was chosen, not a maximal one, because of the known ineffectiveness of sulfonylureas in potentiating insulin secretion at maximal glucose concentrations[11–13].

This inhibitory effect after exposure to glibenclamide on insulin release has recently been found by Borg[13], after cultivation of isolated islets from albino mice for 7 days at 5.5 mmoles/l glucose and glibenclamide, but not by Schatz et al.[7], who reported a stimulating effect after glibenclamide exposure. But a direct comparison with the latter work is not possible because the investigations of these authors were performed 1. with rat islets and 2. the incubation period in the presence of glibenclamide was considerably shorter (2 h).

Our results regarding the β-cell secretion hypofunction after exposure to glibenclamide are also in accordance with data described for tolbutamide and glibenclamide treatment in vivo,[4,5,14]. The low insulin content per islet (13 ng per islet) is not the only reason for the diminished insulin release, since also at unaltered insulin content (treatment with sulfonylureas in vivo) this secretory hypofunction was observed[4,5].

Pancreatic islets contain 50% of insulin only after culture with glibenclamide, compared with untreated controls (figure). Nevertheless, the incorporation rate of ^{3}H-leucine into (pro)insulin does not differ from controls and glibenclamide treated islets (table). Whether the presence of glibenclamide for 48 h provoked a reduction of insulin biosynthesis as described in short-term experiments[5,15,16] cannot be clearly answered at the moment. It is a fact, however, that the loss of stored insulin due to the stimulating effect of glibenclamide on hormone secretion is not compensated for by insulin biosynthesis (figure). The insulin stores are first filled up in the following 48 h period (without glibenclamide), since the insulin biosynthesis is glucose sensitive during diminished insulin release (figure). Our findings suggest that glibenclamide enhances the insulin secretion during long-time incubation in culture as described for short-time experiments too. The glucose-induced insulin release, however, is diminished after exposure to glibenclamide whereas the effectiveness of glucose on insulin biosynthesis is not restricted, i.e. glibenclamide pretreatment provoked a dissociation of glucose-responsiveness of insulin biosynthesis and release. This impairment of glucose-induced insulin release is reversible by cultivation with high glucose and is partly prevented if the glucose concentration is enhanced up to 20 mmoles/l glucose during the glibenclamide loading (unpublished data).

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Haemolymph ecdysteroids level following the injection of ecdysone or ecdysterone; its relation with tegument and midgut response in *Aeshna cyanea* (Insecta, Odonata)

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Summary. The haemolymph ecdysteroid level after injection of ecdysone or ecdysterone in *Aeshna cyanea* larvae has been determined by a radioimmunoassay method. The rate of excretion appears to be dependent on both the ecdysteroid injected and the time of injection. In case of ecdysone injection, the secretion of the epidermis cuticle and the differentiation of the imaginal midgut epithelium occur when the ecdysteroid level remains low for many days.

In an attempt to analyse the control of post-embryonic events in insects some investigators[1-4] have recently tried, by means of an elegant quantification of moulting hormone, to relate the amount of ecdysteroids to developmental events.

In a same way, in larvae of *Aeshna cyanea* injected with ecdysone or ecdysterone we have first determined from day to day the variations of the haemolymph ecdysteroids level, and then tried to correlate these levels to the response of both the epidermis and the midgut. It was also expected that this study would provide some information about the rates of excretion of exogenous ecdysteroids.

Material and methods. 116 last larval instars of *A. cyanea* were injected on day 1 or 5 with 20 µg of ecdysone (Simes)

or ecdysterone (Sigma) as a 10% alcoholic solution. 3 or more larvae were killed daily in the 10 days following the ecdysteroid administration and both midgut and 5th abdominal tergit fixed for light microscopy. Previously, 100 µl or more of haemolymph had been collected from each experimental animal. The hormonal levels were then determined by a radioimmunoassay (RIA) method[5]. In addition, analysis for ecdysone and ecdysterone in 2 samples from larvae injected on day 5 with ecdysterone was carried out by high-performance liquid chromatography (HPLC) on a µm-Bondapak-C 18 reverse phase column. Elution was performed in a continuous gradient of a mixture of methanol and water (initial conditions: 40% methanol, 60% water; final conditions: 90% methanol, 10% water) during 30 min. The debit was 1 ml/min throughout the experiment. Under these conditions, the retention time for ecdysterone is 11 min and for ecdysone 18 min. The eluted fractions were then tested individually by the RIA method.

Results. 1. Ecdysteroid level. The last larval instar, which lasts 24 days in the most favourable conditions, shows a pattern with 2 ecdysteroid peaks after the first 3rd of the instar [6]. In our experiments, the injection of ecdysone or ecdysterone consequently occurred before the endogenous ecdysteroid peaks. Figure 1 shows the variations of the ecdysteroid level in the haemolymph. In the case of an ecdysone administration, the hormone titer falls quickly so that on and after day +4, the amounts of ecdysteroids in experimental animals are closely related to the values of the hormone titer in controls, before the endogenous ecdysteroid peak.

