part of the body—that is, the lumbo-sacral region of the spine. The penalties of the upright position are many, and this is one of them.

I must not close these remarks without paying a tribute to the Boston school of orthopaedic surgeons for the work they have done on this subject. It is due to them, and chiefly to Goldthwait, that a proper system of examination has been established and that these cases, so far as the orthopaedic aspect of them is concerned, are now capable of scientific analysis, classification, and treatment.

THE XENOPUS PREGNANCY TEST

BY

EDWARD R. ELKAN, M.D.

(WITH SPECIAL PLATE)

The discovery of what is now known as the xenopus pregnancy test is based on experiments conducted by Hogben (1930, 1931), who observed that hypophysectomy produced ovarian retrogression, and the injection of anterior pituitary extracts ovulation, in the female South African clawed toad. Further experiments on these lines were carried out by Charles, Slome, and Zwarenstein in Capetown, and later by Bellerby, Zwarenstein, and Shapiro in London, and it is due to the collaboration of these authors that the initial difficulties which stood in the way of a standardization of the xenopus test were overcome. This is particularly true with regard to Bellerby's work on the laboratory conditions necessary for sustained reproductive activity, which work made it possible to improve greatly the reliability of xenopus as a test animal. While there has so far been no difficulty in obtaining regular supplies of these toads from South Africa our next aim must nevertheless be the breeding of xenopus in this country. Bles attempted this as early as 1905, and sporadic successes have been recorded by other authors (Bles, 1906); Bellerby, 1933 ; Vanderplanck, 1935 ; Elkan, 1938). It is to be expected that in the near future we shall have not only a standardized xenopus pregnancy test but also a standardized method of breeding this animal.

Weisman (1938) recently reviewed the tests for the detection of early pregnancy which have been devised since Aschheim and Zondek's discovery eleven years ago. Apart from the Aschheim-Zondek reaction only Friedman's technique has become widely popular ; the xenopus test, though in some points superior to both the Aschheim-Zondek and the Friedman techniques, has remained more or less unnoticed despite the enthusiastic reports which have appeared in various journals in recent years. These three pregnancy tests are the only ones to deserve, in Weisman's opinion, the term "excellent." In the course of the last twelve months I have done 295 xenopus tests on 2,112 toads, and I fully endorse Weisman's judgment. Some of the objections to this test raised by earlier observers seem to have become invalid, and as the early detection of pregnancy remains important from the psychological and the gynaecological points of view a method which allows the diagnosis to be made within a few hours should be welcome.

Biological Considerations

Xenopus laevis Daud., a toad of the genus Aglossa, is fairly common in all the tropical parts of Africa (Fig. 1, Special Plate). The toad is exported from Capetown, where the animal dealers seem to have no difficulty in catching as many as are required. My own experience with these exporters does not confirm fears expressed by Crew (1937) and Weisman (1938) as to the availability of the animal. So far supplies seem to be unlimited and export unrestricted, and since the animals survive the passage from Capetown to London I see no reason why they should not be shipped to any part of the country. I have at present tadpoles in my laboratory bred in an open-air tank this summer. It remains to be seen whether these tadpoles can be brought up in sufficient numbers and whether their growth is rapid enough to make the breeding of xenopus an economic proposition. My present tadpoles look very much like young fish. They stand on their heads most of the time, and feed on a mixed diet of infusoria, particularly flagellates, and raw liver emulsion, of which a little is poured into the aquarium every day.

The adult clawed toads received from Capetown vary greatly in size, the smallest ones measuring 2 inches, the largest 4½ inches, from mouth to anus. I find those of medium size most suitable for tests. The small toads are mature, but do not stand the injections so well ; the larger specimens tolerate the injections well, but they require very big jars for observation. It is easy to distinguish the males from the females. They are identical in colour, but the external opening of the cloaca in the female has three labia—two dorsal and one ventral—which do not, however, form a receptaculum for semen ; these labia are absent in the male (Fig. 2, Special Plate). Fully grown males do not reach the same size as fully grown females. The females also acquire a characteristic shape through the bulging lungs being pushed aside by the ovaries, which protrude on both sides of the abdomen like cushions.

