the end of the experiment. The titre of this antiserum determined radioimmunochemically was 1:2000. A radioimmunoassay for dDAVP was developed which permitted routine detection of 30 pg dDAVP/ml.

A study was made of the capacities of various analogues of AVP to inhibit the binding of $[^{125}I]$-dDAVP to the antibodies. The figure shows the dependence of the binding of labelled hormone on the unlabelled peptide concentration on logarithmic scale. The binding affinity of each substance to the antiserum relative to the binding affinity of DAVP is expressed as a percentage, i.e. 100 × [the amount of DAVP required to displace 50% of labelled hormone/amount of analogue required to displace 50% of labelled hormone], the values being presented in the table.

It is evident from the table that deamination did not reduce the affinity of the analogue to the antibodies. On the other hand, immunoreactivity of the natural hormones AVP, LVP and OT was lower. It can be seen from these data that the strong basicity and specific steric conformation of the amino acid in position 8 of the peptidic chain are important factors in the interaction with the antibodies. The specificity of porcine antibodies to DAVP shows the same regularity as the specificity of porcine antibodies to AVP[9]. We may conclude that, even though the sensitivity of the developed radioimmunoassay for dDAVP is 10–50 times lower than the sensitivity of recently developed RIAs for AVP[12–14], it can be useful for distribution, binding and metabolic studies of the dDAVP. The antibodies are specific enough not to interfere with the content of AVP, AVT and OT in biological material.

[12] G. R. Robertson, E. A. Mahr, S. Athar and T. Sinha, J. clin. Invest. *52*, 2340 (1973).

[13] C. G. Beardwell, G. Geelen, H. M. Palmer, D. Roberts and L. Salamonson, J. Endocr. *67*, 189 (1975).

[14] H.-D. Freisenhausen, H. Frahm, T. Čabrijan and G. Wiethold, Acta endocr. *83*, 50 (1976).

Endocrine significance of critical periods during insect development: Analysis of ligation experiments with *Pieris brassicae* last instar larvae[1]

R. Lafont, B. Mauchamp, J.-L. Pennetier and M. L. De Reggi[2]

Ecole Normale Supérieure, Laboratoire de Zoologie, F-75230 Paris Cédex 05, and Centre d'Immunologie de Marseille-Luminy, F-13288 Marseille Cédex 2 (France), 23 May 1977

Summary. Ecdysone haemolymph levels have been analyzed in neck- or thorax-ligated *Pieris* larvae in order to explain the physiological significance of critical periods. It appeared that head critical period corresponds to an incomplete activation of prothoracic glands, while the thoracic critical period is related to the secretion of a minimal amount of ecdysone necessary for moulting. During *Pieris'* last larval instar, there is no evidence for any noticeable synthesis of ecdysone in isolated abdomens.

Ligation experiments have been widely used with Lepidopteran larvae, due to particularly favourable morphological features. They led to the 'classical scheme' of moulting control by 2 hormones, a brain neurosecretion or prothoracicotropic hormone (PTTH) and a prothoracic gland (PG) steroid hormone, ecdysone. Ligatures define 2 critical periods, a head critical period related to PG activation by PTTH, and a prothoracic critical period corresponding to ecdysone synthesis and release by PG. While many experiments have confirmed the validity of this scheme, firstly established with *Bombyx mori*[3], some more recent observations invite caution, essentially due to the presence of noticeable PTTH activity in *Bombyx* abdomens[4], and the possibility of ecdysone synthesis outside PG in both sexes of several insect species[5–8]. Moreover, the position of critical periods appears to vary much more than expected, as for instance a critical period may take place in the foregoing stage[9]. It is therefore essential to get more direct information about the significance of critical periods, that is to determine the actual hormonal titers in ligated animals.

Materials and methods. We used last instar larvae of *Pieris brassicae*, where ecdysone levels and critical periods had previously been determined[10, 11]. In the present work, animals carefully staged were ligated at various ages before and after the respective critical periods, and their haemolymph collected at regular intervals after ligations. The time of pupal ecdysis, when it occurred, was also recorded. Due to the small number of animals used for each haemolymph sample, ecdysone levels were determined using a radioimmunoassay (RIA) procedure[12].

