals have been used eight or nine times and to date 5 rabbits have survived twelve tests. Relatively little time is required for the operation; not more than from seven to ten minutes for observation of one ovary or from fifteen to twenty minutes if both ovaries must be exposed. Experience with 800 tests has shown that routine pregnancy diagnosis can be handled without highly trained technicians and with but little equipment or outlay of sterile supplies. The cost of the test is not prohibitive.

The lumbodorsal approach to the ovaries, as developed in this laboratory, had been applied in more than 300 tests when a similar technic was described by Goodale and Flanagan.[21] The two procedures are fundamentally the same although they differ in certain details. The latter may perhaps account for the fact that the test animals of the Goodale and Flanagan colony can be used for no more than three tests, while those of our colony are seldom used less than eight or nine times. With observance of the injection technic described above, no rabbits have been discarded because of occlusion of the ear veins. Neither have we encountered granulation tissue with involvement of the uterine horns or ovaries in the wound. A detailed outline of the lumbodorsal technic as used in this laboratory may be of value.

Female rabbits of the standard chinchilla breed are kept in a warm laboratory for one week before use in the tests. The mature does are known to have been kept in separate cages at least three weeks before delivery by the dealer. Individual cages are also provided for them in the laboratory. It is not essential that the immature rabbits be isolated from other females. The test animals are injected as described in a previous section of this article. Twenty-four to thirty hours after the first injection of test urine, the results are read in the ovaries exposed for observation by the following procedure.

C. Technic of the Lumbodorsal Approach: Ether or sodium amytal anesthesia are equally successful. The latter is preferred when there is no assistant to serve as anesthetist. Best results are obtained with the use of 60 mg. of sodium amytal per kilogram of body weight, made up freshly in distilled water and injected intravenously one-half to three-quarters of an hour before the incision is made.* This time is used to advantage in preparing the animal and in arranging instruments and equipment in readiness for use during the surgical procedure. The instruments may be sterilized by boiling or by placing them in a container with a quantity of 2 per cent phenol sufficient to cover them, rinsing in 70 per cent alcohol and sterile water just before use.

When the rabbit has become fairly drowsy, it is stretched, ventral surface down, upon an appropriate operating table. The lumbar region of the animal is elevated by means of the lumbodorsal support (Fig. 1). The hair is closely shaven or chemically removed from a fairly large rectangular area which extends laterally over

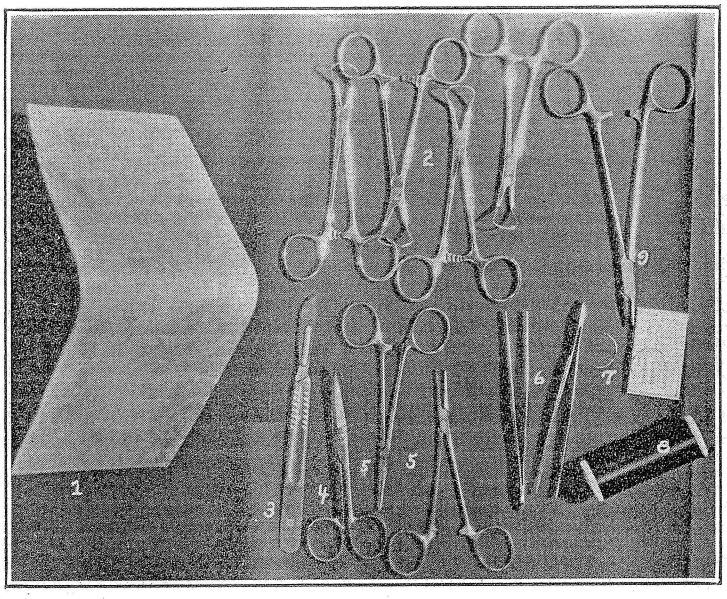

Fig. 1.—Equipment and instruments. *1*, Lumbodorsal support (galvanized iron strip, 12 cm. x 26 cm., angle of 110°, rounded rather than sharply bent). *2*, Backhaus towel forceps, Rochester pattern (an aid but not essential). *3*, Bard-Parker operating knife—No. 4 handle with No. 21 blade. *4*, Mayo Dissecting Scissors, curved, 5½ inch. *5*, Crile hemostatic forceps, straight, 5½ inch. *6*, Tissue or dressing forceps, 5 inch. *7*, Suture needles, ⅜ circle, No. 16. *8*, Surgical silk, black, twisted, No. 9. *9*, Needle holder.

