

## STUDIES ON THE PITUITARY

### IX. CHANGES IN BLOOD CALCIUM FOLLOWING INJECTION OF ANTERIOR LOBE EXTRACTS AND SEXUAL EXCITEMENT IN FEMALE RABBITS

BY LANCELOT HOGBEN AND ENID CHARLES.

(From the Department of Social Biology in the University of London.)

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#### I. INTRODUCTION.

IN a previous communication of this series (1) it has been shown that removal of the pituitary gland in Amphibia results in retrogression of the ovaries and extensive changes in the calcium and magnesium content of the serum. At all stages after removal a fall of calcium was recorded. The effect of hypophysectomy on the magnesium in the blood was different in observations made upon animals from which the gland had been removed according to the time after operation and therefore possibly according to the degree of retrogression of the ovaries. These results were obtained after removal of the entire gland or the anterior lobe alone. Injection of anterior lobe extracts and anterior lobe transplants elicited ovulation in normal animals. To the surprise of the authors it was found that injection of anterior lobe extracts resulted in lowering the blood calcium of *Xenopus laevis*. Owing to the departure of the authors from Cape Town, where the foregoing experiments were carried out, the observation specified last was not repeated. No reference has hitherto been made to it because it was intended to explore the fruits of investigations on the effects of pituitary removal in the light of certain other observations. These are in particular: (a) those of Mirvish and Bosman (2) who have shown that ovarian extracts prepared by the method of Allen and Doisy injected into rabbits produces a fall of serum calcium; and (b) observations of Watchorn (3) suggesting significant disturbances in magnesium metabolism correlated with menstruation in the human subject.

The present enquiry began with a study of the effects of injecting fresh anterior lobe extracts upon the calcium and magnesium content of the serum in rabbits. It has led to certain definite conclusions which have a suggestive bearing upon the connection between coitus and ovulation. Only female rabbits were employed. The diet was uniform throughout the experiments which were continued from the beginning of June till the end of August. Three breeds were used, namely chin-chillas, chocolate havanas and sables. The experiments were conducted in such a way that in general controls and experimental animals in each group were of the same breed. A large number of analyses satisfied us that there is no significant

difference between the calcium content of the sera of the three breeds employed. The ages of the rabbits varied from 11 to 16 months. All rabbits used in these experiments had been segregated previously for at least a month in separate cages to ensure that the ovaries were in a quiescent condition, *i.e.* contained no freshly ruptured follicles or newly formed corpora lutea.

Our own experience has confirmed that of previous investigators who have noted a disturbance of the blood calcium level after bleeding. Successive bleeding was not adopted in these experiments for that reason. All the experiments here recorded involve analysis of the blood of parallel control and experimental groups. The analytical methods have been referred to in the previous communication (1). Spayed females were used in some experiments. In all cases bleeding was not attempted till 6 weeks after removal of the ovaries, so that healing was complete. When the same animals were bled for more than one experiment an interval of at least a week elapsed between two successive experiments. The expenses of this research were borne by a grant from the Trevelyan Fund of the Royal Society.

## II. THE INJECTION OF ANTERIOR LOBE EXTRACTS.

Attention was first directed to the effects of injecting anterior lobe extracts upon the serum calcium level of the rabbit. Our observations agreed with the previous unpublished experiments upon *Xenopus* referred to above. For this purpose it was necessary to employ concoctions injected within a few hours of killing. In the experiments which follow, the extracts were made by grinding the fresh anterior lobes with quartz into a uniform pulp. To this pulp saline was added. After thorough stirring the coarser material was removed by centrifuging. The amount injected into each individual corresponded to the juice exuded from about 4 gm. of fresh anterior lobes. The glands were collected by the authors at the abattoir. The injection occurred within 4½ hours of killing. As the glands were collected they were frozen hard and kept in a thermos flask in that condition during the inevitable delay involved in transporting them from the abattoir to the laboratory. We wish to lay particular stress upon the necessity for these precautions. In researches of the senior author extending over a period of ten years it has become abundantly clear that the active materials of the anterior lobe are highly unstable in the condition in which they exist in the gland itself. There can be little doubt that conflicting testimony and much disappointment results from the use of stale glands which have not been collected under strict personal supervision. Misunderstanding would be avoided if investigators explicitly stated the exact time which elapsed between the slaughtering of the animals and the preparation and injection of their extracts.