The animals are easy to feed. They will accept daphnia, earthworms, newts, tadpoles (*Rana esculenta*), minced meat, or minced liver, but flatly refuse to touch bread or potatoes. It is a little difficult to say authoritatively how often they should be fed. They will certainly accept food every day, but they never show any signs of being particularly hungry, and if not fed for a week they do not seem to be the worse for it. If they grow at all in the laboratory they do so extremely slowly, even if they are well fed. It is wiser not to feed those specimens which are to be used for tests in a day or two. The animals have a short alimentary canal. They eat whenever they happen to come across anything edible, but do not seem to make much use of what they have eaten. The water becomes dirty from their excreta soon after they have been fed, and must then be changed. It does not seem to matter—at least so far as the tests are concerned—whether feeding takes place once, twice, or three times a week if only the animals are given as much food as they will accept at each feeding time.

During the cold season all the animals are kept at the laboratory in a specially constructed tank, and at a temperature of 23° to 26° C. In the summer only the animals actually needed for tests are kept in the laboratory. The others, particularly those who are having their "resting time," are kept in outdoor tanks, where they become comparatively tame ; when it rains they can be seen leaving the water and climbing on to stones provided for them. Hundreds of xenopus can be kept in a comparatively small space, and since the females of this species do not make any noise their presence is not a nuisance to the neighbourhood.

I cannot yet answer a question often asked by visitors: How long do these animals live, and how often can they be used for tests? I have lost a few during the winter from a disease which I cannot diagnose ; it causes paralysis of the hind legs so that the toad cannot come up to the surface to breathe, and is consequently drowned if not rescued in time. This condition affected both males, which are not used for tests, and females, but almost disappeared with the approach of the warm season. The toads frequently suffer from an infection with taenia, but I have been unable so far to ascertain if these tapeworms are the cause of these sporadic losses. Toads very rarely die in the course of a test ; some of my earliest arrivals must have been used three or four times without showing any signs of weakness. I keep the toads mixed, so that arrivals of all ages are used in each test.

A statement made by Zwarenstein and Shapiro (1934) that toads which have been kept under laboratory conditions undergo a process of "desensitization" and can

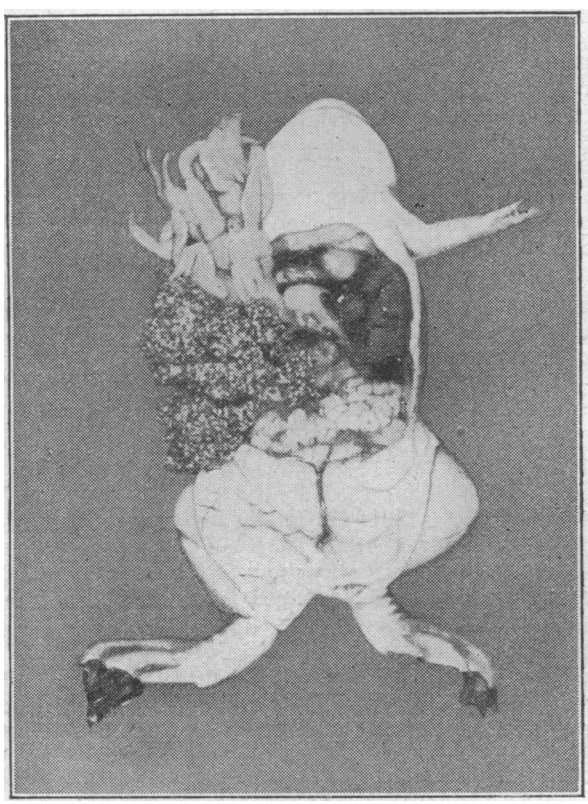

FIG. A.—Dissection of a female *Xenopus laevis* which was kept in captivity for over six months and then killed after having given a positive pregnancy test reaction. Note the well-developed ovaries and oviducts and the absence of any signs of degeneration of the reproductive system.

