PREGNANCY DIAGNOSIS
AN EVALUATION OF NEW LABORATORY METHODS
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Synopsis
A short history of the laboratory diagnosis of pregnancy is followed by an evaluation of four immunological methods of pregnancy diagnosis. The immunological methods based upon haemagglutination-inhibition and slide latex agglutination, gave a 100% accuracy. It is concluded that these techniques are suitable for the diagnosis of pregnancy after the 35th day following the last menstrual period.

Introduction
Man has shown great ingenuity in his attempts to make an early diagnosis of pregnancy. His methods have ranged over hormonal (gonadotrophic, oestrogenic, progestational, chromatophoric), enzymic (The Abderhalden reaction, diamino-oxidase, and histaminase), immunological (allergic skin tests and serological methods), biochemical and cytological. The great majority of these methods were proved to be inaccurate with the production of many false results. Only the techniques demonstrating a great increase in human chorionic gonadotrophin (H.C.G.) in urine in pregnancy have stood the test of time.

In 1926 Zondek and Aschheim showed that the implantation of anterior pituitary gland into immature female mice caused oestrus, i.e., the formation of haemorrhagic follicles and corpora lutea in the ovaries. The same two workers in 1927 showed that the inoculation of urine from pregnant females as early as the 35th day after the last menstrual period into immature female mice also resulted in oestrus. This test required 5 mice for each specimen and the ovaries were examined after 100 hours either by naked eye or by low power microscopy. This technique of pregnancy diagnosis (A-Z test) was immediately accepted and a voluminous literature extending from 1929 until 1950 stands testimony to its value. Its average accuracy was of the order of 98%.

The test was modified in many ways during these years; "Detoxication" methods on urine Zondek (1931)—depended on their bactericidal effects. Concentration of urine HCG by precipitation and by adsorption of the HCG on kaolin and subsequent elution by NaOH Scott (1941) were utilised. The mice underwent many empirical procedures such as splenectomy, unilateral ovariectomy, total hysterectomy and whole body irradiation in attempts to make the test more accurate and above all, more rapid. Rapid methods were introduced Aschheim and Zondek (1928) Zondek (1930) Hirsch and Hoffman (1932). These included the inoculation of larger amounts of urine and the histological examination of the ovaries for early signs of oestrus. Unfortunately these rapid methods were only reliable if positive.

Heape in 1905 had demonstrated that ovulation in the young female rabbit occurs only after copulation but it was in 1929 before Friedman wrote of a pregnancy test.
demonstrating that inoculation of human urine containing raised levels of HCG produced ovulation with the presence of recent corpora lutea. The test was read after 48 hours and had a similar accuracy to the Aschheim-Zondek test. Once again a voluminous literature from 1931 onwards supported its usefulness and confirmed an accuracy similar to that given by the A-Z test. Modifications to the test were almost solely concerned with shortening the time of the test and reducing the wastage of young rabbits. Beohm (1941) inserted a "zip" fastener in the abdominal wall to allow frequent inspection of the ovaries. Suchet (1946) examined the ovaries endoscopically. Allen and Priest (1932) transplanted the ovaries to the anterior chamber of the eye to make frequent inspection possible.

Reiprich (1933) showed that the injection of urine containing raised levels of HCG produced macroscopic hyperaemia of immature rats' ovaries after 24 hours. The test gave a similar accuracy to the A-Z test and became known as the "Rat hyperaemia" test. Female guinea-pigs were found to be of no value, Jares (1931). Engle (1929) showed that the accessory sex glands of immature male rats increased in size following inoculation of urine containing raised HCG. Maurizio (1930) utilised male guinea-pigs. These tests using male rodents were popular in some centres but the tests were less accurate than the A-Z test. Hens, Sato and Kimura (1930) and pigeons, Roberto (1935) were found to be unsuitable for pregnancy diagnosis.

Hogben (1930) and Hogben et al (1931) showed that injections of pituitary gonadotrophin could produce ovulation and oviposition in the South African clawed toad (Xenopus laevis). Bellerby in 1933 confirmed these findings and in 1934 successfully used Xenopus in a rapid pregnancy test oviposition occurring 5-18 hours after an inoculation of urine into the dorsal lymph sac.

