EXPERIMENTAL STUDIES ON THE SEXUAL CYCLE OF THE SOUTH AFRICAN CLAWED TOAD (*XENOPUS LAEVIS*). III

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(With Three Text-figures)

I. INTRODUCTION

Previous papers (Alexander & Bellerby, 1935, 1937) from this laboratory have described experiments which demonstrated the importance of nutrition in the regulation of the reproductive cycle of *Xenopus laevis*. It therefore seems that quantitative seasonal variations in food supply are mainly responsible for seasonal variations in the condition of the gonads. In a further communication (Bellerby, 1937) it was shown that seasonal variation in light intensity apparently plays little if any part in the controlling mechanism. These enquiries emphasized the extrinsic factors in the reproductive cycle of Amphibia. Little attention was paid to the anterior lobe of the pituitary or to any possible connexion between this gland and the interrelated processes of somatic and ovarian growth.

It had been shown previously by Hogben (1930) and more extensively by Hogben *et al.* (1931) that removal of both lobes of the pituitary of *X. laevis*, or of the anterior lobe alone, results in retrogression of the ovaries. The first-named author (Hogben, 1930) also reported oviposition following transplantation of the pituitary of *X. laevis* or injection of extracts of the anterior lobe of the ox pituitary. Later Bellerby (1934 *a*) adopted *Xenopus* as a test animal for the assay of anterior lobe pituitary extracts and other gonad stimulating preparations. In 1933, Zwarenstein & Shapiro, while also noting involution of the ovaries consequent on pituitary removal, found that normal females of *Xenopus* responded to their particular laboratory routine by less pronounced though uniform ovarian retrogression. They described this condition of their animals as "the captivity effect"; and cast some doubt upon the significance of pituitary removal. As can be seen from the condition of the control group described in a series of experiments by Hogben *et al.* (1931), the captivity effect of the treatment to which Zwarenstein & Shapiro allude had not intruded into the experience of one of the writers (L. H.) in Cape Town. Apparently it has escaped the notice of other investigators, who have kept large numbers of toads in this country for experimental work.

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One of the main conclusions which emerged from previous experiments described in this series is that the ovary of *Xenopus* acts as an organ of food storage against the surprisingly prolonged periods during which the animal can go without food. Thus the relation of the pituitary to body growth in females of *X. laevis* can only be satisfactorily elucidated if concurrent changes in the weight of the ovary are separately recorded. This being so, a more comprehensive investigation of the effects of pituitary removal has been undertaken with special attention to growth rates and concurrent food intake. The enquiry has also been extended for the first time to include the relation of the pituitary to the male as well as to the female sexual cycle.

II. TECHNIQUE AND MATERIALS

Four series of toads were used in the experiments to be described. All were kept in white enamelled tanks of the same size, and in the same volume of water. Since they were all kept in the same room, they were thus subjected to the same daily variation in room temperature and to the same differences in the duration and intensity of normal or artificial light. Previous to the experiment the toads had been kept under conditions appropriate to a healthy state of the ovaries and testes. The females were selected from a batch, which had ovulated a short time previously after injection of extract prepared from the anterior lobe of the pituitary. They were fed once a week on the same day and weighed in bulk at monthly intervals; one week being allowed to elapse between the last feeding and weighing.

The pituitary was removed by the method first described by Hogben (1923) for *Rana* and later for *Xenopus* (Hogben & Slome, 1931), and which subsequently has been modified with successful application to birds (Hill & Parkes, 1934). Food intake was determined during the last 10 weeks of the investigation by weighing the animals of each group in bulk before and after the weekly meal. The animal is a voracious feeder, normally devouring a ration of meat often attaining 10 per cent of its body weight so that the mean food intake is easy to record by this means.

Tissues were fixed in Bouin's fluid. Sections were stained with haematoxylin or toluidin blue and eosin. Serial sections were cut of the testes, the oviducts and of the pituitary body in both series. A small surrounding area of brain was examined with the latter organ.

