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ADDENDUM.

As this paper was nearing completion two further contributions appeared dealing with the subject of clearance tests. Hinden (*Lancet,* 1934, 2, 553) gives a very exhaustive summary of earlier papers and reports favourably on the urea clearance test after a brief trial at St. George's Hospital.

Cope (*Lancet,* 1934, 2, 799) brings forward evidence that xylose, sucrose (by injection) and glucose (after injection of phloridzin) also behave similarly to urea and creatinine, and enable clearances to be calculated. He considers the correspondence between the results obtained with these diverse substances strong evidence that a real estimate of at any rate one of the partial functions of the kidney is being made.

In connection with the necessity for placing a load on the kidney when determining the clearance, Cope points out that, while good agreement is found between the values of the maximum urea clearance, when diuresis is forced by administration of urea and water, and the creatinine clearance (in which creatinine is always given), such is not the case when the urea is omitted from the former test. He quotes Hok Lan Ong ("Vergelykende Ondersoek over verschillende Nierfunctieproeven," Amsterdam, 1932) as being, on the contrary, unable to find any parallelism. In the comparison of simultaneous urea and creatinine clearances by Hayman, Halsted and Seyler (ref. [6] above), creatinine but not urea was administered, and in some of their patients no diuresis above two ml. per minute occurred. They concluded from their results that there is a reasonable degree of correspondence.

In this connection it seems to us that the onus lies on those who advocate the need for loading to explain why in very many instances (Fowweather found 75 per cent.) kidneys give a high clearance value even when not working under a load.

A Test for the Early Diagnosis of Pregnancy on the South African Clawed Toad (*Xenopus Lævis*).

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INTRODUCTION.

The Zondek-Ascheim test on rats or mice, or its modification by Friedman using the rabbit, is based on the occurrence in the urine of pregnancy of an anterior pituitary-like gonad-stimulating hormone.

Hogben[1] has shown that injection of extracts of the anterior lobe of ox pituitary into the female South African clawed toad (*Xenopus lævis*) produces extrusion of ova through the cloaca within 18 hours.

These considerations led us to investigate the possibilities of *Xenopus lævis* (the Platanna) as a test animal for the early diagnosis of pregnancy.

Xenopus is a suitable test animal, as it does not ovulate spontaneously under laboratory conditions. In approximately 1,000 toads fresh from the vlei examined during the breeding season no ova were detected in the oviducts, although seasonal variations in the size of the ovaries were well marked (Shapiro and Shapiro[2]). These seasonal variations do not interfere with the use of the animal for test purposes throughout the year.

This paper is an extension of a preliminary investigation communicated to the Royal Society of South Africa by the present authors[3] in October, 1933. Subsequently Bellerby[4] also described a test for pregnancy on *Xenopus lævis*. His results will be discussed in the sequel. A short account of the test as developed by the present writers[5] has also been communicated to *Nature*.

TECHNIQUE OF THE TEST.

(*a*) *Collection of Urine.*

About 5 to 6 ounces of early morning urine are collected in a glass bottle which has previously been cleaned thoroughly by washing with ordinary tap-water several times. It is important to stress that the collecting vessel must be clean. The early morning urine represents a sample concentrated overnight, and no fluids (water, tea, coffee, etc.) should be taken by the woman after supper the night before. No preservatives should be added to the urine. If it is inconvenient to perform the test immediately, the urine can with safety be stored in an ice-chest for two to three days.

We have frequently observed that ecbolics, and soporifics such as luminal, when ingested by the patient,

are excreted in the urine, and thus exert a toxic effect on the test animal. Drugs should therefore rigidly be excluded for two to three days before the urine is collected.

(b) Preparation of Urine Extract for Injection.

The urine is detoxicated, precipitated and concentrated according to the method of Zondek.[6]

The urine, if not acid, is acidified with a few drops of glacial acetic acid until its reaction is acid to litmus, and then filtered. 130 c.c. of the filtrate is transferred to a 500 c.c. separating funnel, and about 350 c.c. of ether are added. The mixture is then shaken for five minutes, the glass stopper being removed at frequent intervals at the beginning.

