Juvenile Hormone: Inhibition of Ecdysis in Larvae of the German Cockroach, *Blattella germanica*

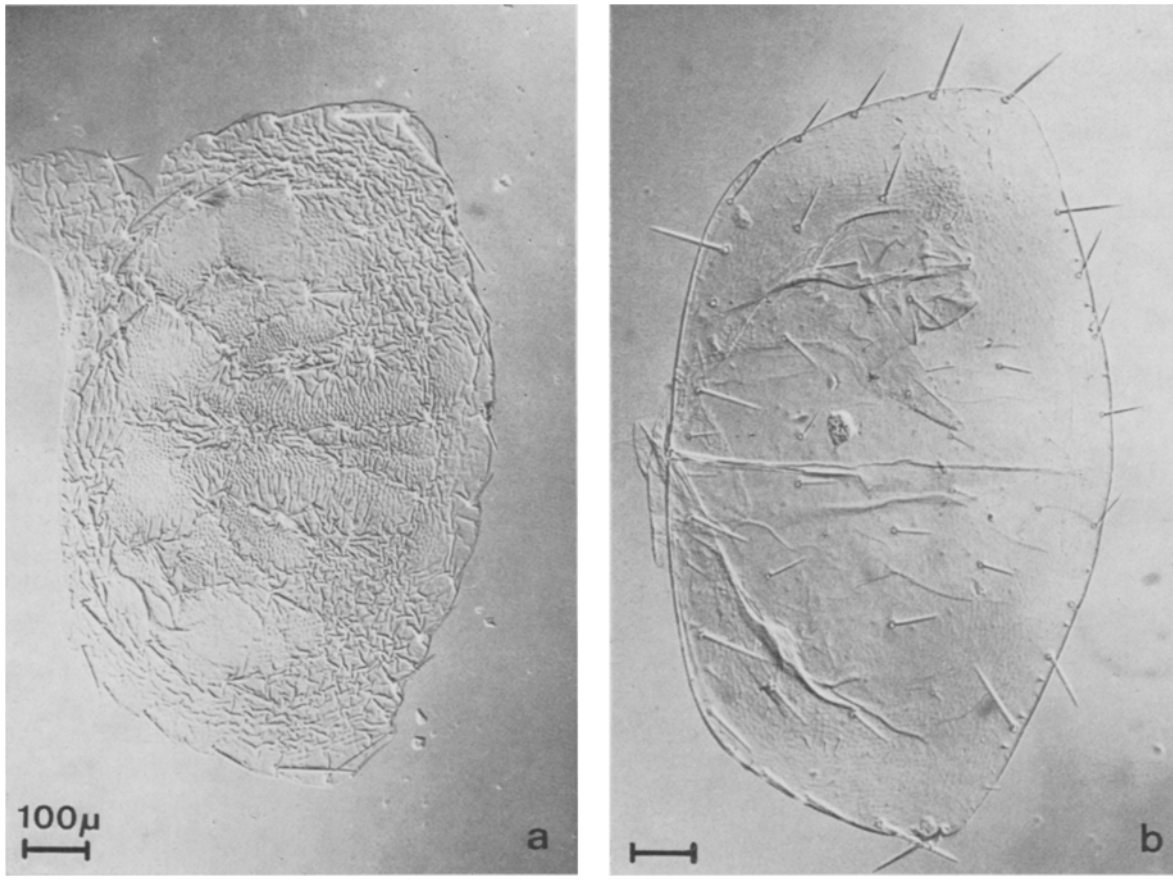
The capacity of juvenile hormone (JH) and many of its analogues to disturb, prevent or interrupt metamorphosis of many insect species is well known. The insects affected during metamorphosis very often exhibit serious difficulties in ecdysing from the old skin. These difficulties are not necessarily connected with the morphogenetic effect mentioned but could as well be the result of an independently working ecdysis-inhibiting effect of JH. If this assumption is correct, the proposed effect should clearly become manifest during the larval moult because the larval stages are subject to no or very limited morphogenetic transformation. We wish to report here that the *Cecropia* hormone (JH = methyl 10, 11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; *cis/trans* mixture) and one of its analogues (JHA = 6,7-epoxy-1-/*p*-ethyl-phenoxy/-3,7-dimethyl-2-octene; *cis/trans* mixture[1,2] are able to prevent ecdysis in any larval stage of the German cockroach, *Blattella germanica* (L.)[3].

Five freshly hatched larvae of the 1st, 2nd, 3rd or 4th instar of *Blattella germanica* were confined in 25 cm^3 plastic cups together with a filter paper disc (10 cm^2) folded to form a tunnel. Ten freshly hatched penultimate or last stage larvae were placed in 200 cm^3 cups with 2 paper discs (20 cm^2 each) arranged similarly as above. The active substance was applied in an acetone solution either to the paper or to the surface of the uninjured abdominal tergites. The first larval stage of treated as well as untreated animals lasts 9–10 days and apolysis takes place at the 7th day followed by the deposition of the new cuticle. Even at a dose of 100 µg JH or JHA/cm^2 filter paper or 10 µg of JH applied topically each 2nd day, the animals behaved quite normally during the first week. There were absolutely no signs of disturbed development or of any poisoning. After 8 days, some animals started to exhibit uncoordinated leg movements, often while laying helplessly on their back. By the 10th day all treated larvae died. At about the same time the untreated and the acetone treated animals underwent a normal ecdysis into the next stage. A more detailed examination of the dead larvae showed that they have formed a perfectly tanned and hardened new cuticle underneath the old one. In some animals, the old cuticle was ruptured along the dorsal suture. Most of them, however, died within the totally intact old integument, being obviously unable to undergo ecdysis. After the removal of the old, smooth and shining cuticle, the wrinkled and coriaceous cuticle of the next stage became visible (Figure). This structure of the new

[1] Stauffer compound No. R-20458.