Results. 1. Cephalic critical period. The diagram of figure 1A reminds the main events which occur during the last larval instar. The head critical period takes place during the feeding period. It corresponds to animals which have reached the $^2/_3$ of their maximum weight and coincides with the end of obligatory feeding period. If the larvae are ligated after the critical period, about 50% them normally ecdyse and transform themselves into headless pupae. The latter can survive for several months as long as they are maintained in moist conditions (the pupal cuticle is thinner than in controls) and they never give any sign

[1] Acknowledgment. This work was partially supported by CNRS, ATP 'Mécanisme d'action des Hormones'.

[2] M. L. D. R., Centre d'Immunologie de Marseille-Luminy, F-13288 Marseille Cedex 2 (France).

[3] S. Fukuda, J. Fac. Sci. Tokyo Univ. Sect. 4 *6*, 477 (1944).

[4] J. Nishiitsutsuji-Uwo and M. S. Nishimura, Experientia *30*, 1105 (1975).

[5] M. Gersch and J. Stürzebecher, Experientia *27*, 1475 (1971).

[6] K. Nakanishi, H. Moriyama, T. Okauchi, S. Fujioka and M. Koreeda, Science *176*, 51 (1972).

[7] F. Romer, H. Emmerich and J. Nowock, J. Insect Physiol. *20*, 1975 (1974).

[8] J. P. Delbecque, J. Delachambre, M. Hirn and M. L. De Reggi, in press.

[9] J. P. Delbecque, Thesis, Université de Dijon (1976).

[10] R. Lafont, B. Mauchamp, J. L. Pennetier, P. Tarroux, L. De Hys and J. P. Delbecque, Experientia *30*, 1241 (1975).

[11] R. Lafont, B. Mauchamp, C. Blais and J. L. Pennetier, J. Insect Physiol. *23*, 277 (1977).

[12] M. L. De Reggi, M. H. Hirn and M. A. Delaage, Biochem. biophys. Res. Commun. *66*, 1307 (1975).

of adult development. Figure 1B shows that the ecdysone peak which precedes pupal ecdysis is deeply affected by such ligatures. The hormonal release is progressive. The median value of the peak is delayed (figure 2) and strongly reduced (an effect amplified by the lack of synchronism of operated animals, especially when they were ligated shortly after the critical period). Not only the peak value is reduced, but the whole amount of ecdysone produced is also strongly reduced (figure 3).

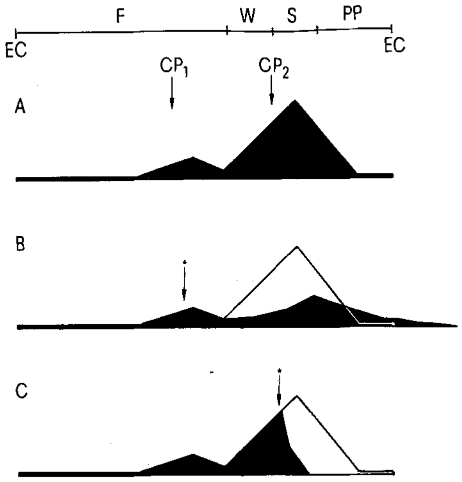

Fig. 1. Diagrammatic representation of the main developmental events and of ecdysteroid haemolymph levels during the last larval instar of *Pieris*. *A* Control animals, with the position of the 2 critical periods (CP_1 and CP_2); *B* neck-ligated animals (arrow indicates the time of ligation); *C* thorax-ligated animals. Time-scale between ecdyses is divided according to: feeding stage (F), wandering stage (W), spinning (S) and pharate pupa (PP).