no longer be relied on after three to four weeks has since been repeated by Crew (1937) and Weisman (1938), and is one of the reasons why workers in this field have been slow in taking up this otherwise useful and reliable test. I cannot endorse this statement from my own experience. I have received consignments of toads from South Africa with the animals' ovaries in a state of quiescence. This may be identical with the state of "desensitization," but after these animals had been well kept and fed for three to four weeks they gave reliable results. Others gave reliable results from the day of arrival. A study of Bellerby's papers (1929, 1933, 1938) on the biology of these toads may explain this puzzling behaviour. In my experience no desensitization takes place in these animals if properly kept, and I see no reason why toads should not be used again and again so long as they are allowed proper resting periods between tests. (See Fig. A.)

The Pregnancy Test

The xenopus test, like all other biological pregnancy tests, depends on the response of the animal's gonads to anterior-pituitary-like hormone present in the urine of pregnant women. I am not quite sure as to the correctness of the term "anterior-pituitary-like," because I have tried commercial pituitary extracts in considerable doses without obtaining a response in these toads. Further experimental work will be necessary to show if the toads react in the same way to anterior pituitary extracts, extracts from serum, and extracts made from urine. The mature female ovaries contain hundreds of eggs in various stages of development. Under the hormonal stimulus numerous eggs enter the oviducts (ovulation) and are discharged from the cloaca (oviposition). Under normal conditions these eggs, which are covered with a sticky gelatinous mass, are one by one fertilized by the male as they leave the cloaca and are then stuck on to water plants by the female. In the test the eggs fall through a platform to the bottom of the test jar, where they can easily be seen with the naked eye (Special Plate, Fig. 3).

The test proper starts with the collection of the urine. It seems wise to limit the patient's intake of fluids so far as possible on the day before collecting the specimen. She thus concentrates her own urine and thus increases the reliability of the test ; no drugs should be administered during this period. Some 6 oz. of morning urine are collected in a clean—not necessarily sterile—bottle and sent to the laboratory. Here the investigator can easily determine whether his directions have been followed or not. Not infrequently the patients drink quantities of Vichy water or tea to be sure that they produce the necessary amount of urine in the morning. An estimation of the specific gravity allows these valueless specimens to be discarded. Personally, I do not expect reliable results from urines with a specific gravity below 1015 ; figures from 1020 to 1030 are desirable. Slight turbidity is not important, but if the urine is very turbid it should be filtered.

For the test itself untreated urine or an extract made by Zondek's alcohol and acetone precipitation method may be used. Bellerby (1933) thinks that the process of ovulation follows quantitative rules and does not depend on a "trigger action." I am not convinced that this can be accepted as a general rule, since I see every day that different toads injected with exactly the same dose of extract lay very different numbers of eggs. Crew (1936) uses extracts only and does not regard as reliable tests with untreated urine. I, too, have seen negative results from untreated urine and positive results on using extracts of the same urines, but in other cases both methods have given positive results, and I could find no constant relation between these latter results and the stages of the respective pregnancies. More experimental work is needed to clarify this point. The use of untreated urine would seem more economical, and in addition we do not know how much hormone becomes denatured in the course of the precipitation and concentration. However, since Zondek's method affords a certain means of concentration, and since we want to make the test as reliable as possible, this method seems at present the better one. This does not mean that tests with untreated urine cannot be perfectly reliable, and some laboratory workers might find it best

to test untreated urine first in all cases, and to test a second time with the extract only in those cases in which untreated urine gave a negative finding.

Technique of Testing

For the test 2 c.cm. of untreated urine or 1 c.cm. of extract is injected into the lymph sac under the dorsal skin of as many female toads as one cares to use. Some observers inject into the leg or into the peritoneal cavity. The toads are slippery and difficult to hold; I find that the easiest way of dealing with them is to hold them in a coarse meshed net and to inject through the meshes. There is no immediate reaction to ordinary urine. If extract is used which still contains a trace of alcohol or acetone the toads react by secreting mucus from the skin glands in the neighbourhood of the injection. The question of the "toxicity" of the urine seems to arise very rarely. Specimens which had been in the post for several days have been tested, and while I am not sure that the hormone content remains constant in such specimens they seem to do no harm to the animals.

