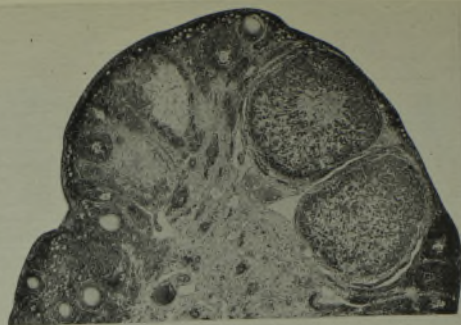
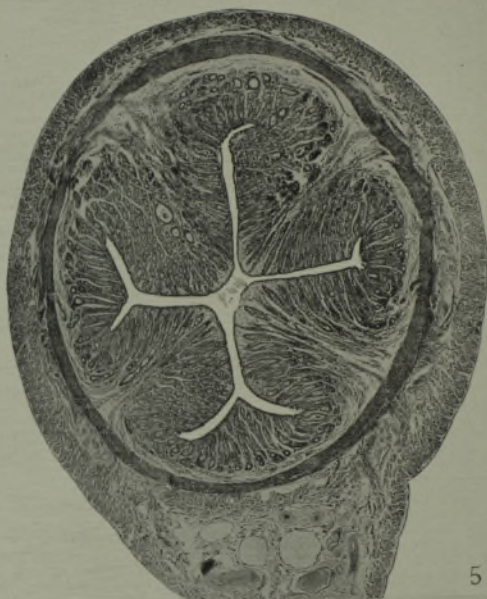


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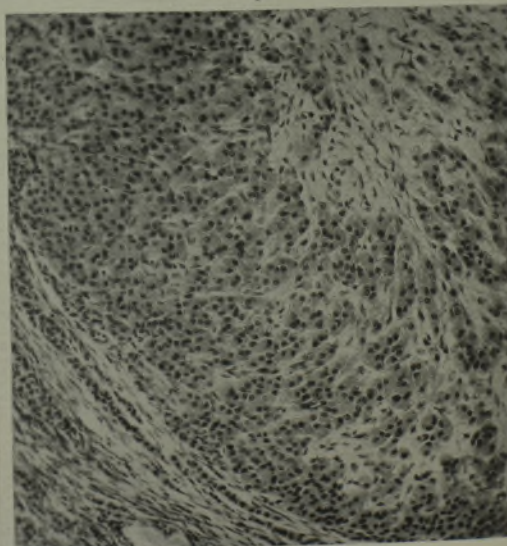


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*Studies on the Hypophysectomised Ferret. III.—Effect of post-coitus hypophysectomy on ovulation and the development of the corpus luteum.*

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(Communicated by Sir Henry Dale, Sec. R.S.—Received August 25, 1932.)

[PLATE 4.]

I. *Introduction.*

In animals which ovulate only after copulation it is necessary to suppose that nervous stimulation of the pituitary body results in the liberation of the ovulation-producing substance. Fee and Parkes (1929) were able to show that in the rabbit removal of the pituitary body (by decerebration) later than 1 hour after copulation did not prevent the ensuing ovulation; removal earlier had an inhibitory effect, which could, however, be overcome by injection of anterior lobe substance (Hill and Parkes, 1931). These results indicated that following copulation, the ovulation-producing hormone was secreted into the circulation with great rapidity. In addition, the removal of the pituitary body before ovulation was found not to derange the initial development of the corpus luteum so far as this could be followed on decerebrate preparations (Deanesly, Fee and Parkes, 1930).

Smith and White (1931) confirmed these results on rabbits hypophysectomised as an operation. They were not able to complete the removal sufficiently soon after copulation to inhibit ovulation, but they extended the work to a more prolonged study of the development of the corpus luteum. The growth of this body in the hypophysectomised rabbit was normal for about 2 days, but afterwards became atypical.

The technique described in Part I of the present series has enabled us to extend this type of work to the other mammal definitely known to ovulate only after copulation—the ferret. This animal has the advantage over the rabbit that the time between copulation and ovulation is rather more than three times as long, *i.e.*, about 36 hours, and that pseudo-pregnancy, during which the life-history of the corpus luteum may be studied, lasts about 6 weeks (Hammond and Marshall, 1930). On the other hand, the fact that copulation takes at least three-quarters of an hour makes it difficult to remove the pituitary

within less than about  $1\frac{3}{4}$  hours of the commencement of the act. No attempt will be made here to describe the ultimate effects of hypophysectomy on the ferret ovary.

## II. *Material and Technique.*

The hypophysectomy technique has been described in Part I, together with the method of examination at autopsy. In the animals dealt with in the present paper, sections through the base of the skull showed no tissue which could positively be identified as hypophyseal, though one ferret (RHF 15) had two small pieces of tissue which were probably remains of anterior lobe, and two other animals each had a minute fragment which may possibly have been of pituitary origin. The remaining five ferrets so far as could be seen were completely devoid of pituitary tissue.

