Synthesis of ecdysone-^{14}C and ecdysterone-^{14}C from cholesterol-^{14}C in cockroaches (Periplaneta americana) without molting glands[1]

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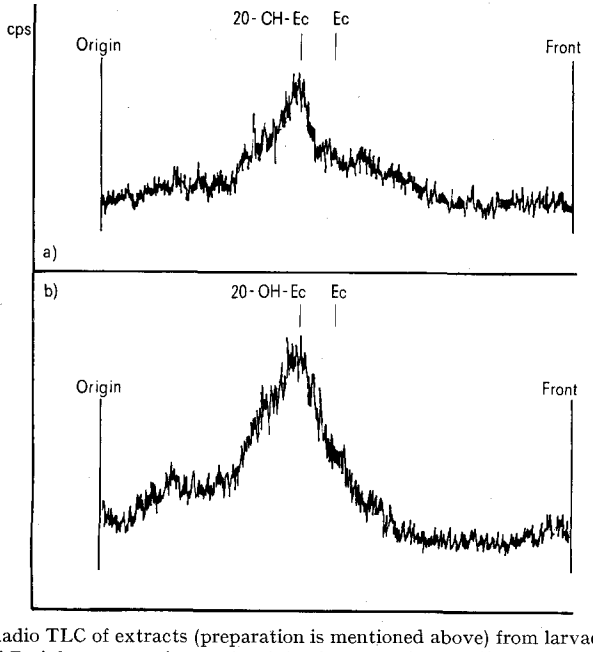
Summary. The synthesis of molting hormone of cockroaches of which the prothoracic glands had previously been extirpated was investigated after injection of cholesterol-^{14}C. It could be proved by means of radio thin-layer chromatography of extracts that ecdysterone and in small amounts ecdysone are synthesized in Periplaneta americana larvae without prothoracic glands. The results demonstrate that other tissues are also able to perform the molting hormone besides the molting gland itself.

The importance of the prothoracic glands of insects for the synthesis of the molting hormone and for the control of developmental processes is generally acknowledged. On the other hand, some experiments demonstrate that molting occurs after extirpation of the molting gland[2–5]. The main reason for these studies were detailed findings on the molting process in larvae of the cockroach Periplaneta americana after extirpation of the prothoracic glands[6]. Therefore it was tested whether or not ecdysone or/and ecdysterone is synthesized in those larvae.

To settle these problems, larvae of Periplaneta were used, the prothoracic glands of which had been removed 2–3 days after molting. Subsequently they were kept isolated under normal living conditions for 40 days. In these animals, no molting took place during this time. In 20 individuals of these '40-day animals' without prothoracic glands 0.1 μCi of cholesterol-^{14}C (suspension in 10% ethanol with Triton X100 added) was injected. After 24 h, these animals were homogenized in chloroform/methanol 2:1. For comparison, normal larvae of Periplaneta americana, which had molted 26 days before, were treated in the same way. The chloroform/methanol extracts of both charges were treated as follows: After evaporation of the chloroform/methanol extract, the residue was dissolved in 65% methanol. The centrifuged methanol phase was evaporated to dryness followed by a n-butanol-water distribution. After removing the n-butanol, the residue was dissolved in ethanol, centrifuged and separated by means of thin-layer chromatography on silica gel (Kieselgel G, Merck).

The following solvents were subsequently used: 1. chloroform/ethanol 4:1 – 3 passages (preparative DC); 2. chloroform/methanol/25% ammonia/water 12:7:1:1 – 1 passage (preparative DC); 3. chloroform/methanol 9:1 – 4 passages; 4. chloroform/methanol 9:1 – 2 passages subsequently chloroform/methanol 9:2 – 2 passages. In each chromatogram, unmarked ecdysone and ecdysterone were added as markers. The ranges corresponding to the R_f-values of ecdysone and ecdysterone were rechromatographed in the order mentioned. Radio thin-layer chromatograms of both charges after the fourth separation are demonstrated in the figure. The chromatograms show that in both extracts cholesterol metabolites are present. Concerning their solubility and thin-layer chromatography R_f-values, they may be considered as the molting hormones ecdysterone (chromatograms a and b) and, in small amounts, ecdysone (chromatogram a). After elution these ranges were biologically active in the Musca test[13]. From the findings that ecdysone and ecdysterone are synthesized in larvae without prothoracic glands, one may conclude that other tissues must also be able to form the molting hormone besides the molting gland itself[5–10]. Some indications show that ecdysone may be produced in the ovary[11,12]. Part of the present contradictory results might be explained in this way. In further studies, the site of ecdysterone synthesis in the cockroach will have to be clarified.



Radio TLC of extracts (preparation is mentioned above) from larvae of Periplaneta americana after injection of cholesterol-^{14}C. *a* Larvae the molting gland of which has been extirpated 40 days before; *b* normal larvae 26 days after preceding molting. The arrows locate the points of the standards ecdysone (Ec) and ecdysterone (20-OH-Ec). Apparatus: Berthold LB 2722/LB 242K, scanning speed 12 mm/h, meas. range 3 cps, time const. 100 sec, thickness 250 μm.

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