After the injection has been given the toads are put into test jars (Special Plate, Fig. 3), where they sit on perforated platforms, so that they have no chance of eating their own spawn. This may be an unnecessary precaution. I have left toads in their jars for days and have never seen them eat their spawn; in fact, as a reaction to the close confinement, which they seem to dislike, they vomit up everything they have eaten shortly before the test. It is for this reason that Bellerby (1929) prefers to leave unfed for a full week those toads he is going to use for tests. I have a strong suspicion, however, that the xenopus when at liberty is cannibalistic. A male and a female left in a breeding tank for a few weeks this summer produced only three tadpoles; since it is unlikely that the female should have laid only three eggs my suspicion is, I think, justified.

During the test the jars are kept at a temperature of 26° C. The shortest time I have so far observed between injection and oviposition was four hours and fifty minutes, the longest twelve hours. Figures midway between these two extremes are more usual. I have no definite proof as to whether the concentration of the hormone in the extract or the temperature at which the animals are kept during the test has anything to do with the reaction time, since of a group of toads used for one test no two ever start laying eggs at the same time. While the hormone concentration and the temperature are certainly of some importance in this respect, the animals themselves may provide a third factor which we cannot at present determine.

The eggs—little round balls, half black, half white, and of about 1 mm. in diameter—are covered with a sticky gelatinous substance. Normally these animals do not lay their eggs in bulk but distribute them over a wide area, sticking them on to water weeds one by one. In the test jar the eggs either stick to the underside of the platform or fall to the bottom of the jar. Their number varies enormously. Anything from five to six eggs upwards can be counted as a positive reaction. Tests in which only one or two eggs have been laid by one or two toads should be repeated. They will usually be found to be negative. In the absence of males these eggs are, of course, unfertilized. It should be emphasized that spontaneous ovulation in this species does not take place in captivity or under laboratory conditions. Females, even if kept under the best possible conditions, will never ovulate except in the presence of a male. It is difficult enough to obtain fertilized eggs even if the males and females are kept in a special aquarium. After many unsuccessful attempts, I have recently succeeded in obtaining a batch of about 200 tadpoles, but I had to inject the female with pregnancy urine extract to induce ovulation.

If nothing is known about the stage of the presumed pregnancy or if the urine comes from a patient who has not yet missed her period an extract is made by Zondek's method. The technique is as follows:

METHOD OF EXTRACTION

Sixty c.cm. of filtered urine are acidulated with acetic acid and mixed in a separating funnel with enough alcohol and/or acetone to bring down a precipitate. The quantity of alcohol or acetone necessary varies from 150 to 300 c.cm. It seems that in some cases an excess of alcohol redissolves the precipitate. The alcohol should therefore be added slowly and only up to the point of maximum turbidity. The mixture is then shaken vigorously for a few minutes and centrifuged. If no centrifuge of sufficient capacity is available the precipitate may be allowed to settle; it can be separated off after thirty minutes, and must then be centrifuged thoroughly to free it from the alcohol–urine mixture. It is important to get rid of as much of the alcohol as possible because it has an unfavourable effect on the toads. It makes no essential difference whether alcohol or acetone is used to bring down the precipitate. Acetone is the more expensive, but it is useful in so far as it brings down precipitates from very dilute urines and, after centrifuging, leaves a residue much "drier" than that which can be obtained by alcohol precipitation. When the precipitate has thus been separated from the alcohol–urine mixture it is stirred up and shaken vigorously with distilled water (1 c.cm. for each animal injected) and glass beads. This mixture is again thoroughly centrifuged and the supernatant fluid is used for injection. The whole procedure takes perhaps 20 minutes. As for the choice of a centrifuge I have found that the small clinic model with a 1/16-h.p. motor is not sufficiently strong. A larger model with a 1/8-h.p. motor is preferable; this should be fitted with 50-c.cm. buckets and attain a rate of at least 3,000 revolutions a minute.