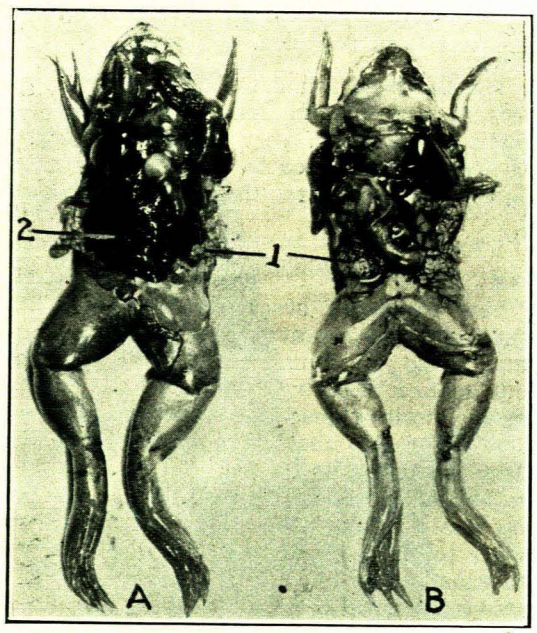

Fig. 1.

A. Toad showing oviducts (1), ovaries (2), *in situ*.
B. Toad with ovaries removed to show oviducts (1) more completely.

After shaking, the mixture is allowed to settle, and 120 c.c. of the urine is run off.

This is transferred to a second separating funnel, and three times the volume of rectified spirits is added. The mixture is again thoroughly shaken for five minutes and then allowed to stand for half an hour. A flocculent precipitate settles down. The precipitate with some of the supernatant fluid is collected in a centrifuge tube and centrifuged for two to three minutes. If the precipitate has not settled completely, the whole mixture must be centrifuged. The supernatant liquid in the centrifuge tube is discarded, and the flocculent residue is next stirred thoroughly with a glass rod, after adding 15 to 20 c.c. ether. It is then centrifuged for about one minute and the supernatant ether discarded.

The precipitate is broken up with a glass rod and carefully dried in a current of air. It must not be heated, as the hormone is not thermostable. When no ether can be detected by smell, 12 c.c. of distilled water are added to the dry precipitate in the centrifuge tube and thoroughly stirred for about three minutes with a glass rod. The mixture is centrifuged for five to seven minutes, and the supernatant liquid is decanted into a small glass dish and used for injection.

(c) Injection into Female Toads.

The female toad can easily be distinguished from the male externally by the presence of anal labia (see Fig. 1). Females are generally also larger than males.

The animals should, if possible, be freshly collected from the vlei, or if kept in the laboratory, their laboratory age should not exceed three to four weeks. We have observed that if the toads have been kept in the laboratory for longer than four weeks they appear to undergo a desensitization to the hormone, when consequently incorrect negatives may be obtained.

1.5 c.c. of the aqueous extract is injected intraperitoneally into each of seven female toads. The needle should first be passed for a short distance under the skin, and then through the muscular abdominal wall obliquely downwards. Care must be taken not to puncture the abdominal vein which lies in the mid-line. Each injected toad is transferred to a canning jar partially filled with tap-water and covered with a perforated top.

(d) Reading of the Test.

The animals are examined 16 to 18 hours after injection. This is at a room temperature of 18° C. At higher temperatures the reaction is speeded up considerably, and in a warm bath at 27° C. the present authors have been able to induce ovulation as soon as five to six hours after injection.

Positive Reaction.—This is indicated either by—(1) extrusion of macroscopically visible ova through the cloaca—the eggs will be seen lying free in the water; or (2) by the presence of eggs in the oviducts. In this latter case the animal is pithed and its abdominal contents exposed. A bilateral paramedian incision about 1 inch long is made with a sharp scissors. The oviducts (whose position and appearance are indicated in Fig. 1) are thus brought into view, and must be carefully examined in a good light for the presence of eggs in the translucent tubes. Each oviduct is examined throughout its entire length from near the root of the lungs to the pars uteri near the cloaca. Occasionally intra-oviducal debris may be confused with ova. Unless the ova in the tube are unequivocally

recognizable as such, the tube is divided below the suspected ovum, which is then carefully expressed and compared with the ova in the intact ovary. The test is only to be regarded as positive if recently released, fresh-looking ova are found in the ducts.