In the first experiment, of which we present the protocol, three experimental animals injected with pituitary extract and three controls injected with saline were bled 4 hours, 10 hours and 19 hours after injection. The injections in this experiment and in all others recorded subsequently were intraperitoneal. The full details are as follows :

Table I.

Time	Controls		Experimental series	
	Ca in mg. %	Mg in mg. %	Ca in mg. %	Mg in mg. %
4 hours	(1) 15.07	2.91	(4) 15.72	3.57
	(2) 18.38	2.22	(5) 14.93	1.93
	(3) 15.42	2.96	(6) 12.64	2.56
10 hours	(7) 15.07	3.46	(10) 11.29	4.79
	(8) 14.91	3.71	(11) 14.28	2.22
	(9) 15.67	2.63	(12) 13.78	2.58
19 hours	(13) 16.17	1.81	(16) 16.97	2.36
	(14) 15.50	2.19	(17) 12.84	1.86
	(15) 16.20	2.51	(18) 14.28	2.90

An autopsy made 48 hours after injection on all these animals revealed no ruptured follicles, corpora haemorrhagica or corpora lutea in the rabbits belonging to the control series. Large corpora haemorrhagica were present in both ovaries in all the rabbits injected with pituitary extract except Nos. 5 and 16. The mean values, excluding 5 and 16, on which the extract produced no visible somatic effect are therefore as follows:

Table II.

	Control series		Experimental series	
	Calcium	Magnesium	Calcium	Magnesium
4 hours	16.29	2.70	14.18	3.07
10 hours	15.22	3.27	13.12	3.20
19 hours	15.96	2.17	13.54	2.38

The results of this experiment indicate no effect upon the magnesium content of the serum. They suggest a fall of calcium beginning about 4 hours after injection.

All the animals used in the first experiment were chinchillas. In the second experiment some months later sables and havanas were also used. The normal calcium level was lower. This may be correlated with the unusually cold and rainy weather in August, when the experiment was carried out. Four series of animals were used. These were (a) normal controls injected with saline, (b) normal rabbits injected with pituitary extract, (c) spayed females as controls to (d) spayed females injected with pituitary extract. The animals were bled 12 hours after injection. An autopsy was made 48 hours after to reveal the presence of ruptured follicles in series (b). The full details are given in Table III.

Contrary to our expectation this experiment showed that a fall in calcium occurs after the injection of pituitary extract into spayed as well as normal rabbits. The data suggest that the fall is not so great. Thus the fall in the normal rabbit may be partly reinforced by the liberation of the ovarian autocoid which Mirvish and Bosman (2) have studied. It seems clear, however, that pituitary extracts evoke a fall independently of their effect on the ovaries.

Table III.

	Calcium	Magnesium	Condition of ovaries
<i>(a) Normal controls</i>			
Chinchillas	13·87	3·92	} No ruptured follicles or corpora lutea
	14·57	3·15	
	14·69	—	
	13·57	2·33	
Sables	14·22	—	
	14·57	—	
	15·21	—	
Havanas	14·86	—	
	14·27	—	
	15·33	—	
Mean values	14·52	3·13	
<i>(b) Normal rabbits injected with pituitary extract 12 hours before</i>			
Chinchillas	12·69	2·65	Corpora haemorrhagica
	11·27	2·47	One ovary
	10·03	6·17	Both ovaries
	12·10	5·70	Both ovaries
	11·45	2·10	One ovary
	14·57	1·81	Both ovaries
			Absent
Mean values	12·02	3·48	—
	11·51*	3·28*	—
<i>(c) Ovariectomised controls</i>			
Chinchillas	13·10	3·68	—
	14·22	2·80	—
	15·17	2·14	—
Sables	14·75	2·96	—
	14·93	2·96	—
Havanas	13·86	2·25	—
	14·63	1·81	—
Mean values	14·38	2·66	—
<i>(d) Ovariectomised rabbits injected with pituitary extract 12 hours before</i>			
Chinchillas	12·75	1·40	—
	13·69	2·82	—
	13·28	2·41	—
Sables	12·57	2·17	—
	12·66	1·51	—
	10·74	1·95	—
Havanas	13·39	—	—
	13·34	2·30	—
Mean values	12·80	2·08	—

\* Mean value excluding last in which ovulation did not occur.

