THE EFFECT OF CAPTIVITY UPON THE REPRODUCTIVE CYCLE OF THE SOUTH AFRICAN CLAWED TOAD (XENOPUS LAEVIS)

BY S. S. ALEXANDER AND C. W. BELLERBY.

(Received 31st January, 1935.)

(With One Plate and Two Text-figures.)

INTRODUCTION.

The South African clawed toad (Xenopus laevis) can be used with advantage to study the nature of the chemical co-ordination existing between the ovary and the anterior lobe of the pituitary (Bellerby, 1933). It can also be employed as a test animal for the rapid diagnosis of pregnancy (Bellerby, 1934b). During the past three years many hundreds of these toads have been kept in this Department for the purposes of this work, and it has been necessary to pay some attention to the conditions under which they can be kept in a healthy condition.

Quite recently Shapiro and Zwarenstein have reported that Xenopus laevis cannot be kept in captivity in South Africa for any length of time without the occurrence of a series of adverse changes to which they have given the name "captivity effect". The nature of these changes can be best seen by citing from one of their papers: "The ovaries of captive females were severely atrophied after six months' captivity and often presented the appearance of gelatinous masses in which individual ova were no longer discernable on naked eye examination... These changes in serum calcium are accompanied throughout the period of captivity by a progressive involution of the ovaries. The ovaries after ten months' captivity are gelatinous masses in which no individual ova can be seen microscopically. Males in captivity show no microscopic gonadal changes nor any significant decrease in the serum calcium level. In striking contrast to the females which have become very emaciated and weak by the ninth or sixteenth month of captivity despite regular feeding and change of water, the males appear healthy and in good condition" (Zwarenstein and Shapiro, 1933).

Ovarian atrophy is not confined to the captive animal. Under natural conditions after ovulation in July or August the ovary declines steadily in weight until about the following January. It is then followed by a period of steady growth until ovulation again occurs. The toads used by Zwarenstein and Shapiro in their experiments were kept in deep slate-lined tanks covered with fine meshed wire netting. No details about the illumination of the room in which they were kept are given, but it appears that they were "exposed to a dim light on a light-absorbing background".
Effect of Captivity upon the South African Clawed Toad

The animals were fed twice a week with raw minced meat and their water was changed thrice weekly. Zwarenstein and Shapiro concluded from their observations that variation in the amount of solar radiation was the main factor influencing the normal periodicity of the ovary under natural conditions, and that the retrogressive change which they found to take place in the ovaries of captive toads was due to inadequate illumination.

During the course of other work carried out on *Xenopus laevis* in this Department, several observations have been made pertaining to the "captivity effect", but in some cases they do not support the contentions of Zwarenstein and Shapiro. Hogben, Charles and Slome (1931) in their earlier work on the changes following hypophysectomy found that no atrophy of the ovaries of their control animals took place even after 5 months' captivity. Zwarenstein and Shapiro, however, explain the difference between these results and their own by the fact that Hogben, Charles and Slome kept their toads in a room in which the electric lights were left on day and night. It will be realised that the existence of a "captivity effect" as described would seriously detract from the practical utility of *Xenopus laevis* as an experimental animal. The present paper deals with the possible factors concerned in its production.

**EXPERIMENTAL.**

The first experiment was carried out on a batch of five *Xenopus* received in exchange from the Zoological Gardens, London, where they had been in captivity for a period of over 7 years. The animals had grown from a small size and when obtained in March 1934 weighed 95.5, 83.0, 98.5, 108.5 and 102.3 gm. respectively. All these five animals ovulated after a single injection of extract prepared from the anterior lobe of the pituitary. The number of ova shed in each case was the largest ever observed in this laboratory and averaged 3000-5000 per toad. Since it has been shown that the production of ovulation by one or two injections of extract is definite proof that the ovary is fully mature (Bellerby, 1933), it is evident that no "captivity effect" had occurred in these animals even after this long period of time.

In direct contrast to this experiment is one which at first appears to confirm the observations of Zwarenstein and Shapiro. In it fifty females which had been in captivity for well over 6 months were kept under standard conditions from December 1933 to April 1934. *Xenopus* with mature ovaries were used exclusively, only those which had ovulated after a single injection of extract being selected. During the period of the experiment the water in which they were kept was maintained at an average temperature of 27°C, and the animals irradiated with a 100-watt lamp at a distance of 3 ft. At the end of the 4 months the toads were injected for a second time with the same equivalent dose of extract, and those which did not ovulate were killed for examination. The results are given in Table I. A series of fifty females which had just arrived from Cape Town and which had been injected with the same dose of extract is included for comparison.