the full width of the body and anteriorly from the intercrest line† (Fig. 2) for a distance of 10 or 12 cm. Through the thin skin, the lateral edges of the longissimi dorsi are plainly discerned. If the animal is so stretched that all the vertebrae are in a practically straight line, the ovaries usually lie approximately 6 cm. cephalad the intercrest line and slightly less than 2 cm. laterad the longissimi border. Upon ascertaining the probable location of the ovaries, the site of the prospective incisions may be marked out with mercurochrome or tincture of metaphen. The entire depilated area is cleansed with a liberal application of 70 per cent alcohol.

*We are indebted to Mr. Frank Maresh of the Department of Physiology of the Medical School for kindly communicating to us the results of his careful investigations as to the dose of amytal best tolerated by rabbits as well as the optimum time required for effective anesthetization of the animals.

†The term "intercrest line" is here used as defined by Schultz (Anat. Rec. 52: 106, 1932) as "the line passing through the right and left iliac crests at the juncture of each lateral ridge with that of the crest.".

A working area of 4 to 6 sq. cm., with the site for one of the incisions at its center, is draped with large sterile flats held in place by towel forceps. Stretching the skin taut between the thumb and finger, a straight incision 2 cm. in length is made with the Bard-Parker knife. With a very sharp knife a single quick, firm stroke should incise the skin and superficial fascia down to the fibers of the external oblique abdominal muscle layer. The tips of the curved dissecting scissors are forced between the bands of both the external and the internal oblique muscle layers. By spreading the blades of the scissors, the muscle fibers are stripped apart the full length of the skin incision. The margins of the skin and of the muscle layers are grasped in the hemostats, the weight of which, falling on either side of the body, serves to spread the opening for exposure of the peritoneum. The latter is cut with the curved dissecting scissors. The ovarian and uterine fat now lies exposed and may be easily pushed aside with the tissue forceps disclosing the ovary. The ovarian fat and the ovary are drawn through the incision. The ovary is held in view by firmly pinching the fat at its base in the tissue forceps. After observing all surfaces of the ovary, it is tucked back into its former position and

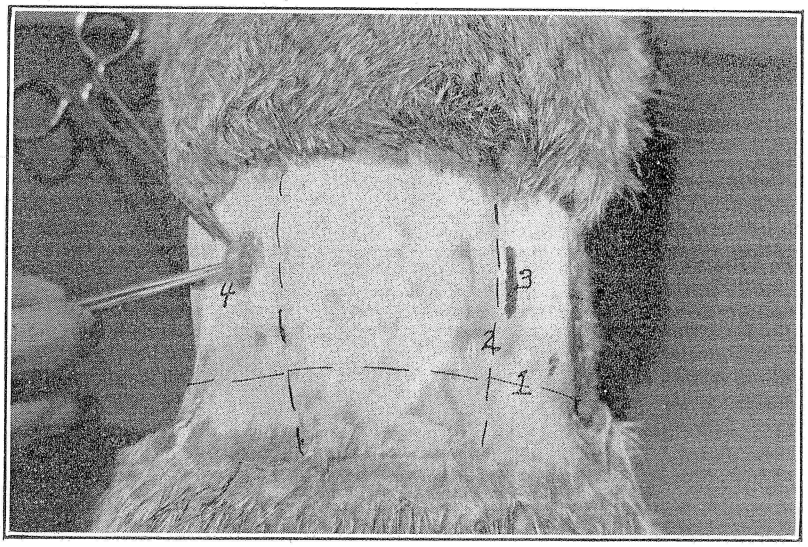

Fig. 2.—Operating field and exposure of ovary. *1*, Intercrest line. *2*, Lateral border of longissimus dorsi. *3*, Probable site of ovary. *4*, Ovary exposed for examination.

covered with a fat pad. The wound is closed in two layers. The peritoneum is caught up with the muscle layers, which are drawn together with one or two sutures of black silk. The skin and fascia are similarly sutured. The wound is then dressed with tincture of metaphen and is coated with flexible surgical collodion.