III. THE CALCIUM LEVEL OF THE SERUM AFTER SEXUAL EXCITEMENT.

The foregoing observations encouraged us to investigate the possibility of changes in serum calcium following coitus. This enquiry was reinforced by two other considerations. One was the experiments of Fee and Parkes (4) who have brought forward evidence that the reflex activation of pituitary secretion by the sexual act supplies the stimulus to ovulation in the rabbit. The other was an observation of Grant and Gates (5), who found that the calcium level is lower in rabbits kept together in pens than when they are segregated in cages. For penned animals they give a value of 11.64 mg. per cent. Our own experience confirms this observation as applied to female rabbits. It is well known that female rabbits indulge in Lesbian practices, and that such practices may evoke ovulation (Hammond) like heterosexual intercourse or coitus *sensu stricto*.

We have therefore determined the serum calcium of previously segregated females at varying periods after association with vasectomised bucks. In each case the buck was placed with the doe sufficiently long to permit him to "jump" the doe several times. In several cases the doe refused to lift her tail, so that true intercourse was not consummated. Ovulation in such cases did not occur. An autopsy was carried out on every individual. Three series of females were used. Series A were sables, series B were havanas and series C were chinchillas. The protocols are as follows:

Table IV. Series A. Sables.

	Ca in mg. %	Mean value	Condition of ovaries
Before coitus	1 14.22	14.67	—
	2 14.57		—
	3 15.21		—
4 hours after	4 13.14	13.25	—
	5 13.34		—
	6 13.17		—
	7 13.26		—
12 hours after	8 13.97	13.34	Ruptured follicles in both ovaries
	9 13.46		Ruptured follicles in both ovaries
	10 12.42		—
	11 13.51		—
24 hours after	12 11.56	11.89	—
	13 12.54		—
	14 11.90		—
	15 11.56		Ruptured follicles in both ovaries
10 days after	16 13.10	14.72	—
	17 13.10		—
	18 17.23		—
	19 15.46		Large corpora lutea in both ovaries

Table IV. *Series B. Havanas.*

	Ca in mg %	Mean value	Condition of ovaries	
Before coitus	20	14·86	14·82	—
	21	14·27	—	—
	22	15·33	—	—
8 hours after	23	12·54	12·68	—
	24	12·45	—	—
	25	13·43	—	—
	26	12·28	—	—
16 hours after	27	11·62	12·54	—
	28	13·00	—	Ruptured follicles in both ovaries
	29	11·96	—	—
	30	13·97	—	—
20 hours after	31	12·11	11·76	—
	32	9·98	—	One doubtfully ruptured follicle
	33	12·59	—	—
	34	12·36	—	Ruptured follicles in both ovaries
48 hours after	35	11·88	12·01	—
	36	12·36	—	—
	37	11·39	—	New corpora lutea
	38	12·42	—	—

Table IV. *Series C. Chinchillas.*

	Ca in mg. %	Mean value	Condition of ovaries	
Before coitus	39	14·99	14·07	—
	40	14·04	—	—
	41	13·57	—	—
	42	13·66	—	—
8 hours after	43	13·57	12·86	—
	44	12·74	—	—
	45	12·27	—	—
12 hours after	46	14·16	14·49	—
	47	15·22	—	Ruptured follicles in both ovaries
	48	13·69	—	Ruptured follicles in both ovaries
	49	14·87	—	—
20 hours after	50	12·60	13·17	—
	51	14·32	—	Ruptured follicles in both ovaries
	52	13·01	—	—
	53	12·84	—	—
5 days after	54	13·92	11·64	—
	55	11·39	—	—
	56	10·86	—	—
	57	10·64	—	—

A comparison of the mean values of these series may be made from the data presented in Table V.

