5 J.P. Delbecque, M. Prost, B.F. Maume, J. Delachambre, R. Lafont et B. Mauchamp, C. r. Acad. Sci. Paris *281*, 309 (1975).
6 A. Bouthier, J.L. Pennetier, B. Mauchamp et R. Lafont, C. r. Acad. Sci. Paris *280*, 1837 (1975).
7 R. Lafont, B. Mauchamp, C. Blais et J.L. Pennetier, J. Insect Physiol. *23*, 277 (1977).
8 B. Calvez, C. r. Acad. Sci. Paris *282*, 1367 (1976).
9 M. Lagueux, J.M. Perron et J.A. Hoffmann, J. Insect Physiol. *22*, 57 (1976).
10 N.L. Young et P.R. Young, Insect. Biochem. *6*, 169 (1976).
11 B. Calvez, M. Hirn et M. De Reggi, FEBS Letters *71*, 57 (1976).
12 R.B. Hodgetts, B. Sage et J.D. O'Connor, Devl Biol. *60*, 310 (1978).
13 J.A. Hanaoka et E. Ohnishi, J. Insect Physiol. *20*, 2375 (1974).
14 J.M. Legay, B. Calvez, M. Hirn et M. De Reggi, Nature *262*, 489 (1976).
15 M. Hirn, M. Coulon et M. De Reggi, C. r. Acad. Sci. Paris *284*, 2147 (1977).
16 M. Coulon, Devl Growth Differ. *19*, 181 (1977).
17 M.L. De Reggi, M.H. Hirn, M.A. Delaage, Biochem. biophys. Res. Commun. *66*, 1307 (1975).
18 M. Hirn, Thèse Etat Marseille, sous presse.
19 C. Hetru, M. Lagueux et J.A. Hoffmann, Life Sci., sous presse.
20 J.N. Kaplanis, W.E. Robbins, M.J. Thompson et S.R. Dutry, Science *180*, 307 (1973).
21 T. Mizumo et E. Ohnishi, Devl Growth Differ. *17*, 219 (1975).
22 M. Lagueux, Thèse, Etat Strasbourg, 1978.

Glycogen contents in the rat uterus: Response to *Hibiscus rosa-sinensis* Linn. extracts[1]

Anand O. Prakash[2]

School of Studies in Zoology, Jiwaji University, Gwalior 474002 (India), 22 September 1978

Summary. Ethanolic extracts (50%), as well the benzene extracts, of *H. rosa-sinensis* Linn. have reduced significantly the glycogen contents in the uterus of adult rat. Both the extracts exhibit a clear-cut dose-response relation. The inhibition in glycogen contents increases as the dose is increased. Of the 2, benzene extract seems to be more potent. The results are due to antiestrogenic nature of the extracts.

Contraceptive property of *Hibiscus rosa-sinensis* Linn. (flowers) reported by Batta and Santhakumari[3] has been confirmed in rats[4–7]. In view of the antiestrogenic activity of 50% ethanolic and benzene extracts[8], the present communication deals with their effect on uterine glycogen contents in adult rats.

Materials and methods. 50% ethanolic and benzene extracts of *H. rosa-sinensis* (flowers) were obtained by soxhlet apparatus as described earlier[6]. Dried extracts were separately macerated with gum acacia suspended in distilled water at doses of 75, 150 and 300 mg/kg b. wt.

Colony-bred Swiss adult female rats, 3–4 months old (165±15 g), were maintained under uniform husbandry conditions and were given 'Hindustan Lever' palleted diet and water ad libitum. The vaginal smear of each rat was examined daily for 18 days to select animals of regular cycles. The rats showing proestrus stage were selected and divided into 4 groups separately for each extract. Each dose was administered orally for 6 days (1 complete cycle), 12 days (2 complete cycles) and 18 days (3 complete cycles) to different groups of adult rats (table). Control rats in each group received vehicle only in a similar manner. Vaginal smear of each rat was examined daily in the morning. The animals were sacrificed in diestrus stage and 48 h after the last dose, i.e. on 8th, 14th and 20th day respectively. The uteri carefully dissected out, trimmed, blotted on filter paper and weighed quickly to the nearest 0.1 mg and processed for glycogen estimation using anthrone reagent method[9]. The results were statistically analyzed using analysis of variance.