III. EXPERIMENTAL DATA

The original intention was to confine the investigation to the effects of anterior lobe removal alone. This was not possible for several reasons. The pituitary of *Xenopus* differs from that of *Rana* and conforms to the Urodele type in one important respect. The pars tuberalis in the adult of a typical Anuran is detached from the anterior lobe at metamorphosis and lies as two flat plaques on the sides of the tuber cinereum well in front of the rest of the adult hypophysis. That of *Xenopus* forms a thin lip projecting forwards from the anterior lobe and attached to the tuber cinereum. In consequence it easily breaks off where it is continuous with the pars anterior. So unless large animals are used it is easy to leave behind the pars

tuberalis, or part of it, with or without traces of anterior lobe. Furthermore in some operations a negative pressure sufficient to detach the lip also removes the posterior lobe whilst in others the lip is left behind because the pressure must be kept low to prevent removal of the posterior lobe. In previous work undertaken at Cape Town where an abundant supply of large females was available the operation was carried out with a binocular microscope. If there was any doubt about the success of it, the animal was discarded. In the investigations here described, the supply of large females was limited so most of the toads used were relatively small. This was *a fortiori* true of the males, always decidedly smaller than females of the same age.

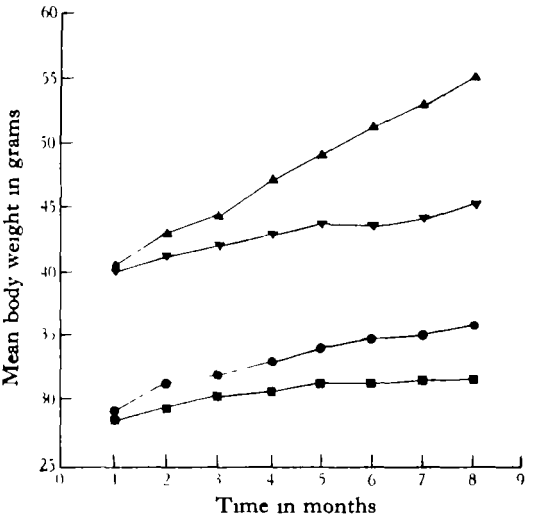

Fig. 1. ■ hypophysectomized ♂♂; ● control ♂♂, ▼ hypophysectomized ♀♀; ▲ control ♀♀.

Because of the shortage of toads a drastic sacrifice of small or unsuitable animals could not be made. The final series of thirty males and thirty females therefore included equal numbers of totally hypophysectomized and toads which all retained the posterior lobe, and some of them, in addition, the pars tuberalis. Autopsy showed that detectable regeneration of anterior lobe tissue occurred in several toads belonging to the last class. Presumably this was because traces of anterior lobe tissue adhered to the pars tuberalis. Appreciable regeneration is also indicated by the spread of the frequency distribution curves for gonad and body weight, when compared with corresponding curves for the two control series. After operation four males and six females died in the course of the experiments.

The growth curves of the four series of toads is given in Fig. 1. The data obtained from the autopsy carried out at the end of the 7 months' period of the experiment are set forth in Table I.

With reference to the retrogression of the gonads, the fall off in weight is specially striking in the hypophysectomized males. Fig. 2 shows their frequency distribution with that of the control group. There is little overlap. The mean testes weight is somewhat smaller in those animals (12) from which the whole gland had

been removed than in those (14) with the posterior lobe intact. Since several animals in the latter group showed detectable signs of regeneration of anterior lobe tissue no special significance can be attached to this discrepancy.

Table I

Series	Mean body weight in g.	Mean weight of fat bodies in g	Mean weight of gonads in g.
Normal ♀♀ (24)	51·16	2·67	5·89
Hypophysectomized ♀♀ (24)	41·12	2·89	1·73
Normal ♂♂ (26)	33·33	1·68	0·16
Hypophysectomized ♂♂ (26)	30·97	1·58	0·05

