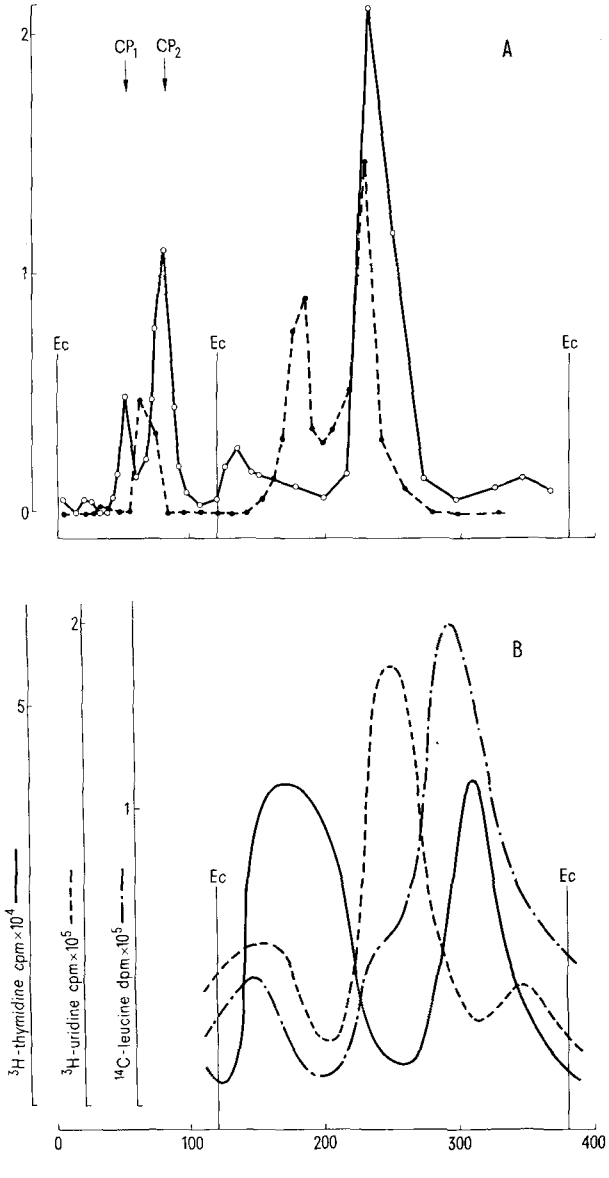
α- and β-Ecdysone Levels in Insect Haemolymph: Correlation with Developmental Events

Previous studies of moulting hormone levels during insect development have shown the existence of 1 peak before each moult and of 1 or 2 peaks during the nymphal stage[1]. They commonly used biological assays of ecdysone mixtures extracted from whole animals. Recently, techniques have been developed using either radioimmunoassays[2] or coupled gas-liquid chromatography/mass fragmentography[3]. The first one has been used, for instance, in the case of *Drosophila*[4]. The specificity of radioimmunoassays is not absolute, so that they measure 'ecdysone-like materials', but with a very good sensitivity, and this technique is very powerful for preliminary studies. Chromatographic techniques provide a more specific tool, although less sensitive and were used for our experiments.



A) Ecdysone levels during development. Time scale starts at the last larval-larval ecdysis. ○—○, β-ecdysone; ●- - -●, α-ecdysone (μg/ml haemolymph); EC, ecdysis. CP_1 and CP_2 are the critical periods respectively defined by post-cephalic and post-thoracic ligatures. B) Macromolecular syntheses during development. ——, ^{3}H-Thymidine incorporation in DNA (10 μCi/animal, 18 h labeling); – – – –, ^{3}H-Uridine incorporation in RNA (5 μCi/animal, 18 h labeling); ·—·—·—, ^{14}C-Leucine incorporation in protein (0.25 μCi/animal, 4 h labeling).

While some species contain mainly β-ecdysone and little α-ecdysone[4,5], others contain noticeable amounts of α-ecdysone, the first identified compound[6]. Recent data have shown evidence that prothoracic glands synthesize α-ecdysone from cholesterol[7], and that conversion of α-ecdysone to β-ecdysone occures in several tissues[8]. As α-edysone shows little activity in vitro on organs that cannot transform it into β-ecdysone, it is considered by several authors to be a prohormone[4,9]. On the contrary, others consider α-ecdysone as a true hormone, whose effects are specific and different from those of β-ecdysone[10,11].

We attempted to analyse such a problem without using an in vitro system, where tissue behaviour might have been non-physiological. We tried two sets of experiments, an analysis of ecdysone levels in haemolymph during development, and a study of the in vivo behaviour of imaginal wing discs, that cannot transform α-ecdysone[12]. Thus we studied the last larval instar and pupal-adult development of the cabbage butterfly, *Pieris brassicae*.

Samples of 2–6 ml of haemolymph were collected for separates analyse of both hormones. Tritiated standards were used in order to estimate the recovery after purifications. Ecdysones were then determined with an LKB 9000 apparatus, as previously described[13]. In vivo metabolism of DNA, RNA and proteins was studied by the incorporation of labelled precursors[14].

