STUDIES IN THE REPRODUCTIVE PHYSIOLOGY OF THE AMPHIBIA.

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INTRODUCTION.

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The Reproduction Of Xenopus Laevis.

Scientific interest in the reproduction of Xenopus laevis was aroused over 70 years ago. Gray (1864) described various stages in the development of the Xenopus tadpole. These studies were extended by Leslie (1890) and Bles (1904).

Another early observation concerning this amphibian was the cyclical appearance of nuptial excrescences on the forelimbs of the male (Leslie, 1890). These were seen to make their appearance just before the breeding season.

The nuptial excrescences were described again by Bles (1901, 1904). In this case they were observed during attempts to breed Xenopus laevis experimentally by simulating natural weather conditions.

The reproduction of Xenopus laevis was investigated later by Hogben (1930), who showed that injections of ox anterior pituitary extracts were able to produce ovulation.

More recent workers have added much to the knowledge of reproduction in Xenopus laevis. In 1934 Shapiro and Shapiro described the seasonal cycle in the ovaries. This work included histological studies of the changes occurring in the ovaries of Xenopus. The same workers postulated on experimental evidence that the cycle was under the control of the anterior pituitary. Subsequent investigations confirmed the important role of the

anterior pituitary in the sexual cycle of Xenopus laevis, although some doubt exists as to the exact mechanism at work. [Alexander and Bellerby (1935, 1938); Bellerby (1939); Bellerby and Hogben (1938).]

The readiness with which Xenopus responds to pregnancy urine and other gonadotrophic extracts was employed by Shapiro and Zwarenstein, (1933 a, 1934, 1935) in devising a new technique for the diagnosis of pregnancy. This test has the advantage of rapidity in obtaining results, and ease and low cost in carrying out the test. The degree of accuracy is superior to that of other tests for pregnancy. Modifications in the execution of the test have been described by Bellerby (1934) and Zwarenstein (1936,b).

The next advance in the knowledge of Xenopus reproduction was the experimental induction of coupling in Xenopus laevis (Shapiro 1935, 1936.a, 1936.b). This was accomplished by injections of gonadotrophic extracts prepared from both sheep pituitaries and from human pregnancy urine. Later investigations by the same worker (Shapiro 1937 a) showed that this reaction depended on the presence of mature gonads in the experimental animals.

The most striking results in the field of Xenopus reproduction were obtained by Shapiro (1936 c), Zwarenstein (1936 a), and Shapiro and Zwarenstein (1937). They showed that steroids identical with those found in the ovaries, corpora lutea and testes

of mammals were capable of producing ovulation in Xenopus laevis.

During the course of the work described above it was noted that when Xenopus females were activated by simulating natural weather conditions or by gonadotrophic preparations, the cloacal labia became swollen and hyperaemic (Bles 1901, 1904, Shapiro and Zwarenstein 1935, Shapiro 1936). Subsequent work showed that this hyperaemia is a secondary sex character of the female Xenopus, dependent on a secretion from the ovary for its appearance. Using this as a test object, Shapiro (1936 c) demonstrated that the action of the mammalian sex hormones was a gonadotrophic one in Xenopus laevis and that these steroids were inactive as regards oestrogenic activity in Xenopus. In a similar way Shapiro (1936 c) demonstrated the ovulation-producing property of many steroids closely related chemically to the mammalian sex hormones.

The cyclical sexual physiology of Xenopus laevis has been demonstrated both by observation and experiment. Leslie (1890), Beddard (1894), Rose (1926) and Shapiro (1936 a, 1936 b) noted that mating in Xenopus laevis is seen only during the latter half of winter and early spring, approximately July to September. Experimentally, Shapiro (1936 a) showed that Xenopus laevis is more sensitive to gonadotrophic extracts of sheep anterior pituitaries during the winter months than during the summer months. The amount of extract required to produce coupling demonstrated this fact in a striking manner.

Subsequent work by Shapiro (1938) using methyl testosterone and Zwarenstein (1938) using progesterone, both employing ovulation as an index of gonadotrophic activity, confirmed the seasonal changes in sensitivity described above.

More recently, Gitlin (1939) described a cycle in the oviduct of Xenopus laevis, which he demonstrated to be under the control of an anterior pituitary - ovary hormonal mechanism.

The present study was undertaken to investigate various aspects of the cyclical reproductive physiology of Xenopus laevis. The secondary sex characters of the male Xenopus are investigated experimentally for the first time. The seasonal cycle in the nuptial excrescences of the male is described in detail together with experiments designed to elucidate the nature of the control of these cycles. A difference in the sizes of the forelimbs of Xenopus males and females is described for the first time and the presence of a cycle in the size of the forelimbs of the male is established. The control of this cycle is also investigated experimentally.

The second portion of this work consists of an investigation into the secondary sex characters of the female Xenopus. As discussed above these are seen to occur cyclically when the female is sexually active.

The third portion deals with coupling, which also occurs cyclically under natural conditions. Studies in the experimental induction of coupling have been advanced.

Arising out of these studies on the cyclical sexual activity

of Xenopus laevis are certain problems of importance in the understanding of Xenopus reproduction. The nature of the Xenopus ovarian and testicular hormones has been investigated biologically, an analysis of the mating reflex has been attempted and the paths taken by the male and female gametes from the gonads to the exterior has been described.

The last problem discussed is the role played by various factors in the production of the cyclical reproductive physiology.

The relevant literature is dealt with more fully in the appropriate sections.

Summary.

1). A brief account is given of the development of the knowledge of the reproductive physiology of Xenopus laevis.

2). The scope of the present work is outlined.

MATERIALS AND METHODS.

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I. Collection Of Pairs Known To Couple.

In various parts of this work the following phrase will be encountered - "Pairs of frogs which coupled previously". To save repetition, this will be explained here.

Large numbers of male and female frogs are collected from the ponds and allowed to stay in the laboratory for at least one day before being used. These frogs are injected intraperitoneally with 0.5 ml. of an extract of sheep anterior pituitary, 1 ml. of which is equivalent to approximately 700 mgs. of fresh anterior lobe. They are then placed in smaller containers, 2, 3 or 4 males and females per container, as described below. Injections are made usually between 5.0 p.m. - 7.0 p.m.

At 8.30 a.m. - 9.0 a.m. the following morning the first observations are made. Coupling pairs are gently removed and placed in separate containers, one pair in its own container. These containers are numbered and in further experiments each pair is confined to its own container. Observations are made at regular intervals throughout the day and on the following days.

Later on in the experiments, on the basis of the findings described in the section headed "Permutations", coupling pairs were transferred to a large bath of water and thus a stock was collected of males and females which were known to respond to a certain quantity of sheep anterior pituitary extract by exhibiting the mating reflex. These frogs were used in further

experiments.

Coupling usually ceases at the end of the first day after injection, i.e. 24 - 36 hours after injection. On the second day after injection, new couples are very rarely found, but pairs which coupled on the previous day may not infrequently still couple. Coupling has not been seen on the third day without reinjection.

Thus frogs that couple are kept till at least the 4th day after injection, before being used in further experiments, in which it is necessary to eliminate the effects of previous injections. The frogs are not fed during this time.

II. Containers.

Glass containers are used for the experiments on coupling. These are round, rectangular, or oblong with rounded ends. They are about 4 - 5 inches deep. Glass slabs are used as covers and these are placed so that there is a small opening to allow air to enter the container, but not large enough for the frogs to escape. Weights are placed on the covers to prevent them from slipping and thus enlarging the opening. They are filled with tap water to a depth of 1 1/2 inches. The water is changed daily.

The round containers vary in diameter and hold a corresponding number of frogs.

There are 12 & 10 inch containers holding 4 prs. of frogs each.
" " 9 & 9 1/2 " " " 3 " " " "
" " 7 & 8 " " " 2 " " " "

The rectangular containers measure 11 1/4 inches x 5 1/2 inches and hold 3 pairs of frogs each.

The oblong containers with rounded ends measure 15 1/2 inches x 8 1/2 inches and hold 4 pairs of frogs each.

The same containers were used for isolating coupling pairs.

III. Operations.

1. Castration was performed according to the method of Shapiro & Zwarenstein (1933 b).

The animals were anaesthetised with a mixture of ether and water, 1 : 5. The ventral skin was divided by a one inch median incision and the ventral abdominal muscles were divided by a paramedian incision half an inch long. This was done in order to avoid the ventral abdominal vein which runs in the midline closely applied to the inner surface of the ventral abdominal wall. The incision in the muscle is held open with a small retractor.

The fat body of one side is exposed and drawn out of the incision. The testis, to the upper pole of which the fat body is attached, is grasped with a forceps and delivered through the wound so that its mesentery is exposed. This is cut through with an electric cautery or a needle heated in a flame, care being taken to avoid the blood vessels supplying the fat bodies as these run close to the upper pole of the testis.

The intestine is drawn aside and the second testis is exposed and removed in the same way.

The muscle is closed with a continuous cotton suture, care being taken to avoid the ventral abdominal vein. The skin is closed by interrupted cotton sutures.

Ovariectomy is performed along similar lines, except that it is found to be more convenient to grasp the ovary with the hand after it has been delivered through the wound.

The frogs are washed and placed in shallow water until they have recovered and then are transferred to deep water. This procedure was found to decrease the postoperative mortality rate and to assist healing of the wound.

The animals stand the operation well, especially the females. The post-operative mortality rate is low.

2. Hypophysectomy. (Fig. 1).

The procedure is a modification of the method of Hogben (1923) and is identical for males and for females.

The animals are lightly anaesthetised as before and placed on their backs. The mouth is held open by a forceps, one blade of which is narrow and is held in one internal naris. The other blade is broad and is held against the roof of the mouth, behind the opening of the eustachian tube.

A small hole is drilled in the roof of the mouth with a dental burr, through the mucous membrane and bone just in front of the opening of the eustachian tube. The drilling is continued until the anterior lobe of the pituitary can be seen shining through the remaining thin plate of cartilage. This is reflected with a thin pointed knife and the anterior lobe is removed by gentle suction through a glass tube of 1.5 mm. bore attached to a water suction pump. If suction is too vigorous, the posterior lobe is removed as well.

The animals are allowed to recover in shallow water and are transferred to white porcelain sinks with a running water supply. The removal of this anterior or of both lobes of the pituitary can

HYPOPHYSECTOMY.

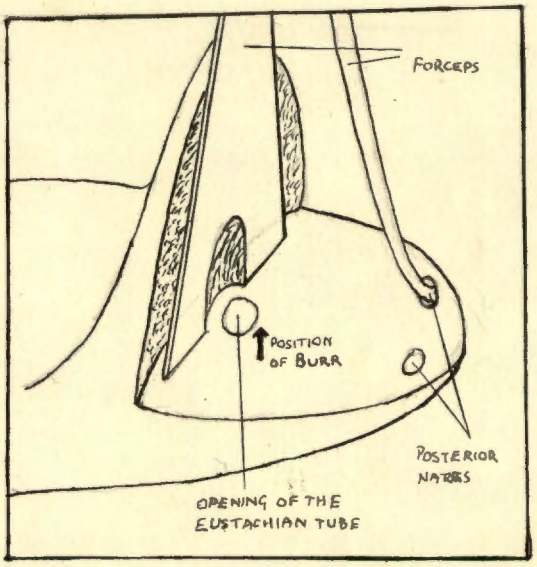

Fig: 1.

be recognised by the post-operative colour changes described by Hogben & Slome (1931).

IV. Extracts.

1. Preparation Of Human Pregnancy Urine Extracts.

Extracts of the gonadotrophic principles of pregnancy urine were made according to the method of Katzman & Doisy (1932).

1 1/2 - 2 litres of urine from an early pregnancy is used. Pregnancy in the donor was usually diagnosed by the Shapiro-Zwarenstein test (1933 a).

The urine is acidified by the addition of a few drops of glacial acetic acid to pH 4.0 - 5.0. Methyl red is used as indicator.

The acidified urine is filtered through a Buchner suction filter and 50 ccs. of acetone saturated with benzoic acid (B.A.A.) per litre of urine is added as follows. The urine is transferred to a tall cylinder and stirred rapidly with a long glass rod. The B.A.A. is added slowly, a little at a time. The urine is allowed to stand overnight in a refrigerator at about 4°C.

When the B.A.A. is added to the urine, the benzoic acid is precipitated and adsorbs on to itself various substances from the urine, including the gonadotrophic factors.

The next morning the urine is filtered through a Buchner filter. The benzoic acid precipitate is collected and dissolved in an amount of acetone equal to the volume of B.A.A. originally added to the urine. The filtrate is retained. The solution is well stirred to dissolve all the benzoic acid and then is

centrifuged. The supernatant acetone solution is retained. The deposit is washed with acetone and recentrifuged. Then it is dried by means of a current of air, or is spread out and allowed to stand until no acetone can be detected by smell. The deposit is often powdery, but occasionally is very viscid and sticky. The latter type of deposit is easily dried in a current of air, but the powdery form is easily lost. Then the best method is to have the powder at room temperature until the acetone has evaporated.

The deposit may be collected and stored as a powder in a refrigerator. Potency is retained longer than in a fluid extract.

Solutions can be made up immediately or afterwards from the powder.

The dry deposit is weighed and distilled water is added to it, 25 mls. of water per litre of original urine. It was found that adding the requisite amount of distilled water in three portions increased the amount of substance going into solution. The deposit is broken up into fine particles in the water and then the solution is centrifuged and the supernatant fluid is collected and measured. The water is removed from the insoluble residue of the deposit by the additon of alcohol and centrifuging and then the alcohol is removed by ether. The ether is allowed to evaporate off and the deposit is weighed again. In this way

the amount of deposit that went into solution is determined and the amount of deposit or powder per ml. of the extract can be estimated.

The acetone in which the benzoic acid precipitate was dissolved is resaturated and added to the urine from which the benzoic acid precipitate has been filtered. A second extraction of the urine proceeds along the lines laid down above. It is found that the second extraction often yields an extract as potent as or sometimes more potent than the first extraction.

2. Preparation Of Acid Extracts Of Sheep Anterior Pituitary.

Acid extracts of sheep anterior pituitaries are prepared according to the method of Bellerby (1933).

Sheep heads are obtained from the Maitland abattoirs which are situated half-an-hour by motor car from the laboratories. Anything from 1 - 11 1/2 dozen heads are collected as the animals are slaughtered.

Immediately upon arrival at the laboratories the heads are sawed open, the brain is moved aside and the pituitaries are removed together with and enclosed in the dura mater lining the sella turcica, and the diaphragma sellae. The pituitaries are immediately placed in a clean dry dish which is surrounded by ice.

The pituitaries are carefully shelled out of their dural investment and the posterior lobes and the infundibula are removed, care being taken to leave only anterior lobes.

These anterior lobes are now dried and as much blood as possible is removed from their surfaces. They are weighed, cut up into fine pieces and transferred to a mortar. An equal volume of clean, dry, coarse sand is added. The pituitaries are ground up until the sand is a fine powder and no large pieces of anterior lobe tissue are recognisable.

The mash is transferred to a small beaker and 1% acetic acid is added, 1.5 mls. for each gramme of anterior lobe. The mixture is stirred well and left overnight in a cool dark place,

with the mouth of the beaker covered by a small glass slab.

The following morning the mixture is centrifuged and the supernatant fluid is collected. It is found that stirring of the deposit in the tube and recentrifuging yields a small additional quantity of extract.

The extract is made neutral to litmus by the addition, drop by drop, of a 40% solution of NaOH. At the neutral point a fine flocculation occurs. This is removed by centrifuging the extract. The volume of the supernatant fluid is measured and is used for injection.

The extract is kept in small Erlenmeyer flasks in a refrigerator. The main body of the extract is kept in the ice chamber of the refrigerator where it freezes. Smaller portions required for immediate use are kept liquid at a temperature of about 4°C.

These extracts are designated in this work by the letter "S", e.g. S. 3., S. 18. The strengths of the extracts are given in protocol I.

3. Preparation Of Pyridine Extracts Of Sheep Anterior Pituitary (Rowlands 1937).

The anterior lobes of sheep pituitaries are collected according to the method described above. They are dessicated in acetone. Three changes of acetone are used, and the anterior lobes are kept for two to three days in each change of acetone in a refrigerator at 4°C. The acetone is then poured off and the

anterior lobes are dried in a current of air and ground up into a fine powder.

The powder is extracted with 50% solution of pyridine in distilled water for two hours. 250 mls. of the pyridine is used per 10 grammes of powder.

The extract is centrifuged and the supernatent fluid is collected. This is precipitated with four volumes of 96% alcohol and recentrifuged. The precipitate is collected and dried in a current of air until no pyridine is left and then ground up.

The powder is dissolved in the required amount of distilled water which has been slightly alkalinised by the addition of a few drops of 40% NaOH. The extract is neutralised with concentrated HCl and stored in the upper compartment of a refrigerator so that it freezes.

Each 1.0 ml. of the extract was equivalent to 534 mgs. of fresh anterior lobe tissue.

4. Preparation Of Ox Anterior Pituitary Extracts.

Ox heads were also obtained from the Maitland abattoirs. In the case of these, however, the heads were sawn open and the pituitaries were removed at the abattoirs, immediately after the ox was killed. The pituitaries were transported on ice to the laboratories.

The shelling out of the anterior lobes and the extraction with 1% acetic acid is identical with the method of preparing an

extract of sheep anterior pituitaries described above.

Three extracts were made, called 0.1., 0.2., and 0.3. These were small in amount and were used almost immediately after preparation. Therefore they were not frozen. Their strengths are given in protocol 1.

5. Xenopus Pituitaries Preparations.

Method Of Collecting Pituitaries.

1. When Xenopus pituitaries were required the animals were hypophysectomised by the method described above. The hypophysectomised frogs were then retained for further experiments.

A frog is anaesthetised by immersion in a mixture of ether and water. It is placed on its back and its mouth held open by a specially designed forceps. A hole is drilled in the skull by means of a small burr, in the middle line, just in front of the opening of Eustachian tube. When the hole is sufficiently deep the anterior lobe of the pituitary can be seen shining through the remaining thin cartilaginous base of the skull. This layer is carefully reflected by means of a sharp - pointed knife and the pituitary is thus clearly exposed.

A narrow glass tube is used to collect the pituitary. This tube has its one end drawn out to a fine tip of sufficient diameter to take the Xenopus pituitary, i.e. the bore of the tube at this end is 1.5 mms. About one inch from the pointed

end of the tube is a small bulb, 2.0 inches long and 0.5 inches in diameter. The other end of the tube is connected to a water suction pump.

The anterior lobe of the pituitary is now removed by gentle suction through the glass tube described above. Gentle suction results in only the anterior lobe coming away.

The anterior pituitary is trapped by the bulb on the glass tube, from which it is easily washed out by disconnecting the glass tube and sucking up water by mouth. The pituitary is carried out as the water is allowed to drain on to a piece of filter paper lying on a porcelain slab. Thus the pituitary is collected on filter paper. The moistness of the latter prevents the pituitary from sticking to the paper. If the whole pituitary comes away the anterior lobe is separated off easily with the point of a fine forceps. The pituitaries are used almost immediately so that very little loss of active principle by solution in the water is likely to occur.

2. A more rapid method of collecting pituitaries is used when the hypophysectomised animal is not required. A frog is pithed from the level of the cervical vertebrae downwards and then the head is cut off with a scissors, one blade of which is held in the mouth of the animal. The severed head is held upside down and steadied by means of a lung forceps clamped across the nose. With a sharp pointed scissors the cranial cavity is opened by two incisions, one on each side, through the base of the skull, starting at the severed end of the spinal column and ending in front of and

lateral to the opening of the Eustachian tube. The base of the skull is reflected forwards and the pituitary is exposed. The anterior lobe can now easily be lifted off by means of a fine forceps.

The pituitaries are kept on moistened filter paper for a few minutes until used.

Method Of Implantation Of Anterior Lobes.

The skin on the dorsum of the experimental frog is held up by a toothed forceps and a small 3 mm. incision is made. The anterior lobes are carefully lifted up from the moistened filter paper by means of a fine forceps and placed in the dorsal lymph sac of the frog through this incision, as far from the latter as possible.

The incision is closed by a single cotton suture.

In the female, the incision is made as far anteriorly as possible so that the ligature will not interfere with the application of the male to the dorsum of the female in any coupling that may take place.

In the male this precaution is not necessary and the incision is made lower down on the dorsum of the frog where the skin is looser and the implantation is thus easier.

6. Dogfish Pituitaries Preparations.

Dogfish are obtainable in Table Bay, Cape Town, in large quantities. A trip was made into the bay. The dogfish were caught by hooks baited with mackerel and killed as soon as they

were taken out of the water by a blow on the back of the cranium.

The head is removed immediately and the base of the skull is reflected. The pituitary is located easily and removed by cutting through its attachments to the base of the brain.

The pituitaries are transferred immediately to a tube surrounded by ice in a thermos flask, where they are kept until used about three hours later.

The dogfish pituitaries were implanted under the dorsal skin in same manner as described above in the case of the Xenopus pituitaries.

Summary.

1). The method of collecting "pairs of frogs known to couple" is described.

2). The containers used in the experiments on coupling are described.

3). The operations of castration and hypophysectomy are described.

4). Methods are described for preparing extracts of the following substances:-

Human pregnancy urine.

Anterior pituitaries of sheep, ox, Xenopus laevis and dogfish.

THE SECONDARY SEX CHARACTERS OF THE MALE XENOPUS.

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I THE PADS.

Introduction.

The development of nuptial excrescences on the forelimbs of male Xenopus in the breeding season has been noted by Leslie (1890), and Bles (1901 and 1904). According to Leslie (1890) the excrescences cover the palmar aspect of the fingers and hands and the ventral aspect of the arms as far as the elbows. Bles (1901) considered that the excrescences developed on the dorsum of the fingers and hands and extended up the ventral aspect of the forelimbs as far as the axillae. He also described the excrescences as consisting of closely set spikes.

Nuptial pads consisting of black epidermal spines covering a swelling produced by acinous glands with granular cytoplasm occur in the prepollex region of each hand of many frogs and toads (Noble 1931). Pigmented spines also occur on the ventral surfaces of breeding males in some discoglossids, pipids and ranids and in a few discoglossids, pelobatids, bufonids and ranids these spikes extend to the chest and chin (Noble 1931). Pope (1931) has shown that some species of Rana, breeding in mountain torrents in China, have spines developed on their chest to assist the male to maintain his position on the back of the female. The situation of the spikes in some species, however, is not

always correlated with this type of advantage in mating and the spikes develop in almost any part of the body.

These spikes assist in sex recognition in some species where the latter occurs by trial embrace, as in Rana Sylvatica (Noble and Farris, 1929).

Studies in Rana have shown that the thumb pads described above grow as a result of testicular activity. The role of the pituitary in this reaction has not been investigated. Also, nuptial pads have been made to develop in castrated female toads by testicular transplantation (Ponse 1923; Welti 1925). The presence of nuptial pads thus appears to depend on the action of a testicular hormone.

In the present work the nuptial excrescences of male Xenopus have been studied anatomically as well as histologically. The seasonal cycle in their appearance and the factors controlling this cycle have been studied. The question of the identity of the amphibian sex hormones is discussed.

For the sake of convenience the nuptial excrescences are referred to here as "pads".

SEASONAL CYCLE IN THE PADS OF XENOPUS LAEVIS UNDER NATURAL CONDITIONS.

Males.

All the freshly-caught male Xenopus brought into the laboratory between September, 1937, and September, 1938, were examined for the presence of pads. From the beginning of 1938 up to and including May no pads were seen. The first month in 1938 in which fully-developed pads were seen was June. By the first week in July all animals showed fully-developed pads. In September pads were still seen in all the males, but in November the pads had disappeared for the most part. By January the pads were absent.

Thus there are four phases in the seasonal cycle in the presence of pads.

I. January to May. No pads are present during this phase.

II. May to July. During these two months the pads become fully-developed. This phase immediately precedes the onset of the breeding season.

III. July to September. This is the breeding season during which the pads are maintained at their full development.

IV. September to January. The pads gradually disappear from the forearms of the males.

Females.

The forelimbs of female Xenopus were examined at all times of the year. No pads were ever seen on the forelimbs of the

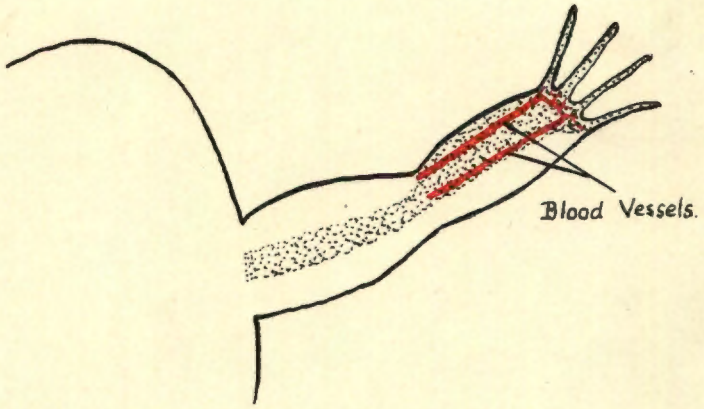
female frogs.

A fuller consideration of these observations is given below.

Appearance of the Forelimbs of Male Xenopus during the Cycle.

I. January to May. No pads are seen on the fore-arms of Xenopus males. The area on which the pads later develop is recognisable, especially on the ventral surface of the forearm.(Fig.2). Rather more than half of the radial side of ventral surface of the forearm has an opaque appearance, contrasting markedly with the smooth pale colour of the remaining ulnar side of the ventral surface of the forearm. This patch extends on to the palmar aspect of the hand and all four fingers. Proximally, it is not always seen on the arm. When it is present it is a narrow strip comprising between a quarter and a third of the ventral surface of the arm, running down the centre of this surface from the junction of the arm and the trunk to the elbow, where it joins the patch on the forearm. Two thin blood vessels can be seen on the forearm. One runs under the pad area and the other runs at the junction of the pad area with the rest of the ventral skin of the forearm. These vessels are rather blue in appearance and end by supplying the fingers. This patch, in addition, feels rough to the touch. It lacks the slimy feel of the skin of the rest of the body and remaining parts of the arms.

II. May to July. The pads are first seen during this period. Not all the arms show pads. The pads that are present differ markedly in appearance. Three distinct types can be distinguished.



VENTRAL SURFACE OF THE FORELIMB OF THE MALE XENOPUS SHOWING THE PAD AREA.

Fig. 2.

(a) In the early part of this phase most of the pads seen extend from the finger-tips as far as the wrist. This area is black in contrast with the pale colour of the rest of the ventral surface of the arm, and feels rougher to the touch than the pad area of the forearm and arm. This pad area on the forearm is either similar in appearance to that seen between January and May, or it may assume a grey colour, when the vessels become red and wider in calibre than those seen in the first phase of the cycle. The pad area on the arm is either not seen at all or has the same appearance as before.
The features described are the same on the two forelimbs.
In the early part of this phase the majority of animals show this type of pad. As the breeding season approaches this type of pad becomes rarer and in the last stage of the cycle only a few frogs show this appearance.

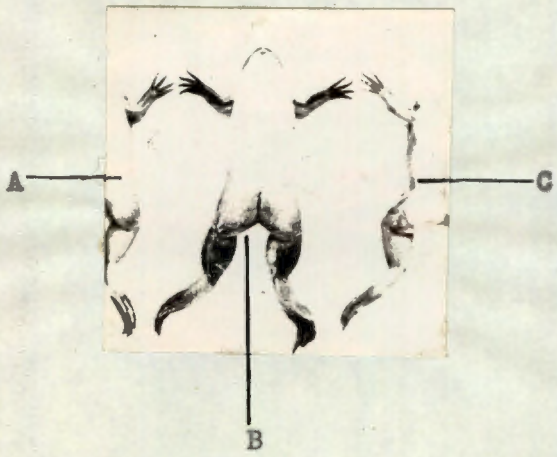
(b) In the second type of pad, the dark area covers the forelimb from the tips of all the fingers as far as the elbow, where it ends sharply. This dark area replaces the pad area and obscures the blood vessels described above. In some of the animals this patch is jet black and shiny and very rough to the touch. In these animals the pad area on the arm is grey to light brown in colour. In other animals the pad area is dark brown in colour and the arm shows a grey pad area. The two forelimbs of the animal show the same appearance.
This type of pad accounts for most of the pads that do not fall

into type (a) at the commencement of this phase of the cycle. As the phase progresses this type of pad becomes more frequent. In the late part of this phase this type of pad again is seen less frequently.

(c) The third type of pad extends the whole length of the arm from finger-tips to axilla, lying in the position of the pad area described above. (Fig 3). In most of the animals it is dark black and shiny in appearance and very rough to the touch. In others the portion on the arm is dark brown, rather than black. Again the two forelimbs show the same appearances. The third type of pad is seen only occasionally at the beginning of the second phase of the pad cycle, but increases progressively throughout the two months, until in the last part of this phase it is the type seen most frequently.

III. During the breeding season the pads seen are mainly of the third type, extending the whole length of the ventral surface of the arm, black in colour, shiny and rough to the touch. Pads extending as far as the elbow are also seen, but frogs with pads on the fingers and palms only or with no pads at all are infrequent.

IV. Between September and January the pads are seen progressively less frequently. In the early part of the phase the extent of the pads is very little changed, but the shiny appearance is lost. The colour is not as dark as before and the pads, in addition, are easily dislodged and peel off the



A Uninjected male. Note the blood vessels of the forearm.

B Injected male showing pads.

C Female.

Fig. 3.

Fully developed pads.

forelimbs in a complete sheet when the animals are handled, leaving behind an area lighter in colour but still rough to the touch.

As this phase goes on the pads are seen to extend for the most part only from finger-tips to elbow and become progressively paler in colour; brown, then grey, and at the end of this phase, opaque and pale in appearance as in the first phase of the cycle.

Throughout, the two forelimbs show the same appearances.

Appearance of the Forelimbs of Female Xenopus during the Cycle.

The appearance of the forelimbs of femal animals is constant throughout the year. They never show pads or an opaque area. The skin of the ventral surface of the females' forelimbs is smooth, pale and slimy and differs in no way from the skin on the ventral surface of the abdomen. The two blood vessels described above are not seen.

Discussion.

The pads, clearly a secondary sex character of the male Xenopus, develop just prior to the breeding season. The three different types of pads seen suggest that the development takes place in the following steps:

(i) The pads develop on the fingers and hands. During this time development of pads commences on the forearm, as shown by the grey colour.

(ii) The pad on the forearm progresses and becomes brown, while the pad on the fingers and palms is maintained in a fully-developed condition.

(iii) The pad now reaches completion as far as the elbow, as evidenced by the black shiny appearance, the portion on the arm commencing to develop showing a grey colour.

(iv) The pad as far as the elbow continues at full development while the development of the portion on the arm progresses and becomes dark brown in colour.

(v) The pad is completely developed when the whole area from finger-tip to axilla is black, shiny and rough.

After the breeding season the pads disappear, probably by successive exfoliations, which occur in the case of the thumb pads of Rana (Rostand 1934). The readiness with which the pads peel off makes this suggestion most likely. The point is discussed again later.

Summary.

1) The seasonal cycle in the pads of male Xenopus is described.

2) Four phases are recognised.

a) January to May. The pads are absent.

b) May to July. The pads develop.

c) July to September. The pads are maintained at their full development.

d) September to January. The pads disappear.

3) Three types of pads are described.

a) Pads covering the fingers and palms.

b) Pads covering the forelimb as far as the elbow.

c) Pads covering the forelimb as far as the axilla.

4) Five stages in the development of the pads are described. The chief stages are as follows:-

The pads are developed first as far as the wrist. Next the forearms show pads and finally the pad is completed.

5) The pads disappear after the breeding season by successive exfoliations.

6) Pads are never seen on the forelimbs of female Xenopus.

EXPERIMENTAL INVESTIGATION OF THE FACTORS CONTROLLING THE SEASONAL CYCLE IN THE PADS.

In all the experiments on the pads, large numbers of male Xenopus were collected from the ponds and their arms inspected. Only those which showed complete absence of pads were used.

Animals with traces of pads were not injected and were used as controls. To avoid repetition, it may be stated here that no development of pads occurred in uninjected animals. On the contrary, the traces of pads which were present at the beginning of the experiments were lost and complete absence of pads was found at the end of the experiments. The experiments were performed between November 1937 and February 1938.

I. THE ROLE OF THE ANTERIOR PITUITARY AND THE TESTIS.

Because of the fact that the nuptial pads are a secondary sex character of the male Xenopus, it was thought profitable to investigate of the roles of the testis and of the anterior pituitary in their development. Attempts were thus made to induce the development of pads by injections and implantations of anterior pituitary and testis preparations.

A. The Effect Of Acid Extracts Of Sheep Anterior Pituitary On The Development Of The Pads.

Experiment 1.

Injection Into Normal Males.

Four males without pads were selected from a batch of freshly-caught frogs. They were injected daily intraperitoneally with 0.5 ml. of an acid extract of sheep anterior pituitary for five days. The forelimbs were examined daily throughout the duration of the experiment and on the day after the last injection. Four control males were uninjected.

Date	Dose per Frog.	Equivalent in fresh tissue.
31.10.37	0.5 ml. S. 20	330 mgs.
1.11.37	0.5 ml. S. 20	330 mgs.
2.11.37	0.5 ml. S. 17	325 mgs.
3.11.37	0.5 ml. S. 17	325 mgs.
4.11.37	0.5 ml. S. 17	325 mgs.
	Total dose:	1.635 grammes.

Table I. Injection of acid extract of sheep anterior pituitary into normal males.

Results.

5.11.37. Each of the four injected males showed pads fully developed, extending up the arms as far as the axilla.

Injections were discontinued on the sixth day of the experiment when the pads were complete.

Discussion.

Sheep anterior pituitary extracts prepared according to the method of Bellerby (1933) contain a substance which causes the development of pads on the forelimbs of Xenopus males, either by a direct or an indirect action. The amount of anterior pituitary required to produce the pads in this experiment was 1.635 grammes, divided into 5 daily doses. The rate of development of the pads in this experiment agrees with the findings of Bles (1901), who observed the formation of pads under simulated natural conditions.

The above experiment was repeated on a larger number of animals. A larger dosage of anterior pituitary was used and the mode of administration was altered.

Experiment 2.

Injection Into Normal Males.

50 males were used in this experiment. 25 of these were injected with 0.8 ml. of an acid extract of sheep anterior pituitary intraperitoneally on alternate days for 4 injections. The forelimbs were examined on the 9th day of the experiment, the second day after the last injection.

A control series was run on the remaining 25 males. These frogs received no injections and their forelimbs were examined

on the 9th day of the experiment.

Date.	Injection per Frog.		Equivalent (mgs fresh tissue.
14.12.37	0.8 ml.	S 23	528
16.12.37	0.8 ml.	S 23	528
18.12.37	0.8 ml.	S 22	456
20.12.37	0.8 ml.	S 19	528
	Total dose:		2.04 grammes.

Table II. Injection of acid extract of sheep anterior pituitary into normal males.

Results and Discussion.

22.12.37. During the course of the experiment the injected animals became unhealthy. They were sluggish in their movements and felt stiff and dry. In addition, many of the injected animals developed ulcers on the dorsum of the thighs and several fatalities followed. On the ninth day of the experiment there were 16 survivors and the experiment was stopped.

The controls remained healthy and no deaths occurred. No pads developed on the forelimbs of the control animals.

The findings in the injected animals explain one of the discrepancies between the statements of Leslie (1890) and Bles (1901, 1904). Only 8 of the animals showed pads covering the fingers, forearms and arms. In 6 of the animals the pads extended only as far as the elbows and in the remaining two

animals the fingers alone were covered with pads. It appears, therefore, that the development of the pads occurs first on the fingers. Next the pads develop on the forearms, the pads on the fingers being maintained in the meanwhile in a complete state. Lastly, the part of the pad which is found on the arm as far as the axilla is developed and the pad becomes complete. Hence, while Bles (1901, 1904) observed fully-developed pads, those seen by Leslie (1890) were only in an intermediate stage of their development.

This finding explains, also, the difference in appearance of the pads on the forearms of the pond males examined between May and July. The three types represent various stages in the development of the pads.

The results recorded here confirm the conclusions of the previous section, namely, acid extracts of sheep anterior pituitary contain a substance which causes the development of the nuptial excrescences. In this case the total dose received by each frog was 2.04 grammes of fresh anterior lobe, in four divided doses on alternate days.

The results of injection of sheep anterior pituitary extracts show that the pads can develop at any time of the year, provided the appropriate stimulus is applied. The first experiment was performed at the beginning of November, and the second at the end of December when the pads in the ponds are either already lost or are in the process of being shed.

The role of the testis in the development of the pads was next investigated.

Experiment 3.

Injection Into Castrated Males.

12 males of a batch of frogs castrated by the method described above were injected into the dorsal lymph sac with 0.8 ml. of an extract of sheep anterior pituitary on alternate days until 4 injections had been made.

The forelimbs were examined every day during the course of the experiment and on the 2 days following the last injection.

Results:

Each frog received a total of 2.016 grammes of fresh anterior lobe. There was no development of pads whatsoever. No blackness or roughness of the palmar skin, or of the inner surface of the fingers, occurred. Very little ill-health occurred in these animals.

Discussion.

This experiment was performed between the 22nd and 30th of December, 1937, the week following the previous experiment. It is unlikely, therefore, that there was any alteration in the sensitivity of the tissues of the animals in this experiment to the sheep anterior pituitary substance. The total dose received by each animal was the same as that given to the normal

frogs and the injections were divided up and spaced out in the same way as before. Also, the animals were kept under identical conditions.

Thus it can be concluded that the testis is essential for the development of pads on the forelimbs of male Xenopus. In the absence of the testis the anterior pituitary is unable to induce pad development. The action of the anterior pituitary on the arms, therefore, is not a direct one, but an indirect effect through the testis. The nuptial pad of the male Xenopus is a true secondary sex character, dependent for its appearance on a secretion from the testis. The testis, in turn, is activated by the anterior pituitary, which acts in this instance by virtue of its gonadotrophic properties.

B. The Effect Of A Pyridine Extract Of Sheep Anterior Pituitary On The Development Of The Pads.

A large quantity of pyridine extract of sheep anterior pituitary was prepared according to the method of Rowlands (1937) described above. 1 ml. of extract was equivalent to 10.3 mgs. of powder or 534 mgs. of fresh anterior lobe.

Experiment 1.

Injection Into Normal Males.

15 males selected as described above were injected into their dorsal lymph sacs on alternate days with 0.5 ml. of the pyridine extract until 6 injections had been given.

The forelimbs were examined on the 1st, 5th, 8th and 13th days of the experiment, care being taken to avoid touching the forelimbs since the developing pads are easily torn off.

A control series consisting of 15 females was treated in exactly the same way.

Results:

Males.

In this experiment more careful observations on the extent to which the pads had developed were made. On the fifth day 3 animals showed pads on their fingers and hands only; 8 had pads extending as far as the elbow and the remaining four frogs

already had complete pads. By the eighth day of the experiment each of the 15 males showed pads extending up as far as the axillae. In all cases the extent of the pads was the same on the two arms.

Females.

Ovulation occurred but there was no development of pads on the forelimbs of the female frogs.

The animals remained in good condition until the last two days of the experiment, when ulcers developed on the dorsum of the thighs in a few of the animals.

Discussion.

Pyridine extracts of sheep anterior pituitary also contain a substance which is capable of causing the development of pads on the arms of normal male Xenopus. No such effect is seen in the females. It is noteworthy that in 4 of the 15 males the pads were completely developed 4 days after the first injection, after only 534 mgs. of fresh anterior lobe tissue had been administered. In the remaining animals 7 days elapsed before the development of pads was complete and in this case each animal received extract equivalent to 1.068 mgs. of fresh sheep anterior pituitary.

Once the pads reached full development they were maintained in that condition until the end of the experiment (13th day). The results confirm the findings in the previous sections, namely, that the development of pads occurs first on the fingers and palms, then on the forearms, and finally the arms show pads right up to the axillae.

Experiment 2.

Injection Into Castrated Males.

A batch of padless males selected as described above was castrated by the method described previously.

A batch of females was ovariectomised and collected in the same way.

15 castrate males and 15 castrate females were injected into the dorsal lymph sac with 0.5 ml. of the pyridine extract of sheep anterior pituitary described above on alternate days for 6 injections.

The forelimbs of all the frogs were examined on the 1st, 3rd, 5th, 7th, 8th, 9th, 10th, 11th, 12th and 13th days of the experiment.

Results:

There was no development of pads in either the males or the females. These animals also remained in good condition.

Discussion.

The results in this experiment confirm the findings in the previous sections. The injections were identical in amount and were injected at the same intervals of time. The anterior pituitary acts indirectly through the agency of the testis.

The female Xenopus has never been observed to develop pads.

Experiment 3.

Injection Into Hypophysectomised Males.

A batch of selected males was hypophysectomised by the method described by Hogben (1923). The animals were kept for several days after operation in containers with white sides and white bottoms. 15 black frogs from this batch were used in this experiment. Thus these had only the anterior lobes of their pituitaries removed.

A control group of 15 anterior lobe hypophysectomised females was collected in the same way.

Each animal in the two groups received injections of 0.5 ml. of the pyridine extract into the dorsal lymph sac on alternate days for 6 injections.

The forelimbs of both groups were examined on the 1st, 3rd, 7th, 9th, 10th, 11th, 12th and 13th days of the experiments.

Results:

Males.

Readings were made of the extent of development of the pads in each animal. The results are summarised in table III.

Total No. of injections given.	Day of Experiment.	No. of Males showing pads on the Fingers and hands.	As far as Elbow.	Up to Axilla.
0	1	0	0	0
1	3	4	1	0
3	7	2	5	0
4	9	5	9	1
5	10	3	2	10
5	11	0	5	10
6	12	0	0	15
	13	0	0	15

Table III. Injection of pyridine extract of sheep anterior pituitary into hypophysectomised males.

Females.

No pads developed on the forelimbs of the females. Ovulation into the water did occur.

The animals again remained in good condition until the last two days of the experiment.

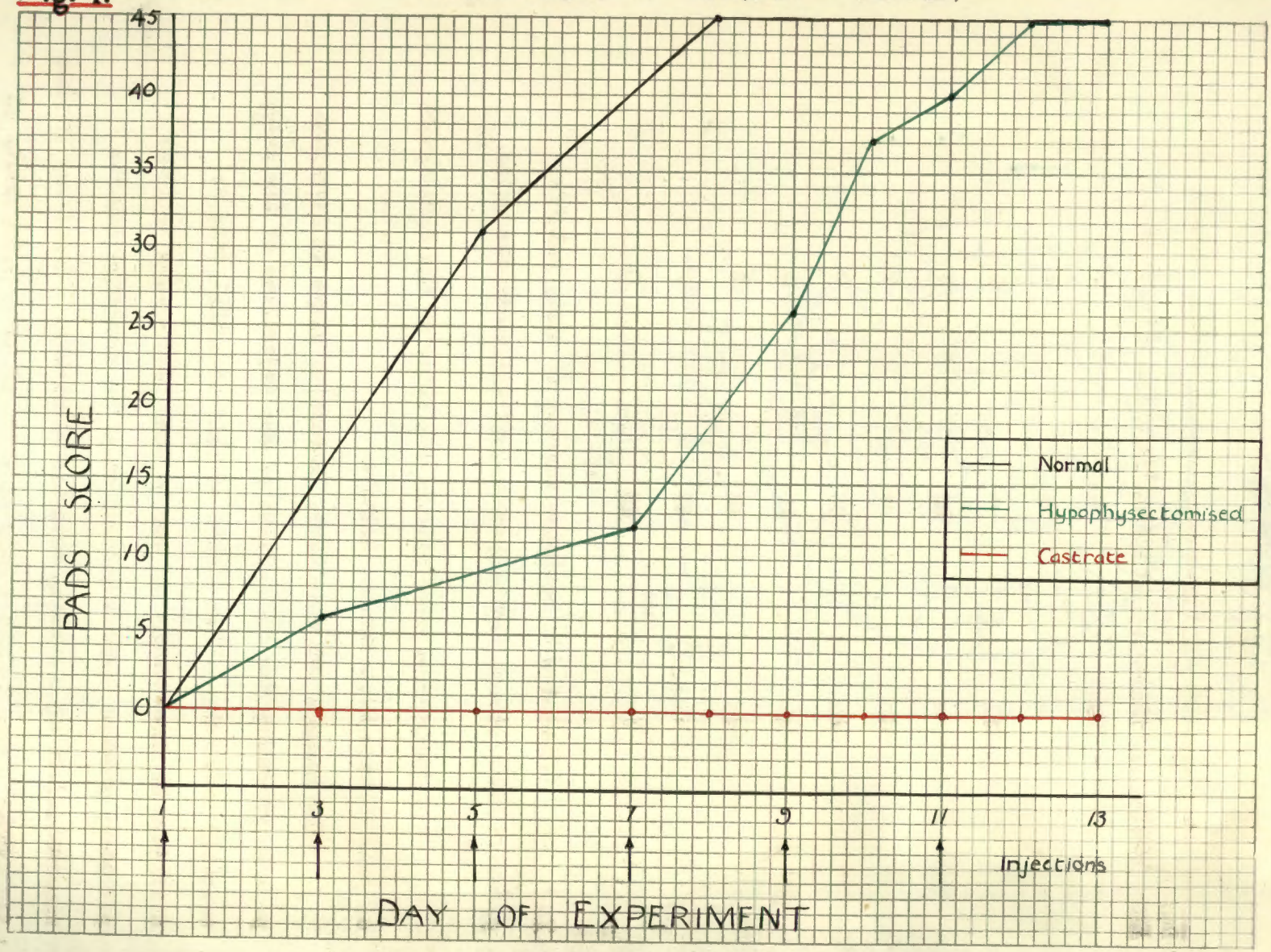
Discussion.

In the absence of the anterior lobe in the male, pyridine extracts of sheep anterior pituitary are still capable of causing the development of pads. The development of pads, however, seems to be delayed in the anterior lobe hypophysectomised frogs, as compared with the normals. Thus, on the 5th day of the experiment, after 534 mgs. of fresh anterior lobe, no animals

showed completely-developed pads, whereas in the normal animals 26.7% of the animals had pads extending as far as the axillae. Again after the administration of 1.068 grammes of fresh sheep anterior lobe tissue only 1 of the animals with their anterior pituitaries removed showed completely-developed pads (6.7%), whereas in the normals all the animals (100%) showed the full response. The different rates of development of pads in normal, hypophysectomised and castrate males was, therefore, analysed in the following way:

1 point was scored for each animal showing pads on the fingers and hands only; 2 points for each animal with pads extending as far as the elbow; 3 points for animals showing pads completely developed. The figures are given in table IV below and are represented graphically in Fig. 4. The maximum score for each group is 45.

Fig. 4.


