Critical period and ecdysone titers in the pupae of *Pieris brassicae*

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Summary. The ecdysone titer in the haemolymph of pupae of Pieris brassicae shows a sharp peak (1.90 µg/ml) on days 5 and 6 of the pupal stage. Isolated abdomens were able to form adult abdomens at 72 h, this being 2 or 3 days before maximum ecdysone levels and at a time when the concentration of hormone in the abdominal haemolymph was only 0.23 µg/ml.

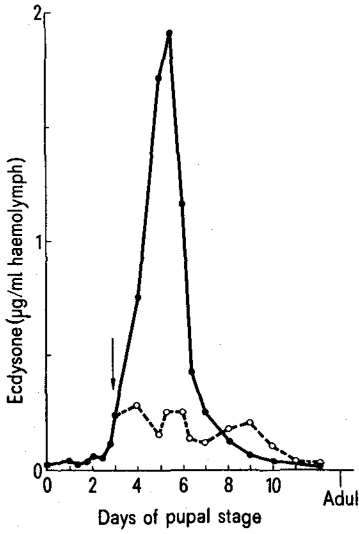
At each stage in the development of insects, ecdysis occurs only after a period of secretion of moulting hormone by the prothoracic glands. Assays for ecdysone during the larval stages in various species have given similar results. The levels of ecdysone, which are low at beginning of each stage, increase and reach a maximum value a short time before ecdysis. The general opinion is that the moult is initiated by this sharp peak in ecdysone levels[3]. In this work we have determined the critical time at which an isolated pupal abdomen of Pieris brassicae is able to metamorphose independently, and we have analyzed ecdysone titers in their haemolymph during development.

Material and methods. Large white butterflies were reared at 20 °C, under a 16 h light: 8 h dark regime. Pupae were cut in the middle of 3rd thoracic segment to separate the abdomens. A radioimmunoassay[4] for ecdysones (the sum of α and β ecdysones) was performed on haemolymph obtained from either whole pupae or from pupal abdomens isolated on the 3rd day (critical period). These abdomens continue their development although the maximal values of ecdysones in whole pupae haemolymph are not reached until days 5 and 6.

Critical period determination

Age*	Number of operated pupae	Number of adults
48 h	12	0
52 h	15	0
64 h	20	2
66 h	14	2
68 h	17	9
72 h	88	83

*Counted from pupal ecdysis.



Ecdysone levels in the haemolymph of pupa (●) and isolated abdomens (○). The arrow indicates the time of isolation of the abdomens. Each point corresponds to an assay performed on a pool of 6 to 10 samples of haemolymph after methanolic extraction.

Results and discussion. None of the abdomens isolated before 64 h was able to develop, although they survived for a long time (table). With a longer period before isolation of the abdomens, the percentage undergoing metamorphosis increased, reaching 94% for the abdomens isolated at 72 h. These abdomens were well developed and indistinguishable from abdomens of whole butterflies. Thus the anterior part of the animal, containing the prothoracic glands, was no longer necessary after the 3rd day of development. However, the rate of development of the abdomens isolated at the critical period was slightly modified. The pupal stage lasted for 15.2 ± 1.32 days (SD) for 78 isolated abdomens compared with 13.1 ± 0.54 days for 21 reference animals.

Ecdysone levels in the whole pupae are low until the 3rd day (figure). After this time the levels increase, reaching a maximum value between the 5th and 6th day, after which the amount of ecdysone decreases rapidly. In the haemolymph of abdomens isolated at 72 h, ecdysone levels fluctuate for several days between 0.1 and 0.3 µg/ml. In these cases the synchronization of development disappears but this alone is not sufficient to explain the absence of the peak of hormone level.

There is a relationship between the alterations of ecdysone levels in whole animals and changes in the secretory activity of the prothoracic glands[5,6]. Thus the circulating hormone levels should be a reflection of this activity. This data is therefore surprising: the prothoracic glands are no longer necessary after the 3rd day of pupal stage even though they have produced less hormone than in untreated animals. In the final larval stage of Pieris, the critical period for the prothoracic glands is close to the time where the levels of ecdysone are maximal[7,8]. The same is true for the caterpillar of Manduca sexta: the critical period for the pupal moult takes place between the 7th and 8th days[9], which is when the main peak of

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[3] W. W. Doane, in: Developmental Systems: Insects, vol. 2, p. 291. Academic Press, 1973.

[4] P. Porcheron, J. Foucrier, Cl. Gros, P. Pradelles, P. Cassier and F. Dray, FEBS Lett. *61*, 159 (1976).

[5] N. Agui and S. Yagi, Appl. Ent. Zool. *8*, 239 (1973).

[6] W. E. Bollenbacher, W. V. Vedeckis and L. I. Gilbert, Dev. Biol. *44*, 46 (1975).

[7] R. Lafont, B. Mauchamp, J. L. Pennetier, P. Tarroux, L. De Hys and J. P. Delbecque, Experientia *31*, 1241 (1975).

[8] B. Calvez, C. r. Acad. Sci., Paris *282*, 1367 (1976).

[9] J. W. Truman and L. M. Riddiford, J. exp. Biol. *60*, 371 (1974).

ecdysone occurs. In contrast, the imaginal ecdysis in Pieris can take place when the ecdysone levels is 8 times lower than its maximum value. It is apparent that the amount of hormone required for imaginal ecdysis is low and considerably less than the highest concentration attained. This results brings to mind experiments performed in the larvae of Manduca sexta[10], where infusions of small doses of ecdysone were more effective in eliciting a normal physiological response than a single large dose of the hormone. The prothoracic glands may secrete an excess of hormone which is not necessary for the imaginal moult but may have some other function than moulting. The critical period for the pupal stage (72 h) coincides with the 1st peak of α-ecdysone described in Pieris by Lafont et al.[7] who used a gas liquid chromatography and mass spectrometry method. We have not found this distinct peak in our result.

