As reported previously,[8] the porphyria-precipitating drugs such as phenobarbital and tolbutamide markedly stimulate, and DDC, one of the porphyria-inducing chemicals, strongly inhibits the activity of microsomal cytochromes (P-450 and b_5) and drug metabolizing enzyme system. Other porphyria-inducing chemicals such as AIA, griseofulvin and C_6Cl_6 have dual actions of stimulation and inhibition on the activity of microsomes. Furthermore, many steps in cholesterol biosynthesis and its catabolism are demonstrated to take place within the microsomes.[9] These facts and the present seemingly anomalous results seem to indicate that the rate of cholesterol biosynthesis could be regulated by the activity of the microsomes and that cholesterol catabolism in the microsomes might be more strongly influenced than its biosynthesis by both porphyria-precipitating and -inducing chemicals. In the latter chemicals the inhibition of cholesterol catabolism seems to play an important role in the production of hypercholesterolemia in the experimental porphyria.

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Inhibition of the hepatic metabolism of amphetamine by desipramine*

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RECENT studies have shown that the enhanced action of amphetamine seen in rats after the administration of desipramine (DMI) is a consequence of the inhibition of the metabolism of amphetamine by DMI.[1, 2] This interaction, however, has not been demonstrated in liver preparations.The studies reported here were undertaken to investigate the inhibitory action of DMI on the metabolism of amphetamine both in hepatic microsomes and in the isolated perfused rat liver.

MATERIALS AND METHODS

Incubation studies. Male Sprague–Dawley rats weighing 200 g were used Animals were killed by cervical dislocation. Their livers were removed and chilled immediately on crushed ice All subse-

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quent manipulations were carried out at 2–4°. The livers were homogenized in a motor-driven Teflon-glass homogenizer with 3 ml of isotonic KCl (1·15%) per gram of tissue. The homogenate was centrifuged at 10,000 *g* (av.) for 30 min in an International model HR-1 centrifuge (rotor No. 856). The supernatant (1·5–2·0 ml), obtained from 375–500 mg liver, was incubated with 1·7 ml of 0·2 M potassium phosphate buffer (pH 7·4) containing glucose 6-phosphate (50 μmoles), NADP (0·5 μmole), nicotinamide (100 μmoles), and $MgCl_2$ (150 μmoles); semicarbazide (60 μmoles) was added in demethylation studies One of the following drugs was added to this mixture: aminopyrine (5·0 μmoles), hexobarbital (1·9 μmoles) or amphetamine (1·4 μmoles). The incubation mixture was adjusted to a final volume of 6 ml with distilled water. The reaction mixtures were incubated for 30 min at 37° under an atmosphere of oxygen.

The metabolism of hexobarbital and amphetamine was determined by measurement of the disappearance of substrate.[3, 4] The demethylation of aminopyrine was measured by estimation of the amount of formaldehyde formed by the Nash procedure.[5]

Liver perfusion studies. Male Sprague–Dawley rats (280–300 g) were used. *d*-Amphetamine-H^3 sulfate (generally labeled, 4·23 c/m-mole) was obtained from the New England Nuclear Corp. The drug was assayed by a modification of the method of Axelrod[4] as previously described.[1] Details of the perfusion procedure[6] and apparatus[7] have been published previously. The perfusion medium consisted of 20 ml of defibrinated rat blood, 500 i.u. heparin and sufficient Krebs–Henseleit bicarbonate buffer, pH 7·4, to make a final volume of 100 ml. The drugs were added to the medium in the recycling system after equilibration. The livers were perfused for 1 hr.

RESULTS AND DISCUSSION

Axelrod has described the metabolism of both *d*- and *l*-amphetamine by the microsomal fraction of rabbit liver.[8] In the present studies, neither *d*- nor *l*-amphetamine was metabolized by microsomal preparations of rat liver which did, however, metabolize both aminopyrine and hexobarbital (Table 1). Since *d*-amphetamine is rapidly metabolized in the isolated perfused rat liver, this preparation was used to study the inhibitory action of DMI on the metabolism of amphetamine. The results

TABLE 1. DRUG METABOLISM BY THE 10,000 *g* SUPERNATANT FRACTION OF RAT LIVER

Substrate	Enzyme activity* (μmoles/g liver)
d-Amphetamine	0·01
l-Amphetamine	0·04
Hexobarbital	2·67
Aminopyrine	0·78

* Enzyme activities are expressed as μmoles substrate metabolized or formaldehyde formed by 1·0 g liver in 30 min. The values are the average of three incubation mixtures and are typical of two experiments.

reported in Table 2 show the striking inhibition of the metabolism of amphetamine which was measured in the perfused liver. The addition of 0·5–2·0 mg of DMI to the perfusion system evoked the maximal inhibition of the metabolism of amphetamine, which was in excess of 80 per cent. The metabolism of amphetamine was also markedly inhibited by 0·1 mg or about 10 μg of DMI per gram of liver. Under these conditions the metabolism of amphetamine was reduced by almost 60 per cent.

DMI has recently been shown to inhibit not only the metabolism of tremorine, oxotremorine,[9] guanethidine[10] and propanolol[11] by liver microsomes, but also the metabolism of amphetamine *in vivo*. The present studies demonstrate that the inhibition of the metabolism of amphetamine which is seen after the administration of DMI is also a consequence of an impairment of its metabolism by hepatic enzymes.

TABLE 2. INHIBITION OF THE METABOLISM OF AMPHETAMINE BY DESIPRAMINE (DMI) IN THE ISOLATED PERFUSED LIVER

	Amphetamine metabolized (μg/g liver)	Inhibition (%)
Control	96·7	—
+ 2·0 mg DMI	14·5	85
Control	95·6	—
+ 1·0 mg DMI	14·0	85
Control	110·2	—
+ 0·5 mg DMI	18·9	83
Control	95·4	—
+ 0·1 mg DMI	40·3	58

* DMI was added to the perfusion system 10 min before ^{3}H-*d*-amphetamine sulfate (1·08 mg, 3×10^7 dpm). Livers were perfused for 1 hr. The data are derived from the amount of amphetamine recovered from liver, perfusate and bile. The results are expressed as the mean values obtained with two livers.

The finding that amphetamine is metabolized in the isolated perfused rat liver, but not by microsomal preparations of rat liver, underscores the value of the perfusion technique as a tool for the investigation of hepatic drug metabolism. Moreover, this observation is of particular interest since the drug is metabolized by rabbit liver microsomes. It has, however, been shown that there are marked differences in the metabolism of amphetamine in rats and rabbits.[4] For example, amphetamine is extensively deaminated in the rabbit, whereas the metabolism of the drug in the rat mainly involves hydroxylation to *p*-hydroxyamphetamine. It thus appears that our inability to detect any metabolism of amphetamine in microsomal preparations of rat liver may be attributed either to a high degree of lability of the hydroxylase enzyme or to a deficiency of a necessary cofactor in the incubation system.

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