With regard to (1) above, ovulation in any one of the test animals is a positive reaction. Consequently post-mortem examination of the remaining animals is unnecessary. In the case of (2) above, the presence of one egg in one oviduct in one animal is a positive reaction. Usually several eggs are found in one or both oviducts (Fig 2, B). Sometimes the reaction has gone further. In such cases, although the oviducts

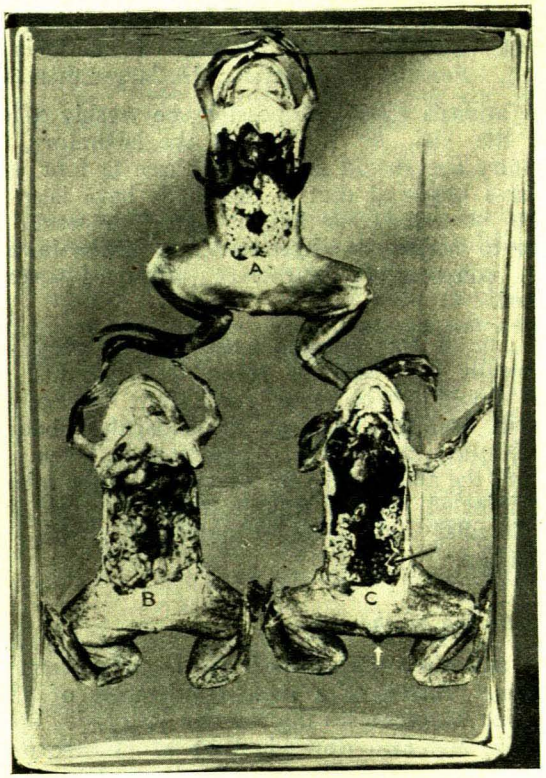

FIG. 2.

A. *Negative reaction.* Toad injected with non-pregnancy extract. No ova in oviducts.

B. *Positive reaction.* Toad injected with P.U. extract. Several eggs in ducts on both sides

C. *Positive reaction.* Later stage. Eggs filling *pars uteri* of oviducts (black arrow). One egg can be seen being extruded from the anus (white arrow).

proper are clear, the pars uteri (*i.e.*, the lower dilated end) is stuffed full with ova (Fig. 2, C). This is the stage which precedes actual extrusion of the ova. In most cases of positive reactions the anal labia are seen to be markedly congested.

Negative Reaction.—No extrusion of ova into the water occurs. The animals are pithed and the oviducts examined in the usual way. The absence of ovulation or of ova in the oviducts is a negative reaction (Fig. 2, A).

Note.—A negative test is always repeated with a new sample of urine on a further batch of seven animals before the negative diagnosis is made.

The present authors have noticed that in spite of treating the urine with ether, urines from non-pregnant women much more often kill the test animals than urines from pregnant women. If the toxic action of ecbolics and other drugs can be excluded, a toxic urine *per se* strongly *suggests* a negative reaction. This rule is by no means invariable.

RESULTS.

In a series of 132 cases investigated by this method to date 64 correct positive and 68 correct negative findings have been recorded.

The test has been found to be of great use in the diagnosis of long-standing amenorrhœas not due to pregnancy, ectopics and very early pregnancy. The earliest pregnancy detected by this method was a case investigated five days after the first missed menstrual period.

DISCUSSION.

We have repeatedly observed that it is impossible to obtain a positive reaction by injecting untreated pregnancy urine into female toads. We are therefore unable to confirm Bellerby's[4] subsequent statement that he obtained positive reactions by injecting 1 c.c. of untreated urine into the test animals. His further statement that extrusion of ova in at least 50 per cent. of ten animals injected is necessary for a positive reaction leads to many and serious errors in diagnosis. A further serious source of error is Bellerby's omission to examine the oviducts of animals which have not extruded ova through their cloaca. It is necessary to emphasize that ovulation in *any one* of the test animals, whether through the cloaca or into the oviducts, is a positive reaction.

