HISTOLOGICAL CHANGES IN THE OVARIES AND OVARIAN BLOOD VESSELS OF *XENOPUS LAEVIS* ASSOCIATED WITH HYPOPHYSECTOMY, CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE

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(With One Plate and One Text-figure.)

INTRODUCTION.

Hogben (1930) and Hogben, Charles and Slome (1931) have shown that hypophysectomy results in involution of the ovaries in *Xenopus*. In the course of a different series of experiments in this laboratory (Zwarenstein and Shapiro, 1933) it was observed that "animals which had been kept in the laboratory for several months showed a progressive involution of the ovaries according to the length of captivity," and that the involution was not as marked as that observed after hypophysectomy in Hogben's experiments or in the experiments of Shapiro and Zwarenstein (1933).

In order to unmask this captivity effect on the ovaries, it was necessary to compare captive toads with fresh animals taken from the ponds (called "vleis" in South Africa) so as to take account of possible variations under natural conditions.

In this paper it is proposed to investigate also, for the first time, the detailed histological changes in the ovaries of *Xenopus laevis*, following hypophysectomy and captivity; and the significance, if any, of the time factor involved.

EXPERIMENTAL.

All the toads used throughout the period of these experiments were obtained from the same pond in the Cape Peninsula. Some 500 animals were collected in January, 1932, and transferred to a large open-air tank, to investigate the effect of captivity. Another batch of toads was hypophysectomised by the method of Hogben, one half of the batch having their anterior lobes, and the other half of the batch having both lobes removed. These animals were also placed in open-air tanks. The water in the tanks was changed three times a week, and the animals were fed on meat twice weekly. On each occasion when vlei material was collected and killed for examination, corresponding samples of captive and hypophysectomised material were investigated in the same way. The animals were pithed and then immediately weighed, after which the ovaries were removed and also weighed.

A useful numerical index of the condition of the ovaries is obtained from the ratio of ovary weight/body weight.

Typical samples of the ovaries were prepared for histological examination.

RESULTS.

The gonad ratio.

The figures in Table I represent mean values of the ovary weight/body weight ratios of toads killed in batches of ten at intervals throughout the year. Figures recording the body weights of the corresponding batches of animals are also included.

In the pond. An analysis of Table I will show, in the first instance, the important fact that there are seasonal variations in the condition of the ovaries of *Xenopus laevis*. These changes have not been described before, nor has the breeding season

Table I. *The figures represent the body weights of the animals as well as the mass of the ovaries relative to their body weight*, i.e. *the gonad ratio.*

	(1) Vlei toads		(2) Captive toads	
	Body weight	Gonad ratio	Body weight	Gonad ratio
Jan.	46 ± 1·5	0·075 ± 0·006	46 ± 1·5	0·075 ± 0·006
Feb.	43 ± 3·0	0·064 ± 0·014	59 ± 1·0	0·070 ± 0·005
March	—	—	56 ± 5·8	0·062 ± 0·007
July	37 ± 6·3	0·113 ± 0·024	30 ± 6·6	0·043 ± 0·008
Sept.	39 ± 6·1	0·105 ± 0·025	32 ± 5·1	0·035 ± 0·005
Dec.	45 ± 8·1	0·052 ± 0·020	19 ± 2·4	0·030 ± 0·007

	(3) Anterior lobe hypophysectomy		(4) Total hypophysectomy	
	Body weight	Gonad ratio	Body weight	Gonad ratio
Jan.	46 ± 1·5	0·075 ± 0·006	46 ± 1·5	0·075 ± 0·006
Feb.	Animals hypophysectomised			
March	—	0·058 ± 0·012	—	0·055 ± 0·010
July	41 ± 5·3	0·013 ± 0·006	42 ± 6·1	0·011 ± 0·006
Sept.	37 ± 7·0	0·008 ± 0·004	51 ± 9·5	0·010 ± 0·004
Dec.	—	—	—	—

of *Xenopus laevis* been clearly established. It must therefore be reported that copulating couples were observed in the material collected from the vlei in July and September, thus indicating the probable breeding season, which would correspond to mid-winter and early spring in South Africa and to the rainy season in the Cape Province. In conformity with this tadpoles undergoing metamorphosis, *i.e.* 3–4 months old, were observed in the pond material collected in December. Further, the ratio of the mass of the ovaries to their body weight, *i.e.* their relative mass, is highest in the late winter and early spring months. This coincides with and is substantiated by the fact that during these months the ovaries are full of large mature eggs ready for extrusion, whereas in the summer months the ovaries are smaller and lighter.