INJECTION of SHEEP A.P.E.
PADS SCORE
45
40
35
30
25
20
15
10
5
0
1
3
5
7
9
11
13
Injections
DAY OF EXPERIMENT
Normal
Hypophysectomised
Castrate

	PAD SCORE.		
Day of Experiment.	Normals.	Hypophysectomised.	Castrates.
1	0	0	0
3	-	6	0
5	31	-	0
7	-	12	0
8	45	-	0
9	45	26	0
10	45	37	0
11	45	40	0
12	45	45	0
13	45	45	0

Table IV. Injection of pyridine extract of sheep anterior pituitary into normal, hypophysectomised and castrated males.

The graph shows very clearly the delay in the development of pads in hypophysectomised animals.

The animals own pituitary, therefore, plays an essential part in producing the full effect of the injections of the sheep anterior pituitary. The nature of this essential role is difficult to understand. It may be that the Xenopus anterior pituitary produces some substance which is necessary for the maximum effect of the sheep anterior pituitary. Or it may be that the animal's own pituitary maintains the state of sensitivity of

the arms to pituitary injections. The normal and hypophysectomised animals were kept in the laboratory for the same length of time before the experiments were commenced (approximately 2 weeks). It has been shown that captivity results in diminished anterior pituitary activity in Xenopus laevis (Shapiro & Shapiro 1934), but hypophysectomy removes the remaining portion of the pituitary activity and hence a lessened sensitivity of the animal's tissue might be expected in the hypophysectomised animals, i.e., it is possible that after two weeks in an anterior-lobe-less condition, the testis of Xenopus laevis is less sensitive to injected sheep anterior pituitary substance than the testes of normal animals kept in a laboratory for an equal length of time.

The delay in the development of pads appears to occur in the early part of the experiment, since the score reaches 12 in the hypophysectomised animals only on the 7th day, whereas the normals pass the 12 mark between the 2nd and 3rd days. After this the two curves run more or less parallel, so that the rate of development from 12 to 45 takes 5 days in the hypophysectomised animals as compared with 5 - 5 1/2 days in the normals.

This finding is in keeping with the second suggestion above viz: that the sensitivity of the testis of hypophysectomised animals is lowered, so that when identical doses of sheep anterior pituitary extract are administered, a smaller response occurs, as

judged by the development of pads. In the hypophysectomised animals it appears that during the first seven days the lowered sensitivity of the testis is increased, after which development of pads proceeds at the same rate as in the normal animals. The role of the animal's own pituitary seems, therefore, to be the maintenance of the testis of the animals in a state of sensitivity to injected anterior lobe substance, rather than to provide a substance which acts together with the sheep anterior pituitary.

The findings in this section agree with the results of similar experiments on the arm size of male Xenopus (see later).

Again the pituitary is seen from the graph to act because of its gonadotrophic properties.

The ill-health of the animals that occurred with repeated injections of the acid extracts of sheep anterior pituitary appears to have been due to the fact that this extract is cruder than the pyridine extract and thus probably contains more toxic substances than the latter extract.

C. The Effect Of Xenopus Pituitaries On The Development Of The Pads.

Experiment 1.

Injection Into Normal Males.

The pituitaries were removed from male frogs by the method of reflection of the base of the skull previously described. The donors had been kept in captivity for 2 - 5 months. They were fed once a week. During their captivity they displayed no sexual behaviour. No croaking was ever heard and no coupling took place. Each injection was prepared as follows:

Four anterior lobes were collected in a small flat-bottomed tube containing 0.5 ml. of distilled water. The pituitaries were then macerated by crushing them with a glass rod with a flat base slightly smaller than the inside of the tube. Crushing was continued until no particles of tissue could be seen with the naked eye. An opaque solution is produced. This is taken up into a syringe through a needle with a large bore and injected into the experimental animal. 0.5 ml. of distilled water is again added to the tube, taken up into the syringe and injected.

Anterior lobes from female frogs were prepared and injected in the same way. The females had also been kept in captivity for 2 - 5 months, during which time they were fed once a week and displayed no sexual behaviour.

Four animals were used in this experiment. Two received injections of male Xenopus anterior lobe aqueous suspensions and two received injections of female anterior lobe aqueous suspensions as follows:-

Into the ventral lymph sac on the 1st day of the experiment.

" " dorsal " " " " 3rd " " " "

" " ventral " " " " 6th " " " "

The ventral and dorsal lymph sacs were used alternately to provide an adequate amount of surface for absorption of the macerated anterior lobes.

The forelimbs were examined daily until the eighth day of the experiment.

Results:

On the eighth day of the experiment the pads reached full development in each of the four animals used. Ulcers developed on the dorsum of the thighs of the injected animals.

Discussion.

Xenopus pituitaries contain a substance which is capable of producing pads on the forearms of male Xenopus. This result is confirmed below. This factor is present in the anterior pituitaries of both male and female frogs. The third significant point is that the donors of the pituitaries had, themselves been in captivity for periods ranging from 2 - 5 months. During this time no sexual behaviour was observed to take place. Although

the males and females were kept in the same tanks there was never any coupling; the females showed no hyperaemia of the cloacal labia, nor did they oviposit; the males were never heard to croak and no pads were present on the arms of these male donors.

Thus the pituitary substance that induces the development of pads shows no sex specificity, the anterior lobes of both male and female Xenopus pituitaries being potent in this respect.

The inhibition of the anterior pituitary of Xenopus laevis in captivity has been described by Shapiro & Shapiro (1934). The fact that active principles can be demonstrated in the anterior pituitaries of animals captive for 2 - 5 months shows that hormone production by the gland does not cease in captivity. The upset appears to be the lack of the appropriate atimulus for the release from the gland of active principle, which is, therefore, stored in the gland. Its presence can be demonstrated by implantation of the gland into the lymph sacs of other animals. The latter finding is abundantly confirmed later in this work.

A total dose of 12 such pituitaries from either male or female frogs is capable of causing the development of pads.

The crudeness of the administered pituitary again seems to to account for the development of the ulcers on the thighs.

Experiment 2.

Injection Into Castrated Males.

Five males were used in this experiment. Each injection consisted of two male and two female Xenopus anterior lobes, prepared according to the method described above.

Each frog received 4 daily injections. These were all given into the dorsal lymph sac, on account of the operation wound on the ventral surface of the body.

Observations were made daily for five days. The forelimbs were examined for pads.

Results:

There was no development of pad whatsoever.

Discussion.

In the absence of the testis, Xenopus anterior pituitaries are incapable of producing pads on the forelimbs of male Xenopus. This finding agrees with the results of injection of acid or pyridine extracts of sheep anterior pituitaries. The total dose received by each frog in this experiment was 16 anterior lobes, collected, prepared and injected in exactly the same way as in the case of the normal animals. The negative result here must, therefore, be explained on the lines laid down above. It is not likely that it is due to insufficient dosage with the anterior lobes. Thus the pituitaries of Xenopus laevis contain a

gonadotrophic hormone which stimulates the testis, which, in turn, stimulates the production of pads.

D. The Effect Of The Injection Of Testis Suspensions Into Male Xenopus On The Development Of The Pads.

Twelve males were injected with a suspension of Xenopus testes prepared in the following way:

Each day 90 testes were collected from animals freshly brought in from the vleis and pithed immediately before being opened up. These 90 testes were collected in 30 mls. of water and ground up in a mortar until all the large particles were broken up. The suspension was taken up into a 10.0 ml. syringe through a needle with a large bore and injected into the peritoneal cavity of the experimental animals.

Each frog received 2 ml. of the suspension (≡ 6 testes) daily for 6 days, commencing on the 22/12/37. The arms were examined daily until the end of the 7th day of the experiment.

Results.

The fourth day of the experiment 2 frogs died; 4 died on the 6th day of the experiment and on the 7th day there was only one survivor. The frogs died of acute peritonitis due to the presence of the particles of injected testis, which could be seen post-mortem in the peritoneal cavity and which were decomposing and very foul-smelling. The last survivor was very ill with acute peritonitis.

On the second day of the experiment there was some greying of the pad areas of the forearms but no further change took place.

Discussion.

The greying of the pad areas indicates commencing development of pads, but owing probably to the sick condition of the animals, further activity of the injected suspension was not seen.

Thus there appears to have been some slight development of pads starting, but no definite conclusions can be shown from this unsatisfactory experiment. Attempts should be made to extract the active principle of the testis and to produce pads with the extracted hormone injected in some easily absorbable form.

II. The Mode Of The Development Of The Pads.

The mode of development of the pads has already been described. In the experiment below the macroscopical observations were repeated and analysed and, in addition, the fully-developed pad and the stages through which it passes to reach full development were investigated histologically.

Fourteen male frogs were used in this experiment. These were chosen because their forelimbs were entirely without pads.

Two of these were pithed and their forelimbs removed. The

skin and muscle was removed from the arms and forearms of each forelimb of each frog and histological sections cut in a transverse plane. Thus no decalcification was necessary. The two middle digits were also sectioned transversely. Here the skin is closely adherent to the underlying structures and the bone, and could not be removed. The digits,therefore, were decalcified before being prepared for section.

In this way numerous sections were cut of each of the four arms, forearms and digits and stained with haematoxylon and eosin. The histology is described below.

The remaining frogs received daily injections into the dorsal lymph sac of 2 male and 2 female Xenopus anterior pituitaries prepared and injected according to the methods described above.

The forelimbs were examined daily.

Each day 2 of the frogs were pithed and histological sections of their arms, forearms and middle two digits were cut, in the manner described above. This was continued until the development of pads was complete.

Results:

(i) Macroscopic Findings.

On the day injections were started there were no signs of the presence of pads, the frogs being specially selected from a batch collected at the end of January, to eliminate all doubt as regards the initial condition.

1st day after injections were commenced. The fingers and palms showed a brown colour on their palmar aspect, while the two vessels of the forearm became red and swollen and the pad area of the forearm became grey and hyperaemic. The arms showed no change.

2 nd day. The fingers and palms were dark brown on the second day after injections commenced. The forearms were brown in the pad area and the arms showed greying of the pad area.

3 rd day. The fingers and hands became black and shiny, but the parts of the pads on the forearms had not yet reached full development. The pad area on the arm had become darker.

4 th day. The pads were fully-developed as far as the elbow. The portions of the pads on the arms were now brown in colour.

5 th day. The pads reached completion on the fifth day after injections commenced. They extended from finger-tips to axilla and were black and shiny.

These results are summarised in table V and represented graphically in Fig. 5.

The scores were judged as follows:-

0 opaque.

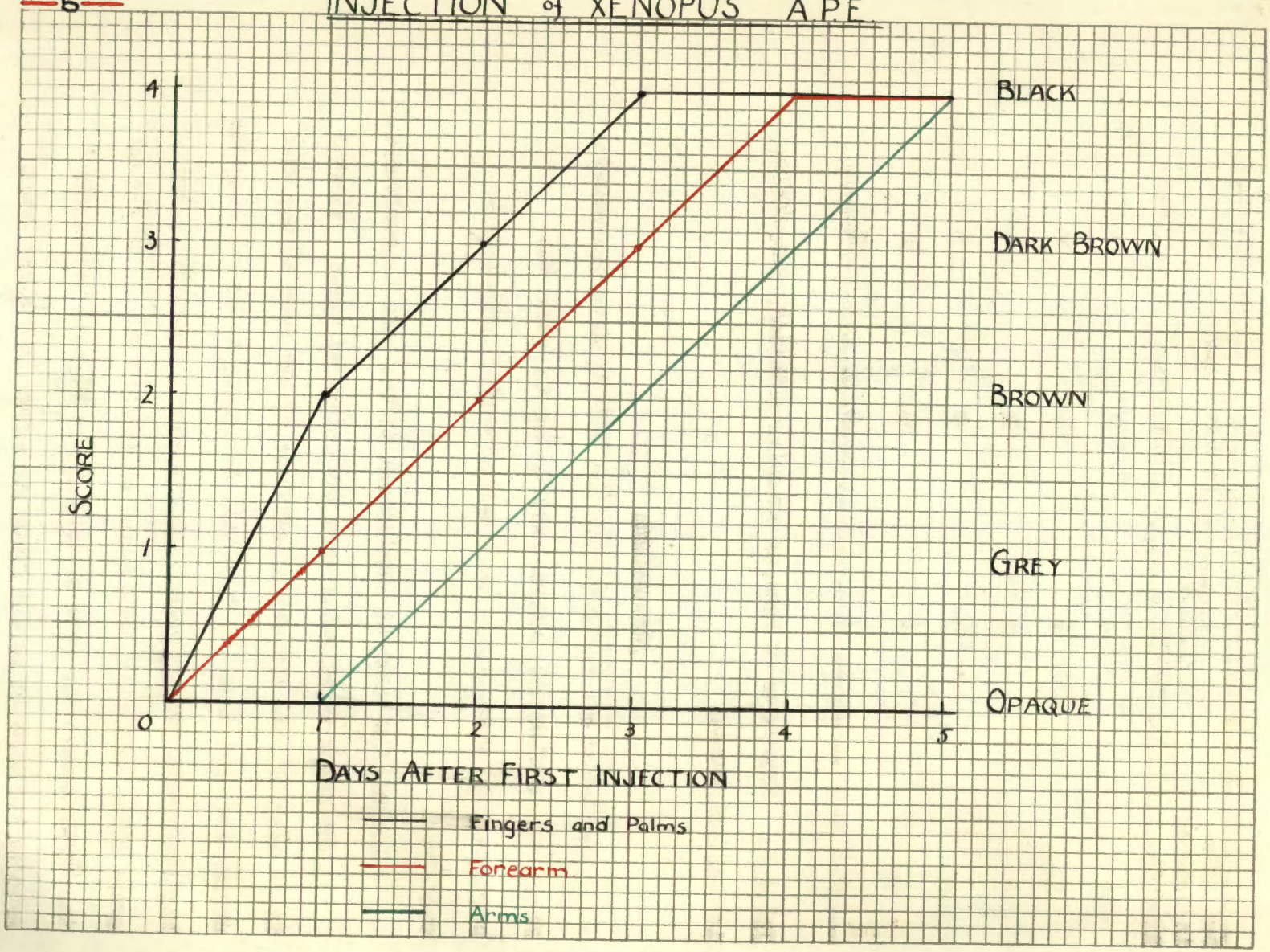
1 grey.

2 brown.

3 dark brown.

4 black and shiny.

Fig. 5.


INJECTION of XENOPUS A.P.E.
4
3
2
1
0
SCORE
1
2
3
4
5
DAYS AFTER FIRST INJECTION
BLACK
DARK BROWN
BROWN
GREY
OPAQUE
Fingers and Palms
Forearm
Arms

Day after injections started.	SCORE.		
	Fingers and Palms.	Forearms.	Arms.
0	0	0	0
1	2	1	0
2	3	2	1
3	4	3	2
4	4	4	3
5	4	4	4

Table V. Injection of Xenopus anterior pituitary suspension into normal males.

Discussion.

Two important confirmations are made by this experiment. First, the ability of Xenopus pituitaries to cause the production of pads, i.e., their gonadotrophic activity, is confirmed.

Second: The mode of development of the pads is confirmed.

The graph is of value from two points of view: First, it is easy to read off the appearances of the arms at any time during the experiment. This will be useful in correlating the histology with the macroscopical findings. Secondly, the graph shows that once development of the pad starts in one of the three areas, the rate of development in that particular area is the same as in the other two areas (the lines being parallel). Thus in this experiment the forearm is one day

behind the fingers and palms and one day ahead of the arm, development in each area proceeding at the same rate.

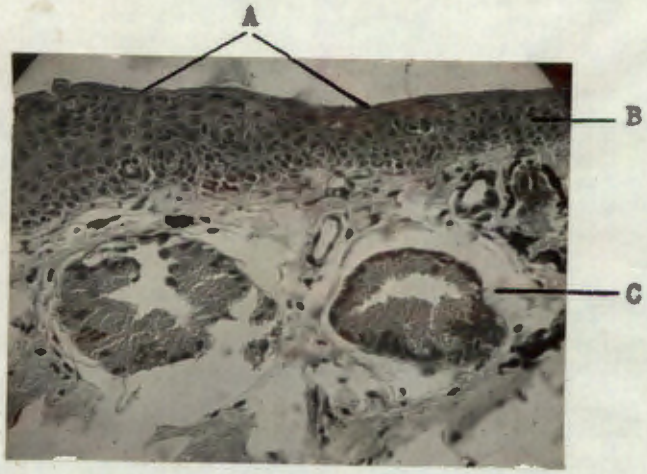
(ii) Histological Findings.

The histological appearance of the skin of the ventral surface of the forelimbs before injections were begun differs in several important respects from that of the dorsal skin described below. (See "Secondary sex characters of the female Xenopus").

Only three layers are seen, since the melanophore layer of the dorsal skin stops short at the junction of the lateral and ventral aspects of the forelimb and is absent from the ventral surface. (See fig. 6).

1) The stratified squamous epithelium is similar to the epithelial layer of the dorsal skin, except that on the pad area its surface shows a thin layer uniformly stained with eosin - a stratum corneum. This layer is interrupted by the ducts of skin glands.

2) The glandular layer is entirely different from that of the dorsal skin. The connective tissue is greatly increased in amount and contains numerous capillaries. There is only one type of gland present beneath the pad area, and this differs from the glands seen on the dorsal skin and the rest of the ventral skin of the forelimb. The gland is simple saccular in type, composed of a single layer of large columnar cells which rest on



A Stratum corneum.

B Stratified epithelium.

C Glandular layer.

Fig. 6.

Histology of the pad area. (x 100)

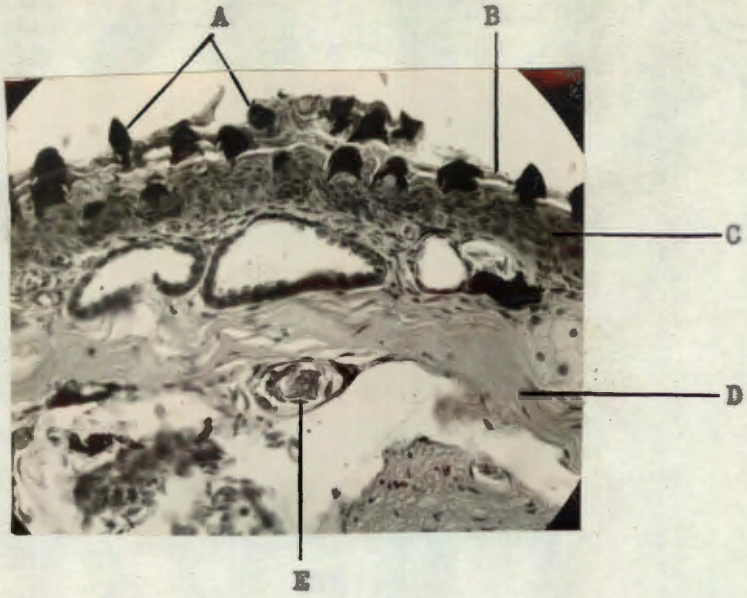
a basement membrane. The cells contain irregularly-shaped basally-situated nuclei and are filled with large eosinophil granules. The glands open on to the surface of the skin through a duct lined by cuboidal cells with large basal nuclei.

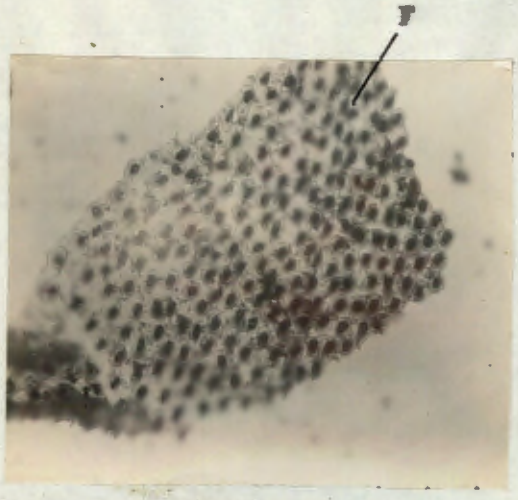
3) A smooth muscle layer is present, similar in appearance to that of the dorsal skin.

In the loose areolar tissue connecting the skin to the muscles of the arm, the large blood vessels described above can be seen.

On the fifth day after injections are begun, the pads are complete macroscopically. Changes occur especially in the first two layers. (See Fig. 7).

1) The epithelial layer appears entirely different. It is thrown into closely set papillae. The cells forming the papillae are arranged in the form of cones. Each cone is surmounted by $\wedge$ - shaped black spines, the point of the $\wedge$ projecting beyond the surface of the skin. As many as four superimposed spines may surmount a single cone. The limbs of the spine completely encircle the cone, so that in thosepapillae that are cut transversely a ring of black pigment can be seen enclosing a few epithelial cells. The stratum corneum persists and the spines appear to be developed on this layer. In some parts of the sections this layer has become detached from the rest of the skin, the spines remaining attached to the former.





A Spines.
B Stratum corneum with spines.
C Stratified epithelium.
D Smooth muscle layer.
E Congested blood vessel.
F Desqramated stratum corneum with spines (Surface view).

Fig. 7.

Histology of the fully-developed pad. (x 100)

2) The glandular layer also shows marked changes. The connective tissue becomes hyperaemic, and numerous congested capillaries are seen between the glands. The cells of the glands have lost most of their granular material and are reduced to a small basal strip containing the nuclei. The lumen of the gland is correspondingly increased. The vessels beneath the muscular layer are markedly congested.

The intermediate stages of these changes are clearly seen in the sections of the limbs on the first four days after injections were commenced. Thus, in the skin of the forearm the following changes are seen. On the first day after injection the increased vascularity of the glandular layer is evident. Some of the glands show the cells breaking down, the granular material being lost and the lumen becoming larger. The vessels below the muscular layer show some congestion. The epithelial layer shows a few cones surmounted by a single spine. On the following three days these changes progress. More spine-capped cones are seen and more spines are laid down on previously developed cones. The glands continue to lose their granular material and are gradually reduced to the appearance described above.

In the fingers cones and spines are seen on the day injections were commenced, the appearance being similar to that seen on the forearm on the first day after injections were begun. The changes proceed in the same way as in the forearm.

The skin on the arm shows the same series of changes. The

changes, however, begin only on the second day after the commencement of the injections.

Discussion.

The histological findings confirm the macroscopic findings except in the case of the fingers at the time that injections commenced. From the curves in Fig. 5 it seemed likely that at that time the fingers would show a grey appearance. Histologically this probability is borne out by the fact that the fingers show the same appearance as the forearms and arms show when they are grey in colour.

It seems likely that the changes in the colour of the pad area, grey, brown, dark brown and black are due to the laying down of successive spines on the epithelial cones. It is difficult, however, to determine whether the successive colours follow the addition of each layer of black spines accurately.

It is also difficult to understand the part played by the changes in the glands in the development of the pads. The increase in spines occurring together with the loss of granules from the glands suggests that the secretion from the glands provides the material from which the spines are derived.

The histological appearance of the pads explains adequately their rough character.

III. THE EFFECT OF CAPTIVITY ON THE PAD CYCLE.

At the commencement of the investigation of the pad cycle under natural conditions (September 1937), large numbers of male and female frogs were collected and kept in captivity in large galvanised iron tanks. The frogs were fed twice a week with raw minced meat and the water of the containers was changed three times a week.

On each occasion when frogs were collected from the ponds for the study of the naturally occurring cycle on the pads, the forelimbs of the captive animals were examined for the presence of pads. The experiment ended in September, 1938.

Results:

At the commencement of the experiment the forelimbs showed the appearances seen in the fourth phase of the pad cycle. (see above). By January 1938, all traces of pads disappeared from the forelimbs of the males and the forelimbs remained pad-less throughout the remainder of the experiment.

No pads were seen on the forelimbs of the females.

Discussion.

Captivity in Xenopus laevis has been shown to inhibit the anterior pituitary. (Shapiro & Shapiro 1934) The results above show that the anterior pituitary, acting via the testis, is capable of inducing the development of pads in Xenopus males.

Thus in the absence of anterior pituitary activity no pads develop and injections of anterior pituitary cause pads to develop. It is likely, therefore, that the action of the anterior pituitary is an hormonal one. It is further likely that the pad cycle occurring under natural conditions is under the control of the anterior pituitary.

On the basis of these results, the four phases in the naturally occurring cycle can be interpreted as follows:-

(i) In the first phase the anterior pituitary is quiescent and does not stimulate the testis to produce its androgenic hormone. (January to May).

(ii)Between them and July the anterior pituitary activity commences, causing development of the pads to the breeding season level.

(iii) During the breeding season, the climax of anterior pituitary gonadotrophic activity, the pads are maintained in a fully-developed condition.

(iv) After the breeding season the anterior pituitary activity diminishes and the pads disappear from the forelimbs of the male frogs.

THE FUNCTIONAL SIGNIFICANCE OF THE PADS.

The pads would appear to play a role in helping the male to maintain a firm grip on the female during mating. The pads are developed just prior to the breeding season and are maintained until the end of the breeding season and then gradually disappear. The frogs are always kept moist and slightly slimy by the water in which they live and by a certain amount of secretion of mucus from their skin glands. In addition, the application of some foreign body to the skin evokes an increased secretion of mucus, so that there must be considerable difficulty for the male to maintain his clasp around the female. The pads seem to be constructed to facilitate this procedure. Their distribution coincides accurately with the portions of the arms in contact with the female, the palmar aspect of the fingers and hands being in contact with the anterior abdominal wall, the ventral surfaces of the forearms being applied to her flanks and the ventral side of the upper arm touching the female along the dorso-lateral borders of her lumbar region. Furthermore, inspection of a histological section of the pads gives the impression that they would form an efficient means of assisting clasping. A male with well-developed pads can easily be lifted up by one forelimb, the forelimb being held very lightly and against the struggling of the animal. Lastly, if the finger is passed along the pad the dry roughness of the excrescence contrasts

markedly with the slimy character of the skin elsewhere.

This theory, however, is upset by the fact that coupling can be induced experimentally in Xenopus laevis at all times of the year (Shapiro 1936 and see below). Even between the months of January and May, when the pads are almost non-existent, the male is still able to manifest the clasping reflex and mate with the female. The injection which is given to induce the mating reflex cannot produce pads in the 10 - 12 hour latent interval before coupling commences, so that the male remains padless. The clasps on these occasions are as tenacious as those that are induced when the pads are fully developed and coupling lasts for the same length of time. The pads, therefore, appear to be totally unnecessary since the male manages to clasp the female and to maintain that clasp without their aid, even though his brachial musculature is smaller than at any other time during the year (see below).

On this account no essential functional significance can be attached to the pads. At the most they may play a minor role as a refinement in the mating reflex under natural conditions.

IV. THE ACTION OF CERTAIN STEROIDS ON XENOPUS MALES.

It has been shown above that the pads develop as a result of the androgenic activity of the testis. The testis, in turn, can be activated by gonadotrophic substances. Thus, using the development of pads as an index of androgenic activity, or of gonadotrophic activity in suitably-arranged experiments, various substances may be tested for either of the two properties.

The experiments were performed during January and February 1938.

Methyl Testosterone.

Experiment 1.

Intramuscular Injection Of Methyl Testosterone Into Castrated Males.

Five padless males were castrated according to the method described previously.

A dilution of the stock solution of methyl testosterone (5 mgs per ml) was made so that there were 250γ in 0.2 ml.

Four daily injections of 0.2 ml. were made into the gastrocnemii, using the left leg for the 1st and 3rd injections and the right leg for the 2nd and 4th injections.

The forelimbs were examined daily.

Results:

There was no sign of pad development in these animals.

Discussion.

One mg. of methyl testosterone administered intramuscularly is incapable of producing pads in castrated males. Thus, in the doses given methyl testosterone is not androgenic in Xenopus males. The dose used in each injection produces a 45% ovulation in female Xenopus (Shapiro, 1938) in January, and is thus fairly potent in the female. The intramuscular route of injection ensures a high percentage of utilisation of the injected hormone. The inactivity of the hormone in castrated males appears to be due, therefore, to the lack of ability of methyl testosterone to stimulate pad development.

Experiment 2.

Intraperitoneal Injection Of Methyl Testosterone Into Normal Males.

A dilution of the stock solution of methyl testosterone was made. The strength of the stock solution was 5 mgs. per ml. 1 ml. of this was added to 19 mls. of nut oil. The strength of the diluted solution thus was 250 y per ml.

18 males were injected intraperitoneally on alternate days for 5 injections with 1 ml. of the diluted solution (250 y).

250 y of methyl testosterone produces a 45% ovulation in January, the month during which the experiments were performed.

The forelimbs were examined daily until the second day after the last injection.

Results:

There was no development of pads whatsoever. The fingers and palms were especially carefully examined, but no trace of pads could be detected either by sight or by touch.

Discussion.

In doses of 1.25 mgs. methyl testosterone produces no pads in normal animals. The hormone, in the doses given, is not gonadotrophic to the male Xenopus.

Experiment 3.

Intramuscular Injection Of Methyl Testosterone Into Normal Males.

The solution here was made up as follows:-

1 ml. of stock was added to 3 ml. of nut oil, thus making the strength of the solution 250 y in 0.2 ml.

Intramuscular injections of 0.2 mls. of diluted solution were made on 7 occasions, the 1st, 3rd, 5th, 7th, 9th, 12th and 14th days of the experiment. The solution is injected into the gastrocnemius of the frog, the needle entering through the tendo calcaneus. The maximum dose which can be injected into a gastrocnemius is 0.25 ml. Injections were made into alternate legs, starting with the right, to allow sufficient time for absorption.

Six padless males were used and daily observations of the forelimbs were made until the 16th day of the experiment.

Results:

There was no development of pads.

Discussion.

1.75 mgs. of methyl testosterone was administered to each animal in this experiment. This is a larger dose than that used in the previous experiments and, in addition, the injections

were made intramuscularly to ensure a high utilisation of the hormone. In spite of this, no gonadotrophic activity was demonstrable.

The results obtained with methyl testosterone contrast markedly with the results of injections of this steroid into mammals and into female Xenopus.

In mammals methyl testosterone is a bisexual hormone. It has an androgenic and oestrogenic activity and is progestrational in high doses. In the Xenopus male it is inactive androgenically.

In Xenopus female, methyl testosterone is gonadotrophic (Shapiro 1936 c), whereas in the male it shows no gonadotrophic activity.

Testosterone Propionate.

Experiment 1.

Intramuscular Injection Of Testosterone Propionate Into Castrated Males.

5 padless males were castrated as described previously. Each received daily intramuscular injections into alternate legs of 0.25 ml. (250 y) of the stock solution (1 mg.per ml.) of testosterone propionate for 4 days.

The forelimbs were examined daily.

Results:

There was no development of pads.

Discussion.

1.0 mgs. of esterified testosterone is inactive androgenically in the male Xenopus. Intramuscular injection of esterified hormone ensures maximum utilisation of dissolved substance (Parkes, 1936). Thus it is likely that most of the injected hormone was made use of by the animals, but no pads developed. In the doses given testosterone propionate is not androgenic.

Experiment 2.

Intramuscular Injection Of Testosterone Propionate Into Normal Males.

The strength of the stock solution of testosterone propionate was 1 mg. per ml. Thus 0.25 ml. contains 250 y and, therefore,

no dilution was required for intramuscular injection.

20 padless males received intramuscular injections of 0.25 ml. of the stock solution on alternate days for 5 injections, a sixth injection on the third day after this and a seventh injection 2 days later. Left and right legs were injected alternately.

Observations were made of the forelimbs daily until the 16th day of the experiment.

Results:

No development of pads occurred.

Discussion.

Testosterone propionate is not gonadotrophic in the male Xenopus. Gonadotrophicity in the male Xenopus is manifested by the development of pads. This failed to occur under the influence of 1.725 mgs. testosterone propionate.

Testosterone propionate has a similar action in mammals to that of methyl testosterone. Its action, however, is greater and more prolonged than that of testosterone, due to the delay in absorption and excretion by the animal. The intramuscular route of administration further delays absorption and thus prevents rapid excretion of the hormone.

In Xenopus males, however, even the intramuscularly-injected esterified testosterone is inactive both androgenically

and gonadotrophically. This contrasts markedly with the gonadotrophic action of testosterone propionate in Xenopus females (Shapiro, 1936 c).

Oestradiol Benzoate.

Experiment 1.

Intraperitoneal Injection Of Oestradiol Benzoate Into Normal Males.

"Progynon B oleosum forte" prepared by Messrs. Schering-Kahlbaum was used in this experiment. It is set up in ampoules of 1 ml. of oil containing 5 mgs. of oestradiol benzoate. 1 ml. of this was added to 19 mls. of nut oil, the strength of the dilution thus was 250 y/ml.

19 padless males were injected intraperitoneally on alternate days with 1.0 ml. of the diluted solution, until 5 injections had been made.

The forelimbs were examined daily until two days after the last injection.

Results:

There was no development of pads in this experiment.

Discussion.

1.250 mgs. of oestradiol benzoate is inactive in male Xenopus. The esterification of oestradiol results in greater activity of the hormone in mammals, but still no effect is seen in Xenopus males. It can be concluded from this experiment that oestradiol benzoate shows no gonadotrophic or androgenic activity in male Xenopus.

Experiment 2.

Intramuscular Injection Of Oestradiol Benzoate Into Normal Males.

"Progynon B oleosum Forte" was again used. 1.0 ml. was added to 3.0 mls. of nut oil. The strength of the solution used was thus 250 y in 0.2 ml.

6 padless males were injected intramuscularly with 0.2 ml. of the diluted solution (250 y) on alternate days for 5 injections. The 1st, 3rd and 5th injections were made into the right leg and the 2nd and 4th injections into the left leg.

The arms were examined daily until the second day after the last injection.

Results:

There was no development of pads.

Discussion.

Further delay in excretion of injected oestradiol benzoate by intramuscular administration fails to produce androgenic activity of the hormone. This result confirms the findings above, namely, that oestradiol benzoate is neither gonadotrophic or androgenic to the male Xenopus.

Oestradiol benzoate in mammals is a bisexual hormone with mainly gynaecogenic activity. In Xenopus males there is no such activity. The action of oestradiol benzoate in Xenopus females is considered in a later section. Shapiro & Zwarenstein (1937)

found that it had no gynaecogenic activity in Xenopus females. It would appear, therefore, that oestradiol benzoate is inactive in Xenopus laevis.

Progesterone.

Experiment 1.

Intramuscular Injection Of Progesterone Into Castrated Males.

"Proluton" prepared by Messrs. Schering-Kahlbaum was used. Each ampoule contains 5 mgs. of progesterone dissolved in 1.0 ml. of oil. 1.0 ml. is added to 3.0 mls. of nut oil, the resulting dilution containing 250 y of progesterone in 0.2 ml.

Five frogs castrated according to the method described previously received daily intramuscular injections for 4 days.

The forelimbs were examined daily during the experiment and on the day following the last injection.

Results:

There was no development of pads.

Discussion.

1.0 ml. Progesterone in divided doses over 4 days is inactive androgenically in Xenopus males. The intramuscular route was again chosen to increase the effectiveness of the hormone, but no activity resulted.

Experiment 2.

Intramuscular Injection Of Progesterone Into Normal Males.

"Proluton" diluted as described above was used again. Each injection consisted of 0.2 ml. of the diluted solution (250 y of progesterone).

250 y of progesterone were injected in this oily solution intramuscularly into 20 padless males. Injections were made on alternate days on five occasions into alternate legs.

250 y of progesterone is a dose which produces ovulation in 50 % of animals in January, the month in which these experiments were performed.

The forelimbs were examined daily until the 11th day of the experiment.

Results:

There was no development of pads.

Discussion.

The results obtained in this experiment confirm the finding above, that progesterone is not androgenic in male Xenopus. Also, no gonadotrophic activity was demonstrated with the dose used, which was 1.25 mgs. The longer time allowed for the action of the progesterone by injection on alternate days did not alter the results.

Again the difference between the effects of progesterone in mammals and Xenopus females on the one hand, and Xenopus females on the other, is striking. Progesterone in mammals is the only purely unisexual sex hormone. It has a progestational effect on the females. In Xenopus females progesterone is gonadotrophic (Zwarenstein, 1937) and causes ovulation and through the agency of the ovary it produces hyperaemia of the female cloacal labia. (Shapiro & Zwarenstein 1937). In Xenopus males progesterone is inactive. There is no gonadotrophic activity.

Summary Of Results.

The results obtained with the steroids used above are summarised in table VI below.

Steroid.	No. and type of animals.	Injections.	Route of administration.	Total dose per frog (mgs).	Pads.
Methyl	5 Castrate	Daily for four days.	Intramuscular	1.00	-
Testo-sterone	18 Normal	Alternate days for five injections.	Intraperitoneal	1.25	-
	6 Normal	1st,3rd,5th, 7th,9th, 12th and 14th days.	Intramuscular	1.75	-
Testo-sterone	5 Castrate	Daily for four days.	Intramuscular	1.00	-
Propi-onate.	20 Normal	1st,3rd,5th 7th,9th, 12th and 14th days.	Intramuscular	1.75	-
Oestra-diol.	19 Normal.	Alternate days for five injections.	Intraperitoneal	1.25	-
Benzo-ate.	6 Normal.	Alternate days for five injections.	Intramuscular	1.25	-
Progest-erone.	5 Castrate	Daily for four days.	Intramuscular	1.00	-
	20 Normal	Alternate days for five injections.	Intramuscular	1.25	-

Table VI. Injection of certain steroids into Xenopus males.

Discussion.

From the results recorded above it appears that the male sex hormone of Xenopus is different from the testicular hormone of mammals - testosterone. The results of injection with sheep and Xenopus anterior pituitaries indicate that the testicular hormone of Xenopus is capable of producing the development of pads on the arms of the males. Testosterone, on the other hand, has no such action in males, even when more potent derivatives are used, or when the hormone is esterified and injected intramuscularly to ensure a high percentage of utilization of the full hormone. Provided that the dosage is not too low, this affords definite evidence of a difference between the two hormones.

In mammals it has been shown that testosterone exerts an inhibitory effect on the ovary. (Zuckerman 1937). In Xenopus females, on the other hand, testosterone is gonadotrophic and activates the ovary (Shapiro, 1936 c), causing ovulation and hyperaemia of the cloacal labia. This work demonstrates a difference in the reaction of the male and female Xenopus to the same substance. This difference may take one of two forms; (a) the female may react to testosterone, while the male does not; (b) it is possible that a very much larger dose is required to stimulate the male than that needed to stimulate the female, i.e., that the dosage used in the experiments above are too small.

Confirmation of the first suggestion would support the view that the Xenopus and mammalian testis hormones are different.

If the second suggestion is valid, it is a gonadotrophic effect that is to be expected and it is extremely unlikely that the Xenopus testis hormone is capable of stimulating the Xenopus testis.

Thus, on the basis of this evidence, it seems fairly apparent that the Xenopus testis hormone is not testosterone, the mammalian testis hormone.

Summary.

1). Acid and pyridine extracts of sheep anterior pituitary induce the development of pads in normal but not in castrated males.

2). Pyridine extracts of sheep anterior pituitary induce pad development in anterior lobe hypophysectomised males, but the rate of development of the pads is slower than in normal males.

3). The above finding is probably explained by the loss of sensitivity of the hypophysectomised animals to gonadotrophic injections.

4). Anterior pituitaries from both male and female Xenopus produce pads in normal Xenopus males.

5). Xenopus anterior lobes do not cause pad development in castrate males.

6). Captivity results in the abolition of the pad cycle.

7). The evidence presented indicates that the pad cycle under natural conditions is under the control of the anterior pituitary acting via the testis.

8). The development of the pad is described macroscopically and microscopically.

9). The inhibition of the anterior pituitary by captivity (Shapiro & Shapiro 1934) consists in the prevention of the pouring

out of the gland of its gonadotrophic principles. Secretion is not inhibited.

10) The pads play no essential role in the mating reflex in Xenopus laevis.

11) Using the development of pads as a test-object for androgenic and gonadotrophic activity in the male Xenopus, methyl testosterone, testosterone propionate, oestradiol benzoate and progesterone are shown to possess neither of these two properties.

12) The evidence presented suggests that the Xenopus testis hormone differs from the mammalian testis hormone testosterone.

SECONDARY SEX CHARACTERS OF THE MALE.

II. The Forelimbs.

Introduction.

Sexual dimorphism in the forelimbs of amphibia is a well known phenomenon. (Gaupp 1886; Arom 1926; Noble 1931; Rostand 1934). In addition in the case of Rana it is known that this difference is due to the fact that the flexor carpi radialis muscle and the upper head of the extensor carpi radialis muscle are very much larger in the male than in the female. Associated with this difference in these muscles of the forearm, there is a ridge on the medial side of the humerus in the male from which these muscles take origin (Gaupp 1886). This ridge is not present on the humerus of female frogs.

It has also been noted by many workers (inter alia Gaupp 1886) that there is a cyclical change in the size of the forelimbs of male frogs, the largest size being associated in point of time with the breeding season. Gaupp(1886) also records that the ridge on the medial side of the humerus is enlarged at the time of the breeding season.

Smith (1938) has followed the course of the hypertrophy and decline of the brachial musculature in frogs and toads by measuring the strength of clasps artificially produced at different times of the year. He has also traced a correlation between the cycle in the strength of the artificial clasps and the cycle in the interstitial tissue of thetestis.

The investigation here was undertaken with a view to determining whether a similar cycle exists in the forelimbs of the male Xenopus laevis. A distinct difference in the forelimbs of male and female Xenopus can be seen, the male forelimb being somewhat larger than that of the female. The studies of the forelimb size in Xenopus laevis were, therefore, placed on a quantitative basis by estimating what percentage the forelimbs are of the total body weight.

Determination Of The Arm/Body Weight Ratio.

The frogs are killed by immersion in a mixture of ether and water for 5 to 10 minutes, and are then dried and weighed. The weight is read correct to the nearest 0.5 gm.

Both arms are removed and are weighed separately on a torsion balance. The mean of the two readings is the "arm weight" and is divided by the total body weight to give the arm/body weight ratio. This figure is expressed as a percentage.

Technique Of Removing The Arms.

The blades of a scissors are placed against the side of the frog, one above and one below the axilla. The arm is cut off by approximating the blades of the scissors, keeping them against the side of the body all the time.

Seasonal Changes In The Arm/Body Weight Ratio Of Pond Animals.

Male and female frogs freshly collected from the ponds are kept for 2 days in the laboratory before being used, to allow the intestinal contents to be passed. On the third day the arm/body weight ratios are estimated according to the method described above.

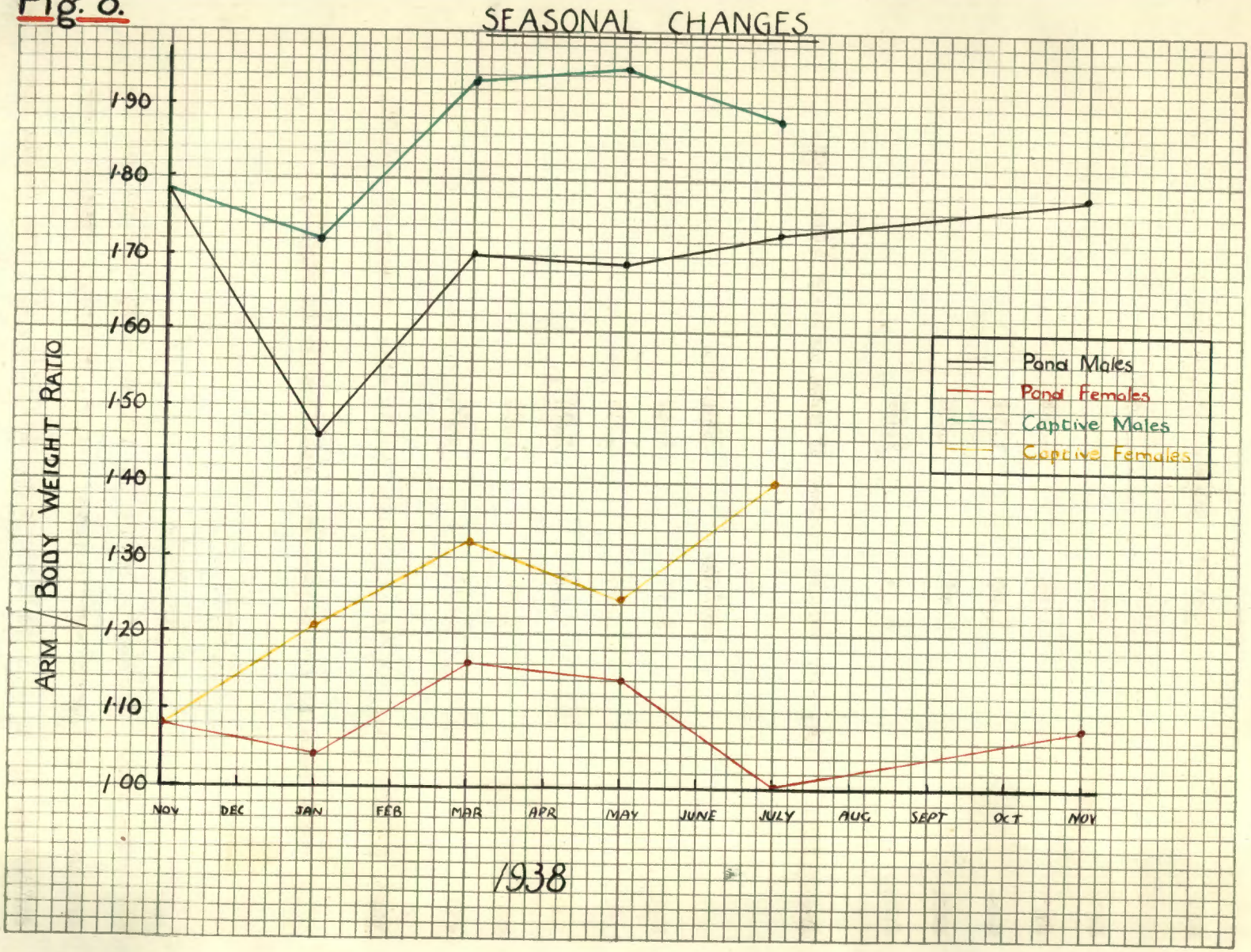
Readings were taken during the first week of every second month - November, 1937, January, March, May and July 1938. 15 males and 15 females were used on each of these occasions.

In addition, in November, 1937, large numbers of male and female frogs were collected and kept in captivity for 1 year. They were fed with raw minced meat once a week and the water of their containers was changed twice a week.

The arm/body weight ratios of 15 "captivity" males and 15 "captivity" females were estimated at the same time as those of the freshly caught frogs.

Results: (Protocols 2 - 19).

Fig. 8.


SEASONAL CHANGES
ARM / BODY WEIGHT RATIO
1·90
1·80
1·70
1·60
1·50
1·40
1·30
1·20
1·10
1·00
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG.
SEPT
OCT
NOV
Pond Males
Pond Females
Captive Males
Captive Females
1938

Month.	Arm/Body Weight Ratio.(%).	
	Males.	Females.
Nov.	1.78	1.08
Jan.	1.46	1.04
Mar.	1.70	1.16
May.	1.69	1.14
July.	1.73	1.00

Table VII. Arm/body weight ratios of pond animals 1937-1938.