Our results are illustrated in Figures A and B. We may firstly note that ecdysone pattern is far more complex than expected from classical data, the most intriguing facts to our mind being the double peak of β-ecdysone during the last larval instar and the peak of α-ecdysone 60 h after larval-pupal ecdysis.

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From data obtained by ligaturing experiments[15] it seems that, during the last larval instar, prothoracic glands are needed until the second peak of β-ecdysone in accordance with their role in ecdysone biosynthesis[7]. The two peaks of β-ecdysone reported here agree well with the existence of two periods of prothoracicotropic hormone (PTTH) release by the brain[16], that correspond to the transition to wandering stage and moult to pupa. This problem will be discussed in more detail elsewhere, and we shall consider here the case of pupae only.

We have previously reported that there was a good correlation between β-ecdysone levels and ribosomal RNA synthesis in pupal wings[18]. In vitro experiments using *Galleria*[17] or *Drosophila*[18] imaginal discs showed that β-ecdysone enhanced both precursor uptake and net RNA synthesis, while α-ecdysone would perhaps increase precursor uptake only[17]. In our experiments, whole wing RNA decreased and precursor incorporation was reduced during the first pupal peak of α-ecdysone. All these results argue against a possible stimulation of ribosomal (stable) RNA synthesis by α-ecdysone.

The control of DNA synthesis seems more complex, because the published data are not in good agreement[10,12]. It is reported that in the pupa ^{3}H-Thymidine incorporations is much lowered in the presence of high levels of β-ecdysone, according to in vitro data for *Galleria*[10], and in vivo experiments with Saturniid pupae, that showed an inhibition of wing scales development by injections of high doses of various ecdysones[19], as with inhibitors of DNA syntheses[20,21]. High doses of α-ecdysone are not reported to have such an inhibitory effect. Our experiments do not show a close relationship between α-ecdysone and DNA syntheses, as would be expected from experiments using *Galleria* wing discs[10] and *Drosophila* cell lines[22]. In *Drosophila* imaginal leg discs cultured in vitro with α-ecdysone, mitoses do occur, as sockets and bristles differentiate[23]. In the case of *Pieris*, polyploidization in trichogen cells begins during the first peak of α-ecdysone. Thus it seems possible that both low levels of β-ecdysone – as recently suggested with *Galleria*[24] – and α-ecdysone are able to stimulate DNA synthesis. The problems could differ according to animal species[9], organs or developmental stages. Further experiments seem to be needed for a better understanding, because there is actually no evidence for the absence of $\alpha \rightarrow \beta$ conversion in culture experiments using α-ecdysone.

Some other data are in favour of a specific role of α-ecdysone. At low doses, it is capable of reinforcing β-ecdysone effects on cuticle synthesis[25], that in *Pieris* pharate adult occurs after the (α + β) peak. Moreover, testes of diapausing *Samia* respond to low (0.1 µg/ml) doses of α-ecdysone. In *Chironomus* salivary gland cells in vitro, the two hormones induce different puffs[26]. α-Ecdysone-binding-proteins ('receptors') have been described in *Drosophila* salivary glands[27].

For all these reasons, we think that both α-ecdysone and β-ecdysone are true hormones, evoking specific responses in target organs. However, only β-ecdysone have noticeable effects at low doses on some processes as rRNA or cuticle synthesis. The existence of other active hormones[28] and the possible need of cofactors in ecdysone action[24,29,30] render such studies even more diffciult.

Résumé. Les taux d'α- et de β-ecdysone ont été déterminés dans l'hémolymphe de *Pieris brassicae* au cours du dernier stade larvaire et de la métamorphose. Parallèlement, les taux de synthèse d'ADN, d'ARN et de protéines ont été mesurés dans les ébauches alaires. La comparaison de ces données a été discutée en fonction du mode d'action possible des deux hormones.

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Radioisotopic Studies of Human Chorionic Gonadotrophin in the Mouse Ovary

The ovary of the intact (non-hypophysectomized) mouse has been employed as a target organ to study the physiologic activity of radiolabeled human chorionic gonadotrophin (HCG)[1,2]. The uptake of ^{125}I-labeled HCG in the rodent ovary has been described by several investigators[3,4] including the concentration of labeled HCG in the ovary of hypophysectomized rodents[5]. It was also reported that ^{125}I-HCG localizes equally well in mouse theca cell carcinoma of the ovary as in the normal ovary[6]. The importance of such animal model systems has been accentuated by the rapid advancement of the radioligand receptor hormone assays[7,8]. The present study was implemented to further evaluate the tissue localization of ^{125}I-labeled HCG in the mouse ovary. Our findings indicate that ^{125}I-HCG concentrates consistently in the thecal and interstitial cells, but differentially in the corpus luteum of the intact mouse ovary.

Materials and methods. HCG (Antuitrin-S, 1700 IU/mg) was kindly supplied by Dr. MERRITT R. CALLANTINE, Parke-Davis, Ann Arbor, Michigan. Human growth hormone (HCH), used as a protein and trophic hormone control for the HCG studies, was provided through the