After a 20-µg ecdysterone injection, the titer of the ecdysteroids decrease less quickly; its value remains above the endogenous main peak of the controls until day +6 or +7. Comparisons made on day +1, using the Student's test, show that there were significant differences (table) in the ecdysteroid titer according to the type of ecdysteroid and the time of injection.

24 h after the injection of ecdysterone, the HPLC analysis of the RIA results in a single peak that cochromatographs with standard ecdysterone. 5 days later, 2 peaks are obtained, the larger (55%) corresponding to ecdysterone and a smaller peak (45%) which represents a less polar compound with a slightly longer retention time. This retention time is, however, inferior to that of standard ecdysone.

2. Target organ response. The detachment of the cuticle is observable in situ 36 or 48 h after the hormonal supply, particularly in the wings[7] whatever the type of ecdysteroid or the time of injection. From day +2 to +4, numerous cell divisions occur, particularly on day +3 (figure 3). A new cuticle is secreted by the epidermis on and after day +6 or +7 (figure 4) and specialized structures such as bristles appear on day +7 or +8 (figure 5).

In the midgut a stimulation of DNA synthesis occurs in the days following the injection of ecdysone or ecdysterone. Such an increase in the number of the regenerative cells leads to the differentiation of an imaginal epithelium beginning on day +7 (ecdysterone injection) or on day +9 or +10 (ecdysone supply)[8].

Discussion. 1. Rate of excretion of exogenous ecdysone. It is well known that, in insects, injected ecdysteroids are degraded rapidly[9-11] and that ecdysone is converted to ecdysterone[12-16].

In *A. cyanea*, as in all insects studied so far, ecdysone is converted to ecdysterone[17] but the hydroxylation of ecdy-

Comparison of the ecdysteroid titers observed 24 h after the injection to the type of ecdysteroid and the time of injection

Day of the injection	Type of ecdysteroid injected	Mean value of the ecdysteroid titer (nM ecdysteroids/ml haemolymph)	Differences $t_{001} \cdot SD$ between the mean values
5	Ecdysone	3.36	
			16.30 > 6.70
5	Ecdysterone	19.66	
			8.76 > 6.27
1	Ecdysterone	28.42	

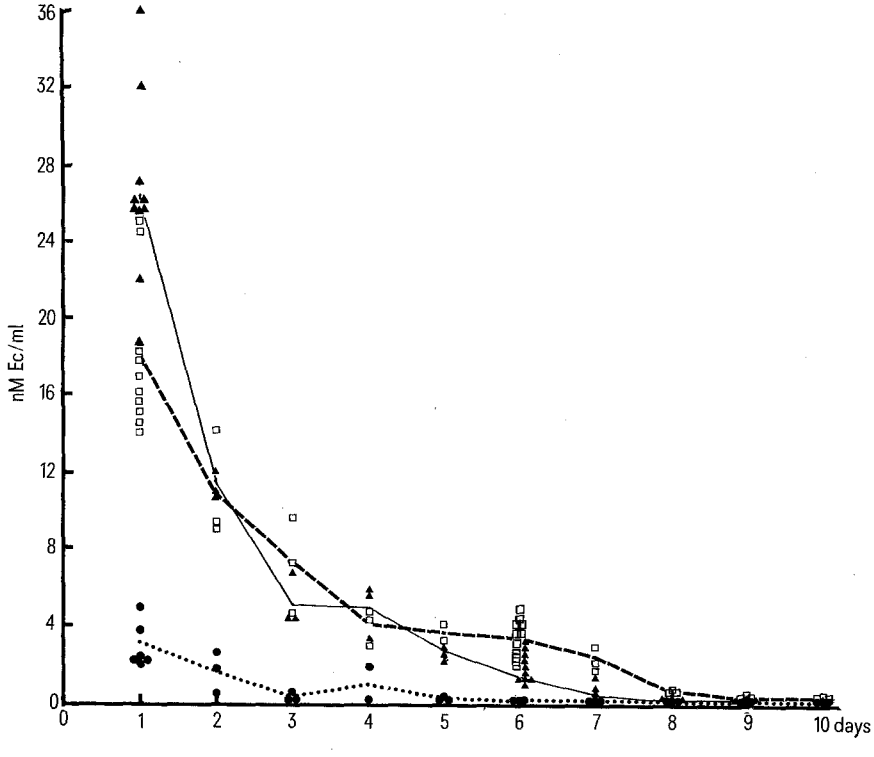


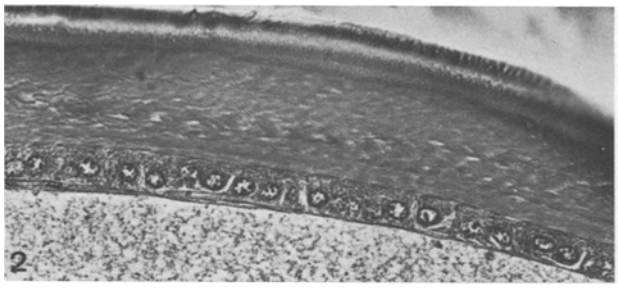
Fig. 1. Changes of the haemolymph titer in the 10 days following the injection of 20 µg of ecdysone on day 5 (black circles), of ecdysterone on day 1 (black triangles) or 5 (withe squares) of the last larval instar.