This test was to become a most popular, accurate and economical pregnancy test. Difficulties in breeding, maintenance and care of Xenopus were its main disadvantage. Robbins et al (1947) demonstrated that the male Xenopus was even more sensitive to gonadotrophin in urine with the production of spermatozoa in the cloacal fluid 2 hours after its injection and Galli-Mainini (1947) showed that the male Bufo arenarum Hensel produced spermatozoa within 2-3 hours of inoculation of positive urine. In 1,354 tests on women in the first 4 months of pregnancy, Galli-Mainini demonstrated a 99.5% accuracy J.A.M.A. (1948).

In 1960 a complete break from the traditional use of laboratory animals was made when Wide and Gemzell demonstrated an immunological test of pregnancy based on the Boyden technique. Boyden (1951) had found that a protein could be adsorbed on to the surface of red cells previously treated with tannic acid, and that the cells agglutinated in the presence of homologous anti-protein serum in a "Salk pattern". Salk (1944) i.e., the cells being disposed over the bottom of the tube. In the presence of non-homologous anti-protein serum the cells sedimented in a sharply demarcated central red button. Wide and Gemzell (1960) made use of this reaction by treating sheep red cells with tannic acid, prior to adsorption of H.C.G. Rabbits were inoculated with H.C.G. to produce anti H.C.G. The combination of the H.C.G. coated red cells and the anti H.C.G. resulted in a "Salk pattern". This reaction was inhibited by the addition of urine containing raised H.C.G. to the anti H.C.G. prior to the addition of H.C.G. coated red cells. The result being that the cells sedimented in a central
red button (Haemagglutination-inhibition). Thus a different “Salk pattern” could be demonstrated between a urine containing normal levels of H.C.G. (non-pregnant) and those containing high levels of H.C.G. (pregnant). A negative result could not be considered reliable until 38 days after the last menstrual period (L.M.P.). On testing 2,230 urines from non-pregnant and from women with a normal pregnancy more than 37 days after the LMP the accuracy was 99.8% Wide (1962).

Keele et al (1962) demonstrated a latex agglutination reaction in which latex particles coated with H.C.G. were mixed with urine specimens. To the latter rabbit anti-H.C.G. had been previously added. In a pregnancy urine containing raised levels of H.C.G. the anti-H.C.G. was neutralised and no agglutination of the latex particles took place. A negative urine gave an agglutination reaction having failed to neutralise the anti-H.C.G.

Other immunological techniques were used, among which, the following are of interest:—

Brody and Carlstrom (1960) used a complement fixation technique.

McKean (1960) used a gel-precipitation technique and the Ouchterlony technique was used by Rao and Shahani (1961) and the Preer technique by Keele et al (1962). Midgley et al (1961) used immunoelectrophoresis and Midgley and Pearce (1962) used a fluorescent antibody technique.

The haemagglutination-inhibition reaction and the latex agglutination reactions are the only techniques which have been utilised commercially for the laboratory diagnosis of pregnancy.

The Army Medical Services with the responsibility to family dependents in many parts of the world were extremely anxious to benefit from a laboratory procedure which would be accurate, and rapid and which would dispense with the tedium of animal husbandry. As the usefulness of the tests had not definitely been accepted at this stage it was decided to carry out a small Army trial to evaluate the accuracy of four commercial preparations.

Methods of Trial

Samples of urine in plain universal containers, the majority being the first specimens of the day, were sent to the laboratory from the ante-natal clinics, out-patient departments and from the wards. No preservatives were added. These specimens were mainly from first visit ante-natal patients but there were two ectopic pregnancies, three ‘doubtful’ incomplete abortions, five abdominal tumours in association with doubtful menstrual histories, nine cases in which difficulty as to diagnosis of menopause or pregnancy was present. Negative samples of urine were obtained from women between the ages of 16 and 45 who were known not to be pregnant.

To control the results of the tests it was necessary to obtain a careful follow-up for clinical assessment. Due to the co-operation of my medical colleagues and the presence of a closely-knit military community this proved a simple task.

Each urine was tested by one or more of the following techniques:—

1. **Pregnosticon (Organon Laboratories)**

   This test depends on the Wide and Gemzell (1960) technique of haemagglutination inhibition.
Pregnancy Diagnosis

2. **Prepuerin Diagnostic Reagent (Burroughs Wellcome and Co.)**
   This is also an haemagglutination inhibition test.

