Crustecdysone is Without Estrogenic or Antiestrogenic Activity in the Rat

Crustecdysone (β-ecdysone, 20-hydroxyecdysone or ecdysterone) occurs naturally in insects[1–4] and crustaceans[5] and is capable of inducing moult in most arthropod groups[6,7]. It also occurs in plants in very large quantities[8–10]. Ecdysone has been reported to stimulate protein synthesis in mouse liver[11] and to inhibit growth of sarcoma and embryonic mouse fibroblasts[12]. Since the ecdysone in plants and animals is likely to find its way into cattle and humans through their food, it was thought worthwhile to scan this compound for its possible estrogenic and antiestrogenic activity.

An inbred strain of 80–120-day-old, 4-day cycling, adult mature white rats on an LD regime of 12:12, was used for the present study. The animals were maintained on Bengal gram and water supplemented with milk. Estrogenic activity was studied by Allan-Doisy test of vaginal cornification[13] and antiestrogenic activity was studied by the vaginal response method[14]. The animals were spayed and when the endogenous estrogen completely disappeared, as shown by absence of vaginal cycling (a phase contrast microscope was used for examination of the smear), a priming dose of 1 µg of estradiol dipropionate (Ovocyclin, Ciba India) in 0.1 ml olive oil was injected s.c. into each rat. When the estrogen completely disappeared from the blood stream, as shown by vaginal cycling method, a total of 30 µg of a solution of ecdysterone (obtained through the kind courtesy of Dr. D. KING to Dr. G. C. UNNITHAN and from Dr. W. E. ROBBINS) in 0.02 ml of 50% aqueous glycerol was delivered into the vagina of each rat in 2 doses at 10.00 h on 2 consecutive days through a steel canula attached to a syringe, to a group of 10 rats. An equal number of controls were given glycerol only. Smears were taken and read at 10.00 and 17.00 h on the 3rd day. Smears of all the experimental and control animals were found equally negative. Into another group of animals, 0.6 µg of the estrogen contained in 0.1 ml olive oil was injected s.c. at 09.00 h and 2 doses of ecdysterone in 0.02 ml of 50% aqueous glycerol (total dose, 0.5 mg) was administered intravaginally at 09.00 and 17.00 h, whereas the controls received 0.02 ml of 50% aqueous glycerol. Examination of the smear after 56, 64, and 72 h of first administration indicated no difference between the controls and the experimentals, both being positive. Hence it may be seen that ecdysterone does not have either estrogenic or antiestrogenic activity as detectable by the methods employed. The absence of estrogenic activity to ecdysterone is in accord with the structure of estrogenic steroids which have a phenolic A-ring and a carbon atom in position 18 but not in position 19[13].

Zusammenfassung. Es wird mittels Vaginalzytologie bei Ratten gezeigt, dass Ecdyson keine Östrogenaktivität und bei östrogenbehandelten Tieren auch keine Anti-oestrogen-Aktivität entfaltet.

V. K. K. PRABHU and K. K. NAYAR[15]

Department of Zoology, University of Kerala, Kariavattom (Trivandrum, India), 20 August 1973.

[1] P. HOCKS and R. WEICHERT, Tetrahedron Lett. *26*, 2989 (1966).
[2] H. HOFFMEISTER and H. F. GRUETZMACHER, Tetrahedron Lett. *33*, 4017 (1966).
[3] D. H. S. HORN, E. J. MIDDLETON and J. A. WUNDERLICH, Chem. Commun. *11*, 339 (1966).
[4] P. KARLSON, Vitam. Horm. *14*, 227 (1956).
[5] F. HAMPSHIRE and D. H. S. HORN, Chem. Commun. *2*, 37 (1966).
[6] A. KRISHNAKUMARAN and H. A. SCHNEIDERMAN, Nature, Lond. *220*, 601 (1968).
[7] A. KRISHNAKUMARAN and H. A. SCHNEIDERMAN, Biol. Bull. *139*, 520 (1970).
[8] H. JIZBA, V. HEROUT and V. SORM, Tetrahedron Lett. *18*, 1869 (1967).
[9] G. B. STAAL, Proc. K. med. Akad. Wet. Ser. C, *70*, 409 (1967).
[10] G. B. STAAL, Meded. LandbHoogesch. Opzoekstns. Gent. *32*, 393 (1967).
[11] W. C. BURDETTE and R. L. CODA, Proc. Soc. exp. Biol. Med. *112*, 216 (1963).
[12] W. C. BURDETTE and R. C. RICHARDS, Nature, Lond. *189*, 667 (1961).
[13] C. W. EMMENS, in *Methods in Hormone Reserach II* (Academic Press, New York 1962), p. 774.
[14] R. I. DORFMAN, in *Methods in Hormone Research II* (Academic Press, New York 1962), p. 774.
[15] A grant from the Ford Foundation and the Indian Council of Medical Research is gratefully acknowledged.

Activité protéosynthétique des glandes prothoraciques et titre d'ecdysone chez des larves permanentes de *Locusta migratoria* obtenues par irradiation sélective du tissu hématopoïétique

L'irradiation sélective du tissu hématopoïétique de jeunes larves de stade V (dernier stade larvaire) de *Locusta migratoria* bloque le déterminisme endocrine de la mue suivante, les insectes évoluant en larves permanentes; une irradiation témoin de régions ventro-abdominales de surface équivalente n'affecte pas la mue[1]. Dans la présente étude nous avons suivi l'évolution des glandes prothoraciques de larves devenues permanentes à la suite d'une radiolésion du tissu hématopoïétique, en choisissant comme critère la vitesse d'incorporation d'un acide-aminé dans les protéines synthétisées pendant un laps de temps donné. D'autre part nous avons suivi l'évolution du taux d'ecdysone à la suite de la radiolésion du tissu hématopoïétique, en la comparant à celle des larves normales, décrite dans un travail précédent[2].

Des larves normales et des larves à tissu hématopoïétique irradié (25000 R, anticathode en tungstène, durée de l'irradiation: 10 min, tension appliquée 42 kV; intensité du courant de chauffage: 32 mA; distance source-objet: 15 cm) reçoivent une injection intraabdominale de leucine tritiée (activité spécifique: 30 Ci/mMol; injection unique de 30 µl par insecte d'une solution correspondant à une radioactivité totale de 30 µCi par injection); les glandes prothoraciques des insectes en expérience sont prélevées aux temps 0, 10 min, 20 min et 40 min dans de l'acide perchlorique; après addition d'albumine bovine et centrifugation à 4000 *g*, le culot est traité à l'acide perchlorique à chaud en vue de l'extraction des seules protéines. Des lavages répétés à l'éthanol permettent de retirer la leucine radioactive non liée aux protéines. La radioactivité liée

[1] J. A. HOFFMANN, C.r. Acad. Sci., Paris, *273*, 2568 (1971).
[2] J. A. HOFFMANN, J. KOOLMAN, P. KARLSON et P. JOLY, Gen. comp. Endocr. *22*, 90 (1974).