The advantages of the test are as follows:—

1. The test animal is cheap, easily available in South Africa, and inexpensive to maintain.
2. It is not necessary in the majority of cases to kill the test animal as it is with rats, mice and rabbits.
3. There are no special precautions with regard to age, weight, isolation (as with mice or rabbits).
4. The extremely short time taken for the test.
5. The simplicity of the end reaction.
6. Small volumes of aqueous extract are injected into the test animal in a single dose, repeated and divided doses being unnecessary.

Goldberger *et al.*[7] have clearly stated the limitations of all pregnancy tests. They say: "For the clinician it is important to remember that a positive test means merely that the patient is excreting anterior pituitary-like hormone which is formed in response to the presence of viable chorion, that the positive test does not indicate whether the fœtus is alive or dead, that the test will remain positive in missed and in incomplete abortions as long as viable chorion is attached to the uterine wall, and that a negative test does not exclude an ectopic pregnancy." With reference to the last point (concerning ectopics), they find that false negatives in ectopic pregnancy are correlated with either degenerated or necrotic villi.

Mazer and Goldstein,[8] in a lucid discussion of the differential diagnosis of pregnancy with animal tests, have summarized their conclusions as follows:—Incorrect positive reactions may be obtained in the cases of women at or near the menopause. Hyperthyroidism is occasionally associated with a positive reaction. Also primary ovarian failure, ovarian cysts, hydatidiform mole and chorion epithelioma give positive reactions.

Summary.

The advantages of a new, simple and rapid test for early pregnancy, using the South African clawed toad, *Xenopus lævis* (Platenna), are described.

In a series of 132 cases investigated to date, an accuracy of 100 per cent. has been recorded.

We are indebted to the Research Grant Board (Carnegie Fund) for defraying the expenses of this investigation. We thank Mr. D. G. Duncan for technical assistance, and Mr. B. McManus for the photographs.

We wish to thank those medical practitioners who so kindly supplied us with urine samples for investigation.

In collaboration with Dr. A. I. Goldberg, the test is being applied to the investigation of cases of endocrine anomalies.

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The Zeiss "Pijper Blood-cell Tester."

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In my work on diffraction I have been assisted by a grant from the Research Grant Board of the Union of South Africa.

Purpose.

This apparatus has been built quite recently by the firm of Carl Zeiss, Jena. As its name ("blood-cell tester") implies, its purpose is the investigation of certain qualities of red-blood cells, both human and animal. These qualities are: the mean diameter, the variation in diameter (in other words, the anisocytosis), which is a feature of every blood, and the presence of poikilocytosis (variation in shape).

The apparatus is intended for:

(1) Diagnosis and differential diagnosis of anæmias.

(2) Following the course of anæmias, especially judging the efficacy of treatment.

History.

The apparatus applies the technique of diffraction-measurement of red-blood cells, as described by me in 1929.[1]

The principle of diffraction-measurement is that a beam of light, sent through a grating, is diffracted, and produces a pattern of light. From this pattern conclusions can be drawn as to the dimensions of the elements constituting the grating.

In 1918 I found that cultures of bacteria provide suitable gratings.[2] In 1919 I employed films of red-blood cells as gratings, and calculated their mean diameter from the pattern produced, which consists of a series of concentric coloured circles.[3] In 1924 I applied this measuring method to the diagnosis of pernicious anæmia.[4] In 1929 I improved the method by instituting a direct comparison of normal blood with the blood under investigation, and by realizing that more could be learnt from the diffraction circles than just the mean red-cell diameter.[1]

During these years various appliances for diffraction-measurement of red cells were marketed by different firms. The improvements of 1929 are not embodied in any of them. I fear this has hindered the more general adoption of the method.

Now the co-operation of Professor Siedentopf, of the scientific staff of the firm of Zeiss, apart from fully confirming the sound optical foundation of the method, has resulted in the construction of a simple and efficient apparatus, which places the full advantages of the diffraction method at the disposal of everybody, who can get a properly-made thin blood-smear.