To induce synchronous ovulation, 4 groups of rats were injected s.c. with 20 IU of PMS (Sigma chemicals). Animals were sacrificed at 0 h, 24 h, 48 h, and 72 h by over-exposure to ether vapors. The ovaries were removed immediately, dissected free from the bursa and extraneous tissues, weighed and analyzed. The procedure utilized for the analysis of ovarian 5-HT was developed by Shellenberger and Gordon[6]. The Aminco-Bowman Spectrophotoflurometer was used at a wavelength of 385 nm for activation peak and 490 nm for emission. The data was subjected to analysis of varience using t-test.

Results. In the 1st experiment, the figure shows the results of assaying for ovarian 5-HT content which reveals comparatively high content, fluctuation of ovarian 5-HT content was observed. The peak for 5-HT was noticed at estrus, which was significantly higher than of that observed during diestrus ($p < 0.05$). No significant difference ($p > 0.05$) was detected between diestrus and metestrus or between estrus and metestrus. In experiment 2, involving immature rats injected with 20 IU of PMS, there was no detected ovarian 5-HT using this procedure. Both experiments were repeated twice and the same results were obtained both times.

Discussion. The increase in ovarian 5-HT level in the cyclic rat during ovulation might indicate the importance of this neurotransmitter in the ovulation process. Injections of 5-HT antagonist in mature animals was found to inhibit ovulation[5]. Meanwhile, large doses of 5-HT inhibit ovulation in the immature PMS-treated animals as well as adult animals[7–9].

Recently it was indicated that the inhibitory effect of 5-HT on ovulation in the adult rat is peripherally mediated rather than centrally[10]. The results presented here indicate a definite physiological difference between the immature induced ovulated animals and the mature cyclic rat. Recently, the pharmacological responsiveness differences have been observed between the 2 different groups of animals[11]. Data from this experiment might indicate the lack of complete innervation in PMS-treated animals which can explain the high number of ova shed in those animals. Follicular innervation might be involved in the control of the number of ova shed to the oviduct. The 5-HT effect on the peripheral level as well as its availability in appreciable amounts in the ovary does not deny its importance in maintaining delicate balance of brain biogenic amines in regulating the release of the hypothalamic gonadotropin releasing factors. This role has been documented previously[9,10]. The present experiment indicates that the mature ovary is probably another site for 5-HT effect. As previously indicated, 5-HT[7–9] and its antagonist[5] can cause the same effect, inhibition of ovulation. It may be that the female rat has 2 5-HT-sensitive areas, one in the brain and the other in the ovary. The pharmacological differences between the spontaneous ovulating mature rats and PMS-induced immature rats may be due to the lack of 5-HT receptors in ovaries of the latter. It can be suggested from this investigation that the 5-HT and serotoninergic fibres of the ovary play an important role in the ovulation process in the cyclic rat, but not in the PMS-induced ovulating rats.

6 M. K. Shellenberger and J. H. Gordon, Analyt. Biochem. *39*, 356 (1971).
7 W. K. O'steen, Endocrinology *77*, 937 (1965).
8 C. A. Endersby, J. M. Robson, F. M. Sullivan and C. Wilson, J. Endocr. *48*, VIII (1970).
9 A. P. Labhsetwar, Nature, Lond. *229*, 203 (1971).
10 C. A. Wilson and P. G. Mc Donald, J. Endocr. *60*, 253 (1974).
11 M. Markó and E. Flückiger, Experientia *30*, 1174 (1974).

Effects of precocene II and juvenile hormone III on the activity of neurosecretory A-cells in *Oncopeltus fasciatus*[1]

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Summary. Application of precocene II, topically or by contact method, appears to inhibit the synthetic activity of neurosecretory A-cells of the pars intercerebralis of *Oncopeltus fasciatus*. Treatment of precocene-treated insects with JH III apparently stimulates the secretory activity of these cells.

Treatment of female *O. fasciatus* larvae with the chromene derivative, precocene II[3], induced precocious metamorphosis and sterility[3,4]. In adult females it inhibited egg maturation and corpus allatum (CA) growth[5] and induced degeneration of the CA[6]. The effects of precocene II on ovarian growth can be reversed by the application of exogenous juvenile hormone (JH)[3,4]. It is not known whether precocene II affects the CA directly or whether its effects are mediated through other components of the neuroendocrine system. In *O. fasciatus* the median neurosecretory cells are known to influence the CA growth and reproduction[7]. Therefore we studied the effects of precocene II on the dynamics of the A-cells of the pars intercerebralis of adult female *O. fasciatus* by microspectrophotometry of paraldehyde fuchsin (PF) stained material.

Materials and methods. Newly emerged adult females (0–1-h-old) of *O. fasciatus* were treated topically with 50 µg precocene II (ZR 2448, Zoecon Corporation, Palo Alto, California) dissolved in acetone. Brains from these, and acetone treated control insects were fixed in Bouin's fluid, 2, 4 and 6 days after treatment, and processed for paraffin sections. Another group of newly emerged female insects were treated with precocene II by contact method (15 µg/an²)[6] and then 5 days after were treated topically

