tained only the germarium and the follicle had not descended even on day 9 (fig. C). In contrast, the ovary of a similar control female contained a fully chorionated egg. Some female offspring which emerged from 4th-cycle pupae of precocene-treated females had no oocyte in either the right or the left ovary, while others carried a fully chorionated egg in the right ovary on day 9 (table 1), comparable to the egg observed in control females of the same cycle. In these experiments between 11 and 14 treated, larvipositing females were examined. The low numbers of F_1 females observed in table 1 are due to emergence of male offspring and non-emergence of some adults. As table 2 shows, treatment with juvenile hormone III restored complete oocyte maturation in offspring of the 1st cycle and reduced the incidence of sterility in 2nd, 3rd and 4th cycle females produced by treated mothers.

Discussion. Precocenes do not affect the reproductive capacity of treated females. However, the effect manifests itself in some females of the F_1 generation. Results in table 1 suggest that precocenes have a delayed action in *G. m. morsitans;* it appears that the offspring are affected, by the action of precocenes on either the mother or the mature oocyte during ovulation. In other insects precocenes are metabolised rapidly[10]; hence it is unlikely that precocenes have a cumulative effect in tsetse flies. When the pregnancy cycle was unduly prolonged - probably due to the premature expulsion of an egg which passed unnoticed (hence the next ovulated egg was not exposed to precocene), the resulting females in the 4th cycle (table 1) had a normal oocyte in the right ovary. Therefore, the time of application of precocenes, relative to ovulation, may be important in determining their action on tsetse flies. This may explain the absence of gross abnormalities in the ovaries of F_1 females of the 1st cycle (table 1), since the time of ovulation varies considerably. The 'sterilising' effect of precocenes is specific to female offspring, while males of the F_1 generation are able to mate and reproduce[11]. Results in table 2 suggest that lack of juvenile hormone may be a causative factor in the retardation of oocyte maturation and sterility in the F_1 generation. Among other factors, the time of application and dose of juvenile hormone could be critical in restoring oocyte development. The corpora allata of precocene treated female parents and their F_1 offspring do not differ in size or structure from those of corresponding control females under the light microscope.

1 Acknowledgments. The authors wish to thank Dr G.C. Unnithan for helpful discussions.
2 D.L. Denlinger and W.C. Ma, J. Insect Physiol. *20,* 1015 (1974).
3 G.C. Ejezie and K.G. Davey, J. Insect Physiol. *22,* 1743 (1976).
4 M.F.B. Chaudhury, in: Advances in Medical, Veterinary and Agricultural Entomology in East Africa, p. 7. Ed. C.P.F. DeLima E.A. Lit. Bereau, Nairobi 1977.
5 W.S. Bowers, T. Ohata, J.S. Cleere and P.A. Marsella, Science *193,* 542 (1976).
6 G.C. Unnithan, K.K. Nair and W.S. Bowers, J. Insect Physiol. *23,* 1081 (1977).
7 G.C. Unnithan, K.K. Nair and A. Sayed, Experientia *36,* 135 (1980).
8 M.H. Landers and G.M. Happ, Experientia *36,* 619 (1980).
9 M. Mackauer, K.K. Nair and G.C. Unnithan, Can. J. Zool. *57,* 856 (1979).
10 B.J. Bergot, K.J. Judy, D.A. Schooley and L.W. Tsai, Pest. Biochem. Physiol. *13,* 95 (1980).
11 M. Samaranayaka-Ramasamy, in preparation.

Delayed effects of juvenile hormone analogues on metamorphosis of *Pyrrhocoris apterus* are not mediated by the corpus allatum

R. Socha and M. Hodková

Institute of Entomology, Czechoslovak Academy of Sciences, Na Folimance 5, 12000 Praha 2 (Czechoslovakia), 3 February 1981

Summary. While it is clear that juvenile hormone analogues disturb metamorphosis, there is some controversy about their effect on the activity of the corpus allatum in the course of metamorphosis. The present experiments showed that juvenile hormone analogues applied to eggs did not prevent inactivation of the corpus allatum at the onset of the last larval instar of *Pyrrhocoris apterus.*

Riddiford[1] reported that metamorphosis of the bugs *Pyrrhocoris apterus* and *Oncopeltus fasciatus* is inhibited by the application of juvenile hormone analogues (JHa) to the late embryonic stages 4 weeks earlier. It was shown later by transplantation experiments that the corpus allatum (CA) of the treated insects fails to stop secreting the hormone at the onset of the last larval instar[2]. The authors conclude that the delayed effects of JHa on metamorphosis are produced by the interference of JHa with the programming of the embryonic CA.

As the sternites of some genetically marked *O. fasciatus* reared with insects treated with JHa in the egg stage showed traces of juvenile cuticle after adult ecdysis, it was suggested that the delayed effects could be due to the persistence of the applied JHa through larval life[3]. Later, Sláma and Socha[4] used white mutants of *P. apterus* as 'victims' of juvenoid contamination through treated red individuals. They proved that metamorphosis is inhibited only when JHa applied to eggs persist outside and/or within body until metamorphosis. It is not clear, however, whether in this case the function of the CA is also influenced by JHa in addition to its direct effect on metamorphosis. There is evidence that JHa present only at the embryonic and early larval stages do not produce delayed effects on the activity of CA[4]. Moreover, JHa does not prevent inactivation of CA at the onset of the 5th (last) instar when present as late as in the course of the 3rd and/or 4th instar[5]. We have examined here whether the delayed effects on the function of CA observed by Riddiford and Truman[2] are due to the continuous presence of JHa from the embryonic or early larval stage until metamorphosis.