From these data it seems justifiable to conclude that a fall in blood calcium occurs after sexual excitement in the doe whether ovulation occurs or not. This fall is already evident 4 hours after congress with the buck. Ovulation occurs in

the rabbit about 10 hours after coitus. Our figures interpreted in the light of the data derived from autopsy indicate that there is a secondary rise immediately after the time at which ovulation occurs, and possibly only if ovulation does occur. This secondary rise is followed by a further and prolonged fall which subsides before the corpora lutea reach their maximal growth.

Table V.

	Series A and B	Series C
Before coitus	14·75	14·07
4 hours after	13·25	—
8 hours after	12·68	12·86
12 hours after	13·34	14·49
16 hours after	12·54	—
20 hours after	11·76	13·17
24 hours after	11·89	—
48 hours after	12·01	—
5 days after	—	11·64
10 days after	14·72	—

These findings prompted us to test the effect of placing spayed does with bucks. Only eight animals (chinchillas) were available, four of which served as controls. The other four were jumped repeatedly, though true coitus did not occur. Unfortunately one of these females had been deprived of its uteri as well as its ovaries, a circumstance that suggests further enquiry at a later date, since the calcium level of this animal was exceptionally high. Blood was taken 20 hours after the buck was introduced. The calcium content of the serum in mg. per cent. of these two series was as follows:

(a) Jumped by buck: 11·95, 11·60, 13·86, 15·11 (uterus removed also).

(b) Controls: 13·33, 13·92, 14·40, 13·01.

The results of this experiment are too meagre to interpret with assurance. It is to be noted that two values for the jumped does are lower than occurred in other experiments in our laboratory on spayed females (twenty in all) during this period. The data are at least consistent with the conclusion stated earlier.

#### IV. THE CALCIUM AND MAGNESIUM LEVEL IN SPAYED FEMALES.

During the period in which this investigation has been carried out we have made determinations of the magnesium and calcium content of the serum following the injection of various endocrine extracts into normal and spayed animals. Of these it may be of interest to other workers to remark that we could detect no change in the magnesium content of the blood following injection with parathyroid extracts kindly supplied by the Eli Lilly Co. and Messrs Parke Davis<sup>1</sup>.

In view of the observations recorded by Mirvish and Bosman and by Miss Watchorn it is perhaps desirable to place on record a summary of all our observations comparing the serum calcium and magnesium content of the blood of

<sup>1</sup> The amount injected was five times the quantity required to raise the serum calcium of a 20 kilo dog 1 mg. per cent.

normal female rabbits and rabbits from which the ovaries had been removed after sexual maturity. They provide no evidence that removal of the ovaries produces any significant effect of blood calcium or magnesium. The data are summarised below. All the figures given refer to does which had been kept in separate cages for at least one month before bleeding.

Table VI.

	A. Ovariectomised females		B. Normal females	
	Calcium	Magnesium	Calcium	Magnesium
(a) Sables	14.27	—	14.22	—
	13.92	—	14.57	—
	14.27	—	15.21	—
	14.75	2.96	—	—
	14.93	2.96	—	—
Mean value	14.43	2.96	14.67	—
(b) Havanas	13.22	—	14.86	—
	12.99	—	14.27	—
	14.04	—	15.33	—
	13.86	2.25	—	—
	14.63	1.81	—	—
Mean value	13.75	2.03	14.85	—
(c) Chinchillas	13.80	4.43	13.87	3.92
	14.99	3.03	14.57	3.15
	14.52	4.23	14.69	—
	13.93	3.07	13.57	2.33
	13.80	2.74	14.76	4.31
	13.10	3.68	15.29	2.58
	14.22	2.80	14.93	3.08
	15.17	2.14	13.09	2.16
Mean value	14.19	3.26	14.35	3.08
Mean values for all breeds taken together:				
	14.13	3.01	14.52	3.08

