Results and discussion. In the first set of assays (Figure) the glands were only homogenized in Ringer solution and the homogenates then applied to the test specimens. On the 4th, 5th and 6th days after moulting, a distinct ascent in hormone activity can be observed, followed by a decline towards the end. In prepupae which already show akinesis, there is also a significant ascent. The corresponding values, not listed in the diagram, are 3 and 4.5 respectively 5 and 6 CU/assay. The values immediately after moulting until the second day are unexpectedly high.

In a second set of assays, the homogenates were shortly heated to more than 70°C to denaturate possibly effective proteins. The activity in the prothoracic glands was weak within the first days, then reaching a maximum shortly before the middle of the moulting cycle, relaxing and then showing a new increase near the end. The oenocytes showed within the first half of the moulting cycle approximately the same level, increasing towards its end.

Comparative assays with similar lots of fat body resulted in an evidently smaller hormone content ranging within 0–20% of that found in oenocytes.

The common result of the 2 sets of assays was that in oenocytes as well as in prothoracic glands, within the whole moulting cycle puparium-inducing substances were detected. The values of prothoracic glands not denaturated are greater than in heated ones. This applies in particular to those stages at which peculiar changes in the ultrastructure could be seen [15]. Except for the excessive value one day past moult, the oenocytes show an almost constant level of activity. In the second set of assays, the activity of the prothoracic glands within the first days of the moulting cycle is markedly reduced in comparision to untreated homogenates. Nevertheless it does not cease. Therefore the hypothesis by which prothoracic glands would synthesize a peptide hormone activating the oenocytes [7], has little probability so far as it concerns a peptide with a high molecular weight. It may be possible that the homogenates of the prothoracic glands not denaturated are further effective in the test specimens. Within the first part of the moulting cycle the activity of denaturated homogenates of oenocytes remains remarkably constant; it seems not at all variable. In the last third of the cycle it has even increased. Regarding the volumes of the organs the prothoracic glands must develop an activity that is at least 10 times greater than in oenocytes.

The question whether prothoracic glands are able to build ecdysone from cholesterol, or only a precursor of ecdysone, could not be clearly answered with the experiments here reported. In prothoracic glands of the American cockroach, the conversion of cholesterol to 7-dehydrocholesterol, a precursor of ecdysone, was shown [20]. In *Tenebrio* these glands incorporate C-3 cholesterol [9, 19], and they produce substances with puparium-building effects in the *Calliphora* bioassay. The possibility remains that a precursor made in prothoracic glands is completed in oenocytes to the real hormone. Besides this the oenocytes may be able to synthesize the hormone alone, as supposed by investigations in *Bombyx* [8] and *Mamestra* [6], where isolated abdomina converted cholesterol to ecdysone.

The problem now is how the titre of free hormone within a moulting cycle is accomplished, either by discontinuous secretion or by an inactivating system that shows a different activity within the moulting cycle [15]? Because the prothoracic glands and oenocytes do not cease synthesizing puparium-inducing substances in *Tenebrio* at any point of the moulting cycle, it is probable that both factors, a different synthesis activity and the inactivating system, determine the hormone titre.

Zusammenfassung. An isolierten und homogenisierten Prothorakaldrüsen und Oenocyten von *Tenebrio* wurde der Gehalt an Häutungshormonen mit Hilfe des Calliphoratests bestimmt. Dabei stellte sich heraus, dass sowohl Prothorakaldrüsen als auch Oenocyten während des gesamten Häutungszyklus verpuppungsaktive Stoffe, wenn auch in unterschiedlicher Menge, enthalten. Die Ergebnisse werden mit jenen verglichen, bei denen der Hormontiter durch Extraktion ganzer Tiere bestimmt wurde. In Verbindung damit wurde das Zusammenspiel von Synthese und Abbau der Hormone diskutiert.

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Cellular and Subcellular Localization of ^{3}H-Estradiol or its Metabolites in the Pituitary of the Neonatal Female Rat

The development of a reciprocal interaction between the pituitary and ovary has long been considered a prerequisite for cyclicity. Attempts to demonstrate a preferential uptake of ^{3}H-estradiol by the neonatal pituitary using biochemical techniques have resulted in conflicting reports. While one laboratory [1] reported a preferential uptake of estradiol in the pituitary of the 5-day-old female rat, others [2–5] have not been able to demonstrate selective uptake by the pituitary in vivo before day 10. The following study was carried out to determine the target cells for estrogen in the neonatal pituitary using a more sensitive technique, namely autoradiography.

Methods. 2-day-old female rats (n = 3) were injected s.c. with 1.0 µg of estradiol-17β-2,4,6,7-^{3}H(106 Ci/m*M*) per 100 g body wt. and killed 2 h later. A second experiment was carried out to determine the nature of the radioactivity in the labeled cells. 15 min prior to the

