METABOLIC CHANGES ASSOCIATED WITH ENDOCRINE ACTIVITY AND THE REPRODUCTIVE CYCLE IN *XENOPUS LAEVIS*

I. THE EFFECTS OF GONADECTOMY AND HYPOPHYSECTOMY ON THE CALCIUM CONTENT OF THE SERUM

By H. A. SHAPIRO and H. ZWARENSTEIN.

(Department of Physiology, University of Cape Town and Department of Social Biology, University of London.)

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(With One Text-figure.)

I. INTRODUCTION.

A definite relationship between the pituitary and the calcium content of the serum was first established by Charles (1931), who showed that removal of either the anterior lobe alone or of both lobes in *Xenopus* caused a persistent fall in serum calcium. The difference between total removal and anterior lobe removal was not regarded as being significant and it was concluded that the anterior lobe is the main if not the only part of the pituitary which determines changes in the serum calcium.

In a later communication Hogben, Charles and Slome (1931) on the basis of further experiments came to the conclusion that "the relation of the pituitary to calcium metabolism is complex and that both lobes exercise some influence, direct or indirect, upon calcium metabolism, since the fall in calcium content following on removal of the anterior lobe alone is greater than the fall consequent upon removal of both lobes."

Hogben has further reported that removal of the pituitary results in involution or retrogression of the ovaries. Consequently it became necessary to determine whether the effect of hypophysectomy on serum calcium is a direct one or a secondary effect through the ovaries, *i.e.* whether this effect resulted only in so far as it was correlated with ovarian retrogression.

In the investigations reported in this paper the work of Charles was repeated and extended and the effect of gonadectomy on serum calcium was determined as a first contribution to the solution of the problem.

Work reported in the literature on the influence of castration on serum calcium is scanty and contradictory. The literature on the relation between the ovaries and serum calcium is summarised by Suzuki (1931). Suzuki found that ovariectomy in rabbits caused a fall in calcium at the end of the second week after operation, after which there was a slight rise. Transplantation of ovary immediately after castration unaccountably led to a considerable decrease. Hogben and Charles (1932) found that removal of the ovaries in rabbits does not produce a significant effect on blood

calcium. Frei and Emmerson (1930) reported that castration in cattle lowers calcium in males but raises it in females. Leites (1924) obtained a slow gradual rise in male rabbits after castration and a similar though less marked increase in females. Only one animal was used as a control. All work on the calcium content of the serum in rabbits must be reconsidered in the light of recent work, which has shown that diet and caging markedly influence serum calcium in these animals.

Gerschman (1931) found that hypophysectomy in dogs has no effect on plasma calcium even 117 days after extirpation.

II. THE EFFECTS OF GONADECTOMY.

The operative procedure for the removal of ovaries or testes was as follows. The animal is lightly anaesthetised in a solution of ether in water. A small skin incision is made in the mid-abdominal region and the muscular wall is cut through half an inch lateral to this. A small retractor is inserted to keep the abdominal muscles apart, thus giving easy access to the gonads, which have a mesenteric attachment along the ventral surface of the kidneys. By means of an electric cautery the mesentery is cut through as near its renal attachment as possible, care being taken not to remove the fat bodies which lie near the upper poles of the kidneys. Muscular and cutaneous sutures complete the operation. About 75 per cent. of the females and about 95 per cent. of the males survived the operation. Deaths occurred usually on the second or third day after the operation.

Micro-determination of serum calcium.

A few of the earlier determinations were carried out by the method of Kramer and Tisdall as modified by Clark and Collip (1925). This necessitated bleeding about ten animals to obtain sufficient serum for one estimation. The above method was therefore modified and the following procedure, slightly adapted from Groak (1929), was finally adopted:

1·5–2 c.c. blood are collected in a centrifuge tube, the clot plus serum centrifuged the next day and 0·4 c.c. serum transferred to a 15 c.c. centrifuge tube. 0·4 c.c. distilled water is added using the same pipette. 0·35 c.c. saturated ammonium oxalate and two drops of 2 per cent. ammonia are added, the tube gently shaken and allowed to stand for 2 hours. It is then centrifuged for 5 minutes, the supernatant fluid poured off by inverting the tube and the tube wiped dry with a piece of folded filter-paper. 0·5 c.c. of 2 per cent. ammonia is added, the tube again centrifuged for 5 minutes and the supernatant liquid poured off. 2 c.c. of N H_2SO_4 are added and the tube placed in a boiling water bath for 3–5 minutes. $N/250$ potassium permanganate is used for the titration.

