NOTES FROM A PREGNANCY DIAGNOSIS LABORATORY (1935)

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During 1935 6,031 pregnancy diagnosis tests were undertaken in this laboratory. Of these 3,014 belonged to the local authority, insured, or hospital categories, the remaining 3,017 being "private" cases. The tests were distributed as follows:

	Number of Tests	Confirma- tions	Errors reported
1. Aschheim-Zondek	5,081	1,174	15
2. 24-hour Friedman fol- lowed by A.-Z.	339	106	3
3. 48-hour Friedman	567	185	6
4. Not completed	44	—	—
Totals	6,031	1,465	24

Occasions for the Test

According to our records the clinician seeks the assistance that these tests can provide mainly in cases in which:

1. The ordinary physical examination for pregnancy is difficult. In no fewer than 434 of the cases it was intimated that the patient was unmarried. In many others excessive obesity or vaginismus made examination impossible.

2. There is unusual and unexplained amenorrhoea or vomiting.

3. Foetal death is suspected.

4. There is difficulty in the differential diagnosis between pregnancy and other varieties of abdominal tumour (carcinoma, cyst, mole); between ectopic pregnancy and other varieties of the acute abdomen; between pregnancy and pseudocyesis; between pregnancy plus fibroids and fibroids alone.

5. A diagnosis of mole or chorion-epithelioma has been made, or in which a recurrence is feared following expulsion or removal.

6. Medico-legal interests are concerned.

7. For some purely personal or domestic reason it is important that the patient shall know at the earliest possible moment whether she is pregnant: for instance, a woman proposes to accompany her husband to some tropical station if she is not pregnant; if she is, she will stay in this country, but the boat leaves in four days' time.

8. If pregnancy exists therapeutic abortion is to be performed—for example, in women with severe tuberculosis, a history of eclampsia associated with a previous and recent pregnancy, exophthalmic goitre, or calcification of the pericardium.

Methods and Procedures

These have been described in previous reports.* Experience, however, has led to the introduction of certain modifications. It was found that immature rabbits were most unsatisfactory, not so much because of their behaviour in the test, but because so many of them were ill with coccidiosis when received from the dealer. It was not unusual for two to four out of a batch of twenty-five to die before injection within a week of receipt. For this reason it became necessary for us to breed our own rabbits and to use only adult females in the tests.

To meet the varying demands and needs of the clinicians two varieties of the Friedman test were made available. In the twenty-four-hour Friedman test one

rabbit is killed at the end of the twenty-four hours; the second either at the same time, if the reaction of the first is sufficiently marked, or else later, at thirty-six or even forty-eight hours. A confirmatory Aschheim-Zondek test is performed concurrently. In the forty-eight-hour Friedman test only one rabbit is used.

There is no doubt whatever that if the rabbit itself as a source of error is to be eliminated the method elaborated by Bishop (1933)† is the ideal one. Under the conditions that obtain in this laboratory, however, it has been found impracticable to perform routine exploratory laparotomies both before and after injection. Since the combination of the Friedman (in which concentrated urine is used) and the Aschheim-Zondek tests demands so much less surgical skill and gives so high a percentage of accuracy, it was decided to employ it whenever possible. If the first rabbit gives a positive reaction then all is usually straightforward. If the result is negative or doubtful the clinician is advised to await the result of the confirmatory Aschheim-Zondek test. The use of the forty-eight-hour Friedman test is restricted to those cases in which some urgency exists and in which the patient cannot afford the more expensive test.

It is seen that, as in previous years, in only about a quarter of the cases do we obtain the clinician's comments upon the reports he receives. This being so it is, of course, very hazardous to speak of the percentage of accuracy of these tests. It is also impossible to include the majority of the results in any hopeful analysis of the records, and equally impossible for us, against the background of our accumulated records, to proffer any firm suggestions relating to differential diagnosis or prognosis. It is to be assumed that errors are much more likely to be commented upon than are those reports which prove to be in harmony with the subsequent clinical history. It is not improbable, therefore, that the number of errors stated is more or less correct.

