REVIEW

PREGNANCY DIAGNOSIS

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INTRODUCTION

It is commonly assumed that non-clinical methods for the diagnosis of pregnancy are of recent origin, but this is not so. Some 3000 years ago the Egyptians attempted to diagnose pregnancy in the human, by watering the seeds of wheat and other cereals with the urine of women. If these seeds germinated, the woman was considered to be pregnant. These, and many other tests are reviewed by Bayon (1939) and Forbes (1957). Henriksen (1941) tried out this early Egyptian test for pregnancy with fifty urines. He found that 75% were correctly positive and 85% correctly negative. This degree of accuracy was no worse than many, and better than some, of the proposed tests for pregnancy which have appeared in the last 40 years.

The first reliable test for pregnancy was that described by Aschheim & Zondek (1928a, b) using the immature female mouse. Since then many laboratory procedures have been developed as diagnostic tests for pregnancy in the human. Some have been based upon the detection of human chorionic gonadotrophin (HCG). Others depend upon the estimation of oestrogenic steroids, progesterone, pregnanediol and melanophore stimulating hormone. Observations of withdrawal bleeding after progesterone–oestrogen therapy, and biochemical reactions of many kinds have also been tried (Cowie, 1948). This review is concerned with these tests which detect HCG in serum or urine. They are the most reliable tests for the diagnosis of early pregnancy.

BIOLOGICAL TESTS

Aschheim–Zondek test: Immature female mouse

In 1928, Aschheim and Zondek published the results obtained with urines from a series of known pregnant and non-pregnant women. The biological test agreed with the clinical diagnosis in 98.5% of the cases.

When urine containing HCG is injected into immature mice, the following reactions occur: (1) stimulation of ovarian follicles, ovulation and the induction of oestrus; (2) ovarian hyperaemia and the production of haemorrhagic follicles; and (3) some follicles become luteinized and corpora lutea atretica are formed. The test was considered positive if reactions 2 and 3 occurred in one or both ovaries, in one or more of the test animals. Because these reactions were similar to those which could be produced by implants or extracts of the anterior lobe of the pituitary, it was thought that the gonadotrophin in pregnancy originated from the hypophysis. We now know that the hormone is produced by the placenta, and Hamburger (1933) proposed the term ‘chorionic

hormone' because of its occurrence when living chorionic tissue was present. Evans (1935) suggested the name 'chorionic gonadotrophin hormone' or 'chorionic gonadotrophin' to distinguish it from pituitary gonadotrophin.

It is now known that the injection of urines, in which there are large amounts of hypophysial gonadotrophin, into non-hypophysectomized rats and mice, may produce an effect upon the ovaries similar to that of an injection of pregnancy urine. In this way a false positive AZ reaction can be produced with the urine from menopausal and post-menopausal women. The injection of hCG into immature rats or mice, in amounts sufficient to cause uterine enlargement without the formation of haemorrhagic follicles or corpora lutea, may produce an effect similar to that of injected hypophysial gonadotrophin. A year after the publication of Aschheim and Zondek's results Professor F. A. E. Crew and Dr B. P. Wiesner set up the first laboratory concerned with the diagnosis of pregnancy. Eight hundred and thirty AZ tests were done in the first year with an accuracy, based upon the clinical diagnosis, of 97.8% (Crew, 1930). Further encouraging reports on the test continued to come from the laboratory with the inevitable consequence that more and more requests for pregnancy diagnosis were received (Wiesner, 1931, 1932, 1933). From this beginning there was developed an organization capable of testing 25,000 urines a year. The AZ test was used successfully in this laboratory for 20 years until it was replaced by the Hogben test in 1948.

Dilution or semi-quantitative tests

Dilution tests are usually done when the pregnancy is thought to be abnormal, and a hydatidiform mole or chorionepithelioma is suspected. These tests are the same as pregnancy tests, except that the original urine, or an extract made from it, is diluted before injection into the test animal.