The loss of prothoracic glands parallels the loss of the large peak in ecdysone levels, but there is a persistant low level which varies between 0.1 and 0.3 μg/ml. Life of ecdysone in vivo is about one or few h[10–13]. The constant low level of ecdysone could be due to a synthesis by an organ other than the prothoracic glands, or to the liberation of stored ecdysone from tissues. In some insects, isolated abdomens have been shown to synthetize ecdysone[14–17], as do oenocytes[18] and ovaries[19–22].

Abdomens isolated at 72 h developed more slowly and with greater variability than the control animals. In Samia cynthia[23] and Galleria mellonella[24], injection of physiological large amount of ecdysone accelerated development. In Locusta migratoria, partial removal of the ventral glands during stages IV and V increased the length of these stages, and implantation decreased it[25]. There seems to be a relationship between rate of development and amount of ecdysone, and this could explain in part the lengthening of the pupal stage for isolated abdomens.

10 H. F. Nijhout, J. Insect Physiol. *22*, 453 (1976).
11 P. Karlson and C. Bode, J. Insect Physiol. *15*, 111 (1969).
12 T. Ohtaki and C. M. Williams, Biol. Bull. *138*, 326 (1970).
13 E. Shaaya, Z. Naturf. *24b*, 718 (1969).
14 M. Gersch and J. Sturzbecher, Experientia *27*, 1475 (1975).
15 K. Nakanishi, H. Moryama, T. Okauchi, S. Fujioka and M. Koreeda, Science *176*, 51 (1972).
16 T. H. Hsiao, C. Hsiao and J. de Wilde, Nature *225*, 727 (1975).
17 G. Studinger and A. Willig, J. Insect Physiol. *21*, 1793 (1975).
18 F. Romer, H. Emmerich and J. Nowock, J. Insect Physiol. *20*, 1975 (1974).
19 H. H. Hagedorn, J. D. O'Connor, M. S. Fuchs, B. Sage, D. A. Schlaeger and M. K. Bohm, Proc. nat. Acad. Sci. USA *72*, 3255 (1975).
20 C. Bordereau, M. Hirn, J. P. Delbecque and M. de Reggi, C. r. Acad. Sci., Paris *282*, 885 (1976).
21 M. Lagueux, M. Hirn, M. de Reggi and J. Hoffmann, C. r. Acad. Sci., Paris *282*, 1187 (1976).
22 J. M. Legay, B. Calvez, M. Hirn and M. de Reggi, Nature *262*, 489 (1976).
23 C. M. Williams, Biol. Bull. *134*, 344 (1968).
24 F. Sehnal, Acta ent. Prag *69*, 143 (1972).
25 M. C. Strich-Halbwachs, Annls Sci. nat. Zool. *1*, 483 (1959).

Effects of constant light exposure and blindness on the oxidative metabolism of selected brain areas in male rats

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Summary. Male rats were housed in continuous illumination or blinded when 21 day-old and killed 69 days later. The continuous illumination exposure increased the weights of testes and sex accessory organs and reduced the pineal gland weight. Blindness decreased weights of testes, sex accessory organs and anterior pituitary. The oxygen consumption rate of the hypothalamus was higher in the blinded animals than in the controls and lower in the continuously illuminated rats. No one of such groups showed significant changes in the oxygen consumption by either the amygdala or the hippocampus.

Some data show evidence that the oxidative metabolism of the hypothalamus and several areas of the limbic system can be influenced by the pituitary-gonadal axis activity. Thus, the oxygen consumption rates of the hypothalamus, the amygdala and, to a lesser extent, the hippocampus, has been reported to be changed by the oestrus cycle phases[1,2] and after experimental manipulations such as castration[1,3,4], hypophysectomy[5] and in vivo treatment with gonadal steroids and gonadotropins[3,4,6]. Data from in vitro works suggest that such effects are mainly due to changes in the pituitary gonadotropin output, which in turn would act directly on the above-mentioned brain areas[1,4,7]. This point constitutes one of the main supports for the gonadotropins 'short feed-back' theory[8].

On the other hand, the exposure of rats to constant illumination has been said to result in an enhanced gonadotropin secretion, and both the blindness or the exposure to constant darkness had the opposite effect (see Fraschini et al.[9] and Reiter[10] for review).

The present experiment was planned in order to see whether changes in light manipulations, with the associated changes in gonadotropin secretion mentioned above, could also affect the hypothalamus, the amygdala or the hippocampus oxygen consumption rates.

Material and methods. 21 day-old male Wistar rats were placed under different experimental conditions. All the animals were maintained in a temperature-controlled room (20 ± 3 °C) and given tap water and commercial chow ad libitum. Animals were divided into the 3 groups shown in table 1. Rats of group 1 were maintained under 14 h daily of artificial illumination, and those of group 2 were under constant illumination during the experiment. Rats of group 3 were blinded and maintained under constant darkness. When the animals were 90 days old, they were killed by decapitation and their endocrine organs dissected out and weighed. The amygdala, hypothalamus and hippocampus were dissected out. The oxygen consumption was determined by Warburg manometry method[11] in vessels in 12–15 ml capacity containing