0·5–1 c.c. serum was usually obtained from one animal, so that by the above method individual results could be obtained and often duplicate determinations carried out. With smaller toads it was necessary to bleed two or three animals. Estimations on the same serum by the original method and by the modified micro-method did not differ by more than 0·4 mg. per cent.

Some months before the castration experiments were begun it was noticed that freshly collected material from ponds in the neighbourhood of Cape Town did not always possess normal well-developed ovaries. As the work proceeded and fresh batches of toads were brought into the laboratory at monthly intervals, it was found that ovarian retrogression varied with the season and that normal ovaries were present only at the beginning and during the breeding season. Further, animals which had been kept in the laboratory for several months showed a progressive involution of the ovaries according to the length of captivity. Serum calcium estimations of fresh pond and captivity animals showed that there was a close correlation between the state of involution of the ovaries and the serum calcium content. Results, up-to-date, indicate that ovaries and calcium in pond animals undergo a seasonal variation, and that in captivity a progressive retrogression of the ovaries occurs which is correlated with a gradual decrease in serum calcium. The serum calcium of males (pond and captivity) remained remarkably constant. A detailed account of these observations will be published later.

Table I. *Effect of castration in males and females.*

6 months' captivity } 30. vi. 31 to 8. i. 32.
6 months' castration }

Captivity		Castrated	
♀♀	♂♂	♀♀	♂♂
9·5	8·7	7·5	7·0
9·0	8·0	7·5	7·0
8·5	8·3	7·5	—
9·5	—	—	—
9·1	8·3	7·5	7·0

Table II. *Effect of castration in females.*

4½ months' captivity, 20. i. 32 to 2. vi. 32.
2½ months' castration, 20. iii. 32 to 2. vi. 32.

Captivity		Castration
20. iii. 32	2. vi. 32	2. vi. 32
9·8	10·6	7·8
12·2	9·8	8·2*
11·6	11·6	7·2
11·9	11·0	7·8
9·2	9·6	—
10·6	10·0	—
11·8	10·0	—
—	9·2	—
11·0	10·1	7·7

* Small portion of ovary found on post-mortem.

The serum calcium of one female after 3½ months' castration (5½ months' captivity) was 7·6 mg. per cent.; that of animals after 5½ months' captivity was 9·0 mg. per cent.

Table III. *Effect of castration in males.*

5½ months' captivity, 20. i. 32 to 6. vii. 32.
3½ months' castration, 20. iii. 32 to 6. vii. 32.

Captivity		Castration
20. iii. 32	6. vii. 32	6. vii. 32
8·8	7·7	7·6
9·0	8·2	8·4
7·6	8·4	8·0
7·8	8·0	7·8*
7·6	8·3	7·8*
9·2	8·2	7·6
9·4	7·6	—
8·2	8·0	—
8·4	8·0	7·9

* Small portion of testis found on post-mortem.

One batch of animals was castrated a few days after being brought into the laboratory (Table I) and two batches after two months' captivity (Tables II and III). On account of the correlation between length of captivity and decrease in serum calcium, determinations were made on captivity material (1) at time of castration except in Table I, and (2) at the same time as calcium of castrates was estimated (Tables I, II and III). In this way the captivity effect was controlled. Since the captivity effect sets in only after three months the figures under (1) represent the normal calcium level, *i.e.* equal to pond level, for that time of the year. Figures under (2) show the typical captivity effect on serum calcium.

In the above and following tables all the figures refer to mg. Ca per cent. In Table I calcium was estimated by Clark and Collip's method; in all other tables by the micro-method described above.

The following conclusions can be drawn from the data presented above:

(1) Castration caused a persistent fall (17–24 per cent.) in the calcium content of the serum in females.

(2) In males there was no effect after 3½ months' castration but a fall (16 per cent.) after 6 months' castration.

(3) The effect appeared earlier in females than in males. This may be correlated with the captivity effect, which takes much longer to appear in males than in females.

III. THE EFFECTS OF HYPOPHYSECTOMY.

The pars tuberalis in *Xenopus* consists of a single triangular shaped median lip attached to the superior margin of the pars anterior and inserted by a tapering process into the cleft extremity of the tuber cinereum (Hogben and Slome, 1931). It is ordinarily removed more or less completely when the anterior lobe is excised. As its tip adheres to the tuber cinereum the whole of the pars tuberalis does not always come away with the pars anterior and may regenerate. To avoid confusion, it must be emphasised that throughout this discussion the term anterior lobe is used to include both the pars anterior and the pars tuberalis.

