STUDIES ON THE PITUITARY

VIII. THE RELATION OF THE PITUITARY GLAND TO CALCIUM METABOLISM AND OVARIAN FUNCTION IN *XENOPUS*

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I. INTRODUCTION.

THE suitability of *Xenopus laevis* for experiments involving protracted survival after operative removal of the ductless glands has made it possible in this laboratory to undertake, during the last four years, an extensive co-operative investigation of metabolic changes and other phenomena associated with the removal of the pituitary body and the exercise of the chromatic function. In this communication some new data referring especially to calcium metabolism and the ovarian function are placed on record.

It has now been firmly established that pigmentary effector activity in Amphibia is a delicate indicator of pituitary secretion. The recent work of two of the present authors has shown that colour response in *Xenopus laevis* and in all probability in other Amphibia is determined by two endocrine agencies. One of these is a specific entity found in extracts of the pituitary of all vertebrates ("melanophore stimulant"). It is manufactured by the posterior lobe of the gland as shown by the work of Swingle (1921) and Hogben and Winton (1922–3) and is not identical with the oxytocic and pressor substances as shown by Smith (1924) and Hogben and Gordon (1930). The other endocrine agency is directly or indirectly associated with the pars anterior or pars tuberalis of the gland. In exploring the rôle of the latter component in the control of amphibian colour change and the metabolic accompaniments of pigmentary effector activity large stocks of operated animals

have been kept in the laboratory under uniform conditions over a period of three years. *Xenopus laevis* is endowed with a very striking capacity for colour change, predominantly determined by light reflected from the surface which occupies the field of vision, influenced only to a slight extent by temperature and ordinarily unaffected by humidity, since it is an aquatic animal. In a white container the melanophores are fully contracted in light and the animal is pale. In a black container they are expanded and the animal is dark. In darkness the melanophores are partially expanded and the animal assumes an intermediate hue. The same is true of animals which have been deprived of the eyes or subjected to section of the optic nerves. As in previous publications dealing with *Xenopus*, we shall refer to the three conditions specified as the white background response, the black background response and the intermediate condition. Analysis of the time relations of colour change in *Xenopus* shows that the physiological condition characteristic of an animal in transition from the white to the black background condition is not identical with that which accompanies the intermediate condition associated with complete darkness or removal of the eyes.

II. THE EFFECTS OF HYPOPHYSECTOMY ON THE CALCIUM AND MAGNESIUM CONTENT OF THE SERUM.

Among other variables investigated in connection with pigmentary effector activity in *Xenopus* the calcium and magnesium content of the serum has been made the subject of enquiry. In a preliminary contribution by one of us (Charles, 1930), it has been shown (i) that there is a very considerable difference between the calcium and magnesium content of the serum in males and in females; (ii) that the calcium and magnesium content of the serum of hypophysectomised females shortly after the breeding season was lower than that of controls. Indications of seasonal variations, however, made it necessary to reserve judgment on the significance of this discovery until the issue had been investigated more comprehensively. Since the methods of bleeding, operative procedure and chemical estimation have already been set forth in the communication referred to, it will be sufficient to refer the reader for further details to the earlier investigation.

Xenopus breeds during October in the vicinity of Cape Town. In the previous work analysis of the serum was carried out on batches of animals which had been hypophysectomised by Hogben's method or deprived of the eyes at least a year before they were killed for the purpose of investigation. They were kept in the laboratory with controls subjected continuously to white and black backgrounds and fed simultaneously with meat at regular intervals. Since then estimations have been carried out on two other sets of animals. One lot consisted of twelve females from which both lobes of the pituitary had been removed four months previously, maintaining their post-operative pallor on a black "background" during the whole of that time, together with dark controls also kept in black containers over the same period and fed simultaneously. The blood of these animals was estimated for calcium and magnesium content of the serum in July. A second group of animals was used

