were gently resuspended and the samples examined by differential interference microscopy at 400× magnification. Cells binding more than 3 erythrocytes were scored positive.

ANAE activity of lymphocytes was detected according to the method of Knowles et al.[2]. The lymphocytes, subjected or not to the E-rosette test, were smeared on glass microscopic slides and then immediately fixed in buffered formalin acetone (pH 6.6) at 4 °C for 10 min. After washing, the slides were kept moist at all times and never allowed to dry. The demonstration of esterase activity was performed using α-naphthyl acetate as substrate and hexazotized pararosaniline as coupler. The fixed cell smears were also incubated with an irreversible inhibitor, diethyl-p-nitrophenyl phosphate (E 600, Sigma) at 37 °C for 1 h before ANAE staining by the method of Ranki[4]. The slides were incubated for 21 h at room temperature, washed with distilled water and then counterstained with 0.5% toluidine blue for 30 min.

Lymphocytes ($1 \sim 5 \times 10^6$/ml) from thymus or peripheral blood were suspended in RPMI-1640 culture medium containing 20% fetal calf serum (Gibco). The cells were incubated with PHA-P (10 μg/ml, Difco) or Con A (10 μg/ml, Miles-Yeda) in short test tubes (14 × 100 mm) at 37 °C for 72 h in a humidified atmosphere with 5% CO_2. The percentages of PHA or Con A blastoid cells were determined according to the morphological method previously described. A microculture method using plastic test plates (0.2 ml, Nunch) containing 2×10^5 cells in the medium was also performed. 16 h before harvest, each well (0.2 ml) received 0.2 μCi ^{3}H-TdR (45 Ci/mmole, The Radiochemical Centre, Amersham, England). The cells were harvested on glass filter paper (Whatman, CF/A). Radioactivity was measured in a Beckman liquid scintillation counter.

Results and discussion. ANAE activity was detected in lymphocytes and monocytes but not in polymorphonuclear leucocytes. The majority of ANAE positive lymphocytes displayed a prominent, red-brown, dot-like staining pattern adjacent to the cell membrane whereas monocytes contained diffuse, cytoplasmic red-brown reaction products. This ANAE reaction was completely inhibited by preincubation of the cell preparation in a 10 mM concentration of E 600. The percentages of ANAE positive lymphocytes in the thymus were much higher than those in the peripheral blood (fig. 1) although the pattern and intensity of ANAE staining of both lymphocytes were the same. In preliminary experiments we also observed high ANAE activity in the thymus lymphocytes from mouse, rat and guinea-pig. These results differ from those reported in human and mouse by some authors[2-4]. We obtained a close correlation of percentages of ANAE positive cells and E-rosette forming cells in the thymus from 10 monkeys: 91.7 ± 1.3% of thymus lymphocytes were ANAE positive and 87.6 ± 19% formed E-rosettes (fig. 1).

In the present studies ANAE activity in lymphocytes forming E-rosettes was demonstrated directly. The intense, dot-like reaction product in thymus lymphocytes forming E-rosettes was localized at the periphery of the cells (fig. 2). These results suggest that the E-rosette forming cell population appears in most instances to be entirely contained within the ANAE positive cell population. The role of esterases in cellular metabolism is not fully understood. However, the distinctive staining characteristic of thymus lymphocytes and T-lymphocytes suggests that the histochemical method may be of great use for identification of T-lymphocytes. In lymphocyte cultures the percentages of PHA or Con A blastoid cells in the thymus were markedly lower than those in peripheral blood (fig. 1). The number of cells carrying out DNA synthesis as estimated by ^{3}H-TdR incorporation into thymus lymphocytes in PHA or Con A cultures was also lower than that of blood lymphocytes obtained from the same donor in 3 cases (PHA; thymus 7477 cpm, blood 19,410 cpm, Con A; thymus 11,109 cpm, blood 28,381 cpm). This is in agreement with the result obtained in human thymus[1]. It is concluded that there is no definite correlation between the ANAE activity or E-rosette formation capability of thymus lymphocytes and their in vitro lectin responses.

1 S. Kato, K. Yamaguchi, K. Awaya and M. Nishioka, Acta haem. Jap. *41*, 45 (1978).
2 D.M. Knowles, H.T. Hoffman, M. Fovarini and H.G. Kunkel, Cell. Immun. *35*, 112 (1978).
3 R. Obrist, R. Albercht and G.A. Nagel, Experientia *34*, 660 (1978).
4 A. Ranki, Clin. Immun. Immunopath. *10*, 47 (1978).
5 S. Kato, Bull. Yamaguchi med. Sch. *24*, 1 (1977).