Two stages in the post-hypophysectomy changes in the ovaries have been obtained from most of the animals by performing a unilateral ovariectomy soon after ovulation should have taken place, and afterwards killing the animal during what would have been the ensuing pregnancy. In some cases where the ruptured follicles were abnormal serial sections of the Fallopian tube were cut. The presence of tubal ova was taken as a definite indication that ovulation had occurred.

The usual histological technique was employed.

## III. *Effect on Ovulation.*

Table I shows the effect on ovulation of removal of the pituitary gland up to 15 hours post-coitum. It is immediately apparent that ovulation is not inhibited by even the earliest ablation achieved—1 hour 50 minutes after the commencement of copulation. It is doubtful if the operation can be speeded up appreciably, and it is most probable, therefore, that it will not be possible to inhibit ovulation in the ferret by post-coitus hypophysectomy. This result, however, implies no criticism of the view that the anterior lobe is directly responsible for ovulation in the ferret as in other animals; the position is the same as in Smith and White's work on the rabbit, *i.e.*, a complicated operation is not sufficiently rapid to remove the pituitary body before it has secreted the relevant substance into the circulation. Ferrets probably fail to ovulate if copulation is interrupted before the end, and it might thus be justifiable to regard the stimulation of the anterior lobe as dating from the end of copulation. In this case the rate of secretion of the ovulation-producing substance would be astonishingly rapid compared with the time taken for its



Table I.—Effect of Hypophysectomy on Ovulation and the Development of the Corpus Luteum.

Number of animal.	Time of hypophysectomy after copulation (hours).	Left ovary.		Right ovary—Autopsy.		Condition of uterus at autopsy.	Condition of pituitary fossa.
		Time removed after copulation (hours).	Ovulation.	Time after copulation (days).	Average diameter corpora lutea (mm.).		
RHF 13	10·15	58	7 r.f.	18	0·77 (5)	No pseudopregnant development	Possible fragment of A.P.
RHF 14	4·00	70	1 r.f.	28	0·78 (6)	"	Devoid of A.P.
RHF 15	15·15	58	6 r.f.	21	1·22 (4)	"	Fragment of A.P.
RHF 16	12·00	83	6 r.f.	17	0·86 (4)	"	Devoid of A.P.
RHF 18	7·00	78	1 r.f.	11	0·91 (4)	"	"
RHF 19	2·30	116	8 r.f.	8	1·05 (3)	"	Possible fragment of A.P.
RHF 22	1·50	96 (both ovaries)	7 r.f.	—	—	"	Devoid of A.P.
RHF 23	1·50	96 (both ovaries)	5 r.f.	—	—	"	"

A.P. = anterior pituitary lobe. r.f. = ruptured follicles.

effect to appear. It is likely, however, that in the ferret stimulation of the anterior lobe by the act of copulation is a much slower process than the trigger mechanism of the rabbit, and we have therefore preferred to adopt the more comprehensive method of dating the experiments from the beginning of copulation. On this reckoning, the ovulation-producing substance is secreted into the circulation in under 2 hours after the first stimulus to the pituitary body. The actual time may be very short. Even on this over-estimate, the time is relatively less than in the rabbit, in which the pituitary body is necessary after copulation for a period equivalent to rather less than 10 per cent. of that taken for ovulation to ensue. The corresponding figure in the ferret is certainly not more than 5 per cent. at the maximum. The significance of this difference is not obvious.

#### IV. *Effect on the Development of the Corpus Luteum.*

Although segmenting ova were found in the left Fallopian tubes when the left ovaries were removed after ovulation, none of the hypophysectomised animals showed any signs of being pregnant at autopsy. This sterility is probably due to the lack of development of the corpora lutea described below.

According to Hammond and Marshall (1930) the corpus luteum of the pregnant or pseudopregnant ferret reaches its maximum diameter of about 2 mm. in the 3rd to 5th weeks after copulation, fig. 2, Plate 4. Our own experience entirely agrees with this. The diameter of the ruptured follicle 48 hours after coitus is of the order of 1.1–1.2 mm. and the changes in the corpus luteum during its development therefore represent a volume increase from about 0.8 c.mm. to about 4 c.mm. This growth curve is roughly indicated by the upper line on fig. 1.

In the hypophysectomised animals we have obtained a series of corpora lutea up to 4 weeks after copulation, hypophysectomy having been carried out at the times after copulation stated in Table I. The largest corpora lutea were found in the right ovary of RHF 15 obtained 21 days post-coitum; the average diameter of the four corpora lutea present was 1.22 mm., representing a volume of about 0.95 c.mm. These corpora lutea were thus about one-quarter the normal size. This animal, however, probably had two fragments of anterior lobe left in. The remaining five stages obtained, at 8, 11, 17, 18 and 28 days post-coitum, showed greater degeneration of the corpus luteum, figs. 3 and 4, Plate 4. The least atrophic corpora lutea were obtained from the right ovary of RHF 19 and had a mean diameter of 1.05 mm. (volume 0.616 c.mm.).



