invertebrates should be identical to the vertebrate hormone. In fact use of 25-hydroxy and 1,25-dihydroxycholecalciferol did not promote incorporation of $^{45}Ca^{++}$ into the shell of B. glabrata whereas under the same conditions ecdysterone did[3]. Hemolymph Ca^{++} levels were not affected in *Helix pomatia* by infusion of ecdysterone, c-AMP, dibutyryl c-AMP or an ionophore (A23187. Eli Lilly)[19]. However, ecdysteroids or their metabolites positively promote calcification in crustaceans[8] and even in an insect[9], although the mechanism might possibly be a passive one accompanying promotion of glycoprotein synthesis and growth.

The existence of a growth hormone from the light green cells of *L. stagnalis*[13] does not, we think, preclude that penta- or hexahydroxy forms of vitamin D could be involved (with calcium binding protein) in calcification of the matrix of the exoskeleton of molluscs or crustacea. A glycoprotein isolated by gel filtration from the mucus of *B. glabrata* does indeed bind $^{45}Ca^{++}$ effectively (Whitehead, unpublished report to Ministry of Overseas Development, U.K. (1972)). In view of the economic importance of invertebrates possessing a calcified exoskeleton, greater effort should be made to understand calcification and the role hormones play.

1 We wish to thank Professor P. Karlson and Dr J. Koolman (Phillips University, Marburg-Lahn, FRG) for providing facilities for RIA. The initial study was begun at the University of Sussex and continued at the Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, while D.L.W. was supported by the Ministry of Overseas Development, London and the Medical Research Council of Great Britain (grant G974/525/T). We thank Mr P. Mellon for the mass spectrum and Dr G.B. Russell for his interest in the earlier part of this study. We also thank Professor E. Scherer and Dr Q. Uwe (University of Essen, FRG) for their help in the irradiation of *L. stagnalis*.

2 Gauss Str. 82, D-4630 Bochum 5 (Federal Republic of Germany). Research results using *L. stagnalis* were submitted for the 'Philips Europa Wettbewerb für junge Forscher und Erfinder' (1979) in a thesis entitled 'Zur Frage der Existenz von Steroiden und Gonadoliberin bei der Grossen Schlammschnecke *(Lymmaea stagnalis L.)*: ein Beitrag zur Evolution der Insekten- und Wirbeltierhormone'.
3 Whitehead, D.L., Comp. Biochem. Physiol. *58C* (1978) 137.
4 Muftic, M., Parasitology *59* (1969) 365.
5 Bayne, C.J., Parasitology *64* (1972) 501.
6 Takemoto, T., Ogawa, S., Nishimoto, N., and Hoffmeister, H., Z. Naturforsch. *22b* (1967) 681.
7 Horn, D.H.S., in: Naturally occurring insecticides, p. 333. Eds M. Jacobson and D.G. Crosby. Marcel Dekker, New York 1971.
8 Carlisle, D.B., Gen. comp. Endocr. *5* (1965) 366.
9 Fraenkel, G.S., and Hsiao, C., J. Insect Physiol. *13* (1967) 1387.
10 Whitehead, D.L. and Saleuddin, A.S.M., Comp. Biochem. Physiol. *59C* (1978) 5.
11 Whitehead, D.L., Bull. ent. Res. *64* (1974) 223.
12 Borst, D.W., and O'Connor, J.D., Steroids *24* (1974) 637.
13 Geraerts, W.P.M., Gen. comp. Endocr. *29* (1976) 61.
14 Koolman, J., Hoffman, J.A., and Dreyer, M., Experientia *31* (1975) 247.
15 Reum, L., and Koolman, J., Insect Biochem. *9* (1979) 135.
16 Horn, D.H.S., Wilkie, J.S., Sage, B., and O'Connor, J.D., J. Insect Physiol. *22* (1976) 901.
17 Imai, S., Toyosato, T., Sakai, M., Sato, Y., Fujioka, S., Murata, E., and Goto, M., Chem. pharm. Bull., Tokyo *17* (1969) 335.
18 Voogt, P., in: Chemical zoology, vol. 7, chapter 9, p. 245. Eds M. Florkin and B.T. Scheer. Academic Press, New York 1971.
19 Burton, R.F., Comp. Biochem. Physiol. *57C* (1977) 135.