The use of dilution tests, as a means of distinguishing between normal and abnormal proliferation of chorionic tissue, resulted from the observations of Zondek (1929) and Aschheim (1930) that women with hydatidiform mole excrete more gonadotrophin than women with normal pregnancies. Meyer (1930) reported that the level of urinary gonadotrophin was also increased in chorionepithelioma. Zondek (1931) tested various dilutions of filtered urine in women suspected of having a hydatidiform mole or chorionepithelioma. If the AZ test was positive in a dilution of 1 : 100 the diagnosis of abnormal proliferation of chorionic tissue was considered certain. Despite evidence to the contrary, the impression was created that urines from women with a hydatidiform mole or chorionepithelioma would always give a positive 'pregnancy test' in a dilution of 1 : 100. This is now known not to be true, and weak positive and even negative reactions may be obtained with undiluted urines from such cases (Hobson, 1952a). Work done in the Edinburgh Laboratory shows that a positive reaction in a dilution of 1 : 100 or more was given by 87% of molar pregnancies, 35% of chorionepitheliomas and 10% of normal pregnancies. Generally speaking, dilution tests must be regarded as providing additional information, when attempting to differentiate between pregnancy, hydatidiform mole and chorionepithelioma. Dilution tests are most useful in the follow-up period after the removal of a hydatidiform mole or a primary choriocarcinoma. The

continued and rising excretion of HCG will indicate the proliferation of chorionic tissue, which may be far removed from the original site in the uterus. We have some evidence that there may be a difference between the biological and immunological ratio of the HCG activity in urine from women with pathological trophoblast and from those with normal pregnancy (Wide & Hobson, 1964).

Friedman test: Immature female rabbit

Friedman (1929) demonstrated that an intravenous injection of urine from pregnant women into the rabbit produced ovulation and corpora lutea formation. The injection of urine from non-pregnant women did not produce these reactions, and Friedman suggested that the method might be useful as a test for pregnancy. Shirai (1930) and Schneider (1930, 1931) confirmed this. Friedman & Lapham (1931) published results obtained after testing ninety-two urines from pregnant and non-pregnant women and claimed 100% accuracy for the test. Numerous workers have since confirmed the accuracy and reliability of this test (Mack & Agnew, 1934; Lewin, 1935; and Feresten, 1937). Like the AZ test, the Friedman gives false results and these may be due to a variety of factors. These, and many other aspects of the rabbit test, were comprehensively reviewed by Anklesaria (1937). Although the Friedman can be done within 36 to 48 hr, compared with the 5 days required for the AZ test, it has never been such a popular test. Crew (1936) gave reasons why it was only occasionally used in the Pregnancy Diagnosis Laboratory at Edinburgh.

*Hogben test: Female South African Clawed Toad, *Xenopus laevis**

In 1930 Hogben showed that the injection of extracts of the anterior lobe of the ox pituitary (ALP) stimulated ovulation and oviposition in *Xenopus laevis*. Bellerby (1933) confirmed these observations, and showed that *Xenopus* injected with ALP will lay eggs even outside the normal breeding season. Bellerby (1934) and Shapiro & Zwarenstein (1934) reported on the successful use of *Xenopus* for the diagnosis of pregnancy. The reliability of the Hogben test was confirmed by Bosman (1937), Crew (1937), Elkan (1938), Landgrebe (1939), Scott (1940), Weisman & Coates (1944a) and others. The results of 10 years experience with this test were summarized by Weisman & Coates (1944b) in their monograph. In 1948, the Edinburgh Pregnancy Diagnosis Laboratory discontinued the AZ test, and until 1964, when the Hogben test was replaced by the Pregnosticon test, 350,000 Hogben tests were done. During these 17 years the accuracy of the test, based upon the subsequent clinical diagnosis in 71,000 tests, has never been less than 99.3%. In spite of the numerous publications on the use of *Xenopus* for pregnancy diagnosis many workers seem unable to maintain the toad in good condition, and do not get consistently good results. Largely for these reasons, the test has had a limited appeal, except in the few laboratories which specialized in pregnancy diagnosis. In this laboratory, only those female *Xenopus laevis* which lay eggs after a priming injection of 70 i.u. of HCG are used in the Hogben test. This ensures that toads of a known sensitivity are used, and also that their ovaries contain mature eggs. Because the female toad is less sensitive than other animals used in biological tests, any HCG in the urine is concentrated, before injection, by the method developed by Scott