The effect of captivity. The ratios of the captive material show a steady diminution with increasing duration of captivity. This is correlated with a steady increase in the degree and number of involuted ovaries in the samples examined. This phenomenon probably explains the fact that *Xenopus* cannot be induced to breed in captivity. It must be recorded that occasional toads, about one out of every ten, did not show the ovarian regression so characteristic of captivity animals after 5 or 6 months. In spite of regular feeding and change of water, a marked degree of emaciation was observed in the females in the later months of captivity, whereas all the male toads appeared to be quite well and in good condition.

Anterior lobe hypophysectomy. Removal of the anterior lobe of the pituitary results in an ovarian regression qualitatively similar to that of captivity only much more rapid. The ovaries regress from a healthy state to a gelatinous mass in which individual ova cannot be distinguished macroscopically. This is clearly demonstrated by comparing the gonad ratios of captive animals in column 2 with gonad ratios of anterior lobe hypophysectomised animals in column 3 in Table I.

Total hypophysectomy. The gonad ratios for the first 6 months following total hypophysectomy are slightly, but not significantly, lower than the gonad ratios for anterior lobe removal over the same length of time. By the seventh month after total hypophysectomy, however, the gonad ratios for totally hypophysectomised animals were slightly but not significantly higher than the ratios for animals which had had only their anterior lobes removed.

Hogben and co-workers were able to show significant differences between the ratios of animals which had been deprived of both lobes of the pituitary as compared with animals which had had only their anterior lobes removed. The present authors were able to report a tendency in this direction, but the differences cannot, in the experiments above, be called significant differences.

All the above results are summarised in Text-fig. 1.

Histology.

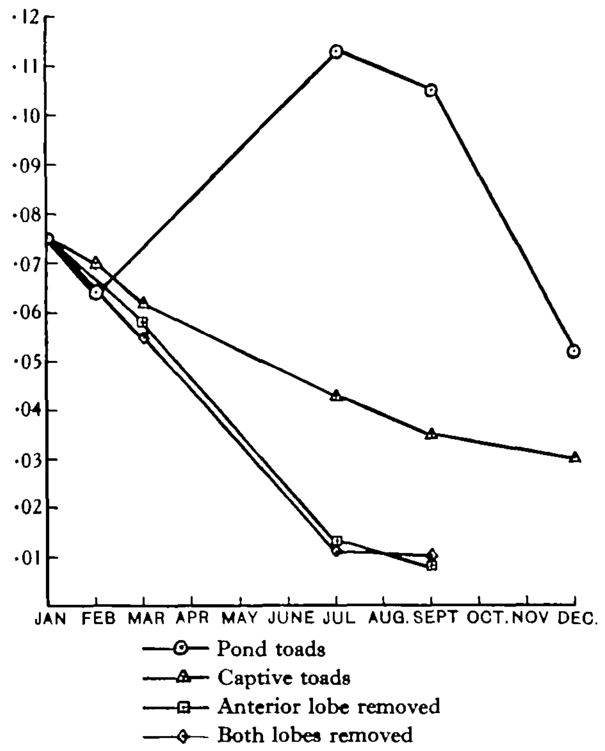
Numerous sections of the ovaries of different animals were prepared and stained. Haemotoxylin and eosin, Mallory's stain, and van Gieson's stain were employed in all the preparations made.

The normal ovary. For a typical example of the normal ovary of *Xenopus laevis*, see Fig. 1, which is a photomicrograph of a normal ovary stained with Mallory's stain. The ovary was removed from a copulating female.

In *Xenopus* during the breeding season the ovaries are large and fill to distension the abdominal cavity. The mass of the ova, which for the most part comprises the ovary, is made up of mature as well as immature eggs, black at one pole and yellow at the opposite pole. The ova are easily visible to the naked eye, while the ovary itself has a mesenteric attachment along the length of the kidney, and is invested with a very thin connective tissue capsule sending in septa which constitute a framework supporting the ova. The connective tissue capsule of the ovary contains small blood vessels with thin muscular walls.

The ova themselves may be divided into two groups: (1) large central, and (2) small peripheral ova.

The large central eggs are characterised by pale, clear staining nuclei. The cytoplasm consists of ovoid discs, the largest of which are arranged around the nucleus, becoming smaller towards the periphery. A well-marked layer of black



Text-fig. 1. Graph to show the relative mass of the ovaries of the South African clawed toad in the pond, in captivity and after hypophysectomy.

pigment, several granules deep, can be seen lying most peripherally in those eggs which have been cut through the pigmented pole. This granular layer is absent in the yellow hemisphere of the ovum. There appears to be a delicate connective tissue reticulum supporting the ovoid discs of the cytoplasm.