The distribution of these errors has been peculiar. One practitioner had, in quite a short period of time, no fewer than three errors in a series of six cases. My correspondence makes it clear that both practitioners and patients are now inclined to take it for granted that the results of these tests are invariably correct. They have been impressed with the statement that the percentage of accuracy is not less than 98.5, and have forgotten that this still leaves 1.5 per cent. of errors. Yet if these tests are, at their present stage of evolution, associated with 1.5 per cent. of error it follows that in a series of 6,000 cases there are going to be ninety mistakes—a very small number when considered in relation to the total. But any appeal to statistical theory can do little to assuage the bitterness of the adverse criticism to which the clinician is exposed. To discuss errors in a report such as this can be instructive, even interesting, but to explain one away to an individual patient who has been misled can be an agonizing and even costly experience. The clinician should always safeguard himself, dwelling rather on the small percentage of error than on the high percentage of accuracy. These tests are amazingly accurate, and bear comparison with any others used in medicine, and they must not be blamed for difficulties which are created by misunderstanding.

Errors

1. The Aschheim-Zondek procedure gave thirteen false negatives; seven of these proved to be instances of early pregnancy (less than eight weeks), and the test, repeated after an interval of ten days, returned a correct positive result. As a general rule the Aschheim-Zondek test carried out during the second to sixth weeks of pregnancy is reliable, but the observed differences in respect of hormone

* *British Medical Journal*, 1930, i, 662; 1931, i, 860; 1932, ii, 759; 1933, ii, 296. No report for 1934 was presented.

† *Guy's Hospital Reports*, lxxxii, 308.

concentration in the urine in different women at this stage of pregnancy are such as to make a negative result in any given case somewhat untrustworthy. Furthermore, the general endocrine disturbance commonly associated with puberty, with the climacteric, and with the state of anxiety in "feared" or "wished" pregnancy can, it seems, produce effects which can cloud the result. In all these conditions, as well as in early pregnancy in some women with a relatively low gonadotropic hormone concentration in the urine, it is not uncommon to find that there is an enlargement of the uterus and vagina in the mice in the test, but no naked-eye ovarian changes. Whether this is an effect produced by anterior pituitary, oestrin, or gonadotropic hormones has not yet been determined, but it is our rule, born of experience, to advise repetition of the test in any case in which pregnancy remains a probability. In those cases in which this result is obtained a second test, almost without exception, gives an unequivocal result. In the six other cases of false negatives no history was given, so that it remains possible that several of these were also cases of early pregnancy in women with an unusually low urine hormone content.

The Aschheim-Zondek procedure returned two false positives. One of these related to a woman in whom the condition proved to be menopausal; the other one, repeated three months later, gave a definite negative, and possibly may have been a case of death of the embryo.

2. The combined Friedman and Aschheim-Zondek tests returned two false negatives and one false positive. In all three cases the results of the two tests were in agreement. In the absence of any sufficient clinical history I have no comment to offer.

In twenty-three cases the Friedman and Aschheim-Zondek results did not agree. In sixteen the Friedman was positive and the Aschheim-Zondek negative, and the former proved to be correct. In fourteen of these cases it is to be assumed that, either for the reason that the pregnancy was a very early one, or else that the hormone concentration in these particular women was unusually low, the Aschheim-Zondek was not sufficiently delicate, for it seems to be the case that the modified Friedman test, in which concentrated urine is used, picks up a concentration of hormone which escapes the Aschheim-Zondek procedure. In seven cases abortion had recently occurred. In the remaining two cases the positive Friedman reaction was incorrect and the negative Aschheim-Zondek reaction was correct. One was a non-pregnant girl aged 12 years and 10 months, and the other a non-pregnant woman at the time of the climacteric.

In five cases the Friedman reaction was negative and the Aschheim-Zondek one positive; the latter proved to be correct. But, since these tests were performed at the time when the rabbit material was unsatisfactory, these disagreements merit no comment. In one other case of this kind a second Aschheim-Zondek test gave a negative reaction, and thus came to agree with the Friedman.

3. The forty-eight-hour Friedman test returned six false negatives, in two of which no history was forthcoming. In two others the Aschheim-Zondek test, a fortnight and three weeks later, gave definite positives. One specimen came by air mail from Spain, was stale, and killed all the mice in two Aschheim-Zondek tests before giving this false negative Friedman reaction. In this case the fault lay with the urine, for a second specimen later gave a definitely positive Aschheim-Zondek reaction. The remaining error was contributed by a woman who was known to be suffering from pituitary inactivity, was pregnant at the time of the test, and in whom subsequently parturition was delayed.

In addition to these errors there were five cases in which the result obtained was not sufficiently definite and in which a repetition of the test was advised. Doubtful

negatives were reported in three of these cases. One referred to a specimen taken fourteen days after the known date of intercourse, and a further test, carried out fourteen days later, gave a definite positive. Two doubtful positives were reported. One of these referred to a non-pregnant menopausal woman, and the other to a case in which a fibroid was shortly afterwards removed from the wall of the uterus, no evidence of pregnancy being then discerned.