## V. DISCUSSION.

It will be evident to the reader that the information disclosed in the present communication raises more problems than it is capable of solving. Nevertheless a few suggestions may be deemed permissible at this stage of the enquiry. Perhaps the most striking positive outcome of the rapid progress which has been made in the physiology of mammalian reproduction during the past decade has been the increasing recognition that the underlying factors which co-ordinate the cyclical activity of the reproductive organs in different species are essentially similar, however different the manifestations of the reproductive cycle may appear to be at first sight. The characteristic features which the reproductive cycle displays in any given species appear to represent an exaggeration of features which are essentially universal in all mammals and, as our own work has shown, are represented by analogous phenomena in the lower vertebrates. In one mammalian species the vaginal changes, in another the uterine events, in another the characteristics of pseudo-



pregnancy associated especially with the mammary glands may be the most prominent visible indications of the internal mechanism which determines the periodic nature of the reproductive function. We might express this by saying that reproductive cycles are made up of a series of somatic responses to periodic changes in the internal environment of the circulatory fluids. The relative sensitivity of these responses to the co-ordinating stimuli of internal secretion varies from species to species. The essential components are the same in all and the necessary stimuli which bring them into operation are the same in all.

Adopting provisionally this attitude to the problem of mammalian reproduction approached from the standpoint of comparative physiology we may be permitted to speculate on the relation of such observations as we have recorded in this communication to the interpretation of the reproductive cycles of other mammals. The rabbit is peculiar (like the ferret) in that ovulation does not occur without the stimulus of sexual excitement. Parenthetically we may remark that our experiments show that profound internal changes are consequent upon sexual excitement involving the muscular movements associated with coitus, even if no actual penetration of the vagina occurs. This is significant in relation to the observations of Fee and Parkes (6), who claim that copulation induces ovulation in females after anaesthesia of the vagina. The most plausible way of interpreting the phenomena of reproductive activity in the rabbit is suggested by the following hypothesis. We may presume that in the rabbit as in other mammals there exists the same potential sequence of internal secretion as in other mammals. It is not unlikely to suppose that in other mammals pituitary secretion is reflexly activated by sexual excitement to some extent. The rabbit may be peculiar only in so far as the secretion of the anterior lobe hormone or hormones involved in activating the ovaries may never reach the requisite threshold for ovulation unless the additional stimulus of sexual excitement is superadded.

This approach to the problem suggests a field for further enquiry into the mechanism of periodicity. In order that a series of events such as occur in the ovarian cycle may form a closed chain with regular recurrence, each phase of the cycle must be the visible response to some stimulus which also activates the mechanism which is responsible for the manifestations characteristic of the succeeding phase. At present we have good reason to believe that pituitary secretion activates the ovaries. We know that certain changes in the accessory reproductive organs are the result of ovarian secretion. We also know that a normal consequence of the rupture of a ripe follicle is the production of a corpus luteum. Thus a simple possible hypothesis to account for the existence of a closed chain of sequences is that the secretion of the corpus luteum stimulates the pituitary to increased secretion, thus repeating the preceding sequence. This possibility could be tested experimentally in a large number of ways.

These reflections prompt us to ask whether the action of ovarian extracts discovered by Mirvish and Bosman is due to oestrin or to an autocoid of the corpus luteum. Mirvish and Bosman used ovaries with ripe corpora lutea. In a private communication Dr Mirvish informs us that he obtained better results when he

used luteal substance alone. A suggestive circumstance is the fact that the drop in blood calcium which followed injection of ovarian extracts in their experiments was only manifest after a latent period of twenty-four hours. Our experiments clearly show that the fall of serum calcium which follows injection of anterior lobe preparations is not a secondary consequence of stimulating the ovaries. On the other hand, the protracted latent period of the result obtained by Mirvish and Bosman as contrasted with the rapid onset of the effect which we have described is fully compatible with the supposition that the effect of ovarian extracts is not a direct one. It may be due to the stimulation of the pituitary by the luteal autocoid. At this stage we put forward this possibility merely as a basis for further enquiry.

#### VI. SUMMARY.

1. The injection of fresh saline suspensions of ox pituitary produces a prolonged fall in the calcium content of the serum of normal female rabbits and rabbits deprived of their ovaries.
2. After sexual excitement there is a fall of blood calcium in the female rabbit beginning within 4 hours from association with the buck, followed by a secondary rise at the time when ovulation occurs with a further fall subsiding by the time the corpus luteum is fully formed.
3. The removal of the ovaries from sexually mature rabbits does not produce any significant change in the calcium or magnesium level of the serum.

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