If the first ovary bears one or more ovulation points, the reaction is considered positive and the other ovary need not be observed. The mature rabbits are returned to individual cages after the operation and are not used again for tests until three weeks have elapsed. In the immature rabbit the wounds heal more quickly, and the effects of ovary stimulation disappear sooner; hence it is our practice to use the seventeen- or eighteen-week-old rabbits for three successive tests with an interval of but fourteen days between tests. After the third test the animal is placed in an individual cage, is given four to six weeks of rest, and is then used as a mature rabbit.

D. Criteria for Judgment of Results: The majority of the positive reactions are easily diagnosed. A few findings among both the positives and the negatives are decidedly puzzling and require careful consideration or repetition of the test before a final report can be given with confidence. It is occasionally necessary to

withhold judgment until a second specimen can be obtained for test. Tests in which large clear follicles are the only finding in the ovaries of injected mature rabbits are reported as definitely negative. The absence of large follicles in the ovaries of a mature rabbit is accepted as evidence that the animal was not in heat at the time of the injection and, therefore, not physiologically in condition to react positively to pregnancy urine. The test is then repeated in another mature rabbit. Failure to induce ovulation in the mature rabbit does not necessarily prove the patient nonpregnant but it does prove that, in the particular specimen injected, the concentration of active principle was insufficient to cause rupture of the follicles and liberation of the ova. A negative test on a second specimen, after an interval of two or three weeks, is usually to be depended upon as eliminating pregnancy.

In this laboratory it has been frequently noted that the injection of urines from cases of endocrinopathy in the nonpregnant may produce corpora hemorrhagica and hemorrhagic follicles in the ovaries of mature test rabbits. Many indeterminate results, in which there is evidence of marked stimulation but no ovulation in the ovaries of the mature rabbits, are clarified upon retesting the urine by the immature rabbit or the immature female rat tests. A markedly positive reaction by either or both of these methods insures the accuracy of a positive report, while failure to obtain a definite response confirms the result as truly negative for pregnancy.

In this series there are 236 pregnancies reported as having terminated in normal deliveries. Among them there was but one urine which failed to cause ovulation in the ovaries of the mature rabbit used for test. In two abnormal pregnancies, which terminated in abortion, ovulation points were not observed, although there was marked ovarian stimulation resulting in many fresh corpora lutea. It would seem safe to assume that the injection of urines from normal pregnancies usually causes follicular rupture as well as luteinization in the ovaries of the mature rabbits.

However, the presence of ovulation points in the ovaries of the test animals is not indisputable proof of pregnancy. Correlation of the laboratory observations, recorded at the time of the tests, with subsequent clinical histories reveals that ovulation occurred as a result of the injection of urine from 12 nonpregnant individuals. Cases of real pathology, other than carcinoma, hydatidiform mole, and chorionepithelioma, yielded many of these false positives. Hence they represent limitations inherent in pregnancy tests based on the presence of gonadotropic substance in urine. Such errors are not easily remedied through laboratory management. They might be somewhat reduced in number if more complete history accompanied the specimen. Urines from patients with histories similar to those previously found to give false positive results might then be routinely subjected to testing by more than one method.

Immature rabbit tests have been used in this laboratory largely for the purpose of deciding the final report to be given in problem cases. Except in very early pregnancy, the accuracy of a mature rabbit test is doubted if the results of the immature rabbit test fail to corroborate it. A third test, by the immature female rat method, is then run. The final report is based on the results obtained by two agreeing tests. Negative tests by the immature rabbit method do not seem wholly reliable in very early pregnancy.