There is no doubt that the errors in these tests are concentrated at the beginning and at the end of the reproductive phase of life. The results obtained in those cases in which the patient is a young unmarried girl aged under 20 or a woman of 43+ are much less reliable than they are in the case of women between 20 and 40. This fact should be borne in mind by those who have the more

difficult task of harmonizing the reported results with individual clinical histories.

There were forty-four specimens in which no result was obtained, for the reason that the injections killed the animals. It still remains a very difficult matter to explain the reasons for this action of the urine and the behaviour of the rabbit and mouse. In a number of cases it has been possible to reduce the lethal quality of the urine by precipitating the surfeit of protein material by the use of hydrochloric acid before the sulpho-salicylic acid itself is added. It is almost the rule for a specimen from a patient suffering from some chronic cachectic condition such as tuberculosis or nephritis to prove to be extraordinarily lethal. Furthermore, it is significant that the specimens derived from single women who have reason to wish to avoid pregnancy is very commonly lethal. Whether this is a reflection of the general emotional state or whether it is an indication of some attempted chemical interference it is impossible to say, but the examination of a specimen from such a case is invariably a difficult and usually an unsatisfactory task.

Age Distribution of Cases

In 1,496 cases the age of the patient, which ranged from 12 to 53, was intimated. The graph shows the age distribution. In twenty-six cases in which the age was 12 to 16 there were only six positives. These figures have a medico-legal interest. Between the ages of 17 and 40 the number of positives was at every age always in excess of negatives. Thereafter the relative number of negatives progressively increased until, out of twenty-two cases at 47, only four were positive; forty-five specimens were from women between the ages of 48 and 53, and not one of these gave a positive reaction. The high peak at 24 in the graph presumably refers to the common age at first pregnancy among the middle classes. The peak at 35 is of interest, but its significance escapes me. The third peak at 42 is, according to my records, associated with the difficulty of distinguishing between menopause and pregnancy. Since only twenty-four of the sixty-two specimens from women at this age proved to be positive, it would seem to suggest that menopausal symptoms at this age are not uncommon.

Abortion

In 102 cases the question of foetal death was raised. These included a certain number of instances of habitual abortion, in which it is the practice of this laboratory to advise that the Aschheim-Zondek test should be performed at fortnightly intervals around the time of the expected abortion, for it has been found that the transformation of a positive into a weak positive has commonly sounded a warning note which has permitted the clinician successfully to take appropriate measures. In three of the cases evidence was sought concerning the completion or otherwise of a criminal abortion, but in the majority of cases the clinician was seeking confirmation of his own tentative diagnosis of foetal death, based on such evidence as haemorrhage, cessation of foetal movements and heart sounds, or disharmony between the length of gestation and uterine enlargement.

There can be no doubt that the provision of a reliable test for the recognition of foetal death shortly after this has occurred is urgently needed. So far the Friedman and Aschheim-Zondek procedures have not proved to be very helpful in this regard, for with the Aschheim-Zondek test a positive reaction has been obtained anything from one to three months after the time of the death of the foetus (Aschheim, 1933¹; Bué, Palliez, and Gernez, 1932²; Bulliard, 1932³; Brouha and Hinglais, 1931⁴; Spielman, Goldberger, and Frank, 1933⁵; Stewart, 1931⁶), while the Friedman in Bishop's (1935⁷) series of eleven cases also gave in the exceptional case a positive as long as six weeks after foetal death.

This is very unsatisfactory, but, after all, it is too much to expect that these tests should invariably give clear-cut results in these cases, for the results obtained are not supposed to depend upon the presence of a living embryo, but on the presence of living functional chorionic tissue. Furthermore, the strength of the reaction is not a true indication of the amount of placental tissue present, and the amount of gonadotropic hormone present in the urine varies not only with the stage of pregnancy—being stronger during its early stages—but also from woman to woman.