(1940) and modified by Landgrebe & Hobson (1949). The equivalent of 30 ml of urine is injected into each animal, and this allows the detection of HCG when it is excreted in amounts of 2500 i.u. and more per litre of urine. In this laboratory the number of false negative results is less than 1% and the majority of these occur after the 14th week from the date of the LMP (Hobson, 1952a). False positive results have been occasionally reported in the literature (Landgrebe, 1939). We have been unable to produce egg-laying in *Xenopus* with purified and biologically active extracts made from the urine of non-pregnant women or men (Hobson, 1958, 1965b). Shapiro (1936), Shapiro & Zwarenstein (1937) and Landgrebe (1939) found that certain steroids would induce ovulation and oviposition in *Xenopus*. The doses required to effect this are larger than the amounts excreted by non-pregnant women. Spontaneous oviposition may occur if the conditions under which the toads are kept in the laboratory are not standardized (Hobson, 1952a). The most likely reason for 'false positives' is that some toads will lay eggs up to 5 days after an injection, and the toads must be used systematically, and not haphazardly. Marks & Shackcloth (1963) have observed an occasional false positive when urines from non-pregnant women receiving chlorpromazine are tested on female *Xenopus*.

Although the Hogben test is no longer used by this laboratory for routine pregnancy diagnosis, it is used when the immunological test gives a doubtful reaction. These occur in less than 0.1% of all tests. The Hogben test is also done when specimens of urine contain blood. The dilution test is still done with *Xenopus*.

Rat ovarian hyperaemia test

Ovarian hyperaemia, which follows an injection of pregnancy urine into immature rats and mice was described by Zondek in 1926. This response in the immature rat was used by Reiprich (1933) as a pregnancy test, and an accuracy similar to that of the AZ test was obtained. Hyperaemia of the rat ovary appears within 2 hr after an injection of HCG. Zondek, Sulman & Black (1945) carried out the ovarian hyperaemia test on urines from pregnant women, and non-pregnant women with amenorrhoea. The end point of each test was read at 2, 6 and 24 hr after injection. They found that the test had an accuracy of 87% at 2 hr, and of 99% at 24 hr. Many modifications of this technique have been made, and these have been reviewed by Berman (1956).

Galli-Mainini test: Male frog or toad test

Galli-Mainini (1947) used the male toad, *Bufo arenarum*, in a test for the diagnosis of pregnancy, based upon the expulsion of spermatozoa by the animal, after an injection of pregnancy urine. The test had an accuracy of 94 to 95% and both false positive and negative reactions occurred. Robbins, Parker & Bianco (1947) suggested the use of male *Xenopus laevis* as an animal in a new test for pregnancy. Although release of spermatozoa was brought about by injecting gonadotrophins of pituitary and chorionic origin into the toad, they did not use pregnancy urine. Hobson (1952b) regarded this reaction of male *Xenopus* as non-specific, and did not recommend its use in pregnancy diagnosis. The appearance of spermatozoa in the urine of male anura, after the injection of

pregnancy urine is not confined to any one species. Berman (1956), in a comprehensive review of the Galli-Mainini test, has presented objections to its use for pregnancy diagnosis. Perhaps the most important of these is the over-sensitivity of males during the breeding season resulting in spontaneous spermiation and false positive results. Similarly, in the summer months, a decreased sensitivity to HCG causes many false negative results. In recent years there have been reports of false positive pregnancy tests occurring when the Galli-Mainini test is used with urines from non-pregnant women receiving tranquillizers, especially phenothiazine compounds (Foxworthy & Lehman, 1957; Hilbert, 1959; Hodgson, 1959). Allison (1955) has provided information about the sensitivity and speed of reaction of several species of male anura to HCG. The most sensitive species was the tree frog, *Hyla arborea*, which will shed spermatozoa when injected with 1.0 i.u. One of the least sensitive species was the edible frog, *Rana esculenta*, in which 16 to 30 i.u. are needed to produce spermiation. The speed of the reaction is related to the amount of HCG injected and can occur within 15 min in *Bufo bufo*.