to determine the calcium and magnesium content of the serum at the beginning of September (*i.e.* immediately before the breeding season begins). This group consisted of six classes, each composed of ten females, viz. (i) anterior lobe only removed five months previously, kept on white background, (ii) entire pituitary gland removed five months previously, kept on black background, (iii) eyes removed five months previously, (iv) eyes and whole pituitary gland removed five months previously, (v) and (vi) pale and dark controls kept on white and black backgrounds respectively for the same period. In Table I are set forth all the determinations of calcium content of the serum for July and September contrasted with previous estimations in February, already published (*op. cit.*). The method employed (Clark and Collip's modification of that of Kramer and Tisdall) was the same in all three groups. The blood of ten females, average weight approximately 75 gm., throughout sufficed for three determinations in which the discrepancies were never greater than 5 per cent.

Table I. *Calcium in mg. per cent.*

	February	July	September
A. White background:			
Pale controls...	9·65	—	9·02
Hypophysectomised (anterior lobe)	6·23	—	1·47
B. Black background:			
Dark controls	9·99	7·66	7·26
Hypophysectomised (both lobes) ...	7·22	6·00	6·00
C. Eyeless:			
Controls	9·85	—	7·21
Hypophysectomised (both lobes) ...	—	—	1·67

In Table II the corresponding figures for the magnesium content of the serum in mgm. per cent. are given. The method was that of Bell and Doisy, modified by Briggs and Benedict and Theis. This has been found to give results that agree within 5 per cent.

Table II. *Magnesium in mg. per cent.*

	February	July	September
A. White background:			
Pale controls...	2·35	—	2·42
Hypophysectomised (anterior lobe)	1·68	—	3·15
B. Black background:			
Dark controls	2·25	1·76	2·56
Hypophysectomised (both lobes) ...	1·72	2·28	3·34
C. Eyeless:			
Controls	2·63	—	2·41
Hypophysectomised (both lobes) ...	—	—	3·08

An inspection of the figures contained in Tables I and II will show that there is a lowering of calcium in the serum of *Xenopus* following removal of the pituitary. The fall is detectable throughout the year. On the other hand, the magnesium

content of the serum of hypophysectomised animals may be lower or higher according to the season of the year. The data suggest 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. This is shown in seeing individuals both in February and September. As previously recorded, it will be observed that no certain correlation between calcium and magnesium content of the serum and colour response is deducible.

III. THE EFFECTS OF HYPOPHYSECTOMY ON THE OVARIES.

In the course of experiments on colour change involving the injection of anterior lobe extracts (ox) and grafting of the pituitary body from other individuals of the same species into females of *Xenopus laevis*, it was observed that ovulation could be induced at any time of the year. This result was never obtained with extracts of other tissues or after implantation of thyroids or suprarenals. This observation was made more than two years ago and was recorded in a preliminary note by one of us (Hogben). Therein it was mentioned that a very striking retrogression of the ovaries was found in females killed one or two years after the removal of the pituitary gland. The importance of this circumstance demanded a more rigorous investigation of the relation of the pituitary to the ovaries. A careful examination of the ovaries was made in each individual of the group of females employed for the determination of calcium and magnesium content in September, at the time when the ovaries should be hypertrophied in anticipation of the breeding season which ensues at the end of September or the beginning of October. As stated, all the animals had been previously kept for a period of five months under uniform conditions with regular feeding and change of water. In all the classes of individuals with the pituitary intact, both eyeless and normal, the ovaries were uniformly composed of large black ova ready for extrusion. For descriptive purposes the entire group of classes will be arranged in three sub-groups each consisting of an "experimental" and "control" series.

Table III. *Series I. Black background.*

(*a*) Normal dark controls.

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	63	12·4	2·50	0·197
2	100	16·6	1·10	0·166
3	70	8·3	2·70	0·118
4	49	6·7	0·60	0·137
5	77	8·2	0·50	0·107
6	62	9·4	0·80	0·151
7	54	9·0	0·20	0·158
8	72	7·5	1·60	0·104
9	42	3·0	0·01	0·071
10	37	4·9	0·01	0·132
Mean values	62·6	8·6	1·0	0·134±0·011

Table III (*cont.*).

