('cytochrome c reductase') and neotetrazolium reductase were measured as described earlier in this journal[12]. Significance was calculated by Student's t-test[14].

In order to study the effects of hexobarbital on aminopyrine N-demethylase and cytochrome c reductase in vitro, we measured the activities of these enzymes in a 12,000 × g-supernatant prepared from the liver of an untreated rat, in the presence of varying concentrations of hexobarbital.

Results and discussion. In the livers of the mice treated for 2 days with hexobarbital, all enzyme activities were increased significantly due to enzyme induction, while in the mice treated for 1 day, only aminopyrine N-demethylase was elevated (see figure 1). In the 2-day-experiment, sleeping times were shortened at the second day as compared to those of the first day of treatment due to accelerated metabolism of hexobarbital brought about by enzyme induction. So we had to administer the barbiturate more often on the second day (namely 1 × 100 mg/kg plus 16 × 50 mg/kg, as an average) than on the first day (namely 1 × 100 mg/kg plus 11 × 50 mg/kg, as an average) so that the animals received 139% more sodium hexobarbital on the second day than the first day. Relative liver weights increased significantly after only the 1-day-treatment, namely from 3.85 ± 0.20% of b.wt with the control group to 6.33 ± 0.71% with the treated group, in all other cases they did not differ significantly from the control group liver weights.

So it is quite possible to induce the biosynthesis of drug-metabolizing enzymes by hexobarbital in the mouse, without any other interfering substance.

30 min after the hexobarbital administration, demethylase is inhibited but the 2 reductases are constant (see figure 1). This corresponds well with the in vitro measurement of enzyme activities where hexobarbital inhibits only the demethylase but not the reductase (see figure 2). The inductive effect of hexobarbital is a typical diphasic one[8–13]. This seems to be an argument in favor of Goldstein and Goldstein's hypothesis[15] that induction of enzyme biosynthesis is a direct consequence of enzyme inhibition caused by the inducing agent. At least in the case of hexobarbital and the substance HOE 17 879[12], it seems as if it were necessary to maintain, for a minimum time, a certain concentration of the inducing substance in liver until this enzyme inhibition results in an induction of enzyme biosynthesis. If a gene codes more than one enzyme, not only the enzyme inhibited by the inducer is induced but the others too, which may be the case with the 2 reductases which are induced but not inhibited by hexobarbital besides the demethylase.

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Effect of desipramine on the contents of some free amino acids of mouse brain[1]

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Summary. I.p. injections of desipramine-HCl (100 mg/kg) produced decreases in the contents of several amino acids of mouse brain after 1 h. Using a 10–100 mg/kg range of doses, these effects appeared to be dose-dependent for α-alanine and aspartate. These changes may be due, in part, to a decrease in cerebral oxidative metabolism (Krebs cycle activity) which occurs secondarily to desipramine-induced hypothermia.

The mechanism of action of tricyclic antidepressants on the mammalian CNS is not well understood. Relationships of their antidepressant actions with availability of norepinephrine at central synaptic sites[2–4] and with central cholinergic mechanisms[5–7] have been suggested. More recently, Patel et al.[8] showed that injections of desipramine produced elevations in the GABA content of mouse brain and that this effect was related to the hypothermia produced by the drug. In contrast, hypothermia produced by administration of allylglycine (a blocker of glutamate-α-decarboxylase (GAD) activity) was associated with a decrease in hypothalamic GABA content in rats[9]. In the latter case, GAD-blockade apparently outstripped the hypothermic effect on brain GABA.

The present study was undertaken to determine the extents to which various doses of desipramine can influence some other cerebral amino acids, some of which, like GABA, are candidate-transmitters.

Material and methods. Male Swiss-Webster mice, weighing 20–30 g, were injected i.p. with 10–100 mg/kg desipramine-HCl (Lakeside Labs, Inc.) dissolved in 0.154 M NaCl, and were decapitated 1 h later. All animals had food and water ad libitum before experiments. 'Acidic and neutral' amino acids were determined, using a Beckman Model 121C amino acid analyzer after deproteinization and preparation of free amino acid extracts[10, 11].

