to be between the 7th and 9th days, which correlates well with the time at which the 1st ecdysone peak is observed in the haemolymph. These observations suggest that, in *P. megistus,* as in other insects[3,5,6], the 2nd ecdysone peak is not involved in the moulting process.

Cauterisation of the PI on the 7th, 8th, 9th or 10th dABM. Destruction of the PI was carried out on the 7th, 8th, 9th or 10th dABM. While moulting occurred in larvae operated on the 9th and 10th days, it was suppressed when the operation was performed on the 7th or even on the 8th dABM (figure 1,B). The critical time at which neurosecretory activity is required is therefore very close to that at which thoracic gland activity is necessary.

Specific ablation of the A cells in the PI on the 7th dABM. The PI of *P. megistus* contains at least 4 neurosecretory cells types[7]. The best characterized are the A cells, which were first described in *Rhodnius prolixus* in 1938[8]. Selective ablation of A cells was carried out on the 7th dABM, when complete ablation of the PI suppresses moulting. This operation did not suppress the moult, which indicates that the A cells are not involved in the secretion of the factor which activates the thoracic gland.

Specific ablation of the A cells in the PI at 24 h ABM. Ablation of the A cells 24 h after the blood meal however, did suppress moulting, indicating that the activity of these neurosecretory cells is also essential for the process to occur, but is required only at very early times.

Cauterisation of the PI followed by ecdysterone injection. We have previously found that cauterisation of the PI carried out on the 7th dABM inhibits moulting. When cauterisation is performed earlier (48 h ABM), it has the same effect. In the 1st case, ecdysterone injection relieves this inhibition, but when the operation is carried out at earlier times no such relief is observed. This indicates that the A cells do not act through the thoracic gland.

The neurosecretory cells which secrete the thoracic gland activating factor (brain hormone) remains to be identified. A histophysiological examination of A and A′ cells from 5th instar larvae suggests that the A′ cells, which release their neurosecretory material later than A cells, may be responsible[7].

Conclusion. Experiments on *P. megistus* show that 2 separate factors, both produced in the PI, are required for moulting to occur. 1 is probably secreted by the A′ cells cells and, as suggested in the classical model, activates the thoracic gland. The other is secreted by the A cells, and acts at an early stage in the process, probably directly. It may act to induce mitoses preceding apolysis, as indicated by investigations of the regulation of the initial stages of oogenesis[2] (figure 2).

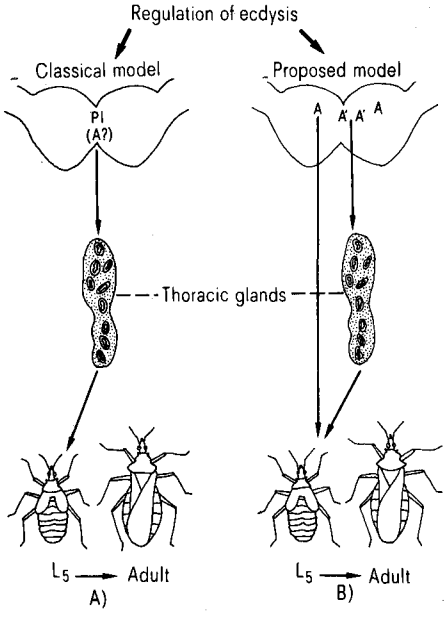

Fig. 2. Regulation of ecdysis. *A* Classical model; *B* proposed model. L_5: 5th stage larva.

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Effective dose present in cockroach larvae exposed continuously to a juvenile hormone active insect growth regulator[1]

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Summary. Larvae of the German cockroach exposed to filter paper impregnated with juvenile hormone (JH) active substance contain, at the middle of the last instar, about one-hundredth of the dose applied to 1 cm^2. The amount of metabolized substance rises sharply before and disappears rapidly after the ecdysis into the supernumerary instar.

