was estimated with Zeiss particle size analyzer. The ovary of the other side was dehydrated without any fixation in polyethylene glycol 1000, embedded in the same substance[8] and cut into 10-μ sections which were incubated according to WATTENBERG[9]. For control of the specificity of the enzyme, some sections were at times incubated without substrate (dehydroepiandrosterone).

In the immature ovary of *A. pulcher* (controls and other untreated fishes) there is little but distinct activity of steroid-3β-ol dehydrogenase (Figure 2). The enzyme is mostly bound to such eggs as seem to be oocytes I or II by their size. In these structures, there is also a large amount of RNA. 'Corpora atretica' could not be detected in these ovaries.

On the first day after injection of LtH (0.05 IU/g body weight, 1 day) the amount of RNA is distinctly increased in comparison with controls. Also, in this case, the oocytes I and II are stained especially intensely. The activity of steroid dehydrogenase was somewhat weaker than in controls. 2 days after injection there was no change in the result. 3 days after injection and a further application of the same dose on the second day (0.1 IU/g body weight, 3 days) the RNA was weaker than in controls, merely oocytes I and II were stained somewhat faster. The nucleoli were enlarged and partially the membrane of the nuclei seemed to disintegrate. Hypertrophy of the nuclei could not be observed unambiguously. The activity of steroid dehydrogenase however was very strong (Figure 3). It may be concluded that the amount and, by this, its activity, is increased. The ovaries of the fourth group (0.1 IU/g body weight, 4 days) showed similar results but the RNA was somewhat increased. It may also be stated that in the epithelial cells of the oviducts of group 3 and 4 there is a distinct activity of steroid dehydrogenase which was never observed in controls.

It may be concluded that ovine prolactin is able to stimulate short-termed activity of steroid-3β-ol dehydrogenase in fish ovaries, and that there is an influence on the nuclei of immature eggs. It induces an increase of RNA synthesis in the ovary which is possibly correlated directly with the formation of an enzyme which has a decisive function at the beginning of steroid synthesis. Because the lack of distinct 'corpora atretica' in all ovaries examined, it is possible that the endocrine activity of these structures proceeds in a very early stage of development which is not to be distinguished clearly from normal immature eggs. That means that the sort of structures which are normally described as 'corpora atretica' may possibly be depots only. Otherwise it is possible that, in our case, the immature eggs are not changed at all in 'corpora atretica', but have a secretory phase influenced by prolactin and are developed normally thereafter[10].

Zusammenfassung. Injektionen von Schafprolaktin (0.05–0.10 IE/g Körpergewicht) lösen bei unreifen weiblichen Buntbarschen der Art *Aequidens pulcher* innerhalb von 2–3 Tagen eine starke Erhöhung der Aktivität der 3β-ol-Steroiddehydrogenase in den Ovarien aus. Dies deutet darauf hin, dass auch bei Fischen LtH oder ein ähnliches Hormon in die Regulation des Gonadensteroidstoffwechsels eingreift.

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Preliminary Indications for the Presence of a Hypothalamic Follicle Stimulating Hormone Synthesizing Factor

CORBIN and STORY[1], who had used a modification of the in vivo procedure of DAVID et al.[2] for the evaluation of hypothalamic follicle stimulating hormone releasing factor (FSH-RF) activity, reported the time course of pituitary FSH depletion and resynthesis in the rat after a single i.v. administration of stalk-median eminence (SME) extract. It was reported that maximum pituitary FSH depletion occurs 45 min after the injection, with normal pituitary FSH levels being restored approximately 4 h after treatment. If an FSH synthesizing factor (FSH-SF) were present in the hypothalamic extract, then a second injection of the extract, given at the time of maximum pituitary FSH depletion (45 min), might induce the resynthesis of hypophysial FSH at a rate faster than that seen in the animal that received only a single injection of the hypothalamic extract. This report provides preliminary data on the effect of 2 injections of SME extract on the resynthesis of pituitary FSH. The results suggest that the hypothalamus may contain a factor responsible for the synthesis of pituitary FSH.

The SME's were derived from 60- to 70-day-old, intact, normally cycling Sprague-Dawley (S-D) female rats. They were homogenized in 0.1*N* HCl, centrifuged, and the supernatant material was immersed in a boiling water bath for 10 min and then diluted with the acid to a concentration corresponding to 2 SME/ml. Intact, mature male rats (S-D, 200 ± 7 g) were used as the recipients of the SME extracts (5 rats/group). The material was injected into the jugular vein; a second treatment was administered 45 min after the initial injection. The rats were decapitated at varying intervals after treatment: the pituitaries were removed, weighed, pooled, homogenized and suspended in physiological saline for employment in the FSH assay of STEELMAN and POHLEY[3]. A

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2 + 2 design was used (5 rats/dose): the NIH-FSH-S3 reference standard was used at 50 and 100 µg, total dose, and the male pituitary homogenate at 1.5 and 3.0 mg wet weight, total dose. The results are expressed in terms of NIH-FSH-S1. 2 identical replications were performed.