	Males.	Females.
Nov.	1.78	1.08
Jan.	1.72	1.21
Mar.	1.93	1.32
May.	1.95	1.25
July.	1.88	1.40

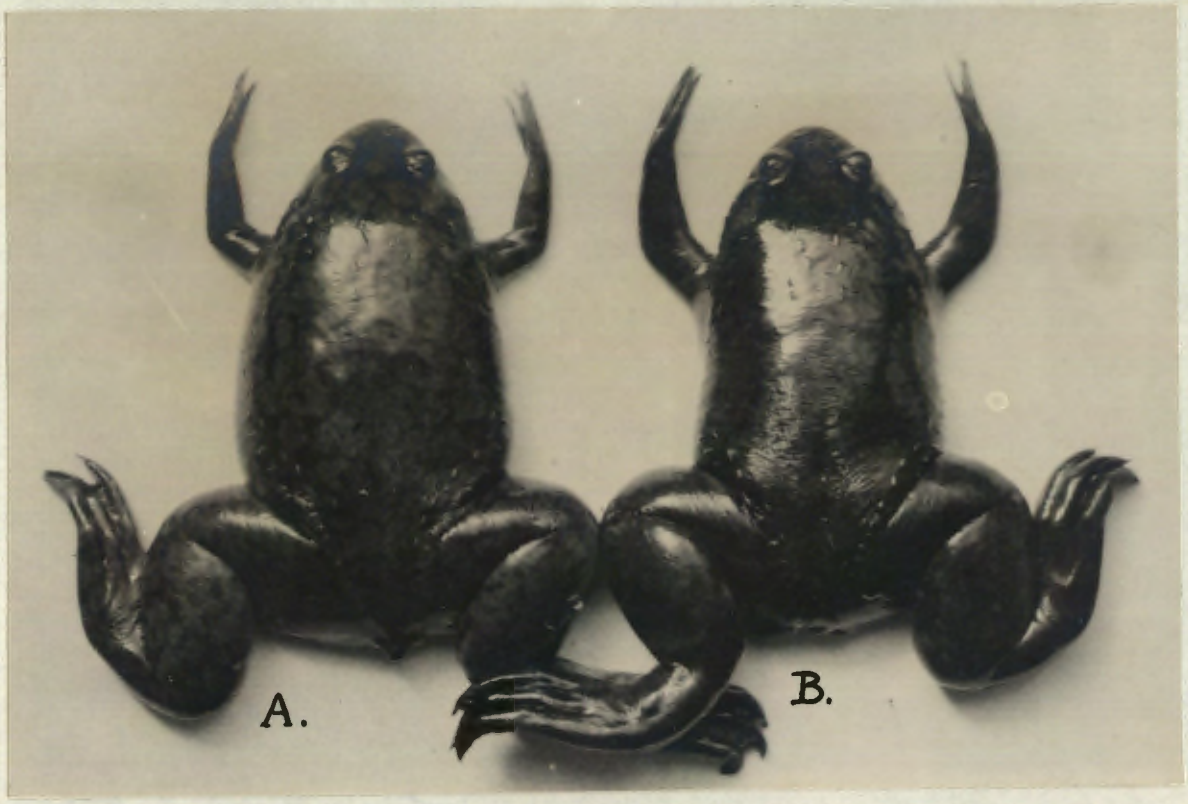
Table VIII. Arm/body weight ratios of captive animals 1937-1938.

These results are represented graphically in Fig. 8.

Discussion.

In The Pond.

The first striking point is the marked difference in the arm/body weight ratios of the males and the females. This difference can be seen with the naked eye (Fig. 9). The female can be recognised by the presence of the cloacal labia. Each of the



A. Female (Note the cloacal labia).

B. Male.

Fig. 9.

Sexual dimorphism in the forelimbs of Xenopus laevis.

frogs in Fig. 9 weighed 38.0 gms. The photograph was taken in January, when the difference between the forelimbs of the males and females is at its lowest level. The ratio, however, forms a quantitative means of studying this difference in the sexes.

Thus it can be seen that this sex difference is maintained throughout the year, being most marked during the breeding season and least in January, midway between two breeding seasons.

Another prominent feature is the well-marked seasonal variation that occurs in the ratios of both the males and the females.

Seasonal Cycle Of Arm/Body Weight Ratio In Male Xenopus.

The curve for the arm/body weight ratio of the males can be conveniently divided into 4 phases:-

(i) In January, the mid-point between the breeding seasons, the forelimbs are smallest, relative to the weight of the whole animal.

(ii) From January onwards there is an increase in the forelimbs until the breeding season (July to September).

(iii) During the breeding season the ratio is maintained at a high level.

(iv) Then a decline occurs to the summer value.

Seasonal Cycle Of Arm/Body Weight Ratios In Female Xenopus.

In the female arm there are 3 phases to be seen.

(i) The forelimbs of the females are smallest during the breeding season.

(ii) During the next phase of the cycle, the arm/body weight ratio rises to a somewhat higher level until January.

(iii) From January until July, the onset of the breeding season, the forelimbs of the females are still further increased relative to the total body weight.

Thus the highest point of the curve for the forelimbs of the males corresponds with the lowest part of the curve for the females' forelimbs. The lowest point on the males' forelimbs curve occurs at the same time as the slightly increased phase of the cycle in the forelimbs of the female. The second phase in the males' forelimbs is associated with the highest position of the curve representing the arm/body weight ratios of the females.

In Captivity.

The effect of captivity on the arm/body weight ratio cycles in the male and female is discussed in a later section.

Factors Influencing The Arm/Body Weight Ratio.

An increase or decrease in the arm/body weight ratios can occur in many ways.

An increase can be due to an increase in the size of the arm, while the total body weight remains constant; or an apparent increase in the arm can occur when the total body weight falls and the arm weight remains constant.

A decrease in the ratio is produced by the opposite changes i.e. a decrease in the weight of the arm, or an increase in the total body weight of the animal.

Various combinations of the above factors may occur and the effect on the arm/body weight ratio will depend on the degree of change in the arm weights and in the total body weights relative to each other.

The most important factor that affects the total body weight is the state of nutrition of the animals. Thus during a period of lowered food intake and assimilation, the ratio may be expected to rise and when food intake and assimilation are increased, the ratio will fall.

The amount of food and faeces actually in the gastro-intestinal tract will also affect the total body weight of the animals. Since this may amount to 2 to 3 grammes, each animal was opened up after being weighed, and the contents of the stomach

and intestine were weighed separately and subtracted from the previous reading to give the true total body weight. Bellerby (1938) has reported that starvation of the animals for a week previous to the weighing eliminates this difficulty. Observations of another worker (Gitlin,private communication) show that even after a week without food the gastro-intestinal contents may weigh up to 2 grammes.

In the female an important factor is the weight of the ovary, which itself undergoes marked seasonal variations in pond animals (Shapiro and Shapiro 1934). Since the ovaries constitute about one tenth of the total weight of the females, this is an important consideration. In the male the testis accounts for much less of the weight of the animal and any variations which may occur are not detectable by inspection. The testes thus are unimportant in connection with the arm/body weight ratio.

Wasting of the muscles of the forelimbs is a factor that may produce a decrease in the arm/body weight ratio.

The difference in the arm/body weight ratios of the males and females indicates that the larger arm size of the male is a secondary sex character of the male. Therefore, an investigation into a possible pituitary-gonadal influence on the arms of the males was undertaken.

Experiments were performed to determine the role of the factors mentioned above in the seasonal cycles in the arm/body weight ratios of the males and females.

Summary.

1) The method of calculating the arm/body weight ratio is described.

2) The arm/body weight ratio of the male is larger than that of the female.

3) Seasonal cycles are seen in the arm/body weight ratios of both male and female Xenopus. These cycles are described.

4) The cycle in the male shows four phases.

a. The arm/body weight ratio is smallest in January.

b. The ratio increases until July.

c. During the breeding season the ratio is maintained at a high level.

d. A decline to the summer level follows the breeding season.

5) 3 phases are seen in the arm/body weight ratio cycle in the female.

a. The lowest level is seen during the breeding season.

b. From then until January the ratio is higher.

c. The highest level is seen between January and July.

Experimental Investigation Of The Factors Controlling The Seasonal Cycle In The Arm/Body Weight Ratio.

I. The Role Of The Anterior Pituitary And The Testis.

Experiment 1.

The Effect Of The Injection Of Acid Extracts Of Sheep Anterior Pituitary On The Arm/Body Weight Ratio Of The Male Xenopus.

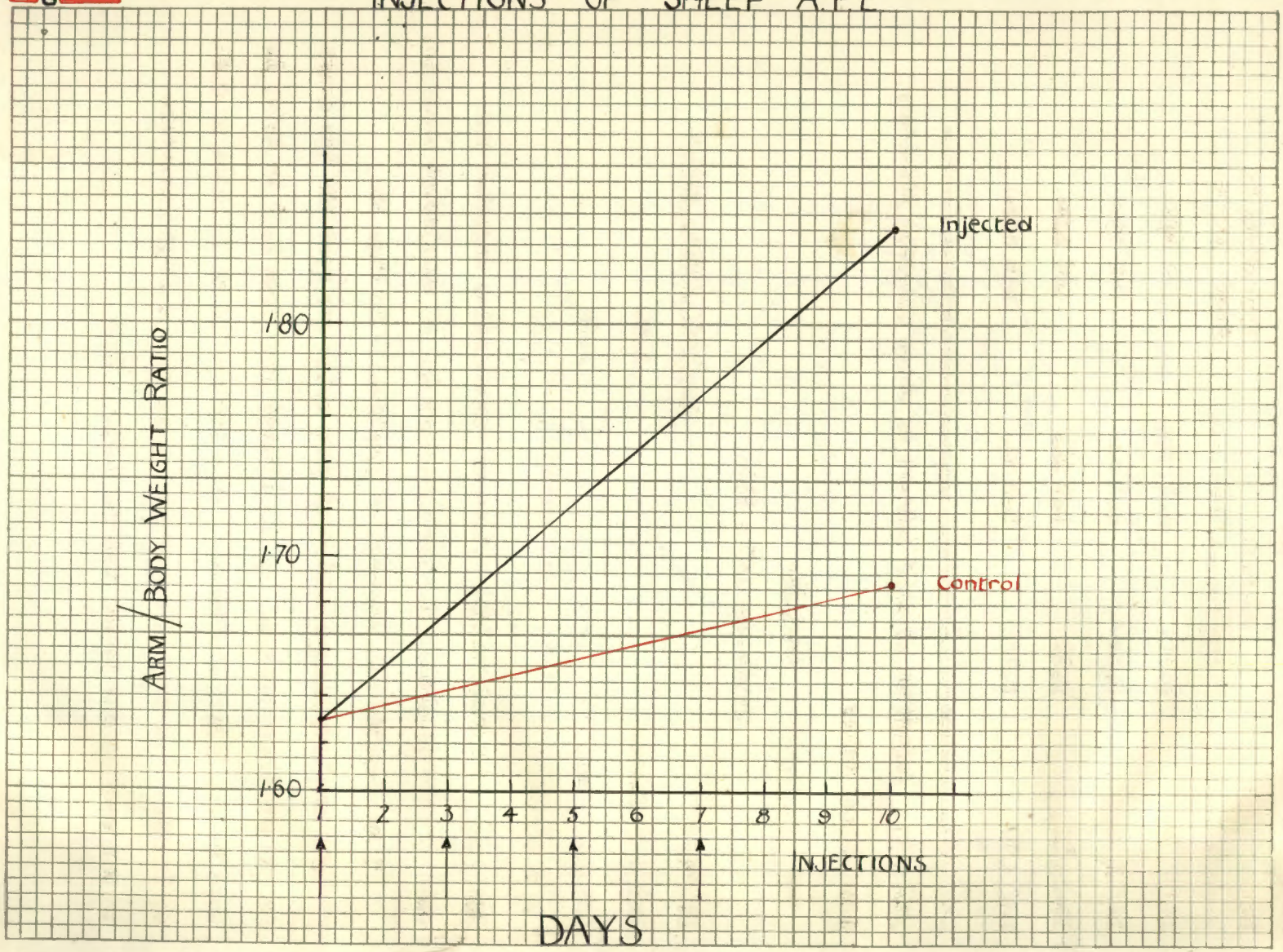
This experiment was commenced on the 14th of December 1937. 32 freshly caught males were used. 16 of these were injected with a single dose of 0.8 ml. of acid extracts of Sheep Anterior Pituitary intraperitoneally on alternate days until 4 injections had been made. On the tenth day (23/12/37) the frogs were killed by immersion in a mixture of ether and water and their arm/body weight ratios were estimated.

The remaining 16 males were used as a control. They were kept in a bath identical in shape and size with that containing the experimental frogs and filled with tap water to the same level, but they received no injections.

At the end of the experiment the frogs were killed and their arm/body weight ratios were determined.

Neither of the two groups of frogs was fed during the experiment.

Fig. 10.


INJECTIONS OF SHEEP A.P.E.
ARM / BODY WEIGHT RATIO
1·80
1·70
1·60
Injected
Control
1
2
3
4
5
6
7
8
9
10
INJECTIONS
DAYS

Date.	Extract. Number.	Vol.	Equivalent Of Fresh Tissue.
14/12/37	S.23	0.8 ml.	528 mgs.
16/12/37	S.23	0.8 ml.	528 mgs.
18/12/37	S.22	0.8 ml.	456 mgs.
20/12/37	S.19	0.8 ml.	528 mgs.

Table IX. Injection of acid extract of sheep anterior pituitary into normal males.

Results.

Protocols (20 & 21).

Normals.	Arm/Body Weight Ratio.
Injected Males.	1.84
Control Males.	1.69
Pond Males.	1.63

Table X. Arm/body weight ratios of normal males injected with acid extract of sheep anterior pituitary and of uninjected controls.

These results are represented graphically in Fig. 10.

The arm/body weight ratios of pond males are read off the graph in the previous section. A significant rise from 1.63 to 1.84, is seen in the injected animals. This represents an increase of 0.21 or 12.9%. The control uninjected males show a slight increase in arm/body weight ratio, 0.06 or 3.7%. This

is not a significant change.

Discussion.

Males injected intraperitoneally with acid extracts of sheep anterior pituitary extract show a rise in the arm/body weight ratio. Control uninjected animals fail to show this rise. Thus an increase in the ratio is produced by the anterior pituitary. It is clear also from the absence of a rise in the control animals that the increase in the ratio is not due to a decrease of total body weight, therefore, anterior pituitary injections either directly or indirectly produce an increase in the weight of the arms of the male.

It is remarkable to note the rapidity with which the weight of the arm rises - 12.9% in 10 days.

During the course of the experiment the injected animals were seen to develop pads on their forelimbs. This fact demonstrated that the extract was gonadotrophically active.

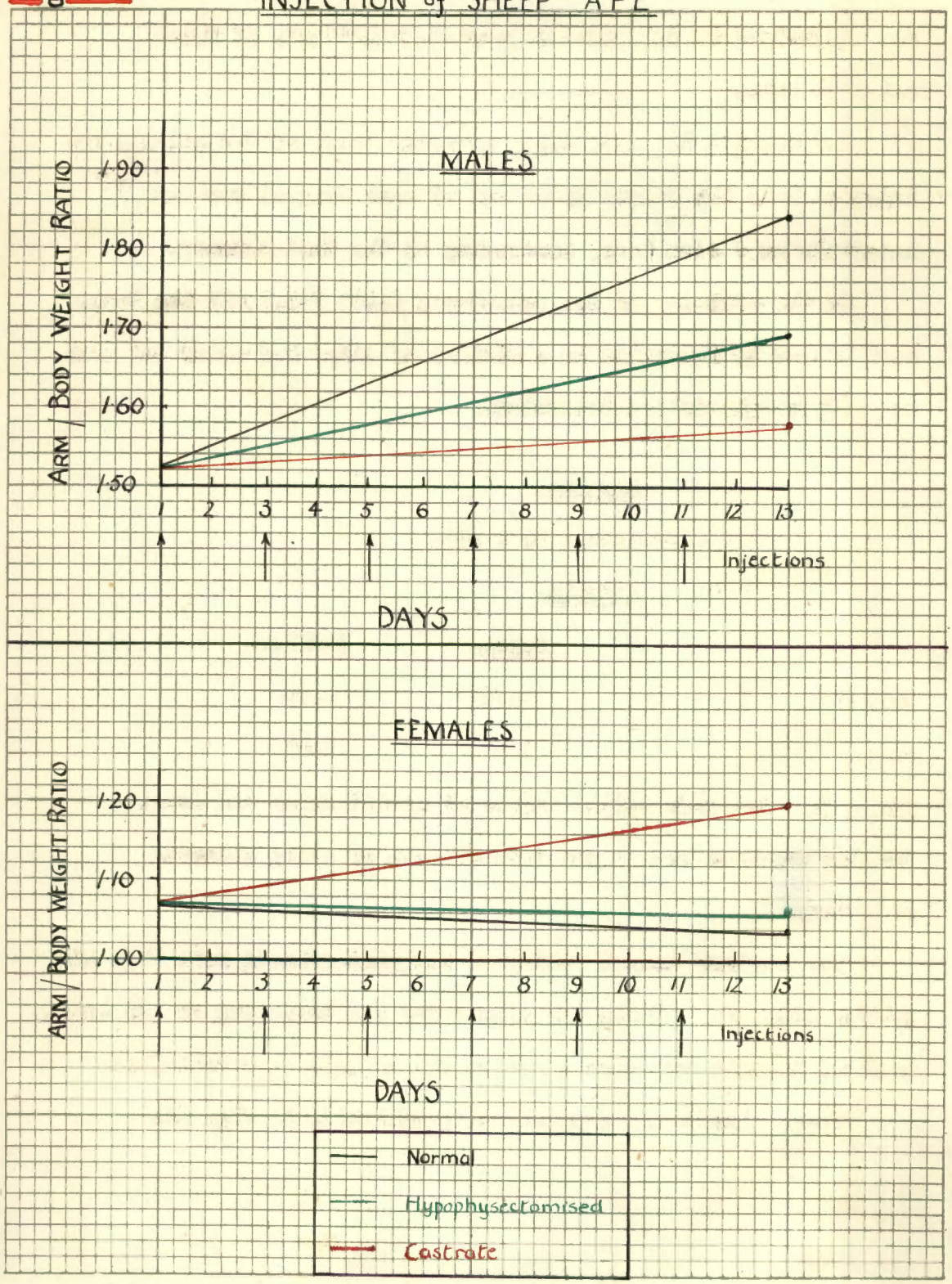
Experiment 2.

The Effect Of The Injection Of Pyridine Extract Of Sheep Anterior Pituitary On The Arm/Body Weight Ratios Of Normal Males And Normal Females.

6 males and 12 females were used in this experiment. They came from a large batch of frogs collected between the 14th and 19th of January, 1938.

A pyridine extract of sheep anterior pituitary prepared

Fig. 11.


INJECTION of SHEEP APE
MALES
ARM / BODY WEIGHT RATIO
1·90
1·80
1·70
1·60
1·50
1 2 3 4 5 6 7 8 9 10 11 12 13
Injections
DAYS
FEMALES
ARM / BODY WEIGHT RATIO
1·20
1·10
1·00
1 2 3 4 5 6 7 8 9 10 11 12 13
Injections
DAYS
Normal
Hypophysectomised
Castrate

according to the method of Rowlands (1937) was used. 1.0 ml. was equivalent to 10.3 mgs. of powder or 534 mgs. of fresh anterior lobe.

0.5 ml. (≡ 267 mgs. fresh anterior lobe) of the extract was injected into the dorsal lymph sac of each frog at two-day intervals on 6 occasions, commencing on the 31st January.

On the 13th day of the experiment (12th Feb.) all the frogs were killed by immersion in a mixture of ether and water and their arm/body weight ratios determined.

Results.

(Protocols 22 & 23).

Normals.

	Males.	Females.
Injected.	1.84	1.04
Pond.	1.52	1.07

Table XI. Arm/body weight ratios of normal males and normal females injected with a pyridine extract of sheep anterior pituitary.

The arm/body weight ratios of the pond animals were read off graphs (Fig. 8). It is the figure for the beginning of the third week in January, the time at which the animals were collected.

These figures are represented graphically in Fig. 11.

Males.

The injected males show an increase in the arm/body weight ratios from 1.52 to 1.84. This is a rise of 0.32 or 21% and is significant.

Females.

There is a slight decrease in the arm/body weight ratios of the injected females, from the pond value of 1.07 to 1.04. This is 0.03 or 2.8% of difference and is not outside the range of normal variation.

Discussion.

Males.

The rise in the arm/body weight ratio of the males produced by injections of pyridine extract of sheep anterior pituitary confirms the findings in the previous section, namely, that the anterior pituitary produces an increase in the weight of the forelimbs of the male.

The route of administration of the extract in this experiment (dorsal lymph sac) did not alter the result of the injections.

Again, there is a remarkable rapidity in the increase in the weight of the arms - 21% in 13 days.

Also, these animals have developed pads on their forelimbs and thus the extracts contained gonadotrophic substances.

Females.

The arm/body weight ratios of the female animals undergo no change as the result of injections of pyridine extracts of sheep A.P.E. The males and females were kept under identical conditions during this experiment and the same extract was used in each case. Also it is unlikely during the short course of the experiment that there was a loss of total body weight and a proportional loss of arm weight, leaving the ratio unaltered. The anterior pituitary, therefore, seems to have no effect on the forelimbs of the female Xenopus.

Ovulation and hyperaemia of the cloacal labia occurred during the experiment, so that the sheep anterior pituitary extract definitely contained gonadotrophic substances. The loss of weight due to extrusion of ova, however, is not large enough to alter the arm/body weight ratio significantly.

There was no development of pads on the females' forelimbs.

Experiment 3.

The Effect Of The Injection Of A Pyridine Extract Of Sheep Anterior Pituitary On The Arm/Body Weight Ratios Of Anterior Lobe Hypophysectomised Males And Females.

15 males and 12 females from the same batch of frogs were hypophysectomised by the method described by Hogben (1923). Only the anterior lobes were removed.

0.5 ml. of the extract described in the previous section was injected into the dorsal lymph sac of each frog at two-day intervals for 6 injections, commencing on the 1st of February, 1938. The frogs were killed on the 13th day (13th February) and the arm/body weight ratios estimated.

Results.

Protocols 24 & 25.

	Males.	Females.
Injected.	1.69	1.05
Pond.	1.52	1.07

Table XII. Arm/body weight ratios of hypophysectomised males and females injected with a pyridine extract of sheep anterior pituitary.

The arm/body weight ratios of pond animals were read off the graph (Fig. 8) in the same way as described above.

These results are represented graphically in Fig. 11.

Males.

The hypophysectomised injected males show a rise in the arm/body weight ratio of 0.17 or 11.2% as compared with the 21% rise that is found in normal injected males collected and injected at the same time. The same doses of the same extract being used. (see above).

Females.

Again the drop in the arm/body weight ratio is too small to be of any significance (0.02 = 1.9%).

Discussion.

Males.

The animals used were kept under exactly the same conditions as the normal animals in the previous section. Hypophysectomised males injected with pyridine extracts show a rise in arm/body weight ratio, but this rise is about half as large as the rise that occurs in normal animals treated in exactly the same way.

Also pad development occurred, but a longer time elapsed before they were complete than in the case of the normal injected animals.

Thus the male Xenopus is dependent upon its own pituitary for the full effect of the pyridine extract of sheep anterior pituitary on its forelimbs. The hypophysectomised animals showed full expansion of their melenophores on a white background, thus only the anterior lobes were removed from these animals. From this it may be concluded that the anterior pituitary of the male Xenopus contains a substance which assists the action of the injections of sheep anterior pituitary in increasing the weight of the forelimbs. This substance is not essential for the action of the sheep anterior pituitary extract, which, therefore, does not exert its effect through the mediation of the anterior lobe of the animal's pituitary.

The results recorded in the section on the pads support the conclusion that the sheep anterior pituitary extract does not act through the animal's own pituitary. In addition, they show that

the action of the sheep anterior pituitary injections is retarded by the absence of the anterior lobe of the animal's own pituitary and thus confirms the conclusion that the latter assists the action of the former. The lack of acceleration of the effect of the sheep anterior pituitary accounts for the smaller rise in the arm/body weight ratios of anterior lobe hypophysectomised males as compared with a rise twice as great in the normal animals.

Females.

In the females there is again no change in the arm/body ratio, which supports the conclusion that the sheep anterior pituitary is without effect upon the forelimbs of the female Xenopus. There was no development of pads, also no inhibition by the anterior lobes of the pituitaries of female Xenopus of some possible action of the sheep anterior pituitary injections on the forelimbs of females occurs. Ovulation and hyperaemia of the cloacal labia occurred in this experiment, again demonstrating the gonadotrophicity of the injections.

It is interesting to note here that injection of acid extracts of sheep A.P.M. into hypophysectomised male and female Xenopus induced the mating reflex after the usual latent post-injection interval of 10 to 12 hours. The percentage response was also unaffected by hypophysectomy (Shapiro 1937 a). On the other hand, repeated injections of pyridine extracts of sheep anterior pituitary produced a larger rise in the oviducts in normal female Xenopus than in those that had their anterior lobes

removed. (Citlin, 1939).

Experiment 4.

The Effect Of The Injection Of A Pyridine Extract Of Sheep Anterior Pituitary On The Arm/Body Weight Ratios Of Castrated Males And Females.

13 males and 11 females from the batch of frogs collected between the 14th - 19th of January, 1938, were castrated according to the methods described above.

Injections were made into the dorsal lymph sacs of the animals to prevent loss of extract through the operation wounds.

Each animal received 6 injections of the extract previously described. 0.5 ml. was injected at two-day intervals, commencing on the 2nd of February. The frogs were killed on the 13th day of the experiment (14th Feb.) and their arm/body weight ratios estimated.

Results.

Protocols 26 & 27.

	Males.	Females.
Injected.	1.59	1.20
Pond.	1.52	1.07

Table XIII. Arm/body weight ratios of castrated males and females injected with a pyridine extract of sheep anterior pituitary.

The arm/body weight ratios of the pond animals were again read off the graph in an earlier section. (Fig. 8).

These figures are represented graphically in Fig. 11.

Males.

There is a slight rise in the arm/body weight ratios of injected male castrates. The size of this increase is 0.07 or 4.6% and is too small to be of any significance.

Females.

A larger increase, 0.13 or 12.1% is seen in the castrated injected females. This is a significant increase.

Discussion.

Males.

There is no significant change in the arm/body weight ratio of castrate males treated in exactly the same way as the normal and hypophysectomised animals. Thus in the absence of the testis, injections of sheep anterior pituitary extracts fail to produce any change in the forelimbs of male Xenopus. Injections were made in this experiment, as in the normal and hypophysectomised animals, into the dorsal lymph sacs of the males. This precludes the possibility of the loss of the injection through the ventral abdominal wound. There was no development of pads.

The sheep anterior pituitary injections thus act on the forelimbs by stimulation of the testis. This stimulation of the testis can be produced by the sheep pituitary injections alone, but it is facilitated and accelerated in the presence of the anterior lobe of the animal's own pituitary.

Females.

In the females the arm/body weight ratio rises by 12.1%. It has been shown before that the sheep anterior pituitary injections

do not produce any change in the arms of the female Xenopus.

Thus some alteration in the total body weight must have occurred. The ovaries of female Xenopus at the time that they were caught (about the middle of January) form 7% of the total weight of the animal. (Shapiro & Shapiro 1934). The arm/body weight ratio at the same time of the year in pond females is 1.07. Thus as a result of castration the expected arm/body weight ratio is 1.15. This value is .05 different from the value recorded in the experiment, i.e. a difference of 4.2% between the calculated result and the figure obtained in the experiment. This difference is too small to be of any significance and thus the high figure obtained for the castrate females is due solely to the removal of the ovaries.

There was no development of pads.

Again the experiment shows that there is no inhibition by the ovaries of any possible action of the injections of sheep anterior pituitary on the forelimbs of the females.

Thus it can be seen that the forelimb weight of the male is under the hormonal control of the anterior pituitary acting by stimulating the testis which in turn causes an increase in the size of the forelimb of the male.

The female forelimb is unaffected by the secretions of the anterior pituitary or the testis.

II. The Effect Of Captivity, Starvation, Hypophysectomy And Castration On The Arm/Body Weight Ratio.

A. The Effect Of Captivity On The Seasonal Cycles Of Male And Female Frogs Under Natural Conditions (See Fig.8).

Males.

In captivity the normal cycle in the arm/body weight ratio fails to occur. Instead a curve with two phases is obtained.

(i) In the first two months of captivity there is little change in the arm/body weight ratio.

(ii) For the next four months the arm/body weight ratio rises to its highest point, which is the highest value yet recorded for an arm/body weight ratio in Xenopus laevis, and is maintained at a high level.

Females.

In the captive females, also, the normal cycle is not seen. The curve commences to rise immediately and continues to rise.

All the animals were observed to become progressively thinner, in spite of the fact that they were fed with raw minced meat twice per week throughout and were kept in large containers with adequate amounts of water to avoid overpopulation.

B. The Effect Of Starvation, Hypophysectomy And Castration.

The factors mentioned in connection with the seasonal cycles influence the arm/body weight ratios of captive animals also. Thus experiments were devised to deal with each of these separately as far as possible.

Large numbers of males were collected between the 14th and 19th of January. These were divided into four groups.

Group I.

The frogs in this group were kept in large tanks and were fed twice a week according to the methods of Alexander & Bellerby (1938).

Group II.

The second group of animals was kept in tanks the same size as those containing the frogs of Group I, but they were never fed.

Group III.

These animals were hypophysectomised according to the method of Hogben (1933). After recovery from the operation they were placed in baths with white bottoms and white sides. Only those animals showing maximal expansion of their melanophores were used. These thus had only the anterior lobes of their pituitaries removed. The animals in this group were fed in the same way as those in Group I.

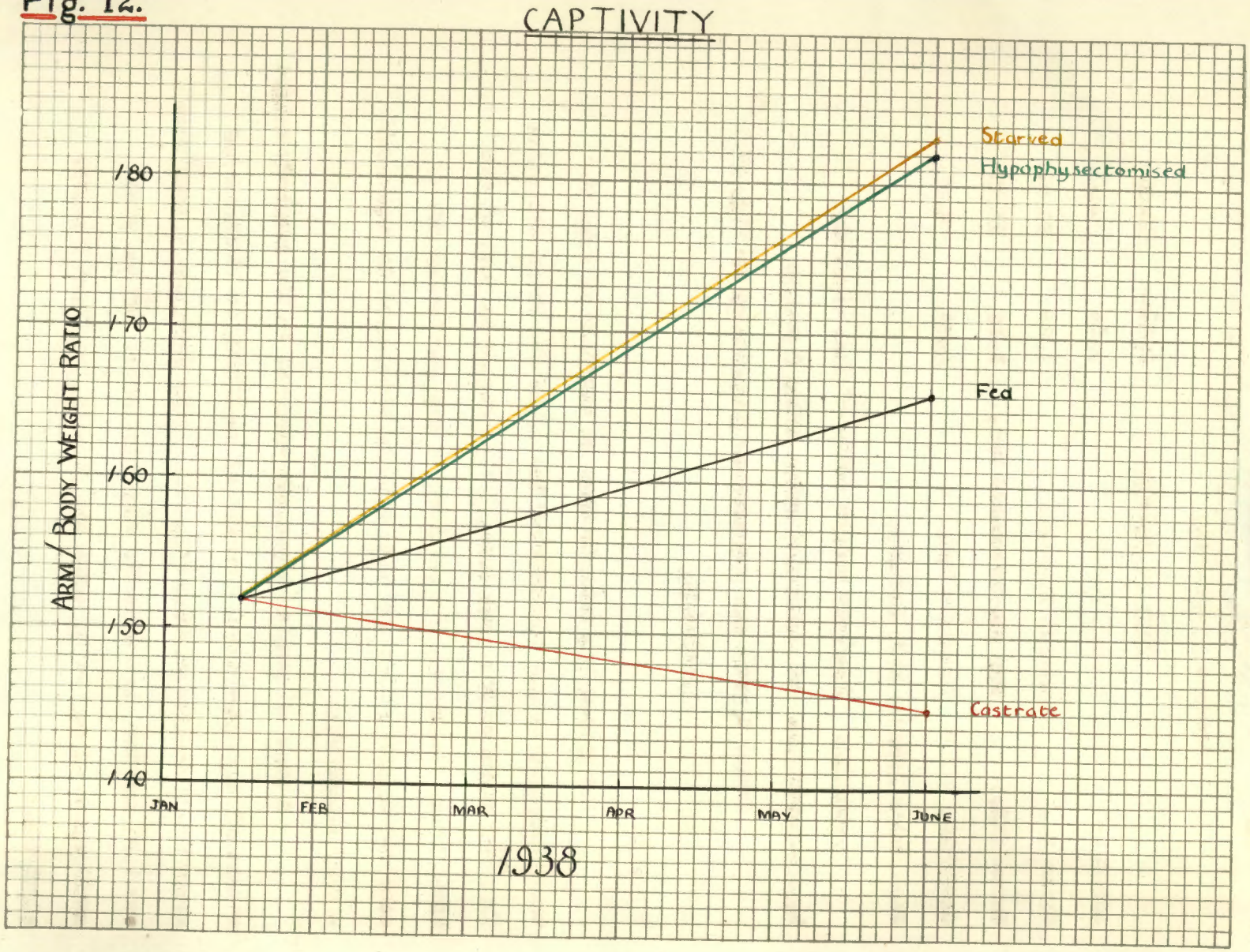
Group IV.

The last group of frogs was castrated according to the method described above. These animals were fed during the course of the experiment in the same way as the frogs of Groups I and III.

The frogs of Groups III and IV were also kept in tanks the same size as those containing Groups I and II.

5 months later 12 frogs in each group were killed by immersion in a mixture of ether and water and their arm/body weight ratios were estimated.

Fig. 12.


CAPTIVITY
ARM / BODY WEIGHT RATIO
1·80
1·70
1·60
1·50
1·40
JAN
FEB
MAR
APR
MAY
JUNE
Starved
Hypophysectomised
Fed
Castrate
1938

Results.

Protocols 28 - 31.

	Type of animal.	Ratio.	Change.
Group I.	Normal Fed.	1.66	9.2%
Group II.	Normal Starved.	1.83	20.4%
Group III.	Hypophysectomised Fed.	1.82	19.7%
Group IV.	Castrate Fed.	1.45	4.6%
Pond males 14th to 19th January.		1.52	

Table XIV. Arm/body weight ratio of fed, starved, hypophysectomised and castrated males after 5 months.

These figures are represented graphically in Fig. 12

Discussion.

Group I.

The Normal fed animals show an increase of 9.2% in their arm/body weight ratios over the value for frogs collected from the ponds at the same time. This figure is just within the highest limits for the readings in any particular set of arm/body weight ratios and thus must be considered to represent a very slight rise.

A rise of similar magnitude occurred in the arm/body weight ratios of the males after 5 months of captivity as shown on the graph in an earlier section (Fig. 8). The increase in that case was 9.0% and the fact that the ratio increased further with continuance of captivity makes the rise in the first 5 months significant.

This rise can be explained by the fact that the animals became somewhat thinner in spite of the feeding. A larger rise in the ratio was probably prevented by the drop in the size of the forelimb. The evidence for the latter statement is as follows. Captivity has been shown in Xenopus laevis to result in depression of the anterior pituitary of the captive animals (Shapiro and Shapiro 1934). In a previous section it has been demonstrated that injections of anterior pituitary produce an increase in the weight of the forelimbs of the male Xenopus. Thus it is probable that the depression of the pituitary caused by captivity resulted in a decrease in the forelimb weight.

Thus taking the two factors together, a very slight rise, occurs in the arm/body weight ratio due to captivity.

Group II.

Starvation of the animals results in a severe loss of body weight, which can be seen easily by inspection of the animals. Any drop in the forelimb weight is too small to offset the decrease in the total body weight, and the ratio thus rises in 5 months by 20.4%.

Taking the results in Group I and Group II, we find that the arm/body weight ratio of the starved animals is 10.3% above the ratio of the fed animals. Since there is no reason for a different degree of drop in the forelimb weights in the two groups, the difference must be explained by a drop in the total

body weight.

Group III.

Hypophysectomised animals that are adequately fed become thinner than the captive fed animals, being approximately as thin as the starved animals. Thus in spite of the fact that a slightly larger drop in forelimb weight than in the captive fed or starved frogs is to be anticipated because of the total absence of the pituitary, the ratio can be expected to be similar to that of the starved animals. The rise of 19.7% is about the same as the rise in the starved animals.

The thin condition of the animal in spite of regular feeding can be explained by the fact that in hyphophysectomised animals the food intake is greatly decreased. (Bellerby 1938). Hypophysectomy thus is complicated by the fact that the animals are in a more or less starved condition, and the effect on the arm/body weight ratio cannot be detected.

Group IV.

The castrates show a slight drop in arm/body weight ratio. This drop is only 4.6% and thus is not significant. These animals also become somewhat thinner than they were before, being similar to the fed captives in appearance. Thus a corresponding drop in the weight of forelimb must have occurred. It is to be expected that a large drop in the size of forelimb will occur when the testis, which is the last link in the pituitary-gonadal control of the forelimb size of the male, is removed. There is the further

evidence that the ratio for the normal fed animals is 14.5% above the value for the castrates. The food intake of the castrates is unaffected, thus this difference in the ratios must be due to a drop in the size of the forelimb of the castrates.

Castration therefore results in a drop in the size of the forelimbs of male Xenopus.

This finding fits in with the results of the injections of anterior pituitary extracts into normal and castrate animals.

These data suggest that the anterior pituitary - testis mechanism is a hormonal one, controlling the size of the forelimbs of the male.

Summary.

1) Injections of acid or pyridine extracts of sheep anterior pituitary into normal male Xenopus results in an increase in the weight of the forelimbs.

2) Under identical treatment, hypophysectomised males also show an increase in the weight of the forelimbs, but this increase is less than in the case of the normal males.

3) This difference is probably due to the loss of sensitivity following hypophysectomy.

4) Anterior pituitary injections fail to alter the weight of the forelimbs of castrated males.

5) From this it is concluded that the anterior pituitary acts on the forelimbs of the males through the intermediation of the testis.

6) No change is produced in the weight of the forelimbs of the females by the injection of anterior pituitary extracts.

7) Captivity alters the naturally occurring seasonal cycle in the arm/body weight ratios of male and female Xenopus.

8) Starvation of the captive animals leads to an increase in the arm/body weight ratio of males due to loss of total body weight.

9) The effect of starvation due to decreased food intake masks the effect of hypophysectomy on the arm/body weight ratio.

10). Castration results in a decrease in the size of the forelimbs of the male frog.

11). An hormonal anterior-pituitary-testis control of the forelimb size in Xenopus males is postulated.

12). The important factors influencing the arm/body weight ratio are as follows.

a.In both sexes undernutrition causes an increase in the ratio.

b.In the female, changes in the ovary weights are important.

c.In the male, the anterior pituitary-testis mechanism controls the size of the forelimbs.

INTERPRETATION OF THE SEASONAL CYCLES IN THE ARM/BODY WEIGHT RATIOS OF MALE AND FEMALE XENOPUS OCCURRING UNDER NATURAL CONDITIONS AND IN CAPTIVITY.

I. THE CYCLE IN THE ARM/BODY WEIGHT RATIO OF CAPTIVE FEMALES.

There is a steady increase in the arm/body weight ratios of captive females. It has been shown that no change is produced in the arms of the female by the hormones of the pituitary or the testis. Thus this increase in the ratio must be due to a progressive decrease in the body weight of the females.

During captivity the ovaries of the female retrogress (Shapiro and Shapiro 1934). The extent to which this lowering of the total body weight changes the arm/body weight ratios can be worked out from the figures for the gonad ratio in captive females given by Shapiro and Shapiro (1934).

(i) 7.5 = the gonad ratio at the beginning of captivity.

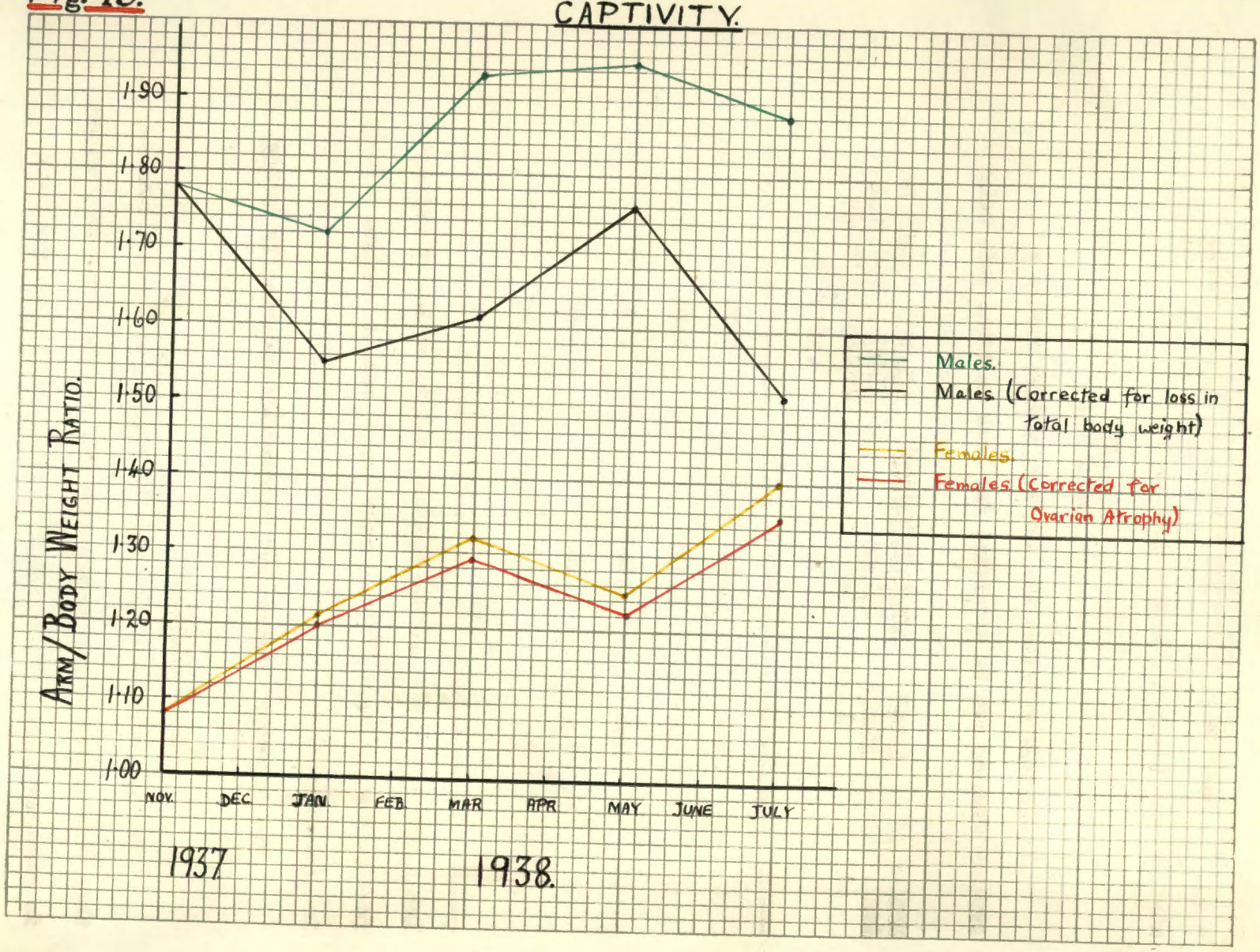
(ii) Let y = the gonad ratio after m months of captivity. Then at the beginning of captivity animal weighs 92.7 + 7.5. After m months of captivity animal weighs (100 - y) + y.

If there were no ovarian atrophy, the animal after m months of captivity would weigh (100 - y) + 7.5 = 107.5 - y.

Let z = the arm/body weight ratio after m months of captivity and let 100 = the total body weight after m months of captivity.

When body weight is 100, arm/body weight = z.

Fig. 13.


CAPTIVITY.
ARM/BODY WEIGHT RATIO.
1·90
1·80
1·70
1·60
1·50
1·40
1·30
1·20
1·10
1·00
NOV.
DEC
JAN.
FEB.
MAR.
APR.
MAY
JUNE
JULY
1937
1938.
Males.
Males (Corrected for loss in total body weight)
Females.
Females (Corrected for Ovarian Atrophy)

. . if body weight were 107.5 - y, i.e. if no ovarian atrophy took place arm/body weight ratio

$$= \frac{z \times 100}{107.5 - y}.$$

Using this equation the arm/body weight ratios of captive females can be corrected for loss in total body weight.

Months of Captivity. (m)	Gonad Ratio. (y)	Arm/body Weight Ratio. (z)	Arm/body Weight Ratio Ratio Corrected For Ovarian Atrophy. $\frac{(z \times 100)}{(107.5 - y)}$.
0	7.5	1.08	1.08
2	6.2	1.21	1.20
4	5.2	1.32	1.29
6	4.4	1.25	1.22
8	3.6	1.40	1.35

Table XV. Correction of the arm/body weight ratios of captive females for the loss of total body weight due to ovarian atrophy in captivity.

The corrected figures are represented graphically in Fig. 13.

Discussion.

The progressive atrophy of the ovaries of captive females thus contributes to the progressive fall in the total body weight in these animals. This factor, however, only accounts for a small part of the total fall and thus the remaining portion of the

fall must be due to a state of relative undernutrition. The captive animals did, in fact become recognisably thinner during the course of the experiment.

THE CYCLE IN THE ARM/BODY WEIGHT RATIO OF CAPTIVE MALES.

In the males only two factors of importance influence the arm/body weight ratio. These are.

(1) The effect of the state of nutrition on the total body weight.

(2) The effect of the pituitary and the testis hormones on the forelimbs.

(1) The State Of Nutrition.

The males also became thinner during the course of the experiment and since the males and females were kept together in the same tanks and were thus subject to the same conditions throughout the course of the experiment, it is reasonable to conclude that their total body weight dropped proportionately the same amount as that of the females.

From the corrected curve of the captive females' arm/body weight ratio it is possible to determine the amount of increase in arm/body weight ratio during the period of captivity caused by the fall in total weight. It is thus possible also to correct the curve for the arm/body weight ratio of the captive males, in order to allow for the decrease in total body weight due to the state of nutrition of the animals.

Let x = the arm/body weight ratio of the male captives n months after commencement of captivity.

Let y = the percentage increase in the arm/body weight ratio in the female captives due to loss in total body weight

due to the lowered state of nutrition, after n months of captivity.