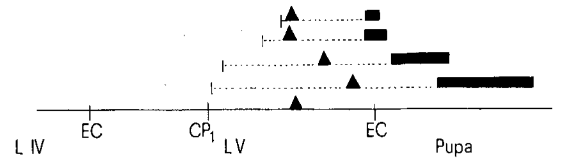

Fig. 2. Effects of neck-ligations at various ages upon development rate. The vertical bar indicates the time of ligation, the triangle ecdysteroid peak and the black rectangle the time of pupal ecdysis (very asynchronous when animals are ligated shortly after the critical period). EC = ecdysis; L IV, L V: larval stage IV resp. V; CP_1 = first or cephalic critical period.

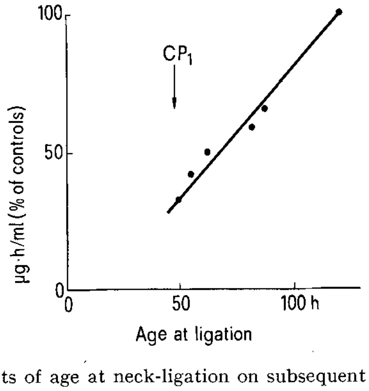

Fig. 3. Effects of age at neck-ligation on subsequent ecdysone synthesis, estimated by integration of ecdysone level curves between last larval-larval ecdysis and larval-pupal ecdysis.

2. Thoracic critical period. It takes place about at the end of the wandering period, before the haemolymph ecdysone titer has reached its maximum value. If post-thoracic ligations are set after this period, ecdysone concentration immediately falls down and rapidly becomes quite undetectable, at a rate corresponding to an half-life of 3 h (figure 1C). During the same time, ecdysone can reach a high concentration in the anterior part of the body, but the survival of this part is too short for precise analyses.

Discussion. Post-cephalic and post-thoracic ligaturing experiments lead to the notion of ecdysone efficiency for larval-pupal moult which can be expressed as mole h/ml, in accordance with the formulation previously established by Marks[13] in the case of in vitro cuticle synthesis by explanted epidermal fragments. We observed that in *Pieris brassicae* the larval-pupal moult can take place in animals which show strongly reduced amounts of ecdysone. Thus, in normal physiological conditions, the whole hormonal release is at least twice that required for ecdysis. However, it should be pointed out that reduced amounts of ecdysone result in delaying the moulting process and that pupal cuticle synthesis is diminished. As a consequence, both cephalic and thoracic critical periods appear to correspond to the secretion of a minimal amount of ecdysone, in fact much less than physiologically observed.

On the other hand, we note that prothoracic gland activation is progressive in the case of *Pieris*. This could mean that PTTH production and release is a rather continuous process in *Pieris*, in contrast with *Manduca sexta*[14], where PTTH release proceeds during short gates. Our hypothesis is supported by the fact that a neck ligation decreases more or less the amount of ecdysone produced. As a consequence, the cephalic critical corresponds to a partial activation of PG, as previously observed in 4 instar *Manduca* larvae[15]. However, we must note the long lag which separates the neck ligation and ecdysone peak. The PTTH storage in the nervous chain (outside the brain) would perhaps give the basis for an explanation for this problem and the variability observed in the position of the 2 critical periods among species[9,16,17]. Abdominal synthesis of ecdysone, while found with other species of Lepidoptera during the last larval instar[5,6], does not appear significative in our case, as in isolated abdomens ecdysteroid level remains below 5 ng/ml. However, the situation is very different during the pupal stage, where noticeable syntheses (de novo or from an inactive derivative) seem to take place in isolated abdomens[18].

[13] E. P. Marks, Biol. Bull. *142*, 293 (1972).
[14] J. W. Truman, J. exp. Biol. *57*, 805 (1972).
[15] M. J. Fain and L. M. Riddiford, Gen. comp. Endocr. *30*, 131 (1976).
[16] J. W. Truman and L. M. Riddiford, J. exp. Biol. *60*, 371 (1974).
[17] M. Locke, Tissue Cell *2*, 197 (1970).
[18] J. Claret, P. Porcheron and F. Dray, Experientia *33*, 1389 (1977).