Results. The table shows the results. 1. 50% ethanolic extract. It reduces the uterine glycogen contents in rats significantly. The effect depends upon the dose and the period of treatment; 75 mg/kg dose is only effective to decrease the glycogen contents when administered for 18 days (vs. control $p < 0.01$). Similarly, 150 mg/kg dose when applied for 12 and 18 days reduces the glycogen concentration significantly (vs. respective control $p < 0.02$ and < 0.01); 300 mg/kg dose is effective at every schedule, but it is highly significant at 18 days schedule (vs. control $p < 0.001$). Interestingly, every dose is statistically significant at 18 days level (vs. control $p < 0.01$, < 0.001).

2. Benzene extract. It exhibits more consistent results, where the inhibition in glycogen contents is highly significant. The effect is dose- and duration-dependent. The suppression in glycogen concentration increases as the dose is increased. 75 mg/kg dose reduces the glycogen contents when administered for 18 days only (vs. control $p < 0.005$). Both 150 and 300 mg/kg doses are significant to reduce the uterine glycogen contents when applied at any of the 3 schedules; however, the activity is highly significant at 18 days level (vs. control $p < 0.001$).

Effect of 50% ethanolic and benzene extracts of *Hibiscus rosa-sinensis* Linn. on uterine glycogen in adult rats

Extract	Dose (mg/kg/day)	Treatment period (days) 6	12	18
Control (vehicle only)	–	34.72±1.58* (5)	37.90±1.37 (4)	36.54±1.59 (5)
50% ethanolic	75	32.76±1.54 (5)	33.20±1.36 (5)	28.07±1.56[c] (4)
	150	31.27±1.56 (4)	29.47±2.44[d] (4)	25.48±1.64[c] (5)
	300	24.70±3.00[c] (5)	21.86±2.00[a] (5)	20.10±1.78[a] (5)
Control (vehicle only)	–	35.95±1.24 (4)	32.92±1.17 (4)	38.38±1.42 (5)
Benzene	75	30.15±1.63 (4)	29.95±1.19 (4)	26.92±1.18[b] (4)
	150	28.60±1.55[d] (5)	25.25±1.52[d] (4)	22.88±1.94[a] (5)
	300	22.20±1.93[b] (4)	18.40±1.39[a] (4)	18.40±1.24[a] (5)

*Values are mean ± SE. Number of rats used in parentheses. p-Values vs. their respective controls: [a] <0.001; [b] <0.005; [c] <0.01; [d] <0.02.

Discussion. Physiological and biochemical changes in the uterine tissue are governed by the ovarian hormones, estrogen and progesterone[10], which are in turn regulated by pituitary gonadotrophins and hypothalamic-releasing factors[11]. Progesterone, an active antiestrogen, has been reported to reduce the uterine glycogen in rats[12,13]. On the contrary, it elevates the glycogen contents in the uterus of Macaque[14]. However, it does not alter the glycogen level in the uterus of rat[15-17]. In the present investigation, both 50% ethanolic and benzene extracts of *H. rosa-sinensis* Linn. reduce the uterine glycogen contents significantly (table). Moreover, the inhibition in glycogen level increases as the dose is increased which further confirms its dose-response relation. The inhibition in the glycogen contents in the uterus of rat under the influence of these plant extracts is due to their antiestrogenic activity[8] which may be further accounted for its inhibition in motor activity of the uterus. However, the antifertility activity of these plant extracts, due to change in uterine contractility, is yet to be elucidated.

1 This investigation was supported by a grant from Council of Scientific & Industrial Research, New Delhi.

2 Present address: Division of Endocrinology, Central Drug Research Institute, Lucknow 226001, India.

3 S.K. Batta and G. Santhakumari, Indian J. med. Res. *59,* 717 (1971).
4 S.D. Kholkute and K.N. Udupa, J. Res. Indian Med. *9,* 99 (1974).
5 S.D. Kholkute, V. Mudgal and P.J. Despande, Planta med. *29,* 151 (1976).
6 A.O. Prakash and R. Mathur, Indian J. exp. Biol. *14,* 623 (1976).
7 A.O. Prakash and R. Mathur, J. Jiwaji Univ. *4,* 79 (1976).
8 S.D. Khokute and K.N. Udupa, Indian J. exp. Biol. *14,* 175 (1976).
9 S. Seifter, S. Dayton, B. Novic and E. Muntwyler, Archs Biochem. *25,* 191 (1950).
10 A.V. Nalbandov, in: Reproductive Physiology, 2nd edn, p. 119, Ed. A.V. Nalbandov. D.B. Taraporewala, Bombay 1970.
11 S.M. McCann, in: Pharmacology of Reproduction. Ed. E. Diczfalusy. Pergamon, New York 1968.
12 A.T. Gregoire, H. Ramsay and A. Adams, J. Reprod. Fert. *14,* 231 (1967).
13 I.C. Dutta, J.N. Karkun and A.B. Kar, Acta biol. med. germ. *20,* 155 (1968).
14 F.C. Lendrum and F.L. Hisaw, Proc. Soc. exp. Biol. Med. *34,* 394 (1936).
15 E.G. Boettiger, J. Cell comp. Physiol. *27,* 9 (1946).
16 W.J. Bo and W.B. Atkinson, Anat. Rec. *113,* 91 (1952).
17 L.M. Demers, K. Yoshinaga and R.O. Greep, Biol. Reprod. *7,* 297 (1972).