Then z = true ratio + y x true ratio.

100 z = 100 (true ratio + [y x true ratio]).

$\therefore$ True ratio = $\frac{100z}{100+y}$.

The arm/body weight ratio of the males at commencement of captivity was 1.08.

Months Of Captivity.	Actual Increase.	% Increase.
0	0.00	0
2	0.12	11.1
4	0.21	19.5
6	0.14	12.9
8	0.27	24.1

Table XVI. The percentage increase in the arm/body weight ratios of captive females due to loss in total body weight caused by undernutrition.

Months of Captivity.	Uncorrected Arm/Body Weight Ratio. (z)	Percentage Increase in Arm/body Weight Ratio. (y)	Corrected Arm/Body Weight Ratio. $\frac{(100\ z)}{(100+y)}$
0	1.78	0.0	1.78
2	1.72	11.1	1.55
4	1.93	19.5	1.61
6	1.95	12.9	1.73
8	1.88	24.1	1.51.

TABLE XVII.

---- TABLE XVII

Table XVII. Correction of the arm/body weight ratios of captive males for the decrease in total body weight caused by undernutrition.

These figures are shown graphically in Fig. 13.

Discussion.

The curve now represents the changes in the weight of the arm with captivity and hence it is an index of anterior pituitary and testicular activity during captivity, i.e. the state of relative undernutrition has been eliminated.

The curve shows a definite tendency to decrease so that with captivity there is a decrease in the size of the arm due to lessened anterior pituitary action. This supports the findings of Shapiro and Shapiro (1934), who also concluded that captivity leads to depression of the anterior pituitary, as judged by progressive atrophy of the ovaries.

THE SEASONAL CYCLE IN THE ARMS OF FEMALE XENOPUS.

Three factors influencing the arm/body weight ratio must be considered.

1. The pituitary-gonadal mechanism.
2. The seasonal cycle in the ovaries.
3. The state of nutrition of the animals.

1. The Pituitary-Gonadal Mechanism.

This has been shown by injection to play no part in the control of the arm/body weight ratio of the female Xenopus.

2. The Seasonal Cycle In The Ovaries.

This factor can be allowed for in the following way. The ovaries are largest in July (Shapiro & Shapiro 1934). The ovaries at this time of the year form 11.3% of the total body weight. Let the total body weight in July be 100.

When gonad ratio is x, total body weight is (100 - x)+ x. If no change had occurred in the ovary, then the (body - ovary) would still be 100 - x, but the ovary would be 11.3, thus the total body weight would be 100 - x+11.3 = 111.3 - x.

Let the arm/body weight ratio be y.

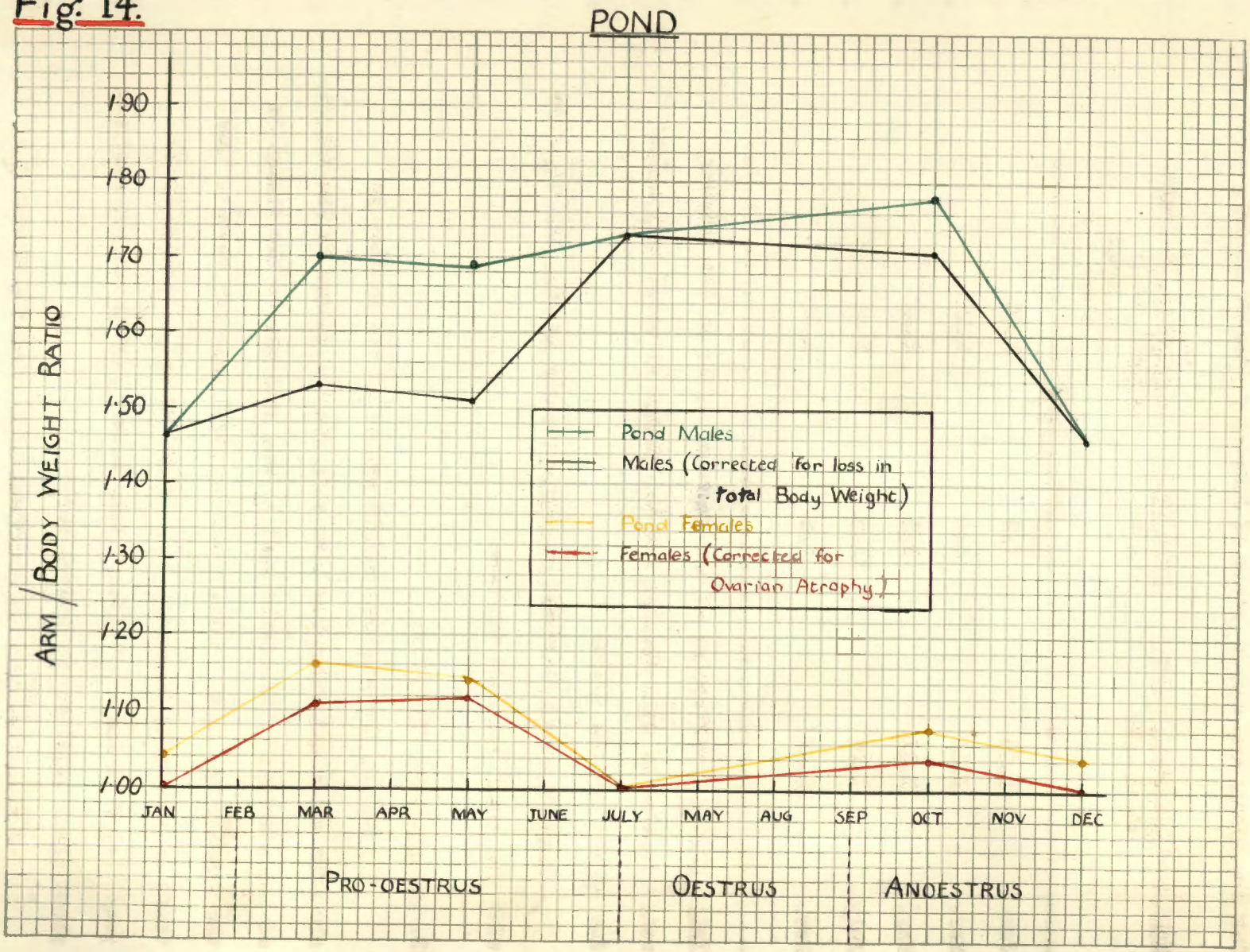
Now when total body weight is 100 (uncorrected), ratio = y.

∴ When total body weight is 111.3 - x (corrected),

$$\text{ratio} = \frac{y \times 100}{111.3 - x}.$$

Using this formula, the curve for the arm/body weight ratio can be corrected for the change in the ovaries, using the curve

Fig. 14.


POND
ARM / BODY WEIGHT RATIO
1·90
1·80
1·70
1·60
1·50
1·40
1·30
1·20
1·10
1·00
Pond Males
Males (Corrected for loss in total Body Weight)
Pond Females
Females (Corrected for Ovarian Atrophy)
JAN
FEB
MAR
APR
MAY
JUNE
JULY
MAY
AUG
SEP
OCT
NOV
DEC
PRO-OESTRUS
OESTRUS
ANOESTRUS

for the ovary/body weight ratio of Shapiro & Shapiro (1934).

Month.	Arm/Body Weight Ratio y.	Gonad Ratio x.	Corrected Arm/Body Weight Ratio $\frac{y \times 100}{111.3 - x}$.
Jan.	1.04	7.5	1.00
Mar.	1.16	6.4	1.11
May.	1.14	9.3	1.12
July.	1.00	11.3	1.00
Nov.	1.08	7.0	1.04.

Table XVIII. Correction of the seasonal cycle in the arm/body weight ratios of pond females for the change in total body weight due to the ovarian cycle.

These figures are represented graphically in Fig. 14. together with the cycle for pond animals.

3. The State Of Nutrition.

A cycle in the arm/body weight ratios of the females is observed after allowance has been made for the change in the weight of the ovaries. This cycle must be due entirely to the change in the total body weight, since the arm weight does not change. Thus there is a drop in the total body weight on two occasions in the year. (a) From January to July. This occurs during the pro-oestrous phase (Berk 1938) of the sexual cycle.

(b) A smaller drop in November, soon after the breeding season. The change in the arm/body weight ratio here is only 4%

and thus is not significant. The drop during pro-oestrous corresponds in point of time with late summer at the Cape, during which time the ponds contain very little water and vegetation. Thus it is quite easy to see how the food intake of the animals is reduced, since Xenopus laevis is totally aquatic and thus depends upon the foods in the pond for its nutrition.

The cycle in the arm/body weight ratio of the female Xenopus in the ponds is dependent therefore mainly upon the state of nutrition of the animals, the largest increase in the ratio being 12 (in May). The part played by the cycle in the ovaries is never more than 5% of the arm/body weight ratio, but it appears to account for the second phase in the cycle of the arm/body weight ratio, i.e. the slightly increased level during anoestrus. This latter increase is abolished by allowing for the change produced by the cycle in the ovary.

The Seasonal Cycle In The Arm Of Male Xenopus.

The male Xenopus also is purely aquatic and thus is subject to the same varying conditions of food supply as is the female Xenopus.

In the male only two factors are of importance in the control of the cycle in the arm/body weight ratio. They are:-

1. State of nutrition.
2. The anterior pituitary-gonadal mechanism.

1. State Of Nutrition.

Due to reduced food supply the arm/body weight ratio of the female is increased. Since the food supply for both sexes is the same, the ratio of the male is increased proportionately. Thus, using the curve of the cycle in the arm/body weight ratios of the females corrected for the ovarian cycle, the curve for the arm/body weight ratios of the male can be corrected for the change in the state of nutrition.

Let x = the increase in the arm/body weight ratio of the female produced by a poor state of nutrition expressed as a percentage of the true arm/body weight ratio.

Let 100 = the true arm/body weight ratio of the female.

$\therefore$ 100 + x = the arm/body weight ratio of the female corrected only for the ovarian change.

Let ratio uncorrected for nutrition = y.

i.e. when $100 + x = y$

Ratio corrected for nutrition $= \frac{y \times 100}{100 + x}$.

Using this formula, the curve for the arm/body weight ratio of the male can be corrected for undernutrition.

Month.	Uncorrected Arm/Body Weight Ratio (y).	Error In Female Ratio Due To State Of Nutrition (x). (From table 14).	Corrected Male Arm/Body Weight Ratio. $\frac{100y}{100+x}$.
Jan.	1.46	0	1.46
Mar.	1.70	11	1.53
May.	1.69	12	1.51
July.	1.73	0	1.73
Nov.	1.78	4	1.71

Table XIX. Correction of the arm/body weight ratios of pond males for the changes due to undernutrition.

The corrected curve is shown in Fig. 14, together with the curve for the arm/body weight ratio of pond animals.

2. Pituitary-Gonadal Mechanism.

After correction of the curve of the arm/body weight ratio for the state of nutrition in the males, a well-marked cycle is seen. The only remaining factor that can account for this cycle is the anterior pituitary-gonadal mechanism.

The cycle shows several phases.

1. There is a high level during the breeding season and shortly

after it. (July to November).

2. Then the curve drops to a low level. (November to January).

3. This low level is maintained until May.

4. The arm/body weight ratio now rises between May and July to the high breeding season level.

The curve is corrected for changes in the total body weight and thus it represents the cycle in the size of the forelimbs of the males. Forelimb size in the males is increased by the anterior pituitary, which acts by stimulation of the testis. Castration leads to a fall in the size of the arms. Captivity abolishes the seasonal cycle in the forelimbs of the male, and causes a progressive drop in the forelimb weight due to diminished anterior pituitary activity.

Thus it is probable that the cycle in the arms under natural conditions is under the control of the anterior pituitary, acting through the testis.

During the breeding season the anterior pituitary of the male appears to be active in the production of gonadotrophic hormone. This results in the maintenance of a large forelimb. The mating reflex also occurs during this period due to a pituitary-gonadal mechanism.

After the breeding season the activity of the anterior pituitary diminishes and the forelimbs decrease in size. In the male anterior pituitary activity only commences again after May, and produces the rise in the arm to the breeding season level.

The discrepancy in the curves for the incidence of pads and for the arm/body weight ratios of pond males is thus explained. The fact that the arm/body weight ratio of the males in the ponds commences to increase from January, whereas in the case of the pads development starts only after May seemed to indicate that there are two pituitary-testis mechanisms at work, one producing an increase in the forelimbs and the other the development of pads. After correction of the arm/body weight ratio curve for the state of nutrition, it can be seen that in both cases the curves start to rise after May. It is likely, therefore, that both the increase in the forelimbs and the development of pads occur as the result of the same mechanism. This mechanism is the hormone of the testis produced by stimulation of the testis by the anterior pituitary.

It is difficult to decide whether the changes in the forelimbs and the development of pads on the one hand and the clasping reflex on the other hand are produced by the same mechanism. In both cases the changes are a function of the anterior pituitary, acting by causing the liberation of a hormone from the testis. The difficulty is that the forelimb and pad changes occur during the two months previous to the onset of coupling. Thus several possibilities arise.

1. There may be one pituitary hormone liberating one testis hormone.

(a) The testis hormone may be liberated between May and July in doses too small to produce mating, and then from July to September an increased production of the testis hormone occurs.

(b) There may be varying thresholds to a constant amount of testis hormone.

2. There may be one pituitary hormone liberating two different testis hormones, one from May to September, producing pads and an increase in the forelimbs, and one from July to September, resulting in coupling.

3. Two such testis hormones may be liberated by two different pituitary factors.

The evidence presented below on the factors controlling the cyclical reproductive physiology of Xenopus laevis favours suggestion 1 above.

Summary.

1) The arm/body weight ratio of captive females rises progressively because of undernutrition. Ovarian atrophy also contributes to the rise.

2) The arm/body weight ratio of the males rises progresively because of undernutrition.

3) Elimination of this factor reveals that the forelimbs of Xenopus males decrease during captivity.

4) This decrease is due to diminished anterior pituitary-testis activity.

5) After correction of the arm/body weight ratios of pond females for the changes in the ovary, a cycle still exists.

6) This cycle is due to a state of relative undernutrition, causing an increase in the arm/body weight ratio during pro-oestrous.

7) Correction of the arm/body weight ratios of pond males for the state of relative undernutrition results in a curve showing four phases. This curve represents the seasonal changes occurring in the weight of the forelimbs of the male.

a. The arms are largest during the breeding season.

b. A decrease follows until January.

c. From January to May the arms remain at their smallest size.

d. The increase in the size of the forelimbs to the breeding season level of the males occurs between May and July.

8) This cycle corresponds with the cycle in the pads.

9) The cycle in the forelimbs of the males is controlled by the anterior pituitary acting through the testis.

THE SECONDARY SEX CHARACTERS

OF THE FEMALE.

The Secondary Sex Characters Of The Female.

The Cloacal Labia And The Oviducts.

Introduction.

In the female Xenopus the cloaca is guarded by three cloacal labia. There are two large lateral labia and one small one situated dorsally in the middle line, interposed between the two lateral labia. This labium is much thinner than the other two labia and also projects a shorter distance from the body of the frog. In the inactive state the labia are in contact with one another, the two lateral labia touching each other ventrally, at their tips and in their distal part dorsally. Proximally in the dorsal part the small dorsal labium is interposed between the two lateral labia.

The oviducts of Xenopus laevis, one on each side, can be divided into three portions. The first half inch is a straight slightly red-coloured tube, the pars recta. The second portion is the longest portion of the oviduct, extending from the pars recta to the pars uteri. This portion is pale and convoluted - the pars convoluta. The third portion is the pars uteri, or ovisac, which opens into the cloaca.

Histological Study Of The Secondary Sex Characters Of Female Xenopus.

A. The Cloacal Labia.

Sections of inactive labia from uninjected animals were cut together with sections of the dorsal skin of a female frog for the purpose of comparison.

Sections were cut also of labia activated by endocrine preparations (see below).

Histology Of The Skin.

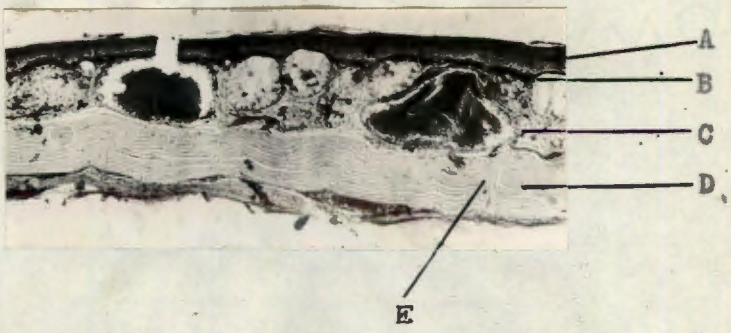
Histologically the skin shows the following layers (Fig.15).

(a) A stratified epithelium. This layer is 7 - 8 cells deep and its continuity is interrupted on the surface by the openings of the skin glands.

(b) A layer of melanophores interposed between the stratified epithelium and the next layer.

(c) A glandular layer. Two types of saccular glands are present and both types have ducts which open on to the surface of the skin. The commoner glands are mucous, showing different stages of activity (Fig. 16). The other type of gland is lined peripherally by a single layer of flattened cells, the lumen of the gland being occupied by a mass of pink staining material which consists of granules and of long needle-like crystals. (Fig. 17). Gunn (1930) observed that the secreting glands are of a simple saccular type, lined with one layer of columnar epithelium.

(d) A well-defined smooth muscle layer forms the third stratum. This layer is interrupted at intervals by fibrous septa carrying



A. Stratified epithelium.
B. Melanophore layer.
C. Glandular layer.
D. Smooth muscle layer.
E. Septum carrying blood vessels.

Fig. 15.

Histology of the dorsal skin. (x 60)

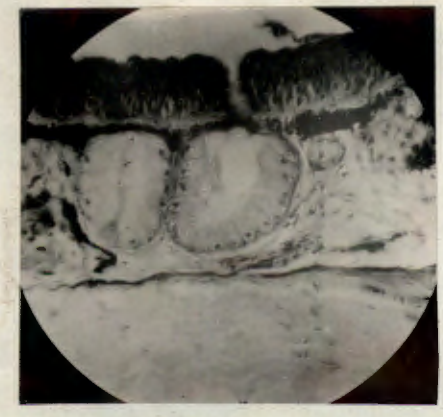


Fig. 16.

Mucous gland. (x 140)

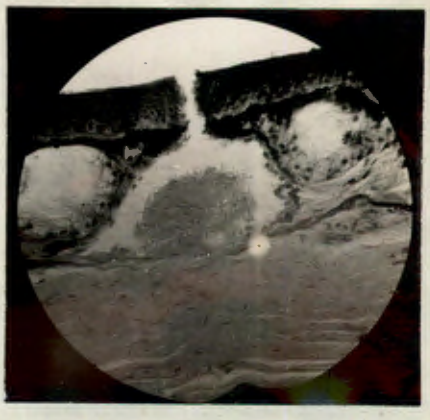


Fig. 17.

Granular gland. (x 140)

blood vessels. The septa run at right angles to the skin surface. The septa are generally situated opposite granular glands. (Fig. 15).

Below the muscle layer is the lymph space with loose areolar connective tissue running from the skin to the masculature of the animal's body.

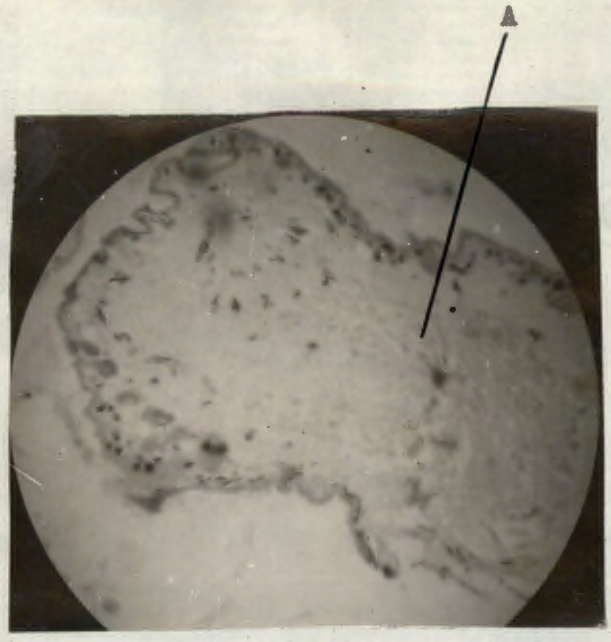
Histology Of The Inactive Labia.

The labia show the same fundamental histological characters, the epithelial, the melanophore and the glandular layers being similar to those in the skin (Fig. 18). The cloacal aspect of each labium, however, is lined by a layer of ciliated epithelium.

A striking difference is the marked increase in the vascularity of the smooth muscle layer, which, in addition, contains large amounts of fibrous tissue. The smooth muscle layer in the distal portion of the labia is not continuous as it is in the skin, but it is broken up into fairly thin irregular bundles (Fig. 18). Proximally at the base of the labia the smooth muscle is gathered into a sphincter consisting of thicker bundles (Fig. 19).

Distally the labia are very thin and filamentous. The smooth contour is lost and the outline of the tip of the labia is markedly irregular.

When the female is sexually activated, the labia become swollen and red and no longer hang limply. They project distally and the canal between the labia is seen to be opened up. Active labia were removed for histological section and the following changes were noted.



A. Irregular bundles of smooth muscle.

Fig. 18.

Histology of the inactive cloacal labia. (x 38)

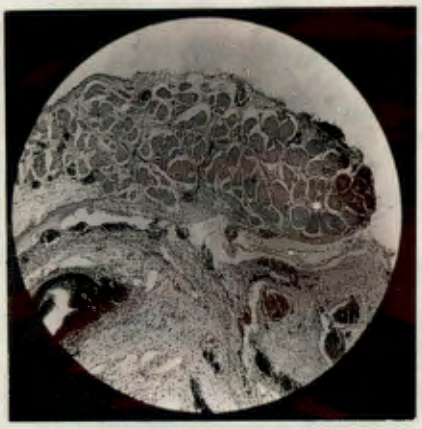
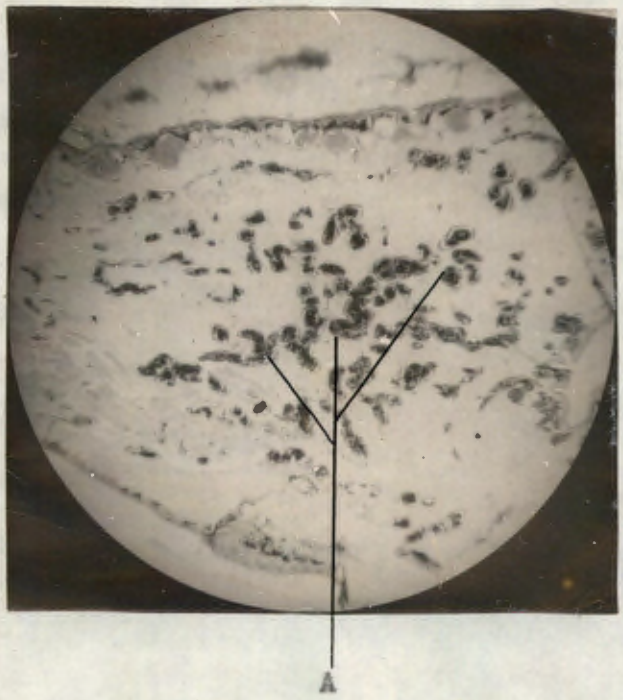


Fig. 19.

The cloacal sphincter. (x 38)

Histology Of Activated Labia.

There is no change in the epithelial, melanophore and glandular layers. The important change is in the blood supply of the fourth layer. (Fig. 20). The vessels are markedly congested and, in addition, many capillaries that are closed in the inactive state are opened up and are filled with red blood corpuscles. In the active state the lining endothelium of the blood vessels can be seen clearly. It consists of a single layer of flattened cells with flattened nuclei. This endothelium forms a continuous lining to the blood vessel. The red blood corpuscles are all intravascular, no diapedsis occurring. The congestion is especially well marked at the tip of the labia.



A. Congested blood vessels.

Fig. 20.

Histology of the activated labia. (Cf. Fig. 18).

(x 38)

B. The Oviducts.

The pars convoluta of inactive oviducts from uninjected females and of oviducts activated by endocrine preparations were removed for histological section.

Histology Of The Inactive Oviduct.

Histologically the pars convoluta shows the following layers from without inwards. (Figs. 21 & 22).

(a) A thin smooth muscle layer invests this portion of the oviduct circularly.

(b) A glandular layer. This consists of simple tubular glands bound together by septa of connective tissue carrying blood vessels. The cells of these glands contain basophil granules and the nuclei are small and irregularly-shaped and are situated basally.

(c) A layer of ciliated columnar epithelium. This layer is interrupted by the openings of the glands, the ciliated epithelium being thrown into folds opposite the interglandular septa. These folds lie at right angles to the long axis of the oviduct. The blood vessels from the interglandular septa run into the concavity of these folds.

In the inactive state only an occasional red blood corpuscle is seen in the capillaries.

OVIDUCT.

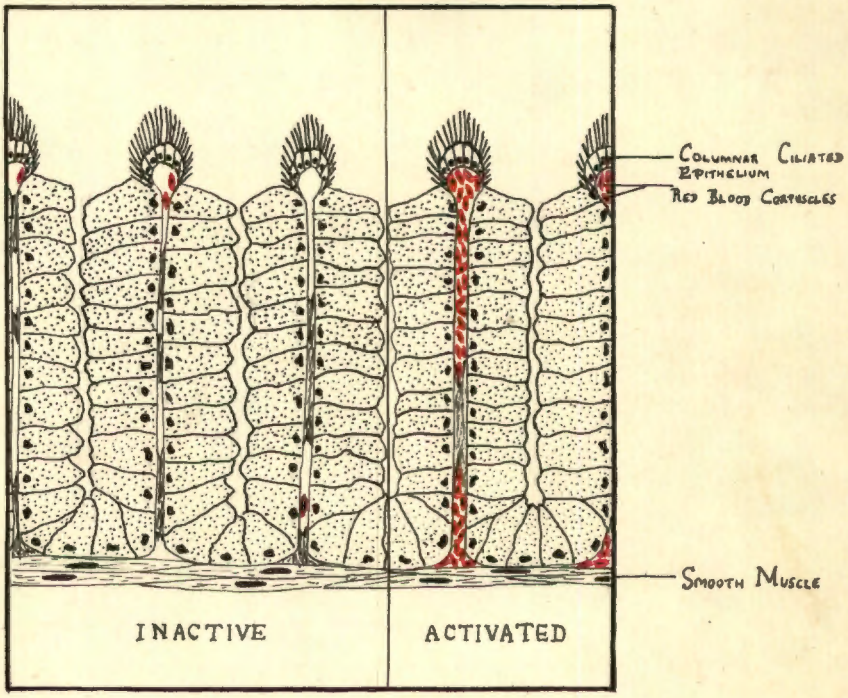
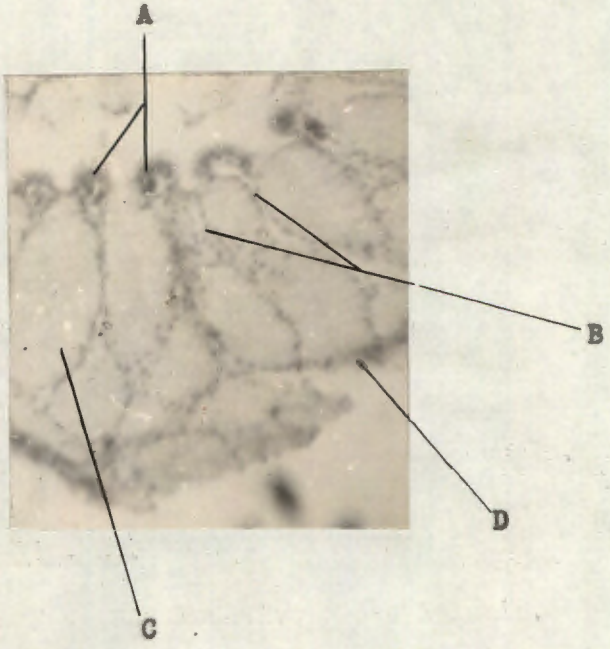


Fig. 21.



A. Epithelial folds.
B. Empty capillaries.
C. Mucous gland.
D. Smooth muscle layer.

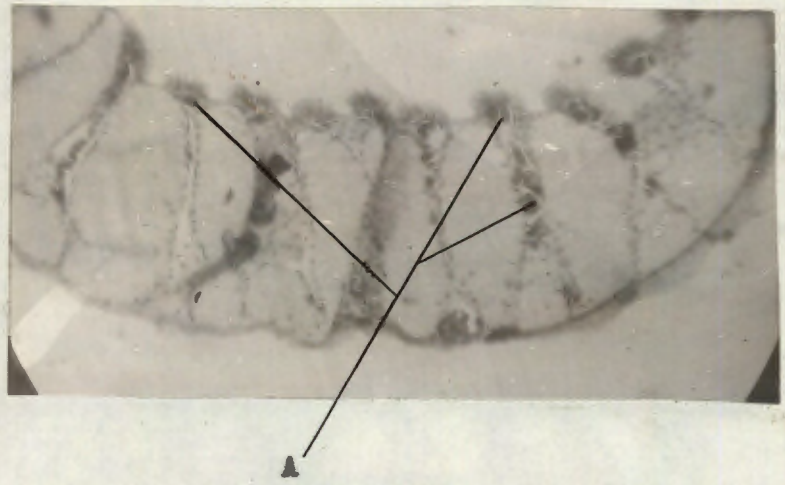
Fig. 22.

Histology of the inactive oviduct. (x 175)

Histology Of The Activated Oviduct.

In the active state the chief change in the oviduct again takes place in the blood supply. (Fig. 23). The inter-glandular capillaries, many of which were previously closed, are opened up and are filled with red blood corpuscles. The concavity of the epithelial folds are also filled with numerous red blood corpuscles. In the interglandular septa in such preparations the lining of the capillaries is seen clearly. It consists of a single continuous layer of flattened endothelial cells with small flattened nuclei, slightly wider than the cells, so that they bulge somewhat out of their cells. Again the red blood corpuscles are all intravascular and no diapedesis occurs.

The increased vascularity of the oviducts can be recognised macroscopically.



A. Red blood corpuscles.

Fig. 23.

Histology of the activated oviduct. (x 175)

II. Experimental Activation Of The Cloacal Labia And The Oviducts.

Hyperaemia of the cloacal labia of female Xenopus has been observed during sexual activation induced experimentally by the injection of gonadotrophic substances (Shapiro & Zwarenstein 1935; Shapiro 1936 c; Zwarenstein 1936 a). Bles (1901, 1904) has also noted labial hyperaemia when the mating reflex was induced by simulating natural conditions.

Shapiro & Zwarenstein (1937) also reported that certain steroids have a gonadotrophic action in female Xenopus at present indistinguishable from the action of anterior pituitary preparations. It was also shown in this work that activation of the female cloacal labia occurs through the mediation of the ovaries, since no hyperaemia of the cloacal labia occurred in animals that had had their ovaries removed.

Thus this hyperaemia of the labia may be used as a test-object for the detection of gonadotrophic substances. In testing substances for gonadotrophic activity it is important to specify clearly the nature of the test-object employed. Many apparent differences in gonadotrophic preparations may be due to the different biological test-objects used. In the present section the test-object was rigidly standardised. The hyperaemia of the cloacal labia and of the oviducts was used as a criterion of gonadotrophic activity and various substances were investigated in this way.

All injections in this section were made into the cephalic end of the ventral lymph sacs of females whose laboratory age did not exceed two weeks. This route of administration was chosen because intraperitoneal injections or injections into the dorsal lymph sacs may in some cases produce labial and oviducal hyperaemia by reflex irritation. Since captivity results in pituitary inhibition in Xenopus laevis, animals were used within the first two weeks after they were brought in from the ponds to eliminate a possible loss of sensitivity to gonadotrophic substances.

The experiments were performed during January and February 1938.

1. Activation Of The Cloacal Labia By Human Pregnancy Urine Extract.

Extracts of human pregnancy urine from cases 1 - 3 months amenorrhoea, diagnosed by the Shapiro & Zwarenstein pregnancy test and confirmed by later clinical evidence, were prepared according to the benzoic acid/acetone method of Katzman & Doisy (1932).

16 freshly-caught females were each injected into the ventral lymph sac with 0.5 ml. of the above extract (20 mls. original urine). This dose produces coupling in 70% of pairs at this time of the year. (Shapiro 1937 a).

The animals were placed in glass containers, 2 - 4 animals per container, with sufficient tap water to cover them.

Injections were made at 6.00 p.m. and readings were taken the following morning at 9.00 a.m. and every hour after that throughout the day.

Results.

Hyperaemia of the labia was observed in 11 animals in this group after the usual overnight latent interval. Ovulation also occurred.

Discussion.

Human pregnancy urine contains a substance which causes hyperaemia of cloacal labia of the female Xenopus, i.e., a substance is produced in man which is capable of stimulating the ovaries of Xenopus. The gonadotrophic action is further demonstrated by the ovulation that took place. This ovulation reaction is the basis of the Shapiro & Zwarenstein test for pregnancy (1933 a).

2. Activation Of The Cloacal Labia By Extracts Of Mammalian Anterior Pituitaries.

(a) Sheep Anterior Pituitary Extract.

10 females freshly brought in from the ponds in January were injected with 0.5 ml. of sheep anterior pituitary extract (= 365 mgs. fresh tissue) into the ventral lymph sac. Injections and readings were made at the same times as before.

Results.

After 14 hours hyperaemia of the labia was well marked in 5 animals.

After 28 hours 7 animals showed marked hyperaemia of the labia and the remaining 3 showed slight hyperaemia.

Oviposition into the water of the containers was well-marked.

(b) Ox Anterior Pituitary Extract.

10 females collected at the same time as the animals in the previous section were injected into the ventral lymph sac with 0.5 mls. of an extract of ox anterior pituitary (= 365 mgs. fresh tissue). Injections were again made at 6.00 p.m. and the animals were kept in glass containers, 2 - 4 frogs per container with sufficient tap water to cover them. Readings were made every hour on the following morning, starting at 9.00 a.m.

Results.

4 of the animals showed hyperaemia of the labia after the

usual overnight interval. Ovulation occurred in some of the containers.

Discussion.

The anterior lobes of sheep and bovine pituitary both contain substances gonadotrophic to the female Xenopus. Judging by the fewer number of females showing labial hyperaemia and the smaller amount of ovulation it appears that the ox anterior pituitary is less active gonadotrophically than the sheep anterior pituitary. The small numbers of animals used do not warrant any definite conclusion on this point. The question is more fully dealt with later.

The ovulation produced by ox anterior pituitary confirms the results of Hogben (1930).

3. Activation Of The Cloacal Labia By Amphibian Anterior Pituitaries.

The anterior lobes of the pituitaries of male and female Xenopus were collected according to the method described previously. Varying numbers of anterior lobes were implanted under the dorsal skin of freshly-caught females, at the level of the forelimbs. The donors of the pituitaries were animals that had been kept in captivity for approximately two months. These animals never ovulated, nor did any mating take place in the laboratory. The males were never heard to croak.

Implantations were made at 6.00 pm. and observations were made on the following morning as in the previous sections.

Results.

No. of Females.	No. of Male Pituitaries Implanted.	Number Showing Hyperaemia.
5	3	5
8	2	3

No. of Females.	No. of Female Pituitaries Implanted.	Number Showing Hyperaemia.
4	2	3
4	1	2

Table XX. Implantation of Xenopus anterior pituitaries into Xenopus females.

Hyperaemia of the labia is produced by the implantation of 2 - 3 anterior lobes from male pituitaries, or by 1 - 2 anterior lobes from female pituitaries. Ovulation occurred both with male and with female anterior lobe implants.

Discussion.

The anterior pituitary of Xenopus laevis males and females contains substances gonadotrophic to the female Xenopus. There is thus no sex specificity in the action of the amphibian gonadotrophic hormone.

It can also be seen that while all sexual activity of the female ceases in the laboratory, the production of gonadotrophic hormone by the pituitaries of the animals does not cease. Thus the inhibition of the anterior lobe of the pituitary of Xenopus laevis in captivity (Shapiro & Shapiro 1934) does not consist in

the inhibition of secretion by the gland. Captivity results in the prevention of release from the gland of the hormone, which is thus retained in the anterior pituitary and can be absorbed when the gland is implanted subcutaneously. Gonadotrophic substances are still detectable biologically in the anterior pituitary of both male and female frogs after two months captivity.

These results confirm the findings in a previous section.

4.Activation Of The Cloacal Labia By Fish Anterior Pituitaries.

The anterior lobes of the pituitaries of dogfish collected as described previously were implanted under the dorsal skin of Xenopus females at the level of the forelimbs. Varying numbers of dogfish anterior lobes were used and the implantations and observations were made at the same times as in the case of Xenopus anterior lobes.

Results.

No. of Females.	No. of dogfish Pituitaries Implanted.	No. showing Hyperaemia.
2	3	0
1	4	0
1	5	0

Table XXI. Implantation of dogfish anterior pituitaries into Xenopus females.

No ovulation occurred.

Discussion.

In the doses given the dogfish pituitary is not gonadotrophic to Xenopus females. This is further borne out by the absence of ovulation. The fact that the pituitaries were removed from the animals as soon as they were killed and that they were kept on ice from that time until they were used (2 - 3 hours) precludes the possibility of decomposition of any possible gonadotrophic factors which they might have contained.

With hyperaemia of the labia as a criterion of gonadotrophic activity in Xenopus females, it is found that this gonadotrophic factor has a wide distribution in the vertebrates; amphibia, several mammals including man, are found to produce substances gonadotrophic to Xenopus laevis. It is noteworthy that attempts so far to detect a gonadotrophic substance in the anterior pituitary of an elasmobranch fish have been unsuccessful.

5. Activation Of The Secondary Sex Characters Of Xenopus Females By Certain Steroids.

a. Methyl Testosterone.

16 females were used in this experiment. Each was injected into the ventral lymph sac with 500 y of methyl testosterone, a dose which produces a 70% ovulation in January, the month in which the present experiment was performed. (Shapiro 1938). The reasons for injecting into the ventral lymph sac have been stated above.

24 hours after injection the animals were pithed and the following observations were made:-

The labia were examined for the presence of hyperaemia. Then the abdomen of the frog was opened from the front and the ovaries, oviducts and ovisacs were examined for hyperaemia. Lastly, the condition of the ovary was noted, i.e., the presence or absence of large mature ova was recorded.

Results.

No.	Hyperaemia of Labia.	Hyperaemia of Ovaries	Hyperaemia of Oviducts	Hyperaemia of Ovisacs	Condition of Ovaries.
1	-	-	-	-	-
2	+	-	+	-	+
3	+	-	+	-	+
4	+	-	+	-	+
5	+	-	-	-	+
6	-	-	-	-	-
7	+	-	-	-	+
8	+	-	+	-	+
9	+	-	+	-	+
10	++	-	+ E	-	+
11	+	-	-	-	+
12	-	-	+	-	+
13	+	-	-	+ E	+
14	-	-	+	-	+
15	+	-	+ E	-	+
16	+	-	+	-	+
Total Positive:	12	0	10	1	14

E = Eggs.

Table XXII. Injection of methyl testosterone into Xenopus females.

Labia.

Hyperaemia of the labia occurred in 12 of the animals injected. Of the remaining 4 animals 2 showed atrophic ovaries,

i.e., the animals were "hypogonadal". (Shapiro, 1937 a).

Ovaries.

There was no hyperaemia of the ovaries. 2 of the animals had atrophic ovaries.

Oviducts.

10 of the animals showed hyperaemia of the oviducts. 8 of these animals had, in addition, hyperaemic labia. In the remaining two animals the labia were inactive. Two of the animals (Nos. 10 and 15) had eggs present in the oviduct.

Ovisacs.

One animal had hyperaemic ovisacs containing eggs.

Ova were seen in the water of the containers.

Discussion.

Methyl testosterone in doses of 500 y produces hyperaemia of the labia and oviducts and thus is gonadotrophic. The two negative results in the hypogonadal animals is in keeping with the findings of Shapiro & Zwarenstein (1937), who showed that the activation of the labia occurs through the mediation of the ovaries. The possibility of a direct irritant action on the labia and oviducts is eliminated by the fact that the injections were made into the ventral lymph sac. The action of methyl testosterone is thus a hormonal one.

This result contrasts markedly with the actions of methyl testosterone in the male Xenopus and in mammals. In the latter this steroid has androgenic, oestrogenic and progestational activity, but no gonadotrophic activity has been demonstrated.

In the male Xenopus methyl testosterone is inactive, both as regards gonadotrophic and androgenic actions.

b. Testosterone Propionate.

16 females were injected with 500 y testosterone propionate into the ventral lymph sacs.

24 hours after injection the labia were examined for hyperaemia and the frogs were returned to their containers.

24 hours later, i.e., 48 hours after injection, the animals were pithed and the same series of observations were made as in the previous experiment.

Results.

24 hour reading.

9 of the 16 animals showed hyperaemia of the labia.

48 Hour Reading.

	Hyperaemia of the Labia.	Hyperaemia of Ovaries.	Hyperaemia of Oviducts.	Hyperaemia of Oviscacs.	Condition of Ovaries.
1	+	+	-	-	+
2	+	-	-	-	+
3	-	-	+	-	+
4	-	-	+	-	+
5	-	-	-	-	-
6	-	-	-	-	+
7	-	-	+	-	+
8	-	-	-	-	-
9	-	-	+	-	+
10	+	-	+	-	+
11	-	-	+	-	+
12	+	-	+	-	+
13	+	-	+	-	+
14	-	-	+	-	+
15	-	-	+	-	+
16	Dead.				
Total	5	1	10	0	13

Table XXIII. Injection of testosterone propionate into Xenopus females.

Labia.

24 hours after injection 9 of the 16 animals gave a positive response with testosterone propionate. 24 hours later there were

only 5 positives (out of 15). Again there was no hyperaemia of the labia in hypogonadal animals.

Ovaries.

Only one animal showed hyperaemic ovaries 48 hours after injection. The ovaries of two animals were atrophic.

Oviducts.

10 of the 15 animals showed hyperaemic oviducts. No eggs were seen in the oviducts.

Ovisacs.

There were no eggs present, nor was there any hyperaemia.

Ovulation occurred in this experiment.

Discussion.

Testosterone propionate in doses of 500 γ has a gonadotrophic action in Xenopus females, resulting in hyperaemia of the oviducts and the labia. In the case of the labia, hyperaemia was more marked on the first than on the second day. The oviducts, however, seem to be activated more powerfully and for a longer time than the labia.

Testosterone propionate in mammals has the same actions as methyl testosterone, except that its action is greater and more prolonged due to the esterification. In the male Xenopus it is inactive. In the female Xenopus it is gonadotrophic. In mammals, testosterone propionate has been shown to inhibit the ovaries, on account of its inhibitory action on the anterior pituitary.

c. Progesterone.

16 females were used in this experiment. Each received an injection of 500 γ of progesterone into the ventral lymph sac. This dose of progesterone produces ovulation in approximately 50% of frogs in January, the month in which this experiment was performed (Zwarenstein 1938).

The animals were killed 24 hours after injection and the same series of observations were made as in the two previous sections.

Results.

	Hyperaemia of Labia.	Hyperaemia of the Ovaries.	Oviducts.	Ovisacs.	Condition of Ovaries.
1	-	-	+	-	+
2	-	-	+	-	+
3	-	-	-	-	-
4	+	-	+	-	+
5	+	-	+	+	+
6	+	-	-	-	+
7	+	-	+	-	+
8		Male			
9	-	-	+	-	+
10	-	-	+ E	-	+
11	+	-	+	+	+
12	+	-	+	-	+
13	+	-	+	-	+
14	-	-	-	-	-
15	+	-	+	-	+
16	-	-	+	-	+
Total.	8	0	12	2	13

E = Eggs.

Table XXIV. Injection of progesterone into Xenopus females.

Labia.

13 animals had ovaries in good condition. Of these 8 showed hyperaemia of the labia.

Ovaries.

No hyperaemia was seen.

Oviducts.

12 animals showed hyperaemia of the oviducts. These animals all had mature ovaries. Of the remaining three animals 2 had atrophic ovaries. Eggs were present in the oviducts of Number 10.

Ovisacs.

2 animals had hyperaemic ovisacs.

There was oviposition into the water of the containers.

Discussion.

Progesterone activates the labia and oviducts of female Xenopus and produces ovulation. It is thus gonadotrophic to Xenopus females in 500 γ doses in 24 hours.

Progesterone in mammals is the only sex hormone with a purely female activity. It is progestational, but is not oestrogenic or androgenic. Further, it has been shown that progesterone is gonadotrophic in the rat, stimulating the formation of corpora lutea (McKeown & Zuckerman 1937). Corpus luteum formation does not take place in Xenopus females, so that no progestational activity can occur. The gonadotrophic activity of progesterone in rats and Xenopus females, however, suggests some similarity in action in the two species.

On the other hand, there is a striking difference between the action of progesterone in the male and female Xenopus. In the males

progesterone shows no activity at all. Progesterone is also inactive in the female Bufo Arenarum (de Allende 1938).

d. Oestradiol Benzoate.

A batch of females freshly caught from the ponds in the second week of July were examined for hyperaemia of the labia. 40 animals showing inactive labia were used in this experiment and divided into two groups.

Group I.

20 animals. Each animal received daily intramuscular injections of 500 y of Oestradiol Benzoate on 5 successive days. "Progynon B oleosum Forte" (Schering-Kahlbaum) was used. Each ampoule contains 5 mgs. Oestradiol Benzoate in 1.0 mls. of oil. Each animal thus received 0.1 ml. of the solution in each injection. The gastrocnemii were used alternately. Injections were made at 5.00 p.m. and readings were taken at 11.00 p.m., 9.00 a.m. and every hour until 5.00 p.m. on each day until the third day after the last injection. The labia were examined for hyperaemia.

Group II.

20 animals. Each animal received daily intramuscular injections into alternate legs on 5 successive days at the same times as the animals in Group I. The control solution consisted of 19.0 mgs. of sodium benzoate dissolved in 10.0 mls. of distilled water. The concentration of the benzoate radicle is thus the same as in the case of "progynon B oleosum forte".

Distilled water was used because sodium benzoate is insoluble in nut oil. However, the effect of the oil can be disregarded, since Zwarenstein (private communication) has shown that even large intraperitoneal doses of nut oil do not produce hyperaemia of the labia. Observations were made at the same timesas in the case of Group I.

Results.

There was no hyperaemia of the cloacal labia of any of the animals in the two groups.

No ovulation occurred.

Discussion.

Oestradiol benzoate in doses of 2.5 mgs. given intramuscularly over 5 days is inactive in female Xenopus.