IMMUNOLOGICAL TESTS

Zondek & Sulman (1942) contributed to the early attempts to measure HCG by serological methods. More recent progress in immunological techniques, and the improvement in the preparation of purified protein hormones, has contributed to the present development of immuno-assay methods, but as Brody & Carlström (1965) have pointed out the necessary conditions for the immuno-assay of HCG have been available for nearly 20 years.

Boyden (1951) found that a protein could be adsorbed on to the surface of erythrocytes, which had previously been treated with tannic acid, and that these cells agglutinated in the presence of homologous anti-protein serum. Agglutination could be inhibited by the addition of the same protein. If these reactions are allowed to take place in test tubes which are hemispherical at the bottom, agglutination can be identified by the particular pattern the cells make at the bottom of the tube. This forms the basis of the agglutination inhibition tests for pregnancy.

In 1960, Brody & Carlström, using a complement fixation test, demonstrated the presence of antibodies in the sera of rabbits injected with HCG and showed that the method could be used, with serum or urine, for the diagnosis of pregnancy. McKean (1960) used the standard precipitin testing technique, and demonstrated that a ring of precipitation occurred with HCG in the presence of HCG antiserum. Wide & Gemzell (1960) and Swierczynska & Samochowiec (1961) also described methods using the haemagglutination inhibition reaction to detect HCG in urine or serum. In 1961, the Ortho latex immunological test for pregnancy was marketed. Since then several firms have produced immunological tests for use in the laboratory and a great many publications have appeared about their use.

Agglutination inhibition tests

Urine containing HCG will inhibit agglutination of HCG sensitized blood cells, or latex particles by rabbit-anti-HCG sera. Untreated urine from non-pregnant

women does not usually inhibit the agglutination reaction. The anti-serum is prepared by injecting HCG, dissolved in an adjuvant, into a rabbit. Sheep erythrocytes are stabilized with formalin, and treated with tannic acid, and HCG adsorbed onto the cell surface to produce HCG sensitized blood cells.

Some urines contain substances which prevent the inhibition of agglutination by HCG. For this reason, in some tests, a 'urine control' is done. This consists of formalin preserved erythrocytes, stabilized in normal rabbit serum, but not sensitized with HCG. The agglutination of this control should always be inhibited by urines from both pregnant and non-pregnant women. If the agglutination is not inhibited, non-specific agglutinins must be present, and these can be eliminated by acetone precipitation of the urine (Wide, 1962). The precipitate produced, after washing with ether and alcohol, is dried and re-suspended in a phosphate buffer solution. This solution is centrifuged, and the supernatant liquid tested again.

Laboratory haemagglutination inhibition tests

The initial success of the test developed by Wide and Gemzell has been substantiated by Wide (1962) and many other workers who prepared their own reagents for the haemagglutination inhibition reaction. Hamburger (1963) compared the immunological test with the male toad, Friedman and the AZ tests. In 3000 cases the immunological test was correct in more than 99% of cases, and Hamburger concluded that it could replace both male toad and Friedman tests, but that it was not superior to the AZ test. Bertini (1965) examined 35,000 urines by the haemagglutination inhibition test, using reagents made in his laboratory. The sensitivity of his system was such that a minimum concentration of 600 i.u. HCG was detectable. The test was accurate in 97.7% of cases. There were 805 incorrect results and the majority of these were false negatives. Thirteen false positive results were obtained with urines from women with oligomenorrhoea, menopause praecox and post-partum amenorrhoea. This degree of accuracy is similar to that found by other investigators (Table 1).

Commercial haemagglutination inhibition tests

The following commercial preparations are available, and have been widely reported on.

Pregnosticon (Organon Laboratories Ltd). This test is done in a disposable ampoule. The sensitivity of the method is such that a concentration of 1000 i.u. HCG/l of urine will produce a positive reaction. 0.1 ml of urine is required for the test, but in women over 40 years old, half this amount is used. The test is read, by observing the pattern of the sedimented cells, either directly from underneath the tubes, or by means of a mirror, 2 hr after the reagents are mixed together. This test can be used as early as the 35th day after the last menstrual period. In the past 18 months the Edinburgh Laboratory has done 31,648 Pregnosticon tests, and in our most recent estimate of the accuracy of this test, based upon subsequent clinical diagnosis, there were forty-eight negative reactions from 2902 pregnant women, giving an accuracy of 98.0%. More than 80% of these false negative results occurred between the 35th and 70th day after the LMP. There were three false positive reactions from 1965 women who were not