Out of this series of toads only two of the non-ovulating females were found to have the completely atrophic gelatinous ovaries described by Zwarenstein and
Sharpiro (Pl. I, fig. 4). In the majority of the animals killed for examination only a certain degree of atrophy had occurred. In a few cases the ovaries contained ripe ova, but in the great majority the ova were immature and about half the size of the normal ripe egg (Pl. I, figs. 2 and 3). The ovaries on the whole were well developed and contained very many immature eggs, but the degree of atrophy had apparently been sufficient to prevent the toads from ovulating even after two injections.

Table I.

<table>
<thead>
<tr>
<th>Series</th>
<th>No. ovulating</th>
<th>No. failing to ovulate</th>
<th>Results of post-mortem examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After one injection</td>
<td>After two injections</td>
<td>No. with normal ovaries</td>
</tr>
<tr>
<td>Old females</td>
<td>9</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>New females</td>
<td>41</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

These experiments show that the retrogression of the ovaries had resulted from some difference in the conditions under which the toads in Series 1 and 2 had been kept. This could not be attributed to insufficient light or low temperature because in this respect the conditions in the Department had been more favourable than those at the Zoological Gardens. In the Reptile House where the toads had been kept the temperature of the water varied according to the external temperature which was within the limits of that in the Department. The tanks also had never been exposed to direct sunlight, light being admitted through a frosted skylight and from a distant doorway. The water in the tank was changed twice weekly by flushing. No data could be obtained as to its pH, but that in the Department averaged 7.4.

Apart from light and temperature there was apparently no significant difference in the two environments, but there was a marked difference in the number of times a month that the toads were fed. The Zoological Society's toads had been fed regularly twice a week with raw minced meat, whilst those in the Department had been fed "occasionally" according to custom. It must be admitted that in the past the word "occasionally" had been interpreted in its most liberal sense so that it is doubtful whether the toads in Series 2 received more than four meals throughout the experiment. The main difference between the two experiments therefore seemed to lie in the frequency of feeding, and a further series of experiments were designed to test whether diet alone could account for the difference in the condition of the ovaries.

Two preliminary experiments were first carried out on a batch of toads that had already been in captivity for over a year. Most of these were emaciated and post-mortem examination of a sample showed that in the majority of cases the ovaries were much atrophied. The batch was divided into two series consisting of fifty and seventy-five females respectively, which were then fed twice weekly for 6 months on raw minced beef. The toads were allowed to feed for 24 hours and the water was then changed. At every feeding sufficient meat was thrown in the water to ensure that a surplus remained the next day. The series of fifty toads (Series 3) was weighed
in one batch at monthly intervals. A week was allowed to elapse between weighing and previous feeding so that the animals were not weighed with their stomachs distended with food. The growth curve of this series is shown in Text-fig. 1. In the other series of seventy-five toads (Series 4) a random sample of ten toads was killed every month and the ovaries weighed and examined. At the end of the 6 months the remaining toads were injected with a single dose extract for the reason given previously. The data resulting from this experiment are presented in Table II. The period selected for these two experiments was from July to December because it was during this time that the maximum decline in ovary and body weight of the free-living *Xenopus* was reported to occur. During the whole period of feeding the temperature of the water in which the animals were kept did not vary more than 3.1°C from the mean. The daily temperature actually varied from 12.0 to 17.6°C, the average for the 6 months being 14.5°C. The toads were kept in open tanks in an

![Graph](image)

Text-fig. 1. Growth curve of Series 3. Fed twice a week.

<table>
<thead>
<tr>
<th>No. of months fed</th>
<th>Month when killed</th>
<th>Average weight of ovary in gm.</th>
<th>Average body weight in gm.</th>
<th>Ovary-body weight %</th>
<th>No. of toads with atrophic ovaries</th>
<th>No. of toads with normal ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>July</td>
<td>0.71</td>
<td>20.2</td>
<td>34</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>September</td>
<td>2.21</td>
<td>28.3</td>
<td>7.4</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>October</td>
<td>1.67</td>
<td>24.7</td>
<td>6.8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>November</td>
<td>2.69</td>
<td>30.8</td>
<td>8.8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>January</td>
<td>2.39</td>
<td>32.0</td>
<td>7.4</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>
underground room placed about 5 ft. away from two windows which faced a brick wall. Consequently they never received any direct daylight. Speaking in general terms the room could be described as cold and dark, and some idea of its lighting can be gathered from the fact that it is usually necessary to put on the electric lights even in the summer in order to carry out detailed work.