(*b*) Hypophysectomised (both lobes).

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	44	1·05	1·15	0·024
2	105	3·70	3·75	0·035
3	122	0·65	1·20	0·005
4	63	0·65	2·60	0·010
5	63	2·00	0·10	0·032
6	57	5·50	1·20	0·096
7	112	1·25	1·15	0·011
8	65	5·10	2·60	0·078
9	60	1·75	0·80	0·029
10	54	3·05	0·60	0·056
Mean values	74·5	2·47	1·51	0·039 ±0·009

Series I consists of individuals kept on a black background, being composed of (*a*) normal dark animals, and (*b*) pale animals from which both lobes of the pituitary had been removed five months previously. The protocols are as above.

It will be seen that the two series only slightly overlap and that there is very evident macroscopic retrogression of the ovaries in half of the individuals of series (*b*). In nos. 3 and 4 of this series no ova of visible dimensions were present. The histological and macroscopic appearance of the ovary, which was greyish-yellow in hue, can be most explicitly described as "embryonic."

Table IV. *Series II. White background.*

(*a*) Normal pale controls.

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	94	17·5	1·50	0·165
2	72	9·5	0·65	0·132
3	100	13·9	0·90	0·139
4	107	9·4	1·70	0·088
5	101	10·7	1·70	0·106
6	67	5·3	1·25	0·079
7	76	9·9	0·25	0·130
8	79	7·9	0·80	0·100
9	67	8·7	2·10	0·130
Mean values	84·8	9·2	1·2	0·110 ±0·009

(*b*) Hypophysectomised (anterior lobe only).

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	73	1·10	0·80	0·015
2	83	2·20	1·40	0·027
3	93	0·50	0·35	0·005
4	91	0·95	1·90	0·010
5	72	1·05	1·50	0·014
6	66	1·60	2·40	0·024
7	44	0·70	0·01	0·016
8	92	3·70	1·80	0·019
9	57	1·50	0·01	0·026
10	56	1·30	0·01	0·023
Mean values	72·7	1·41	1·02	0·018 ±0·002

Series II is composed of individuals which had been kept on a white background, consisting of (*a*) normal pale animals, and (*b*) dark animals from which the anterior lobe alone of the pituitary had been removed five months previously. It may here be mentioned that the removal of the anterior lobe alone in *Xenopus*, as shown by Hogben and Slome, has the opposite effect of removing both lobes. After removal of the anterior lobe alone the animal remains dark when subjected to conditions which would otherwise produce pallor.

In all the females from which only the anterior lobe of the pituitary gland had been removed there was evident retrogression of the ovaries. The term retrogression is here used advisedly because the ovaries in most cases were not only less developed than those of the controls when the animals were killed, but were actually less developed than the ovaries of normal toads at the time when the operation was performed five months previously. In nos. 1, 3, 4, 5, 7 and 10 there were no ova of visible dimensions. The ovaries of these animals in macroscopic and microscopic appearance might be described, like no. 3 in Series I (*b*), as "embryonic." To nos. 2 and 6 the same adjective might be applied, but a few ova of visible dimensions were still to be seen. In nos. 8 and 9 fully developed ova were present, but there were signs suggestive of resorption of ova and the ovaries were relatively of much smaller dimensions that in any of the control series.

Table V. *Series III. Eyeless females.*

(*a*) Controls—Intermediate condition.

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	94	10·7	0·35	0·113
2	98	6·4	0·60	0·065
3	47	3·4	0·05	0·072
4	46	3·6	0·15	0·078
5	68	2·8	0·01	0·040
6	56	3·4	0·10	0·061
7	55	2·9	0·01	0·053
8	57	4·5	0·10	0·078
9	60	3·3	0·30	0·055
10	64	2·8	0·30	0·044
Mean values	64·5	4·38	0·20	0·066 ±0·006

(*b*) Hypophysectomised pale females (both lobes).