pregnant, an accuracy of 99.8%. This is a considerable improvement over the first report from this laboratory in which 97% of the tests were correct. Most of these inaccurate tests gave false positive results (Hobson, 1963a). The test material at this time would detect HCG in a concentration of 600 i.u./l. When the sensitivity of the test was lowered, there was an inevitable slight increase in the number of 'false negative' reactions. However, false positive results are now rare. In other laboratories similar good results have been obtained and from

TABLE 1

ACCURACY OF THE IMMUNOLOGICAL TESTS IN NORMAL PREGNANCY

Test	Total No. of tests	Pregnant			Not pregnant			Accu- racy of all tests	Refer- ences†
		No.	Accuracy (%)		No.	Accuracy (%)			
			Of test	Range		Of test	Range		
Gravindex	6687*	3485	92.4	70 to 99	3202	98.4	92 to 100	95.3	A
Hyland	497	390	63.0	50 to 67	107	94.3	93 to 100	69.8	B
Ortho latex	2941	1591	82.2	47 to 95	1350	92.7	84 to 100	87.9	C
Pregnosticon	13752	7574	98.4	86 to 100	6178	99.2	97 to 100	98.7	D
Prepuerin	3205	1787	98.9	96 to 99	1418	96.7	94 to 100	97.9	E
U.C.G.	1980	1051	93.6	69 to 97	929	96.3	90 to 100	94.9	F
Wide & Gemzell	39418	21501	96.3	96 to 100	17917	99.8	91 to 100	97.9	G

* Results of Tietz (1965) and Sato & Greenblatt (1965) not included.

- † A: Baumgarten *et al.* (1964), Felton *et al.* (1964), Gusdon (1964), Hobson (1965, unpublished), Hutcherson *et al.* (1964), Islami *et al.* (1964), Jacobson & Davis (1965), Keller & Pletscher (1965), Lang (1964), Mayo & Thompson (1965), Mendel *et al.* (1965), Noto & Miale (1964), Rimbach & Dacic (1965), Sato & Greenblatt (1965), Soost *et al.* (1965), Taymor *et al.* (1965), Weiser (1965), Witt (1965), Yahia & Taymor (1964).
- B: Asher (1963), Hobson (1965, unpublished), Mayo & Thompson (1965), Mendel & Haberman (1964).
- C: Barnett (1963), Eden & Black (1963), Fukumoto *et al.* (1963), Goldin (1962), Henry & Little (1962), Hobson (1965, unpublished), Mendel & Haberman (1964), Olson & Adduci (1963), Sharman & Pearson (1964), Smith (1964), Spadoni *et al.* (1964), Stewart & Welshman (1963), Tyler *et al.* (1964), Weiser (1965).
- D: Bélanger *et al.* (1964), Benoit & Guffroy (1964), Bertini (1965), Daume (1965), Hepp (1965), Hobson (1965, unpublished), Keller & Erb (1963), Keller & Pletscher (1965), Mayo & Thompson (1965), Oliver (1963), Raj *et al.* (1963), Stewart & Welshman (1963), Tietz (1965), Venning (1965), Weill & Dancourt (1965), Weiser (1965), Witt (1965).
- E: Barr (1963), Fulthorpe *et al.* (1963), Politzer (1963), Shea & Warrack (1963), Shuttleworth (1963), Stewart & Welshman (1963), Venning (1965).
- F: Bisley & Kay (1964), Hobson (1965, unpublished), Keller & Pletscher (1965), Mayo & Thompson (1965), Mendel *et al.* (1965), Michelin *et al.* (1964), Sato & Greenblatt (1965), Southam *et al.* (1963), Tietz (1965), Venning (1965).
- G: Bertini (1965), McCarthy *et al.* (1963), Pesonen (1963), Salzberger & Nelken (1963), Stewart & Welshman (1963), Swierczynska & Samochowiec (1961), Waynforth *et al.* (1964), Wide (1962), Wide & Gemzell (1960).