The main facts which emerge from these two series of experiments can be summarised as follows. Series 3 shows that no further loss in body weight occurred during the period of the experiment, but instead the toads grew steadily and after 6 months increased in weight by 71.3 per cent. From Series 4 it can be seen that no atrophy of the ovaries was apparent even after a year and a half's captivity, because there had been a rapid regeneration of ovarian tissue within 2 months of regular feeding being commenced. The figures show that within this time the ovary-body weight percentage had increased from 3.4 to 7.4 per cent. The latter figure is also greater than that (5.0 per cent.) given by Zwarenstein and Shapiro for a series of free-living pond animals killed by them during December in Cape Town. At the end of the experiment no females were found to possess the completely atrophic gelatinous ovaries described by these authors. The injection experiment showed that the ovaries were mature (Pl. I, fig. 1) in all the remaining twenty-five toads which were not killed for autopsy, twenty-three of them ovulating after a single dose of extract.

Since these experiments suggested that the condition and sensitivity of the ovaries resulted directly from the amount of food available a further series of four experiments was carried out on a batch of toads freshly arrived from Cape Town. No information can be given about the length of time they had been in captivity before shipment, but as the journey takes 3 weeks it can be safely assumed that they had been kept in tanks for a period longer than this. Twenty toads were used in each series which were fed as follows: Series 5 twice a week; Series 6 once a week; Series 7 once a fortnight; Series 8 once a month. Every series was weighed in one batch every month taking the same precautions as before. The growth curves of the four series are shown in Text-fig. 2. The period of feeding was from July to December as previously. All the four series were injected with the same equivalent dose of extract at the end of this time. The data obtained is summarised in Table III. The main conclusions of these series confirm those of the previous experiments, but several fresh facts emerge. Firstly Series 7 shows that some regressive change in ovaries took place leading to a decreased sensitivity in response to injection of extract although the average body weight had increased by 53.6 per cent. It shows that atrophy of the ovary can occur in toads which received sufficient food to enable them to grow at a normal rate. Since this effect was most pronounced in the series fed once a month and absent in the three series fed one or more times a week it seems that the ovarian change can be attributed to insufficient feeding. Secondly, the results of Series 6 show that one feeding a week is sufficient to maintain both ovary and body in the optimum condition. The absence of any significant difference in the growth curves of Series 5 and 6 is probably due to the fact that the food intake was the same in both. The slow process of digestion in Xenopus does not permit it to fill
its stomach more often than once a week and observation showed that the actual amount of food eaten by both series was the same although the number of feedings per week was different. Thirdly, Series 3, 5 and 6 show that it is not necessary to keep the toads at a high temperature or in a strong light in order to maintain the

Text-fig. 2. Growth curves of Series 5, 6, 7 and 8. ● fed twice a week. ▲ fed once a week. ▲ fed once a fortnight. ■ fed once a month.

Table III.

<table>
<thead>
<tr>
<th>No. of series</th>
<th>No. of feedings per month</th>
<th>% increase in weight</th>
<th>No. of toads ovulating</th>
<th>Results of post-mortem examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After one injection</td>
<td>After two injections</td>
<td>No. with normal ovaries</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>71.3</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>56.9</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>58.3</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>53.6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>25.5</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
ovaries in a mature state. Once again in this group of experiments none of the ovaries observed had atrophied to the advanced stage reported by Zwarenstein and Shapiro. The condition of the ovaries of those animals which had undergone some atrophy was similar to those previously described (Pl. 1, figs. 2 and 3).

CONCLUSIONS.