Precocene treatment of the female tsetse fly *Glossina morsitans morsitans* sterilises her female offspring

M. Samaranayaka-Ramasamy and M.F.B. Chaudhury[1]

The International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi (Kenya), 19 January 1981

Summary. When adult female *Glossina morsitans morsitans* were treated with either precocene I or precocene II, females of the F_1 generation, emerging from pupae of the 2nd, 3rd and 4th reproductive cycles failed to develop oocytes.

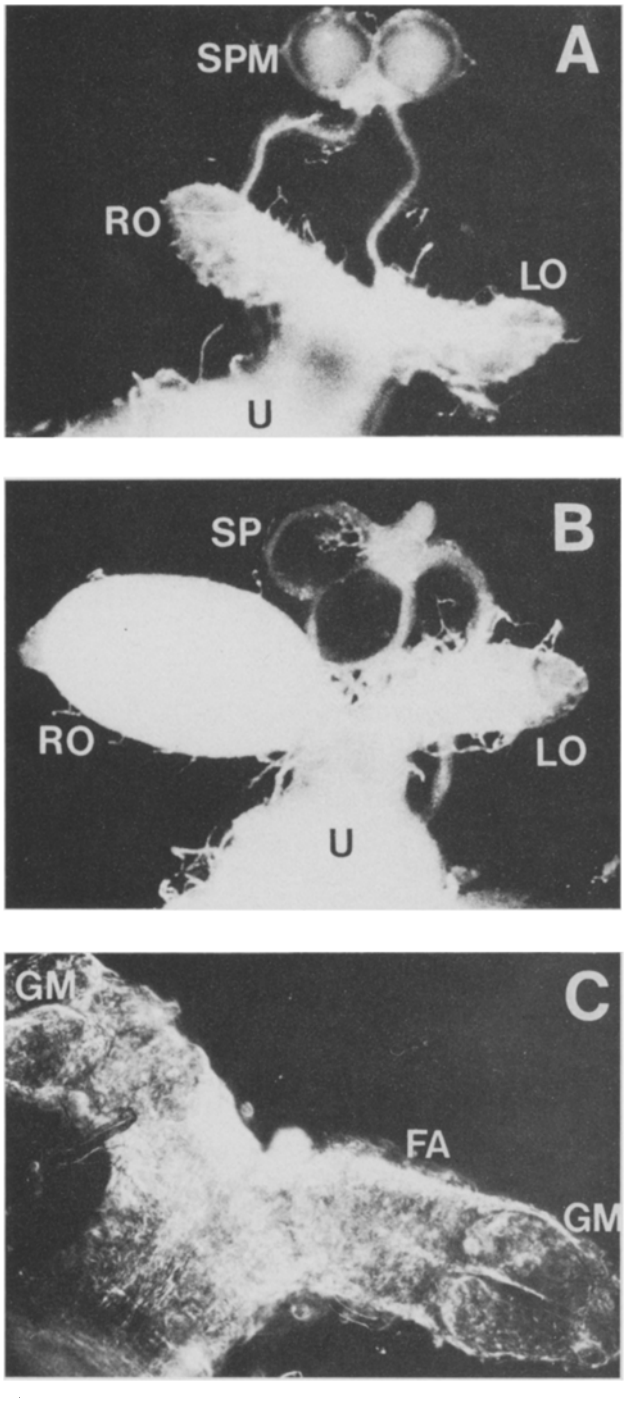
Tsetse flies (*Glossina* spp.) reproduce by adenotropic viviparity. To achieve this end, the female tsetse fly produces a single egg, in the right ovary, which ovulates on day 9 of adult life in the case of *G. morsitans*[2]. The fertilized egg undergoes embryogenesis and development is completed within the mother, the developing larva being nourished by secretions of the modified accessory glands. During this time, the 2nd egg develops in the left ovary. Following larviposition, the 3rd instar larva soon pupates, the pupal stage lasting about 30 days, after which adult emergence occurs. 1-2 h after the 1st larviposition[2], the 2nd egg ovulates and the cycle repeats itself. The corpus allatum is known to be involved in regulating the reproductive cycle of female tsetse flies[3,4].