Inhibition of steroidogenic activity in the adrenal cortex of rats fed benzene hexachloride (hexachlorocyclohexane)

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Summary. Feeding various dosages of benzene hexachloride (100, 250, 750 and 1500 ppm) in the diet to weanling male albino rats for 90 days resulted in marked hypertrophy of the adrenals with large, vacuolated cells in the cortex at 750 and 1500 ppm. Accumulation of cholesterol-positive lipids and marked reduction in the activities of steroidogenic enzymes such as Δ^5 3β HSDH, 11β HSDH, G-6-PDH and SDH were seen using histochemical methods in the adrenal cortex of rats fed 750 and 1500 ppm. The results are suggestive of steroidogenic inhibition at 750 and 1500 ppm of dietary BHC while 100 and 250 ppm did not produce any discernible changes in the adrenal cortex.

Nelson and Woodward[2] were the first to report that chronic administration of o,p'-DDD [1,1-dichloro-2,2-bis(chlorophenyl)ethane], a derivative of the insecticide DDT, causes atrophy of the adrenal cortex of dogs and it was later confirmed by others[3,4]. It was shown that the atrophied gland secretes less than normal levels of corticosteroids in response to ACTH[5,6]. Later studies revealed that o,p'-isomer of DDD inhibited ACTH-induced corticosteroid production by interfering with the ACTH-dependent conversion of cholesterol to pregnenolone in the mitochondria[7-9]. These observations were confirmed by Hart et al.[10], who found degenerative ultrastructural changes in the mitochondria in the inner cortical zones, which were correlated with the inhibition of steroidogenesis in dogs given DDD isomers. This pharmacological property of DDD was exploited for therapeutic use in man to check excessive production of corticosteroids in adrenocortical carcinoma and Cushing's syndrome[11,12].

All these reports deal mainly with the effect of DDD on the dog adrenal, and surprisingly little is known about the effects of other organochlorine pesticides on the adrenal cortex of experimental animals[13]. This is important in view of the fact that the adrenal is one of the principal sites for accumulation of organochlorine pesticide residues[14,15].

Benzene hexachloride (BHC or hexachlorocyclohexane), a widely used organochlorine insecticide, has low acute toxicity and high chronic toxicity[16,17]. Short-term feeding of BHC at various dietary levels is known to produce histopa-

Effect of BHC feeding on the adrenal cortex histochemistry of rat

Dietary level of BHC** (ppm)	Relative adrenal weight (mg/kg b.wt)	% hypertrophy	Histochemical profile of the adrenal cortex* Sudanophilic lipids	Schultz reaction	SDH	G-6-PDH	Δ^5 3βHSDH
0(Control)	152.00± 17.00	-	+ +	faint	+ + + +	+ + + +	+ + +
100	150.00± 12.00	-	+ +	faint	+ + + +	+ + + +	+ + +
250	155.00± 10.00	-	+ +	faint	+ + + +	+ + +	+ + +
750	213.00± 13.00[a]	40	+ + +	strong	+ +	+ +	+
1500	281.00± 17.00[b]	85	+ + + +	strong	+	+	+

*Histochemical activity of enzymes, based on the amount of formazan formed, is graded on a subjective scale: + = faint; + + = low; + + + = high; + + + + = intense. [a]$p < 0.01$; [b]$p < 0.001$. **n = 10 in each group (Student's t-test).

thological, histochemical and biochemical lesions in the liver, kidney and testes of rats[18,19]. The present work deals with the effects of feeding various dietary levels of BHC on the adrenocortical histophysiology of the laboratory rat with special reference to steroidogenic enzymes.