It is difficult to give the exact reason for the difference between these experiments and those of Zwarenstein and Shapiro because the latter authors lay particular emphasis on the light factor. No precise comparison can therefore be made, since in both investigations no data are available concerning the intensity and nature of the light received by the toads. If we exclude, for the time being, the question of light and temperature difference the present results suggest strongly that the “captivity effect” observed in Cape Town was due to insufficient feeding. Zwarenstein and Shapiro state that their toads were fed twice a week, but they do not say how much food was given. Their animals may have been fed at regular intervals, but this does not necessarily mean that they received an adequate supply of food. The absence of a “captivity effect” in the male *Xenopus* could also be explained on the same grounds. The fully grown male is much smaller than the female and reaches maturity far more rapidly. The female often grows to three or four times the size of the average male and takes much longer to reach the full-grown state. It will be realised that a given amount of food which would be sufficient to maintain a certain number of males in a healthy state would be insufficient for the developmental needs of the same number of females. The reason why the influence of food supply escaped observation in previous experiments on the “captivity effect” is easy to see. The large ratio of ovary to body weight signifies that constant body weight or even a steady increase is not an adequate criterion of the sufficiency of a diet to ensure that the ovary will respond to the stimulus inducing ovulation. Furthermore, the extraordinary power of the female clawed toad to abstain from food over long periods receives a possible explanation. Since the ovary undergoes resorption when the supply of food is cut off, the ovary may be regarded as a capacious storage organ for lipins, proteins, etc. The investigation also permits us to advance a theory to account for the cyclical phases of growth and retrogression in the ovary of *Xenopus laevis* on the basis of periodic fluctuations in the food supply.

The female *Xenopus* ovulates spontaneously in July or August when the ponds are full. After ovulation the ovaries gradually decline in weight until the following February. It is during this period that the ponds dry up slowly, so that presumably a decrease in the food supply of the toad occurs. In about March or April the rainy season begins and the ponds fill up again. An increasing food supply then becomes available and the ovaries gradually gain in weight until ovulation occurs once again in July. It is not suggested that ovulation itself is induced solely as a result of increase in the food supply. Other work has shown that more than one factor is involved in this particular process (Hogben, Charles and Slome, 1931; Bellerby, 1933). The ecological and experimental data available at present simply demon-
strate that periodic phases of growth in the ovary coincide largely with the amount of food available for the toad in its natural habitat and that the requisite sensitivity of response to the ovulation producing stimulus can only be attained by adequate nutrition. This theory is supported by the present experiments because they show that the developmental condition of the ovary is directly dependent upon the amount of food available. If an abundant supply of food is at hand the ovary remains in a mature and sensitive condition all the year round and no periodic phases of growth and retrogression occur. The female *Xenopus* therefore resembles the rabbit. In this mammal no alternating periods of oestrus and anoestrus occur if it is kept under optimum conditions of nutrition and environment. Ripe follicles are always present in the ovaries throughout the year and the doe remains on oestrus indefinitely, in this way it contrasting with the wild female which has a definite breeding season.

The results of the above experiments agree closely with those obtained from investigation of the effects of a diminished food supply—as distinct from Vitamin deficiency—upon ovarian activity in the mammal. In the guinea-pig, for instance, a decrease in the amount of available food produces changes in the ovaries, which, though not visible, lead to delayed ovulation and consequent increase in the length of the oestrous cycle. It appears that a high level of nutrition is essential for the efficient working of the ovary-pituitary mechanism, and that increased activity of the anterior lobe of the pituitary alone cannot compensate for any deficiency in ovarian function resulting from inadequate nutrition.

**SUMMARY.**

1. The South African clawed toad (*Xenopus laevis*) can be maintained in captivity in a healthy condition provided that it is adequately fed.
2. No atrophic changes are found to occur in the ovary as reported by Zwarenstein and Shapiro for animals kept in captivity in Cape Town.
3. The developmental condition of the ovary depends directly upon the amount of food available.
4. The normal periods of ovarian growth and retrogression which occur in the toad under natural conditions can be explained on the basis of fluctuation in food supply.

The cost of these experiments was defrayed from a grant made to the Department by the Medical Research Council. One of us (C. W. B.) is in receipt of a full-time grant from the latter.

**REFERENCES.**

EXPLANATION OF PLATE.

(Portions of the ovaries were fixed in Bouin's fluid. Sections were cut at 10\mu and stained with haematoxylin and eosin.)

Fig. 1. Mature ovary, showing fully developed ova. (M.O.)

Figs. 2 and 3. Semiatrophic ovaries. Larger ova are degenerating. The cytoplasm is becoming vacuolated. (V.C.)

Fig. 4. Atrophic ovary. All ova are degenerating, and there is well-marked vacuolisation of the cytoplasm in the larger ova (V.C.). A gelatinous exudate is present in the interoval spaces (G.E.). (All magnified about 45 diameters.)
ALEXANDER AND BELLERBY—THE EFFECT OF CAPTIVITY UPON THE REPRODUCTIVE CYCLE OF THE SOUTH AFRICAN CLAWED TOAD (XENOPUS LAEVIS) (pp. 306—314).