In the ordinary routine work the animals are injected within an hour of the urine being received. They are left in their test jars overnight and the results are read the next morning. During the test the jars should be kept at about 26° C.; in the absence of a suitable incubator this can be done by putting the jars back into the tank. The patient knows the result of the test in less than twenty-four hours. The animals, after the result has been read, are kept in the resting tank for a week if the test was negative, for a month in the case of a positive test.

Reliability of Xenopus Test

Among the 295 tests which I have done so far and in which 2,112 frogs were used I have not seen one clear positive that did not indicate a pregnancy. There were a few negative results which when repeated after a fortnight became positive, but I do not think that these can be regarded as failures. What we test is the hormone concentration in the patient's urine. If the urine is sent in for examination at a time when no hormone or only a trace of hormone is present the test naturally appears to be negative. Nor would it seem advisable to make the test too "sharp" by using methods of extreme concentration. The normal urine of a non-gravida contains a varying amount of anterior pituitary hormone. If the test is too "sharp" it may fall within the limits of the normal hormone concentrations, which vary in different women and are not necessarily constant in any one patient. It would seem better only to test urines from

women who have gone at least two or three weeks over the first "missed" period. By that time the concentration of hormone in the urine has always risen so far above the normal that no false negatives need be feared with the ordinary extraction method.

The tests I have performed myself are still too few to allow any final conclusions being drawn. Disregarding my first ninety tests, the results of which may have been influenced by the fact that I was learning the method, I can report that of the next 150 tests sixty-two had a positive and eighty-eight a negative result. No clear positive was found to be incorrect; of weak positive results, which as experience has shown must be counted as negatives, there were ten. Of negatives that became positive at a later date there were three.

These 150 tests were done on 742 toads. Of these, 734 survived the test without apparently being the worse for it. Eight toads died in the course of tests—four during negative and four during positive tests. A number of toads which were not counted, as their deaths obviously had nothing to do with the tests, some of them being males, died during the winter. I do not think that their number exceeded thirty.

Summary

The xenopus test allows a diagnosis of early pregnancy to be made within less than twenty-four hours.

No animals need be killed to obtain the result.

The reliability of the test does not seem to differ from that of the Aschheim-Zondek or the Friedman reaction.

The technique of this test is comparatively simple and very suitable for experimental work on the anterior-pituitary-like hormone of pregnancy.

My sincere gratitude is due to Mr. D. P. Gould of Loughton and Mr. R. Milton for their interest and helpful collaboration in my experiments with xenopus.

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The Henry Saxon Snell Prize was founded to encourage improvements in the construction or adaptation of sanitary appliances, and is awarded by the council of the Royal Sanitary Institute at intervals of three years. The prize in the year 1939 will consist of fifty guineas and a silver medal, and is offered for an essay describing suggested improvements in the construction or adaptation of sanitary appliances. Particulars may be had from the Secretary, Royal Sanitary Institute, 90, Buckingham Palace Road, London, S.W.1.

USE OF THE SKIN OF THE FEMALE BREAST IN PLASTIC SURGERY

BY

J. F. S. ESSER, M.D.

(WITH SPECIAL PLATE)

As the breasts of many women and girls, particularly in countries abroad, are inclined to be too much developed, and the weight of these large glands stretches the surrounding skin, it is sometimes a great advantage to get rid of the superfluous skin, fat, and gland tissue.

Having thought this over seriously, I have for some twenty years advocated the use of this skin for various purposes to supply missing portions. The importance of this from an aesthetic point of view is generally recognized, but few people are aware of the suffering caused by the weight of these heavy glands. Even when not abnormally developed they may be so mobile as to cause serious inconvenience to their possessors, especially where sport is concerned.