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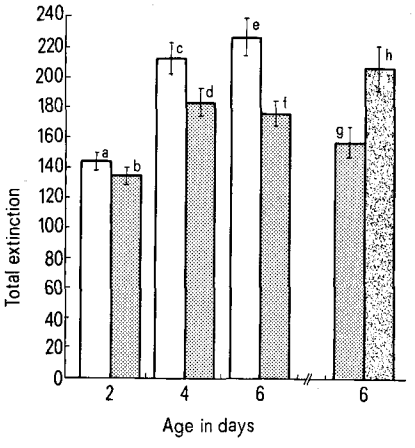
2 We thank Dr G. B. Staal, Zoecon Corporation, Palo Alto, California, for the gift of precocene II.
3 W. S. Bowers, in: The Juvenile Hormones, p. 394. Ed. L. I. Gilbert. Plenum Press, New York and London 1976.
4 W. S. Bowers, T. Ohta, J. S. Cleere and P. A. Marsella, Science *193*, 542 (1976).
5 W. S. Bowers and R. Martinez-Pardo, Science *197*, 1369 (1977).
6 G. C. Unnithan, K. K. Nair and W. S. Bowers, J. Insect Physiol. *23*, 1081 (1977).
7 A. S. Johansson, Nytt Mag. Zool, *7*, 1 (1958).

with 20 µg of JH III (Calbiochem, San Diego, California) or acetone. 24 h later the brains from these insects were processed as described before. 5 µm thick paraffin sections were stained with PF and the amount of PF-stainable material in the A-cell perikaryon was determined microspectrophotometrically[8,9] by using a scanning microscope photometer (Carl Zeiss, Oberkochen, W. Germany) linked to a PDP-12 computer. The measuring apperture was set at 1.6 µm and extinction measurements were taken at 490 nm and 1 µm step size from sections of 15–30 A-cells, from a minimum of 6 insects, for each group. The data were analysed using 1 way analysis of variance or by the t-test.



Amount of PF-stainable material (total extinction) in the neurosecretory A-cells. a, c and e acetone treated control; b, d and f treated with 50 µg precocene II on day 0; g treated with precocene II, by contact method, on day 0, and 2 µl acetone on day 5; h treated with precocene II, by contact method, on day 0, and 20 µg JH III on day 5. Vertical bars represent twice the SE of the mean.

Results and discussion. In insects treated with precocene alone there was no sign of yolk deposition in the oocytes, whereas application of JH III to precocene-treated insects induced yolk deposition. Analysis of the data obtained by microspectrophotometry (figure) revealed that in precocene-treated insects there was a significant reduction in the amount of stainable material 4 and 6 days after the treatment ($p<0.05$; $p<0.01$). There is no evidence to suggest that this decrease is due to an accelerated rate of transport of neurosecretory material from the perikaryon to the neurohaemal site in the aorta. The decrease is probably due to an inhibition in the synthetic activity of the cells. The dynamics of the A-cells in the control insects is comparable to that of normal insects[9]. Within 24 h after JH-treatment of precocene-treated insects, there is a significant increase in the amount of PF-stainable material in the A-cells ($p<0.01$) (figure), indicating an apparent stimulatory effect. Therefore, it is probable that the inhibition of these cells after precocene-treatment may be due to the absence of a positive feed back from the CA. It is pertinent to mention that injection of JH or implantation of CA into allatectomized *Schistocerca gregaria* stimulates the synthetic activity of the A-cells[10,11]. From the present studies it is not known whether the inhibition and subsequent degeneration of the CA, after precocene-treatment[5,6] is mediated through the A-cells, although removal of these cells leads to subnormal size of the CA[7].

8 D. C. Mahon and K. K. Nair, J. Histochem. Cytochem. *23*, 652 (1975).
9 D. C. Mahon and K. K. Nair, Cell Tissue Res. *161*, 477 (1975).
10 A. R. McCaffery and K. C. Highnam, Gen. comp. Endocr. *25*, 358 (1975).
11 A. R. McCaffery and K. C. Highnam, Gen. comp. Endocr. *25*, 373 (1975).

PRO EXPERIMENTIS

Blood volume and extracellular space (ECS) of the whole body and some organs of the rat

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Summary. Methods are described for estimation of blood volume and extracellular space (ECS) in the whole body and in some organs with ^{51}Cr, ^{14}C-thiocyanate and ^{3}H-inulin. A mean blood volume of 47 ml/kg, a thiocyanate space of 350 ml/kg and a inulin space of 288 ml/kg were determined in the rat. The corresponding values of organs are shown in figures 1–3.

Blood volume and extracellular space of rats have already been determined by several authors[1–3]. The total blood volume or the blood content of the organs can be determined either by volume of plasma with Evans Blue (T 1824) and ^{131}J marked albumin, or by the volume of erythrocytes with ^{32}P, ^{55}Fe or ^{51}Cr. If ^{51}Cr is used as tracer, the isotope is absorbed by the erythrocytes and excreted by the kidneys to a very small degree[4], because ^{51}Cr effectively combines with the globins components of the haemoglobines[5]. The object of this paper was to determine the blood volume and the extracellular space in the total animal and in organs as exactly as possible. As we could not find any related data or other details, the point of interest of those experiments was the determination in the organs.

Methods. General. Adult male Sprague-Dawley rats (Zentralinstitut für Versuchstierkunde, Hannover), weighing 170–240 g were used in this investigation. They were fed with pellets (Altromin, Lage/Lippe) ad libitum, but were fasted for 18 h prior to each experiment.

Red cell volume determination. ^{51}Cr was obtained from the Radiochemical Centre Amersham in form of $Na_2{}^{51}CrO_4$. After having sampled the blood with a heparinised capilette, a slight centrifugation is executed, because the ^{51}Cr-absorption in a saline erythrozyte-suspension is more complete than in pure blood[6,7]. The erythrocyte sediment is suspended in a physiological saline to a volume of 1 ml. After having added 15 µCi ^{51}Cr, the mixture was stored for 45 min at room temperature. The incubation was terminated by centrifugation, the erythrocytes were washed twice with 0.9% NaCl solution, the plasma was added again and 80 µl of reconstituted blood were injected into the tail vein. 50 µl of the suspension was applied as standard for the measurements of radioactivity. Blood samples were taken 60 min p.a. for activity measurements and determination of haematocrit;