Moulting Hormone Content in Prothoracic Glands and Oenocytes of *Tenebrio molitor* within a Moulting Cycle

Prothoracic glands have been considered hitherto as the source of the moulting hormone ecdysone[1–5,17]. The investigations of some authors indicate that there must be other sites for the synthesis of moulting hormones besides the prothoracic glands[6–9]. My own investigations with isolated oenocytes of *Tenebrio*[9] showed comparatively great amounts of the moulting hormone in prepupae. Ligature experiments with butterflies[10] suggest the existence of an interaction between prothoracic glands and oenocytes. The results obtained with *Bombyx* and *Calliphora*[11,12] showed an increasing hormone titre only within the second part of the moulting cycle. These hormone titres were worked out on whole animals which were homogenized[13]. For this reason, it is difficult to determine the activity of the moulting hormone-producing organs. The actual hormone titre will be modified by the inactivating system which has a changing activity within a moulting cycle[14–16]. The problems mentioned are investigated by some experiments reported in this investigation.

Material and methods. Because the accumulation of hormone-producing organs and the demonstration of hormones by the method described by KARLSON and SHAAYA[13] are very time consuming, we have tried to work out another one which is easier to handle. In the course of preparation, prothoracic glands and oenocytes of 6 *Tenebrio* larvae with a moulting weight of about 100 mg each, were isolated and homogenized carefully by glass pearls. About 15–20 ligatured animals prepared for *Calliphora* bioassay were injected with these homogenates, and examined 24 h later. The puparium formation as an index for the hormone content was classified by indices (0.00, 0.25, 0.50, 0.75, 1.00) according to the instructions of ADELUNG and KARLSON[18]. The percent values of puparium formation were added and then divided by 0.50 (50% pupation = 1 CU) to receive the real hormone content of the original material. Because a variable percentage of necrotic animals was found within a moulting cycle, the test abdomina were dissected, delivered of attaching organs and examined with the binocular. In this way the percentage of sclerotized animals could be exactly determined.

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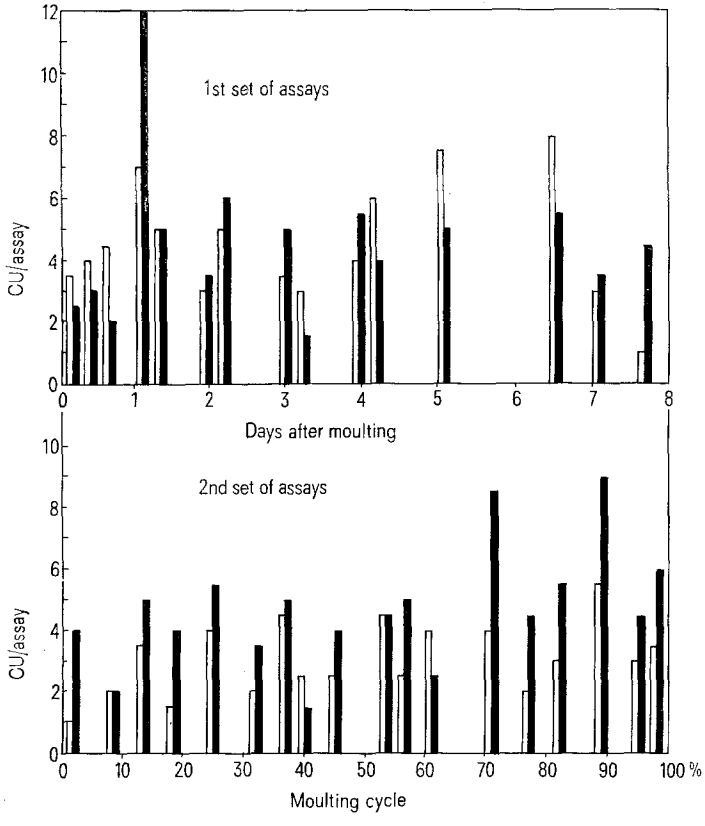

Demonstration of puparium-building substances found in isolated prothoracic glands and oenocytes of *Tenebrio molitor*. For each assay the glands of 6 *Tenebrio* larvae, weighing about 100 mg after moulting, were used. The homogenized glands had been injected to 15–20 ligatured *Calliphorae* and the result of one assay summarized. Clear columns: prothoracic glands, dark columns: oenocytes. In the first set of assays, the homogenates were not denaturated, whereas in the second one they were heated for a short time to more than 70 °C. CU, *Calliphora* units.

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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