13,752 tests, abstracted from the literature, a total of 173 false reactions were reported, an accuracy of 98.7%. False negative reactions are mainly due to the fact that the amount of HCG in the urine is below the limit of the test's sensitivity. Some false negative reactions are due to unidentified substances in the urine which interfere with the inhibition of the haemagglutination reaction. False positives may occur with urines from women at or near the menopause, and these can be reduced if the age of the patient is known before the test is done.

Other factors which can produce false positive results are the presence of non-specific antibodies in the urine. Glassware, contaminated with soap or synthetic detergents, can result in the sedimentation of agglutinated erythrocytes, giving a false reaction.

Prepuerin (Burroughs Wellcome and Co.). This test, although basically the same as Pregnosticon, differs from it in several ways. The filtered or centrifuged urine is diluted 1 : 5, 1 : 10 and 1 : 20 with an isotonic borate buffer. Two series of such dilutions are set up in 1.0 ml amounts in test tubes. A test suspension, containing HCG sensitized blood cells agglutinated by rabbit anti-HCG is added to one series. A control suspension, of erythrocytes not sensitized with HCG in normal rabbit serum, is added to the other series.

After mixing, the test should be left overnight for the reaction to develop. Shea & Warrack (1963) claim that most of the results can be read in a 'few hours', but in our experience positive reactions do not start to develop in less than 8 to 9 hr. A reaction is recorded as negative when there is complete agglutination in all the tubes containing urine and the test suspension. There must not be any agglutination in the tubes containing urine and the control suspension. A positive reaction is one in which inhibition of agglutination occurs in one or more of the tubes containing urine and the test suspension. The control tubes should also show complete inhibition of agglutination. The overall accuracy of this test is not as high as that of Pregnosticon. This difference is almost entirely due to the number of false positive reactions, which would appear to be due to the greater sensitivity of the Prepuerin test and hence the ability to measure small amounts of pituitary luteinizing hormone (LH). The positive reaction in the 1 : 5 dilution is produced when the urinary concentration of HCG is 500 i.u./l. Barr (1963) pointed out that if the results of the 1 : 5 dilutions were ignored the percentage of false positives would be halved. A 'urine control' is done to eliminate false agglutinations due to non-specific substances in the urine. According to Wide (1962) one or two such urines will be encountered in every thousand tested. Despite the fact that a 'urine control' is done with every Prepuerin test, it gives the same percentage of false negative tests as Pregnosticon.

Urinary chorionic gonadotrophin test. The uCG test, which is available from several sources (Denver Laboratories, Wampole Laboratories, and Laboratoires Fumouze), is essentially the same as the two previous tests, and is not as accurate as the Pregnosticon or Prepuerin tests, giving many false negative reactions.

Ortho latex or flocculation test (Ortho Pharmaceutical Corporation). This is a two-stage agglutination test in which centrifuged urine is incubated for 1 hr at 37° C with HCG antiserum. This is followed by the addition of latex particles coated with HCG, and a further incubation at 37° C for 2 hr. The mixture is centrifuged for 2 min at 3000 rev/min. This speed is critical for the success of the test. If the recommended speed and time is exceeded, then a false negative reaction may be obtained. Centrifugation at too low a speed will leave agglutinated latex particles in suspension giving a 'false positive' result. After the

test has been completed the turbidity of the test solution should be compared with a turbidity standard, both tubes being compared against a black background. If the turbidity is equal to, or greater than, that of the standard, the response is recorded as positive, i.e. the patient is probably pregnant. A turbidity less than that of the standard indicates that she is not pregnant. To obtain the best results, only first morning urines should be used, and tested within 12 hr of being collected. The accuracy reported by the Ortho Research Foundation for this test in normal pregnancies, is 97·8%, with 2% false negatives and less than 0·2% false positives. The overall accuracy of nearly 3000 tests done in fourteen laboratories was 79%. Henry & Little (1962) and Spadoni, McLean & Herrmann (1964) were two groups who obtained the expected accuracy of 97·0%. Rosenberg & Sredzinski (1963) did 752 Ortho tests in parallel with tests on the male frog, *Rana pipiens*. The immunological test had an accuracy of 96·3%, and 96·7% of the biological tests were correct. The large number of false negative results were due to the inherent insensitivity of the test. A minimum concentration of 20,000 i.u./l of urine is necessary for a positive response. False negative results may occur with urine having a specific gravity less than 1·020 (Henry & Little, 1962). Goldin (1962) used serum and urine in the test, and concluded that serum could not be recommended for routine use. An increase in turbidity of the test with urines from non-pregnant women, or men after therapeutic doses of aspirin, has been reported (Ortho Research Foundation, not dated; Garden, Miller & Durant, 1963). Barnett (1963) reported two false positive results when the test was done with urine from a non-pregnant woman treated with chlorpromazine.

