generation were altered by methylparaben at a concentration which produced a 50% inhibition of iodide organification.

Previous studies[4-6] have shown that 4-aminobenzoic acid, 2:3- and 2:4-dihydroxybenzoic acid were antithyroid compounds in vivo in the rat at doses ranging from 0.1 to 1 mmole/100 g b.wt. In vitro, 4-aminobenzoic acid was found to be a very active inhibitor of the thyroid peroxidase catalyzed iodination[7]. The concentration of 4-aminobenzoic acid which produced a 50% inhibition was between 5 and 10 μM. This compound had the same potency as methimazole in this system. In the dispersed thyroid cell system, methylparaben is less potent than methimazole to block iodide organification. The concentrations which decrease iodide organification by 50% were about 200 μM and 8 μM for methylparaben and methimazole[8], respectively. Although, methylparaben seems to have a weak intrinsic antithyroid activity compared to that of a representative antithyroid agent, our results indicate that methylparaben included in drug preparation (generally at 0.1% w/v) or food is a potential inhibitor of thyroid functioning. However, it remains to establish its activity in in vivo conditions, where absorption properties and degradation pathways could increase or decrease the observed effect of the compound.

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Increase in the total reducing substances in the hemolymph of the freshwater crab, *Barytelphusa guerini*, produced by a pesticide (DDT) and an indolealkylamine (serotonin)

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Summary. DDT and serotonin produced significant increases in the total reducing substances in the hemolymph of intact crabs, *Barytelphusa guerini*, apparently by triggering release of the hyperglycemic hormone.

DDT and its breakdown products have been accumulating in the environment for many years, and crustaceans are being exposed constantly to chronic, if not lethal, concentrations of these compounds. But relatively little attention has been directed towards determining the effects of DDT on physiological and biochemical parameters in crustaceans.

The neurotransmitter serotonin (5-HT, 5-hydroxytryptamine) has been shown to produce hyperglycemia in crustaceans, apparently indirectly by triggering release of the hyperglycemic hormone (HGH)[1-5]. Evidence in support of this indirect action of 5-HT was obtained by Strolenberg and Van Herp[4] with the crayfish *Astacus leptodactylus* and by Martin[5] with the isopod *Porcellio dilatatus*. In both species not only did 5-HT produce hyperglycemia but also, as observed by electron microscopy, an increase of exocytotic figures in the sinus glands, the pair of neurohemal organs in these crustaceans from which the HGH is released. In view of the fact that DDT induces repetitive discharges in crustacean neurons[6], it is conceivable that DDT could produce secretion of HGH from the neurosecretory cells that synthesize it. This investigation was undertaken to determine a) whether a sublethal dose of DDT can indeed produce an increase in the quantity of total reducing substances (TRS), practically all, if not all, of which are sugars, in the hemolymph of the freshwater crab, *Barytelphusa guerini*, and b) if DDT does have such an effect whether it might involve stimulation or inhibition of the release of HGH.

Materials and methods. Adult, intermolt specimens of *Barytelphusa guerini*, collected in the area of Aurangabad were used in these experiments. The DDT was first dissolved in ethanol and diluted with distilled water so that the final concentration was 0.01 mg/0.05 ml of 0.5% ethanol. DDT control crabs received 0.5% ethanol alone. Reserpine (RSP) was prepared in distilled water in a concentration of 0.1 mg/0.05 ml. Serotonin creatinine sulfate (5-HT) was also dissolved in distilled water, the concentration being 0.01 mg/0.05 ml. Distilled water-injected crabs served as controls for the crabs receiving RSP or 5-HT. The volume of any solution or distilled water injected into each crab was always 0.05 ml.

TRS concentrations were determined by the Nelson-Somogyi method as modified by Varley[7]. All blood samples were removed the same time each day to obviate any possible effect of a circadian cycle in the concentration of the TRS. TRS concentrations were determined 2 h after any particular treatment. Student's t-test was used in the calculation of probability values.

Variations in the concentration of total reducing substances in the blood of *Barytelphusa guerini* after various treatments

Groups of crabs tested	Total reducing substances (mg%) ± SD
Intact crabs	26.2 ± 1.2
Distilled water-injected intact crabs (reserpine and serotonin controls)	33.3 ± 2.2
Reserpine-injected intact crabs	21.8 ± 3.8
Serotonin-injected intact crabs	38.5 ± 1.2
Ethanol-injected intact crabs (DDT control)	31.8 ± 1.7
DDT-injected intact crabs	41.0 ± 4.2
DDT + reserpine-injected intact crabs	28.8 ± 4.2
Eyestalkless crabs	22.4 ± 1.6
Distilled water-injected eyestalkless crabs	25.7 ± 7.5
Ethanol-injected eyestalkless crabs (DDT control)	27.2 ± 2.1
DDT-injected eyestalkless crabs	19.4 ± 3.5
Serotonin-injected eyestalkless crabs	29.5 ± 5.8

Experiments and results. The results obtained with untreated, intact crabs and several different groups of crabs that received various experimental treatments are presented in the table where each value represents the mean ± SD for 5 crabs. The TRS concentration in eyestalkless crabs was significantly ($p < 0.05$) less than in intact crabs. DDT and 5-HT significantly ($p < 0.05$) increased the TRS concentration in intact crabs but not in eyestalkless crabs. However, the TRS-elevating action of DDT in intact crabs was prevented by RSP. Not only was the TRS concentration in the DDT-RSP-injected intact crabs significantly less ($p < 0.05$) than in the DDT-injected intact crabs but also there was no significant change in the TRS concentration in the DDT-RSP-injected intact crabs as compared with the ethanol-injected (DDT control) intact crabs.