Pregnancy Diagnosis by Immature Rat Tests.—It has been found that the Davis and Ferrill[22] immature male rat modification is more liable to error in the hands of the technicians of this laboratory than are the methods in which immature female rats are used as test animals. The

injection of urine concentrates prepared according to Zondek[23] or by the method of Eberson and Silverberg,[24] as a means of shortening the time required for the completion of the tests, has not proved satisfactory when applied to immature female rats of our colony. Wide variations in response of the test animals is noted. Intraperitoneal rather than subcutaneous injection technic also frequently results in marked difference in the response of the individuals of a test group receiving identical doses of the urine.

The Immature Female Rat Test: Immature female rat tests have been run in parallel with mature rabbit tests on many of the specimens tested in this laboratory. They have been found particularly valuable in the diagnosis of pregnancies of less than three weeks' duration. Urines from such cases frequently cause so little reaction in the mature rabbit ovaries that a definitely positive report is not justified. Upon injection of the urine into 3 or more female rats of standard age and weight range, a positive reaction is usually obtained in at least one of the test animals. Aside from the fact that rats rather than mice are used, the test employed in this laboratory differs from that outlined by Aschheim[25] chiefly in that the urine is not injected in graded doses. Each of 3 or 5 female rats, twenty-four days old, weighing from 35 to 45 gm., is given 1 c.c. of urine by subcutaneous injection once each day for five successive days, with autopsy and ovary inspection on the sixth day. The test is considered positive if the ovaries of at least one test animal show one or more definite corpora lutea and weigh 20 mg. or more. Blood points usually appear but occasionally they are not present in ovaries of rats injected with urines of low hormone concentration. In the diagnosis of pregnancy, as well as in establishing the minimal effective dose of pregnancy urine, as described in an earlier publication,[8] it seems less essential that the rats be litter mates than that they be of uniform age and weight.

Of this series 9 cases, in which the results of the mature rabbit test were indeterminate or were slightly suggestive of positive reaction, were correctly diagnosed by means of the immature female rat test. Three of these were cases of pregnancy of less than three weeks' duration, and 6 of them were in nonpregnant patients. The larger number of animals in the immature female rat test group probably accounts for the more definite final result. The difficulties of housing and feeding make the use of several rabbits per test impractical; hence check tests by the immature female rat method are of value in questioned cases.

SUMMARY

Problems encountered in the application of the Aschheim-Zondek principle to pregnancy diagnosis in more than 800 cases are discussed. Technical details which have contributed to the simplification of several published methods and to their adaptation to the requirements of a practical and accurate clinical test method are presented.

A modification of the Friedman rabbit ovulation test is described whereby the results may be read twenty-four hours after injection of the test urine. Operative procedure for exposure of the ovaries by way of a lumbodorsal approach permits the use of a mature rabbit for from eight to twelve tests and requires but little time, technical skill, or

laboratory equipment. The criterion for judgment of test results is based on a comparison of the ovarian picture characteristically produced by injection of urines from normal pregnancies with that observed to follow injection of urines from abnormal pregnancies and from cases of endocrinopathy in the nonpregnant.

Immature rabbit and immature female rat tests are advocated for use as adjuvants of the mature rabbit test when the results of the latter are indeterminate or unusual, and also as a means of reducing the number of false positives in problem cases in which there is a demonstrable quantity of gonadotropic hormone in the urine.

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The author suggests that a uterine hemorrhage, or a blood-tinged discharge following pregnancy (especially a hydatidiform mole) with a positive Aschheim-Zondek test, should arouse suspicion of a chorionepithelioma. While 45.7 per cent of chorionepitheliomas follow moles, only about 1 per cent of moles are followed by chorionepithelioma; therefore, a hysterectomy or large doses of radium are not justifiable in young women with moles. The diagnosis of typical cases of choriocarcinoma from the histologic findings and clinical symptoms should not be difficult. A panhysterectomy should be performed when a diagnosis is made, which should be followed by irradiation. As embryonic cells are very sensitive to radium rays, radium is a good prophylactic and curative agent in chorionepithelioma in selected cases. Repeated Aschheim-Zondek tests following moles and especially following hysterectomy for chorionepithelioma are of paramount prognostic importance. Patients with mole pregnancies should be watched for several months. However, if an Aschheim-Zondek test is negative, one may feel reasonably assured of no further trouble.

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