strains being used for immunization, the titres rose from 1:8 to 1:64, and from 1:8 to 1:32. Howard (1950) noted that antibody titres were definitely associated with immunity as judged by clinical criteria; according to Miller and Ryan (1948) there was no susceptibility where there was a titre, though immunity could exist without there being a demonstrable titre; the attack rate varied inversely with the titre. Paschoud (1952), in mouse-protection experiments, observed that rabbit-immune sera with a high agglutinin titre were also highly effective in protective function, although McGovern (1950) stated that agglutinin value did not correlate closely with the degree of protection afforded (mouse tests). Mishulow *et al.* (1941) had observed that the titres of agglutinating antibodies did not parallel the occurrence of protective antibodies, and stated further that there was no evidence that the antibody titre was an index of resistance to the disease.

In the present investigation laboratory methods demonstrated a specific response to the commercial antigen only, there being no accompanying rise in the titres of the naturally occurring antibodies. Indeed, all but one of these had disappeared by the time the final tests were made at 6 months.

In view of the recognized existence of strain variation and related antigenic changes in *H. pertussis* (Bordet and Sleswyk, 1910; Bordet, 1912; Standfast, 1951; Lacey, 1951), and of the role of *H. paraptussis* in causing much "so-called whooping-cough" (Halpern and Halpern, 1948; Miller and Ryan, 1948), the production of a universally efficient vaccine seems to be a question still open to discussion. It has been noted by Ungar (1954) that pertussis vaccines do not protect laboratory animals against *H. paraptussis* infections.

By the test-tube methods described above, the aluminium-adsorbed vaccine appeared to elicit a better response than the plain suspension.

Summary

A brief review of work on pertussis immunization in babies is given.

Details of an investigation into the possibilities of early immunization, and the laboratory techniques used, are described.

Antibody levels at birth were found in about half the babies tested. All babies but one had lost their natural antibody by the age of 6 months. More than one type of natural antibody occurred in the series.

Appreciable agglutinin titres were obtained in all babies who received the aluminium-adsorbed pertussis vaccine. A poorer response was achieved with the suspended vaccine.

The presence of a naturally occurring antibody in the cord blood did not affect the production of an antibody in response to immunization.

Response to immunization as indicated by laboratory methods was specific for the commercial antigen.

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EVALUATION OF THE RAT OVARIAN HYPERAEMIA TEST FOR PREGNANCY DIAGNOSIS

BY

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Immature rats are more resistant than mice to toxic urines (Evans and Simpson, 1930), and after treatment with gonadotrophin their ovaries show easily recognizable hyperaemia (Zondek, 1926). This reaction was first utilized by Reiprich (1933, 1934) for purposes of a pregnancy test. Since that time the method has aroused considerable interest and numerous workers have reported on it favourably (Walker and Walker, 1938; Kelso, 1940; Frank and Berman, 1941; Salmon *et al.*, 1942a, 1942b; Kupperman *et al.*, 1943; Soman, 1944; Kaminester, 1944; Ramsey *et al.*, 1944; Kupperman and Greenblatt, 1944; Kline, 1944; Aschheim and Varangot, 1945; Sala *et al.*, 1945; Hinglais and Hinglais, 1947; Riddell, 1949). Positive results may be obtained as early as two hours after subcutaneous injection or even one hour on intravenous injection (Sturgis and Haour, 1951). Most workers, however, used longer periods ranging from 6 hours to 18 or 24 hours, and the latter apparently led to more accurate results (Zondek *et al.*, 1945).

Rakoff and Fried (1947) found that the administration of a pituitary extract increased the sensitivity of test animals to urinary chorionic gonadotrophin and suggested this as an improvement of the test (Fried and Rakoff, 1949, 1950). The use of the pituitary synergist has, however, proved unsatisfactory in the hands of other workers (Hoffman *et al.*, 1951). Farris (1944, 1946, 1950) was the first to draw attention to false-positive results: he stated that during the normal menstrual cycle erroneous results may be obtained for three or four days. These findings were disputed by Kupperman and Greenblatt (1946) and Bunde (1947), who found that in their series all the errors were false negatives. However, since then an increasing number of workers have reported false-positive findings in their control material (Behnken *et al.*, 1948; Fried, 1948; Riley *et al.*, 1948; Dubrausky, 1950; Kelsey *et al.*, 1950; Hoffman *et al.*, 1951). Furthermore, different authors used different techniques: the number of rats per test, the strain, the mode of inoculation (subcutaneous, intraperitoneal, intravenous) all varied as well as the total volume of urine injected. Readings were taken at 1, 2, 3, 6, 18, or 24 hours afterwards, and the criteria of accepting a positive result were also at variance. Many of the workers compared their results with the Aschheim-

Zondek or the Friedman test; others found their clinical material difficult to analyse, since false-positive findings could be ascribed to abortion.