Materials and methods. 28-day-old weanling male albino rats (*Rattus norvegicus,* CFT-Wistar strain) were housed in individual cages and divided into 5 groups of 10 animals each. They were fed a standard basal diet containing 0, 100, 250, 750 and 1500 ppm of technical BHC for 90 days. The technical BHC (99% pure) used in this study (Tata Chemicals, Mithapur, India) is a mixture of 5 principal isomers, viz., α (72%), β (5%), γ (13.5%), δ (8%) and ε (traces).

The animals were autopsied under chloroform anesthesia and the organ weights were recorded. The adrenals were fixed in 10% neutral formalin and 6 µm thick paraffin sections were stained with haematoxylin and eosin for histological observations. For lipid histochemistry, adrenals were fixed in cold calcium-formal, and frozen Cryostat sections (16 µm) were stained with Sudan Black B (for general lipids), Fettrot 7B (for neutral lipids) and Nile blue (for acidic and phospholipids) and a few sections were subjected to Schultz test for cholesterol and its esters according to Chayen et al[20]. For enzyme histochemistry, fresh, unfixed adrenals were quickly frozen at −20 °C and 16-µm-thick frozen sections were cut in a Cryostat and incubated at 37 °C aerobically for the following enzymes: Δ^5 3β hydroxysteroid dehydrogenase (Δ^5 3 β HSDH)[21], 11 β hydroxysteroid dehydrogenase (11 β HSDH)[21], Glucose-6-phosphate dehydrogenase (G-6-PDH)[22] and succinate dehydrogenase (SDH)[23]. Control sections were incubated in media lacking the substrate. After incubation, the sections were washed and post-fixed in 10% neutral formalin and mounted in glycerine jelly or PVP mountant.

Results. Daily food intake and weekly body weight gain were normal in all the groups except in that receiving 1500 ppm BHC, in which reduced food intake and consequent growth retardation were evident from the 7th week onwards[17,18]. The average BHC ingested (mg/rat/90 days) was: 80 (100 ppm), 200 (250 ppm), 625 (750 ppm) and 1174 (1500 ppm). There was a marked increase in the relative weights of adrenals at 750 and 1500 ppm (table) and conspicuous hypertrophy and hyperplasia of the cortical cells in all the 3 zones, viz., zona glomerulosa, zona fasciculata and zona reticularis, was evident. The cells were enlarged, with large nuclei and vacuolated cytoplasm. The histochemical profile of the adrenal cortex in 750 and 1500 ppm showed conspicuous changes (table). Accumulation of cholesterol-positive lipids in the adrenal cortex was a characteristic feature in 750 and 1500 ppm; it was marked in the zona glomerulosa and zona fasciculata. Activities of SDH and G-6-PDH in the adrenal cortex were considerably reduced and those of Δ^5 3 β HSDH and 11 β HSDH were markedly diminished. There were no discernible changes in the lipid distribution or enzyme activity in the adrenal cortex at 100 and 250 ppm (table).

Discussion. It is well established that steroidogenesis in the adrenal cortex is under the control of the pituitary ACTH, and hypophysectomy leads to atrophy of the adrenal glands. DDD-induced adrenal atrophy which is similar to that of hypophysectomy is reported to be due to the direct cytotoxic effect involving inhibition of ACTH-mediated intramitochondrial side chain cleavage of cholesterol to pregnenolone, a basic stepiin steroidogenesis[8,9]. Marked hypertrophy of the adrenals of BHC-fed rats could be due to the altered positive/negative feed-back mechanism as a result of reduced corticosteroid production which can lead to stimulation of ACTH secretion from the pituitary.

A close correlation between the lipid content and accumulation of organochlorine residues in various tissues has already been established[24]. Adrenal ranks next after adipose tissue in its fat content (% tissue) and accordingly a high accumulation of various chlorinated pesticides, more than in the kidney and liver, has been reported[15]. Chronic feeding of BHC in dogs has also been reported to result in high accumulation of residues in the adrenal[14]. Hypertrophied and vacuolated cells of the adrenal cortex of BHC-fed rats in this study show intense lipid accumulation which may probably be a reflection of the accumulation/metabolism of BHC residues in the adrenal cortex. Hence, it is likely that the tissues or sites that have a high affinity for these pesticides suffer the toxic damage.