	Body weight (*b*)	Ovary weight (*a*)	Fatty body	Ratio *a*/*b*
1	133	9·90	5·00	0·074
2	56	1·85	2·75	0·033
3	71	4·90	5·05	0·069
4	50	3·20	1·20	0·064
5	66	3·90	1·60	0·059
6	59	2·50	1·30	0·043
7	43	0·45	0·30	0·001
8	51	2·50	0·90	0·009
9	48	0·90	1·20	0·002
10	49	2·20	0·70	0·045
Mean values	62·6	3·23	2·0	0·045 ±0·008

Series III consisted of eyeless animals, one set being otherwise normal and displaying the intermediate condition, while the other set consisted of pale individuals from which both lobes of the pituitary gland had been removed. This series was examined because, as hinted already (Hogben and Slome), the analysis of colour change in *Xenopus* indicates that the removal of the eyes involves changes in the secretory activity of both lobes of the pituitary gland. The protocols are as above.

The discrepancy between these two series is not as striking as the discrepancy between the control and experimental groups in I and II, and this is partly because the ovaries of the eyeless animal are less developed than the ovaries of the normal control female.

It is quite evident from these data that the development of the ovaries is intimately dependent on the activity of the anterior lobe of the pituitary gland. The data suggest, though they do not conclusively prove, that the relation of the pituitary gland to the ovaries is a complex one involving two antagonistic influences. This is indicated by the fact that the effects of removal of the anterior lobe alone are manifestly more striking than the effects of removal of the whole gland.

IV. THE EFFECTS OF PITUITARY GRAFTS AND THE INJECTION OF EXTRACTS.

It has been mentioned in a preliminary note by Hogben that injection of pituitary (anterior lobe) extracts prepared by Bellerby's method has repeatedly been found to induce ovulation. This effect has never been subjected to separate investigation with special reference to the influence of the pituitary on the ovaries; and it is not proposed to submit any formal protocols. However, it may be mentioned that ovulation has been obtained uniformly in batches of twelve individuals injected in this way during the period of the year midway between the breeding seasons. In experiments undertaken over a period of four years involving the injection of several hundred animals no case of spontaneous ovulation has occurred in the laboratory in normal animals nor in animals injected with other tissue extracts as controls. A limited number of experiments on the implantation of pituitary gland reinforces the conclusion that the ovaries of *Xenopus* are stimulated by a specific constituent of the anterior lobe. Thus, two eyeless females into each of which six anterior lobes of other individuals had been grafted at the end of June continued to ovulate for two weeks. In other experiments in which thyroids and suprarenals were likewise implanted in a pocket of the ventral musculature or beneath the urostyle, ovulation was never found to occur.

V. SOME OTHER CONSEQUENCES OF HYPOPHYSECTOMY IN *XENOPUS LAEVIS*.

In the course of investigations into the chromatic function of *Xenopus* a number of other facts bearing on the functional activity of the pituitary gland have come to light. As these have not been placed on record elsewhere, it seems worth while to mention two in particular. These are the relation of the pituitary to skin secretion and to the proliferation of the epidermis.

1. Skin secretion. One of the most characteristic responses of *Xenopus laevis* to handling is the copious slime which exudes from the skin glands, a reaction hardly less redoubtable than the analogous and more familiar secretion of the hag. Anyone who has carried out experiments on *Xenopus* will be familiar with the extreme difficulty of holding the animal in the hand on this account. Hypophysectomised animals do not secrete in response to handling. In consequence the skin assumes a leathery texture. The animal can be readily picked up and held in the hand and a blindfolded person would have no difficulty in distinguishing hypophysectomised from normal individuals swimming in the same tank. The difference might be compared to the condition of the skin in an axolotl and the amblystoma form. Injection of pituitary extracts, especially posterior lobe, induces a copious secretion of slime in *Xenopus* after a few minutes.