In the present inquiry, instead of following-up retrospectively a proportion of the routine tests performed—the approach used by other investigators—known pregnant and non-pregnant healthy individuals were tested. It was felt that a clinical series analysed retrospectively may be misleading, since only a small proportion of cases can be followed up: owing to the moral and personal issues involved the answers obtained are likely to be biased. The positive material used consisted of

Histogram showing periods of gestation of positive controls at time of testing.

early morning specimens of urine obtained from women attending an antenatal department. The time since the last menstrual period varied from 30 to 200 days, with the majority around 50 to 100 (see Figure). The negative material was obtained from healthy female laboratory workers and nurses.

Technique

The tests were carried out by injecting 2 ml. of early morning urine, untreated in any way, subcutaneously into immature female rats 3 to 6 weeks old. The Wistar No. 1 strain was used, and this stock showed unchanged sensitivity to gonadotrophin through a period of several years. Titrations carried out from time to time always showed sensitivity ranging from 2 to 3 I.U. per rat, corresponding to 1,000 to 1,500 I.U. per litre of urine (Albert, 1949). Readings were taken 18 hours after injection. The animals were anaesthetized by chloroform and the abdomen was opened while the animal was alive. The ovaries were exposed and their colour was observed against the peritoneal surface of the abdominal wall and compared with the colour of the Fallopian tubes. The normal ovary of the immature rat has a dull, waxy, yellowish colour. The effect of gonadotrophin consists in varying degrees of hyperaemia and usually some swelling. This contrasts with the background of the abdominal surface, which has the colour of the normal ovary. Occasionally only one of the ovaries shows the positive change. The readings have to be taken immediately after opening the abdomen, and the animal is afterwards killed by exsanguination.

The simplicity of this test is its main advantage. The urine requires no treatment or concentration. In over 300 tests only 3 specimens killed the rats. The reading, however, requires practice and good light. Satisfactory readings cannot be taken by fluorescent light. Unless the ovaries are looked at within one minute of opening the abdomen, false-positive readings may result, as the ovaries turn pink on

exposure to air. False-negative results may be obtained if the animal dies before the reading is taken, as hypostasis may drain away the hyperaemia. Likewise exsanguination blanches the ovaries within seconds, and must be avoided. Occasionally all the abdominal organs are found to be intensely red—in these cases satisfactory readings cannot be taken. Urine from patients treated with penicillin is also said to lead to false-positive results (Blaich and Gerlach, 1950).

Results

The findings obtained, using a single rat per specimen, are shown in Table I. With the "one-rat" test a false-negative finding was obtained in 1 out of 14.2 cases and a false-

TABLE I.—Results of 282 Rat Ovarian Hyperaemia Tests

	Total Tested	Correct	Wrong
Known pregnant	227	211 (93%)	16 (7%)
Normal controls	55	51 (92.7%)	4 (7.3%)

positive finding in 1 out of 13.7 cases. Part of this material was also examined by means of the Hogben test, through the kindness of Dr. H. Schwabacher, and a comparison is shown in Table II. The rat test gave a slightly greater number of

TABLE II.—Comparison of 204 Rat Ovarian Hyperaemia and Hogben Tests

	Known Positives		Negative Controls	
	Correct	Wrong	Correct	Wrong
Rat test	162 (93.1%)	12 (6.9%)	27 (90%)	3 (10%)
Hogben test	150 (86.2%)	24 (13.8%)	30 (100%)	0 (Nil)

correct positives, but while the toad test does not give false positives the "one-rat" test fails in this respect. The greater recovery of correct positives may be due to the nature of the material (see Chart) since many of the specimens were from advanced pregnancies with falling gonadotrophin output at a stage when a test would hardly be required for clinical purposes. The disagreement between the two tests was 18.1%. A positive rat test and negative *Xenopus* test were obtained in 12.8%, while the reverse was found in 5.3%. A false-negative result by both tests was obtained in only 0.5% of pregnancies. In these cases the output of gonadotrophin was probably deficient, but all the other discrepancies must be attributed to variations in the animals used. The false-positive results obtained with the rat test are not due to the injection of urine: such findings were also encountered occasionally with uninoculated control animals.

Discussion

Since the laboratory diagnosis of pregnancy is often invoked for cases requiring operative termination of gestation, even an occasional false-positive finding may be of serious importance and the percentage of positives recovered is not a true measure of the clinical usefulness of the test. It is clear that the rat ovarian hyperaemia test carried out on single animals is unsatisfactory for clinical use, since false negatives will be obtained in about 7% of the cases, and, what is more important still, false positives in about the same percentage. By increasing the number of animals used, the statistical odds of obtaining a correct answer can be greatly improved, however.