Discussion. DDT could have produced a rise in the TRS in the intact crabs in several different ways such as by triggering release of HGH, by mimicking the action of this hormone, or even by directly stimulating glycogenolysis. However, because DDT was not able to produce an increase in the TRS concentration in eyestalkless crabs, it seems most likely that DDT exerted its effect by triggering release of HGH from the sinus glands in the eyestalks. In keeping with the well-known[6] ability of DDT to cause repetitive discharges in neurons, DDT could have produced this increase in the TRS concentration by causing repetitive discharges in neurons in the chain that ultimately synapsed with the neurosecretory cells whose axonal terminals comprise the sinus glands or even in these neurosecretory cells themselves. The action potentials that are recorded from neurosecretory cells presumably trigger release of the neurohormone from these cells[8]. Because of the well-known 5-HT-depleting action of RSP[9] and the fact that there is no evidence that 5-HT is present in the sinus gland of any crustacean, the observation that RSP was able to prevent the TRS-elevating action of DDT would favor the hypothesis that DDT was exerting its effect on presynaptic neurons. RSP presumably lowered the 5-HT level in *Barytelphusa* because in another decapod crustacean, *Uca pugliator*, RSP has been shown to decrease the 5-HT levels in the eyestalks and supraesophageal ganglia after 2 h[10]. If RSP is indeed depleting 5-HT in *Barytelphusa* its action presumably precedes the 'repetitive firing' effect of DDT in the presynaptic neurons and an increased rate of electrical discharge in these presynaptic neurons following the simultaneous treatment with DDT and RSP would be ineffective in eliciting release of HGH because of the reduced availability of neurotransmitter substance to carry the message across the synapse. Support for this interpretation is the observation that RSP alone when injected into intact crabs produced a significant ($p < 0.05$) drop in the TRS concentration. Presumably, once again because of the 5-HT-depleting action of RSP, there was less 5-HT available in these RSP-injected crabs, to trigger release of the amount of HGH needed to maintain the normal level of TRS in the blood. The significant rise in the TRS concentration induced by 5-HT is consistent with the results of the others[2-5] first referred to above. The fact that 5-HT had no significant effect on the TRS concentration in eyestalkless crabs supports the hypothesis that the sinus glands in the eyestalks of this crab are the main release site for HGH, and that 5-HT is normally involved in triggering the release of this hormone.

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Induction of mouse hepatic ornithine decarboxylase by skin application of 12-0-tetradecanoylphorbol-13-acetate[1]

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Summary. Topically applied 12-0-tetradecanoylphorbol-13-acetate on mouse skin was able to induce liver ornithine decarboxylase. Maximum induction occurred 10 h after a single application. Whereas no induction was noticeable at doses of 0.17 and 1.7 nmoles, 17 and 177 nmoles of 12-0-tetradecanoylphorbol-13-acetate caused about 30- and 57-fold increases respectively.

Ornithine decarboxylase, the first and rate limiting enzyme in polyamine biosynthesis, is induced more than 200-fold in mouse epidermis by topical application of the potent tumor promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA)[2]. Such an induction may be one of the essential components of the tumor promotion in mouse skin[2-5]. Besides promoting skin tumor formation in initiated mice[6], topically applied TPA appears to possess a general promotional ability and enhances tumor formation in the internal organs of mice initiated transplacentally with DMBA or urethan. Skin and liver were reported to be the most susceptible to the combined treatment with the initiator and promoter[7]. The observations of Kreibich et al. show that, in fact, TPA applied to skin reaches liver and other internal organs; by 4 h after application of tritium labelled TPA, 0.3% of the total radioactivity applied on the skin was detected in the liver[8].

In the light of the above findings, the induction of hepatic ornithine decarboxylase by topically applied TPA was investigated.

Materials and methods. TPA was obtained from Dr Peter Borchert. Eden Prairie, MN. DL-[1-^{14}C]ornithine hydrochloride (sp.act. 49.9 mCi/mmole) was from New England Nuclear, Boston, MA. Dye reagent for the protein assay was purchased from Bio-Rad Laboratories, Richmond, CA. Female Charles River CD-1 mice, 7–9 weeks of age, were used for these studies. Food and water were given ad libitum. The dorsal skin of the mice was shaved 2–3 days