Advantages of the Method

It seemed logical to me, therefore, to combine the necessity for supplying skin for surgical purposes with the advantage of reducing overdeveloped glands. I carried this idea further by using not only superabundant skin but even skin of normal breasts which could be taken away without causing any damage. One of the greatest advantages of the method is that the glands are often very mobile and the pedicled flaps taken from them can be easily brought to and be used in remote parts. In several instances I employed the skin for covering amputated stumps of the legs, even beneath the knee, as the legs and breasts can be brought into apposition, and also for plastic surgery of the hand and the arm. On one occasion I made practical use of it on a girl who was a pianist and who was unable to pursue her career because of the scar tissue occupying the whole surface of the inner part of the lower arm, the result of serious inflammation with suppuration and necrosis. After removing all the scar tissue I succeeded in making the articulations and muscles mobile, and in covering the gap completely with a large pedicled flap taken from the breast. In her case the skin of the breast was not sufficient to enable me to close the secondary gap on the gland with the remaining skin, so I had to fill in the gap with the help of an epithelial inlay. Epithelial inlay is a method I introduced in plastic surgery, and is so widely used that I need not here enter into all the details concerning it. I will only say that it consists in taking a mould, with Stent's composition, of wounds which have to be covered by free skin grafts. This mould is wrapped in a Thiersch graft and replaced on the wound exactly as it was when taken, and is kept pressed on it for a week.

The flap on the arm healed completely, and the layer of fat in the flap helped to keep the muscles and tendons of the arm mobile: these had lost their surrounding protective tissue and would have formed scars with the skin flap if the layer of fat had not separated them. The patient was able afterwards to continue her career successfully, and the breast had not suffered. The following are other cases in which the method was employed.

JOSEPH S. BARR: INTERVERTEBRAL DISK LESIONS AS CAUSE OF SCIATICA

(For Legends *see* Text)