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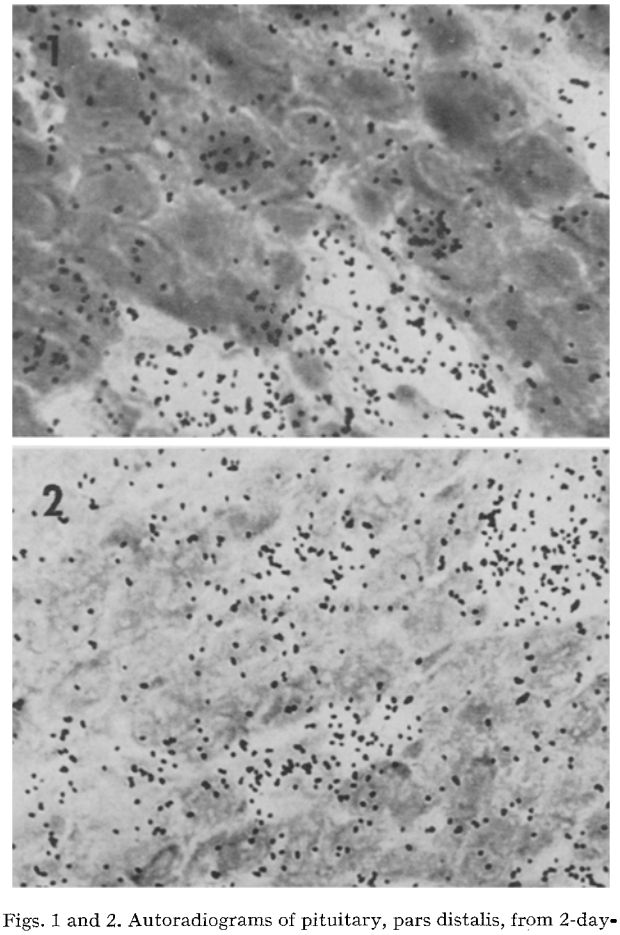
injection of ^{3}H-estradiol, 2-day-old female rats (2 animals per treatment) were s.c. injected with the vehicle only, 0.05 ml of 20% ethanol-isotonic saline, or 10.0 µg of estradiol-17β, and killed 2 h after the injection of labeled estradiol.

The autoradiographic procedure is described in detail elsewhere[6]. Briefly, the pituitaries were excised, placed on brass tissue holders and simultaneously frozen and mounted by immersion in liquefied propane at about –180 °C. 4 µm sections were cut at –35 °C knife temperature in a Wide-Range Cryostat (Harris Manufacturing Co., Cambridge, Mass.). The frozen sections were then either mounted directly on emulsion (Kodak NTB3) coated slides by bringing the slide into brief contact with the upper surface of the knife so that the sections adhered to the emulsion by melting from the heat of the slide or were freezedried with a Cryo-pump (Thermovac Industries Corp., Copiague, N.Y.) for 24 h and then dry mounted by pressure with a teflon support on emulsion coated slides, previously stored over drierite. The slides with the sections adhering to them were exposed at –15 °C between 3 and 6 weeks, then developed for 45 sec at 18 °C in Kodak D19 developer, rinsed, and fixed for 5 min in Kodak fixer, rinsed and stained. For the staining, methylgreen-pyronin was used.

Results. Nuclear concentration of radioactivity was found in a number of cells in the anterior lobe (Figure 1), but not in either the intermediate lobe or the posterior lobe. The majority of labeled cells appeared on the periphery of the anterior lobe with only a scattered few in the interior. Less than 10% of all the cells in the anterior pituitary were found to be labeled. Pretreatment with cold estradiol was found to inhibit the nuclear concentration of radioactivity indicating that the radioactivity in labeled cells is associated with estradiol or its metabolites (Figure 2).



Figs. 1 and 2. Autoradiograms of pituitary, pars distalis, from 2-day-old neonatal female rat, 2 h after injection of ^{3}H-estradiol, showing concentration of radioactivity in nuclei of certain cells (Figure 1) which is prevented after pretreatment with unlabeled estradiol (Figure 2). Exposure time 21 days. 4 µm ×1200. Stained with methylgreen-pyronine. Thaw-mount autoradiographic procedure.

Discussion. The scarcity of labeled cells in the anterior pituitary of the neonatal rat is in contrast to 76 and 86% reported for the immature and mature rats respectively[7]. Since it has been reported that the 'ambiguous' cells of the anterior pituitary, from which the acidophils and basophils develop, rapidly proliferate through mitosis during the first few days after birth[8], the topographical distribution and quantity of labeled cells may reflect the position and number of mature cells in the developing pituitary. Also in contrast to the adult rat[9] where a number of cells in the posterior lobe were found to be weekly labeled, no such labeled cells were observed in the posterior lobe of the 2-day-old female rat. Whether this is due to lack of developed cells, variations in dose or exposure time, or the hormonal state of the animal remains to be determined. The dramatic differences between the topographical distribution and quantity of labeled cells in the pituitary of the adult rat and the neonatal rat is in contrast to the striking similarity we have found in the topographical distribution of labeled neurons in the brain[10]. The autoradiographic results are the first of the existence of estrogen target cells in the pituitary of 2-day-old female rats. The small number of these cells, compared to the adult, may indicate the existence of hormone feedback – albeit limited – at this early age. The tinctorial identification of these labeled cells has not been established yet[11].

Zusammenfassung. Bei 2 Tage alten Ratten wurde markiertes Oestradiol-17β injiziert. Im Hypophysenvorderlappen waren weniger als 10% der Zellkerne markiert. Diese Zellen befanden sich vorwiegend in der Peripherie. Im Zwischen- und Hinterlappen fanden sich keine markierten Zellen. Eine Vorbehandlung mit nicht markiertem Oestradiol hemmte die nukleare Aufnahme der Radioaktivität, was beweist, dass diese mit Oestradiol oder dessen Metaboliten verbunden sein muss.

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