Cerebral neurosecretions and regulation of moulting in a haematophagous insect *Panstrongylus megistus,* (Heteroptera, Reduviidae)[1]

A.F. Furtado

Neuroendocrinologie des Insectes, C.N.R.S. n° 24, Université Pierre et Marie Curie, Paris, 12 rue Cuvier, F-75230 Paris-Cedex 05(France), and Laboratorio de Entomogênese, Dept. de Biologia Geral, C.C.B., Universidade Federal de Pernambuco, 50.000 Recife, PE (Brasil), 16 October 1978

Summary. 2 factors elaborated by distinct neurosecretory cells in the pars intercerebralis control moulting in *Panstrongylus megistus.* The 1st, originating probably in the A′ cells, activates the thoracic gland. The 2nd, produced by the A cells, acts at an earlier stage in a different way.

It is generally accepted that moulting in insects is controlled by the activity of the thoracic glands (moulting glands), which are themselves activated by neurosecretory cells in the pars intercerebralis.

During an investigation of the regulation of the initial phases in oogenesis[2,3], involving different surgical manipulations, we have observed that the mechanism of control seems more complex in the heteropteran *Panstrongylus megistus.* A previous investigation of the varying levels of ecdysones in the haemolymph[4] demonstrated that 2 peaks occur during the 5th larval instar. The ecdysone level peaks at 240 ng/ml on the 8th day after the blood meal (dABM), beginning to decline on the 10th dABM (164 ng/ml). The 2nd peak is considerably larger, reaching a maximum level on the 15th dABM (7000 ng/ml) (figure 1,A).

Materials and methods. The insects used in this investigation were female *P. megistus* larvae in the 5th instar. They were raised in the dark at a temperature of 27±1 °C and a relative humidity of 70±5%. They were fed once, on guinea-pigs, 10 days after moulting. The techniques of electrocoagulation surgery of the pars intercerebralis (PI) have been described previously[2]. A cells were ablated specifically using tweezers. Ablation of the thoracic glands was performed by pulling out the lobe of the fat body where they are joined together. Ecdysterone was injected into the abdomen as a 10-µg/µl solution in oil.

Results. Ablation of the thoracic gland on the 7th and 9th dABM. Ablation of the thoracic gland was carried out either on the 7th or on the 9th day after the blood meal. The 1st operation suppressed moulting while the 2nd did not in 10 of the 13 larvae operated (figure 1,B). The critical time for the action of the thoracic gland therefore appears

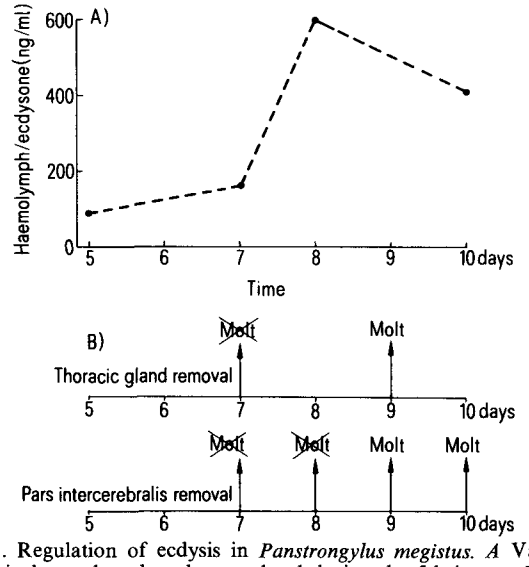

Fig. 1. Regulation of ecdysis in *Panstrongylus megistus. A* Variations in haemolymph ecdysones level during the 5th instar; *B* effects on moulting as a result of ablating of the thoracic gland or the pars intercerebralis at different times.