Of the animals in which the anterior lobe had been removed, only those were kept which showed maximal expansion of melanophores on a white background. After several months the white background response reappeared in about half of these but to a varying degree, some showing a melanophore index of 3–4 and some an index of 2. Hogben and Slome (1931) came to the conclusion that the white background response is directly or indirectly dependent upon a hormone elaborated in the pars tuberalis. The gradual recovery of the white background response is thus explicable on the following assumptions: (*a*) that in some animals, in removing the anterior lobe, a smaller or larger portion of the pars tuberalis was left behind, and (*b*) that after several months this portion regenerated to a different degree in different individuals. Assuming that involution of the ovaries is due solely to the influence of the pars anterior, the fact that the ovaries in the regenerated animals still presented the complete involution typical of total anterior lobe removal is evidence in favour of the view that regeneration involved only the pars tuberalis. For final evidence on this point histological examination of regenerated pituitaries is necessary.

Table IV. *Effect of hypophysectomy and regeneration.*

6 months' captivity
6 months' hypophysectomised.

Captivity	Both lobes removed	Anterior lobe removed	Regenerated. *Melanophore Index* −3
10·4	7·6	6·6	9·0
8·2	7·4	—	9·0
8·0	8·2	—	10·6
8·6	7·6	—	8·0
9·2	6·5	—	—
10·0	7·6	—	—
9·4	7·3	—	—
8·2	6·8	—	—
9·0	7·4	6·6	9·1

Table V. *Effect of hypophysectomy and regeneration.*

8 months' captivity
8 months' hypophysectomised.

Captivity	Both lobes removed	Anterior lobe removed	Regenerated. *Melanophore Index*	
			3 −4	2
9·9	8·8	7·0	7·7	9·2
8·6	8·0	6·2	8·9	10·4
8·2	6·7	7·9	7·4	8·0
8·6	7·6	5·9	7·0	7·0
8·8	8·2	6·2	8·4	8·0
8·8	8·2	7·4	8·1	10·0
9·2	8·1	6·2	9·2	—
9·8	—	—	9·1	—
—	—	—	9·5	—
—	—	—	8·8	—
—	—	—	11·1	—
9·0	7·9	6·9	8·7	8·8

The serum calcium was determined in three classes of hypophysectomised animals: (1) both lobes removed; (2) anterior lobe including pars tuberalis removed; (3) regenerated pars tuberalis. Animals kept in captivity for the same length of time as the operated toads served as controls. This part of the investigation was carried out on females only.

(1) THE ANTAGONISTIC EFFECTS OF THE ANTERIOR AND POSTERIOR LOBES.

An inspection of the data shows that the serum calcium after anterior lobe removal and after complete hypophysectomy is significantly different from the figures for the controls, thus confirming Charles' results. The figures for anterior lobe removal and for total removal form a discontinuous series and the difference is probably significant. The removal of the anterior lobe alone thus leads to a lower calcium level than removal of the entire gland, thus confirming Hogben, Charles and Slome (1931). This difference can be explained on the assumption that the anterior lobe normally maintains a high calcium level and the posterior lobe normally depresses serum calcium. Consequently, if the posterior lobe is present, as in anterior lobe removal, the calcium should reach its lowest level, under the conditions of these experiments; if the posterior lobe is then removed, as in complete hypophysectomy, a higher calcium level should obtain, owing to the removal of the depressant action of the posterior lobe. The above evidence for an antagonistic influence of the anterior and posterior lobes on calcium metabolism is reinforced by the results of the following experiment: A number of toads in which both lobes had been removed six months previously was divided into two batches; the animals in one batch each received an injection of 1 c.c. of antuitrin (Parke Davis) and the animals in the second batch were each injected with 1 c.c. of pituitrin (Parke Davis). Normal animals injected with antuitrin showed maximal pallor on a black background 2–3 hours after injection. Both normal and completely hypophysectomised toads became maximally dark on a white background after injection of pituitrin. Two and a half hours after injection the animals were bled and the serum calcium estimated. In the following table the control figure is the mean of the results for total hypophysectomy in Table IV:

Table VI. *Injection of antuitrin and pituitrin into completely hypophysectomised animals.*

Control	Antuitrin	Pituitrin
See Table IV under "Both lobes removed."	8·6 9·0 8·4 9·4 — —	6·8 6·4 6·8 6·6 6·6 5·8
7·4	8·9	6·5

Injections of histamine had no effect on the melanophores or on the calcium content of the serum.

The above data strikingly confirm the results of hypophysectomy as detailed in Tables IV and V.

(2) THE EFFECT OF REGENERATION.

The figures for regenerated animals are consistently higher than those for partial or complete hypophysectomy, but do not differ significantly from the normal captivity figure. The melanophore index was taken as an indication of the degree of regeneration, but the difference in serum calcium between the two series is not significant.

