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

IV. THE EFFECTS OF INJECTION OF OVARIAN AND PITUITARY EXTRACTS ON THE SERUM CALCIUM IN NORMAL, OVARIECTOMISED AND HYPOPHYSECTOMISED TOADS

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I. INTRODUCTION.

An account of the previous literature dealing with the effects of ovarian and pituitary extracts on the calcium content of the serum will be found in the review by Zwarenstein (1934).

In a previous paper of this series (Shapiro and Zwarenstein, 1933) it was shown that ovariectomy caused a persistent fall in the serum calcium of female *Xenopus*. Removal of the anterior lobe alone, or of both lobes, of the pituitary also led to a significant fall in the serum calcium level. Removal of the anterior lobe alone, *i.e.* pars anterior and pars tuberalis, led to a significantly lower level than removal of the entire gland. Evidence was presented which suggested that when the pars tuberalis regenerates the low serum calcium of anterior lobe removal returns to normal, and that the pars tuberalis and the posterior lobe have antagonistic effects on serum calcium.

In order to obtain further evidence with regard to a relationship between the ovaries, the pituitary and calcium metabolism, thus indicated by the effects of extirpation, the effect of injection of extracts of these glands on the serum calcium was investigated.

II. EXPERIMENTAL METHODS AND DATA.

Two kinds of ovarian extract were used, one prepared from sheep and pig ovaries according to the method of Moore, McGee and co-workers (McGee, Juhn and Domm, 1928; Moore, Price and Gallagher, 1930), and the other an extract of residual ovary (*i.e.* without corpus luteum) prepared by Parke, Davis and Co. Two pituitary extracts prepared by Parke, Davis and Co. were also used: "Antuitrin," an extract of anterior lobe and "Pituitrin," an extract of posterior lobe.

All the animals were injected by way of the dorsal lymph sac, the needle of the syringe being passed first through the muscles of the thigh, an effective barrier

being thus obtained preventing loss of the injected fluid. The animals were killed in most cases 3 hours after injection, and the serum calcium estimated by the micro-method described previously (Shapiro and Zwarenstein, 1933). All the toads were starved for at least 6 days before they were injected.

In the following tables all serum calcium figures refer to mg. per 100 c.c. Unless otherwise stated, all ovariectomised animals were operated on 6 months previously.

(*a*) *Effect of injection of ovarian extracts.*

Table I. *Injection of ovarian extract (Moore and McGee) into normal and ovariectomised animals.*

Normal females	Ovariectomised	Normal plus ovarian extract	Ovariectomised plus ovarian extract
10 0	7·5	10·2	8 0
10 2	7·4	10·0	7·8
10 6	7·8	10·1	7·4
10·8	7·6	10·4	—
—	7·5	10 2	—
10·4	7 6	10·2	7·7

Table II. *Injection of extract of ovarian residue (Parke, Davis and Co.) into normal and ovariectomised animals.*

Normal females	Normal plus ovarian extract	Laparotomised controls	Ovariectomised 2 weeks	Ovariectomised plus ovarian extract
12 6	12·4	11·4	9·8	12·8
10 0	13 8	10·6	9 6	13·2
10·4	11 8	10·8	9·6	12·8
12 6	13·0	10·8	10·2	13·6
12·4	—	11 6	10·0	16 6
11·0	—	10·8	8·4	10·8
12·6	—	11·8	10·4	11·6
—	—	10·8	6 8	13·0
—	—	—	7·4	12·8
—	—	—	9·2	—
11·7	12·8	11·0	9·1	13·0

Each toad injected with 0·75 c.c. extract.

An analysis of Table II reveals the fact that normal females exhibited a serum calcium range of from 10·0 to 12·6 mg. per 100 c.c., whereas normal females injected with ovarian residue exhibited a range of from 11·8 to 13·8 mg. per 100 c.c. This is probably a significant difference, and indicates that this particular extract of residual ovary has the property of elevating the level of the serum calcium. This suggestion is effectively reinforced by the results of injecting the extract into females which had been ovariectomised 2 weeks previously. A striking increase from 9·1 to 13·0 mg. per 100 c.c. occurred. In this experiment the serum calcium values of the ovariecto-

mised controls ranged from 6·8 to 10·4 mg. per 100 c.c., whereas the injected animals exhibited a range of from 10·8 to 16·6 mg. per 100 c.c. serum. The discontinuity in the two series indicates definitely a significant change in the serum calcium level. A comparison of the values for the laparotomised controls with the values for the ovariectomised animals shows that the ovariectomy effect was already setting in as early as 2 weeks after the operation.