Ortho Gravindex-Slide test. This test has replaced the Ortho latex tube test. Latex particles coated with HCG are used instead of sheep erythrocytes. One drop of antiserum is placed on a black slide, and one drop of a first morning specimen of urine is added, and mixed well with an applicator stick for 30 sec. Two drops of the well-shaken latex antigen solution are added, mixed, and the slide gently rocked for 2 min. If agglutination of the latex particles occurs within 2 min then the reaction is interpreted as negative, i.e. the urine is not from a pregnant woman. If agglutination does not take place within 2 min, the reaction is positive, i.e. the woman is pregnant. Agglutination which takes place after 2 min should be disregarded. The enthusiasm with which this test has been received is largely due to the speed with which a result can be obtained, for the accuracy of this test is low (Table 1) and many false negatives occur. It is the least sensitive of the extant immunological tests. The concentration of HCG in the urine needed to produce a positive reaction is about 5000 i.u./l. To obtain the best results the test is done between 41 and 109 days after the LMP otherwise many false negative results will be given. The experiences of Tietz (1965) and Sato & Greenblatt (1965), who deliberately used the test before the recommended time, confirm this. These authors found that 60% of false negative reactions occurred in early pregnancy, but there were no false positives. False negatives may also be due to the reagents being badly mixed. Noto & Miale (1964) reported that the too rapid rotation of the urine-reagent mixture may cause the agglutinating latex particles to disperse, resulting in a

false positive reaction. The experience of Sharman (1965) with Gravindex is exceptional. He has not observed an incorrect result in over 1,200 tests. Mayo & Thompson (1965) obtained 8% false positives in their series of Gravindex tests, but few investigators find more than 1 or 2% of the tests falsely positive.

Gravindex tests must be done individually, and each one takes about 4 min from the time it is set up, to the time when it is read. A technician in our laboratory would therefore spend more than 6 hr doing 100 Gravindex tests in a day. This compares unfavourably with the Pregnosticon tests where, although the result of one test cannot be read until 2 hr have elapsed, 100 tests can be set up and read within 3 hr. The greatest objection to the Gravindex test is its insensitivity.

Precipitin or ring test. McKean (1960), using a standard micro precipitation technique (Boyd, 1956), developed a qualitative pregnancy test. The test is read after 2 hr and the sensitivity is such that urines containing less than 16,000 i.u./l seldom cause the characteristic precipitation. McKean compared this and the Friedman test with urines from nineteen pregnant and twenty non-pregnant women. There were no false results with the immunological test. The Friedman test gave one false positive and two false negative results. A further forty urines known to contain HCG, and twenty-five control urines from five normal males and twenty non-pregnant females, were tested. No false precipitin reaction was obtained.

This precipitin reaction has been utilized by Hyland Laboratories (Asher, unpublished report). Antisera prepared in horses, using a purified HCG as the antigen, are distributed in a melted, buffered agar base, and the mixture poured into plastic trays. Wells are punched into the solidified gel. An extract of the urine to be tested, is put into the well and the agar plate incubated at 37° to 40° C for 4 to 5 hr. A positive and negative control should be done at the same time. If there is enough HCG in the extract, at least 4000 i.u./l in the original urine, then a precipitin reaction develops in the gel. This can only be seen with the aid of a magnifying viewer with the plastic plate illuminated from below, and appears as a halo around the well. A ring of any size indicates the presence of HCG in the urine specimen. This test is the least accurate of all the immunological tests for pregnancy and has little to recommend it.