The relation of the pituitary to skin secretion in *Xenopus* is a very perplexing problem. There is little doubt that the immediate extrusion of slime which follows rough handling is a reflex phenomenon throughout. It is not suggested that it is effectively controlled by an endocrine agency. On the other hand there is indisputable evidence that a very intimate relation exists between the pituitary gland and skin secretion. One possible interpretation of this relation is that it is vasomotor in origin. While it is true that removal of the pituitary in *Xenopus* is accompanied by capillary dilatation such as was originally described by Krogh in the frog, it is difficult to accept this explanation for several reasons. One is that a persistent cessation of skin secretion is characteristic of animals from which the anterior lobe alone has been removed.

2. Proliferation of the epidermis. In all experiments with anterior lobe extracts complete shedding of the skin occurs within a few hours and this may be repeated several times so that several casts are found within the containers. The autacoid is heat stable and ether soluble. A very characteristic feature of metamorphosis in Urodeles is repeated shedding of the larval skin. The transformation of the Mexican axolotl by thyroid feeding is visibly initiated by this event. Hogben (1923) obtained metamorphosis in axolotls by injection of anterior lobe extracts, a result subsequently confirmed by Spaul (1925). There are very good reasons for believing that the influence of the pituitary on amphibian metamorphosis is an indirect one, involving the activation of the thyroid. It was found that thyroid grafts like pituitary injections induce proliferation of the epidermal cells in *Xenopus*. There is thus presumptive evidence that the shedding of the skin after injection of pituitary extracts is due to the substance which is concerned with metamorphosis and involves the activation of the thyroid gland.

VI. SUMMARY AND CONCLUSIONS.

The principal facts presented in this communication may be summarised as follows:

1. Removal of the pituitary gland or of the anterior lobe alone results in involution of the ovaries, lowering of the serum calcium and cessation of skin secretion.

2. Ovulation and skin secretion can be induced in *Xenopus* by injection of extracts of the pituitary gland.

This communication has raised a number of issues which would have been investigated more extensively if circumstances had not forced two of the authors to leave South Africa. It is therefore advisable to bring into sharper relief the two most important conclusions which are justified by the facts recorded.

The first of these is the very conclusive and complete evidence in support of the view that the anterior lobe of the pituitary controls the activity of the ovaries. Zondek and Ascheim (1927), Smith and Engle (1927), Bellerby (1930) and Parkes (1930) have shown that anterior lobe extracts or transplants stimulate the development of the ovaries in mammals. Unfortunately the removal of the pituitary gland in mammals is an operation of extreme difficulty. Conclusive evidence that the pituitary has a functional relation to the ovaries is lacking, unless the effects of operative removal can be brought into harmonious relationship with the effects of injection and implantation. In *Xenopus* it is possible to do this in a very striking manner. It is now therefore justifiable to assert that the results obtained by injection and implantation in mammals are not merely an indication of a new and specific pharmaco-dynamic constituent in the anterior lobe of the pituitary gland but of a genuine functional influence of the pituitary on the normal ovarian cycle. The new evidence presented in this communication reinforces a large volume of previous work on the comparative physiology of the ductless glands pointing to a widespread uniformity of endocrine phenomena within the vertebrate series.

Table VI.

	Ovary/body weight ratio	Calcium mg. %
A. White background:		
Pale controls	0·110	9·02
Hypophysectomised (anterior lobe) ...	0·018	1·47
B. Black background:		
Dark controls	0·134	7·26
Hypophysectomised (both lobes) ...	0·039	6·00
C. Eyeless:		
Intermediate controls	0·066	7·21
Hypophysectomised (both lobes) ...	0·045	1·67

The other conclusion of outstanding importance which emerges from the present study is the influence of the pituitary gland on calcium metabolism. This opens up a field for further research and it would be premature to discuss it except in a

very tentative way. Although there is no evidence at present to indicate that the pituitary gland exerts its influence on calcium metabolism directly, two considerations indicate that the influence of the anterior lobe on serum calcium is independent of the ovary. One is a comparison of the figures given for the eyeless and seeing groups in Table II which summarises the data presented in Sections II and III. The other is that Mirvish and Bosman (1927) have shown that injection of ovarian extracts lowers the serum calcium of rabbits.

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