Again there is a marked difference between the actions of oestradiol benzoate in mammals and in Xenopus. In mammals oestradiol benzoate is a bisexual hormone with mainly female activity. In Xenopus females oestradiol benzoate is inactive. The injections were made intramuscularly and, in addition, an ester was used, so that very high utilisation of free oestradiol must have occurred. Also the experiment was performed in July, when the sensitivity of the animals is high. Yet there was no production of hyperaemia of the labia. Hyperaemia of the labia in Xenopus has been shown to occur as a result of ovarian activity in Xenopus (Shapiro & Zwarenstein 1937). Thus the experiment above forms definite evidence that there is a

difference between the ovarian hormones of mammals and of Xenopus laevis.

In Bufo Arenarum de Allende (1938) showed that oestrone has no effect on the genital tract of the female. This finding, however, does not permit the conclusion reached above to be generalised to include other amphibian female sex hormones, since the same author showed that the anterior pituitary-like factors of human pregnancy urine were also without effect on the genital tract of Bufa Arenarum females. The effect of the mammalian sex hormones has not been investigated in any other amphibians.

Evidence presented in an earlier section shows that the mammalian and Xenopus testis hormones also differ from each other.

6. The Effect Of Xenopus Ovary Preparations On The Secondary Sex Characters Of Xenopus Females.

a. Ovary Suspensions.

The ovaries were removed from 25 freshly-caught females in July, 1938. This is the breeding season in the life of Xenopus laevis, during which the ovaries reach their largest size and are most mature. The ovaries were ground up with sand, and 50.0 mls. of distilled water was added. The mixture was stirred well and allowed to stand in a cool place overnight. The following morning it was centrifuged and the supernatant fluid was collected. This was very viscous and was recentrifuged.

The supernatant fluid was again collected and its volume measured. Almost 75.0 mls. of a dark somewhat viscous suspension was obtained. Each millilitre, therefore, represented 2/3rds of an ovary.

10 freshly caught females were castrated according to the method described previously. All the operated animals recovered. Each was injected into the dorsal lymph sac with 2.0 mls. (equivalent to 1 1/3 ovaries) of the suspension prepared as described above. The labia were examined at hourly intervals for hyperaemia.

Results.

There was no hyperaemia of the labia.

Discussion.

2.0 mls. of a watery suspension of Xenopus ovaries failed to activate the cloacal labia of castrate females.

b. Xenopus Ovary Extract.

An attempt to extract the active principle of Xenopus ovaries was made as follows:

Ovaries from 27 Xenopus were collected in acetone and allowed to stand for some time. The acetone was centrifuged off and retained. The acetone soluble material was collected by evaporation of the acetone and was dissolved in about 15.0 mls. nut oil. Each millilitre of nut oil solution thus represented 3 1/2 ovaries.

2.0 mls. of the nut oil solution were injected into the

ventral lymph sac of each of 4 freshly-caught females (equivalent to 7 ovaries per frog). The labia were examined every hour after injection for hyperaemia.

Results.

There was no hyperaemia of the labia.

Discussion.

The failure to produce hyperaemia of the labia may be explained in many ways. The dose of the final solution may have been too small. The active principle may not be soluble in nut oil, or it may be insoluble in acetone. The hormone content of the ovaries may have been too low to produce any effect on the injected animals. These appear to be the main sources of difficulty in this experiment and the work should be repeated, keeping these factors in mind.

Summary.

1) The anatomy of the cloacal labia and of the oviducts of Xenopus females is described.

2) The histology of the dorsal skin of female Xenopus is described and the cloacal labia are shown to be a more fibrous and more vascular modification of the skin.

3) The histology of the pars convoluta of the oviduct is described.

4) Activation of the cloacal labia and of the oviducts consists in the production of a capillary hyperaemia.

5) This activation of the secondary sex characters has been used as a test-object for gonadotrophic activity in Xenopus females. In this way the following preparations were shown to be gonadotrophic:-

Human pregnancy urine, sheep and ox anterior pituitary, Xenopus anterior pituitaries from both males and females, methyl testosterone, testosterone propionate, and progesterone.

6) Negative results were recorded in the case of dogfish pituitaries and oestradiol benzoate.

7) From the last finding it is concluded that the Xenopus ovarian hormone differs from that of mammals.

8) Attempts to extract the Xenopus ovary hormone were unsuccessful.

EXPERIMENTAL INVESTIGATION OF THE MATING REFLEX OF XENOPUS LAEVIS.

EXPERIMENTAL INVESTIGATION OF THE MATING REFLEX OF XENOPUS LAEVIS.

Introduction.

Observations on sexual behaviour in frogs and toads were made as long ago as the 4th century B.C. Aristotle in his "Historia animalium" gives an account of the factors responsible for the congress of the sexes. According to him the croaking of the males is the stimulus that attracts the females. In addition he states that the eyes shine through the open mouth of the male like lamps, directing the female to the male. Aristotle, however, erroneously stated that the male performs the act of copulation with "an organ in which the ducts converge".

More recently Linnaeus (1707 - 1778) and Oliver Goldsmith (1776) added to the existing knowledge. The genitalia were described correctly. Also the thumb pads of the males were described. The onset of rut was ascribed by Goldsmith to the rise of temperature at the beginning of Spring. Goldsmith also described the axillary clasp of Rana.

The elaborate pattern of motor behaviour making up the mating reflex in amphibia suggests that the nervous system is intimately involved in the response.

Goltz (quoted by Schrader, 1887), Tarchanoff (1887), Steinach (1910), Baglioni (1911) and Lindeboom (1928), have indicated the importance of the central nervous system in this connection. Verworn (1897) has drawn attention to the fact that

the state of tonic immobility which can be induced in amphibians is more marked in the female than in the male. Noble (1931) has suggested that this sexual difference in the neuro-muscular apparatus may have some significance in the mating process, the vigorous grip of the male inducing in the female a state of tonic immobility which prevents her escape. Shapiro (1936 a) does not consider this to be the case in Xenopus laevis. Kahn (1921), Wacholder (1923) and Lullies (1923) have drawn attention to changes in electrical excitability of frogs' tissues during clasping, while Lindeboom (1928) has shown marked differences in muscular excitability in the amphibian forelimbs (flexor carpi radialis muscle) before and after rut.

Steinach (1910) considers that the clasping reflex is normally inhibited by an inhibitory centre which he located between the medulla oblongata and the mid-brain. Langhans, working in Steinach's laboratory, localised this inhibitory centre more accurately in the distal portions of the corpora bigemina and the hind brain. During the breeding season, the inhibitory centre is itself inhibited by an endocrine secretion, possibly from the testes, and the clasping reflex thus appears in the male frog. This hypothesis also explains the appearance of the reflex in an animal which has had its medulla divided experimentally (Busquet 1910).

Shapiro (1936 b) suggests that in Xenopus laevis anterior pituitary-like substance extracted from pregnancy urine acts by inhibiting the centre in the corpora bigemina and the hind brain,

thus releasing the clasping reflex in the male toad. In the female the mode of action of the extract appears to be quite different. She is physiologically depressed whereas the male is stimulated.

It appears further, that the extract does not act directly on the clasping reflex inhibitory centre, but does so only through the intermediation of the gonads.

The role of the endocrine glands in the mating reflex has only been recognised in the present century. Nussbaum (1907), Steinach (1910) and Brossard and Cley (1929) recognised the close relationship of the gonads to the mating reflex. Osima (1937) produced amplexus in Rana with Rana testis extracts.

The part played by the anterior pituitary in coupling forms the most recent advance in the knowledge of the mating reflex in amphibia. Rugh (1935) produced coupling in several species of Rana with anterior pituitaries from various species of Rana and Bufo. Antuitrin - S (pregnancy urine extract) produced coupling in rana catesbiana and bufo fowleri but not in other species of Rana. Whole sheep pituitary extracts produced coupling in bufo fowleri.

Shapiro (1936 a, 1936 b) induced coupling in Xenopus laevis by means of sheep anterior pituitary extract and extracts from human pregnancy urine.

Osima (1937) produced coupling in Rana japonica and Rana negromaculata by using the anterior pituitaries from either of these two species.

The anterior pituitaries have been shown by Shapiro (1937 a) to act in coupling by virtue of their gonadotrophic properties. Xenopus laevis, however, occupies a rather unique position with regard to the effectiveness of mammalian gonadotrophic preparations, as can be seen from the account given below of the work of several authors. Adams (1930 a, 1930 b, 1930 c, 1931 a, 1931 b, 1932, 1933, Adams & Grayer (1938), Bardeen (1932), Bellerby (1929, 1933), Creaser & Corbman (1935, 1936), Creaser & Scholnek (1937), Noble & Richards (1930), O'Neil (Quoted by Adams & Granger 1938), Rugh (1934, 1935, 1937), Wills et al (1933), Wolf (1929 a, 1929 b).

Ovulation is the index of gonadotrophic activity in each case.

Rana is stimulated by preparations from Rana, toad, urodele pituitaries and phyone, but not by pregnant mare's urine or serum, human pregnancy urine, placental extracts or whole sheep pituitary. Beef pituitaries are active in some species of Rana (e.g. Rana vulgaris) but not in others (e.g. Rana pipiens). Conflicting evidence exists with regard to the effect of fish pituitaries in Rana (pipiens).

Toads are activated by preparations from toad pituitaries, but are not stimulated by Rana pituitaries, pregnant mare's urine, human pregnancy urine, beef pituitaries or rabbit pituitaries.

Urodeles have been caused to ovulate by means of Rana and urodele pituitary preparations and also phyone and hebin.

Xenopus laevis, however, has been made to ovulate by means of pituitaries from male and female Xenopus, Rana, Sheep, ox and human pituitaries. In addition, human pregnancy urine and serum are also capable of inducing ovulation in Xenopus females. As has been mentioned above, the hormones of the mammalian testis and corpus luteum, namely testosterone and progesterone are gonadotrophic to Xenopus females, as well as certain synthetic compounds closely related chemically to these two steroids.

In the work on coupling described above, injections were made into both the male and female partners in order to produce the mating reflex. Shapiro (1936 b) induced coupling in Xenopus laevis in 30% of cases by injecting only the female partners with human pregnancy urine extracts. The dose used gave a 75% response when injected into both partners. The effect of this dose thus is roughly the same as that of an amount of sheep anterior pituitary extract equivalent to 360 mgs. of fresh anterior lobe tissue.

The experiments described below were undertaken to elucidate several of the problems concerning the mating reflex in Xenopus laevis.

I. THE EXPERIMENTAL INDUCTION OF COUPLING IN XENOPUS LAEVIS.

A. The Experimental Induction Of Coupling By Extracts Of Ox And Sheep Anterior Pituitaries.

Experiment 1.

The effect of the injection of acid extracts of sheep and ox anterior pituitaries.

125 pairs of frogs, freshly brought in from the vleis were divided into 2 groups. There were 47 pairs of frogs in the first group and 78 pairs in the second group.

The frogs were placed in the containers previously described, 2, 3 or 4 pairs per container.

Group I.

Each frog in the first group was injected with an amount of ox pituitary extract equivalent to 365 mgs. of fresh anterior lobe tissue. Commencing 10 hours after injection, observations were made every half hour. Coupling pairs were removed and placed in separate containers for further experiments.

Results.

Date.	No. Of Pairs Injected.	Pairs Coupling.
10. 9.37	14	2
17. 9.37	18	5
8.10.37	15	4
Total.	47	11

Table XXV. Injection of ox anterior pituitary extract in Xenopus laevis.

Coupling occurred in each of the experiments 10 to 12 hours after injection. 11 pairs of the 47 pairs injected coupled, a response of 23.4%.

Fertilized ova were removed and reared to the tadpole stage.

Group II.

In the second group each frog received an amount of extract of sheep anterior pituitary equivalent to 360 mgs. of fresh tissue. These experiments were carried out at the same time as those of Group I, so that all the conditions of the experiment were the same. Observations were made in the same way.

Results.

Date.	No. Of Pairs Injected.	Pairs Coupling.
10. 9.37	9	5
17. 9.37	16	15
8.10.37	53	38
Total.	78	58

Table XXVI. Injection of sheep anterior pituitary extract into Xenopus laevis.

Coupling occurred in each of the experiments after a latent interval of 10 to 12 hours. 58 pairs out of the 78 pairs injected coupled, i.e. there was a 74.4% response.

Fertilized ova were recovered and reared to the tadpole stage.

Discussion.

Ox and sheep anterior pituitaries both contain substances gonadotrophic to Xenopus. Injection of acid extracts of these glands therefore leads to coupling and ovulation, with the production of fertilized eggs. The results obtained with sheep anterior pituitary extracts confirm the findings of Shapiro (1936 a).

The frogs that did not couple were killed and examined.

Females.

The labia were examined for hyperaemia and then the frogs were opened up and the ovaries were examined for the presence of large mature ova.

Males.

The testes of the males were crushed on a glass slide and a drop of water was added. The smear was examined under a microscope for the presence of motile sperms. The latter was taken as a criterion of testicular maturity and ability to mate.

No. Of Pairs.		Females Showing Labial Hyperaemia.	Females Showing Large Mature Ova in Ovaries.	Males Showing Motile Sperms.
Gr. I	36	4	33	34
Gr. II	20	5	18	15

Table XXVII. Post-mortem findings in the animals which failed to couple.

Thus the difference in the responses to ox and sheep anterior pituitary cannot be explained on the basis of hypogonadism of the animals.

A marked difference in the ability of the two extracts to induce mating in Xenopus laevis is shown. In experiments performed at the same time with the same doses of fresh anterior lobe tissue, ox anterior pituitary extract produces a 23.4% response, whereas sheep anterior pituitary extract injections result in coupling in 74.4% of the pairs used. Sheep anterior pituitary thus has almost 3 1/2 times as much gonadotrophic activity as ox anterior pituitary when doses of 365 mgs. of fresh tissue are used. The average weight of the ox anterior pituitaries used was 1.95 gms. and that of the sheep anterior lobes was 0.573 gms. Thus the ox anterior lobe is approximately 3 1/2 times as heavy as the sheep anterior lobe. Roughly speaking therefore, one sheep anterior pituitary has the same gonadotrophic activity as one ox anterior pituitary.

Experiment 2.

The Gonadotrophic Potency Of Sheep Anterior Pituitaries.

Within a week of the completion of the above experiment, attempts were made to produce coupling with smaller doses of acid extracts of sheep anterior pituitary. 100 pairs of frogs were collected from the ponds and divided into two groups containing 50 pairs in each.

Group I.

Each animal in this group was injected intraperitoneally with 1.0 ml. of an extract of sheep anterior pituitary diluted so that 1.0 ml. was equivalent to 50 mgs. of fresh anterior lobe. Injections were made at 6.00 p.m. and readings were taken the following day every two hours, starting at 9.00 a.m.

Group II.

Each animal received 1.0 ml. of the same extract, diluted in this case so that 1.0 ml. was equivalent to 100 mgs. of original fresh tissue. Injections and observations were made at the same time as in Group I.

Results.

Group I.

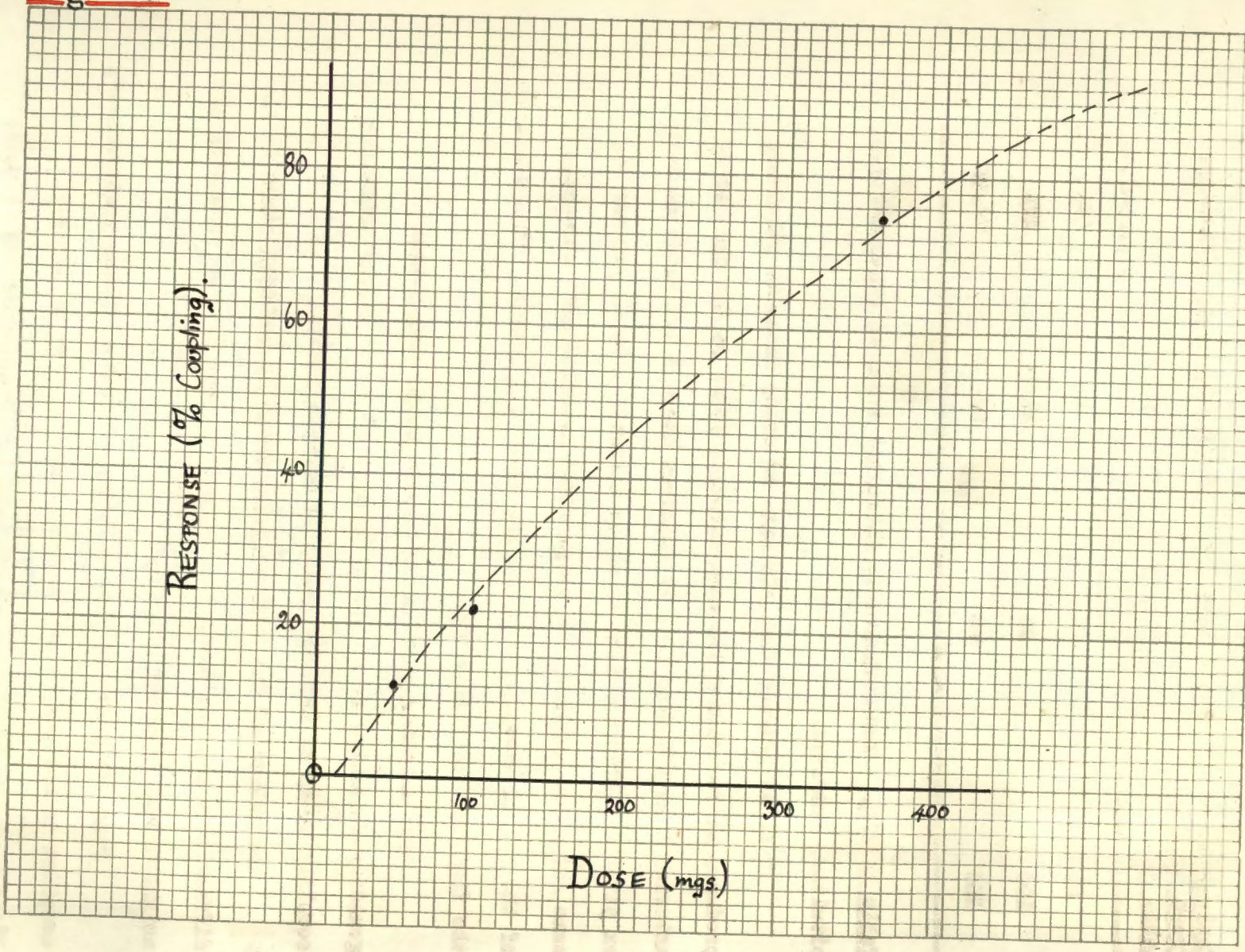
6 pairs coupled on the day following injection - a 12% response.

Group II.

11 pairs coupled on the day following injection - a 22% response.

These results are represented graphically in Fig. 24 together with the result of sheep anterior pituitary extract injections recorded in the last section. (Table XXVIII).

Fig. 24.



Equivalent Of Fresh Anterior Lobe. mgs.	Pairs Injected.	Pairs Coupling.	Response.
50	50	6	12%
100	50	11	22%
360	78	58	74.4%

Table XXVIII. The response to different doses of sheep anterior pituitary extracts.

It is noteworthy that 100 mgs. of sheep anterior pituitary produces approximately the same response (22%) as 365 mgs. of ox anterior lobe (23.4%). i.e. it requires about 3 1/2 times as much ox pituitary anterior lobe as sheep anterior lobe to produce the same response. Again, the comparative gonadotrophic strengths of the ox and sheep pituitaries are about 3 1/2 to 1. This confirms the findings recorded above.

Sheep anterior pituitaries appear to be very potent gonadotrophically, as little as 50 mgs. of fresh tissue producing coupling in12% of injected pairs of Xenopus laevis.

The nature of the dose-response curve is doubtful since it is difficult to get the correct slope without one or more points in the neighbourhood of a 50% response.

From the point of view of assay of gonadotrophic preparations, coupling in Xenopus is not satisfactory for several reasons. Firstly, large quantities of material would be necessary to carry out the tests adequately and secondly, the interval between the

injections and the first observations should be about 10 - 12 hours, i.e. the post-injection latent interval before mating occurs. This is an awkward time to fit into laboratory routine. The ovulation reaction is more suitable. It reduces the amount of material needed by half and readings can be taken after longer latent intervals. The animals would have to be killed to examine for intra - oviducal ovulation, but this is no disadvantage as compared with coupling tests, since in the latter the animals should not be re-used until all possible effects of the first injection have disappeared, by which time the sensitivity of the animals would be considerably lowered by the period of captivity, and the animals would no longer be suitable for assay of gonadotrophic principles.

B. The Experimental Induction Of Coupling By Means Of Xenopus Anterior Pituitary Implants.

The frogs from which pituitaries were taken for implantation (see above) were animals which had been in captivity for varying periods up to one month and which had never coupled spontaneously in the laboratory.

The frogs into which implantations were made were frogs that had previously coupled.

Varying numbers of Xenopus anterior pituitaries were implanted into the males and females of these pairs according to the method described above. Anterior lobes from males were used in separate experiments from those in which female anterior lobes were employed. The results are given below.

Results.

No. Of Anterior Lobes Implanted Into:		No. Of Pairs.	Pairs Coupling.
Male	Female.		
3	3	5	5
4	2	1	1
2	2	7	2
		13	8

Table XXIX. Coupling induced by anterior pituitaries from male Xenopus.

Ovulation and hyperaemia of the cloacal labia of the females occurred. Fertilized ova were recovered and reared to the tadpole stage.

No. Of Anterior Lobes Implanted Into:		No. Of Pairs.	Pairs Coupling.
Male	Female		
2	2	4	3
1	1	2	1
		6	4

Table XXX. Coupling induced by anterior pituitaries from female Xenopus.

Ovulation and hyperaemia of the female cloacal labia were marked. Fertilized ova were recovered and reared to the tadpole stage.

Xenopus Male Anterior Pituitaries.

When each partner received 3 male anterior lobes, coupling took place after 10 to 12 hours in all 5 pairs.

When the male received 4 anterior lobes and the female received 2 anterior lobes, the pair coupled after the same latent interval.

Only 2 pairs out of the 7 pairs in the group which received 2 male anterior lobes coupled. The latent interval was again 10 to 12 hours.

Xenopus Female Anterior Pituitaries.

In the first group, where each partner had 2 female anterior lobes implanted into the dorsal lymph sac , 3 of the 4 pairs coupled after the usual latent interval.

Coupling was also obtained after 10 to 12 hours in 1 out of 2 pairs in which each frog received 1 female anterior lobe.

Altogether 19 pairs of frogs had Xenopus anterior lobes implanted into their dorsal lymph sacs. 12 of these pairs coupled.

Discussion.

Eight of the thirteen pairs implanted with 2 - 4 Xenopus male anterior lobes coupled. Thus the mating reflex can be induced in Xenopus laevis by implantation of male Xenopus anterior pituitaries. This indicates that the Xenopus anterior lobes contain gonadotrophic substances. The male pituitaries stimulate both the male and female gonads. This confirms a finding in an earlier section. Again, the anterior lobes came from animals that had been in captivity for about one month, during which time they evidenced no sexual behaviour. This supports the conclusion in an earlier section that in captivity the gland secretes and stores its gonadotrophic principle, but there is no release of it into the circulation.

4 of the six pairs implanted with 2 female Xenopus anterior pituitaries coupled. The female Xenopus anterior pituitary thus also contains substances gonadotrophic to both the

male and female Xenopus. The absence of sex-specificity in the gonadotrophic principles of the anterior pituitaries of Xenopus laevis is thus confirmed. Again, the donors were captive frogs of one month's standing.

The anterior lobes of Xenopus pituitaries are able to induce coupling in Xenopus laevis. Hypophysectomy leads to cessation of sexual activity and in captivity, when the anterior pituitary of the animals is inhibited (Shapiro & Shapiro 1934) no coupling has ever been observed. Thus it seems likely that the normal stimulus to coupling under natural conditions is an hormonal one from the anterior pituitary of the animals.

C. The Effect Of The Implantation Of Dogfish Pituitaries On The Mating Reflex.

Four pairs of frogs known to couple had dogfish pituitaries implanted into the dorsal lymph sacs in the same way as in the case of Xenopus anterior pituitaries. Three to five dogfish pituitaries were implanted into each pair at 6.00 p.m. and observations were made the following day at 9.00 a.m. and every hour after that throughout the day.

Results.

No. Of Pituitaries Implanted Into:				No. Of Pairs.	Pairs Coupling.
Male	Fresh Tissue.	Female	Fresh Tissue.		
3	17 mgs.	3	23.5 mgs.	1	-
3	26.5 "	3	13.0 "	1	-
4	64.0 "	4	63.5 "	1	-
5	87.5 "	5	69.0 "	1	-

Table XXXI. Implantation of dogfish pituitaries in Xenopus laevis.

No coupling took place in any of the pairs used in this experiment. No ovulation was seen.

Discussion.

In the doses used the absence from the anterior pituitary of an elasmobranch fish of substances gonadotrophic to Xenopus laevis is confirmed.

D. The Experimental Induction Of Coupling In Xenopus Laevis By Means Of Injecting The Female Partner Only.

Shapiro (1936 a, 1936 b) has shown that coupling in Xenopus laevis can be induced experimentally by the injection of only the female partner of a pair of frogs with sheep anterior pituitary extracts. This work was repeated and extended.

Experiment 1.

Injection Of Sheep Anterior Pituitary Into The Female Only.

25 pairs of frogs freshly brought in from the ponds were injected with 0.8 mls. of S.23., each frog thus receiving extract equivalent to 528 mgs. of fresh anterior lobe. Injections were made at 6.00 p.m. and observations were made throughout the following day, starting at 9.00 a.m. This experiment was performed on the 14th and 15th day of December, 1937.

Results.

One pair coupled. Ovulation occurred in most of the containers.

Conclusions.

The result confirms the findings of Shapiro (1936 a, 1936 b), that when the female is injected, coupling occurs, but the response is smaller than when both the male and female partners are injected. The response in the present experiment (4%) is much smaller than that of Shapiro (1936 b) who found a 30% response using doses of human pregnancy urine extract equivalent to 360 mgs. of fresh sheep anterior pituitary tissue. The latter experiments

were performed in July, during the breeding season. It appears, therefore, that the animals undergo a marked loss of sensitivity during the period of aestivation.

This loss of sensitivity to injected gonadotrophic substances during the summer months has been demonstrated clearly by Shapiro (1938) using methyl testosterone, and by Zwarenstein (1938) using progesterone. The test-object in each case being ovulation.

Experiment 2.

The Role Of The Anterior Pituitary Of The Male In The Establishment Of The Mating Reflex.

The role of the pituitary of the male Xenopus in the coupling produced by injection of the female partner only was investigated using testosterone as stimulus.

A large number of male and female frogs were brought in from the ponds during the last week of June 1938. Half of the males were hypophysectomised according to the method described above.

The frogs were then divided into two groups.

Group I.

Consisted of fifty pairs, the male and female partner of each pair being normal. Each female received an intraperitoneal injection of 1.0 mgs. of testosterone dissolved in 1.0 ml. of nut oil. The pairs were placed in containers. Injections were made at twelve o'clock midnight and observations were made at 8.30 a.m. and every hour after that throughout the following day.

Group II.

There were 50 pairs in this group also. The female were normal and were injected with 2.0 mg. of testosterone in 1.0 ml. of nut oil in the same way as the animals in Group I. The males were selected from the hypophysectomised animals. Only frogs that were black on a white background and which, therefore, had only their anterior lobes removed were used. The males were not injected. The animals were placed in containers, these being similar to those in which the animals of Group I were kept.

Results.

Pairs With.	No. Of Pairs. Used.	No. Of Pairs. Coupling.	% Response.
Male Normal	50	6	12
Male Hypophysectomised	50	2	4

Table XXXII. The effect of hypophysectomy of the male partner on coupling induced by the injection of the female partner only.

Of the animals in GroupI, six pairs or 12% coupled.

In Group II, two pairs or 4% coupled.

Discussion.

From previous studies it was not expected that any of the animals in Group II would couple on account of the important part played in coupling by the anterior pituitary. It has, however, been noted in previous experiments that an hypophysectomised male soon after the operation will couple with a female that has been

injected 24 - 48 hours previously. It was thought probable that that coupling could be explained on the theory that a portion of the anterior lobe may be left behind during the operation, that this was absorbed subsequently and the presence of the hormones in the blood induced coupling. This may be the explanation in the present instance, in which case the results are to be interpreted as indicating that the activated female stimulates the anterior pituitary of the male and that amplexus occurs as a result of this. Absence of the anterior pituitary in the male partner inhibits this reaction except in cases where a portion of the anterior lobe is left behind at operation. This conclusion is perhaps supported by the fact that the females in Group II received a dose of testosterone twice as large as that used in Group I.

On the other hand the difference in the results in the two groups is not very large, and may be entirely explained on the basis that the operation disturbed the males sufficiently to prevent coupling.

The experiments ought to be repeated using larger numbers of frogs and a stronger gonadotrophic stimulus, such as human pregnancy urine extracts, which are capable of producing a 30% response in 0.5 ml. doses (Shapiro 1936) when injected into the female partners only during the breeding season.

It is interesting to note that testosterone, the mammalian testis hormone, is capable on its own of producing coupling in Xenopus laevis. This is a result of its gonadotrophic action on the female.

Summary.

1) In doses of 360 mgs. ox anterior pituitary produces coupling in 23.4% of pairs injected and sheep anterior pituitary in 74.4%.

2) 100 mgs. of sheep anterior pituitary produces coupling in 22% of injected pairs.

3) Thus sheep anterior pituitary is 3 1/2 times as potent as ox anterior pituitary. Since the weight of the average ox anterior pituitary used was 3 1/2 times as great as that of the average sheep anterior lobe, one ox anterior pituitary has approximately the same gonadotrophic activity as one sheep anterior pituitary.

4) Coupling is not a satisfactory test-object for the assay of gonadotrophic hormone.

5) Coupling can be induced in Xenopus laevis by anterior pituitaries from male and female Xenopus but not by anterior pituitaries from dogfish.

6) Coupling can be induced by the injection of sheep anterior pituitary extract into the female partner only.

7) Testosterone, the mammalian testis hormone, induces coupling when injected into the female only.

8) A marked loss of sensitivity to injected gonadotrophic substance occurs during aestivation in Xenopus laevis.

9). Coupling occurs under natural conditions as a result of an hormonal stimulus from the anterior pituitary.

II Experimental Analysis Of The Mating Reflex.

Introduction.

Sex recognition in frogs and toads has been studied in many species.

In Salienta, sight and chemical sense play no part in the correct orientation of the partners in the mating reflex. The male clasps all objects, releasing those which have not the silence and stoutness of the female (Banta 1914, Cummins 1920, Miller 1909, Hinsche 1926 and Lullies 1926).

In Bombina variegata the mechanism is more complex (Savage 1932). In this species the male alternately calls to and searches for the female, but the adoption of the correct amplexus position is regulated by a special sound produced by the female. Each partner produces a note that is a sexual stimulant.

In the toad, also, the male maintains his clasp only on females. According to Rostand (1934), the eyes are most important at a distance. In distinguishing between male and female the important factors are that the female is quiet, does not struggle, has a longer and firmer body, a coarser and more warty skin and a peculiar vibration of the flanks. In addition, Rostand considers that there is a special chemical sense that assists in sex recognition.

Rugh (1935) observed in artificially induced amplexus, that the male toad Bufo fowleri is indiscriminate when sexually aroused,

going into amplexus with male toads or female frogs of various species when female toads are not available. Noble and Farris (1929) describe a similar lack of discrimination in Rana sylvatica. This lack of discrimination is not seen in Rana clamitans, catesbiana, pipiens and palustris. (Rugh 1935).

In Rana temporaria the male is attracted to any other frog by means of the sense of sight, but sex recognition occurs by means of the tactile sense (Savage 1934), the female possessing "pearly granulations", described by Huber (1887).

Savage (1934) has observed that sex recognition in bufo bufo depends on the voice possessed by the male, who chirps when clasped, and on a dodging, repulsive action shown by the males when seized. Liu (1931) describes a similar mechanism in Bufo asiaticus. In the latter species the male is also capable of distinguishing between the gait of a male and female.

In Xenopus laevis, Shapiro (1936 a, 1936 b) has emphasised the importance of the passive role of the female in the adoption of the embrace. The male does not sieze its partner as in the species described above, but approaches her slowly and tightens his grip around her very gradually. This finding of Shapiro (1936 a, 1936 b) fails to confirm the observations of Bles (1901) who states that the male makes a sudden dash at the female from about six inches behind her.

Mode Of Adoption Of The Normal Embrace In Xenopus Laevis.

In Xenopus laevis the clasp is lumbar (Shapiro 1936 a). In this respect it resembles that of the tailless Batrachians, Pelobates, Discoglossus, Alytes, Pelodytes and Bombinator (Rostand 1934) and Cystignathidae (Boulenger 1890). It differs therefore from the embrace of the toad Bufo, the common frog and the tree frog, which is axillary. Thomas has suggested that there is a connection between the pupil and the mode of embrace. The horizontal pupil corresponds with the axillary embrace and the vertical or triangular pupil with the lumbar embrace. In Xenopus laevis, however, the lumbar embrace is associated with a circular pupil.

In order to study the mode of adoption of the lumbar embrace, coupling pairs are isolated and the partners are separated. After a variable time of five minutes to several hours, attempts at coupling recommence, and observations can be made. Absolute silence and stillness are necessary, since the animals are easily disturbed and coupling may be delayed for several hours. Shallow glass vessels are best for this work.

In such pairs the female lies motionless, while the male croaks almost continuously, gradually approaching the female. The approach is towards the lumbar region. The male approaches the female more often from her left side than from the right.

In the first place the male loosely clasps the left thigh of the female in the case of the usual approach from the left. His left forelimb is round the left lumbar region of the female and his

right forelimb lies between her thighs. The body of the male is thus at an angle to the long axis of the female. Soon the male begins to move into line with the female. His right forelimb moves up and down, i.e. towards and away from the right thigh of the female, at the same time approaching her body. Finally the right forelimb reaches the right lumbar region of the female.

If disturbed at this stage, the pair separate readily.

Thus it appears that the clasp is still a loose one. From this time the clasp gradually becomes stronger until the reflex is complete. The embrace now is very firm and the partners are separated only with considerable difficulty. Coupling pairs have been transported as far as 5 miles by motor car without separating.

The male croaks almost continuously throughout this manoeuvre.

A. Departures From The Normal Lumbar Embrace.

Nine different abnormal clasps have been observed at various times during the experiments on coupling.

Of these, two clasps are merely stages in the adoption of the normal embrace (1 and 2).

1. Left Femoral. (Fig. 25).

The left forelimb of the male is in the position normally adopted in coupling, but the long axis of the male is at an angle to that of the female and the right forelimb is between the hindlimbs of the female.

This position has been noticed on quite a number of occasions and is a stage in the adoption of the normal embrace. It is soon discarded in favour of the normal embrace. The frogs when in an abnormal clasp separate rapidly when disturbed. This made it difficult to obtain photographic records of abnormal positions.

2. Right Femoral. (Fig. 26).

This position is the mirror image of the left femoral. The right forelimb of the male is in the normal position, and the left forelimb is between the hindlimbs of the female.

This position is seen less frequently than the left femoral and is also a stage in the adoption of the normal embrace and hence is also not maintained for any length of time. This clasp also is not very firm and the frogs separate at the slightest disturbance.

Fig. 25.

Left femoral clasp.

Fig. 26.

Right femoral clasp.

3. Pectoral. (Fig. 27).

This position is further away from the normal clasp and approaches the axillary clasp of Rana. The forelimbs of the male are tightly clasped around the upper part of the trunk of the female.

This clasp is as firm as the normal clasp and may be maintained for several hours. It is seen fairly frequently.

4. Reverse Axillary. (Fig. 28).

In this position the long axes of the partners are in the same line, but the frogs are facing in opposite directions, the forelimbs of the male being round the axillae of the female.

This clasp is seen fairly frequently, and is not a very firm one. It is not maintained for any length of time.

The remaining five clasps are extremely rarely seen.

5. Reverse Lumbar. (Fig. 29).

The frogs face in opposite directions. The forelimbs of the male are clasped round the lumbar region of the female.

This clasp has only been seen on two occasions and is an extremely firm one. In one of the pairs the embrace was observed to be maintained for six hours, after which the frogs separated. Later the normal position was adopted.

6. Double Clasp. (Fig. 30).

Here another male is clasping the male of a normally coupling pair. The clasp of the second male is the same as that of a male normally clasping a female.

This clasp has been seen five times and is firmly

Fig. 27.

Pectoral clasp.

Fig. 28.

Reverse axillary clasp.

Fig. 29.

Reverse lumbar clasp.

Fig. 30.

Double clasp.

maintained for several hours. On both occasions another female was present in the same container. The non-coupling female in each case was completely normal and her ovaries were in good condition.

At post-mortem the three frogs in the clasp were all anatomically normal.

7. Left Reverse Lumbar. (Fig. 31).

This clasp is similar to the reverse lumbar. The long axis of the male is at an angle to that of the female and the frogs are facing in opposite directions.

The left forelimb of the male is around the right lumbar region of the female, but the right arm is around the left flank of the female, about its middle.

This clasp has been seen once and was not a firm one. It was soon relinquished as a result of a slight disturbance.

8. Right Cervico-Axillary. (Fig. 32).

The frogs are facing the same way, but their long axes are at an angle to each other. The right forelimb of the male is around the right axilla of the female, and the left forelimb is around the head of the female.

This position was seen once. The clasp was fairly firm and not relinquished in spite of considerable disturbance. It was maintained for some time.

9. Right Subaxillary. (Fig. 33).

The male frog lies on his right side, clasping the right side of the female, his left forearm being on the female's back and the right

Fig. 31.

Left reverse lumbar clasp.

Fig. 32.

Right cervico-axillary clasp.

Fig. 33.

Right subaxillary clasp.

forearm on the front of the female, near the level of the arms. Both frogs are facing in the same direction.

This clasp was also seen on one occasion only. It was not very firm and was soon given up in favour of the normal embrace.

Discussion.

From the observations described above, it appears probable that two factors of importance in the adoption of the lumbar embrace are the passiveness of the female (as described by Shapiro, 1936) and the tactile sense of the male. The significance of the croak is not clear. The latter question is discussed again later.

The importance of the passiveness of the female is further emphasised by the following observations. Males have only been seen to embrace other males when the latter are moribund or dead or are already embracing a female. Only one exception to the above statement has been observed, both males being perfectly healthy and showing no anatomical abnormalities at post-mortem examination.

The importance of the tactile sense of the male is perhaps supported by the fact that abnormal clasps are seldom maintained for any length of time, except in those cases where the contours of the portion of the female clasped resemble those of the normal position, e.g. the reverse lumbar clasp.

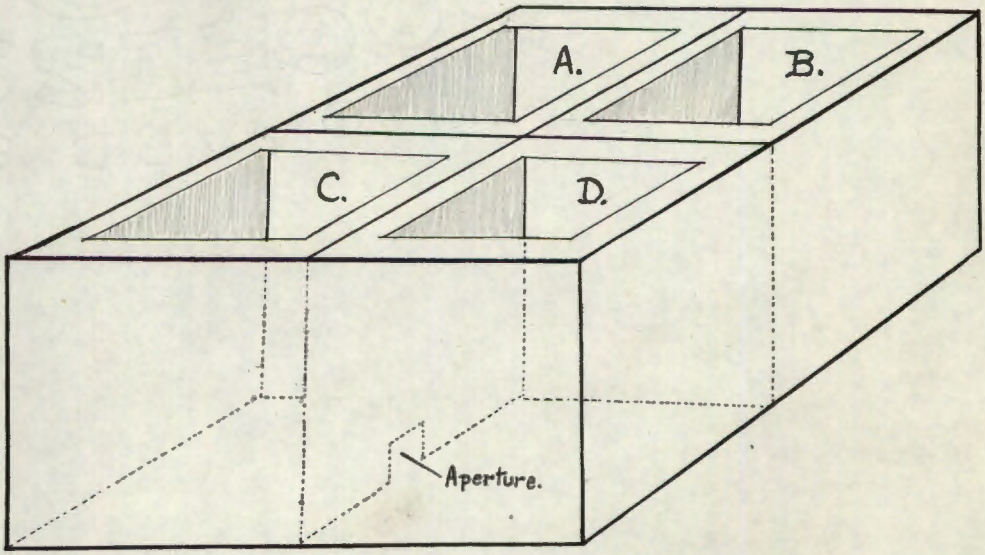
B. The Movements Of The Sexually Activated Male And Female Xenopus.

The investigation below was undertaken to determine how the males and females are brought together before the actual clasping reflex is exhibited.

For these experiments a special tank was constructed. (Fig. 34). The tank was made of galvanised iron and measured 3' x 3'. The depth was 12 inches. A plug was inserted in the floor of the tank for changing the water. The tank was divided into two equal parts by a partition. The two halves being completely separate and water-tight. Each half was again divided into two by a partition at right angles to the first partition. This second partition had a small hole, measuring 2" x 2" in the centre of its lower part, called the "aperture". Thus there were four compartments, A, B, C and D. A communicates with B through an aperture but these two are entirely separate from C and D which communicate only with each other. The compartments were closed for 3 inches from the tops of their walls by sheets of metal to prevent the frogs from escaping by jumping out of their compartments or from leaving their compartments by any way except via the aperture. The tank was filled with tap water to a depth of 1 - 1 1/2 inches and the apertures were closed by blocks of wood.

The tank was kept in a quiet place and the experiments were carried out during the night to exclude noise and other external disturbances as far as possible.

OBSTRUCTION TANK.



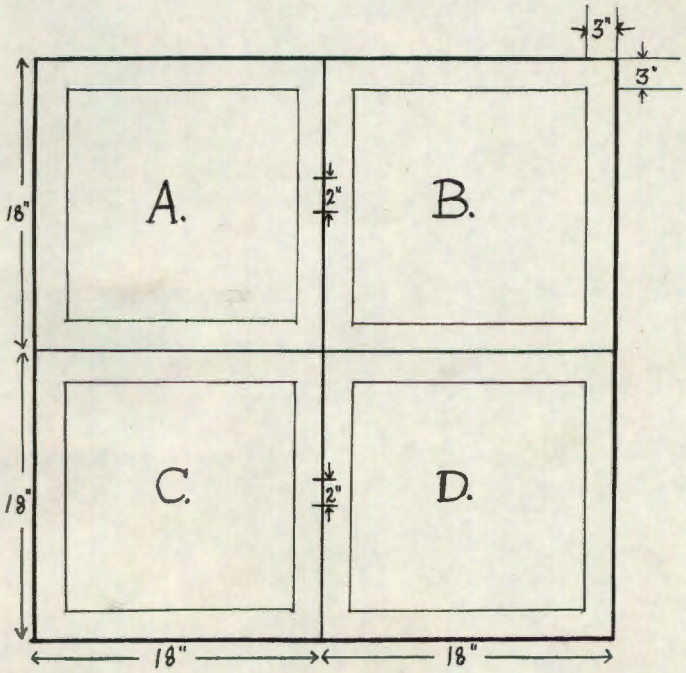
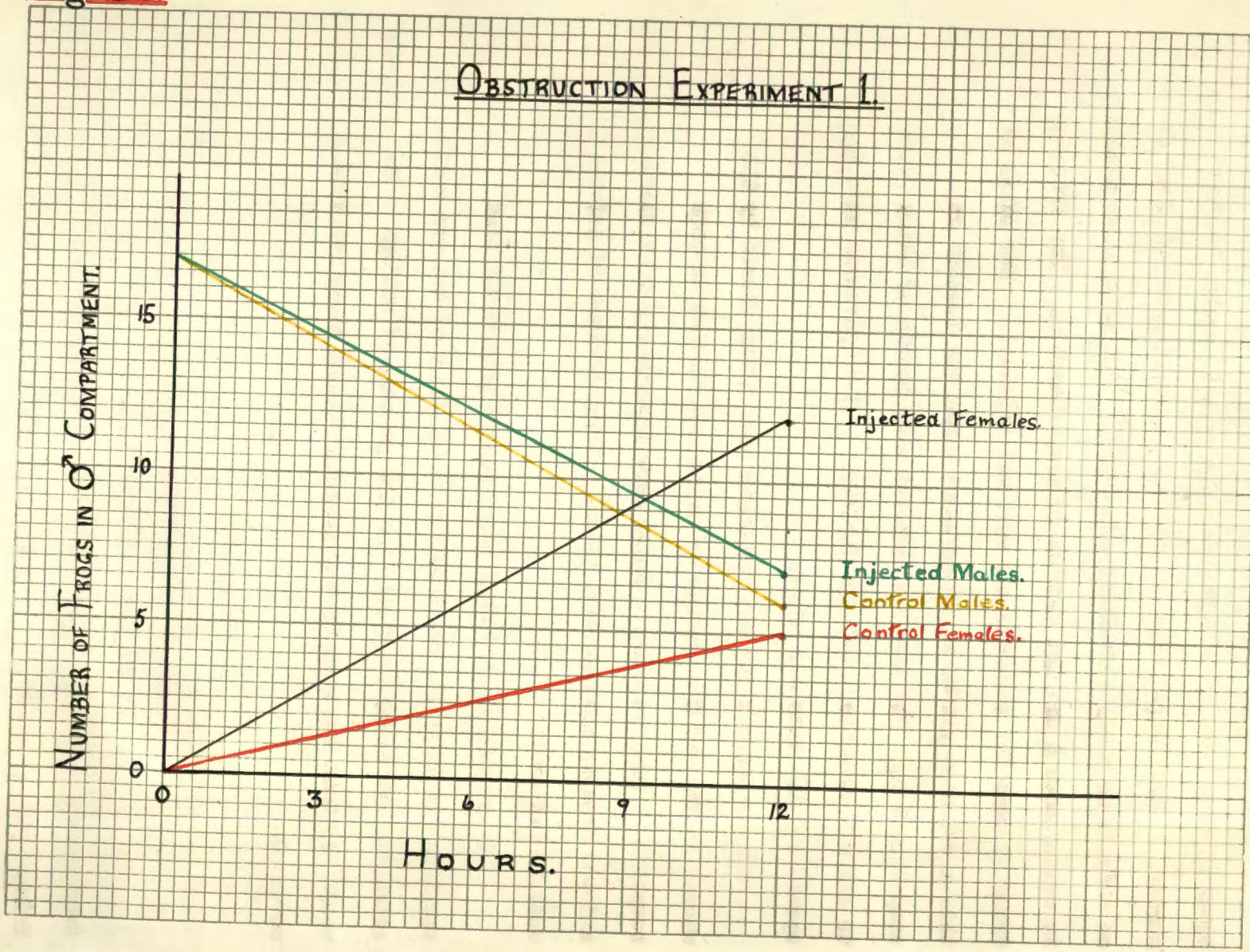


Fig. 34.

Fig. 35.