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1 This study was supported by Centro 'Ramón y Cajal' and Fundacion Juan March.
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Effects of i.p. injections of desipramine on amino acid contents of mouse brain

Amino acid (μmole/g, wet wt)	Control injection (0.9% NaCl)	Desipramine-HCl (mg/kg) 10	25	50	100
Phosphoserine	0.29 ± 0.06 (4)	0.30 ± 0.04 (3)	0.22 ± 0.02 (4)	0.21 ± 0.02 (4)	0.12 ± 0.007 (8) [a]
Phosphoethanolamine	1.71 ± 0.19 (4)	2.11 ± 0.14 (3)	1.78 ± 0.14 (4)	1.60 ± 0.21 (4)	0.92 ± 0.03 (8) [b]
Taurine	9.71 ± 0.66 (4)	11.03 ± 0.75 (3)	9.70 ± 0.43 (4)	8.97 ± 0.48 (4)	6.44 ± 0.17 (8) [c]
Aspartate	3.04 ± 0.14 (4)	3.02 ± 0.14 (4)	2.87 ± 0.21 (4)	2.56 ± 0.09 (4) [a]	2.39 ± 0.05 (8) [b]
Threonine	0.31 ± 0.04 (4)	0.37 ± 0.08 (3)	0.37 ± 0.03 (4)	0.29 ± 0.01 (4)	0.37 ± 0.02 (6)
Serine, glutamine + asparagine	5.29 ± 0.14 (4)	5.23 ± 0.38 (3)	5.18 ± 0.20 (4)	4.65 ± 0.31 (4)	3.98 ± 0.06 (8) [c]
Glutamate	9.17 ± 0.45 (4)	9.99 ± 0.58 (3)	9.13 ± 0.19 (4)	8.66 ± 0.31 (4)	8.34 ± 0.22 (8)
Glycine	0.88 ± 0.05 (4)	0.99 ± 0.04 (3)	0.89 ± 0.03 (4)	0.79 ± 0.04 (4)	0.78 ± 0.03 (8)
α-Alanine	0.48 ± 0.03 (4)	0.49 ± 0.05 (3)	0.40 ± 0.03 (4) [a]	0.35 ± 0.03 (4) [a]	0.30 ± 0.02 (8) [c]

Mice were killed 1 h after injection of the drug; means ± SEM, numbers of animals in parentheses; [a, b] and [c] represent, respectively, $p < 0.05$, $p < 0.01$ and $p < 0.001$, when these values were compared with those for control animals.

Results and discussion. Results shown in the table revealed that the cerebral contents of some amino acids (or derivatives) were decreased 1 h after desipramine injection, especially at the 100 mg/kg dose. However, glutamate and glycine levels showed no changes. The most significant changes; i.e., the decreases in α-alanine and aspartate, were dose-dependent. Hypothermia was evident in all mice which received 25–100 mg/kg, but this effect was not experimentally determined. This study has revealed that the cerebral levels of several amino acids, especially α-alanine and aspartate, which are linked directly with oxidative metabolism, are decreased by the tricyclic antidepressant, desipramine. These effects, as well as the increase in GABA levels produced by this drug[8], are likely to be linked to its hypothermic action. The extent to which these changes in cerebral amino acids are involved in the antidepressant action of desipramine in humans is not known, but it is suggested that such changes should be considered, along with the effects that this agent produces on cerebral noradrenergic and cholinergic mechanisms, in explaining this action.

Polyoxin fungicides: Demonstration of insecticidal activity due to inhibition of chitin synthesis

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Summary. Polyoxin A, a selective inhibitor of chitin synthetase, was found to be insecticidal when injected into the abdomen of grasshoppers nymphs, with an LD_{50} of 1.26 ± 0.20 μg per insect. Symptomatology and the absence of any toxicity toward adult insects indicate that toxicity is due to interference with cuticle deposition.

The polyoxin complex is an antifungal mixture of nucleoside peptide antibiotics elaborated by *Streptomyces cacaoi* var. *asoensis*. The structures of major and minor components of this mixture have been described[2–4]. The polyoxins have been used as practical fungicides in Japan. They act through their ability to be powerful inhibitors of chitin synthesis in filamentous fungi[5]. The structural similarity of the polyoxins to uridine disphospho-N-acetyl glucosamine (UDPNAG), the natural substrate of chitin synthetase, accounts for the competitive nature of this inhibition[6]. The polyoxins have been shown to inhibit in vitro chitin synthesis in insects, both in an organ culture system[7] and in excised abdominal integument incubated under appropriate conditions[8]. To the best of our knowledge there have not been any reports on the in vivo toxicity of polyoxins toward insects. Interest in the possibility of insect control through interference with cuticle deposition, has been recently heightened by the discovery of a new group of insecticides that actually display such a mode of action[9,10]. These are substituted urea compounds and there is no similarity of structure between them and the polyoxin or any natural precursor of chitin. The work to be reported in this note was an adjunct to the development of a simple in vitro assay system for measuring chitin synthesis in insects[8]. It was found that polyoxin A was a powerful inhibitor of chitin synthesis in the system when either glucose, glucosamine, or UDPNAG were used as precursors[8]. It was therefore of interest to see whether or not this compound could affect the process of chitin deposition in vivo.

1 Acknowledgment. I thank Henry Bork for his expert technical assistance.

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