From Table I it appears that the ovarian extract prepared by the method of Moore and McGee did not raise the level of the serum calcium in either normal or ovariectomised animals. It seems that the extract did not contain the active principle which affects the metabolism of calcium. It is interesting to note that although this extract did not affect the mineral metabolism of the clawed toad, it affected the creatinine metabolism of the rabbit very strikingly, as shown by the experiments of Schrire and Zwarenstein (1933).

The results for ovariectomised animals in Tables I and II are, however, not strictly comparable, since the commercial extract was injected into animals which had been ovariectomised 2 weeks before injection, and the other extract into animals ovariectomised 6 months previously.

(*b*) *Effect of injection of antuitrin, pituitrin and histamine.*

Table III. *Injection of antuitrin and pituitrin into ovariectomised animals.*

Ovariectomised	Antuitrin	Pituitrin
See under Table I	10·4 10·2 10 0 9 8 10·6 10·5 —	6·2 7·2 7·0 7 8 6·5 6·2 6·6
7·6	10·4	6·8

Each toad injected with 1 c.c. antuitrin or pituitrin.

Table IV. *Injection of pituitrin into normal females.*

Normal females	Pituitrin	
	0·5 c.c.	1·0 c.c.
See under Table II	8·2 8·2 9·4 8·2 — — —	8·8 9·2 8·6 8·4 8·2 8 0 8·3
11·7	8·5	8·5

Injection of 0·2 c.c. pituitrin into normal females had no effect on the serum calcium level.

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

Completely hypophysectomised toads	Antuitrin 1·0 c.c.	Pituitrin	
		0·5 c.c.	1·0 c.c.
7·6	8·6	6·8	6·8
7·4	9·0	6·8	6·4
8·2	8·4	6·4	6·8
7·6	9·4	6·8	6·6
6·5	—	—	6·6
7·6	—	—	5·8
7·3	—	—	—
6·8	—	—	—
7·4	8·9	6·5	6·5

Injection of 0·5 c.c. pituitrin had no effect after 1½ hours.

Table VI. *Injection of histamine (1 c.c. of a 0·1 per cent. solution) into normal toads.*

Normal toads	Histamine
10·0	10·6
10·2	10·4
10·6	10·4
10·8	10·8
10·4	10·6

Injection of antuitrin raises the serum calcium of ovariectomised toads from 7·6 to 10·4 mg. per 100 c.c. (Table III). This appears to be a direct effect of the anterior lobe extract acting independently of the ovaries, as these had been removed. Injections of pituitrin, on the other hand, significantly depress the serum calcium level in normal animals from 11·7 to 8·5 mg. per 100 c.c. This depressant effect is also observed in ovariectomised toads. Pituitrin depresses the serum calcium level not only in normal and ovariectomised toads, but also in completely hypophysectomised animals (Table V). This effect is, however, produced more readily in the ovariectomised condition.

III. DISCUSSION.

From the recent work of Dixon (1933) it appears that extracts of residual ovary or corpus luteum did not produce an appreciable change in the serum calcium in dogs. Pregnancy or pseudo-pregnancy produced no demonstrable change in the serum calcium 8 days after copulation in rabbits. Injection of crystalline trihydroxyoestrin sufficient to produce uterine distension and vaginal cornification in ovariectomised, parathyroidectomised rats did not produce any marked difference in serum calcium from the control animals.

