treatment with thyroxine (Table). The gain in gland weight is specific since the body weight was similar in both groups of animals; it decreased by about 15–20% in 7 weeks.

The threshold dose to acetylcholine was found to be 0.5–5 μg/kg after hypophysectomy. Treatment with thyroxine seemed to lower the threshold dose; e.g. 0.5 μg acetylcholine/kg or lower was the threshold dose in 40% after hypophysectomy alone but in 90% after treatment with thyroxine.

The maximal secretory responses to chorda stimulation or pilocarpine were increased after treatment with thyroxine by about 150–200% when expressed per gland. The increase in maximal flow rate was more marked than the gain in gland weight in thyroxine-treated animals. Therefore, the maximal secretory responses were also augmented when expressed per unit weight (Table).

The sensitivity of the rat's submaxillary gland to parasympathomimetics is affected by the hypophysial gland; it is decreased after hypophysectomy[4]. The endocrine sensitivity control seems to be at least partly via the thyroid gland since the threshold dose of acetylcholine was decreased in hypophysectomized animals treated with thyroxine.

The maximal secretory responses to chorda stimulation or pilocarpine have previously been estimated in unoperated controls[7]. The maximal flow rate was markedly decreased after hypophysectomy both when expressed per gland and per unit weight. It was increased in hypophysectomized rats after treatment with thyroxine but still somewhat lower than that of controls[7] when expressed per gland. The well-known glandular atrophy after hypophysectomy which is located to the tubules of the gland, was only partly abolished by treatment with thyroxine. Therefore, the maximal secretory responses in thyroxine-treated animals were similar or higher than those of controls[7] when expressed per unit weight. These results indicate an important role for thyroxine regarding the maximal flow rate of the gland and they further strengthen a previous suggestion[8] that the tubules of the gland to a large extent determine the parasympathetic secretion.

Zusammenfassung. Das Gewicht der Submaxillarisdrüse der Ratte nimmt nach Wegnahme der Hypophyse ab; die Gewichtsabnahme wird teilweise nach Behandlung mit Thyroxin verhindert. Die Empfindlichkeit der Drüse gegen parasympathische Substanzen und die maximale Fähigkeit zur Sekretion sind in hypophysenlosen Ratten verringert, aber nach Behandlung mit Thyroxin wieder gesteigert.

P. Ohlin

Institute of Physiology, University of Lund (Sweden), 12 September 1967.

[8] P. Ohlin, Acta Univ. lund. II. 7, 1 (1966).

Effect of Copulation or Vaginal Stimulation on Melanocyte-Stimulating Hormone Content of the Hypophysis

Copulation has been shown to provide a stimulus which triggers off the mechanism that produces liberation of gonadotropins in the so-called 'reflex ovulators' animals like the rabbit[1], as well as in the rat in which ovulation occurs 'spontaneously'[2]. Since copulation and vaginal stimulation were also shown to induce pseudopregnancy, a situation in which the release of ovulating hormones is inhibited and that of luteotrophic hormone (LtH) is enhanced, a dual effect of these stimuli on the hypothalamus could be presumed. Secretion of melanocyte-stimulating hormone (MSH), which is to a certain extent controlled by a similar mechanism to that of LtH, may also provide information about the effect of such stimuli on the hypothalamus.

The effect of coitus on pituitary MSH content was studied in male and female albino rats. To study the effect in males 2 or 3 of them were placed in a cage with 2 or 3 females pretreated with estrogen-progesterone to increase their receptivity. For each animal the sexual behaviour was allowed to proceed for a period of about 10 min after the first mounting, which was taken as the beginning of the test. Only those males which displayed active sexual behaviour with repeated mountings and intromissions during the test period were used as pituitary donors.

Female donor animals were used in the afternoon of the day of proestrus. Two or 3 of them were placed in cages with 3 or 4 vigorous, active males. The first intromission was taken as the beginning of the test and sexual behaviour was allowed to proceed for about 10 min. During this time repeated intromissions occurred and at the end of this period a mucus plug was usually found in the vagina. Only those females receptive to the males and showing lordosis during coitus were used.

The animals were killed with ether 1 h after mating and their pituitaries were taken immediately, weighed, suspended in an appropriate volume of distilled water and kept frozen until the MSH was assayed. Pools of 2 glands were used in each determination.

In a group of rats vaginal stimulation were made by means of a glass rod during 2 min the day after the vaginal smears showed a typical proestrous stage. The animals were killed 30, 60 or 120 min later and the hypophyses were taken for MSH determination.

MSH was assayed in vitro using the skin of toads as test material[3]. The hypophyses were assayed at 2 dose levels and the activity/mg gland was compared to that of control animals in the same stage of the cycle. Control pituitary MSH concentration was also tested at 2 dose levels and taken as 100%. Five to 7 pieces of skin were assigned to each point and the interval between doses was

[1] J. Hillard, J. N. Hayward and C. H. Sawyer, Endocrinology *75*, 957 (1964).

[2] S. Taleisnik, L. Caligaris and J. J. Astrada, Endocrinology *79*, 49 (1966).

[3] S. Taleisnik and R. Orías, Am. J. Physiol. *208*, 293 (1965).

2-fold. Standard statistical methods for parallel lines were used[4].

As Table I shows, 1 h after copulation there was a highly significant drop in MSH concentration of the pituitary in male as well as in female rats. The mean concentration was 61% as compared with the control animals in 3 experiments made in male rats and 48.7% in the female rats. Only in 1 group of male rats the decrease in pituitary MSH activity after copulation was not significantly different from the normal control animals, while in the female rats a highly significant decrease was observed in the 3 groups which were studied.