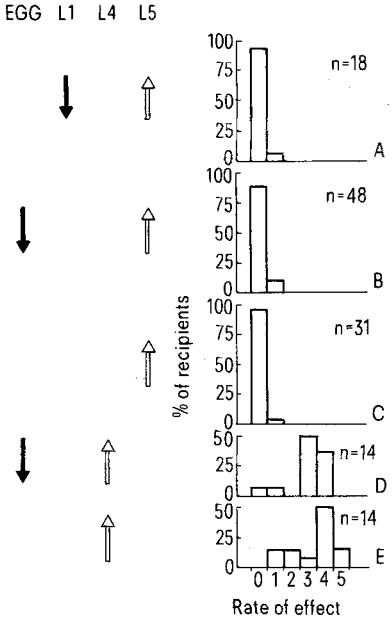
Methods. The larvae of *P. apterus* were reared on linden

seed and water ad libitum at 27 °C and daylengths of 18 h. 'Synthetic mixture' of JHa was prepared according to Vinson and Williams[6] by Dr P. Beran at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences. JH-active material prepared according to the same instructions was used also by Riddiford and Truman[2]. We applied 5 µg of synthetic mixture in 0.2 µl of acetone solution topically to each egg at 5 days old (i.e. about 1.5 days before hatching) or to each freshly hatched 1st instar larva. For removing of unhatched eggs, shells, shed skins, and transfer of larvae (thereafter referred to as treated larvae) to new Petri dishes the experimental procedure of Riddiford and Truman[2] was exactly repeated.

Effects of juvenile hormone analogues (applied to 1st instar larvae or eggs) on metamorphosis of *Pyrrhocoris apterus*

Treatment of insect with JHa (developmental stage)	N	Rate of effect (%)* 0	1	2	3	4	5
1st instar larva	39	5.1	17.9	25.7	17.9	23.1	10.3
Egg	37	5.4	32.4	21.6	29.7	10.8	0

* For classification of adultoids see figure.



Effects of juvenile hormone analogues (applied to 1st instar larvae or eggs) on the corpus allatum activity at onset of 4th or 5th larval instar of *Pyrrhocoris apterus*. Full arrows indicate developmental stages treated with JHa. Open arrows indicate instars from which CC+CA was excised and transplanted into recipients. The effect of CC+CA on recipients was evaluated after their adults ecdysis according to a published scoring system[7]. Juvenilization increases from 1 to 5.

According to Sláma and Socha[4] this treatment assures persistence of JHa from the day of application until metamorphosis of experimental insects.

The endocrine activity of the CA was evaluated after its transplantation by its ability to induce development of the recipients into adultoids instead of adults. The complex of corpora cardiaca + aorta + corpus allatum (CC+CA) was excised under insect saline from donor larvae 24 h after ecdysis. The glands from treated and untreated donors were passed alternatively through a series of 6 dishes with insect saline. Dishes with insect saline were renewed after washing of 6 CC+CA (3 from treated and 3 from untreated larvae). Recipients were never touched by the same forceps as donors. Recipient larvae were deprived of food within a few hrs after ecdysis to 5th (last) instar and operated upon the next day. The CC+CA was implanted through an incission in the abdomen.

Results and discussion. In the first experiment we tried to determine whether the presence of JHa in the embryonic stage is a prerequisite for delayed effects on the function of CA. We applied JHa to 1st instar larvae and evaluated the activity of CA after ecdysis of the treated larvae to 5th instar. The transplanted glands, however, had no juvenilizing effect on recipients (fig. A). This indicates that the CA of treated larvae is inactive at the onset of the last larval instar. Supposing that the results of Riddiford and Truman[2] are correct, we concluded that JHa has to act at a late embryonic stage, in addition to the larval stage, to prevent inhibition of the CA at the last larval instar.

In the next experiment we applied JHa to eggs. Most treated larvae ecdysed to adultoids (table) as in the experiments of Riddiford and Truman[2]. The results of transplantation experiments, however, contradict the finding of the above authors. The CC+CA excised from the 5th instar larvae was not active and did not produce juvenilization of recipients irrespective of whether donors were treated or untreated larvae (fig. B, C). On the other hand, the CC+CA excised from either treated or untreated 4th instar larvae was active and strongly disturbed the metamorphosis of recipients (fig. D, E). This shows that inactivity of the transplanted 5th instar glands (fig. B, C) was not due to the transplantation procedure.

We have determined that JHa applied at a late embryonic stage or the onset of the 1st larval instar and persisting outside and/or within the body until metamorphosis neither impair the function of CA at the penultimate larval instar nor prevent the inactivation of CA at the last larval instar. The persistence of JHa in our experiments is clearly documented by the fact that treated larvae ecdysed into adultoids (table) although they had no active CA in the last larval instar (fig. A, B).

How can the difference between our results and the results of Riddiford and Truman[2] be explained? Being aware of the JHa persistence in treated donor larvae, we have taken special care to avoid contamination of recipients by JHa through any of the equipment or the insect saline (see 'methods'). It is plausible that the results of the above authors were affected by the contamination of recipient larvae by JHa during transplantation experiments.

1 L.M. Riddiford, Science *167*, 287 (1970).
2 L.M. Riddiford and J.W. Truman, Nature *237*, 458 (1972).
3 J.H. Willis and P.A. Lawrence, Nature *225*, 81 (1970).
4 K. Sláma and R. Socha, Acta ent. bohemoslov. *76*, 289 (1979).
5 M. Hodková and R. Socha, Biol. Bull., in press (1981).
6 J.W. Vinson and C.M. Williams, Proc. natl Acad. Sci. USA *58*, 294 (1967).
7 C.M. Williams and K. Sláma, Biol. Bull. *130*, 247 (1966).