SHORT COMMUNICATIONS

EFFECTS OF EXOGENOUS MOULTING HORMONES ON PUPARIUM FORMATION IN CALLIPHORA*

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Pupal sclerotization in holometabolous insects has been widely used as a basis for bioassays of moulting hormone activity (for review see Horn 1969), and there has been considerable interest in the mode of action of these hormones at the cellular level (Karlson and Sekeris 1966). It is surprising that the effects of exogenous moulting hormone on the time and mode of puparium formation in the intact dipteran larva have seldom been reported (except e.g. Fourche 1967, on Drosophila), especially in view of interest in the relative biological activity of the various ecdysone-group steroids in stimulating sclerotization (Ohtaki, Milkman, and Williams 1967).

The work reported here deals mainly with the effect of crustecdysone, the principal moulting hormone of Calliphora (Galbraith et al. 1969), on puparium formation in a blowfly of this genus. The administration of exogenous hormone to insects often proves difficult. Ingestion (Fourche 1967) and cutaneous application (see Horn 1969) provide the easiest means, but in each case uptake may be highly variable from animal to animal. Further, in at least some species, absorption of hormone from the digestive tract may be limited (Staal 1967), and the relative biological activities of ingested moulting hormones may differ markedly from those observed when other methods of administration are used (Robbins et al. 1968). In the present investigation, hormone solutions were injected into the haemocoele so that the effect of varying dosages could be assessed accurately.

Crustecdysone was obtained from the plant Podocarpus elatus (Galbraith and Horn 1966). Two other steroids were tested: α-ecdysone (synthetic; kindly supplied by Dr. P. Hocks, Schering AG, Berlin) and callinedysone A, a steroid which appears to be the major hormone present in the premoult stages of the crab, Callinectes sapidus (Horn et al., unpublished data). Preliminary evidence indicates that callinedysone A is an isomer of crustecdysone and may be identical with inokosterone [isolated by Takemoto, Ogawa, and Nishimoto (1967) from plant material] or one of its optical isomers.

Third-instar larvae of the brown blowfly, Calliphora stygia (Fabr.) Schiner, were reared as described by Neufeld, Thomson, and Horn (1968). Development rates of different batches were checked against the aging criteria listed by Kinnear et al. (1968), who also gave details of the behaviour and crop condition of third-instar larvae. In each case 2 µl of aqueous steroid solution (or distilled water for “treated” controls) was administered by injection following the method of Neufeld, Thomson, and Horn (1968). A mortality of about 5% resulted from handling larvae aged 6 days and younger; larvae aged 7 days or more were unaffected by the injection procedure. Injected larvae were placed in clean dry sawdust at 20°C and examined regularly for signs of sclerotization.

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Preliminary experiments showed that injection of distilled water alone had no detectable effect on the time of onset of puparium formation (Fig. 1; compare water-injected and untreated groups) unless carried out within about 12 hr of its commencement, in which case a delay of some hours became evident. Administration of \( \alpha \)-ecdysone, crustecdysone, or callinecdysone A at a dose of 0.2 \( \mu \)g per larva produces a marked acceleration of puparium formation (Fig. 1). In each case apparently normal adults subsequently emerged with pupal viability remaining at the control level. Much higher doses of these steroids (results for crustecdysone and callinecdysone A shown in Fig. 1) resulted in sclerotization of the majority of larvae without normal contraction to the ovoid puparial form. The cuticle, although of the usual red-brown coloration, remained relatively thin, soft, and crinkled in these animals. Callinecdysone A and crustecdysone appeared to act additively in producing this precocious sclerotization (Fig. 1). In some high-dose groups (Fig. 1), a few larvae formed normal puparia.