The effect of vaginal stimulation with a glass rod on the concentration of MSH of the pituitary was studied in proestrous rats at varying times after the stimulus. As is shown in Table II, no changes in pituitary MSH concentration were observed 30 min after vaginal stimulation, but at 60 min a highly significant decrease took place and an even lower pituitary MSH concentration was found 1 h later.

Table I. Changes in pituitary MSH concentration 1 h after copulation in male and female rats

Experimental group	Experiment No.	Pituitary MSH activity[a]	λ[b]	P[c]
Male rats	1	74.9 (45.5–108.6)	0.1636	> 0.05
	2	43.4 (22.6– 66.3)	0.1321	< 0.001
	3	61.3 (39.2– 84.1)	0.1306	< 0.001
	mean	61.0 (48.3– 77.4)		
Female rats	4	50.4 (34.2– 64.7)	0.1081	< 0.001
	5	52.4 (31.5– 70.4)	0.1345	< 0.001
	6	44.2 (28.8– 57.8)	0.1069	< 0.001
	mean	48.7 (40.1– 59.2)		

[a] % of activity/mg referred to that of control pituitaries. In parenthesis 95% confidence limits. [b] λ, index of precision. [c] P, probability of difference compared with controls.

Table II. Effect of vaginal stimulation on pituitary MSH concentration

Time after stimulation	Experiment No.	Pituitary MSH activity[a]	λ[b]	P[c]
30 min	1	134.0 (92.6–243.2)	0.1677	n.s.
	2	118.3 (83.5–185.8)	0.1551	n.s.
	3	88.7 (54.3–136.5)	0.1642	n.s.
	mean	112.7 (89.9–141.3)		
1 h	4	60.9 (43.2– 77.2)	0.1120	< 0.001
	5	66.3 (40.2– 90.1)	0.1388	< 0.02
	6	58.3 (31.9– 80.9)	0.1440	< 0.01
	mean	61.9 (50.8– 75.3)		
2 h	7	38.3 (28.3– 48.4)	0.0606	< 0.001
	8	53.9 (31.3– 73.1)	0.1280	< 0.001
	mean	42.7 (34.7– 52.4)		

[a] % of activity/mg referred to that of control pituitaries. In parenthesis 95% confidence limits. [b] λ, index of precision. [c] P, probability of difference compared with controls. n.s., non significant.

Release of LH has been shown to occur after mating in male and female rats which results in an increase of the hormone in blood and a decrease in its pituitary content[2]. The present study shows that MSH has to be added to the list of pituitary hormones which can be influenced by this stimulus.

Although no evidence could be obtained of a release of LH after vaginal stimulation in the rat[2], it was shown that ovulation can be induced in the estrous cat[5] and in rabbits primed with estrogen[6]. This fact would indicate that vaginal stimulation is able to induce gonadotropin release. Also prolactin content in the pituitary has been demonstrated to decrease after vaginal stimulation[7]. The results of the present investigation show that MSH release also occurs after such stimuli.

Since the release of LH involves an hypothalamic activation, while, on the contrary, the release of MSH depends on a depression of the hypothalamic activity, the secretion of both hormones could not take place simultaneously. Such a situation is evident during suckling when MSH is released[8] while LH is inhibited[9]. The fact that release of LH and MSH can be activated by copulation or vaginal stimulation does not rule out the possibility that both hormones are released, not simultaneously but one after the other. Unfortunately it is not possible with the data of the present and previous works to draw conclusions about the existence of a sequence in the secretion of LH and MSH. If this were the case, excitatory and inhibitory impulses should arrive at the hypothalamus in a certain sequence within a relatively short period of time. The possibility also exists that copulation and vaginal stimulation activate MSH secretion by stimulating the release of MSH-relcasing factor[3] providing in this way physiological evidences for the existence of this factor[10].

Résumé. Dans le rat, 1 h après la copulation, la concentration de l'MSH de l'hypophyse présente une diminution statistiquement significative, autant chez le mâle que chez la femelle. La stimulation vaginale avec une baguette de verre provoqua 60 et 120 min plus tard une diminution de la concentration de plus de 50% de l'MSH de l'hypophyse. Au bout de 30 min, on n'a observé aucun changement. On en déduit que la copulation ou la stimulation vaginale libère l'MSH.

S. TALEISNIK and MARÍA E. TOMATIS

Instituto de Investigación Médica Mercedes y Martín Ferreyra, Córdoba (Argentina), 17 July 1967.

[4] C. T. BLISS, *The Statistics of Bioassay* (Academic Press, New York 1952).
[5] W. W. GREULICH, Anat. Rec. *58*, 217 (1934).
[6] C. H. SAWYER and J. E. MARKEE, Endocrinology *65*, 416 (1959).
[7] U. HERLYN, H. F. GELLER, I. v. BERSWORDT-WALLRABE and R. v. BERSWORDT-WALLRABE, Acta endocr., Copenh. *48*, 220 (1965).
[8] S. TALEISNIK and R. ORÍAS, Endocrinology *78*, 522 (1966).
[9] S. M. MCCANN, T. GRAVES and S. TALEISNIK, Endocrinology *68*, 873 (1961).
[10] This investigation was supported by grant No. NB 04732-03 from the NIH, US. Public Health Service.