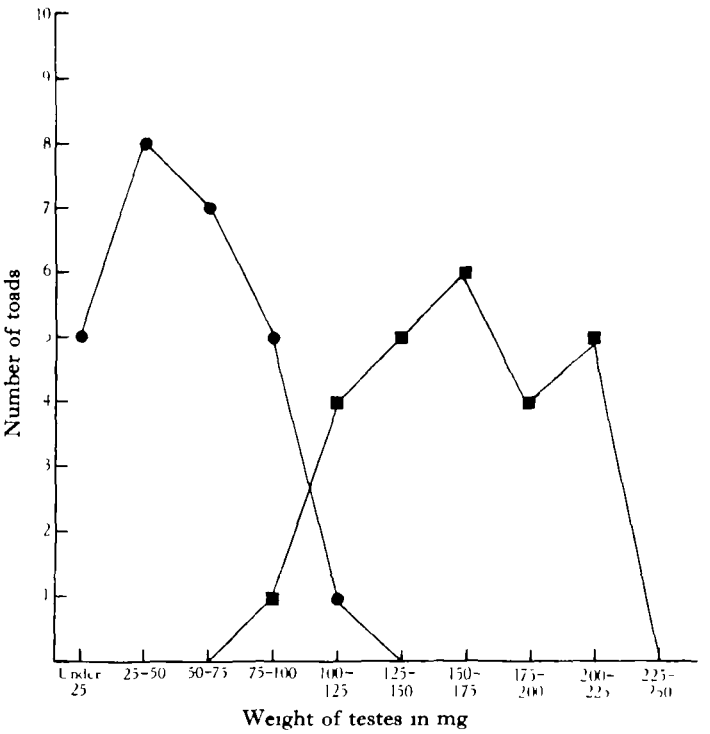

Fig. 2. ● hypophysectomized ♂♂, ■ control ♂♂.

Condition of the ovaries

The frequency distribution for ovary weight is given in Fig. 3. The weight difference is not so striking as the visible appearance at the autopsy. In the control group the ovaries generally presented the usual healthy appearance. In nineteen out of twenty-four they were composed of large black fully formed ova. In twenty-one out of twenty-four in the experimental group they were reduced to a small mass of greyish jelly with very few, if any, oocytes of visible dimensions. This

confirms the results of the previous experiments by Hogben *et al.* (1931). The variation in size of oocytes at different stages of development in both series did not permit us to form any conclusions about the mode of resorption. One striking result of the preliminary autopsy was that in the hypophysectomized females in which retrogression of the ovaries was so advanced, so that no pigmented oocytes

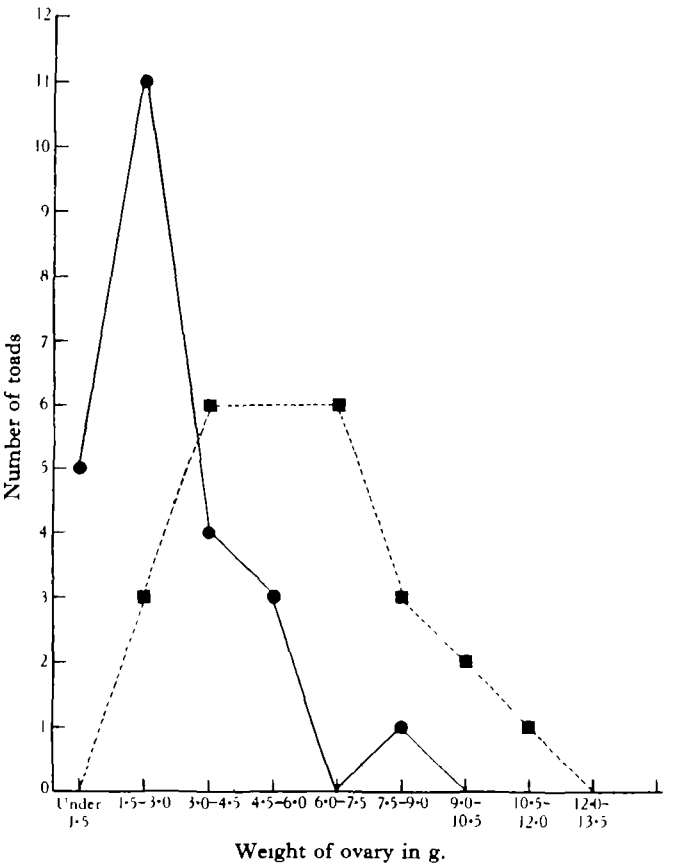

Fig. 3. ● hypophysectomized ♀♀; ■ control ♀♀.

and few oocytes of visible dimensions remained, the corpora adiposa displayed a peculiar slate-grey hue with specks of black pigment barely visible to the naked eye. The natural conclusion to draw is that the egg pigment is not wholly excreted, but is in part reabsorbed and deposited with the fat reserves of the body.

Microscopic condition of the testes

Out of twenty-four control males microscopic preparations of the testes of twenty-two were examined. Those of two animals were useless owing to defective inbedding. In sixteen of these the condition presented a uniform picture. The major cysts were well defined, the centre of each cyst being partially filled with

