determined, by these internal standards, that the quantity of 5HIAA present in the butyl acetate extract of normal urine was identical in all three methods.

Accurate knowledge of all prior medication is a necessity in any study involving urinary metabolites, thus allowing the investigator to choose a method which is not likely to be affected by reported interfering medications.[5,6]

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L. B. Mendel Research Laboratory,
Elgin State Hospital,
750 *South State Street,*
Elgin, Ill. 60120, *U.S.A.*

C. DENNIS AHLBERG

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p-Chloroamphetamine—Species differences in the rate of disappearance and the lowering of cerebral serotonin*†

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THE MAJOR pathway for the metabolism of amphetamine in rats involves *p*-hydroxylation; in mice, however, it appears that deamination is of equal importance.[1,2] Recent studies have shown that the administration of desipramine to rats strikingly prolongs the psychomotor stimulation evoked by amphetamine but not that elicited by its *p*-chlorinated derivative.‡ This prolongation of the central stimulatory action of amphetamine is a consequence of an inhibition of its hepatic metabolism by desipramine which results in a sustained elevation of the levels of amphetamine in both the brains and bodies of rats.[3] The marked prolongation of the half-life of amphetamine which resulted from the inhibition of *p*-hydroxylation suggested that *p*-substituted derivatives, such as *p*-chloroamphetamine, should be metabolized much more slowly than amphetamine in the rat. Moreover, in mice where

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† A preliminary report of this work was presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics, Pittsburgh, Pa. (Pharmacologist **11,** 273, 1969).

‡ F. Sulser and J. V. Dingell, unpublished results.

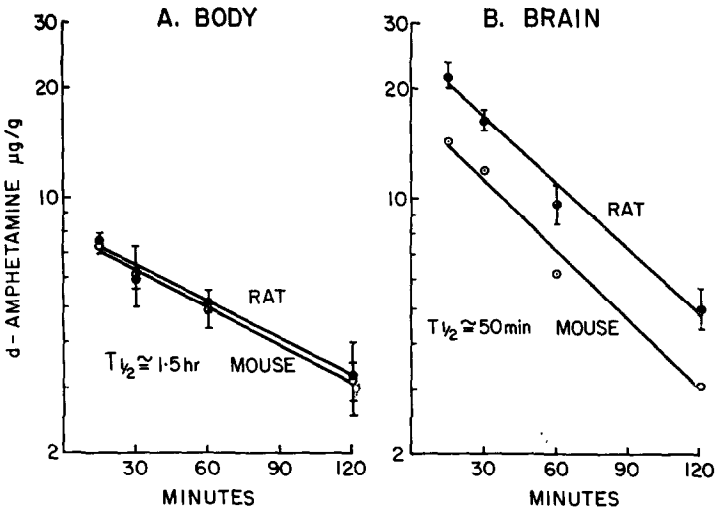

FIG. 1. Half-life of *d*-amphetamine in rats and mice. Each point represents the average value from at least four animals. The values reported for mice are the mean of two determinations, each using the pooled brains of three animals. Lines were drawn according to the method of least squares. Vertical bars indicate the S. D.

hydroxylation is less important, the rate of metabolism of *p*-chloroamphetamine should more closely approximate that of amphetamine. Since these species differences in the metabolism of *p*-chloroamphetamine could explain the reported inability of chlorinated amphetamines to lower cerebral serotonin (5HT) in mice,[4] the rate of disappearance of amphetamine from the tissues of rats and mice was compared with that of its *p*-chlorinated derivative.

Male Holtzman rats (180–200 g) or male Dublin I.C.R. mice (20–25 g) were used in these studies. The drugs were given intravenously; *d*-amphetamine sulfate 10 mg/kg to rats, 8 mg/kg to mice; *d,l-p*-chloroamphetamine hydrochloride, 10 mg/kg to both species. The doses are expressed as the salt. Amphetamine and *p*-chloroamphetamine were assayed by the methylorange procedure of Axelrod,[1] 5HT by the method of Bogdanski *et al.*,[5] and 5-hydroxyindole acetic acid (5HIAA) according to the method of Sharman and Smith.[6]

Although the major pathway for the metabolism of amphetamine in rats differs from that in mice, the drug disappears at the same rate in both species. After the administration of *d*-amphetamine, its levels in the bodies of rats and mice declined exponentially with a half-life of about 1·5 hr (Fig. 1). Moreover, this rate of metabolism was reflected by a half-life of about 1 hr for the disappearance of amphetamine from the brains of these animals (Fig. 1).

In contrast to these findings, the body levels of *p*-chloroamphetamine declined at markedly different rates in rats and mice (Fig. 2). For example, the half-life of *p*-chloroamphetamine in the mouse was 4 hr, which was about twice that of amphetamine in both rats and mice. The 10-hr half-life of *p*-chloroamphetamine in the rat, however, was six to seven times that of amphetamine. These results confirm the report of Fuller and Hines[7] that substitution of chlorine in the *para* position of amphetamine markedly slows the rate of disappearance of the drug from tissues. As was observed with amphetamine, the rate of disappearance of *p*-chloroamphetamine from brain resembled its rate of metabolism in each species (Fig. 2). Moreover, it is noteworthy that 16 hr after the administration of *p*-chloroamphetamine, its presence could not be detected in brains of mice but levels of 10 µg/g were measured in brains of rats.