Investigation of the effect of larval age at the time of treatment led to elucidation of the pattern of hormone sensitivity. The results for two dose levels of crustecdysone are shown in Figures 2(a) (0.16 \( \mu \)g per larva) and 2(b) (1.6 \( \mu \)g per larva). Normal puparia always resulted from doses of the lower amount. Puparium formation did not occur in larvae younger than 8 days, even after starvation (Sin, personal communication, cf. Fourche 1967). At a dose level of 1.6 \( \mu \)g of hormone, larvae aged 6 days at the time of injection began to form normal puparia at day 8; larvae receiving this dose at 7, 8, or 9 days of age showed an increasing tendency to premature
sclerotization without contraction to the puparial shape. Ten-day-old larvae, however, formed normal puparia after receiving the same dose. Flies emerging from these puparia were mostly normal in appearance, but a few with poorly expanded or held-out wings or both were consistently observed. Again some precocious but apparently normal puparia appeared in batches treated with large doses of hormone before day 10 [Fig. 2(b), cf. Fig. 1], suggesting that in these individuals a critical
developmental step had been achieved prior to the treatment. Although development was accelerated when large amounts of hormone were injected early in third instar, sufficient time apparently elapsed before the larvae reached the sensitive stage for breakdown or deactivation to levels below that at which premature tanning could take place.

By selection of larvae at a suitable age and by adjustment of the hormone dose it proved possible to produce abnormally elongate puparia grading in appearance from that of sclerotized larvae to almost normal puparia (Table 1). Thus 0·2 μg crustecdysone per larva at 9·5 days of age results in puparia of normal appearance. At 8·5 days of age, 0·5 μg of this hormone per larva causes all individuals to sclerotize without contraction. The same dose at 9·5 days yields a range of intermediate forms including many elongate puparia, pointed rather than ovoid anteriorly, and often with an exaggeration of the normal slight constriction at the fourth sclerite.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age at Injection (days)</th>
<th>Number Measured</th>
<th>Length (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (S.D.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Water (control)</td>
<td>9·5</td>
<td>25</td>
<td>9·76 (0·163)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9·5–10·1</td>
</tr>
<tr>
<td>Crustecdysone (0·5 μg)</td>
<td>8·5</td>
<td>15</td>
<td>12·35 (0·256)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11·7–12·7</td>
</tr>
<tr>
<td>Crustecdysone (0·2 μg)</td>
<td>9·5</td>
<td>15</td>
<td>10·09 (0·220)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9·5–10·4</td>
</tr>
<tr>
<td>Crustecdysone (0·5 μg)</td>
<td>9·5</td>
<td>15</td>
<td>10·81 (0·605)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>9·6–11·9</td>
</tr>
</tbody>
</table>

* Measured 24 hr after treatment.

Fraenkel (1935) recognized three processes as involved in puparium formation in *Calliphora*: contraction, hardening, and darkening. The latter two he described as "indissolubly coupled". It is clear from Fraenkel's work that presence of the intact nervous system up to a certain stage is required for subsequent contraction. Exogenous moulting hormone will cause hardening and darkening of the cuticle in the absence of the functional nervous system in appropriate preparations (Fraenkel 1935); it does not cause contraction. At the quiescent stage (Kinnear *et al.* 1968), just prior to puparium formation, larvae of *C. stygia* adopt a shortened, rounded posture. At first, such larvae become elongate and active when disturbed, but contraction becomes irreversible once changes in the cuticle itself lead to reduction in surface area (Fraenkel and Rudall 1940). In *Galleria*, after muscular shortening of the abdomen, neural integration of proprioceptive information on posture and nutritional state appears to initiate neurosecretory activity necessary for the pupal moult (Edwards 1966). If the same is true of *Calliphora*, a sharp increase in the level of moulting hormone in the haemolymph before appropriate muscular contraction might lead to premature sclerotization and malformed puparia. The important questions to resolve are those concerning the development and control of competence of the epidermis to undergo sclerotization in response to moulting hormone. It is interesting in this
connection that, in the body wall, the susceptibility of gross protein synthesis to stimulation by exogenous moulting hormone drops after day 8 of development (Neufeld, Thomson, and Horn 1968).

Tanning of the larval cuticle without contraction to the normal puparial form has also been observed in Sarcophaga following injection of juvenile hormone during the third instar (Srivastava and Gilbert 1968) and, in a few instances, after destruction by electrocautery of the ring gland of Calliphora larvae treated near the time of pupation (Pryor 1940). In the latter case, the possibility of operative damage to the nervous system renders interpretation uncertain. The effect of juvenile hormone, however, may be antagonistic to that of the early low level of endogenous moulting hormone which appears to initiate the behavioural and postural changes preceding normal puparium formation.

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References