Insect growth regulators (IGRs) are slow-acting insecticides, which must be present at a certain concentration in the insect body during a sensitive period in order to affect the insect development. A single application (topical or by injection) requires an initial overdose to cope with subsequent inactivation and discharge of the applied substance. Our experiments are based on application of the substance to a substrate which is ingested or stays in permanent contact with the insects. This arrangement assures a long-term treatment. This paper deals with the question of the actual amount of IGR taken up by these insects.

Material and methods. 10 freshly emerged larvae of the last instar of the German cockroach *(Blattella germanica* L.*)* were confined to 180 cm^3 plastic cups, supplied with food and drinking water and incubated at 24 °C, with 60% relative humidity and permanent illumination. 2 folded filter paper discs (Schleicher & Schüll No. 16, 20 cm^2 each) were added. Tritium-labelled JH-active IGR 2,3-^{3}H 6,7-

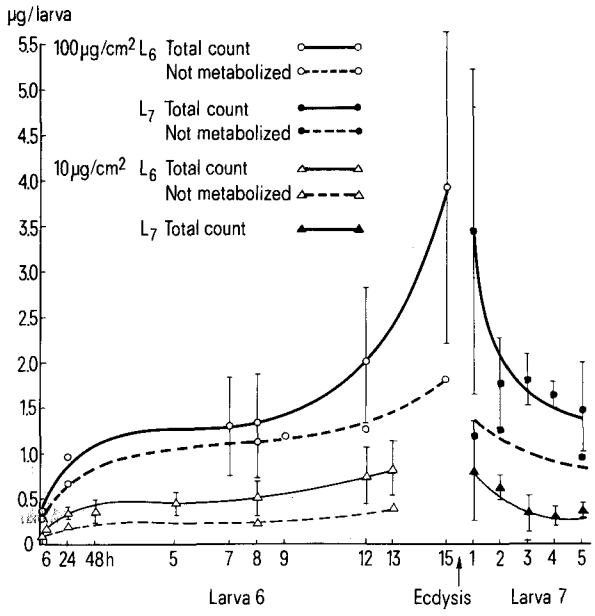
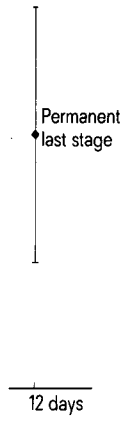


Fig. 1. Total amount of the labelled material and proportion of the unchanged active substance in the body of the larvae of the last (L6) and supernumerary instars (L7) exposed to filter paper impregnated with IGR (standard deviation indicated).



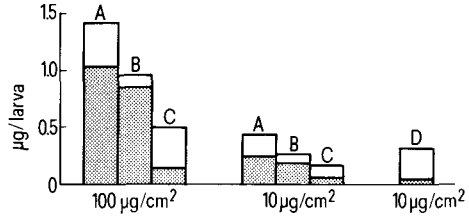


Fig. 2. Total amount of the labelled material and proportion of the unchanged active substance (dotted part of the column) in 8-day-old larvae exposed to filter paper impregnated with 100 or 10 µg/cm² of IGR. *A* Whole body; *B* body without digestive tract and Malpighian tubules; *C* digestive tract with Malpighian tubules; *D* faces collected between the 6th and 12th day of L6 calculated pro larva/day.

epoxy-3-ethyl-1-(p-ethylphenoxy)-7-methylnonane, cis/trans mixture (=Ro 10-3108/000a constituent of epofenonane) dissolved in acetone was applied to each paper disc in dosages of 10 and 100 µg/cm². Each experiment was carried out in triplicate. The specific radioactivity of the IGR for the 2 dosages used was 1.206×10^5 dpm/µg and 8.434×10^5 dpm/µg respectively. The insects (3 pairs per sample) collected at regular intervals during the last and supernumerary larval stages were analysed individually (unit = 1 pair). The material was homogenized in 3 ml of methanol to which 115 µg of the non-radioactive IGR were added in order to protect the small amount of the radioactive material. The radioactivity was measured from an aliquot of the methanol supernatant by liquid scintillation counting and the mean was calculated per insect weight of 8-day-old last stage larva (= 59.8 mg). The radioactivity which remained in the biomass was negligibly small.