While the original Aschheim-Zondek test required six immature mice, the Friedman test was generally carried out on a single rabbit for reasons of economy. All later biological tests followed this trend of economy and suffered in accuracy as a result. This is well illustrated by the statistical analysis of the "one-rat" test, which shows that by increasing the number of animals any desired degree of accuracy can be achieved. The calculations were based on the assumption that all the errors were due to the test animals, and the rare cases where failure of gonadotrophin excretion occurred were left out of account. It must also be stressed that the

estimate of proportion of errors has a fairly large sampling error. The expectations depend on the expansion of the binomial $(p+q)^n$, where p is the probability of obtaining a false result. Table III shows the reliability of the test with

TABLE III

No. of Rats Used	Error if all Animals Give Same Result	Repeat rate (One or More Animals Giving Divergent Results)
2	1 in 173	1 in 7.6
3	1 " 2,250	1 " 5
4	1 " 29,450	1 " 4
5	1 " 400,300	1 " 3.3

increasing numbers of animals if all the animals give either positive or negative results. In a high proportion of cases the answer will not be clear-cut in the sense that one or more animals give divergent results. In these cases a repeat test would be required and the repeat rate has also been calculated.

For most purposes a "two-rat" test would combine a sufficient degree of accuracy with a relatively low repeat rate. If a rapid answer is needed four rats should be used (Table IV). If all agree the chances of error are negligible,

TABLE IV

No. of Rats Used	Result	Chances of Error	Repeat Rate (Two or More Animals Giving Divergent Results)
2	AB	1 in 1	0
3	AAB	1 " 70	0
4	AAAB	1 " 5,536	1 in 32
5	AAAA	1 " 42,750	1 " 19

and if one disagrees the chances of error for the remaining three are still only 1 in 5,500. The 3 to 1 ratio will occur with tolerable frequency, and only very few tests will have to be repeated.

This approach allows a more critical test when clinical urgency or importance warrants the added expense.

Summary

The rat ovarian hyperaemia test was carried out on 282 samples of urine. When one rat per test was used a slightly greater number of correct positive findings were obtained than with the Hogben test, but the rat test gave a considerable number of false-positive results. The single-rat test is unsuitable for clinical purposes. By increasing the number of test animals, however, any desired degree of accuracy can be obtained.

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Medical Memoranda

An Unusual Rectal Injury

The following rectal injury would appear to be so unusual as to merit recording in some detail.

CASE REPORT

A man aged 24 was admitted to hospital on November 5, 1953, complaining of severe abdominal pain of twelve hours' duration. He gave a history of a similar attack of severe pain six weeks previously, and stated that over the past ten years he had been subject to attacks of abdominal pain, lasting two to three hours, and sited around the umbilical region. The evening before admission he began to have diarrhoea, and passed both bright and dark red blood per rectum. Vomiting had occurred on the morning of admission. There was no previous history of melaena, and the bowels had been opened regularly each day.

On examination he was pale and collapsed; pulse 130. The abdomen was distended, with generalized tenderness and guarding, most pronounced in the lower half. The bowel sounds were absent; no tumour could be palpated. Per rectum, bright red blood was present in the anal canal. There was much tenderness, and a "doughy" feeling above the prostate which could have been caused by an intussusception, but exact palpation was not possible because of the great tenderness. Proctoscopy revealed copious fresh and altered blood, and a continual stream of faecal matter which made it impossible to visualize the anal canal or lower rectum.

Operation through a right lower paramedian incision, performed three hours after admission, revealed an extensive retroperitoneal extravasation of blood but no evidence of an intraperitoneal injury, though blood-stained fluid was present, together with considerable bruising of the mesentery of the small bowel in the region of the sacral promontory. The small and the large bowel were normal. In the absence of an intraperitoneal lesion the wound was closed. The rectum was again examined, and, now that the patient was anaesthetized and relaxed, a circular tear the size of a two-shilling piece was felt in the anterior rectal wall above the prostate. Blood clot and fluid faeces were present, and a considerable amount of bleeding was coming from this laceration. There was no evidence of injury to the anal or perianal regions, and the sphincter was of normal tone. It was now clear that this was a traumatic injury. A rubber tube was passed up the anal canal, through the laceration in the rectal wall, and into the perirectal tissues. Immediately there was a copious discharge of altered blood, together with a quantity of turbid and offensive fluid. The tube was sutured to the anal margin to keep it in position. A left inguinal colostomy was established and opened immediately.

Post-operatively the treatment was continuous gastric suction, intravenous fluids, penicillin, and streptomycin. In all, 5 pints (3.7 litres) of blood was given. The discharge from the rectal tube was copious for some days, and it was left in position for a week. Recovery was uneventful, and the patient left hospital nineteen days after admission. The laceration in the rectal wall could still be felt, but healing