The peripheral ova are small and lie in direct relation to the thin connective tissue capsule which invests the ovary and sends in thin septa. Their nuclei contain a large number of dark red staining granules. The cytoplasm of such ova is hyaline and non-granular in appearance, occasionally staining a deep blue colour with haemotoxylin and eosin. These peripheral ova correspond to the small immature ova which can be seen by the naked eye disposed about the periphery of the ovary.

The ovary in captivity. In captivity the ovaries present a totally different appearance. Fig. 2 is a photomicrograph of the ovary of a toad in captivity for $5\frac{1}{2}$ months, and represents the maximal degree of retrogression which occurs in captivity. To the naked eye the ovary appears as a gelatinous mass in which individual ova cannot be detected. There is also an increase in the number of peripheral ova as compared with the normal ovary.

The blood vessels which lie in the connective tissue capsule which invests the ovary have, in some places, slightly thicker muscular walls than in those of the normal ovarian capsule. The latter itself appears also to have undergone a slight thickening or fibrosis in the captive animals. It is important to note that in animals examined after $4\frac{1}{2}$ months in captivity, the central ova could still be made out, but were smaller than those of the pond animals (Fig. 2 *a*).

The ovary after total hypophysectomy. After removal of both lobes of the pituitary gland the typical result is that seen in Fig. 3, which is a photomicrograph of the ovary of a toad hypophysectomised $4\frac{1}{2}$ months previously. It is to be noted that whereas in $4\frac{1}{2}$ months' captivity animals' central ova could still be made out, these have entirely disappeared from the ovaries of animals hypophysectomised for the same length of time.

In the peripheral ova the same hyaline structure occurs as in captivity and normal ovaries described above. In the case of ovaries from pituitary-less toads, however, there is an increased number of peripheral ova as compared with captive or vlei normal ovaries.

A striking feature is the very marked thickening and fibrosis of the connective tissue capsule of the ovary.

In the blood vessels there is a marked proliferation of the intimal connective tissue, as well as a very definite hypertrophy of the muscular media, to such an extent that the lumen of the vessels is almost completely obliterated. This type of change is similar to that observed in cases of arteriosclerosis in man. The marked degree of arteriosclerosis of the ovarian vessels does not occur at all in normal ovaries and only to a very slight extent, if at all, in captive animals after $5\frac{1}{2}$ months.

In all cases the adventitia of the blood vessels is hardly affected. The veins show a marked connective tissue proliferation. Occasionally several small arterioles are included together in an area of arteriosclerotic change, but the lesion may sometimes affect individual blood vessels only (see Fig. 3 *a*).

The above description refers to animals killed $4\frac{1}{2}$ months after total hypophysectomy. In succeeding months, similar but more advanced arteriosclerotic changes supervene, *e.g.* complete obliteration of the vessel lumen 7 months after hypophysectomy (see Fig. 4).

Ovary after anterior lobe hypophysectomy. In all respects the histological changes in the ovary after removal of the anterior lobe alone are the same as those described for total hypophysectomy in the paragraph above.

In view of the pathological significance and the physiological importance of this possible relationship between the hypophysis and the vascular system as a whole, further investigations are being undertaken with respect to such organs as the kidneys, spleen, pancreas, heart, aorta, etc.

DISCUSSION.

It is clear from the graph printed above that there is a definite seasonal change in the ovaries, which are largest and filled to distension with ova during the breeding season, *i.e.* late winter and early spring. The ovaries are lightest and smallest in the summer months.

Shapiro and Zwarenstein (1933) have shown that castration in *Xenopus* leads to a persistent fall in the serum calcium, and in this connection it is interesting to observe that the serum calcium of vlei, *i.e.* pond, material is low when the relative mass of the ovary is low, and rises when the ratio rises, except in the post-breeding season in December, when the serum calcium is high but the ovary ratio is low. A complete correlation between the condition of the ovaries and the level of the calcium in the serum cannot therefore be described. A detailed discussion of the factors involved in seasonal ovarian changes and in ovarian retrogression in captivity will be found elsewhere in this *Journal* (Zwarenstein and Shapiro, 1933).

Hypophysectomy and captivity, after a sufficient length of time, result in a disappearance of the central mature ova, while the peripheral ova remain unaffected. That the disappearance of the mature ova is related to pituitary function is clearly established by an analysis of the time relations of this phenomenon in hypophysectomised, as compared with captive, animals.