3. **Ortho Pregnancy Test (Ortho Pharmaceutical Ltd.)**
   A latex tube agglutination reaction.

4. **Gravindex Pregnancy Test (Ortho Pharmaceutical Ltd.)**
   A latex slide agglutination test.

<table>
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<th></th>
<th>No. of Tests</th>
<th>Positive</th>
<th>Negative</th>
<th>False Positive</th>
<th>False Negative</th>
<th>% Accuracy</th>
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<tr>
<td>Pregnosticon</td>
<td>315</td>
<td>219</td>
<td>96</td>
<td>---</td>
<td>---</td>
<td>100%</td>
</tr>
<tr>
<td>Prepuerin</td>
<td>59</td>
<td>52</td>
<td>7</td>
<td>---</td>
<td>---</td>
<td>100%</td>
</tr>
<tr>
<td>Ortho</td>
<td>93</td>
<td>53</td>
<td>32</td>
<td>3</td>
<td>5</td>
<td>91.3%</td>
</tr>
<tr>
<td>Gravindex</td>
<td>123</td>
<td>75</td>
<td>48</td>
<td>---</td>
<td>---</td>
<td>100%</td>
</tr>
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The Ortho tube agglutination gave only a 91.3% accuracy and was rapidly discarded although various modifications to the original test were made.

The Prepuerin test although giving 100% accuracy in this small group of tests was found to have certain drawbacks, i.e., 3 urine dilutions were required, separate tubes were required and reading was performed after the tubes had stood at room temperature overnight.

The Pregnosticon test was accurate (100%) it did not require separate tubes and could also be read within a two hour period.

The Gravindex test required no tubes, gave a 100% accuracy and the result was available in less than two minutes.

**Discussion**

Urinary chorionic gonadotrophin (HCG) rises rapidly from a normal level of approximately 10,000 units per 24 hours after the 35th day following the last menstrual period to reach a peak between 500,000 and 1 million units per 24 hours towards the end of the 1st trimester. The level then falls rapidly until the end of the 4th month when there is a more gradual fall in the level until the level of 10,000 units per 24 hours is reached post-partum.

The tests discussed all depend on demonstrating this raised level of HCG and a positive test will not be given by normal levels of pituitary gonadotrophin. The Ortho tube test gave only an accuracy of 91.3% which is similar to that found by Garden et al (1963) in a series run in parallel with the Hogben test (93.1% accurate in 148 tests).

Raj et al (1963) found the Pregnosticon test to give a 100% accuracy in 1,105 tests on urines from suspected pregnancy tests performed in parallel with an 88.2% accuracy with the "rat hyperaemia test".

Keller and Erb (1963) compared Pregnosticon with the A-Z test and the rat hyperaemia test and found an accuracy of over 99% with the Pregnosticon, 97.8% with the A-Z test and 97.8% for the rat hyperaemia test.
Shuttleworth (1963) in comparing the accuracy of Prepuerin with the Hogben test found the positive Prepuerin correct in 98% and negative correct in 94% of cases as against the correct positive Hogben of 100% and correct negative in 67%.

Shea, J and Warrack (1963) found the Pregnosticon and Prepuerin tests to be equally accurate and to be more sensitive than the Hogben test.

A comparison of immunological methods and the Hogben tests made by Wort et al (1963) resulted in their conclusion that "immunological " methods are reliable for the diagnosis of pregnancy at least between the 5th and 14th weeks of gestation ".

A letter by Sharman (1963) appears to be the only account at this present time indicating the value of the Gravindex test. After carrying out 247 tests and finding a 100% accuracy he considers his results "suggest that a major breakthrough or revolution in pregnancy testing is at hand and false positive results would appear to be no longer a problem ".

Conclusion

The results of this small series and the findings of other workers suggests that immunological pregnancy tests dependent upon haemagglutination inhibition and latex agglutination (slide) reactions have a high degree of accuracy and reliability and will undoubtedly replace all animal tests in the near future.

The diagnosis of pregnancy is an important part of everyday medical practice and the advantage of these new, truly "rapid" tests is immediately apparent to both clinician and patient.

REFERENCES


REFERENCES—continued.