OBSTRUCTION EXPERIMENT 1.
NUMBER OF FROGS IN ♂ COMPARTMENT.
15
10
5
0
0
3
6
9
12
HOURS.
Injected Females.
Injected Males.
Control Males.
Control Females.

Frogs which had previously coupled were divided into two equal groups, each group containing males and females in equal numbers. The frogs in one group were injected with 0.5 mls. of an extract of sheep's anterior pituitary. The males were placed in compartment A and the females in compartment B. The frogs in Group II were the controls and were not injected. The males of this group were placed in compartment C and the females in compartment D. The tank was covered with sheets of black paper to exclude extraneous stimuli further. The apertures were then opened and the time was noted.

Twelve hours later the apertures were again closed by the wooden blocks and the numbers of males and females in each compartment were counted. Three such experiments were carried out and the results are recorded below. Large numbers of pairs were used in all cases.

It was anticipated that these experiments would shed considerable light on the question as to which of the two sexes attracted the other to itself for the purpose of mating. The controls would indicate how much of the movement taking place was random movement.

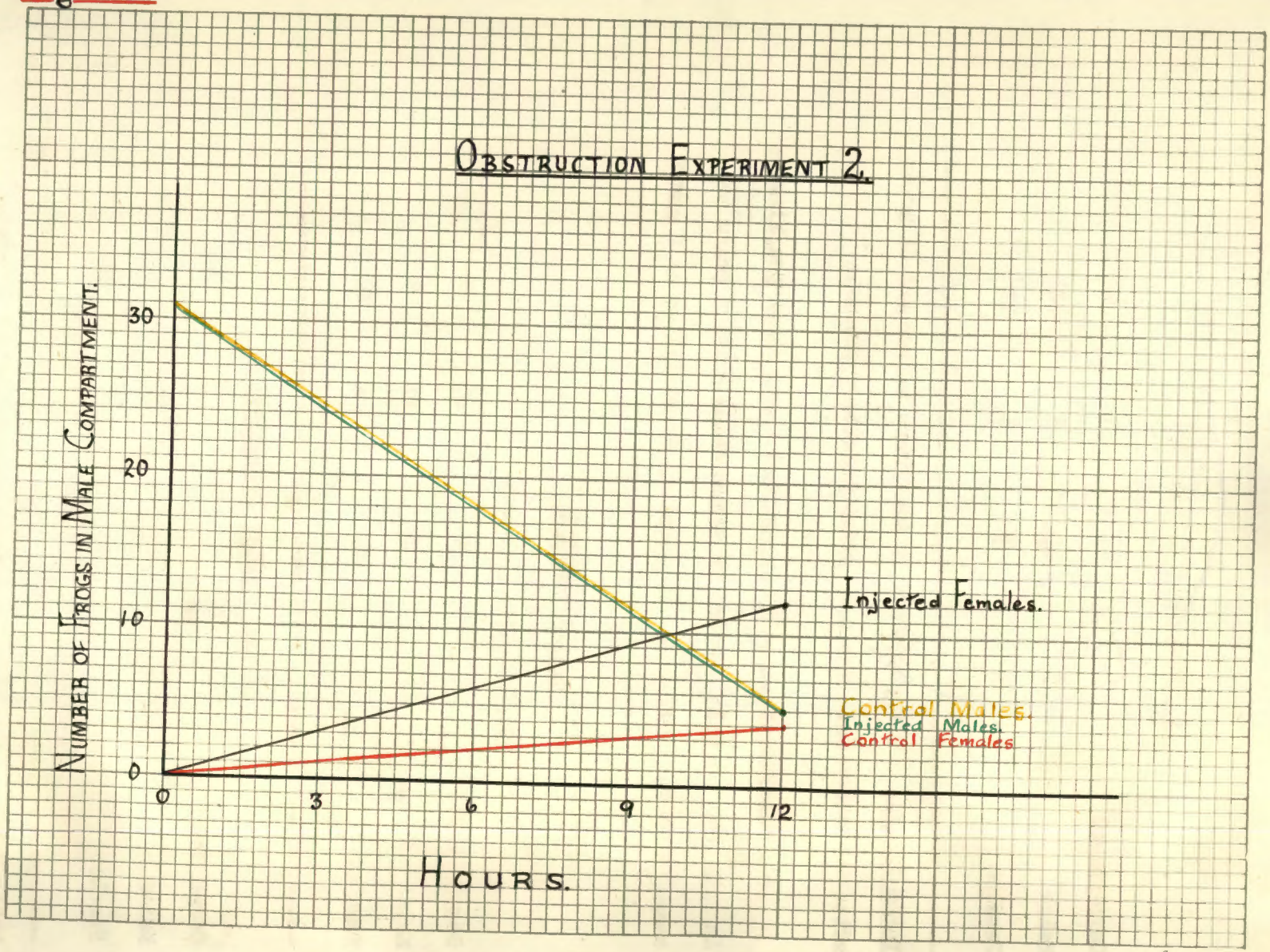
Results.

Experiment 1.

At The Time Of Injection.

Compartment	A	B	C	D
Males	17	0	17	0
Females	0	17	0	17

Fig. 36.


OBSTRUCTION EXPERIMENT 2.
NUMBER OF FROGS IN MALE COMPARTMENT.
30
20
10
0
0
3
6
9
12
HOURS.
Injected Females.
Control Males.
Injected Males.
Control Females

12 Hours After Injection.

Compartment	A	B	C	D
Males	7	10	6	11
Females	12	5	5	12

Table XXXIII. The positions of the frogs before and 12 hours after injection with sheep anterior pituitary extract.

The results obtained in Experiment 1 are shown graphically in Fig. 35. 1 pair coupled in compartment A and 2 pairs in compartment B. There was no coupling or ovulation in C and D.

Experiment 2.

At Time Of Injection.

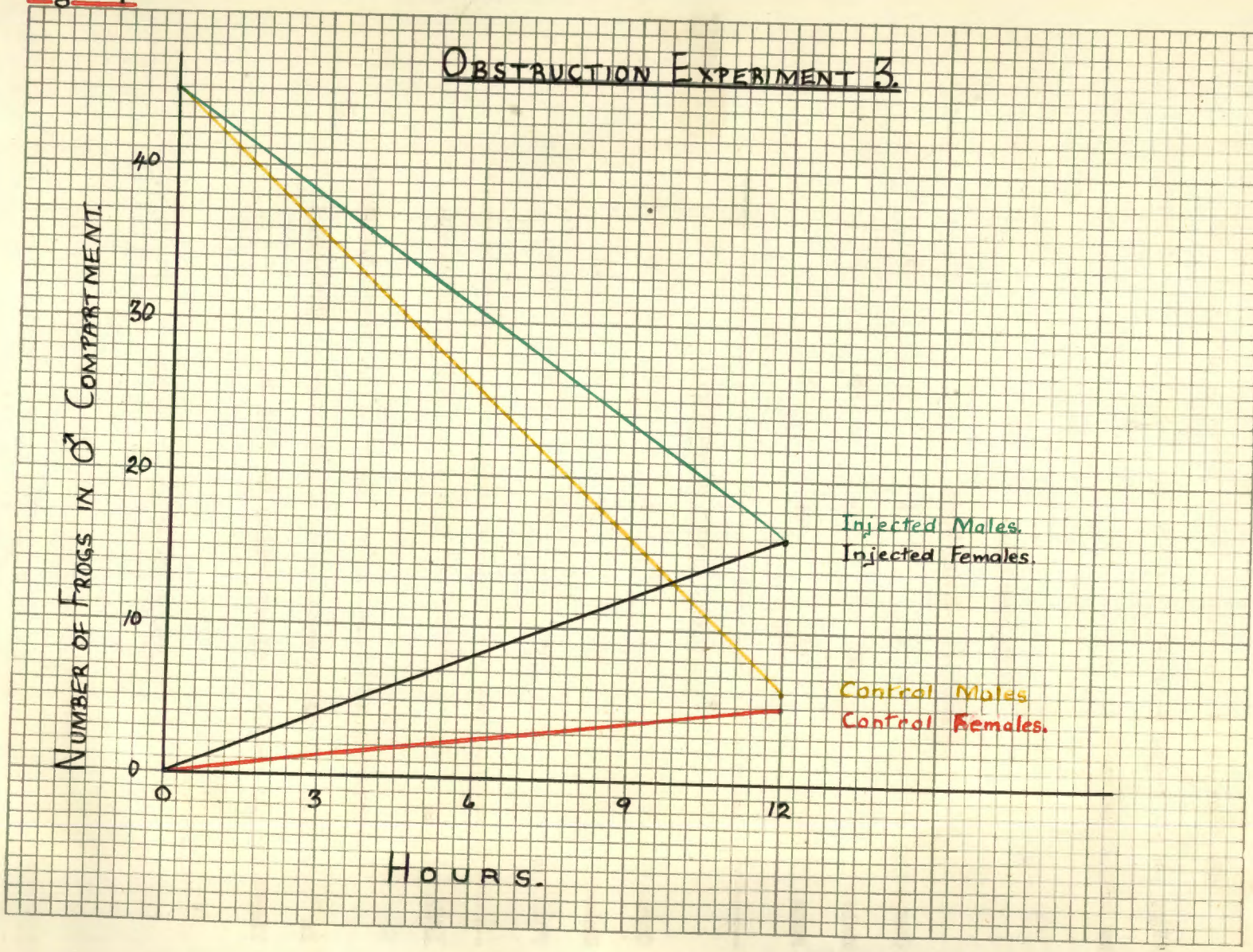
Compartment	A	B	C	D
Males	31	0	31	0
Females	0	31	0	31

12 Hours After Injection.

Compartment	A	B	C	D
Males	5	26	5	26
Females	12	19	4	27

Table XXXIV. The positions of the males and the females.

Fig. 37.


OBSTRUCTION EXPERIMENT 3.
NUMBER OF FROGS IN ♂ COMPARTMENT
40
30
20
10
0
0
3
6
9
12
HOURS.
Injected Males.
Injected Females.
Control Males
Control Females.

The results in Experiment 2 are shown graphically in Fig. 36. 4 pairs coupled in A and 5 pairs in B. No coupling or ovulation in C or D.

Experiment 3.

At Time Of Injection.

Compartment	A	B	C	D
Males	45	0	45	0
Females	0	45	0	45

12 Hours After Injection.

Compartment	A	B	C	D
Males	18	29	6	39
Females	16	29	5	40

Table XXXV. The positions of the males and the females.

The results obtained in Experiment 3 are shown graphically in Fig. 37. 11 pairs coupled in A and 13 pairs coupled in B. There was no coupling or ovulation in C or D.

Fig. 38.

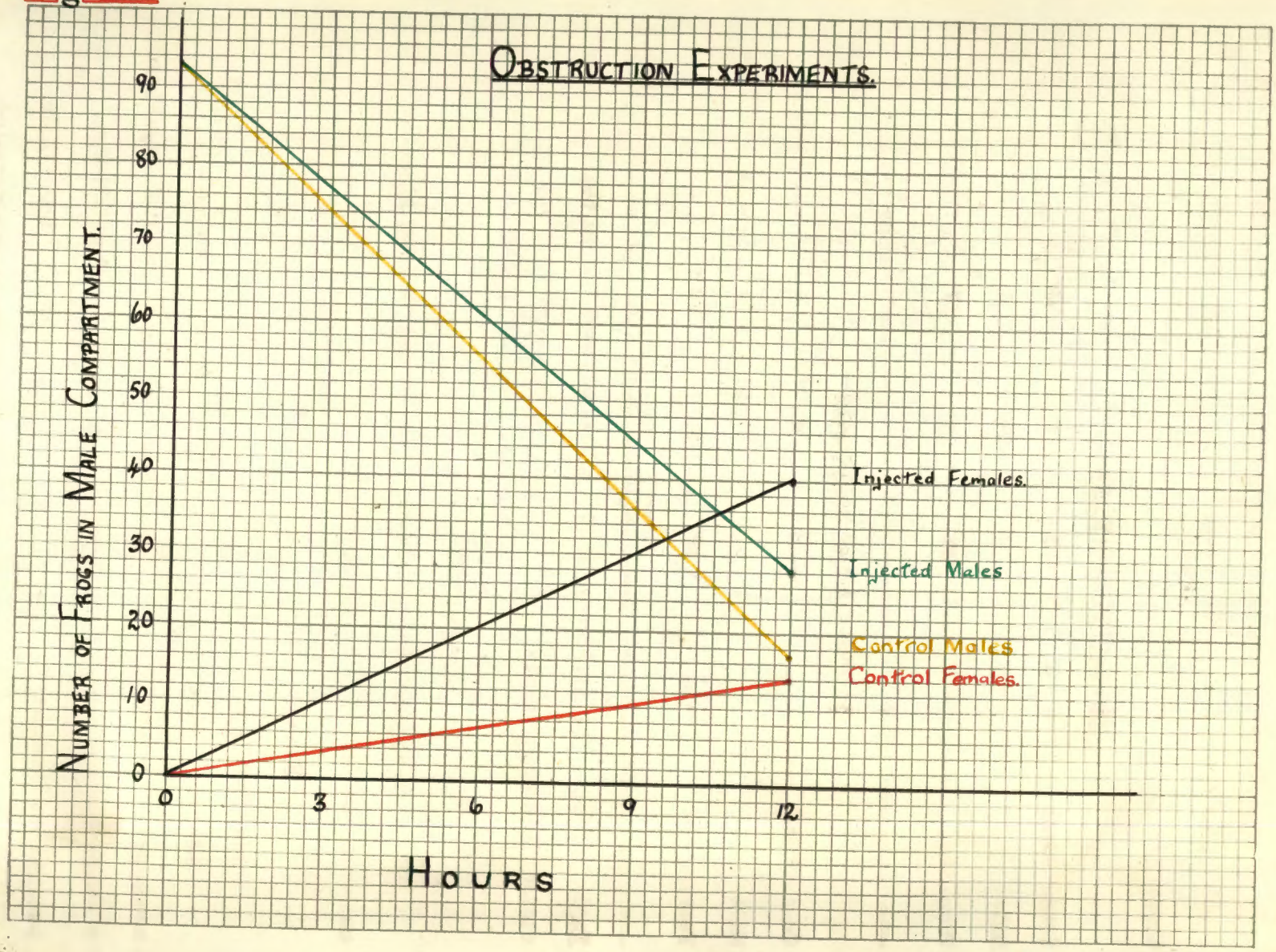

OBSTRUCTION EXPERIMENTS.
NUMBER OF FROGS IN MALE COMPARTMENT.
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
HOURS
Injected Females.
Injected Males
Control Males
Control Females.

Table XXXVI & Fig. 38 represent the total results of the three groups.

At Time Of Injection.

Compartment	A	B	C	D
Males	93	0	93	0
Females	0	93	0	93

12 Hours After Injection.

Compartment	A	B	C	D
Males	23	66	17	76
Females	40	53	14	79

Table XXXVI. The positions of the males and females in the obstruction experiments.

Discussion.

The graphs show the movements of males and females injected and controls in and out of compartment A and C.

Males.

Experiment 1.

Of the fifteen males in compartment A only 7 remained 12 hours after injection. 6 of the 15 controls were left in compartment C after the same time. Thus there is no significant difference between the movement of injected and control males. Thus all the movement of the injected males can be ascribed to random movement.

Experiment 2.

Here there is no difference at all between the movements of the injected and control males, 26 of the 31 males moving into the female compartments in each case. Thus again all the movement of injected males appears to be random movement.

Experiment 3.

In this experiment there is a difference between the curves for injected and control males. 16 of the 45 injected males are left after 12 hours, whereas only 6 of the 45 control males remained in compartment C, i.e. 29 injected males left their compartment as compared with 39 males in the control group. Thus it appears that the injection of sheep anterior extract decreased the movement of the males.

From Fig. 38 it can be seen that 76 males moved into the female compartment in the control experiment, whereas 65 of the injected males moved out, i.e. of the total number of males used, approximately 12% less injected males than control males left their compartment. The injection thus seems to tend to keep the males in their original compartment.

Females.

Experiment 1.

A marked difference is seen in the movements of injected and control females. 12 injected females moved into the male compartment, but only 5 control females were found in the male compartment after 12 hours, i.e. a 71% movement of injected

females and a 29% movement of control females. This suggests that the injection of sheep anterior pituitary extract increases the movement of the females into the male compartment.

Experiment 2.

A similar result is seen in Group II. Here 39% of the injected females moved into the male compartment, whereas only 13% of control females left their compartment. Again the effect of sheep anterior pituitary injections appears to increase the movement of the females into the male compartment.

Experiment 3.

The results here confirm the findings of Group I and II. 36% of the injected females moved into the male compartment compared with the 11% response obtained in the uninjected controls.

Fig. 38 clearly demonstrates the difference in movements between injected and control females. 15% of the control females were found in the male compartment after 12 hours. 43% was the figure obtained in the case of injected females. Thus it may be concluded that sheep anterior pituitary injections increase the movement of females into the male compartment.

The correlation between the results obtained with injection of sheep anterior pituitary extracts into male and female Xenopus is obvious. When the female is activated she moves towards the male. The male, when activated moves less, and thus appears to await the arrival of the female. Coupling is also obtained in the female compartment (B) because if a male by random movement

reaches compartment B, there is no reason why he should not couple there with an activated female.

It is noteworthy that in Rana the males reach the breeding ground first. A few days later the females arrive, attracted, it is thought, by the croaking of the males (Savage 1934). In Xenopus the activated male also croaks so that it is possible that the croak of the male plays an important part in attracting the female to itself. The female never croaks.

This experiment shows that the role of the female in the sexual behaviour of Xenopus is not entirely passive. The female, in fact, plays a most important part in the bringing together of the two sexes. Later, in the actual adoption of the lumbar embrace by the males, the role of the female is passive (Shapiro 1936 a). In the bringing together of the sexes, the male appears to be the passive partner.

C. The Selection Of Partners For Coupling In Xenopus Laevis.

Pairs of frogs were injected with 0.5 ml. of an extract of sheep's anterior pituitary. After a latent interval of about 10 - 12 hours, the usual response was obtained, i.e. about 70% of the pairs coupled.

Coupling pairs were gently removed and placed in separate containers, one pair per container. In this way a stock was collected of pairs of frogs that were known to couple. The containers were numbered. The females of various containers were interchanged and then all the frogs were re-injected with 0.6 ml. of the extract of sheep anterior pituitary and left overnight.

The results are recorded in table XXXVII below.

Male.		Female.	Injection.	Coupling.
1	x	2	0.6 ml. S.2.	-
2	x	1		C
3	x	4		C
4	x	3	≡380 mgs.	C
5	x	6	fresh tissue.	C
6	x	7		C
7	x	5		C
8	x	9		C
9	x	8	0.6 ml. S.4	C
25	x	10		C
11	x	12		C
12	x	11		C
14	x	15	≡380 mgs.	C
15	x	14	fresh tissue.	C
16	x	17		C
17	x	16		C
18	x	19		C
19	x	18		C
20	x	21		C
21	x	20		C
22	x	24		C
23	x	22		-
24	x	23		-

Table XXXVII. Coupling after the first interchange of partners.

Of the 23 pairs injected, 20 coupled.

The remaining three pairs were re-injected with 0.7 ml. of S.2 (Table XXXVIII).

Results.

Male.		Female.	Injection.	Coupling.
1	x	2	0.7 ml. S.2	C
23	x	22		C
24	x	23	≡320 mgs.	C

Table XXXVIII. Injection of pairs which failed to couple after the first interchange of partners.

All three pairs coupled.

A further change of partners was made in the 20 pairs that coupled after the first injection and these frogs were re-injected in the same way as before. (Table XXXIX).

Results.

Male.		Female.	Injection.	Coupling.
2	x	7	0.6 ml. S.2	-
3	x	1		C
4	x	6	≡380 mgs.	-
5	x	3	fresh tissue.	C
6	x	5		C
7	x	4		C
8	x	10		C
9	x	12		C
25	x	9	0.5 ml. S.5	C
11	x	8		C
12	x	14		C
14	x	17	≡320 mgs.	C
15	x	11		C
16	x	15	fresh tissue.	C
17	x	18		C
18	x	21		C
19	x	16		C
20	x	19		C
21	x	24		C
21	x	20		-

Table XXXIX. Coupling after the second interchange of partners.

Of these, 17 pairs coupled.

The three pairs that did not couple after the second interchange of partners were re-injected. (Table XL).

Male.		Female.	Injection.	Coupling.
2	x	7	0.5 ml. S.3	C
4	x	6	≡ 360 mgs.	C
22	x	20	0.5 ml. S.6 ≡360 mgs.	C

Table XL. Injection of the pairs that failed to couple after the second interchange of partners.

The experiment was repeated using extract of human pregnancy urine as stimulus. 6 pairs of frogs were used. (Table XLI).

Results.

Male.		Female.	Injection.	Coupling.
26	x	27		C
27	x	28	0.5 ml. P.U.2	-
28	x	29		C
29	x	30	≡ 2.25 mgs.	C
30	x	26	Solids.	-
31	x	32		C

Table XLI. Coupling after interchange of partners.

Four pairs coupled.

The remaining 2 pairs and another pair made up of male 32 and female 28 were re-injected. (Table XLII).

Results.

Male.		Female.	Injection.	Coupling.
27	x	28	0.5 ml.P.U.2	C
30	x	26	≡ 2.25 mgs.	C
32	x	28	Solids.	C

Table XLII. Injection of pairs which failed to couple after interchange of partners.

All three pairs coupled.

Altogether 50 pairs were injected after interchange of partners. 42 coupled. Of the remaining 8 pairs, all coupled after a third injection. Thus all 50 pairs coupled after interchange of partners.

Discussion.

20 of the males used coupled with three different females (original partner and two interchanges). 10 males coupled with two different females (original partner and one interchange). In the females also, coupling took place with two or three different males.

Thus there seems to be no special selection of partners for coupling in Xenopus laevis. Animals that are sexually activated and ready to mate, do so indiscriminately, the activated male clasping any activated female, and the sexually active female allowing any male to clasp her. However, apart from the exceptions noted above, Xenopus males clasp Xenopus females only. The lack of discrimination observed in Rana, where the male clasps all objects, is not seen.

D. The Role Of The Distance Receptors In The Establishment Of The Clasping Reflex.

1. The Eyes.

Thirty-three pairs of frogs that coupled previously were collected and divided into three groups.

(a) In the first group (fifteen pairs) the eyes of each male partner were removed.

(b) In the second group (thirteen pairs) the eyes of each female partner were removed.

(c) In the third group (five pairs) the eyes of both the male and the female partners were removed.

In order to remove the eyes the animals were anaesthetised by immersion in a mixture of ether and water. The eyes of Xenopus are very prominent and can easily be dissected out. Great care was taken to remove the retina completely. Very little bleeding occurred after the operation. A second injection of the pregnancy urine extract (0.5 ml.) was given intraperitoneally immediately before or after the extirpation of the eyes.

Results.

(a) Eyeless Males Paired With Normal Females.

The animals remained in good condition and showed no signs of shock. Haemorrhage was minimal and gave no cause for concern. Attempts at coupling, accompanied by vigorous croaking on the part of the male, were observed within 1 - 2 hours after the operation, and on two occasions the normal lumbar embrace was adopted by the eyeless males within 40 minutes after the injection had been given.

It was noticed that a considerable number of abnormal positions of coupling was adopted. These abnormal positions are seen only very infrequently when both partners are normal. The eyeless male frequently embraced a hindlimb or a forelimb or even the head of the female. Ultimately, however, such positions were relinquished and the normal lumbar embrace adopted in each of the fifteen pairs injected within the usual 10 - 12 hours.

Fertilised ova were recovered and reared to the tadpole stage.

(b) Eyeless Females Paired With Normal Males.

The female toads appeared to withstand removal of the eyes as well as did the males. The normal lumbar embrace was adopted without difficulty within 10 - 12 hours after injection in each of the thirteen pairs injected.

Fertilised ova were recovered and reared to the tadpole stage.

(c) Eyeless Males Paired With Eyeless Females.

Abnormal positions of coupling as described in group (a) above were again observed. All five pairs used in this experiment, however, coupled in the normal way within 10 - 12 hours after injection.

Fertilised ova were recovered and reared to an advanced tadpole stage.

Discussion.

The fact that the normal lumbar embrace can be adopted by the eyeless male suggests that the eyes do not play an important role in the orientation of the male during clasping. It is likely that the eyes facilitate the adoption of the normal lumbar embrace, as

the adoption of abnormal embraces(see above) was frequently observed in the case of the eyeless males. During such an embrace the male would execute the forward movements characteristic of coupling, even though the lumbar region of the female was not being clasped. The eyes, therefore, act as a refinement which assists in the establishment of the normal embrace. They are not at all, however, essential, as the total time taken did not differ from that for normal pairs. This is further substantiated by the experimental results obtained with the animals in group (c).

The passive role of the female in the actual mating (Shapiro 1936 a) is again emphasised in the experiments in group (b),when eyeless females were paired with normal males. The abnormal positions characteristic of the eyeless males were not observed. The time taken for the establishment of the reflex was normal.

In all cases fertilised eggs were recovered and reared to an advanced tadpole stage.

2. The Nares.

76 pairs of frogs that had coupled previously were used in this experiment.

Technique Of Removing Nares.

The animals were anaesthetised by immersion in a mixture of ether and water, 1 : 20. The nares were removed by section with a pair of scissors along an imaginary line drawn from the posterior part of the anterior (external) nares to the posterior limit of the

posterior (internal) nares. Plasticine was rubbed over the exposed surface, and each pair was then returned to its container. Males and females stood the operation equally well. Bleeding was minimal, and there were no signs of shock.

The animals were divided into three groups.

(a) In the first group (31 pairs) the nares of each male partner were removed.

(b) In the second group (20 pairs) the nares of each female partner were removed.

(c) In the third group (25 pairs) the nares of both the male and the female partners were removed.

Parallel series, 5 pairs each, were run in the control experiments.

When the animals had recovered from the anaesthetic 2 - 3 hours later, the members of the experimental series were injected, males as well as females, with 0.5 ml. of sheep anterior pituitary extract.

The animals in the control series were injected with 0.5 ml. of Brain Extract. l., prepared in the same way as the sheep extract.

Observations were made at 2 hourly intervals during the post-injection interval from 9.0 a.m. until 6.0 p.m. and then again at midnight.

Results.

(a) Noseless Males Paired With Normal Females.

The mating reflex was established in the normal manner in 8 - 10 hours. In a few cases the response was evoked in 40 - 60 minutes, but this was distinctly unusual and may have been because

the second post-operative injection was given within 12 - 15 hours of the first pre-operative one. All the pairs coupled, and fertilized ova were recovered and reared to an advanced tadpole stage.

(b) Normal Males Paired With Noseless Females.

All the pairs coupled in the normal manner and in the usual time. Fertilized eggs were recovered and reared to an advanced tadpole stage.

(c) Noseless Males Paired With Noseless Females.

23 out of the 25 pairs coupled. (Fig. 39). The remaining 2 pairs were re-injected on 3 successive occasions but still failed to couple. These two pairs and 8 which did couple were killed and their gonads examined. In the pairs that failed to couple the males appeared to be normal, but the females showed absence of large mature ova in their ovaries. The ovaries only contained a large number of immature or atrophied eggs.

The ovaries of the females from the 8 coupling pairs were all in good condition.

Fertilized ova were recovered and reared to an advanced tadpole stage.

One pair (a noseless male mated with a normal female) coupled in the control experiments.

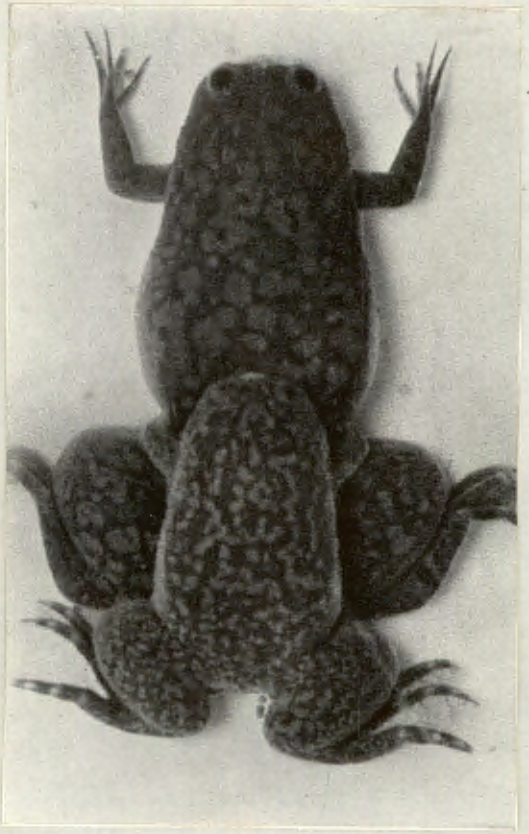


Fig. 39.

Noseless pair coupling.

Discussion.

The normal lumbar embrace was adopted by the noseless males with considerably less difficulty than was the case with the eyeless males described above. The large number of abnormal postures observed in matings attempted by the eyeless males did not occur in the course of the present experiment. It, therefore, appears that the portion of the nares removed in this experiment is unimportant as a receptor organ for some olfactory stimulus which may be provided by the quiescent female, after injection with the pituitary extract. This may be interpreted to mean that the olfactory receptor area may be neglected as a factor providing sensory data to assist in the correct orientation of the male preparatory to and during coupling. The coat of plasticine rubbed over the denuded area after excision of the nares renders it unlikely that there could have been any olfactory reception on the part of the male. It seems unlikely therefore that olfactory stimuli play any role in the mechanism whereby the male is stimulated to perform the very complex neuro-muscular response of coupling with the female.

In all cases fertilized eggs were recovered and reared to an advanced tadpole stage.

It is necessary to explain the fact that one pair of animals in the control series coupled successfully after a post-operative injection with brain extract. It has repeatedly been demonstrated that the brain extracts do not stimulate the gonads.

It has also been observed that occasionally animals which couple after injection with anterior pituitary extract may, after having been separated deliberately, begin to couple again within 24 - 48 hours without further injection. The apparent exception in the control series may be explained on this basis.

Summary.

1. The mode of adoption of the normal lumbar embrace is described.

2. Nine departures from this normal embrace are described.

3. The passive role of the female in the establishment of the mating reflex is confirmed.

4. It is suggested that the tactile sense of the male is of importance in the adoption of the clasp.

5. The activated female moves towards the male.

6. Activation of the male diminishes the amount of movement normally performed by the male.

7. Activated males mate indiscriminately with activated females. The reverse also holds true.

8. The eyes and nares play no essential part in the establishment of the normal lumbar embrace. The eyes, however, act as a refinement which may facilitate the adoption of the normal lumbar embrace.

III. THE TRANSPORT OF THE GAMETES FROM THE GONADS TO THE EXTERIOR.

A. The Path Of The Ovum.

The path of the ovum from the time of maturation to fertilization can be divided into roughly four stages.

1. Release from the ovary.
2. Passage from the ovary to the oviduct.
3. Passage down the oviduct to the ovisac.
4. Release from the ovisac.

1. Release Of The Ovum From The Ovary.

The theories of release of the ova from ripe follicles have been classified by Rugh (1935). Rugh considers that localized degenerative changes, possibly encouraged by enzymatic action are the cause of follicular rupture.

Contractions of the ovaries of pithed frogs were described by Pfluger (1859), Aeby (1861) and Rugh (1935). In Xenopus these contractions occur also. They can be seen at any time of the year both in healthy and in atrophied ovaries, but are especially well marked after the frog has been injected with some gonadotrophic preparation e.g. extract of sheep anterior pituitary. These contractions serve to increase the blood supply to the ovary and those portions of the ovary with the best blood supply release their ova earliest. (Rugh, 1935).

In Xenopus also ovulating ovaries show marked hyperaemia, resulting probably from the increased contractions.

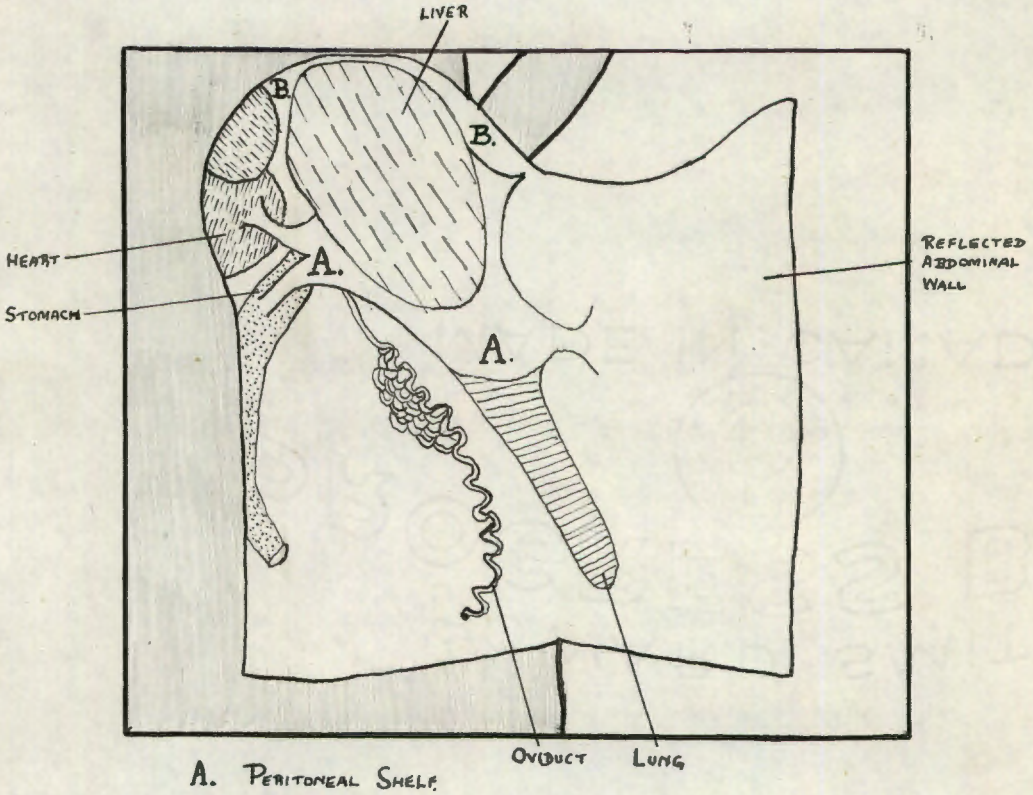
Prior to follicular rupture the mature ova have the appearance of being covered only by peritoneum, the growth of the follicles having brought them to the surface of the ovary. The next stage, macroscopically, shows the ova free from peritoneum but still attached to the ovary. Lastly, ova are found free in the abdominal cavity. Zwarenstein (private communication) has described that in isolated Xenopus ovaries the follicular wall retracts over the ovum. This finding explains why ova before being shed from the ovary appear to be attached to the ovary for some time although they are no longer within their follicles.

2. Ovary To Oviduct.

In order to understand this portion of the path, a description of the anatomy of the abdominal cavity of the female Xenopus is necessary. (Fig. 40).

The peritoneal cavity of Xenopus is divided roughly into two symmetrical parts by the gastro-intestinal tract and its mesentery, which is attached in the mid-line dorsally. On each side near this attachment lies the kidney, from the front of which the ovary hangs by the mesovarium, filling the lower part of the abdominal cavity. Attached to the anterior part of the ovary at its upper pole is the fat body. Lateral to the kidney lies the oviduct which is suspended by a mesentery from the dorsal wall.

PERITONEAL CAVITY OF XENOPUS FEMALE.



A. Peritoneal Shelf.

B. Perihepatic Recess.

Fig. 40.

This mesentery becomes shorter in the upper part of the cavity until it disappears and the oviduct lies under cover of the lung and the liver. Posteriorly the oviduct ends in the ovisac, (pars uteri of the oviduct) which opens into the cloaca.

The lung projects into the abdominal cavity from behind the heart and the pericardium, forming a double shelf of peritoneum which is attached to the dorsal wall and extends from the lateral abdominal wall tothe stomach. This shelf gives the lung its peritoneal investment. The liver peritoneum is continuous with this shelf laterally and with the peritoneum on the pericardium and anterior abdominal wall medially. In the latter fold the abdominal vein runs to enter the liver. Anterior to this shelf is a recess in each half of the abdominal cavity, occupied by the liver, occasionally the portions of the fat body and more rarely, by portions of the ovary. The heart and pericardium together with the fold of peritoneum enclosing the abdominal vein, separate the recess of one side from that of the other. The recesses communicate over the front of the shelf with the general peritoneal cavity.

In order to trace the path taken by the ovum, it was decided to obstruct some known section of the path, so that the ova would be dammed back and thus demonstrate the route taken.

12 female frogs were anaesthetised by immersion in a mixture of about 1 part of ether and 5 parts of water. The frog was laid on its back and the skin opened by a median incision 1 inch long. The muscle was divided by an incision 1/2 inch long to one side of the mid-line in order to avoid cutting the abdominal vein which lies in the mid-line and to prevent its being tied off later in sewing up the incision.

A loop of oviduct was lifted out of the incision and held by a small clip. A piece of cotton was securely tied round this loop, so as to obliterate the lumen of the oviduct. In this way the continuity of the peritoneum was not disturbed. Both oviducts were tied off in this way. The muscle incision was closed by continuous cotton suture, care being taken to avoid the abdominal vein. The skin incision was closed by interrupted cotton sutures. The frog was then turned over and placed in shallow water until it recovered and then was transferred to running water.

The frogs stood the operation very well. There was no haemorrhage and all the operated animals survived. The frogs were divided into two groups.

10 hours later each frog in the first group received 0.8 mls. of a potent extract of sheep anterior pituitary (S.23) equivalent to 530 mgs. of fresh tissue (approximately one anterior lobe). This was injected into the dorsal lymph sac, since intraperitoneal

injection might result in loss of extract through the operation wound.

The frogs in the second group were uninjected controls.

12 hours after injection all the frogs were pithed and examined.

In the injected animals, the oviduct proximal to the ligature was distended with ova and an opaque jelly-like substance. The distal part was empty and normal in appearance and size. At the anterior end, the oviduct, as shown by the eggs, began to get wider. This upper funnel-shaped part continued up under the liver and the root of the lung.

On pulling the liver down, the recess was seen to be filled with eggs. Eggs were also seen free in the general peritoneal cavity in large numbers, lying mostly in the ventral part, in front of ovary, stomach, liver and lung. These eggs were collected and kept in frog's Ringer solution for later experiments.

The region of the top of the oviduct was now more carefully examined. The recess was emptied and the oviduct below the peritoneal shelf was squeezed. In this way eggs were pushed back into the recess. A probe inserted into the oviduct near its upper end appeared in the recess at the lower dorsal border of the liver. Similarly, a probe passed down from the recess entered the oviduct. Pulling on the oviduct below the peritoneal shelf gave the recess a funnel-like appearance.

Dissection from behind, removing the urostyle showed the eggs lying in the recess, dorsal to the liver and ventral to the peritoneum, from where they could be squeezed into the oviduct.

These findings were present in all the injected frogs.

The results in the two groups are referred to again below.

All this evidence clearly demonstrates the fact that the ostium of the oviduct lies dorsal to the liver, in the recess in the upper part of the peritoneal cavity (Fig. 41). This was confirmed by dissection of a normal uninjected female Xenopus. The ostium was found as a narrow slit, 4 mms. long and 1 mm. wide, lying obliquely behind the liver, its anterior end being nearer the mid-line than the posterior end, and facing ventro-anteriorly. Through this a probe was passed into the oviduct.

This ostium opens into a peritoneal funnel which in turn opens into the oviduct proper below the level of the peritoneal shelf.

Thus after release from the ovary, the eggs travel into the recess, then into the peritoneal funnel andthen into the oviduct.

Mechanism Of Transport Of The Egg From The Ovary To The Oviduct.

Several factors must be considered of importance in the carriage of the ova from the ovary to the oviduct.

(a) Cilia.

(b) Contractions of the abdominal muscles.

(c) Movements of viscera.

(d) Gravity.

PERIHEPATIC RECESS.

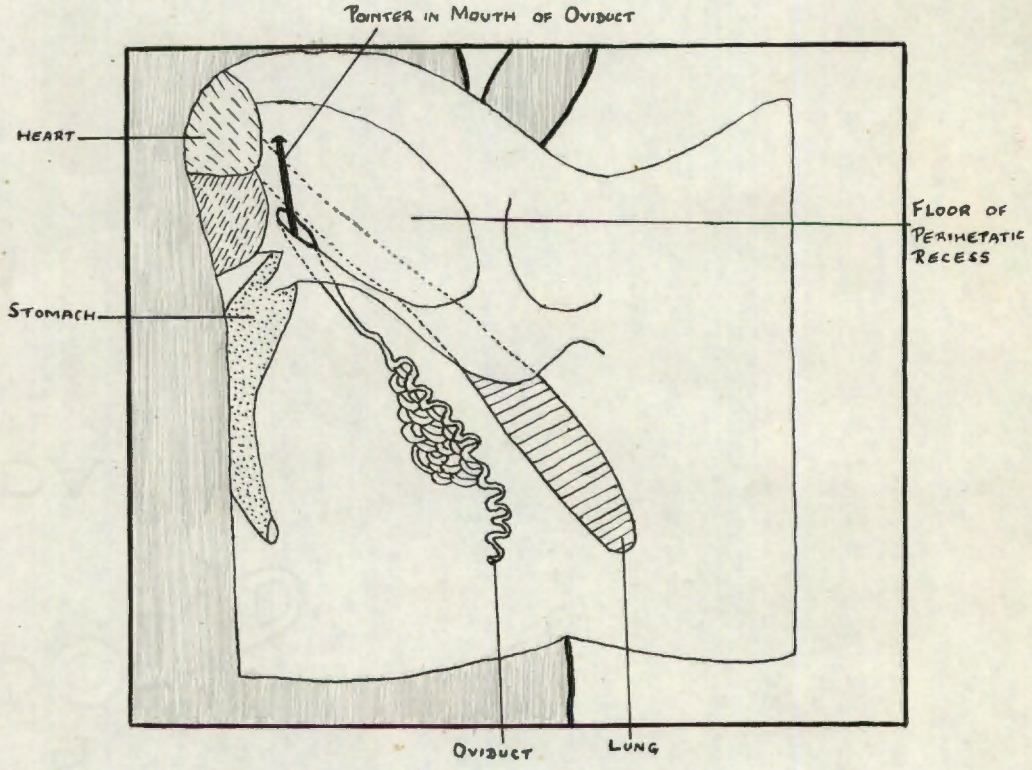


Fig. 41.

Newport (1851) considered that body and visceral movements were adequate to move the eggs from the ovary to the ostium. Smith (1916) considered that the cilia (in Cryptobranchus and Rana pipiens) are not sufficiently powerful to carry the eggs to the oviduct. Lebrun (1891), Nussbaum (1895), Morgan (1897), Kellicott (1913), Noble (1931) and Rugh (1935) are opposed to this view and believe that the ciliary action is sufficient on its own to transport the cilia.

According to Rostand (1934) both factors are of importance.

Several experiments were devised to investigate the importance of the factors mentioned above. First it was necessary to find out whether cilia were or were not present in the peritoneal cavity.

During all these experiments the temperature was more or less constant, never falling below 21°C or rising above 23°C.

Four groups of frogs were examined.

1. Freshly caught females with good ovaries.
2. Freshly caught females with atrophic ovaries.
3. Freshly caught males.
4. Females kept in captivity for three months.

The frog was pithed and its abdomen opened by two incisions. One transversely at the lower end of the abdomen and one longitudinally in the midline extending from the first incision to the xiphisternum and branching from there laterally and upwards on the two sides to open the recesses. The two flaps were reflected and the frog was

placed under the dissecting microscope, so that the light was reflected up the barrel of the instrument, and examined. The surfaces of the peritoneum and viscera were kept slightly moistened with frog Ringer solution.

1. In this way ciliary movement was observed on the whole of the abdominal wall in the freshly caught females with good ovaries. Cilia were also present on all surfaces of the liver and on the peritoneum lining the recess, but were absent from the peritoneal covering of the gastro-intestinal tract, ovaries, oviducts and lungs and from the mesenteries of the gastro-intestinal tract, ovaries and oviducts. (Fig. 42).

The distribution of the cilia in the peritoneal cavity of female Xenopus was confirmed histologically in the following way:

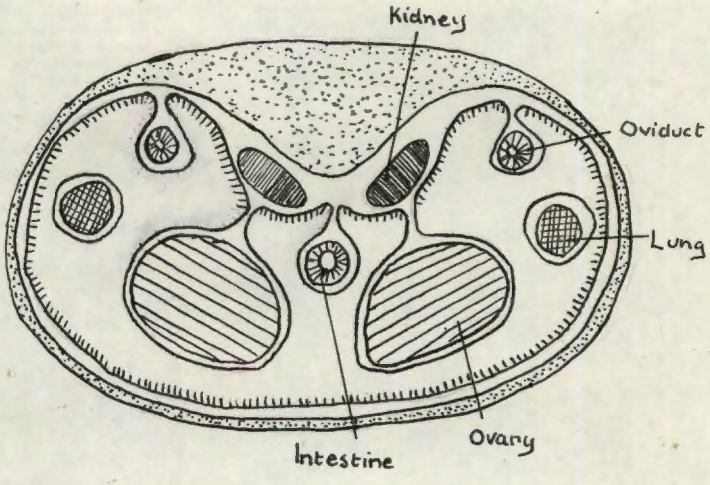
A freshly-caught female with good ovaries was pithed and the liver and oviduct, together with portions of the ventral abdominal wall were removed for histological section.

Liver. The serous coat of the peritoneum consists of a single layer of flattened cells with flattened nuclei, closely adherent to the underlying liver parenchyma. This layer is covered with cilia.

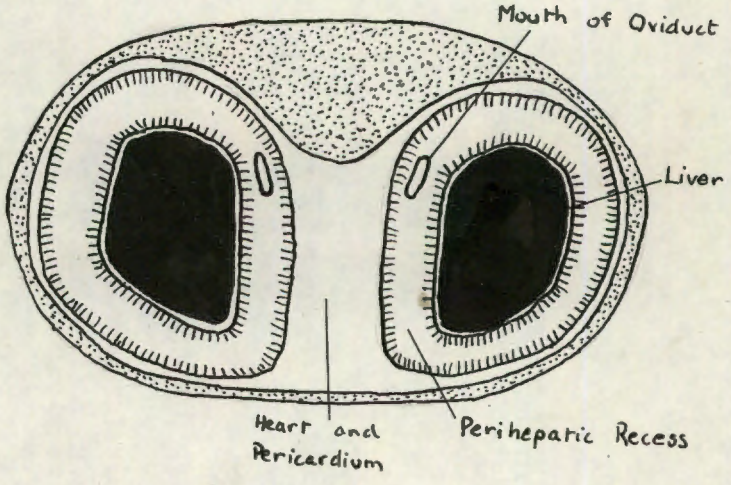
Oviduct. No cilia are seen on the peritoneum covering the oviducts.