Sections of ovaries taken from toads kept captive for 4½ months show central ova which are beginning to undergo regression. Ovaries from animals of the same batch, but hypophysectomised 4½ months previously, show only peripheral ova, and no central ova at all. Consequently it would appear that the more rapid regression of the ova in the hypophysectomised animals is due to the removal of the pituitary gland. Thus, as there is the same qualitative change in the appearance of the ovaries of the two cases as compared with pond normals, the true difference being in the degree or intensity of the change, it may be suggested that the degeneration of the central mature ova is dependent on the absence of pituitary function in hypophysectomised and diminution of pituitary function in captive animals. It is possible, however, that the captivity effect may be independent of the pituitary, and depend on some such factor as nutrition.

Since, in addition to the disappearance of the central mature ova, there is at the same time an increase in the number of immature peripheral ova, the tentative suggestion may be made that the pituitary is concerned in the process of maturation of the ova of the South African clawed toad. Such an hypothesis will explain also the fact that all ovaries, whether of captive, hypophysectomised or normal copulating females, contain peripheral ova which are morphologically identical. The regressive changes described in captive and hypophysectomised animals occur only in the fully matured central ova.

There is a marked disparity in the ratios, *i.e.* the mass of the ovaries relative to their body weight, of vlei animals at the height of the breeding season as compared with the captive animals examined at the same season of the year.

It may be remarked at this stage that, in the experiments of Hogben and co-workers, the ovaries did not regress in captivity. This is probably due to the much greater light ration supplied to their animals. In their experiments, referred to in fuller detail in another contribution in this *Journal* (Zwarenstein and Shapiro), the captive animals were kept in a warm room with the electric light switched on day and night, whereas in the experiments described in this communication the toads were kept in subdued light during the day and in total darkness during the night. That light does affect the mass of the gonads in vertebrates was proved by Bissonnette (1932) who showed that red rays have a stimulating effect on the growth of the testes.

The decrease in the ovary-body weight ratio with captivity appears to be due to the disappearance of the large central ova. With removal of the pituitary there is a still more marked decline in the relative mass of the ovary. (See graph.) This may be correlated with the fact that, in addition to the degeneration of the central ova, severe arteriosclerotic changes supervene in the vascular system of the ovary of the hypophysectomised animal. These changes occur hardly, if at all, in the corresponding captive material. The arteriosclerotic changes would result in a diminished blood supply to the part, thus leading to a general atrophy of the organ concerned. In some cases the sclerotic changes were so severe as to result in a complete obliteration of the vessel lumen 7 months after the operation. The superposed vascular lesion would thus explain the more rapid, as well as the quantitively more severe decline, in the relative mass of the ovary of the hypophysectomised as compared with captivity animals.

Finally, since the histological effects of total or partial removal of the hypophysis are identical, it appears likely that the absence of the anterior lobe alone is the primary factor concerned in initiating the histological changes described.

SUMMARY.

1. Seasonal changes in the ovaries of *Xenopus laevis* (the South African clawed toad) are described.

2. Degenerative changes in the large mature central ova occur more rapidly as a result of hypophysectomy than as a result of captivity.

3. The suggestion is made that the pituitary may be concerned with the maturation of ova in *Xenopus laevis*.

4. Hypophysectomy results in severe arteriosclerotic changes in the ovarian blood supply as early as 4½ months after the operation. Control animals show no such changes.

5. The histological changes observed in the ovaries after hypophysectomy, whether total or partial, are probably due to the removal of the anterior lobe alone.

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EXPLANATION OF PLATE I.

Fig. 1. Normal ovary. *A*, connective tissue capsule; *B*, peripheral ova; *C*, central ovum. (×50.)

Fig. 2. 5½ months' captivity. *A*, connective tissue capsule; *B*, peripheral ova. (×50.)

Fig. 2 *a*. 4½ months' captivity. *A*, connective tissue capsule; *B*, peripheral ova; *C*, small central ova. (×50.)

Fig. 3. 4½ months after hypophysectomy. *A*, thickened capsule; *B*, peripheral ova; *D*, arterio-sclerotic vessel; *E*, accompanying vein. (×50.)

Fig. 3 *a*. 4½ months after hypophysectomy. *D*, arteriosclerotic vessels; *E*, accompanying vein. (×50.)

Fig. 4. 7 months after pituitary removal. *A*, thickened capsule; *B*, peripheral ova; *D*, arterio-sclerotic vessels. (×50.)