It is, thus, of interest that Pletscher *et al.*[4] have reported that 16 hr after the administration of 4-chloro-*N*-methyl amphetamine cerebral 5HT and 5HIAA are markedly lowered in rats but not in mice. The results obtained in the present studies suggest that the more rapid metabolism of *p*-chloroamphetamine in mice could explain the finding that normal levels of 5HT and 5HIAA are present in brain 16 hr after its administration. This hypothesis was further investigated by examining the time course of the depletion of cerebral 5HT by *p*-chloroamphetamine in both rats and mice. The results showed that *p*-chloroamphetamine does indeed lower the levels of 5HT in the brains of mice and rats (Fig. 3). There were, however, differences in the degree and duration of the depletion of 5HT which

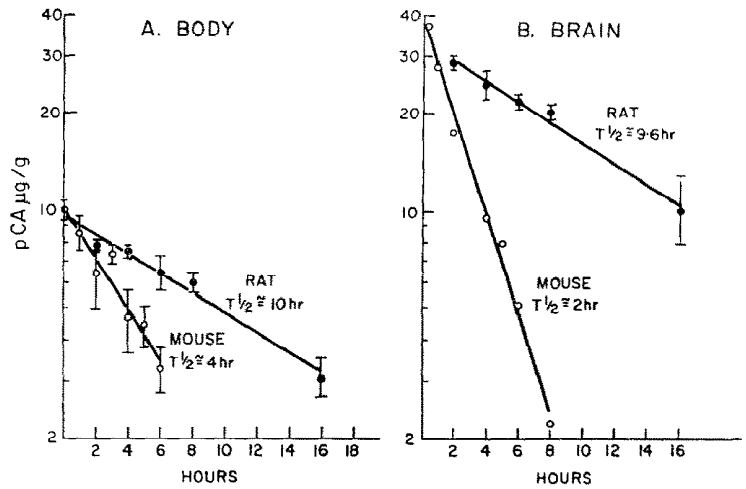

FIG. 2. Half-life of *p*-chloroamphetamine (*p*CA) in rats and mice. Each point represents the average value from at least four animals. The values reported for mice are the mean of two determinations, each using the pooled brains of three animals. Lines were drawn according to the method of least squares. Vertical bars indicate the S. D.

were consistent with the differences in the metabolism of the drug. For example, in rats cerebral 5HT declined to about 20 per cent of the controls 8 hr after the administration of *p*-chloroamphetamine and remained low even after 30 hr. In mice, the levels of 5HT declined to only 60 per cent of the controls after 6 hr and returned to normal within 16 hr.

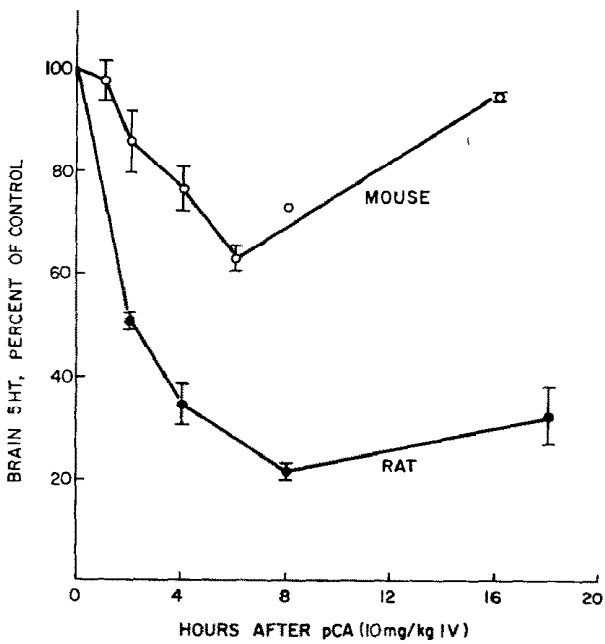

FIG. 3. Effect of *p*-chloroamphetamine on cerebral 5HT in rats and mice. Each point represents the mean value of three determinations. For mice each determination required the pooled brains from two animals. Vertical bars indicate the standard error of the mean. Control levels of 5HT (µg/g): mouse, 0·44 ± 0·02; rat, 0·40 ± 0·01.

TABLE 1. EFFECT OF *p*-CHLOROAMPHETAMINE ON CEREBRAL 5-HYDROXYINDOLE ACETIC ACID (5HIAA) IN MICE*

Time after *p*-chloroamphetamine administration (hr)	5-HIAA	
	μg/g Brain	Per cent of control
Experiment I		
Control	0·18 ± 0·01	
1	0·14 ± 0·01	77
2	0·12 ± 0·01	66
4	0·09 ± 0·01	48
6	0·08 ± 0·01	45
8	0·09 ± 0·01	50
12	0·10 ± 0·01	57
Experiment II		
Control	0·13 ± 0·02	
24	0·12 ± 0·01	95

* Groups of mice were treated with 10 mg/kg i.v. of *p*-chloroamphetamine and sacrificed at various times after the administration of the drug. For each determination, the whole brains of three animals were combined. The values are the average of at least three determinations ± S. E.

Recent findings from this laboratory[8] suggested that the ability of *p*-chloroamphetamine to lower both 5HT and 5HIAA in the brains of rats may be a consequence of an inhibition of cerebral tryptophan hydroxylase. Interestingly, cerebral 5HIAA is also lowered in mice after the administration of *p*-chloroamphetamine (Table 1). It thus appears likely that a similar mechanism may explain the action of the drug in both species.

Evidence presented in this paper suggests that species differences in the metabolism of chlorinated amphetamines can explain the variations in their ability to lower cerebral serotonin in rats and mice. These findings also emphasize the importance of appropriate time–response curves when the action of a drug is investigated in different species.

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Psychopharmacology Research Center,
Department of Pharmacology,
Vanderbilt University School of Medicine,
Nashville, Tenn. 37203, U.S.A.

KENNETH W. MILLER
ELAINE SANDERS-BUSH
JAMES V. DINGELL

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