Abdominal Wall. The serous membrane, with the same histological structure as that covering the liver, is seen to be covered on its peritoneal surface with large numbers of closely-set cilia.

DISTRIBUTION OF THE CILIA.



CROSS-SECTION OF THE PERITONEAL CAVITY.



CROSS-SECTION OF THE PERIHEPATIC RECESS.

Fig. 42.

2. Freshly caught females with atrophied ovaries also showed active cilia.

3. Freshly caught male frogs, examined in the same way showed no cilia present.

4. Female frogs kept in captivity for three months showed cilia present and active. These frogs had severely atrophied ovaries. (The experiments were performed in December, the middle of the non-breeding season).

Thus cilia are present in the female at all times in the sexual cycle but are never present in the male Xenopus. The cilia, therefore, are a secondary sex character of the female Xenopus. They are probably dependent for their development on the anterior pituitary, acting through the ovary, but once they are complete, they are no longer dependent upon any hormone for their maintenance.

Next the strength and direction of ciliary action were investigated. The eggs collected previously were used, because they are in the condition that ova normally are during their transport through the peritoneal cavity.

Frogs were prepared as for examining for cilia, and eggs were placed on the reflected flaps. These eggs were carried at the rate of about 1 inch per minute, and consistently along definite paths. Ova placed on the posterior part of the abdominal wall were carried proximally to the level of the liver and then turned inwards and entered the recess. These ova were recovered

later in the oviduct. Similarly, ova placed anywhere on the flap first travelled anteriorly to the level of the recess and then into the recess and were found later in the oviduct. This is represented diagrammatically in Fig. 43.

Next the flaps were arranged so that the ova, if they were to reach the recess, had to travel uphill. This they accomplished without appreciable loss of speed up fairly steep inclines.

Thus the action of the cilia is extremely powerful and fully capable of moving the eggs without the assistance of abdominal contractions.

This conclusion is supported by further experiments that were undertaken in order to eliminate abdominal contractions. Freshly caught females were anaesthetised in a mixture of ether and water and placed on their backs. A median skin incision was made, 1 inch long. The muscle was divided by a 1/2 inch incision and 12 eggs collected previously were placed on the exposed ovaries and fat bodies. The muscle and skin were sutured with cotton and the frog turned over and placed in shallow water. 1/2 hour after the operation and before they had recovered at all from the anaesthetic, the frogs were pithed and carefully opened up. The eggs that had been placed in the abdominal cavity at the operation were found either in one or other oviduct, i.e. the ova reached the oviducts 1/2 hour after being in the ventral part of the lower abdomen.

CILIARY CURRENTS.

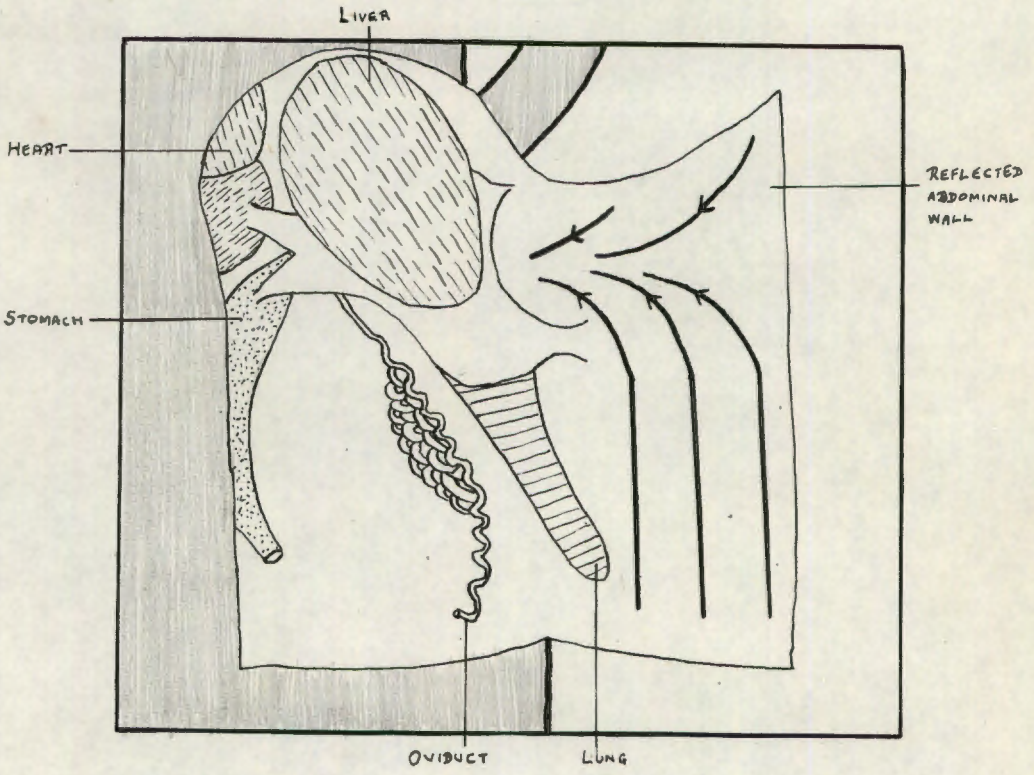


Fig. 43.

The same was done in pithed frogs and the findings were exactly the same.

These results demonstrate several points of importance.

(i) The fact that the animals never recovered from the anaesthetic and therefore never moved, shows that the contractions of the abdominal muscles are unnecessary for the transport of the ova, hence the cilia alone can transport the ova. Abdominal movements may be an accessory factor but this is not very probable. (See section below on the rate of travel of the ovum.)

(ii) The distance travelled by the ova was approximately 2 inches. In the experiment on the reflected flaps, the time taken by the ova to cover this distance was 2 minutes. In the operated animals, the time taken was 1/2 hour. Thus the rate of travel was slowed 15 times. The cause of this slowing obviously must be the viscera, assisted probably by the pressure of the weight of the frog, since the animals were resting on their ventral surfaces.

(iii) The operated frogs were fresh from the pond and never had any injections. Thus the cilia are probably active all the time and are not dependent on any hormonal stimulus for their action.

When eggs were placed in the abdominal cavity on one or other side, after 1/2 hour both oviducts may be found to contain ova. It is thus likely that ova may travel from one ovary to either oviduct.

In all these experiments the ova found in the oviducts could not have been there previous to the experiment because Xenopus is inactive sexually under the conditions present in the laboratory in which the experiments were done. Also, all frogs are allowed at

least one day in the laboratory before use in experiments, so that any ova that might have been liberated from the ovaries while the frogs were in the vlei would have been extruded before the time of the experiments.

In all the experiments already described and in those to be described later, it was found that most of the eggs free in the abdominal cavity after injections of sheep anterior pituitary extracts, lay in close relation to the ventral abdominal wall. Thus it appears that the ova, after being shed from the ovaries, gravitate to the anterior abdominal wall. Gravity, however, does not prevent the ova from travelling dorsally over the liver to the mouth of the oviduct.

Thus, of the factors concerned in transport of the ova from ovary to oviduct, the cilia are all-important and entirely capable of carrying the eggs without assistance. Contractions of the abdominal muscles are unnecessary. The presence of the viscera slows the rate of transport. Gravity brings the ova to the anterior abdominal wall, but has no further influence. Even this action of gravity is not essential, since the entire abdominal wall is covered by cilia.

Mechanism Of Entry Of The Ova Into Oviduct.

Several factors are of importance here.

1. Cilia.
2. Contractions of the abdominal muscles.
3. Suction.

4. Gravity.

Newport's (1851) view is that the ostium gapes open at each heart beat, since its margins are attached to the pericardium. In this way the eggs are sucked into the ostium. Noble (1931) supports this opinion, but considers that the movements of the female also tend to squeeze the eggs into the oviduct.

Rugh (1935) opposes this view. He considers that the ciliary forces are adequate to carry the eggs into the oviducts, the ostium acting as a trap.

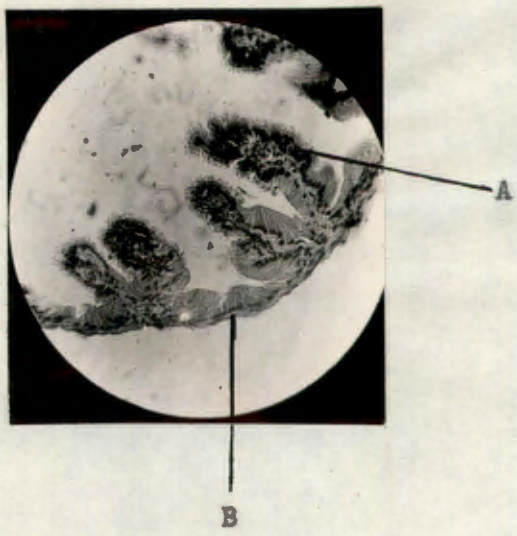
In Xenopus there is no gaping of the ostium to receive the eggs and as in Rana (Rugh 1935) the ova enter the ostium against the slight tension of the ostial walls, carried only by ciliary action. This is borne out by the fact that in the experiments previously described, the eggs entered the oviduct in completely anaesthetised and in pithed animals. Gravity does not hinder the process in any way.

3. Oviduct To Ovisac.

The histology of the oviduct forms a valuable guide as to its functions.

a. Pars Recta.

The histology of the pars recta of the oviduct differs considerably from that of the pars convoluta (see above). The smooth muscle is thicker, the glandular layer is narrow and the epithelial layer is thrown into folds that are considerably longer



A. Ciliated epithelium.

B. Smooth muscle layer.

Fig. 44.

Histology of the pars recta. (x 125)

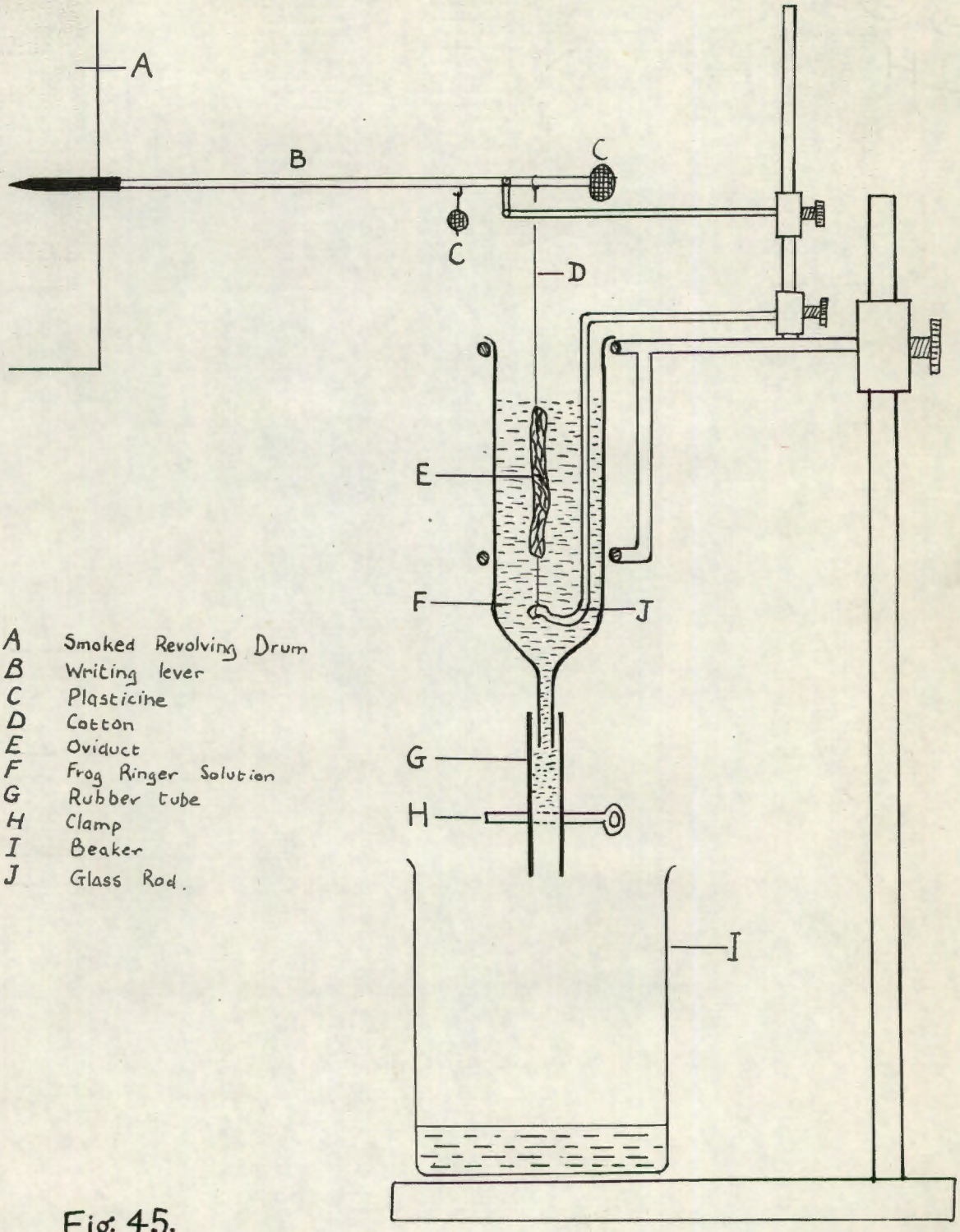
than those found in the pars convoluta (Fig. 44).

In the experiments where eggs were watched as they entered the mouth of the oviduct, it was noticed that the eggs travelled very rapidly down the pars recta of the oviduct. During the time taken for the liver to be replaced in the perihepatic recess in order to expose the pars recta, the eggs had already travelled down this portion of the oviduct and were seen at the junction of the pars recta and the pars convoluta. This rapid rate of progress was attributed to peristalsis of the thick muscular coat assisted by ciliary action. The smooth muscle was accordingly investigated.

The pars recta was carefully freed from its surrounding structures and attachments and suspended in frog Ringer solution through which oxygen was bubbled (Fig. 45). The lower end of the strip of oviduct was connected by a cotton thread to the glass rod (J) and the upper end was connected by a cotton thread to the lever (B). The point of the lever was in contact with a smoked, slowly-revolving drum (A). No spontaneous excursions of the lever took place.

Acetyl choline was added to the Ringer solution, but no contractions occurred. The tissue was washed with fresh Ringer solution and eserine was added and allowed to act for 20 minutes. Acetyl choline administration after this still failed to produce any excursions of the lever.

In spite of the fact that no peristalsis could be obtained in the isolated pars recta, and that acetyl choline failed to



A	Smoked Revolving Drum
B	Writing lever
C	Plasticine
D	Cotton
E	Oviduct
F	Frog Ringer Solution
G	Rubber tube
H	Clamp
I	Beaker
J	Glass Rod.

Fig. 45.

produce contractions, even after eserinisation of the tissue, the theory put forward above still appears to be the most likely one. A positive finding would, of course, have added considerable weight to the suggestion made above.

b. Pars Convoluta.

The histology of this portion of the oviduct clearly demonstrates its functions (See above).

1. Cilia. Observations of the travel of the ova down the oviducts show that the ova are carried in a spiral manner, i.e. they roll in an axis oblique to the long axis of the oviducts. The rolling of the ova suggests ciliary action and this is fully borne out by the histology. The spiral motion is probably determined to some extent also by the folds of the mucous membrane of the oviducts.

2. Smooth muscle. It is doubtful if the smooth muscle in the wall of the oviduct plays much part in the transport of the egg down the oviduct. Three facts support this statement.

(a) The quantity of smooth muscle is not very large and thus the muscular coat is not powerful.

(b) No peristalsis of the oviducts can be seen in oviducts which are full of eggs and down which the ova are moving.

(c) A section of this portion ~~of this portion~~ of oviduct, carefully freed from its mesenteric attachment and straightened out, was tested in the same way as the pars recta. Again no peristalsis was observed and no contractions were obtained with acetyl choline,

even after eserinisation.

3. Mucous Glands. The function of these is to furnish the egg with a gelatinous envelope.

Frogs which had their oviducts tied off by the method described previously, were divided into two groups. The frogs of the first group were injected with sheep anterior pituitary extract and those of the second group were uninjected controls.

At autopsy the uninjected frgs showed slight distention with jelly proximal to the ligature. This finding shows that the glands are constantly active.

The oviducts proximal to the ligature in the injected animals were distended with eggs and a very large amount of jelly. Thus it appears that the mucous glands respond to the presence of the ova by increased activity. The nature of the stimulus is mechanical because drops of oil or other foreign bodies injected into the peritoneal cavity of female frogs are eliminated through the oviducts covered with jelly in the same way as the eggs.

The oviducts of frogs injected with gonadotrophic preparations show hyperaemia. The increased activity in the mucous glands in the injected frogs must be dependent upon, but is not necessarily due to this increased blood supply, i.e. the anterior pituitary may act directly on the oviduct causing disintegrative changes similar to those seen in bufo arenarum (de Allende 1938). Spaul, (private communication) has also described a direct

action of the anterior pituitary on the oviduct in Rana.

Thus the ova reach the ovisac enclosed in jelly envelopes. Here they are held up for some time before extrusion.

4. Release From The Ovisac.

Histologically the ovisac presents three layers (Fig. 46).

(1) A smooth muscle layer. This layer is 3 - 4 cells thick and encloses the whole of the ovisac.

(2) A layer of loose connective tissue. This layer contains muscular bundles irregularly placed in the stroma. The layer is about twice as thick as the muscular layer.

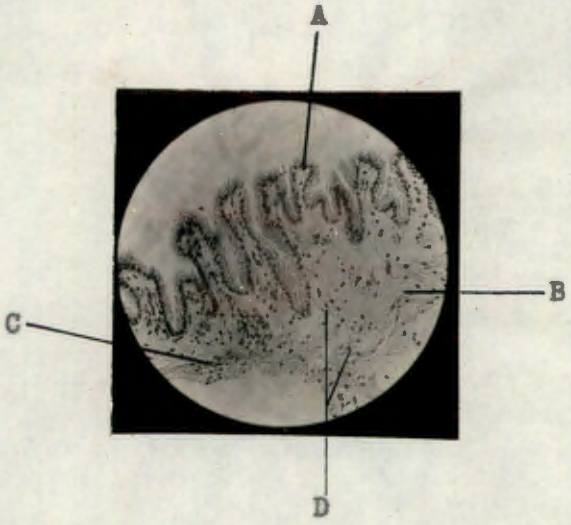
(3) A single layer of columnar ciliated epithelium. In the empty ovisac the epithelium is thrown into folds.

Strips of ovisac were tested in the same way as the oviduct. No peristalsis was obtained from the ovisac and no contractions were elicited by adding acetyl choline to the Ringer's solution in which the tissues were suspended, even after eserinisation for 20 minutes.

Discussion.

In considering this portion of the path of the ovum, the histology of the cloacal labia must be borne in mind. The important facts are that the cloacal canal is lined by columnar ciliated epithelium and that a cloacal sphincter is present.

In oviposition the cloacal sphincter must relax. Two factors must play a role in the transport of the egg to the exterior.



A. Ciliated epithelium.

B. Loose connective tissue.

C. Smooth muscle layer.

D. Smooth muscle fibres.

Fig. 46.

Histology of the ovisac. (x 125)

1. The smooth muscle layer of the ovisac.
2. The cilia lining the ovisac and cloacal canal.

The absence of contractions in the isolated ovisac does not exclude the possibility of a stretch reflex taking place, the ovisac contracting when it is stretched to a certain degree.

The cilia may assist in emptying the ovisac of eggs. In the case of the cloacal canal the cilia appear to be the only means by which the eggs can be passed on.

Several difficulties are presented by a theory of a stretch reflex. In the isolated injected female oviposition takes place, but the eggs are laid singly or in small groups. There is no sudden complete emptying of the ovisac such as is to be expected if the ovisac is stretched to a certain point and then contracts. In addition during coupling the eggs are extruded at the climax of the stimulation of the female by the male. (Shapiro 1936 a). It is difficult to see how the latter finding could be the result of a stretch reflex in the ovisac. To fit the facts concerning oviposition during coupling a mechanism such as the one below would have to be postulated. At the climax of the mating reflex, the female swims forward (Shapiro 1936 a). Part of the reflex set up by the stimulation by the male may be a nervous impulse to the ovisac wall causing it to contract. This mechanism is negatived, however, by the fact that isolated females oviposit.

It appears, therefore, that the eggs are passed from the ovisac to the exterior by the action of both the smooth muscle of the ovisac and the cilia of

the ovisac and the cloacal canal. The exact mechanism and the stimulus that gives rise to it, however, are obscure.

Summary.

1. Pendular movements of the ovary increase the blood supply to that organ.

2. At ovulation the follicle wall retracts over the ovum, but the latter remains attached to the ovary for a while.

3. The anatomy of the abdomen of the female Xenopus is described.

4. Cilia cover the peritoneum on the liver and the entire abdominal wall. They are a secondary sex character of the female.

5. The cilia are the most important factor in transporting the ova from the ovary to the ostium of the oviduct which lies in the perihepatic recess of the peritoneal cavity.

6. Ciliary action is also responsible for the entry of the ova into the oviducts.

7. The muscular peristalsis of the pars recta conveys the ovum rapidly down that portion of the oviduct. The histology of the pars recta is described.

8. Ciliary action is the most important factor concerned in propelling the ovum down the pars convoluta of the oviduct.

9. The cloacal sphincter relaxes to allow the ova to leave the ovisac.

10. The cilia lining the ovisac and the cloacal canal are the most important factors in this portion of the path of the ovum.

B. The Rate Of Travel Of The Ovum.

This investigation was carried out during December, 1937. 60 female frogs were kept in the laboratory for 2 days before being used in the experiments to allow any ova which might have been present in the abdominal cavity, oviducts or ovisacs to be extruded, although the animals were anoestrous and not likely to have ovulated in the ponds.

The animals were divided into two groups, consisting of 25 and 35 frogs each. This was done to prevent overlapping of injections and observations.

Group I.

The animals were injected intraperitoneally with 0.8 mls. (equivalent to 500 mgs. fresh tissue). One animal was injected every five minutes. The time of each injection was carefully noted and each frog was placed in a separate container. The frogs were divided into 5 sub-groups, 5 frogs in each. Each animal in the first sub-group was pithed 3 hours after injection.
Each animal in the 2nd sub-group was pithed 3 1/2 hours after injection.
" " " " 3rd " " " " 4 " " "
" " " " 4th " " " " 4 1/2 " " "
" " " " 5th " " " " 5 " " "

The labia were first examined for hyperaemia. Then the abdominal cavity was opened from the front and the ovaries examined for pendular movement, hyperaemia and for ova which had ruptured through the peritoneal covering of the ovary but were not yet free.

Then the abdominal cavity and recesses were thoroughly searched for free ova. The oviducts and ovisacs were next examined for hyperaemia and ova.

GroupII.

The 35 frogs in the second group were divided into 5 sub-groups of 7 frogs each and each frog was injected intraperitoneally with 0.8 ml. of S.19. (equivalent to 500 mgs. fresh tissue). The frogs were killed after the same time intervals as those in Group I and the same series of observations were made.

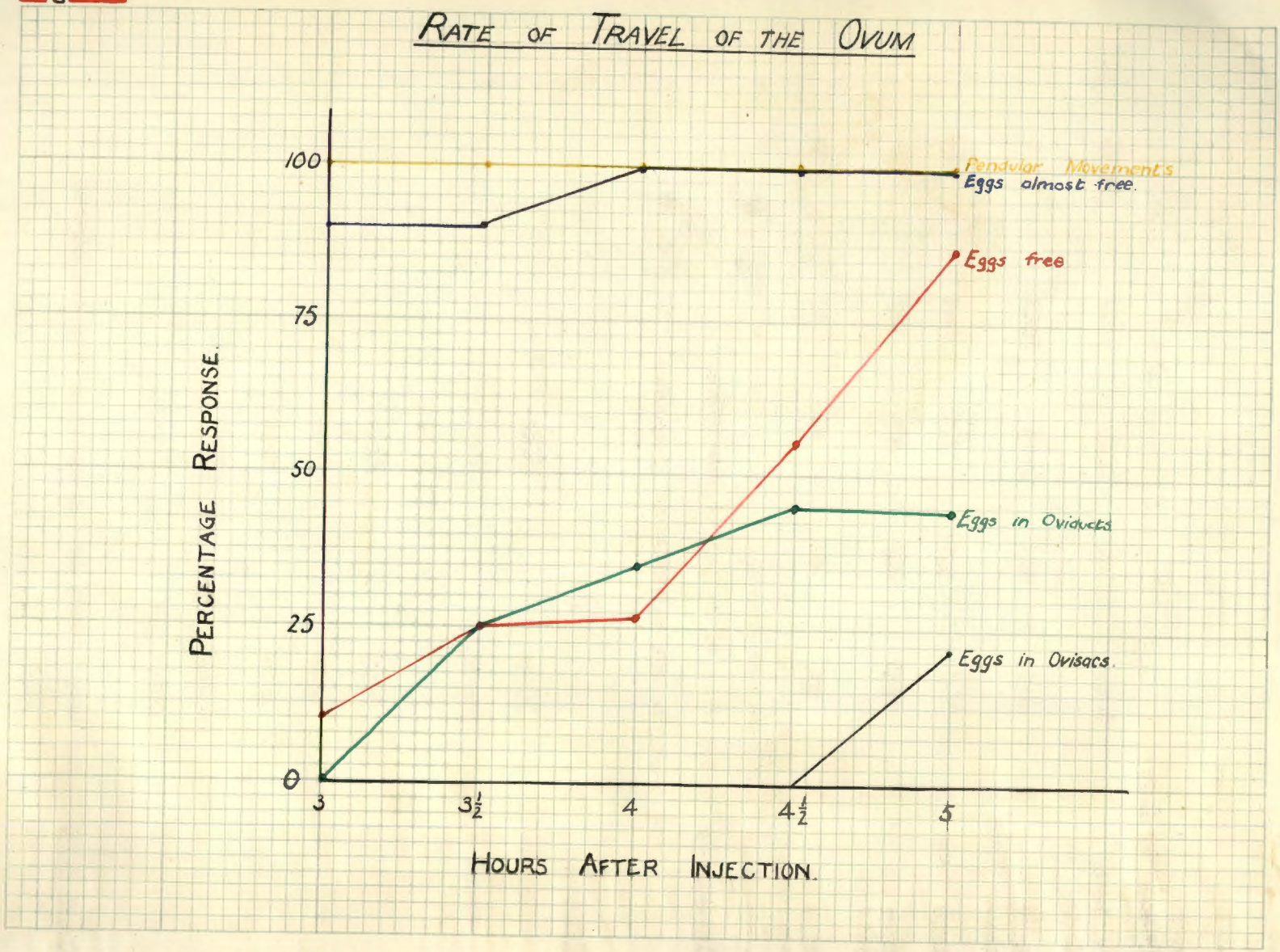
The temperature of the water of the frogs' containers was taken at regular intervals throughout the experiments. (Table XLIII & XLIV.)

Results.

The results recorded below summarise the findings in Group I and Group II.

Note: Animals with atrophied ovaries are not included here.

Fig. 47.


RATE OF TRAVEL OF THE OVUM
100
75
50
25
0
PERCENTAGE RESPONSE.
Pendular Movements
Eggs almost free.
Eggs free
Eggs in Oviducts
Eggs in Ovisacs.
3
3½
4
4½
5
HOURS AFTER INJECTION.

Hours after Injection.	3	3 1/2	4	4 1/2	5.
Pendular Movements	100%	100%	100%	100%	100%
Eggs almost free	90%	90%	100%	100%	100%
Eggs free	10%	20%	27%	55%	89%
Eggs in oviduct	0%	20%	36%	45%	44%
Eggs in ovaries.	0%	0%	0%	0%	22%

Table XLV. The rate of travel of the ovum.

These results are represented graphically in Fig. 47.

Group I.

Time	Temp. (°C).
12.30	22.0
3.0	22.5
3.45	22.5
4.18	23.0
4.50	23.0
5.50	22.7
Mean:	22.6°C

Table XLIV. The temperature of the water of the containers.

Group II.

Time	Temp. (°C).
12.00 p.m.	20.2
1.00 p.m.	20.5
2.00 p.m.	20.6
3.00 p.m.	21.3
4.00 p.m.	21.9
5.00 p.m.	22.4
6.00 p.m.	22.0
7.00 p.m.	21.5
8.00 p.m.	20.6
Mean:	21.2 °C

Table XLIII. The temperature of the water of the containers.

Discussion.

3 hours. All the ovaries showed pendular movements and in 90% of animals follicular rupture had already occurred. Eggs were free in the peritoneal cavity in only 1 animal (10%), but no eggs had yet reached the oviducts. Follicular rupture commences within three hours after injection and three hours after injection eggs are free from the ovaries.

3 1/2 hours. Again all the ovaries showed pendular movements, and in 90% of the animals follicular rupture had occurred. 20%of the animals now showed eggs free in the peritoneal cavity and the eggs had reached the oviducts in 20% of the animals. Thus it is half-an-hour from the time of shedding of the eggs from

ruptured follicles to the time the eggs reach the oviducts. This finding agrees with the experimental placing of eggs in the peritoneal cavity on the ovary as described above. In each case the eggs reached the oviducts in half-an-hour. In the present experiments the animals were alive and unanaesthetised, and moved around freely, so that the contractions of the abdominal musculature appears to play no part in the transport of the egg from the ovary to the oviduct. As has been stated before, the distance between these viscera is about 2 inches and the rate of travel of the ovum is thus 4 inches per hour in this part of its course.

4 hours. Pendular movements were seen in all the ovaries. They were more marked than in the earlier observations. All the ovaries showed ruptured follicles and, in addition, each ovary showed more ruptured follicles than those in the earlier readings. An increased number of animals had eggs free in the peritoneal cavity and more animals showed eggs in the oviducts. These eggs had progressed a considerable distance down the oviducts but none had reached the ovisacs.

4 1/2 hours. Pendular movements and follicular rupture were again prominent features. The number of animals with eggs free in the peritoneal cavity reached a still higher level (55%) and more animals had eggs in the oviducts (45%). The eggs had progressed a further distance down the oviducts, but again none had as yet reached the ovisacs.

5 hours. Pendular movements were very marked and the ovaries showed abundant ruptured follicles. Most of the animals had eggs free in the peritoneal cavity (89%), but the number showing eggs in the oviducts was very little altered. In 2 animals, however, the eggs had reached the ovisacs (22%). Thus it is 1 1/2 hours from the time that the eggs first reach the oviducts to the time that the ovisacs are first found to contain eggs.

To determine the rate of travel of the eggs down the oviduct, the lengths of the oviducts of several animals were measured.

The Length Of The Oviduct.

10 frogs were chosen at random from the previous experiment and the oviducts were carefully dissected out, cutting through the mesenteries close to their attachments to the oviducts. In this way the oviducts were straightened out as far as possible. The "ostium-ovisac" length was measured with a ruler correct to the nearest half-inch.

No.	Ostium-Oviduct Length (Inches). Left Oviduct	Right Oviduct.
1	14.5	15.0
2	14.0	14.5
3	14.5	14.0
4	16.0	17.0
5	12.0	13.0
6	17.5	18.5
7	12.0	13.5
8	16.5	15.0
9	23.0	19.5
10	13.0	12.0
Means	15.3	15.2

Table XLVI. The length of the oviduct.

In 4 of the animals (3, 8, 9 and 10) the left oviduct is longer than the right. In the remaining 6 animals the reverse holds true. Thus there appears to be no constant difference between the lengths of the oviducts of the two sides, the difference being easily accounted for by the difficulty in straightening out the oviduct completely. The average length of an oviduct thus is 15 1/4 inches, although some oviducts may be as short as 12 inches, and others may reach a length of 23 inches. The remarkable degree of coiling of the oviduct becomes apparent when it is seen that the direct distance between

the mouth of the oviduct and the ovisac is approximately 2 inches.

Thus the ovum takes 1 1/2 hours to travel a distance of 15 1/4 inches, i.e., it travels down the oviduct at the rate of about 10 inches per hour.

At the temperatures at which the experiments were performed (± 22°C) oviposition first occurs about 10 hours after injection, so that the ova are retained in the ovisac for a considerable length of time.

Summary.

1. Pendular movements occur all the time in the ovaries of Xenopus laevis (this confirms the findings above), but become more marked 4 - 5 hours after injection with an acid extract of sheep anterior pituitary.

2. Follicular rupture occurs within the first 3 hours after injection and the amount of follicular rupture increases in the next 2 hours.

3. Eggs are seen free in the peritoneal cavity 3 hours after injection.

4. Travelling a distance of about 2 inches through the peritoneal cavity into the perihepatic recess at the rate of 4 inches per hour they reach the oviduct a half-hour later.

5. They pass down the first 1/2 inch (pars recta) of the oviduct in a few seconds and then continue downwards at the rate of 10 inches per hour to reach the ovisac 1 1/2 hours later.

6. There they are retained for 5 hours and then they are passed to the exterior.

7. The average length of the oviducts is 15 1/4 inches.

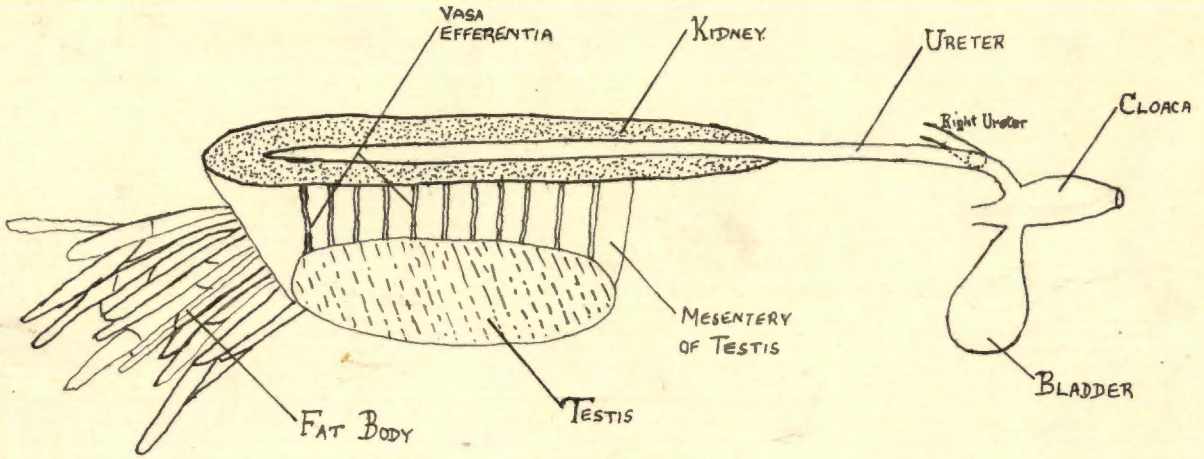
C. The Path Of The Sperm.

It is a wellknown fact that in frogs and toads the vasa efferentia from the testis pass into the kidney. According to Noble (1931) the site of communication between the vasa efferentia and the kidney tubules varies in different species. In some the vasa efferentia join the renal corpuscles, and in others they open into the transverse collecting tubules.

Rugh (1937) was the first to demonstrate the presence of spermatozoa in the kidney of an amphibian, Rana pipiens. A coupling pair in which amplexus has been induced by anterior pituitary injections is separated and the male is killed. Histological study of the kidney shows spermatozoa in the Bowman's capsule and in the tubules. The anterior pituitary injection releases mature spermatozoa from the Sertoli cells (Rugh 1937). Shapiro (1937 b) working on Rana temporaria, found that prolactin - containing extracts of the anterior pituitary which contain some gonadotrophic principle injected for six successive days caused an almost complete disappearance of spermatids from the testis and very few spermatozoa remained visible in the lumina of the seminiferous tubules.

In Xenopus laevis the urogenital system resembles that of other frogs and toads.(Fig. 48.) The testes are suspended from the front of the kidneys by a mesentery. In this mesentery run the vasa efferentia, disappearing into the kidney. Attached to the

PATH OF THE SPERM.



UROGENITAL SYSTEM OF XENOPUS MALE.

Fig: 48.

upper pole of each testis is a fat body. The ureters commence near the upper poles of the kidney and descend on the lateral side of the kidney, closely applied to the latter. The ureters join together a short distance above the cloaca, into which they open by a single orifice. No seminal vesicle is found in Xenopus males. In rana this consists of a dilation of the lower part of the ureters. Another difference is that in Rana the ureters open into the cloaca separately.

The Path Of The Sperm In Xenopus Laevis.

The path of the sperm in Xenopus laevis was investigated in the following way.

Pairs of frogs were injected with sheep anterior pituitary extract. 10 - 12 hours later the mating reflex was established. Coupling pairs were removed from the containers and allowed to embrace for several hours. Then the partners were separated. The males were killed immediately and their abdomens were opened. the genito-urinary organs were examined and then the kidneys were removed for histological section. The ureters were removed from some of the animals and opened with a fine-pointed scissors. Then they were crushed, a drop of water was added and the smears were examined under a microscope. Control un-injected frogs were also opened and examined.

Results.

Testis.

The testes of coupling males were found to be hyperaemic. They were pink in colour and numerous small blood vessels filled

with red blood cells could be seen.

Mesentery.

The blood vessels were congested. The vasa efferentia were seen more clearly than in uninjected males. They stood out as white cords with a beaded appearance.

Kidney.

Histologically spermatozoa were seen in some of the Bowman's capsules of the kidneys and in several of the tubules in large numbers. The blood vessels of the kidney also showed marked hyperaemia.

Ureter.

Motile sperms were seen in the smears made from the ureters.

Discussion.

Under the influence of anterior pituitary injections two sets of changes occur in the genito-urinary organs.

(1) A markedhyperaemia is produced.

(2) From the appearances described above, it can be concluded that spermatozoa leave the testis via the vasa efferentia. They reach the Bowman's capsules in the kidney and pass down the tubules to the ureter. The path suggested by an anatomical consideration is thus confirmed. Furthermore, in Xenopus laevis it seems likely that the vasa efferentia open into the renal corpuscles.

It is also probable that as in Rana pipiens and Rana temporaria, the spermatozoa are released from the testes by the anterior pituitary extracts.

Summary.

1). Spermatozoa are released from the testis by the anterior pituitary.

2). They travel down the vasa efferentia of the mesentery to the kidney.

3). The sperms enter the Bowman's capsules of the kidney.

4. They reach the ureter via the tubules and pass through the cloaca to the exterior.

IV. THE RELATION OF EXTERNAL ENVIRONMENTAL FACTORS TO THE SEXUAL CYCLE OF XENOPUS LAEVIS.

The evidence presented in this work suggests that the cyclical reproductive physiology of Xenopus laevis is dependent upon the action of the anterior pituitary. Thus the fundamental physiological problem that presents itself is the marked rhythmicity of the function of this gland.

It was thought profitable, therefore, to investigate the relation of anterior pituitary activity to climatic events, which themselves undergo profound cyclical changes.

Shapiro and Shapiro (1934) have shown that the cycle in the ovary of Xenopus females is under anterior pituitary control. In their work they presented a curve showing the seasonal changes in the ovary weight, expressed as a ratio of the total body weight, called the gonad ratio. This curve was used as a basis for the study outlined above.

The work of Shapiro and Shapiro (1934) was performed during the year 1932.

The Sexual Cycle Of Xenopus Laevis.

The sexual cycle of Xenopus laevis can be divided conveniently into three stages which are clearly seen in Fig. 49, the curve being constructed from the figures of Shapiro and Shapiro (Table XLVII).

Fig: 49.


GONAD RATIO
0·11
0·10
0·09
0·08
0·07
0·06
0·05
PRO - OESTRUS
OESTRUS
ANOESTRUS
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
1932

Month.	Gonad Ratio.
January	0.075
February	0.064
July	0.113
September	0.105
December	0.052

Table XLVII. The seasonal cycle in the ovaries of Xenopus females.

(1) The stage of anoestrus lasts approximately from September to February. This is the period during which the gonad ratio falls to its lowest value and the frogs are not observed to couple under natural conditions.

(2) The stage of pro-oestrus lasts roughly from February to July. During this period the gonad ratio is steadily increasing due to the maturation of the ova. Coupling is not observed in the ponds during this period either. This phase is an essential one in the cycle, because mature ovaries are necessary both to provide ova for fertilisation and for the establishment of the mating reflex. (Shapiro 1937 a).

(3) The stage of oestrus, or the mating season, lasts from July to September. During this period there is a sudden drop in the gonad ratio due to extrusion of large numbers of ova during mating.

Zahl (1935) found a cycle in the acidophile cells of the anterior pituitary of Rana. These are the important cells and they increase both numerically and in granularity with the approach of the breeding season, during which time they reach their maximum number. The cycle corresponds to the seasonal changes in the

weather. It is possible that the sexual cycle in Xenopus laevis may be due to a similar series of changes in the acidophile cells of its anterior pituitary.

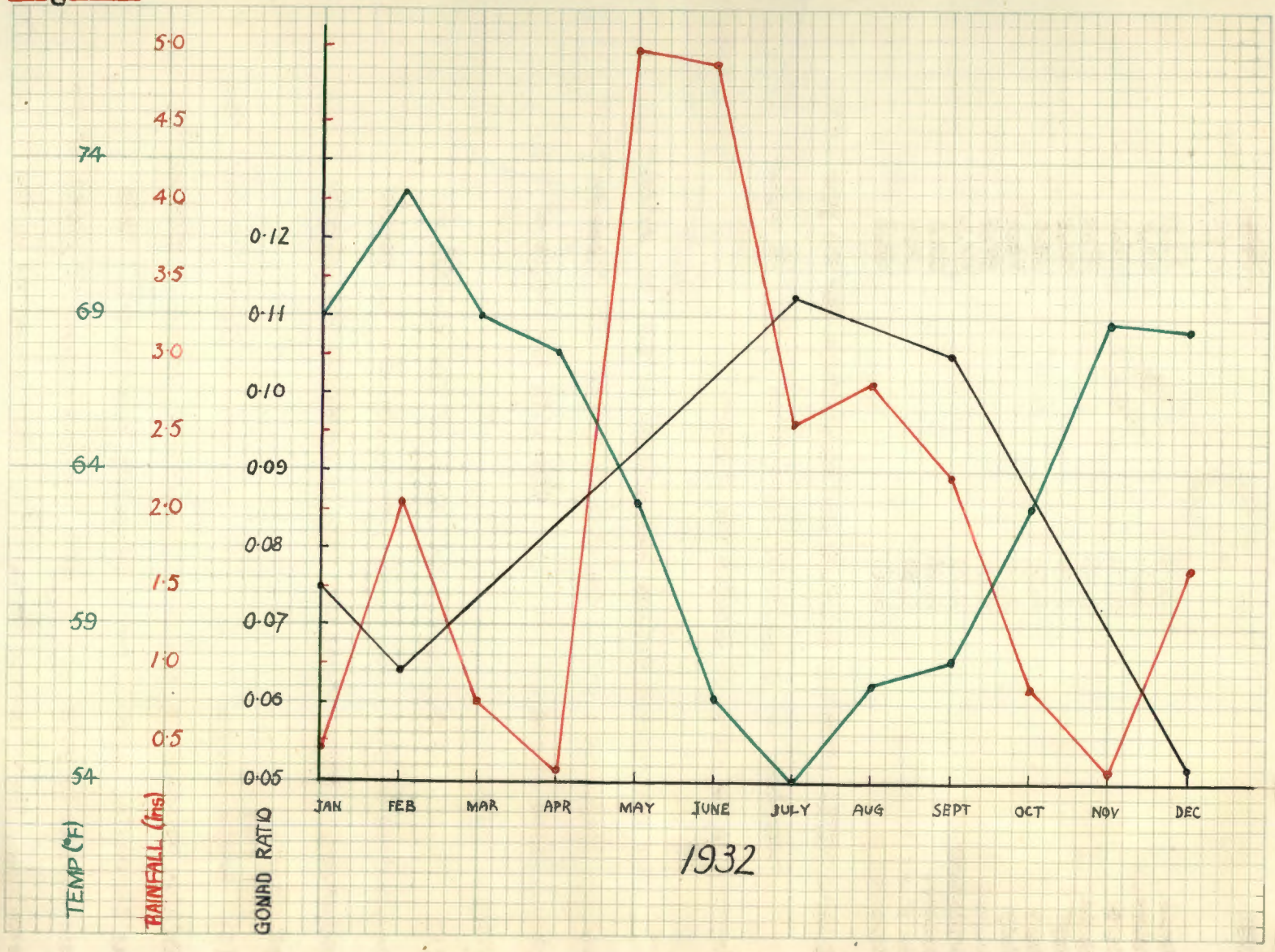
The time of the breeding season as described by Shapiro and Shapiro, namely July to September, corresponds with the findings of earlier observers. Leslie (1890) and Beddard (1894) stated that pairing takes place at the Cape in early spring (August). Shapiro (1936) gives July to September as the breeding season.

The Relation Of Climatic Conditions To The Sexual Cycle.

The period during which mating has been observed to occur in Xenopus laevis under natural conditions corresponds closely with the Cape winter, which is characterised by heavy rainfall and a severe drop in temperature. The super-position of the curves for rainfall, temperature and gonad ratio for the whole of the year in which the ovary changes were recorded would show whether any significance can be attached to the trends of the curves expressing these data.

The data for rainfall and temperature were obtained from the Royal Observatory near Cape Town. The reading were taken at the Observatory, which by a fortunate chance is situated within two miles of the ponds from which Shapiro and Shapiro obtained their frogs. The figures given in Table XLVIII are the average monthly figures for temperature and the monthly figures for total rainfall for 1932, the year in which Shapiro and Shapiro made their investigation.

Fig. 50.


5·0
4·5
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
74
69
64
59
54
0·12
0·11
0·10
0·09
0·08
0·07
0·06
0·05
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
1932
TEMP (°F)
RAINFALL (ins)
GONAD RATIO

1932	Total Rainfall Inches.	Average Temperature (°F)
January	0.45	68.9
February	2.02	72.8
March	0.74	69.0
April	0.33	67.8
May	4.99	63.0
June	4.79	56.6
July	2.56	54.1
August	2.81	57.2
September	2.23	57.8
October	0.83	62.8
November	0.34	68.9
December	1.61	68.6

Table XLVIII. Monthly rainfall and temperature for the year 1932.

These data are graphically represented in Fig. 50 superimposed on the curve of Shapiro and Shapiro.

The interesting feature that emerges is that the rainfall and the temperature cycles can also be divided into three definite phases which correspond closely in time with the stages of the ovarian cycle.

(1) Starting in September, the return of the rainfall and temperature to the summer level corresponds with the period of anoestrus. These conditions fail to stimulate the anterior pituitary.

(2) Sudden changes in the curves are seen at about February. The falling rainfall and the rising temperature of summer give way to an increasing rainfall and a decreasing temperature. This corresponds in point of time with the commencement of growth of the ovaries. These changes in the environment appear to be the stimulus which initiates the activity of the anterior pituitary.