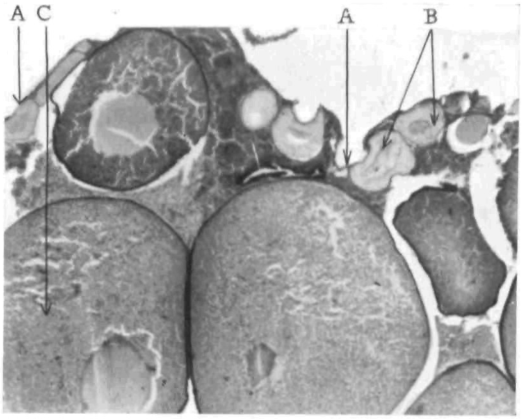

Fig. 1.

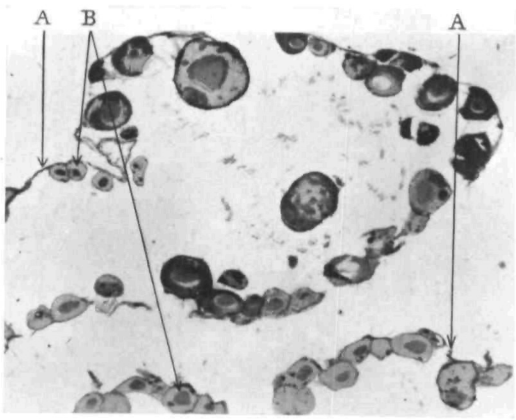

Fig. 2.

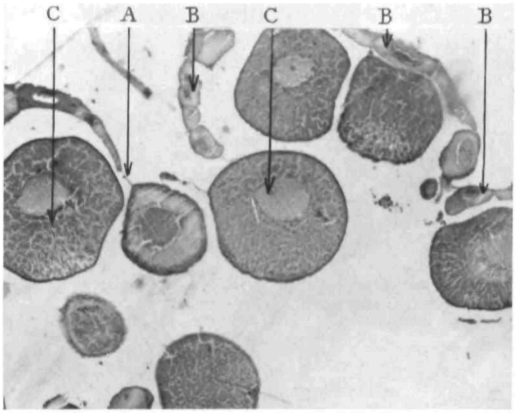

Fig. 2 *a*.

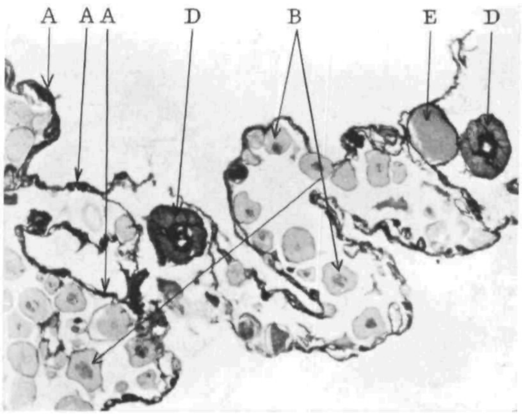

Fig. 3.

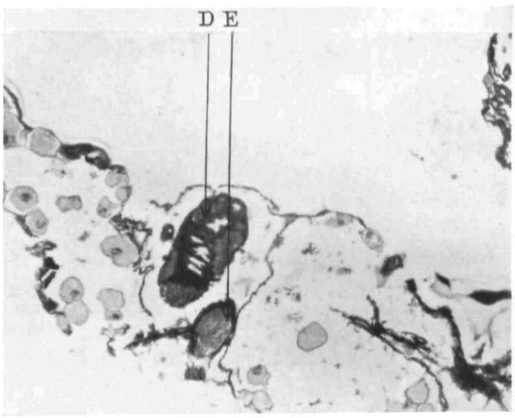

Fig. 3 *a*.

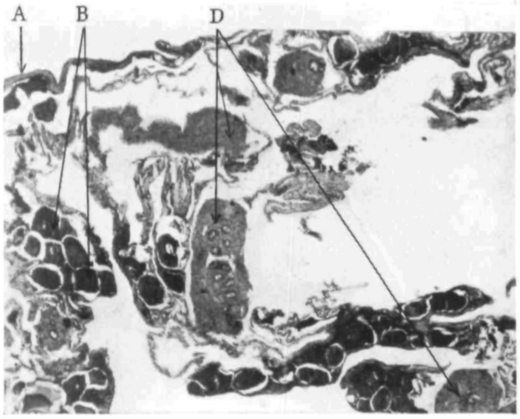

Fig. 4.

SHAPIRO AND SHAPIRO—HISTOLOGICAL CHANGES IN THE OVARIES AND OVARIAN BLOOD VESSELS OF *XENOPUS LAEVIS* ASSOCIATED WITH HYPOPHYSECTOMY, CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE (pp. 73—80).