[2] Both compounds were kindly supplied by F. Hoffmann-La Roche, Ltd., Basel.

[3] Results presented to members of the A.R.C. Unit of Invertebrate Chemistry and Physiology at Brighton (Prof. A. W. JOHNSON, Director) and Cambridge (Dr. J. E. TREHERNE, Deputy Director) on March 19 and 20, 1973.



Wrinkled and coriaceous structure of the pronotum of a pharate 2nd stage larva which died inside the unshed skin of the preceeding stage (a) in comparison to the smooth pronotum of the first stage (b). (10% KOH, dehydration, canada balsam, interference contrast).

cuticle can definitely not be considered a direct effect of the active substance on the epidermal cells because of its similarity to untreated pharate next stage larvae shortly before ecdysis. The new cuticle is apparently stretched directly after ecdysis but before sclerotisation. Because treated animals cannot escape from the old cuticle, sclerotisation of the unstretched cuticle takes place underneath the old one.

In order to determine a possibly existing sensitive phase of this effect, we kept first instar larvae for various periods of time on treated filter paper. We could demonstrate that the larvae have to be in contact with the treated paper for at least 6 succeeding days to get a pronounced anti-ecdysis effect. No clearly defined sensitive phase could be detected in trials, where we kept first instar larvae on treated filter paper between the 3rd and 5th, 5th and 7th, 4th and 6th and between the 3rd and 6th day. 1 or 2 topical applications of 10 µg JH/larvae revealed, however, an increased sensitivity at the 3rd and 4th day of the first instar.

The anti-ecdysis effect could be demonstrated in all larval instars. In the last instar, however, permanent exposure to 10 µg JHA or 100 µg JH/cm² elicited ecdysis inhibition only in about 50% of the larvae. In this case, the anti-ecdysis effect was obviously overridden by the morphogenetic effect signalled by the formation of extra-larvae and all sorts of adultoids. The morphogenetic effect remained strongly expressed up to a dose of 0.1 µg JHA or 1 µg JH/cm². All morphogenetically effected males or females were found to be permanently sterile.

In *Hyalophora cecropia*, the emergence difficulties of adultoids could be explained as the result of a suppression of muscle development (RIDDIFORD[4]). During the larva-larva moult, however, no particular organ reconstruction takes place and consequently, the dissection of larvae of the German cockroach dying in ecdysis did not reveal any particular changes of musculature. DAVEY[5] reported a very interesting case of ecdysis inhibition in last instar larvae of *Phocanema decipiens* (Nematoda) treated with JH. Stimulation of the release of a neurohumoral factor from the brain caused disruption of the function of the exuvial fluid. The larvae died encapsulated in the undigested old cuticle. The moulting difficulties of *Blattella* larvae look very similar. We believe that the inhibition of metamorphosis and ecdysis are two separate effects of JH and its analogues. The ecdysis inhibition is the general effect of JH and can act in any stage of the postembryonic development of roaches. Much lower dosages are required to inhibit metamorphosis. Further experiments are now on the way in this laboratory to understand the mechanism of ecdysis inhibition and its relation to the morphogenetic effect of JH active compounds in both Hetero- and Holometabola.

Zusammenfassung. Das Juvenilhormon von *Cecropia* und eines seiner Analoga bewirken letale Häutungsstörungen in allen Larvenstadien von *Blattella germanica*.

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Urinary Kallikrein from Normal and Hypertensive Rats

According to several reports, there is a significant decrease in kallikrein activity in the urine of hypertensive rats[1,2] and of essential hypertensive patients, as compared with normals[3–5]. Furthermore, evidence has been provided that urinary kallikrein is similar to that contained in renal tissue[6] and that the excretion of this enzyme is correlated with sodium excretion[7,8]. It has been established that a good correlation exists between the esterase, kininogenase and oxytocic activities of the urine of normal rats[9], but no attempts have been undertaken to investigate the enzymatic kinetics of kallikrein obtained from the urine of hypertensive rats. On the other hand, it has not been ruled out that the occurence of an inhibitor could account for the low kallikrein activity.

In the present report, a comparative chemical study of the effect of inhibitors was undertaken between purified kallikrein obtained from the urine of normal and hypertensive rats.

Material and methods. Adult Sprague-Dawley rats were used. Two batches of urine were obtained from hypertensive rats: a) 1 of 860 ml from 17 rats (mean blood pressure, 178 mm Hg); and b) 1 of 820 ml from 11 rats (mean blood pressure, 152 mm Hg). A 3rd batch of 1,450 ml of normal rat urine was used as control. The operation to induce hypertension was performed according to GROLLMAN's procedure[10]. Blood pressure was measured at weekly intervals by the FRIEDMAN and FREED method[11]. For the experiment, only those rats showing a blood pressure consistently over 145 mm Hg were selected. The rats were placed in metabolic cages to collect urine in conditions which prevent contact with feces.

Purification step	KU/mg protein/min		Purification factor	
	Normal	Hypertensive	Normal	Hypertensive
1 Sephadex G-200	4.35	0.14	1	1
2 Sephadex G-200	6.25	0.22	1.48	1.57
CM-52	130.00	6.25	30.00	44.50

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