The results (see Table) demonstrate that, after a single i.v. injection of SME extract, significant ($p \leqslant 0.05$) pituitary FSH depletion occurred at 45 min, with normal FSH stores being replenished between 2 and 4 h. When a second injection of the SME extract was administered at 45 min, no further pituitary FSH depletion occurred; in fact, depletion appeared to be halted, since the FSH concentration was restored to resting levels within 30 min after the second injection, and was similar to that concentration found in rats sacrificed 2 h after a single administration. Furthermore, when a second SME injection was administered at 45 min and the animals were killed 1 h later, the pituitary FSH concentration approximated that of an animal sacrificed 4 h after a single injection of the SME extract. Since the second injection was unable to induce a further pituitary FSH depletion, it appeared that the animals had responded maximally to the FSH-RF component of the initial injection of the extract. The hypophysial FSH concentrations of double injected rats reached maximum values between 2–4 h after the second injection; these values were significantly ($p \leqslant 0.05$) greater than those of the initial cortex-injected controls. Additionally, the resynthesis of pituitary FSH was stabilized within 2 h after the second injection.

These preliminary data appear to indicate the presence of hypophysiotropic factors within the hypothalamus that are capable of activating, in addition to the release, the

Rat pituitary FSH depletion and resynthesis following 1 and 2 jugular vein injections of SME extracts

Treatment	Assay No.	Time interval, treatment to sacrifice	(Male recipient) µg FSH/mg pituitary	% FSH depletion	λ[b]
Uninjected	1	–	19.3 (14.8–24.1)[a]	–	0.173
	2	–	22.0 (12.7–38.3)	–	0.201
Injection No. 1					
Cerebral cortex	1	45 min	20.4 (14.6–30.6)	0 (initial	0.185
	2	45 min	24.7 (13.5–45.2)	0 control value)	0.218
2 SME/rat	1	15 min	17.9 (14.5–24.5)	12.3	0.162
	2	15 min	21.0 (11.9–37.0)	15.0	0.204
			unweighted mean	13.6	
2 SME/rat	1	30 min	16.0 (11.8–21.6)	20.0	0.169
	2	30 min	19.3 (11.2–33.4)	21.9	0.199
			unweighted mean	20.9	
2 SME/rat	1	45 min	11.8 (9.0–15.7)	42.2	0.171
	2	45 min	13.7 (7.9–20.8)	44.5	0.201
			unweighted mean	43.4	
2 SME/rat	1	1 h	17.6 (13.5–22.9)	13.8	0.165
	2	1 h	20.0 (11.6–34.6)	19.0	0.199
			unweighted mean	16.4	
2 SME/rat	1	2 h	20.0 (15.1–26.4)	6.1	0.163
	2	2 h	23.0 (13.5–39.3)	6.9	0.193
			unweighted mean	6.5	
2 SME/rat	1	4 h	21.7 (15.5–30.4)	no depletion (+ 3.7%)	0.173
	2	4 h	25.7 (16.0–46.5)	no depletion (+ 4.0%)	0.209
			unweighted mean	no depletion (+ 3.9%)	
Injection No. 2[c]					
Cerebral cortex	1	1 h	18.8 (13.7–25.8)	7.9	0.163
	2	1 h	24.2 (13.6–44.1)	2.0	0.213
			unweighted mean	4.9	
2 SME/rat	1	30 min	19.2 (13.0–28.0)	5.9	0.180
	2	30 min	24.6 (13.3–45.5)	0.4	0.222
			unweighted mean	3.4	
2 SME/rat	1	1 h	23.6 (17.8–31.4)	no depletion (+ 15.1%)	0.163
	2	1 h	27.0 (15.5–47.0)	no depletion (+ 9.3%)	0.201
			unweighted mean	no depletion (+ 12.2%)	
2 SME/rat	1	2 h	28.1 (19.6–40.0)	no depletion (+ 37.7%)	0.175
	2	2 h	32.0 (18.4–51.6)	no depletion (+ 29.9%)	0.200
			unweighted mean	no depletion (+ 33.8%)	
2 SME/rat	1	4 h	30.0 (23.0–39.0)	no depletion (+ 42.1%)	0.163
	2	4 h	33.4 (17.9–62.1)	no depletion (+ 35.2%)	0.226
			unweighted mean	no depletion (+ 38.6%)	

[a] Mean (95% confidence limits), [b] index of precision, [c] performed 45 min after injection No. 1.

synthesis of FSH. The FSH concentration of the rats sacrificed 4 h after the single injection rose slightly above that of the uninjected or cortex-injected controls; this may not simply be a 'rebound' phenomenon, because the second injection of the SME extract further increased the FSH concentration.