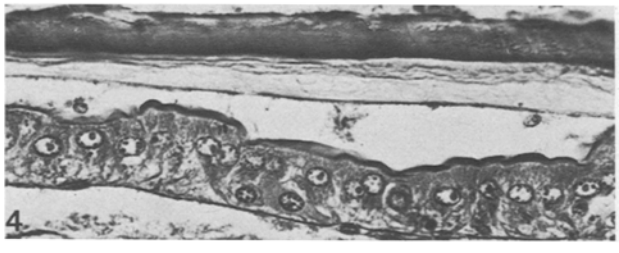
sone into ecdysterone is not the major metabolic pathway when the titer of endogenous hormone is low; ecdysone and its metabolites then being excreted in massive amounts in the faeces[17]. Our results corroborate these reports since more than 98% of the injected ecdysone is excreted in the first 24 h. In addition, the excretion rate is more rapid in animals injected with ecdysone than with ecdysterone and also depends on the time of injection; it is faster when the supply occurs sooner in the instar.

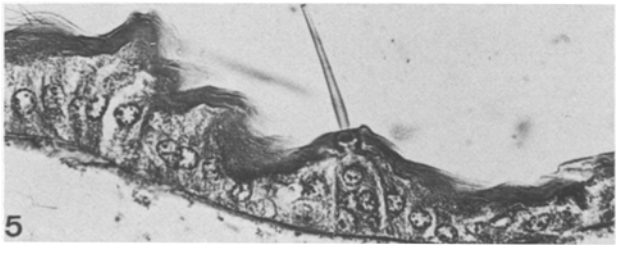
It was also reported that the moulting hormone can stimulate the prothoracic glands[18–20]. The results of the HPLC analysis of the RIA which clearly show that there is no ecdysone in the haemolymph 24 h or 6 days after an ecdysterone supply could suggest that high doses of exogenous ecdysterone inhibit the prothoracic glands. Such results are also in agreement with the observations of Porcheron et al.[21] who have described an inhibitory action of ecdysone on the prothoracic glands of *Locusta migratoria* maintained in vitro.

2. Ecdysteroid level and target organs response. In the last larval instar of *Aeshna cyanea,* apolysis may occur long before the main ecdysteroid peak. This is the case, for example, for the wings or the labium[22]. On the other hand, the secretion of the cuticle of the abdominal tergits starts on day 16 of the instar[23] when the ecdysteroid titer begins to rise rapidly[6] and the genesis of the midgut imaginal epithelium begins on day 18[24] when the ecdysteroid titer reaches a maximum (2 nM/ml)[6]. The superposition of these hormonal levels and morphological events gives us a good idea of the correlation between them. However the problem of whether ecdysteroids are necessary during the entire secretion of the epidermis or the complete genesis of the midgut epithelium, remains open.

In experimental larvae, the ecdysteroid supply triggers first apolysis, secondly, cell multiplication and afterwards cuticle synthesis in the epidermis; first cell multiplication and then differentiation in the midgut. It appears, at least after an ecdysone administration, that the beginning of cuticular synthesis and midgut epithelium differentiation occurs when the hormonal level has dropped to a low value, respectively to 0.10–0.12 nM/ml and 0.04–0.06 nM/ml, and continues in the presence of ecdysteroid titers still at their lowest. Therefore it seems that ecdysteroids at high doses are not necessarily involved during the entire secretion of the epidermis nor during the complete differentiation of the regenerative midgut cells.







Figures 2–5. Cross section of the 5th abdominal tergit after ecdysterone (figures 2–4) or ecdysone (figure 5) supply on day 1 (figures 3 and 4) or 5 (figures 2 and 5) of the last larval instar. ×330. Fig. 2. Appearance of the tegument 24 h after the injection. The old cuticle sticks to the epidermis. Fig. 3. Appearance of the tegument 3 days after the injection. The old cuticle has become detached from the epidermis. A telophasic figure can be observed in the left side of the picture. Fig. 4. Appearance of the tegument 7 days after the injection. A fine layer of new cuticle overlays the epidermis. Fig. 5. Appearance of the tegument 10 days after the injection. The layer of new cuticle is more important and bristles are constituted.

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