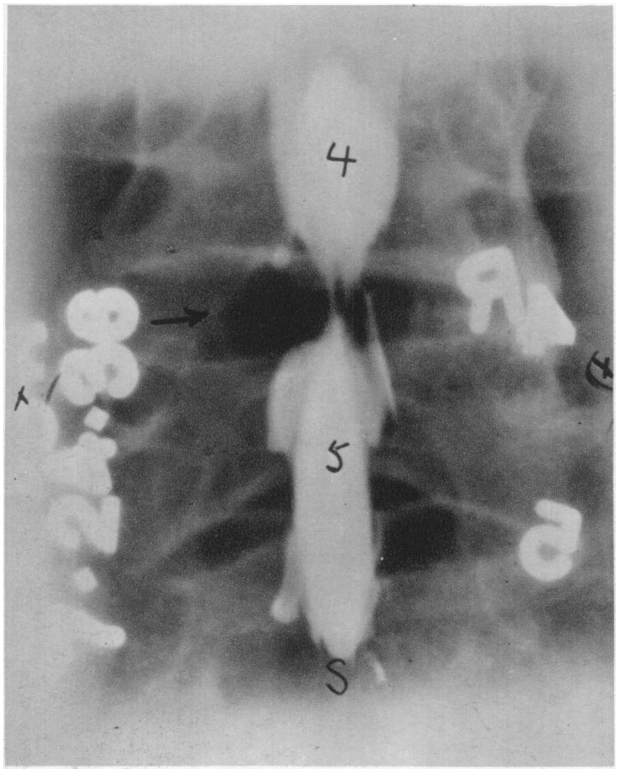

FIG. 6

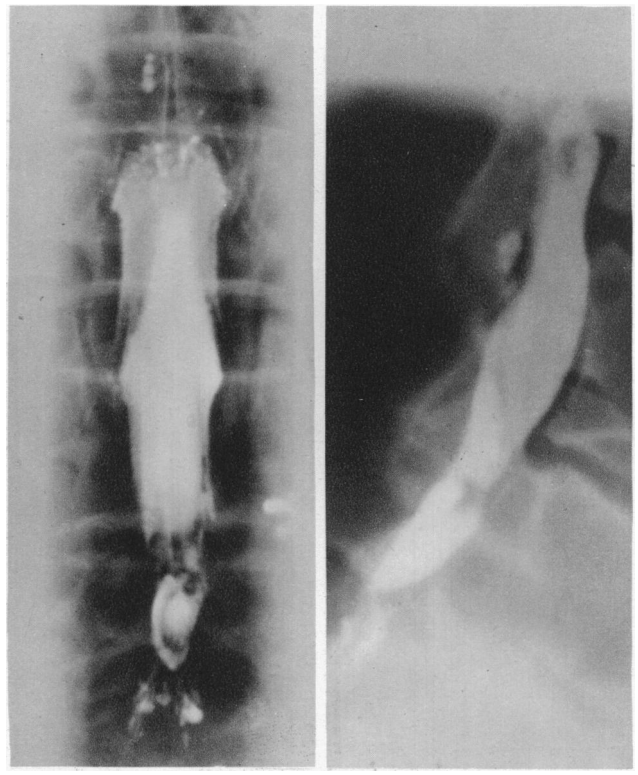

FIG. 7

EDWARD R. ELKAN: THE XENOPUS PREGNANCY TEST

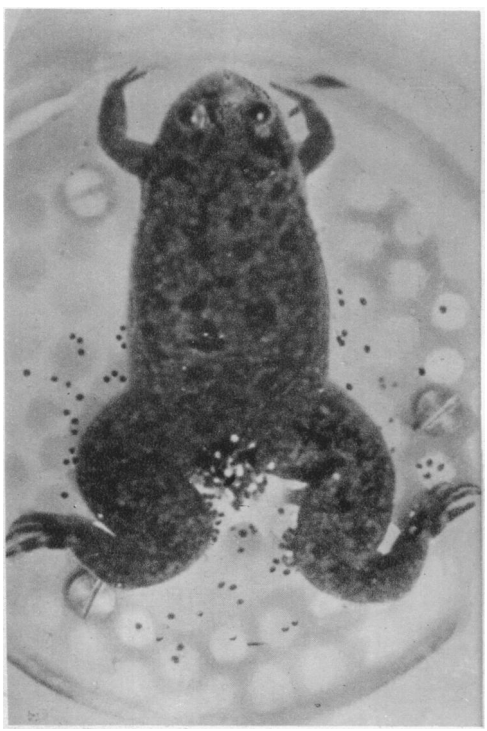

FIG. 1.—*Xenopus laevis* Daud. Female ovulating after injection with pregnancy urine.

FIG. 2A.—*Xenopus laevis* Daud, female. Note valves on both sides of external opening of cloaca.

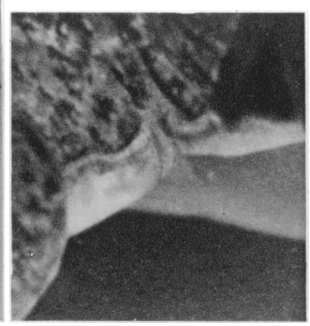

FIG. 2B.—*Xenopus laevis* Daud, male. Note absence of valves at external opening of cloaca.

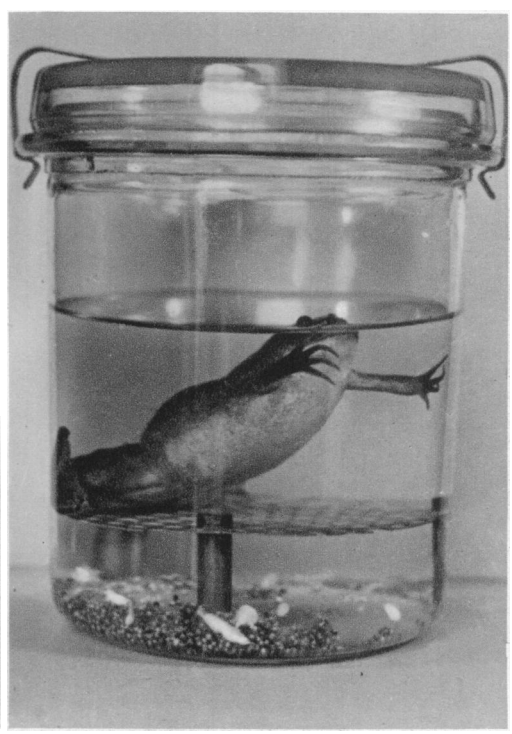

FIG. 3.—Test jar for xenopus pregnancy test. The centre of lid is perforated. The toad sits on a platform: the eggs fall to bottom of jar. Note typical attitude, holding nostrils above surface of water.