The results shown in Table 1 are for normal pregnancies only. The reaction of immunological tests, like the biological tests, depends upon the excretion of a certain amount of HCG. The results from cases of disturbed and ectopic pregnancies have not been included, because they may either be positive or negative indicating the secretory activity of the trophoblast, and not necessarily the viability of the foetus (Table 2).

Similar results are found in disturbed pregnancies. Soost, Mehring & Knot (1965), using the Gravindex test, obtained 116 positive reactions in 203 tests. Hobson (unpublished), using the Pregnosticon test in 147 disturbed pregnancies, found a positive result in 114 cases. Positive reactions will be produced by any urine containing HCG, i.e. from women with a hydatidiform or invasive mole, or a choriocarcinoma of the uterus or ovary. In men, choriocarcinoma of the testis is also associated with the excretion of HCG. Like the biological tests, im-

munological tests can also be used semi-quantitatively in an attempt to distinguish between the presence of normal and pathological trophoblast. Wide (1962), Brody & Carlström (1965) and Mishell, Wide & Gemzell (1963) have published information about the level of HCG in urine and serum in normal pregnancy. A maximum hormone excretion occurs about 10 weeks after the LMP followed by a fall to lower levels, with excretion continuing into the post-partum period. Although there is a growing literature on the HCG levels in urine and serum of women with hydatidiform mole, and men and women with choriocarcinoma as measured by biological methods (Hobson, 1965b), it cannot be assumed that the results obtained by these methods can be compared directly

TABLE 2
RESULTS OF THE IMMUNOLOGICAL TESTS IN ECTOPIC PREGNANCY

Test	Reaction in ectopic pregnancy		References
	Positive	Negative	
Gravindex	24	16	Gusdon (1964), Islami <i>et al.</i> (1964), Soost <i>et al.</i> (1965)
Ortho latex	5	3	Goldin (1962)
Pregnosticon	17	9	Glass & Jesurun (1966), Hobson (1965, unpublished)
U.C.G.	13	6	Southam <i>et al.</i> (1963)
Wide & Gemzell	18	2	Wide (1962)
Total	77	36	

with results obtained by immuno-assays. Wide (1962), and Hobson & Wide (1964), have shown that bio-assay and immuno-assay do not measure the same activity.

Specificity

Wide (1962) found that false positive reactions did not occur provided the urines were not first concentrated, or that the sensitivity of the test was such that it did not detect less than 500 to 600 i.u. HCG/l. Increasing the sensitivity so that 100 to 200 i.u. HCG/l could be measured increased the number of false positive reactions. Moudgal & Li (1961), using the Ouchterlony double diffusion technique, showed that HCG cross-reacted with rabbit antiserum to human LH. Wide, Roos & Gemzell (1961) showed that human pituitary LH inhibited the haemagglutination reaction between anti-HCG and HCG-coated blood cells and that this could be used to estimate the concentration of LH in human hypophysial extracts, and in human urine. Wide & Gamzell (1962) used this reaction to estimate the excretion of LH in the normal menstrual cycle. The sensitivity of their assay is such that LH can be detected throughout the cycle except during menstruation. The concentration of LH was also estimated in the urine of adult men and post-menopausal women. This cross-reaction between human pituitary LH and the anti-HCG sera and HCG-coated erythrocytes, probably accounts for the majority of false positive reactions which are given by all the immunological tests. The more sensitive the tests are made, the more likely it is that false positives will occur.

Immunological tests for pregnancy are very convenient. Much is made of their speed, but, with the exception of the Gravindex test, there are several biological tests which are as quick. Depending upon the species used, the male frog or toad will react positively within 15 min to 2 hr of the injection being made. In some laboratories the rat ovarian hyperaemia test has provided reliable results within 2 hr of injection (Kupperman & Greenblatt, 1946). Pre-puerin is an overnight test, and in this respect it is no quicker than the Hogben test.

The range of accuracy obtained when the immunological techniques are used in different laboratories may seem surprisingly large (Table 1). In small laboratories where tests may only be done on a few occasions, the ability to gain experience with techniques is correspondingly limited. Confirmation of results should be obtained from clinicians, so that some idea may be gained of the standard of accuracy in each laboratory.

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