Using the Aschheim-Zondek test, I have had a correct negative result as early as five days after foetal death, but in others the same test has continued to give a positive for as long as three weeks. The same is true of the Friedman method. In order to overcome these difficulties so far as possible, it is now the practice here to employ, whenever we can, the combined Friedman and Aschheim-Zondek tests in any case of suspected abortion. If both

give a negative reaction the question is answered, but it is more usual in recent cases to get a positive Friedman and a negative Aschheim-Zondek reaction, the uterus and vagina of the mice being enlarged. A review of my records shows that this combination of tests has yielded a high percentage of accuracy when performed seven to fourteen days after the death of the ovum, but exceptionally, using these tests, a false positive is obtained as late as two months after foetal death. It is not improbable that a quantitative blood-oestrin test would prove to be of great value in these cases.

Ectopic Gestation

In fifty-three cases the diagnosis "possible ectopic gestation" was intimated. Clear-cut results were obtained with the Friedman and the Aschheim-Zondek and with the combination of the two. It is in connexion with this condition that the most spectacular and gratifying successes of the tests are achieved. The records show that in several instances the result of the test alone has determined that operative intervention should proceed immediately. It is not too much to say that in these cases the tests have been the means of saving life.

Carneous Mole

In eight cases in which the diagnosis was "possible carneous mole" the Aschheim-Zondek test was used. In two in which the dilution tests were employed the result was weak positive, negative, negative. In all the rest the ordinary Aschheim-Zondek and dilutions procedure were negative. I have no information concerning the final diagnosis in these cases.

Hydatidiform Mole and Chorion-epithelioma

We performed 130 dilution tests. When asked to distinguish between pregnancy and mole—to confirm a tentative diagnosis of mole or chorion-epithelioma, or to test for the recurrence of mole after expulsion or removal—it is the routine practice in this laboratory to use (a) undiluted urine, (b) urine diluted nine times, and (c) urine diluted with ninety-nine times its own volume of distilled water. Since in the majority of these cases details of the clinical history do not accompany the specimen, it is impossible to draw from the results obtained any clear conclusion. However, such evidence as is available permits me to hold the view that the test is reliable, and usually provides information of value to the clinician. In the majority of instances normal pregnancy gives Aschheim-Zondek reaction positive; 1 in 10 dilution negative; 1 in 100 dilution negative; whereas mole and chorion-epithelioma return positives with all three. Very exceptionally a normal pregnancy can give a 1 in 10 positive, but, since it is so very rare, it can be disregarded in practice for the reason that the clinical signs themselves are usually sufficient to prevent any mistake in differential diagnosis on the part of the clinician. Although, exceptionally, both the Aschheim-Zondek and the two dilution tests give negative reactions as early as seven days after expulsion or removal of the mole, it is more usual to get at this time a weak positive, negative, negative. At the end of the second week after expulsion or removal the result obtained is usually a negative in all three tests, though a positive with the ordinary Aschheim-Zondek procedure is not uncommon. After a longer interval than this a series of negative results are usually obtained, though there are many exceptions. In one case the ordinary Aschheim-Zondek reaction was positive up till the end of six months, thereafter being negative. In another case in which a positive, positive, negative reaction series had been obtained the reactions were all negative fourteen days later, and during the interval radium treatment had been exhibited.

The age of the patients was given in forty-seven of these cases; it ranged from 19 to 51. The distribution was as follows:

Age	19	21	22	23	24	25	26	27	28	29	30	31	32	33	35	35
No. of cases	1	3	2	3	4	4	4	3	1	1	1	2	2	2	1	1

Age...	...	37	38	39	40	41	43	44	50	51
No. of cases...	...	1	1	1	1	3	1	1	1	2

It is seen that more than two-thirds of these cases occurred in women of 35 and under. In six cases the women were primigravidae, and in two cases the history showed that a normal pregnancy can occur after the expulsion of an undoubted mole.

Testicular Tumours

In four males in which there were testicular tumours the Aschheim-Zondek test gave negative results. One patient was aged 23 and another 30. In the third case there was a small nodule in the right epididymis, the liver was enlarged, and there was associated jaundice. Operation showed that the nodule was a simple cyst. In the fourth case there was a small testicular nodule with liver enlargement and ascites; the operation revealed a non-malignant tumour. In addition to these the Aschheim-Zondek test gave a negative reaction in the case of a man aged 58 suffering from doubtful hyperpituitarism. In one case in which the sex of the patient was not given, but in which the diagnosis was possible teratoma, the Aschheim-Zondek procedure gave a positive result, as did also the two dilutions.

General

Combined Friedman and Aschheim-Zondek tests gave correct positive results in the case of specimens taken fifteen days and seventeen days after the known date of intercourse, and also correct results in the case of specimens which came by air mail from Kenya, Malta, and Spain.