Thus, on regeneration the serum calcium returned to normal concomitantly with the reappearance of the white background response, *i.e.* with the regeneration of the pars tuberalis. This suggests that it is the regenerated pars tuberalis which caused the rise and that it is only when this portion of the pituitary is removed with the pars anterior that the animal becomes maximally dark and a definite fall in calcium occurs. Both these effects are enhanced by the unantagonised activity of the posterior lobe. The above hypothesis is supported by the results of injection and by the evidence presented below. Hogben has presented evidence that the black background mechanism is located in the pars intermedia and the possibility therefore arises that the depressant effect of the posterior lobe on serum calcium may be referable to a hormone produced in the pars intermedia. The decrease in calcium after injection of pituitrin was accompanied by maximal expansion of melanophores. An analogous relationship holds for the pars tuberalis and its effect on melanophores and serum calcium.

IV. DISCUSSION.

It is well established that removal of the entire pituitary gland leads to a retrogression of the ovaries and that this effect is much more striking after removal of the anterior lobe alone. This suggests, as Hogben has pointed out, that the relation of the pituitary to the ovaries is a complex one involving two antagonistic influences: the anterior lobe stimulating and the posterior lobe inhibiting ovarian growth and activity. In the regenerated animals the ovaries remain in a state of complete retrogression although the melanophores are in a contracted condition. Injection of anterior lobe extracts as prepared by Bellerby's method induces ovulation but has no paling effect on the melanophores (Bellerby, 1932). The evidence supports the hypothesis that regeneration affects only the pars tuberalis and that the pars anterior is the sole agent in the anterior lobe controlling ovarian activity. The possibility that it is the pars nervosa of the posterior lobe which supplies the antagonistic influence on the ovaries is tentatively suggested. The results of castration show that the ovaries influence calcium metabolism. Since hypophysectomy leads to involution of the ovaries, the effects of pituitary removal on serum calcium may be due to a secondary effect through the ovaries. In the regenerated animals, however, the ovaries remain completely involuted but the serum calcium rises to normal. Anterior lobe removal alone also leads to complete involution and yet to a low calcium level. Thus the same extreme degree of ovarian retrogression is associated with both a high and low calcium content of the serum.

In the case of total removal of the pituitary and castration the presence of a severely involuted ovary on the one hand and the total absence of ovarian tissue on

the other are both associated with the same calcium level. It thus seems that the effect of the pituitary on ovarian activity and its effect on calcium metabolism are concomitant but independent activities. It is probable that the former is a direct relationship between the several lobes of the pituitary and the ovaries. The effect of the pars tuberalis on calcium metabolism may be an indirect one through a third gland of internal secretion, *e.g.* the parathyroids or adrenals. The fact that double hypophysectomy and castration both lead to the same calcium values indicates that both the pituitary and the gonads may indirectly influence calcium metabolism through some other endocrine gland.

Most of the evidence presented in this paper is summarised in the following figure, which consists of photographs showing the degrees of ovarian involution occurring as a result of hypophysectomy, regeneration after anterior lobe removal and captivity with the relevant serum calcium values (Table IV). The value for the ovariectomised calcium level is included (Table I).

SUMMARY.

1. A new micro-method for the estimation of serum calcium requiring only 0·4 c.c. serum is described.

2. Castration causes a persistent fall (17–24 per cent.) in the calcium content of the serum in females of *Xenopus laevis* as soon as 2 months after operation. In males there is no effect 3½ months after castration but a fall (16 per cent.) 6 months after.

3. Removal of the anterior lobe (*i.e.* pars anterior and pars tuberalis) of the pituitary leads to a significantly lower calcium level than removal of the entire gland.

4. Injection of antuitrin into completely hypophysectomised animals causes a rise, injection of pituitrin a fall in serum calcium. Injection of antuitrin into normal animals leads to maximal contraction of melanophores on a black background and injection of pituitrin into normal or completely hypophysectomised toads causes maximal expansion on a white background.

5. When the pars tuberalis regenerates the serum calcium returns to normal concomitantly with the reappearance of the white background response, but without regeneration of the ovaries.

6. The influence of the pituitary on ovarian activity and its effect on serum calcium are concomitant but independent activities. It is suggested that the pars tuberalis and the posterior lobe have antagonistic effects on both colour change and serum calcium, and that the pars anterior and the posterior lobe exert antagonistic influences on ovarian activity. It is further suggested that the pituitary directly controls ovarian activity, but that both the pituitary and the gonads control calcium metabolism through some other endocrine gland.

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