The Influence of Prolactin on the Activity of Steroid-3β-ol Dehydrogenase in the Ovaries of the Cichlid Fish *Aequidens pulcher*

As in mammals, the reproduction and parental care of fishes is regulated by hormones, but only a few details are known. Experiments with the Mediterranean fish *Crenilabrus ocellatus*[1] and the tropical Cichlid fish *Symphysodon aequifasciata axelrodi*[2-4] gave some evidence that there is an important hormonal control system in these animals, similar to the lactogenic hormone (prolactin, LtH) in mammals. Especially in the latter species, it is possible to induce arbitrary behaviour patterns of parental care and changes in skin and gonads by injections of mammalian prolactin. The question arises whether LtH alone induces these effects, or whether there is a subordinate hormone gland which is responsible for parental care. By analogy with mammals prolactin possibly influences the ovary and induces the production of gonadal hormones which are steroids in fishes as in mammals[5].

To study these hormonal conditions, it would be necessary to relate an incretory function to a certain histological structure of the ovary. BRETSCHNEIDER and DUYVENE DE WIT[6] described immature transformed eggs in the ovary of *Rhodeus amarus* – so-called 'corpora atretica' – supposing that these secrete a hormone which lengthens the ovipositor before spawning. It is now known that this supposition is improbable, but the function of these 'corpora atretica' as an endocrine organ is still discussed by some authors. This function can only be demonstrated if a metabolic step is available which is bound to endocrine functions, and if the correlation of this with specific influences is studied.

The results presented in this paper may give some further evidence that certain structures in the ovaries of fishes are able to act as endocrine glands. It is presumed that – as in mammals – a characteristic enzyme of steroid hormone synthesis – steroid-3β-ol dehydrogenase – is proof of the formation of gonadal steroids.

In our experiments we used 20 female *Aequidens pulcher* with immature but well-developed ovaries (Figure 1). The animals were divided into 5 groups and kept in separate basins at 27 °C with a 12:12-h photoperiod. The first group served as control, i.e. 1 of the control animals was sacrificed at the same time as the single test groups. On the first day, all test groups received an injection of 0.05 IU ovine (NIH-P-S6)/g body weight into the dorsal musculature. The first test group was checked on the next day, the second 1 day later. At the same time, the 2 remaining groups received further injections of 0.05 IU LtH/g body weight. They were sacrificed on the following 2 days.

The ovary of the one side was embedded in paraffin after fixation in BOUIN's fluid and cut into 7-μ sections. They were stained alternately with aldehyde fuchsine (GÖMÖRI)-GOLDNER and with methylene blue according to PISCHINGER[7] for estimation of RNA. Thereafter, the sections were photographed and the size of the nuclei

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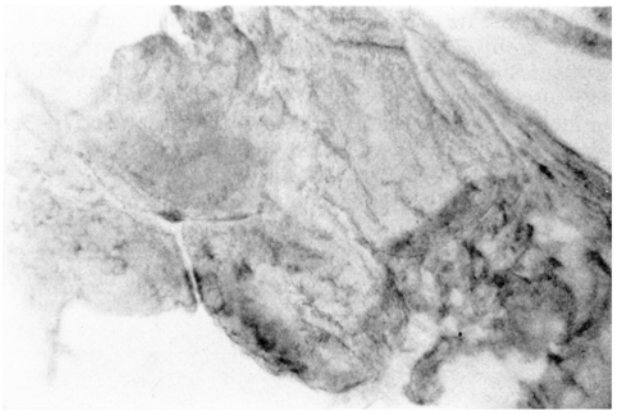

Fig. 2. Histochemical localization of the activity of steroid-3β-ol dehydrogenase in the ovary of an untreated control fish.

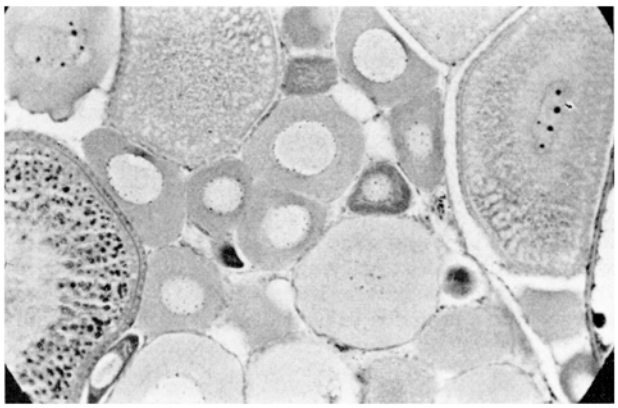

Fig. 1. Histological demonstration of the developmental stage of the ovaries used in our experiments.

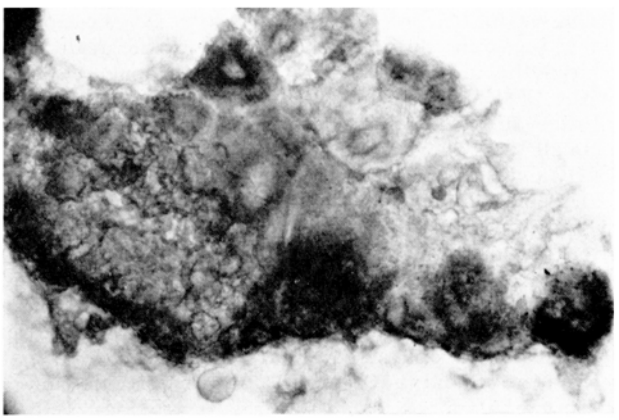

Fig. 3. Histochemical localization of the activity of steroid-3β-ol dehydrogenase in the ovary of an experimental fish, treated twice with 0.05 IU ovine LtH/g body weight, 3 days after the first injection.

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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