Medico-legal interests were concerned in the following cases: six in which mental deficiency was involved; three of criminal abortion; one of rape; and one in which the possibility of pregnancy complicated divorce proceedings.

In one case in which a final diagnosis of carcinoma of the ovary was returned the Aschheim-Zondek gave a definite negative reaction.

An Aschheim-Zondek positive reaction was obtained in a case which was recorded following operation as an instance of "an enormous ovarian cyst."

In a girl aged 13, in which the Aschheim-Zondek result was negative, the uterus was as large as a three-months pregnancy, owing to an imperforate hymen.

A combined Friedman and Aschheim-Zondek test gave a positive reaction in a case in which it was stated that adequate contraceptive measures had been used.

In one case in which the patient had been sterilized, both tubes being cut and tied, she herself was quite convinced that she was pregnant, since her sensations were the same as when previously she had carried her only child. The result was a definite positive, which was confirmed following curettage.

In a case in which the Aschheim-Zondek reaction was positive the patient (pregnant) was suffering from fragilitas ossium. Her mother was crippled by the same defect, and her child, aged 3½, also had had several fractures. This child had been delivered by Caesarean section.

The records, which include the time of intercourse in relation to the last menstrual period, indicate quite clearly that conception can, in a series of women, occur at all times during the intermenstrual period.

Breakage in the post accounts for an average of three specimens a week, and one report a week goes mysteriously astray. The Post Office accepts no responsibility for articles placed in its care, and all broken packages (which may include letters, postal orders, and cheques) are destroyed. If a letter goes astray, then of course its sender is to blame.

To anyone who has read so far it will be exceedingly obvious that any value that the observations made and the very tentative conclusions drawn might have possessed has been largely destroyed, for the sole reason that in two-thirds of the cases it is not known whether or not the result of the test and the subsequent clinical history were in harmony. A great deal of very valuable information concerning sex and reproductive physiology, and social biology also, has not been garnered.

There is a danger lest this work should become commercialized, the doctor, as it were, paying so much for certain information of value in return. This attitude must, I submit, be opposed, for if only clinician and laboratory worker will pool their interests and knowledge there is no doubt that through these pregnancy diagnosis tests much that is now mysterious can be explained and much that is now beyond our powers can be controlled. Accurate knowledge is needed, and this can come in this particular field only through the analysis of great numbers of carefully observed and fully recorded cases. Pregnancy diagnosis, for the present at least, is more than a side-room technique; it should be an essential part of all programmes of research in human and social biology.

REFERENCES

- ¹ *Die Schwangerschaftsdiagnose aus dem Harn*, Berlin.
- ² *Bull. Soc. d'Obstét. et de Gynéc.*, 1932, xxi, 752.
- ³ *Ibid.*, 1932, xxi, 46.
- ⁴ *Gynéc. et Obstét.*, 1931, xxiv, 55.
- ⁵ *Journ. Amer. Med. Assoc.*, 1933, ci, 266.
- ⁶ *Lancet*, 1931, i, 1347.
- ⁷ *Ibid.*, 1935, ii, 364.

Clinical Memoranda

Giant Urticaria following Aspiration of a Pleural Effusion

The following case appears to be of unusual interest. After a careful investigation of the literature no similar one appears to be on record.

A student, aged 24, developed a left-sided pleural effusion in April, 1934. There was a history of slight pain in the left chest attributed to "rheumatism" two weeks before onset, but increasing dyspnoea on exertion was the first symptom noted by the patient. When seen, three days later, the fluid dullness reached the inferior scapular angle on the left side. There was no clinical evidence of intrapulmonary tuberculosis in either lung. His temperature averaged 100° in the morning and 102° in the evening, pulse 120, respirations 30 per minute. Three days later a specimen of clear, amber-coloured fluid containing a few lymphocytes but no tubercle bacilli, was removed, but the fluid slowly increased, and, at the end of a fortnight, reached the second rib, with resultant cardiac displacement and severe dyspnoea. A gas replacement was performed, with considerable relief to the patient, about three pints of fluid being withdrawn. Three days later, however, he complained of intense itching over the forearms and legs, soon followed by numerous areas of erythema, varying in size from a sixpence to a half-crown, which spread centripetally until the whole trunk was involved. These progressed to the formation of large urticarial wheals, which became confluent. The face and neck were only slightly affected.

Under treatment with calcium chloride, 15 grains t.d.s. orally, and calamine lotion locally, the condition subsided in three days and did not recur. The patient made an uninterrupted recovery.