closely packed sheaves of ripe spermatozoa. The periphery of the cysts consisted of several nests of cells, those of each nest being in roughly the same phase of meiosis, i.e. leptotene, diplotene, first and second reduction divisions, etc. Six were anomalous. The testes of Nos. 2, 22, 23 and 24 contained ripe spermatozoa. The major cysts were less clearly demarcated. The peripheral nests of cells were less distinct. There was a noticeable concentration of intercalary tissue, the cells of which possessed spindle-shaped nuclei. The testis of No. 10 was more homogeneous, with few indications of meiotic stages. The existence of separate major cysts was only indicated by isolated clumps of spermatozoa, apparently in process of degeneration. No. 4 showed very little trace of cyst differentiation and very few traces of recognizable spermatozoa. The testes of the hypophysectomized group were less uniform. Those of three (Nos. 1, 10 and 11) hardly differed from the normal condition, and were essentially like those of Nos. 2, 22, 23 and 24 in the control group. Three others (Nos. 14, 19 and 22) showed signs of resorption of spermatozoa in the cavity of the major cysts. The peripheral nests of meiotic stages contained less cells than those of the typical controls. In all the remainder there were relatively fewer sperms. There were definite signs of cavitation in the centre of the major cysts. The nests of meiotic cells were more sharply demarcated and there was extensive development of intercalary tissue. The last remarks apply to 50 per cent of the material. Nine testes (Nos. 24, 17, 25, 7, 3, 9, 18, 2 and 8) contained hardly any trace of recognizable spermatozoa.

The results of the microscopic autopsy as affecting the testes may be summed up by stating that 73 per cent of the controls exhibited large distinct cysts with abundant spermatozoa. Only three of the hypophysectomized group were comparable to them. Of twenty-two testes in the control group twenty-one or 95 per cent contained healthy spermatozoa in comparative abundance. On the other hand in the hypophysectomized group nine out of twenty-six or 35 per cent displayed no signs of healthy spermatozoa. In 50 per cent there were very definite signs of resorption both of spermatozoa and meiotic cells. Pronounced invasion of the space between the major cysts of intercalary tissue was also characteristic of rather more than half the sample.

Microscopic condition of the oviducts

Examination of the oviducts showed that in general the tubular glands of the mucosa in the hypophysectomized females were of smaller girth. The lumen was generally difficult to detect in those of the controls owing to the bulging of the cells. It was generally patent in those of the hypophysectomized animals. The most characteristic distinction was shown by the appearance of the nuclei. In the control group they were relatively inconspicuous and flattened against the outer periphery of the cells, so that they almost seemed to lie between contiguous tubules. In the hypophysectomized group, they lay towards the centre of the cell, stained more deeply and exhibited a spheroidal configuration. The two series showed no noticeable difference in the content of secretory granules.

Food intake

Weekly records of the food intake of the animals during the last 10 weeks of the experiment are tabulated in Table II. These reveal an unexpected fact. For both sexes the food intake of the normal animals was much greater than that of animals from which the pituitary gland had been extirpated.

Table II

Mean weekly food intake in g

Males		Females	
Control	Hypophysectomized	Control	Hypophysectomized
1·32	0 75	3 33	1·60
1·45	1·25	4·26	1·25
1·86	1·66	3·84	1·46
1·62	1 11	5·20	1·25
1·78	1·04	4·63	1·35
1·05	1·08	5·25	1·53
2 23	1 00	4·53	1·96
1·44	1·15	4 28	1·34
2 29	1 46	4·66	1·53
2·41	1·61	4·62	1·81
Mean 1 74 ± 0·04	1·21 ± 0·03	4·46 ± 0·05	1·51 ± 0·02

IV. DISCUSSION

Besides confirming previous work on the female by Hogben *et. al.*, these experiments establish two new conclusions. The first is that the maintenance of reproductive activity in the male as well as the female depends on the secretion of the pituitary gland (anterior lobe). The second is the existence of a close connexion between reproductive activity and the nutritional requirements of the female. Although pituitary removal results in greatly diminished growth, as judged by total body weight, there is no consistent fall off in the fat reserves. At the end of the experiment the mean weight of the corpora adiposa of the hypophysectomized females was actually, though not significantly, greater than that of the females in the control group. This shows that the retrogression of the ovaries is not explained by lower food intake.

The ratio of percentage increase in total body weight for females of the two groups is much in excess of the corresponding ratio for the two groups of males. During the entire period of experiment the normal females increased in body weight from a mean of 37 to 51·12, a difference of 14·12 g. or in round figures 38 per cent. The percentage increase for the experimental group was in round figures 11. The corresponding figures for normal males and males of the experimental group were 15 and 7 per cent. Thus the percentage increase of normal females was about $3\frac{1}{2}$ times and of normal males about twice that of the corresponding group of toads deprived of the pituitary gland. The reduction in growth

rate of females was apparently much greater than the reduction in the growth rate of males.