Δ^5 3 β HSDH catalyses the conversion of Δ^5 3 β hydroxy steroids to their keto forms, a basic rate-limiting step in the biosynthesis of all hormonally active steroids[25]. 11 β HSDH is an adrenal cortex-specific enzyme involved in corticosteroid biosynthesis. The histochemical demonstration of these enzymes is considered to be a reliable visual index of steroidogenic activity in tissue sections[21]. The marked inhibition of these important steroidogenic enzymes and that of G-6-PDH is, therefore, a positive histochemical evidence for inhibition of steroidogenesis in the adrenal cortex of rats fed 750 and 1500 ppm of BHC. Diminished SDH activity in the adrenal cortex denotes the effect on mitochondria which supports the earlier ultrastructural observations by Hart et al.[10]. It remains to be shown whether the steroidogenic inhibition is due to mitochondrial dysfunction or is a consequence of TPNH depletion caused by G-6-PDH inhibition since TPNH is required for the side chain cleavage and hydroxylation reactions in steroidogenesis[26]. It would also be interesting to know whether BHC inhibits ACTH-induced steroidogenesis in the adrenal as in the case of DDD.

1 Acknowledgments. The authors thank Dr Muralidhara, H.P. Ramesh and K. Nanjundiah for their help and Prof. H.B. Devaraj Sarkar, Department of Zoology, University of Mysore, for providing us facilities for histochemical work. We are grateful to Sri C.P. Natarajan, for his keen interest in this investigation.

2 A.A. Nelson and G. Woodward, Archs Path. *48,* 387 (1949).

3 J. Nichols and G. Henninger, Exp. Med. Surg. *15*, 310 (1957).
4 O. Vilar and W.W. Tullner, Endocrinology *65*, 80 (1959).
5 J. Nichols and A.W. Richardson, Proc. Soc. exp. Biol. Med. *104*, 539 (1960).
6 A. Cazorla and F. Moncloa, Science *136*, 47 (1962).
7 N. Kaminsky, S. Luse and P. Hartroft, J. nat. Cancer Inst. *29*, 127 (1962).
8 M.M. Hart and J.A. Straw, Steroids *17*, 559 (1971).
9 M.M. Hart, E.S. Swackhamer and J.A. Straw, Steroids *17*, 575 (1971).
10 M.M. Hart, R.L. Reagan and R.H. Adamson, Toxic. appl. Pharmac. *24*, 101 (1973).
11 R.D. Brown, W.E. Nicholson, W.T. Chick and C.A. Scott, J. clin. Endocr. Metab. *36*, 730 (1973).
12 Y. Toutiou, A. Bogdon and J.P. Louton, J. Steroid Biochem. *9*, 1217 (1978).
13 S.C. Bhatia, S.C. Sharma, V.V. Damodaran and T.A. Subramanian, Indian J. Biochem. Biophys. *8*, 57 (1971).
14 B. Davidow and J.P. Frawley, Proc. Soc. exp. Biol. Med. *76*, 780 (1951).
15 L.J. Casarett, G.C. Fryer, W.L. Yanger and H.W. Klemmer, Archs envir. Hlth *17*, 306 (1968).
16 S.B. dem Barros and A.M. Saliba, Toxicology *10*, 27 (1978).
17 T. Shivanandappa, M.K. Krishnakumari and S.K. Majumder, Proc. 3rd All India Symp. exp. Zool., p.55. Baroda 1981a.
18 T. Shivanandappa and M.K. Krishnakumari, Indian J. exp. Biol. *19*, 1163 (1981b).
19 T. Shivanandappa and M.K. Krishnakumari, Acta pharmac. tox., in press (1982).
20 J. Chayen, L. Bitensky and R. Butcher, Practical histochemistry. Wiley, London 1973.
21 A.H. Baillie, M.M. Ferguson and D.McK. Hart, Developments in steroid histochemistry. Academic Press, New York 1966.
22 M.M. Nachlas, K.C. Tsou, E. de Souze, C.S. Cheng and A.M. Sligman, J. Histochem. Cytochem. *5*, 420 (1957).
23 R. Hess, D.G. Scarpelli and A.G.E. Pearse, J. biophys. biochem. Cytol. *4*, 753 (1958).
24 D.P. Morgan and C.C. Roan, Archs envir. Hlth *20*, 452 (1970).
25 L.T. Samuels, M. Helmreich, M. Lasater and H. Reich, Science *113*, 490 (1951).
26 K. McKerns, Biochim. biophys. Acta *100*, 612 (1965).