In the experiments described above, ovarian residue (*i.e.* without corpus luteum) was injected. It seems therefore that the effect observed cannot be due to a substance elaborated by the corpus luteum. Dixon, moreover, has shown this directly by injection of extracts of corpus luteum, although Mirvish and Bosman (1927) obtained a fall in the serum calcium of rabbits by injection of extracts of residual ovary, corpus luteum and placenta. Reiss and Marx (1928) and Frei and Emerson (1930), in rabbits and in cows respectively, found that injection of commercial extracts of ovary, and a commercial oestrin preparation, caused a slight fall in the serum calcium level. Most of these experiments are vitiated by the fact that rabbits were employed in the investigations, but if any value can be attached to them at all it is that the effects observed in *Xenopus* are not due to oestrin or the corpus luteum. This latter is hardly surprising since the amphibian ovary does not form corpora lutea. It is possible that a different principle in the ovary, other than oestrin or the corpus luteum, may be concerned. On the other hand the difference may be due to the fact that the experiments were performed on amphibians and not on mammals.

Increased absorption of calcium from foodstuffs in the alimentary canal can be ruled out as a factor since all the animals were starved for about a week before the injections were made.

Recent work by Cannavo (1932) and Dixon (1933) suggests that the gonadotropic hormone of the pituitary is not the one concerned with calcium metabolism. Cannavo (1932) failed to establish any significant changes in the serum calcium level after subcutaneous injection of prolan into men, dogs and rabbits. Dixon (1933) found that injections of anterior pituitary extracts (method of preparation not described) into rats in sufficient amounts to cause luteinisation of the ovaries, produced no effect on the serum calcium level. Frei and Emerson (1930) obtained a slight rise in serum calcium 8 hours after injection of a commercial anterior pituitary extract. Hogben and Charles (1932) found that the injection of fresh saline extracts of ox pituitary produced a prolonged fall in the serum calcium of normal and ovariectomised rabbits.

It appears likely that the pars tuberalis rather than the pars anterior may be concerned with the regulation of the serum calcium level (Shapiro and Zwarenstein, 1933). The negative results of Cannavo and of Dixon may be due to the fact that their extracts contained active principles derived solely from the pars anterior. Frei and Emerson's results are in substantial agreement with the data on the effects of antuitrin injections reported above. While it thus appears unlikely that the gonadotropic hormone is the active factor concerned in calcium metabolism, it is necessary to suspend judgment on this point until evidence is available on the effects of injections of extracts of pars tuberalis as well as of pars anterior. The results obtained in *Xenopus* in animals in which the pars tuberalis had regenerated, and also after injection of antuitrin, are suggestive.

As in the case of ovarian extracts the serum calcium rise is probably not due to increased absorption of calcium from the alimentary canal. It is theoretically possible that there was a diminished rate of excretion of calcium, or else that more calcium was being mobilised into the blood-stream from the calcium depôts as a

result of a stimulating action of antuitrin on the thyroid-parathyroid apparatus. These points, however, constitute a separate problem for future investigation.

In connection with the depressant action of pituitrin on serum calcium, it seems fairly certain that this principle is located in the posterior lobe, probably the pars intermedia. This is borne out by the effects of hypophysectomy, complete and partial, and by the injection effects observed in the experiments included in Tables III, IV and V.

The results of ovarian and pituitary extract injection experiments taken in conjunction with the results of ovariectomy and hypophysectomy reinforce the hypothesis advanced in an earlier paper that there is an antagonistic relationship between the two lobes of the pituitary with regard to the regulation of the serum calcium level, and afford evidence of the existence of an endocrine relationship, probably indirect, between the pituitary, the ovaries and calcium metabolism.

IV. SUMMARY.

1. Experiments are described which show that active extracts of ovarian tissue minus corpus luteum can be obtained which significantly raise the lowered level of the serum calcium in ovariectomised toads; and also raise the serum calcium level in normal toads above the normal level.

2. Injection of adequate doses of antuitrin (Parke, Davis and Co.), raises the lowered level of serum calcium in hypophysectomised toads almost to normal; and of ovariectomised toads to the normal level. The effect is obtained more readily in the ovariectomised condition.

3. Injection of pituitrin (Parke, Davis and Co.), depresses the serum calcium level in normal, ovariectomised and completely hypophysectomised toads. The effect is more readily obtained in the normal intact animal than in the ovariectomised or the totally hypophysectomised state, when the ovaries have undergone involution.

4. It is suggested that there is evidence that the principles exerting a hormone-like action on calcium metabolism are not, in the case of the ovary, either oestrin or the luteal hormone, or in the case of the pituitary, the gonadotropic hormone.

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