Leaving out the specific effect of pituitary removal on the gonads themselves, the discrepancy is more apparent than real. The ovaries constitute a large proportion of the bulk of the body. On the other hand the testes contribute only a very small fraction to total body weight. In Table III is shown the increase of body weight in females deprived of the pituitary gland when the contribution of the ovary is eliminated. The ovary weight assigned is calculated on the assumption that the ovary-body-weight ratio in the control group did not change much during the 7-month period.

Table III

	Initial	Final	% increase or decrease
Body weight	37	41·12	+11·1
Ovary	4 14	1·73	−58 2
Body weight without ovary	32 86	39·39	+19·8

To put the issue in another way, we can make the figures cited for females comparable with those for males by adding to the mean weight of the hypophysectomized females what would have been the weight of the ovary, if it had not undergone retrogression. This would make the percentage increase in body weight 20. Leaving out of account the effect of ovarian retrogression, the net percentage increase of both sets of normal animals was roughly twice that of the animals in the corresponding experimental groups.

Comparison of these figures with those for food intake raises three questions. The first is whether the food intake in the experimental groups was a post-operative phenomenon due to a general lowering of vitality. This can be dismissed for several sufficient reasons: (*a*) the records of food intake did not begin till over 4 months after operation, i.e. long after complete healing of the wound; (*b*) the animals had eaten food offered to them from the first week after operation; (*c*) there is no evidence of a trend in the figures to suggest that the food intake would have been greater if the experiment had been continued for a longer period; (*d*) the animals were increasing in body weight. Excluding this possibility two though not necessarily exclusive alternatives remain. One is that the absence of the pituitary results directly in reducing the food demands of the body and that the retrogression of the gonads is an indirect consequence of their established dependence on a dietetic minimum (Alexander & Bellerby, 1935). The other is that the metabolic activity of the gonads themselves creates a greater or less demand for food. That the retrogression of the gonads is not an indirect consequence of reduced food intake is indicated by the fact that pituitary removal did not significantly reduce the relative amount of fat deposited. That the metabolic activity of the gonads themselves is the pacemaker is indicated by the much greater disparity in the two groups of females. This provides unexpected confirmation for the conclusion to which previous experiments by Alexander & Bellerby had led. That is to say, the ovary of *Xenopus* besides being a

gonad is also the principal organ of storage which enables the animal to survive during aestivation in its normal habitat or prolonged fasting under laboratory conditions, when deprived of food. In the laboratory, where a continuous and sufficient food supply is available, females of *X. laevis* have no reproductive "cycle" in so far as this term refers to the condition of the gonads. They can be maintained in a continuous condition of readiness for reproduction if appropriate conditions for mating exist. The presence or absence of ripe ova which is observed when females of *Xenopus* are taken from their natural habitat at different periods of the year can be explained by access to food supply without introducing any other cyclical agency. On the other hand, there has not, as yet, been established any evidence pointing to the external stimulus which determines the exhibition of the mating reflex itself.

These experiments are of particular interest in view of the fact that oviposition in many Amphibia takes place immediately after aestivation or hibernation. We must therefore conclude that the basis of cyclical activity in *Xenopus* is not the same as in other Anura. This is not surprising, because *Xenopus* is in many respects, structurally and physiologically, an anomalous genus. It is probable that in other Anura the pituitary is an important link co-ordinating external stimuli with activity of the gonads. The same humoral mechanism is present in *Xenopus*, and *Xenopus* provides exceptionally favourable material for studying some of its characteristics. On the other hand it does not seem to play an important part in relating seasonal events to the cycle of changes in the gonads.

V. SUMMARY

1. Removal of the whole pituitary gland of *Xenopus laevis* or of the anterior lobe alone is accompanied by

(*a*) reduced food intake,
(*b*) diminished growth of the body as a whole,
(*c*) retrogression of the gonads in both sexes.

2. Since there is no significant reduction in the main fat reserves of the body (corpora adiposa) the retrogression of the gonads is not the direct result of reduced food intake at an all-round lower metabolic level.

3. Since the reduction of food intake is proportionately much greater in females than in males, the metabolic demands of the gonad seems to be the controlling agency of food intake.

4. The relatively greater reduction of growth rate in females is due to the large bulk of the ovaries coupled with the fact that ovarian retrogression accompanies the lowering of growth in the rest of the body.

5. Since the food demand of the active ovary is far in excess of the minimum requisite for body growth, the results of this enquiry provide additional confirmation for the view that seasonal variations of food supply are mainly responsible for seasonal variations in the condition of the ovaries of *Xenopus* in its natural habitat.

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