The proportion of unaltered IGR was determined, using thin layer chromatography on silica gel (0.25 mm) with benzene/ethyl acetate = 19:1. The zone containing IGR was scraped and the radioactivity measured. All degradation products were much more polar than the IGR and were well separated in the benzene/ethyl acetate system.

Results. The development in the last larval instar of the German cockroach lasts 14–15 days, whereupon the adults emerge. The larvae exposed to the highest dose of 100 µg/cm² of the IGR ecdyse on the 15th day into a supernumerary instar. The ecdysis of about 15% of the larvae is considerably delayed, and the last of those permanent larvae[3] were examined 12 days after the adult emergence in the control. The larvae exposed to the lower dose of 10 µg/cm² ecdysed on the 13th day into a supernumerary instar. Still lower dosages cause the formation of intermediate forms between larvae and adults.

The persistence of the IGR on filter paper is very high. Filter discs impregnated with 100 µg/cm² still contained 40% and 34% of the amount applied after 17 or 21 days exposure respectively.

In freshly-hatched larvae exposed to 100 µg/cm² of the IGR, the amount of the labelled material rises steeply during the first h (figure 1). The rate of intake slows down after 24 h, and after reaching the value of 1–1.5 µg/larva or 16–26 µg/g fresh weight, the amount remains almost constant through 2 3rds of the instar. During this period nearly all labelled material consists of the unchanged IGR. A conspicuous accumulation of the labelled material was observed towards the moulting, followed by an abrupt decrease 24 h after the ecdysis. Most of the labelled material which accumulated at the moulting is not the original IGR but some of its degradation products. The permanent larvae, which remain in the last nymphal instar 12 days longer, contain on average relatively high amounts of the labelled material. This is due to few individuals with very high amounts as found in larvae shortly before ecdysis. The majority of the permanent larvae contain, however, about the same amount as the larvae three days before ecdysis.

The larvae exposed to 10 µg/cm² contain less than half the labelled material as compared with the larvae treated with a 10 times higher dose. Not more than half of this material is the unchanged active substance. The accumulation of the labelled material at the time of the ecdysis is in this case less conspicuous.

The distribution of the IGR in the body of the treated larvae was determined at the middle of the instar, on day 8. The alimentary canal with Malpighian tubules contained mostly metabolites (60–80% of the total radioactivity), the rest of the body mostly original substance (90% of the total radioactivity). 10% of the total labelled material detected in faeces from day 6–12 was the unchanged active substance.

Discussion. The amount of IGR present in the insect is the result of the dynamic relationship between intake, metabol-

ism and discharge of the substance. The accumulation of the labelled material at the time of ecdysis is very remarkable. The examination of the limited number of permanent larvae available indicates that this accumulation is due to a special physiological condition, rather than to the duration of exposure to the treated substrate. This accumulation may be connected with the limited excretion caused by the antidiuretic hormone, and the sudden decrease after the ecdysis may be brought about by the increased discharge triggered by the diuretic hormone as described by Mills and Whitehead[4] in the American cockroach. The fact that most of this accumulated material is not the original substance but some metabolite, mostly present in the gut, seems to support this interpretation.

The total amounts of the IGR of 1 and 0.3 µg found in the 8-day-old larvae exposed to 100 and 10 µg/cm^2, respectively, are relatively low, when compared with the doses usually applied by topical treatment. The high proportion of the unchanged active substance in the total amount of the labelled material in larvae exposed to the higher dose indicates the limits of the enzymatic deactivation in the insect. In the larvae exposed to the lower dose, the daily intake and discharge is nearly in equilibrium because the amount of the active compound present in the body equals the amount of the metabolites found in the daily discharged faeces. The presence of unaltered IGR together with its degradation products in the faeces demonstrates that both metabolism and excretion are responsible for the loss of active compound. This is consistent with the results of a study by Erley et al.[5] on the distribution and metabolism of JH-3 in the adult locust.

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Effect of lesions of the locus coeruleus complex on the circadian rhythm of plasma corticosterone in the mouse[1]

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Summary. An apparently transient elevation of basal morning (08.00 h) plasma corticosterone levels in male mice was found 48 h after bilateral electrolytic lesions of the brainstem locus coeruleus complex but was not observed 6 weeks after lesioning.