Support for the concept of a hypothalamic FSH-SF has been provided by Nikitovitch-Winer et al.[4], who employed hypophysectomized females with pituitary autografts under the kidney capsule. Such grafts become cytologically de-differentiated and lose their functional status. Upon infusion of median eminence extracts into the renal artery, the grafts were reactivated, as evidenced by the re-appearance of PAS + 'gonadotrophs' and ovarian follicular development. Thus, both FSH and LH synthesis, as well as release, had occurred in response to the hypothalamic extracts. Critchlow et al.[5], using a similar method, demonstrated the presence of a hypothalamic factor with ACTH-synthesizing activity, while the in vitro study of Sinha and Meites[6] revealed the presence of a thyrotropin-synthesizing substance.

Clementi et al.[7], studying the effects of a single intracarotid injection of hypothalamic extract on the ultrastructure of the growth hormone (GH) producing cells of the rat pituitary gland, have shown that these cells undergo changes which are associated with the release and resynthesis of GH with time. It would be of interest to know if a second injection of the hypothalamic extract could accelerate the ultrastructural alterations observed during the resynthetic phase.

Additional experiments are being designed that will utilize male rats with median eminence lesions, in order to explore further this conjectured FSH-SF phenomenon. It remains to be determined whether the hypothetical FSH-SF is identical with or different from FSH-RF.

Résumé. Après une période de 45 min, une seule injection intrajugulaire des extraits hypothalamiques provoque une chute maximale du niveau de FSH hypophysaire, qui atteste la présence de FSH-RF. Quand une seconde injection des extraits est administrée 45 min plus tard, la FSH hypophysaire est rétablie à une vitesse supérieure à celle observée chez les animaux qui n'ont reçu qu'une seule injection. Les résultats indiquent la présence possible d'un «FSH-Synthesizing Factor» (FSH-SF).

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The Action of Estrogens on the Sebaceous Glands of the Guinea-Pig's Nipple

In acne vulgaris the sebaceous glands play a very important role. On the other hand, the sex hormones are also of importance, as is proved by the occurrence of this disease at puberty. The treatment of acne vulgaris with estrogens in women seems to be indicated: 'Seit den von Ebling, Lasher, Haskin, Rothman zwischen 1948 und 1953 beschriebenen Versuchen ist allgemein bekannt,... dass die östrogene Wirkung zur Atrophie der Talgdrüsen führt'[1].

We controlled, in the nipple of the guinea-pig, the generally accepted fact that there is an atrophy of the sebaceous glands under the influence of estrogens. Estrogens provoke a very marked acanthosis of the nipple. According to our previous experiments[2,3], acanthosis is regularly accompanied by a noticeable increase in the size, and apparently in the number, of the sebaceous glands.

Experiments were made to show whether a nipple with acanthosis due to the action of estrogen, represents an exception to our previous results.

Materials and methods. We administered per os 0.05 µg of hormoestrol (p.p'-dioxydiphenylhexane) every day to 36 male guinea-pigs. The animals were divided into groups and the nipples were excised 1, 2, 5, 10 and 20 days after the beginning of the experiment. We also excised the nipples of 6 control guinea-pigs who received no treatment. The sebaceous glands of both the treated and control animals were examined microscopically for a possible change in size and number.

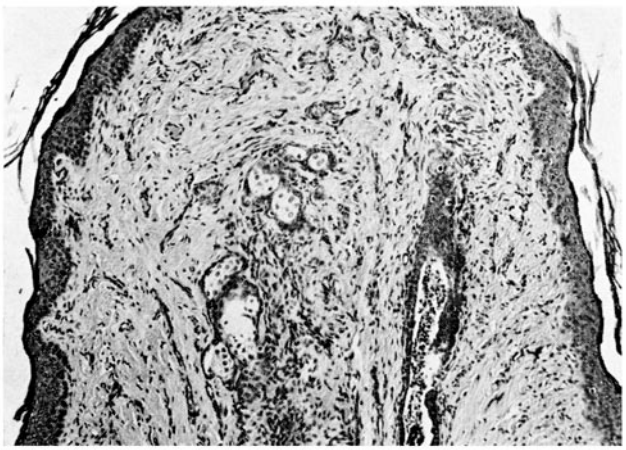

Fig. 1. Nipple of control guinea-pig. Epidermis is normal. Sebaceous glands are small. × 125.

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