Does prolactin control the blood progesterone level on early dioestrus in rats?[1]

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Summary. Bromocriptine treatment on either prooestrus or oestrus in female rats did not affect luteal function on the day of dioestrus 1.

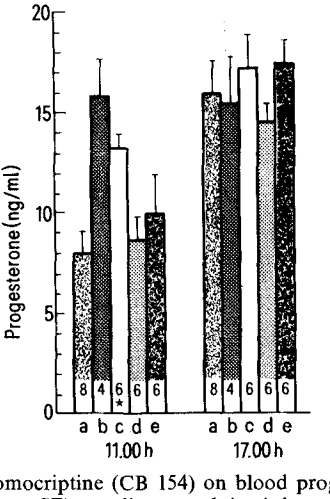
There is some evidence[3] that the corpus luteum can function autonomously during the oustrous cycle in the rat following the preovulatory release of LH on the afternoon of prooestrus. But the role played by the surge of prolactin which also occurs on the afternoon of prooestrus and oestrus remains an unanswered question. Döhler and Wuttke[4] reported that progesterone secretion on the expected day of dioestrus 1 did not differ in prooestrus-mated female rats whose prolactin release had been suppressed by bromocriptine, as compared to nontreated females. However, no data are available at the present time for cyclic animals. The aim of this work was then to determine whether the function of the corpus luteum was dependent on prolactin release during the oestrous cycle in the rat.

Material and methods. Adult virgin 3- to 4-month-old female Wistar rats from our colony, weighing 180–200 g, were used. They were kept under the normal rhythm of natural lighting at a temperature of 22–24 °C. They were fed with a commercial laboratory food and received tap water ad libitum. Oestrous rhythm was monitored by vaginal lavages 6 days a week. Only females which had experienced at least 2 successive 4-day cycles were used. A 4-day sequence consisted of oestrus, dioestrus 1, dioestrus 2 and prooestrus with ovulation taking place during the night following prooestrus[5].

The aim of study 1 was to examine the effects of bromocriptine on progesterone secretion on dioestrus. The females were divided into 5 groups: 1 mg bromocriptine s.c. rendered soluble with tartaric acid was injected on prooestrus, on oestrus and on both prooestrus and oestrus, always at 11.00 h; non-injected animals and tartaric acid injected animals served as controls. All the females were decapitated at the time of the highest activity of the corpus luteum, i.e. on dioestrus 1 at 11.00 h or 17.00 h for the determination of progesterone using a previously described RIA[6].

Blood prolactin values on prooestrus and oestrus in 4-day cyclic female rats injected with bromocriptine (CB 154) at 1100 h on prooestrus and oestrus respectively

Treatment (6 animals per group)	Prolactin (pg/ml) ± SE on prooestrus at 17.00–18.00 h	oestrus at 17.00–18.00 h
CB 154 (1 mg s.c.)	4 ± 2	8 ± 3
Controls	936 ± 283	205 ± 84



Action of bromocriptine (CB 154) on blood progesterone concentration (mean ± SE) on dioestrus 1 in 4-day cyclic female rats: uninjected controls (a); 1 mg tartaric acid at 11.00 h on prooestrus and oestrus (b); 1 mg CB 154 at 11.00 h on prooestrus (c); on oestrus (d); on prooestrus and oestrus (e). * Number of animals in each group.