The most atrophic were obtained from RHF 13 and 14 (mean volumes 0.239 and 0.247 c.mm.) at 18 and 28 days post-coitum. The mean volumes of the corpora lutea of the hypophysectomised ferrets are plotted in fig. 1 in comparison with the approximate growth curve of the normal. There would appear to be a gradual falling off in size from immediately after ovulation; at the time when pseudopregnancy should be fully developed the corpus luteum is less than one-tenth normal size. Since the corpus luteum fails to develop after ovulation, it is rather remarkable that the atrophic structure persists

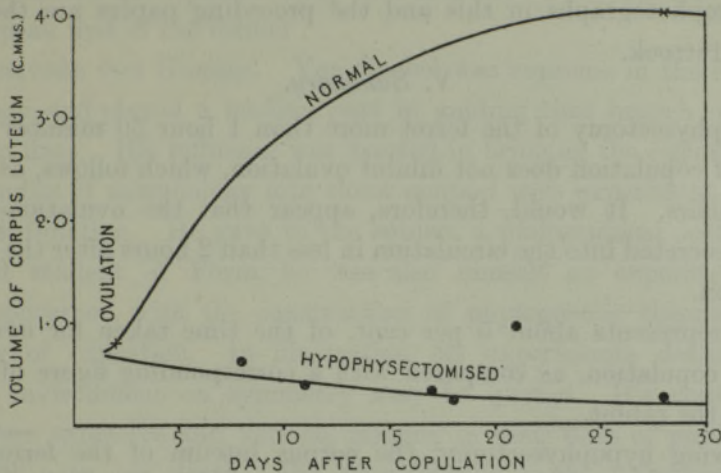


FIG. 1.—Size of the corpus luteum after post-coitus hypophysectomy compared with approximate growth curve of the normal.

for even as long as a month. This persistence of the degenerate corpus luteum after ovulation has, however, been noted by Smith (1930) in the rat, and is probably due to lack of competition from the follicular apparatus. A similar state of affairs was found in the mouse after obliteration of the follicles by X-rays (Brambell, Fielding and Parkes, 1928). We have not yet determined the ultimate fate of the corpus luteum in the hypophysectomised animal.

Histologically, the corpora lutea in the hypophysectomised animal show a curious condition of arrested development. Most of them retain the general structure of the young corpus luteum, *i.e.*, luteal tissue surrounds a central core of fibroblastic tissue, with a general drift of the cells towards this core and the now occluded ovulation point. At 8 days post-coitum the actual cells of the corpus luteum of the hypophysectomised animal are very similar to the cells of the newly ruptured follicle, *i.e.*, little change has occurred in the old granulosa cells. The nuclei are still large and seem in good condition. There is a remarkable lack of cohesion between the cells, a feature which persists in the older corpora lutea. During the next 3 weeks the cells shrink slightly

and the nuclei, in particular, become smaller. At 28 days post-coitum the cells are not more than a quarter the diameter of those of the corresponding normal corpus luteum, fig. 6, Plate 4. The general picture is essentially different from that presented by the corpus luteum degenerating after pseudopregnancy and is typically one of arrested development.

Judging by the lack of pseudopregnancy changes in the uteri of the hypophysectomised animals, fig. 5, Plate 4, the stunted corpora lutea perform no endocrine function.

The microphotographs in this and the preceding papers are the work of Mr. F. J. Pittock.

#### V. Summary.

(1) Hypophysectomy of the ferret more than 1 hour 50 minutes after the beginning of copulation does not inhibit ovulation, which follows, as usual, in about 36 hours. It would, therefore, appear that the ovulation-producing hormone is secreted into the circulation in less than 2 hours after the beginning of copulation.

(2) This represents about 5 per cent. of the time taken for ovulation to occur after copulation, as compared with a corresponding figure of about 10 per cent. in the rabbit.

(3) Following hypophysectomy, the corpus luteum of the ferret fails to develop, and at 1 month after the operation the volume is less than one-tenth that of the normal corpus luteum of the same age.

(4) The animals fail to get pregnant after hypophysectomy before ovulation, and the uterus shows no signs even of pseudopregnant development.

#### DESCRIPTION OF PLATE.

FIG. 2.—Corpora lutea of the ferret on the 28th day of pseudopregnancy.  $\times 20$ .

FIG. 3.—Corpora lutea (median section) of RHF 19, right ovary, 8 days after hypophysectomy carried out 1 hour 50 minutes after copulation.  $\times 20$ .

FIG. 4.—Corpora lutea (median section) of RHF 14, right ovary, 28 days after hypophysectomy carried out 4 hours after copulation.  $\times 20$ .

FIG. 5.—Uterus of RHF 14, showing lack of pseudopregnant development.  $\times 20$ .

FIG. 6.—High power of part of corpus luteum in fig. 4, showing small size of cells and lack of cohesion.  $\times 140$ .

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