Brainstem noradrenergic systems ascending to the hypothalamus can exert an inhibitory effect on corticotropin (ACTH) secretion[2]. For example, electrical stimulation in the locus subcoeruleus (LSC), or in its efferent projection, the ventral noradrenergic bundle, effectively inhibits stress-induced ACTH release in dogs[3]. Similarly, electrical stimulation in the LSC or in the anteroventral locus coeruleus (LC) rapidly and profoundly depresses ACTH release in chloralose-treated cats[4]. The present study examines the effect of lesions in LC cell groups on the basal (non-stressed) pituitary-adrenal diurnal rhythm in mice.

200 male Swiss ICR mice (Flow Research Labs) were housed individually with food and water ad libitum and a 12-h light/12-h dark lighting cycle (lights on 09.00–21.00 h). Mice were anaesthetized with Nembutal (50 mg/kg) and implanted bilaterally with Teflon-insulated platinum-iridium electrodes (125 µm diameter) aimed at the LC nuclear complex as previously described[5] (coordinates: anterior-posterior = −1.3 mm from lambda; medial-lateral = 0.7 mm; ventral-dorsal = 3.5 mm below the brain surface). 7 days after implantation and either 48 h (n = 91) or 6 weeks (n = 46) prior to decapitation the mice were anaesthetized with ether and lesioned (500 µA anodal current for 10 sec) bilaterally in groups of 12 at 1 of 4 different times of day (6-h intervals). Similarly implanted control groups of 12 mice each were also anaesthetized at 6-h intervals but were not lesioned. Following another 48 h (acute) or 6 weeks (chronic) in the controlled environment, the lesioned and sham lesioned mice were killed at (±30 min) 08.00, 14.00, 20.00 or 02.00 h (acute group) or at 08.00 h only (chronic group) by rapid decapitation within 90 sec of removal from the home cage. Trunk blood was collected and assayed in duplicate for plasma corticosterone[6]. Brains were removed for histological verification of electrode placement and lesion damage. Lesioned mice were divided into 3 groups for each of the 2 lesion-decapitation intervals: unilateral LC-lesioned, bilateral LC-lesioned and bilateral non-LC lesioned mice. Substantial bilateral damage to the LC complex was required to qualify an animal for the designation as bilateral LC-lesioned.

Corticosterone values for mice killed 48 h after lesion or sham-lesion manipulations are shown in the figure 1. Analysis of variance (ANOVA) revealed a significant ($p < 0.01$) diurnal variation in the sham-lesioned control animals similar to that previously reported[7]. The bilateral non-LC lesioned mice also showed a significant daily rhythm ($p < 0.025$, ANOVA). The diurnal rhythm of the unilateral LC-lesioned mice appears normal but was not significant ($p > 0.05$) by either ANOVA or by a Student's t-test comparing morning (08.00 h) and evening (20.00 h) corticosterone values. The rhythm within the bilateral LC-lesioned group, however, may be disrupted. A diurnal variation within this group was not apparent by ANOVA, due primarily to the fact that steroid concentrations at 08.00 h were approximately double those of the bilateral non-LC lesioned and sham-lesioned controls at the same time of the day ($p < 0.05$, Student's t-test). Furthermore, when bilateral LC-lesioned and unilateral LC-lesioned groups were combined to increase the error degrees of freedom in the ANOVA, there was no significant diurnal variation ($p > 0.05$). Plasma corticosterone concentrations for the mice killed at 08.00 h 6 weeks after lesioning were as follows (not shown in the figure): unilateral LC = 6.6 ± 1.5 (n = 14), bilateral non-LC = 8.7 ± 2.4 (n = 14) and bilateral LC = 8.9 ± 1.4 µg/100 ml (n = 18). Thus, the possible acute elevation of the basal morning (08.00 h) corticosterone levels 48 h after bilateral LC-lesions was not found in the chronic lesion groups. We suggest that a transient disrup-