Precocenes[5] are known to affect corpus allatum activity[6,7] and oocyte development in insects[5,8]. All reports on the effects of precocenes on insects focus attention on their action on the treated (parental) generation. The only exception is the work on aphids[9], where the effect of precocene II was carried over to the F_1 generation to produce alate

offspring. The present publication constitutes the first report on the ability of precocenes to induce sterility in the F_1 generation following treatment of the female parent.

Materials and methods. *Glossina morsitans morsitans* were obtained from the laboratory colony at ICIPE maintained at 25 °C, 12-h photoperiod, 100 lx light intensity, fed on rabbit ears 6 days a week. Virgin females were treated with either 10 µg/µl precocene I (Calbiochem) per fly, or 5 µg/µl precocene II (Calbiochem) per fly by topical application on the dorsum of the thorax on days 1 and 2 of adult life. Control females were treated similary with 1 µl of acetone per fly. All females were mated on day 2-3, with 6-day-old unmated males. Females were treated again with precocene I, or precocene II or acetone on day 9 and then once within 1-12 h after each larviposition for the first 3 larvipositions. Most larvipositions occurred during the photophase[2], and therefore the larger proportion of tsetse flies received precocene treatment within 1-2 h of larviposition. Mated females were kept individually in 7.6×3.8 cm polystyrene vials, the pupae from each female being collected and observed throughout, until after emergence. In a series of experiments, adult female offspring of the F_1 generation were maintained routinely and dissected on day 9. In another experiment, all females of the F_1 generation were treated topically with 20 µg/2 µl juvenile hormone III (Calbiochem) per fly on day 1 after the 1st blood meal; control flies received 2 µl of acetone per fly. Females were maintained routinely and dissected on day 9. Ovaries were dehydrated in alcohol, cleared in xylene and mounted in DPX for microscopic examination.

Results. In the F_1 generation, all females that emerged from pupae produced in the 1st reproductive cycle of precocene-treated females, had an oocyte in the right ovary. While retardation of oocyte maturation was observed in some of these females (table 1), the right ovary always had a fully chorionated egg on day 9 in the corresponding F_1 offspring from acetone-treated females. F_1 generation females which emerged from pupae produced in the 2nd and 3rd reproductive cycles of precocene-treated females were normal in every respect; these flies could mate successfully, but had no oocyte in either the right or the left ovary (table 1). As figure A shows, the ovaries of such insects appeared 'empty' compared to those of control insects (fig. B). They con-



Ovaries of F_1 generation females which emerged from pupae produced in the 2nd reproductive cycle. *A* Precocene I-treated female. ×47. *B* Acetone-treated female. ×52. *C* Precocene II-treated female. ×216. RO, right ovary; LO, left ovary; U, uterus; SPM, spermatheca containing sperm; SP, spermatheca without sperm; GM, germarium; FA, follicle absent.

Table 1. Score chart for oocyte development in right ovaries in the F_1 generation on day 9 of adult life

Treatment	1st cycle	2nd cycle	3rd cycle	4th cycle
Precocene I	++[a] (3)[d]	++ (0)	All normal	++ (2)
	+−[b] (4)	+− (0)	male	+− (0)
	−−[c] (0)	−− (2)	offspring	−− (1)
Precocene II	++ (3)	++ (0)	++ (0)	++ (2)
	+− (2)	+− (0)	+− (0)	+− (0)
	−− (0)	−− (6)	−− (5)	−− (1)
Acetone	++ (4)	++ (3)	++ (3)	++ (4)
	+− (0)	+− (0)	+− (0)	+− (0)
	−− (0)	−− (0)	−− (0)	−− (0)

[a] Chorionated egg; [b] retardation; [c] no oocyte; [d] number of observations.

Table 2. Score chart for oocyte development in right ovaries after treatment with juvenile hormone

Treatment	1st cycle	2nd cycle	3rd cycle	4th cycle
Precocene I	++[a] (4)	All normal	++ (1)	++ (1)
	+− (0)	male	+− (0)	+− (0)
	−− (0)	offspring	−− (1)	−− (0)
Precocene II	++ (5)	++ (1)	++ (5)	++ (1)
	+− (0)	+− (0)	+− (0)	+− (0)
	−− (0)	−− (2)	−− (0)	−− (2)
Acetone	All normal	++ (2)	++ (2)	++ (1)
	male	+− (0)	+− (0)	+− (0)
	offspring	−− (0)	−− (0)	−− (0)

[a] Scoring as in Table 1.

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