The continued growth of the ovaries accompanies and is very likely dependent upon the continued increase of the rainfall and the progressive decrease of the temperature. These last two factors probably provide the stimulus for continued anterior pituitary activity.

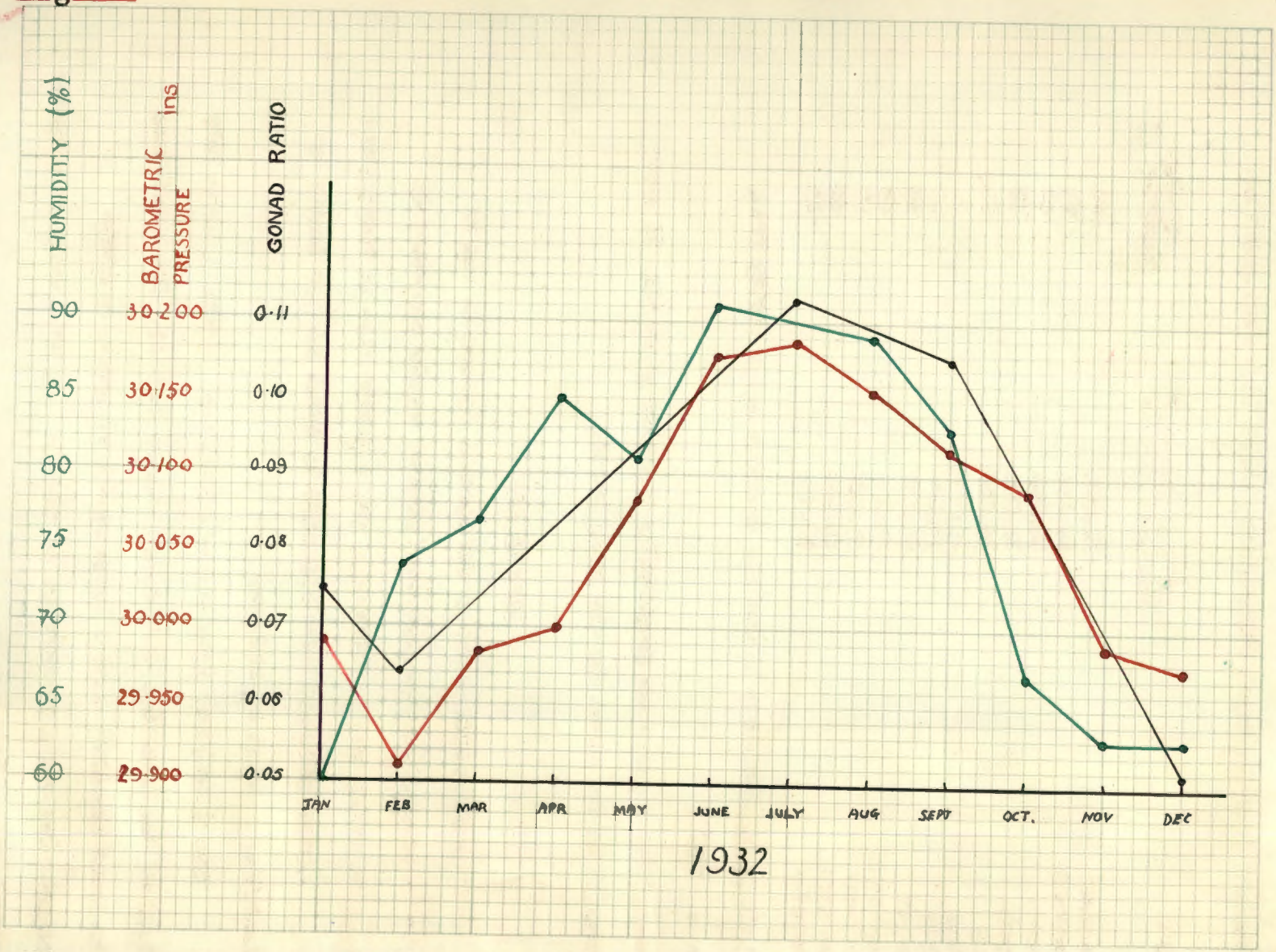
An extensive investigation by Savage (1935) on Rana also emphasises the importance of rainfall and temperature in the period preceding actual mating.

(3) Sudden changes in the curves are again seen at about July. The sharp rise in temperature and the high rainfall at this period correspond closely in point of time with the onset of pairing in the ponds.

Thus when the ovaries have already fully developed, the animal is submitted to a new pattern of stimuli, namely, a sudden increase of temperature occurring at a time of heavy rainfall. This appears to be the climatic situation which, in animals with mature gonads, evokes a mating reflex through the hormonal mechanism of the anterior pituitary. It is not known whether the anterior pituitary is stimulated directly, or indirectly through the hypothalamic nerve centres.

It is of added interest that other climatic data which are obviously closely related to and dependent on the rainfall and the temperature correlate equally closely with the curve for the gonad ratio. The figures in Table XLIX give the average monthly barometer readings and humidity for the year 1932 and were also

Fig. 51.


HUMIDITY (%)
BAROMETRIC PRESSURE ins.
GONAD RATIO
90
30·200
0·11
85
30·150
0·10
80
30·100
0·09
75
30·050
0·08
70
30·000
0·07
65
29·950
0·06
60
29·900
0·05
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT.
NOV
DEC
1932

obtained from the Royal Observatory, where they were recorded.

1932	Average Barometric Pressure (Inches)	Average Humidity (%)
January	29.991	60
February	29.909	74
March	29.985	77
April	30.000	85
May	30.083	81
June	30.176	91
July	30.187	90
August	30.157	89
September	30.119	83
October	30.090	67
November	29.992	68
December	29.978	63

Table XLIX. Monthly barometric pressure and humidity for the year 1932.

These figures are graphically represented in Fig. 51 superimposed on the curve for the gonad ratio.

Discussion.

From a consideration of the natural factors it can be predicted that the two important natural events necessary to produce mating are (1) rainfall, (2) a sudden rise in temperature.

Several attempts have been made to breed Xenopus laevis by imitating natural conditions.

Bles (1901) was able to breed the frogs in the Tropical Lily tank of the Cambridge University Botanic Gardens. The conditions he found necessary were a large amount of space, adequate hibernation and a temperature of 22-23°C. By this means he obtained mating in the late winter.

Bles (1904) kept his Xenopus in a large bell-jar. He obtained pairing in spring if he satisfied three conditions. First, he raised the temperature from 15-16°C. to 22°C; second, the fall of rain was simulated; and third, the food of the container was altered so that it was suitable for the larvae.

Kotthaus (1933) in Greifswald gives similar findings. A large container, a period of hibernation, a temperature of 22-25°C., and food for the larvae were necessary to obtain mating.

Holmgreen (quoted by Zondek, 1935) in Stockholm obtained fertilised eggs in the summer months, provided that the frogs were given a large, well-lit aquarium.

Vanderplank (1936) in Bristol (private communication to Dr. H.A. Shapiro), obtained mating by raising the temperature of the container from 54°F. to 64°F., and simulating the fall of rain. He was also able to produce mating by transferring the frogs to clean rain water, at the same time raising the temperature by 10-15°F.

It is significant that the features common to all the successful attempts at producing artificial mating in Xenopus are the imitation of rainfall together with the production of a sudden rise in the temperature. However, from the work of Vanderplank and Holmgreen it appears that the occurrence of rainfall is not essential, and that the rise of temperature is the all-important stimulus.

The correspondence of the humidity and barometric pressure curves with the gonad ratio curve is probably incidental and

unimportant.

The hibernation described by Bles and by Kotthaus appears to correspond to the pro-oestrous period, the importance of which has already been emphasized. Under natural conditions, however, pro-oestrus in Xenopus laevis is a period of aestivation in South Africa.

Summary.

1. Three phases of the sexual cycle of Xenopus laevis are described.

Oestrus - July to September.

Anoestrus - September to February.

Pro-oestrus - February to July.

2. Certain climatic conditions determine the activity of the anterior pituitary in controlling this cycle. Of these rainfall and especially temperature are important.

3. Curves have been drawn which depict these relationships very clearly.

GENERAL SUMMARY.

GENERAL SUMMARY.

1). A brief account is given of the development of the knowledge of the reproductive physiology of Xenopus laevis.

2). The scope of the present work is outlined.

3). The method of collecting "pairs of frogs known to couple" is described.

4). The containers used in the experiments on coupling are described.

5). The operations of castration and hypophysectomy are described.

6). Methods are described for preparing extracts of the following substances:-

Human pregnancy urine.

Anterior pituitaries of sheep, ox, Xenopus laevis and dogfish.

7). The seasonal cycle in the pads of male Xenopus is described.

8). Four phases are recognised.

a) January to May. The pads are absent.

b) May to July. The pads develop.

c) July to September. The pads are maintained at their full development.

d) September to January. The pads disappear.

9). Three types of pads are described.

a) Pads covering the fingers and palms.

b) Pads covering the forelimb as far as the elbow.

c) Pads covering the forelimb as far as the axilla.

10). Five stages in the development of the pads are described. The chief stages are as follows:-

The pads are developed first as far as the wrist. Next the forearms show pads and finally the pad is completed.

11). The pads disappear after the breeding season by successive exfoliations.

12). Pads are never seen on the forelimbs of female Xenopus.

13). Acid and pyridine extracts of sheep anterior pituitary induce the development of pads in normal but not in castrated males.

14). Pyridine extracts of sheep anterior pituitary induce pad development in anterior lobe hypophysectomised males, but the rate of development of the pads is slower than in normal males.

15). The above finding is probably explained by the loss of sensitivity of the hypophysectomised animals to gonadotrophic injections.

16). Anterior pituitaries from both male and female Xenopus produce pads in normal Xenopus males.

17). Xenopus anterior lobes do not cause pad development in castrate males.

18). Captivity results in the abolition of the pad cycle.

19). The evidence presented indicates that the pad cycle under natural conditions is under the control of the anterior pituitary acting via the testis.

20). The development of the pad is described macroscopically and microscopically.

21). The inhibition of the anterior pituitary by captivity (Shapiro & Shapiro 1934) consists in the prevention of the pouring out by the gland of its gonadotrophic principles. Secretion is not inhibited.

22). The pads play no essential role in the mating reflex in Xenopus laevis.

23). Using the development of pads as a test-object for androgenic and gonadotrophic activity in the male Xenopus, methyl testosterone, testosterone propionate, oestradiol benzoate and progesterone are shown to possess neither of these two properties.

24). The evidence presented suggests that the Xenopus testis hormone differs from the mammalian testis hormone testosterone.

25). The method of calculating the arm/body weight ratio is described.

26). The arm/body weight ratio of the male is larger than that of the female.

27). Seasonal cycles are seen in the arm/body weight ratios of both male and female Xenopus. These cycles are described.

28). The cycle in the male shows four phases.

a. The arm/body weight ratio is smallest in January.

b. The ratio increases until July.

c. During the breeding season the ratio is maintained at a high level.

d. A decline to the summer level follows the breeding season.

29). 3 phases are seen in the arm/body weight ratio cycle in the female.

a. The lowest level is seen during the breeding season.

b. From then until January the ratio is higher.

c. The highest level is seen between January and July.

30). Injections of acid or pyridine extracts of sheep anterior pituitary into normal male Xenopus results in an increase in the weight of the forelimbs.

31). Under identical treatment, hypophysectomised males also show an increase in the weight of the forelimbs, but this increase is less than in the case of the normal males.

32). This difference is probably due to the loss of sensitivity following hypophysectomy.

33). Anterior pituitary injections fail to alter the weight of the forelimbs of castrated males.

34). From this it is concluded that the anterior pituitary acts on the forelimbs of the males through the intermediation of the testis.

35). No change is produced in the weight of the forelimbs of the females by the injection of anterior pituitary extracts.

36). Captivity alters the naturally occurring seasonal cycle in the arm/body weight ratios of male and female Xenopus.

37). Starvation of the captive animals leads to an increase in the arm/body weight ratio of males due to loss of total body weight.

38). The effect of starvation due to decreased food intake masks the effect of hypophysectomy on the arm/body weight ratio.

39). Castration results in a decrease in the size of the forelimbs of the male frog.

40). An hormonal anterior-pituitary-testis control of the forelimb size in Xenopus males is postulated.

41). The important factors influencing the arm/body weight ratio are as follows.

a. In both sexes undernutrition causes an increase in the ratio.

b. In the female, changes in the ovary weights are important.

c. In the male, the anterior pituitary-testis mechanism controls the size of the forelimbs.

42). The arm/body weight ratio of captive females rises progressively because of undernutrition. Ovarian atrophy also contributes to the rise.

43). The arm/body weight ratio of the males rises progressively because of undernutrition.

44). Elimination of this factor reveals that the forelimbs of Xenopus males decrease during captivity.

45). This decrease is due to diminished anterior pituitary-testis activity.

46). After correction of the arm/body weight ratios of pond females for the changes in ovary, a cycle still exists.

47). This cycle is due to a state of relative undernutrition, causing an increase in the arm/body weight ratio during pro-oestrous.

48). Correction of the arm/body weight ratios of pond males for the state of relative undernutrition results in a curve showing four phases. This curve represents the seasonal changes occurring in the weight of the forelimbs of the male.

a. The arms are largest during the breeding season.

b. A decrease follows until January.

c. From January to May the arms remain at their smallest size.

d. The increase in the size of the forelimbs to the breeding season level of the males occurs between May and July.

49). This cycle corresponds with the cycle in the pads.

50). The cycle in the forelimbs of the males is controlled by the anterior pituitary acting through the testis.

51). The anatomy of the cloacal labia and of the oviducts of Xenopus females is described.

52). The histology of the dorsal skin of female Xenopus is described and the cloacal labia are shown to be a more fibrous and more vascular modification of the skin.

53). The histology of the pars convoluta of the oviduct is described.

54). Activation of the cloacal labia and of the oviducts consists in the production of a capillary hyperaemia.

55). This activation of the secondary sex characters has been used as a test-object for gonadotrophic activity in Xenopus females. In this way the following preparations were shown to be gonadotrophic:-

Human pregnancy urine, sheep and ox anterior pituitary, Xenopus anterior pituitaries from both males and females, methyl testosterone, testosterone propionate, and progesterone.

56). Negative results were recorded in the case of dogfish pituitaries and oestradiol benzoate.

57). From the last finding it is concluded that the Xenopus ovarian hormone differs from that of mammals.

58). Attempts to extract the Xenopus ovary hormone were unsuccessful.

59). In doses of 360 mgs. ox anterior pituitary produces coupling in 23.4% of pairs injected and sheep anterior pituitary in 74.4%.

60). 100 mgs. of sheep anterior pituitary produces coupling in 22% of injected pairs.

61). Thus sheep anterior pituitary is 3 1/2 times as potent as ox anterior pituitary. Since the weight of the average ox anterior pituitary used was 3 1/2 times as great as that of the average sheep anterior lobe, one ox anterior pituitary has approximately the same gonadotrophic activity as one sheep anterior pituitary.

62). Coupling is not a satisfactory test-object for the assay of gonadotrophic hormone.

63). Coupling can be induced in Xenopus laevis by anterior pituitaries from male and female Xenopus but not by anterior pituitaries from dogfish.

64). Coupling can be induced by the injection of sheep anterior pituitary extract into the female partner only.

65). Testosterone, the mammalian testis hormone, induces coupling when injected into the female only.

66). A marked loss of sensitivity to injected gonadotrophic substance occurs during aestivation in Xenopus laevis.

67). Coupling occurs under natural conditions as a result of an hormonal stimulus from the anterior pituitary.

68). The mode of adoption of the normal lumbar embrace is described.

69). Nine departures from this normal embrace are described.

70). The passive role of the female in the establishment of the mating reflex is confirmed.

71). It is suggested that the tactile sense of the male is of importance in the adoption of the clasp.

72). The activated female moves towards the male.

73). Activation of the male diminishes the amount of movement normally performed by the male.

74). Activated males mate indiscriminately with activated females. The reverse also holds true.

75). The eyes and nares play no essential part in the establishment of the normal lumbar embrace. The eyes, however, act as a refinement which may facilitate the adoption of the normal lumbar embrace.

76). Pendular movements of the ovary increase the blood supply to that organ.

77). At ovulation the follicle wall retracts over the ovum, but the latter remains attached to the ovary for a while.

78). The anatomy of the abdomen of the female Xenopus is described.

79). Cilia cover the peritoneum on the liver and the entire abdominal wall. They are a secondary sex character of the female.

80). The cilia are the most important factor in transporting the ova from the ovary to the ostium of the oviduct which lies in the perihepatic recess of the peritoneal cavity.

81). Ciliary action is also responsible for the entry of the ova into the oviducts.

82). The muscular peristalsis of the pars recta conveys the ovum rapidly down that portion of the oviduct. The histology of the pars recta is described.

83). Ciliary action is the most important factor concerned in propelling the ovum down the pars convoluta of the oviduct.

84). The cloacal sphincter relaxes to allow the ova to leave the ovisac.

85). The cilia lining the ovisac and the cloacal canal are the most important factors in this portion of the path of the ovum.

86). Pendular movements occur all the time in the ovaries of Xenopus laevis (this confirms the findings above), but become more marked 4 - 5 hours after injection with an acid extract of sheep anterior pituitary.

87). Follicular rupture occurs within the first 3 hours after injection and the amount of follicular rupture increases in the next 2 hours.

88). Eggs are seen free in the peritoneal cavity 3 hours after injection.

89). Travelling a distance of about 2 inches through the peritoneal cavity into the perihepatic recess at a rate of 4 inches per hour they reach the oviduct a half-hour later.

90). They pass down the first 1/2 inch (pars recta) of the oviduct in a few seconds and then continue downwards at the rate of 10 inches per hour to reach the ovisac 1 1/2 hours later.

91). There they are retained for 5 hours and then they are passed to the exterior.

92). The average length of the oviducts is 15 1/4 inches.

93). Spermatozoa are released from the testis by the anterior pituitary.

94). They travel down the vasa efferentia of the mesentery to the kidney.

95). The sperms enter the Bowman's capsules of the kidney.

96). They reach the ureter via the tubules and pass through the cloaca to the exterior.

97). Three phases of the sexual cycle of Xenopus laevis are described.

Oestrus	-	July to September.
Anoestrus	-	September to February.
Pro-oestrus	-	February to July.

98). Certain climatic conditions determine the activity of the anterior pituitary in controlling this cycle. Of these rainfall and especially temperature are important.

99). Curves have been drawn which depict these relationships very clearly.

ACKNOWLEDGMENTS.

ACKNOWLEDGMENTS.

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PROTOCOLS.

EXTRACTS.

Name of Extract.	No. of Pituitaries.	Weight of Anterior Lobes.	Volume of Extract.	Strength of Extract.
	Doz.	Gms.	Mls.	Mgs./ml.
S. 1	1	9.48	11.00	860
S. 2	2	14.47	23.00	630
S. 3	2 1/2	16.63	23.10	720
S. 4	2 1/2	16.76	29.50	580
S. 5	1 1/2	12.11	19.00	640
S. 6	2 1/2	16.87	26.40	640
S. 7	3 1/2	29.45	45.00	655
S. 8	3	19.40	28.4	680
S. 9	3 1/2	23.70	35.3	670
S.10	4	24.50	40.0	610
S.11	3	21.68	30.0	720
S.12	5/6	5.81	6.80	855
S.13	1	6.37	7.80	820
S.14	6	30.33	-	-
S.15	6 1/2	34.83	-	-
S.16	4 1/2	28.36	-	-
S.17	3	17.90	27.6	650
S.18	7 1/2	44.33	-	-
S.19	9 1/2	52.56	80.0	660
S.20	4 1/2	32.35	49.2	660
S.21	3	15.98	27.2	590
S.22	5	22.88	40.2	570
S.23	11 1/2	56.29	85.0	660
S.24	3/4	4.82	7.1	680
S.25	12 1/2	34.32	57.8	594
S.26	2 3/4	8.61	14.0	615
S.27	9 1/4	26.36	-	-
O. 1	1/2	12.33	14.25	865
O. 2	1/2	13.21	18.85	700
O. 3	1/2	9.51	15.00	630

Protocol 1.

Acid extracts of sheep and ox anterior pituitaries.

POND ANIMALS.

November, 1937.

Male.

Body Wt.	Left Arm.	Right Arm.	Mean.	Arm/body Weight Ratio.
Gms.	Mgs.	Mgs.	Mgs.	
29.8	504	516	510	1.69
27.3	472	420	446	1.63
24.4	414	426	420	1.72
23.5	393	408	400	1.70
20.9	365	383	374	1.77
27.8	499	528	513	1.84
25.3	501	532	516	2.04
28.9	526	498	512	1.77
32.3	577	593	580	1.79
32.0	566	580	573	1.79
21.3	385	404	395	1.85
26.7	510	506	508	1.90
30.6	555	523	539	1.76
30.3	564	538	551	1.82
32.5	500	516	508	1.60

Protocol 2.

Female.

26.5	246	266	256	0.97
33.8	324	320	322	0.95
40.6	437	459	445	1.09
57.3	668	671	669	1.16
19.2	194	185	190	0.99
20.3	223	228	225	1.11
37.0	432	464	448	1.21
36.2	385	393	399	1.10
31.9	378	357	367	1.15
20.1	234	242	238	1.18
19.2	218	208	213	1.11
21.7	221	247	234	1.08
37.5	348	374	361	0.96
33.7	368	397	382	1.13
31.4	342	317	330	1.05

Protocol 3.

POND ANIMALS.

January, 1938.

Male.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
36.0	525.5	574	550	1.53
37.0	537.0	560.5	549	1.48
36.5	599	500	550	1.51
40.0	573.5	578.5	576	1.44
25.5	335	333.5	334.2	1.31
38.0	596.5	650.0	623	1.64
36.5	502	497	500	1.37
32.0	434	457.5	446	1.39
46.0	617	657	637	1.39
40.0	593	568	580	1.45
35.5	588	541	565	1.59
37.5	499	534	517	1.36
37.5	540.5	511.5	526	1.40
45.0	693	663	678	1.51
25.5	400.5	388	394	1.54

Protocol 4.

Female.

39.5	378	371	375	0.95
43.0	415	457	436	1.01
35.0	393	361	377	1.08
36.0	442	404	423	1.18
36.0	423	337	380	1.06
34.5	350	350	350	1.01
37.0	398	351	375	1.01
39.0	408	450	429	1.10
32.0	336	307	322	1.01
32.5	385	354	370	1.14
30.5	345	322	333	1.09
34.5	361	334	348	1.01
32.5	301	338	320	0.98
20.0	217	218	218	1.09
28.0	272	276	274	0.91

Protocol 5.

POND ANIMALS.

March 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
41.5	667.	656	662	1.59
40.0	558	578	568	1.42
28.0	446	430	438	1.56
26.5	457	455	456	1.72
43.5	727	697	712	1.64
29.5	483	472	478	1.62
35.5	639	616	628	1.77
28.0	479	431	455	1.62
27.0	472	498	485	1.79
25.5	514	449	482	1.81
29.0	488	500	494	1.70
40.0	730	679	705	1.76
30.5	502	518	510	1.67
22.5	439	475	457	2.03
27.0	501	455	478	1.77

Protocol 6.

Females.

29.0	365	355	360	1.25
35.5	435	398	417	1.17
32.5	342	380	361	1.11
26.0	268	273	271	1.04
43.0	439	413	426	0.99
38.0	477	464	471	1.23
62.5	852	879	866	1.38
29.0	375	325	350	1.21
53.0	607	594	601	1.13
62.5	810	846	828	1.32
30.0	335	346	341	1.13
35.0	Deformed	452	452	1.29
26.0	335	305	320	1.22
28.0	260	270	265	0.94
23.5	237	246	242	1.03

Protocol 7.

POND ANIMALS.

May 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
33.0	625	550	588	1.71
37.0	632	614	623	1.68
30.0	531	478	505	1.68
33.0	583	628	606	1.84
35.5	534	542	538	1.52
34.0	544	480	512	1.51
26.0	428	383	406	1.56
29.0	503	493	498	1.72
40.0	661	624	643	1.61
34.0	607	601	604	1.78
32.5	568	564	566	1.74
31.5	587	554	572	1.82
31.0	575	535	555	1.79
30.5	523	450	487	1.60
30.0	578	545	562	1.87

Protocol 8.

Females.

29.0	311	292	302	1.04
35.5	404	348	376	1.06
30.0	368	345	357	1.19
33.0	392	354	373	1.13
27.0	335	361	348	1.29
34.5	377	383	380	1.10
28.0	299	287	293	1.05
37.0	453	393	423	1.14
36.0	384	334	359	1.00
37.0	420	364	392	1.06
33.0	383	376	380	1.15
32.0	377	371	374	1.17
31.0	383	388	386	1.25
38.5	511	473	492	1.28
26.0	302	300	301	1.16

Protocol 9.

POND ANIMALS.

July, 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
23.5	392	403	398	1.70
24.0	442	447	445	1.85
28.0	494	489	492	1.76
28.5	482	460	471	1.65
36.0	664	602	633	1.76
26.0	460	454	457	1.76
33.0	568	561	565	1.71
37.0	653	648	651	1.76
28.0	502	525	514	1.84
34.0	568	570	569	1.68
30.0	522	471	497	1.66
28.0	495	475	485	1.73
36.0	584	568	576	1.60
31.5	540	496	518	1.65
29.0	528	568	548	1.89

Protocol 10.

Females.

69.0	698	644	671	0.97
56.0	611	558	585	1.04
67.5	743	668	706	1.05
56.0	616	641	629	1.12
88.0	938	829	884	1.00
74.5	728	670	699	0.94
65.5	617	609	613	0.94
55.5	586	569	578	1.04
49.0	513	478	496	1.01
49.0	563	551	557	1.13
51.5	518	458	488	0.95
50.0	501	449	475	0.95
60.0	593	572	583	0.97
60.0	558	587	573	0.96
53.0	482	435	459	0.86

Protocol 11.

CAPTIVES.

January, 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
22.0	364	372	370	1.68
33.0	606	642	624	1.89
25.0	475	479	477	1.91
31.0	448	469	458	1.48
22.5	360	358	359	1.60
32.0	502	567	535	1.67
24.5	502	438	470	1.92
27.0	526	491	509	1.89
26.0	390	397	394	1.52
28.0	485	464	475	1.70
31.5	509	520	515	1.63
27.0	506	429	468	1.73
21.5	355	395	375	1.74
26.0	498	480	489	1.88
31.5	548	485	517	1.64

Protocol 12.

Females.

21.5	235	239	237	1.10
41.0	482	513	497	1.21
29.0	374	400	387	1.33
25.0	299	267	288	1.15
28.5	293	274	288	1.01
26.0	265	239	252	0.97
27.5	290	352	321	1.17
31.5	419	359	389	1.23
21.0	277	377	327	1.56
38.5	457	368	408	1.04
33.0	445	435	440	1.33
32.5	427	404	416	1.29
25.0	325	298	312	1.25
22.5	302	270	286	1.27
24.0	309	285	297	1.24

Protocol 13.

CAPTIVES.

March, 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight. Ratio.
22.0	360	386	373	1.70
20.5	373	379	376	1.83
22.0	443	373	408	1.85
26.0	497	495	495	1.90
23.0	467	454	461	2.00
20.5	425	427	426	2.08
25.0	503	489	496	1.98
18.5	330	334	332	1.79
22.0	406	397	497	2.26
24.5	494	427	461	1.88
24.5	413	372	393	1.60
25.5	546	515	531	2.08
18.0	373	313	343	1.91
15.5	290	304	297	1.91
14.0	196	182	189	1.35

Protocol 14.

Females.

28.5	371	348	360	1.26
40.5	541	464	503	1.24
28.5	417	355	386	1.35
20.0	290	273	282	1.41
18.0	230	249	240	1.33
22.0	305	180	305	1.39
26.0	365	362	364	1.40
21.5	266	258	262	1.22
23.0	288	290	289	1.26
18.5	246	248	247	1.34
23.5	278	272	275	1.17
20.5	281	288	285	1.39
24.0	349	317	333	1.39
25.0	361	340	351	1.40
45.5	626	606	616	1.35

Protocol 15.

CAPTIVES.

May 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
24.0	431	481	456	1.90
17.5	387	337	362	2.07
25.0	549	507	528	2.11
14.5	321	321	321	2.21
22.0	469	457	463	2.10
18.0	350	306	328	1.82
17.5	378	362	370	2.11
21.5	390	394	392	1.82
20.5	415	364	390	1.90
19.0	359	363	361	1.90
19.5	357	352	355	1.82
19.0	348	343	346	1.82
16.0	318	328	323	2.02
22.5	413	407	410	1.82
20.0	373	363	368	1.84

Protocol 16.

Females.

18.0	217	213	215	1.19
22.0	263	256	260	1.18
20.0	242	209	226	1.13
16.5	213	197	205	1.27
17.0	238	221	230	1.35
19.0	247	233	240	1.26
14.0	217	217	217	1.55
18.0	255	273	264	1.47
25.0	302	302	302	1.21
31.0	340	331	336	1.08
15.0	191	190	191	1.27
17.0	194	187	191	1.12
29.5	331	343	337	1.14
23.0	337	318	328	1.43
15.5	203	195	199	1.22

Protocol 17.

CAPTIVES.

July, 1938.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
24.0	463	487	475	1.98
25.0	453	435	444	1.78
25.5	449	428	439	1.72
23.0	482	445	464	2.02
28.5	569	597	583	2.00
26.5	486	495	491	1.85
29.5	538	534	536	1.82
27.0	500	475	488	1.81
18.5	398	382	390	2.11
27.0	486	509	498	1.84
32.0	623	641	632	1.98
26.5	510	508	509	1.92
19.5	360	350	355	1.82
21.5	404	385	395	1.84
24.5	433	425	429	1.75

Protocol 18.

Females.

37.5	507	463	485	1.29
30.5	462	485	474	1.54
24.0	333	331	332	1.38
18.0	243	257	250	1.39
18.0	271	270	271	1.51
30.0	399	417	408	1.36
23.0	327	340	334	1.45
29.5	427	423	425	1.44
26.0	398	379	389	1.49
28.5	402	382	392	1.37
28.5	377	376	377	1.32
27.5	382	404	393	1.43
21.5	277	273	275	1.26
23.0	316	327	322	1.60
26.0	355	349	352	1.36

Protocol 19.

Injection Of Acid Extracts Of Sheep Anterior Pituitary.

Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
28.0	530	539.5	535	1.91
25.0	453	444	449	1.80
26.5	490.5	485.5	488	1.80
26.0	485	499	492	1.90
33.0	627	575	601	1.82
21.5	399	429	414	1.92
30.0	583	576	580	1.93
32.5	570	620	595	1.83
22.5	393	393	393	1.75
25.0	462	474	468	1.87
23.0	383	382	383	1.67
28.0	577	543	560	2.00
21.0	361	393	377	1.80
27.5	509	555	532	1.97
27.5	445	440	442	1.61
23.0	390	419	405	1.76

Protocol 20.

Controls.

28.0	489	490	490	1.75
26.5	515	478	497	1.87
28.0	451	445	448	1.60
24.0	406	403	405	1.69
28.0	506	494	500	1.78
28.5	497	467	482	1.69
32.5	535	506	521	1.60
23.0	340	348	344	1.50
33.0	551	549	550	1.67
28.0	462	448	455	1.63
26.5	474	445	460	1.73
27.5	456	448	452	1.65
26.5	459	484	472	1.78
30.0	516	560	538	1.79
29.0	442	464	453	1.56
27.5	492	499	496	1.80

Protocol 21.

Injection Of Sheep Anterior Pituitary Extract. (Pyridine Extract).

Normal Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
35.5	674	637	656	1.85
27.5	493	496	495	1.80
27.0	550	496	523	1.94
30.5	544	554	549	1.82
27.5	507	486	496	1.81
37.0	693	643	668	1.81

Protocol 22.

Normal Females.

36.0	365	329	347	0.96
38.0	450	471	460	1.21
54.0	653	653	653	1.04
47.5	461	446	454	0.95
52.5	546	518	532	1.01
30.0	309	323	316	1.05
54.5	592	540	566	1.04
50.0	526	501	514	1.03
50.0	528	504	516	1.03
71.0	767	663	715	1.00
51.5	550	517	534	1.04
49.5	567	535	551	1.13

Protocol 23.

Injection Of Sheep Anterior Pituitary Extract.(Pyridine Extract).

HYPOPHYSECTOMISED MALES.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
40.0	699	666	683	1.71
36.5	632	691	662	1.80
33.5	586	540	563	1.68
23.5	429	398	414	1.76
34.5	637	565	601	1.74
37.5	653	622	638	1.70
34.5	569	537	553	1.60
27.0	413	363	388	1.44
27.5	457	465	461	1.68
26.5	465	471	468	1.77
27.0	433	398	416	1.54
29.0	527	509	518	1.79
30.5	553	533	543	1.71
38.0	702	673	688	1.81
30.5	529	500	515	1.69

Protocol 24.

Hypophysectomised Females.

55.5	636	646	641	1.16
61.5	735	694	714	1.14
45.5	423	403	413	0.91
43.0	486	463	475	1.10
44.5	467	427	447	1.00
43.5	475	449	462	1.06
63.5	744	750	747	1.18
52.5	536	533	535	1.02
60.0	524	475	400	0.83
49.5	557	532	545	1.10
51.5	545	528	537	1.04

Protocol 25.

Injection Of Sheep Anterior Pituitary Extract.(Pyridine Extract).

Castrated Males.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
35.0	625	517	571	1.63
32.0	565	519	542	1.69
36.0	566	487	527	1.46
28.5	486	439	463	1.62
26.5	446	417	432	1.63
35.0	585	523	554	1.58
21.0	341	340	341	1.62
25.0	406	361	384	1.54
30.5	524	482	503	1.65
29.0	419	388	404	1.39
27.0	477	462	470	1.74
26.5	403	366	385	1.45
27.0	488	409	449	1.66

Protocol 26.

Castrated Females.

30.5	402	394	398	1.30
43.5	505	467	486	1.12
45.0	593	601	597	1.33
54.0	550	532	541	1.00
46.5	554	570	562	1.21
34.0	440	410	425	1.25
33.5	426	347	387	1.16
39.0	471	464	468	1.20
42.0	513	500	507	1.21
45.0	556	513	535	1.19
46.5	577	542	560	1.20

Protocol 27.

Group I.

Captive Fed.

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio.
25.5	454	398	426	1.67
22.5	394	352	473	2.10
22.5	457	407	432	1.92
28.5	463	445	452	1.58
31.5	502	452	477	1.51
32.0	449	387	418	1.31
25.0	450	435	443	1.77
26.0	429	397	413	1.57
28.0	464	463	464	1.66
25.0	412	413	413	1.65
24.5	385	345	465	1.89
38.0	640	568	504	1.33

Protocol 28.

Group II.

Captive Starvation.

27.0	500	479	490	1.81
28.0	505	523	514	1.84
28.5	522	531	527	1.85
27.0	490	466	478	1.77
27.5	510	484	497	1.81
30.0	590	529	560	1.87
31.5	600	583	592	1.88
34.5	600	626	613	1.77
29.5	583	567	575	1.95
24.0	457	437	447	1.86
22.0	386	371	379	1.72
20.5	380	376	378	1.84

Protocol 29.

Group III.

Hypophysectomised (And Fed).

Body Wt. Gms.	Left Arm. Mgs.	Right Arm. Mgs.	Mean. Mgs.	Arm/Body Weight Ratio
25.5	462	526	494	1.94
29.5	590	519	555	1.88
29.5	595	510	553	1.88
30.0	658	642	650	2.17
35.0	683	685	684	1.95
41.5	657	591	624	1.50
28.5	514	510	512	1.80
30.5	529	560	545	1.80
28.5	412	495	454	1.63
37.5	676	672	674	1.80
40.0	675	650	663	1.66

Protocol 30.

Group IV.

Castrates (And Fed).

28.0	470	495	483	1.72
37.0	598	576	587	1.57
40.0	560	545	553	1.38
45.5	625	594	610	1.35
40.5	546	495	520	1.28
33.5	549	519	534	1.59
32.0	435	403	419	1.31
30.0	439	413	426	1.42
33.0	488	431	460	1.39
27.0	400	390	395	1.44

Protocol 31.

STUDIES IN THE REPRODUCTIVE PHYSIOLOGY OF THE AMPHIBIA.

Summary Of

THESIS

Presented for the degree of

DOCTOR OF PHILOSOPHY

in the

DEPARTMENT OF PHYSIOLOGY

by

LIONEL BERK, B.Sc.

University of Cape Town. 1939.

GENERAL SUMMARY.

1). A brief account is given of the development of the knowledge of the reproductive physiology of Xenopus laevis.

2). The scope of the present work is outlined.

3). The method of collecting "pairs of frogs known to couple" is described.

4). The containers used in the experiments on coupling are described.

5). The operations of castration and hypophysectomy are described.

6). Methods are described for preparing extracts of the following substances:-

Human pregnancy urine.

Anterior pituitaries of sheep, ox, Xenopus laevis and dogfish.

7). The seasonal cycle in the pads of male Xenopus is described.

8). Four phases are recognised.

a) January to May. The pads are absent.

b) May to July. The pads develop.

c) July to September. The pads are maintained at their full development.

d) September to January. The pads disappear.

9). Three types of pads are described.

a) Pads covering the fingers and palms.

b) Pads covering the forelimb as far as the elbow.

c) Pads covering the forelimb as far as the axilla.

10). Five stages in the development of the pads are described. The chief stages are as follows:-

The pads are developed first as far as the wrist. Next the forearms show pads and finally the pad is completed.

11). The pads disappear after the breeding season by successive exfoliations.

12). Pads are never seen on the forelimbs of female Xenopus.

13). Acid and pyridine extracts of sheep anterior pituitary induce the development of pads in normal but not in castrated males.

14). Pyridine extracts of sheep anterior pituitary induce pad development in anterior lobe hypophysectomised males, but the rate of development of the pads is slower than in normal males.

15). The above finding is probably explained by the loss of sensitivity of the hypophysectomised animals to gonadotrophic injections.

16). Anterior pituitaries from both male and female Xenopus produce pads in normal Xenopus males.

17). Xenopus anterior lobes do not cause pad development in castrate males.

18). Captivity results in the abolition of the pad cycle.

19). The evidence presented indicates that the pad cycle under natural conditions is under the control of the anterior pituitary acting via the testis.

20). The development of the pad is described macroscopically and microscopically.

21). The inhibition of the anterior pituitary by captivity (Shapiro & Shapiro 1934) consists in the prevention of the pouring out by the gland of its gonadotrophic principles. Secretion is not inhibited.

22). The pads play no essential role in the mating reflex in Xenopus laevis.

23). Using the development of pads as a test-object for androgenic and gonadotrophic activity in the male Xenopus, methyl testosterone, testosterone propionate, oestradiol benzoate and progesterone are shown to possess neither of these two properties.

24). The evidence presented suggests that the Xenopus testis hormone differs from the mammalian testis hormone testosterone.

25). The method of calculating the arm/body weight ratio is described.

26). The arm/body weight ratio of the male is larger than that of the female.

27). Seasonal cycles are seen in the arm/body weight ratios of both male and female Xenopus. These cycles are described.

28). The cycle in the male shows four phases.

a. The arm/body weight ratio is smallest in January.

b. The ratio increases until July.

c. During the breeding season the ratio is maintained at a high level.

d. A decline to the summer level follows the breeding season.

29). 3 phases are seen in the arm/body weight ratio cycle in the female.

a. The lowest level is seen during the breeding season.

b. From then until January the ratio is higher.

c. The highest level is seen between January and July.

30). Injections of acid or pyridine extracts of sheep anterior pituitary into normal Xenopus results in an increase in the weight of the forelimbs.

31). Under identical treatment, hypophysectomised males also show an increase in the weight of the forelimbs, but this increase is less than in the case of the normal males.

32). This difference is probably due to the loss of sensitivity following hypophysectomy.

33). Anterior pituitary injections fail to alter the weight of the forelimbs of castrated males.

34). From this it is concluded that the anterior pituitary acts on the forelimbs of the males through the intermediation of the testis.

35). No change is produced in the weight of the forelimbs of the females by the injection of anterior pituitary extracts.

36). Captivity alters the naturally occurring seasonal cycle in the arm/body weight ratios of male and female Xenopus.

37). Starvation of the captive animals leads to an increase in the arm/body weight ratio of males due to loss of total body weight.

38). The effect of starvation due to decreased food intake masks the effect of hypophysectomy on the arm/body weight ratio.

39). Castration results in a decrease in the size of the forelimbs of the male frog.

40). An hormonal anterior-pituitary-testis control of the forelimb size in Xenopus males is postulated.

41). The important factors influencing the arm/body weight ratio are as follows.

a. In both sexes undernutrition causes an increase in the ratio.

b. In the female, changes in the ovary weights are important.

c. In the male, the anterior pituitary-testis mechanism controls the size of the forelimbs.

42). The arm/body weight ratio of captive females rises progressively because of undernutrition. Ovarian atrophy also contributes to the rise.

43). The arm/body weight ratio of the males rises progressively because of undernutrition.

44). Elimination of this factor reveals that the forelimbs of Xenopus males decrease during captivity.

45). This decrease is due to diminished anterior pituitary-testis activity.

46). After correction of the arm/body weight ratios of pond females for the changes in ovary, a cycle still exists.

47). This cycle is due to a state of relative undernutrition, causing an increase in the arm/body weight ratio during pro-oestrous.

48). Correction of the arm/body weight ratios of pond males for the state of relative undernutrition results in a curve showing four phases. This curve represents the seasonal changes occurring in the weight of the forelimbs of the male.

a. The arms are largest during the breeding season.

b. A decrease follows until January.

c. From January to May the arms remain at their smallest size.

d. The increase in the size of the forelimbs to the breeding season level of the males occurs between May and July.

49). This cycle corresponds with the cycle in the pads.

50). The cycle in the forelimbs of the males is controlled by the anterior pituitary acting through the testis.

51). The anatomy of the cloacal labia and of the oviducts of Xenopus females is described.

52). The histology of the dorsal skin of female Xenopus is described and the cloacal labia are shown to be a more fibrous and more vascular modification of the skin.

53). The histology of the pars convoluta of the oviduct is described.

54). Activation of the cloacal labia and of the oviducts consists in the production of a capillary hyperaemia.

55). This activation of the secondary sex characters has been used as a test-object for gonadotrophic activity in Xenopus females. In this way the following preparations were shown to be gonadotrophic:-

Human pregnancy urine, sheep and ox anterior pituitary, Xenopus anterior pituitaries from both males and females, methyl testosterone, testosterone propionate, and progesterone.

56). Negative results were recorded in the case of dogfish pituitaries and oestradiol benzoate.

57). From the last finding it is concluded that the Xenopus ovarian hormone differs from that of mammals.

58). Attempts to extract the Xenopus ovary hormone were unsuccessful.

59). In doses of 360 mgs. ox anterior pituitary produces coupling in 23.4% of pairs injected and sheep anterior pituitary in 74.4%.

60). 100 mgs. of sheep anterior pituitary produces coupling in 22% of injected pairs.

61). Thus sheep anterior pituitary is 3 1/2 times as potent as ox anterior pituitary. Since the weight of the average ox anterior pituitary used was 3 1/2 times as great as that of the average sheep anterior lobe; one ox anterior pituitary has approximately the same gonadotrophic activity as one sheep anterior pituitary.

62). Coupling is not a satisfactory test-object for the assay of gonadotrophic hormone.

63). Coupling can be induced in Xenopus laevis by anterior pituitaries from male and female Xenopus but not by anterior pituitaries from dogfish.

64). Coupling can be induced by the injection of sheep anterior pituitary extract into the female partner only.

65). Testosterone, the mammalian testis hormone, induces coupling when injected into the female only.

66). A marked loss of sensitivity to injected gonadotrophic substance occurs during aestivation in Xenopus laevis.

67). Coupling occurs under natural conditions as a result of an hormonal stimulus from the anterior pituitary.

68). The mode of adoption of the normal lumbar embrace is described.

69). Nine departures from this normal embrace are described.

70). The passive role of the female in the establishment of the mating reflex is confirmed.

71). It is suggested that the tactile sense of the male is of importance in the adoption of the clasp.

72). The activated female moves towards the male.

73). Activation of the male diminishes the amount of movement normally performed by the male.

74). Activated males mate indiscriminately with activated females. The reverse also holds true.

75). The eyes and nares play no essential part in the establishment of the normal lumbar embrace. The eyes, however, act as a refinement which may facilitate the adoption of the normal lumbar embrace.

76). Pendular movements of the ovary increase the blood supply to that organ.

77). At ovulation the follicle wall retracts over the ovum, but the latter remains attached to the ovary for a while.

78). The anatomy of the abdomen of the female Xenopus is described.

79). Cilia cover the peritoneum on the liver and the entire abdominal wall. They are a secondary sex character of the female.

80). The cilia are the most important factor in transporting the ova from the ovary to the ostium of the oviduct which lies in the perihepatic recess of the peritoneal cavity.

81). Ciliary action is also responsible for the entry of the ova into the oviducts.

82). The muscular peristalsis of the pars recta conveys the ovum rapidly down that portion of the oviduct. The histology of the pars recta is described.

83). Ciliary action is the most important factor concerned in propelling the ovum down the pars convoluta of the oviduct.

84). The cloacal sphincter relaxes to allow the ova to leave the ovisac.

85). The cilia lining the ovisac and the cloacal canal are the most important factors in this portion of the path of the ovum.

86). Pendular movements occur all the time in the ovaries of Xenopus laevis (this confirms the findings above), but become more marked 4 - 5 hours after injection with an acid extract of sheep anterior pituitary.

87). Follicular rupture occurs within the first 3 hours after injection and the amount of follicular rupture increases in the next 2 hours.

88). Eggs are seen free in the peritoneal cavity 3 hours after injection.

89). Travelling a distance of about 2 inches through the peritoneal cavity into the perihepatic recess at a rate of 4 inches per hour they reach the oviduct a half-hour later.

90). They pass down the first 1/2 inch (pars recta) of the oviduct in a few seconds and then continue downwards at the rate of 10 inches per hour to reach the ovisac 1 1/2 hours later.

91). There they are retained for 5 hours and then they are passed to the exterior.

92). The average length of the oviducts is 15 1/4 inches.

93). Spermatozoa are released from the testis by the anterior pituitary.

94). They travel down the vasa efferentia of the mesentery to the kidney.

95). The sperms enter the Bowman's capsules of the kidney.

96). They reach theureter via the tubules and pass through the cloaca to the exterior.

97). Three phases of the sexual cycle of Xenopus laevis are described.

Oestrus	-	July to September.
Anoestrus	-	September to February.
Pro-oestrus	-	February to July.

98). Certain climatic conditions determine the activity of the anterior pituitary in controlling this cycle. Of these rainfall and especially temperature are important.

99). Curves have been drawn which depict these relationships very clearly.