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

before the experiments and TPA was applied to the shaved areas in a volume of 0.2 ml acetone. Control mice were treated with the same volume of acetone. Mice were killed at specified time intervals after the apllication. The livers were quickly excized and chilled in 0.025 M Tris-HCl buffer, pH 7.2, containing 5 mM dithiothreitol (DTT), 0.1 M EDTA, and 0.1 mM pyridoxal phosphate. Homogenizations were carried out in the above buffer (1:3 w/v) with a Potter-Elvehjem homogenizer. Cytosol fractions were prepared by centrifugation at 105,000 × g for 60 min.

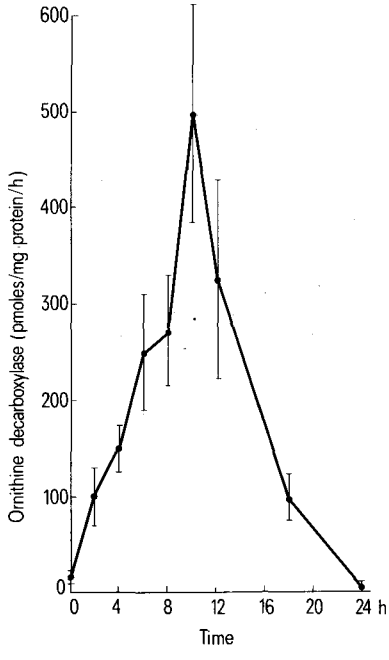

Fig. 1. The effect of a single topical application of 17 nmoles of TPA on hepatic ornithine decarboxylase activity. Mice were treated with either 0.2 ml acetone or TPA in acetone and killed at the times indicated. Each point represents the mean ± SEM of 4 groups, each group containing livers from 2 mice.

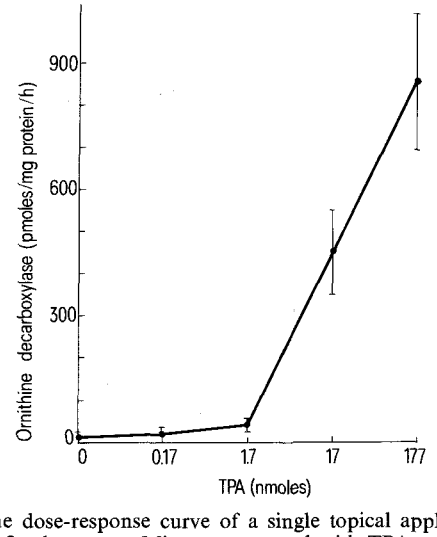

Fig. 2. The dose-response curve of a single topical application of TPA in 0.2 ml acetone. Mice were treated with TPA at the doses indicated and killed 10 h later. Each point represents the mean ± SEM of 4 groups, each group containing livers from 2 mice.

Ornithine decarboxylase was assayed in 0.025 M Tris-HCl buffer at pH 7.2[9]. Protein determinations were done with Bio-Rad dye reagent using crystalline bovine serum albumin as the standard.

Results. The time course of induction of hepatic ornithine decarboxylase activity by topical application of 17 nmoles of TPA is shown in figure 1. Appreciable increases were noticed as early as 2 h after application. The peak of activity, which was approximately 30-fold greater than that of the acetone-treated control, occurred 10 h after application and declined to control values by 24 h.

The effect of various doses of topically applied TPA on the stimulation of hepatic ornithine decarboxylase activity at 10 h after a single application is shown in figure 2. No induction was noticed when TPA doses were 0.17 and 1.7 nmoles, but at 17 and 177 nmoles of TPA the increases were 30- and 57-fold, respectively.

Experiments were also carried out to see the effect of multiple applications of TPA on liver ornithine decarboxylase. In these experiments mice were treated with 17 nmoles of TPA at intervals of 3 days and killed 10 h after the specified number of applications. The results (not shown) indicate that even after 6 applications, the magnitude of the stimulation of liver ornithine decarboxylase did not change appreciably over that caused by a single application.

Discussion. Among a variety of biochemical responses resulting from the application of TPA to the skin of mice is the transient induction of epidermal ornithine decarboxylase[2]. This phenotypic change is one of the essential components of the mechanism of skin tumor promotion[2-5]. Our results indicate that TPA applied topically is capable of inducing hepatic ornithine decarboxylase, which could play a role in the liver tumor promotion by TPA as reported by Goerttler and Löhrke[7]. The magnitude of induction of liver ornithine decarboxylase by TPA application is less compared to that in epidermis but is comparable to that observed in regenerating liver[10].

In epidermal tissue, maximum induction of ornithine decarboxylase occurs 6 h after the application of TPA[2]. Our present results show that the maximum activity in liver is reached 10 h after TPA application (figure 1). Taking into consideration that TPA levels in liver may peak 4 h after topical application[8], the hepatic induction of ODC appears to follow the same time course as that observed in epidermis[2].

Our results also indicate lack of cumulative effect on liver ornithine decarboxylase activity by multiple TPA applications. Lack of such an effect could be possibly due to rapid inactivation of TPA by liver.

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