require Na^+ and K^+ were also examined and are shown in Figs. 3 and 4. Only a slight decrease of activity was observed.

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Stereoselective metabolism of amphetamine*

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SINCE *d*-amphetamine is a potent pharmacological agent—central stimulant, pressor agent and appetite depressant—whereas *l*-amphetamine is inert (or very low in activity), a difference in the fate of the stereoisomers may help to define the biochemical processes that determine the drug's actions. Gunne[1] has presented indirect evidence of stereoselectivity in amphetamine metabolism in man.

Direct evidence for a specific stereoselective pathway, utilizing the amphetamine metabolite *p*-hydroxyamphetamine (α-methyl tyramine), has been demonstrated *in vivo* in rats by Goldstein and Anagnoste.[2] The initial steps in this pathway are hydroxylations at two different positions. The first, hydroxylation of amphetamine at the *para* position on the benzene ring to yield *p*-hydroxyamphetamine, does not discriminate between stereoisomers and is thought to occur in the liver. The second, and stereoselective, step is hydroxylation of *p*-hydroxyamphetamine at the β-carbon of the side chain to yield *p*-hydroxynorephedrine (α-methyl octopamine). This reaction proceeds only for the *d*- and not for the *l*-isomer of hydroxyamphetamine. It is probably catalyzed by dopamine-β-oxidase, an enzyme located in the catecholamine-containing granules of sympathetic nerves.[3, 4] Although the stereoselectivity of the β-hydroxylation has so far been demonstrated in rats only, the β-hydroxylating step has been shown to occur *in vivo* in man,[5] in rats,[6, 7] and in cats;[8, 9] *p*-hydroxylation has been described in rats, dogs and man,[10–12] and in cats.[9]

The present work was designed to study the differential metabolism of amphetamine isomers as revealed by the presence of optically active metabolites in urine. Rats that had been given racemic *d*, *l*-amphetamine were found to excrete less *d*-than *l*-hydroxyamphetamine during the time intervals examined.

Racemic *p*-hydroxyamphetamine hydrobromide and dextro-*p*-hydroxyamphetamine hydrobromide were provided by Smith, Kline & French Laboratories. Trifluoroacetyl-*l*-prolyl chloride was synthetized by Dr. Enoch Gordis according to the method of Weygand *et al.*[13] Amphetamine sulfate injection, USP, and dextro-amphetamine sulfate injection were obtained from Gotham Pharmaceutical Co.; *l*-amphetamine sulfate was obtained from Walker Chemicals. The β-glucuronidase was obtained from Warner-Chilcott.

Twenty male albino rats weighing about 250 g were given 5 mg *d*, *l*-amphetamine i.p. per kg. Eight rats were given 2·5 mg of either *d*- or *l*-amphetamine i.p. per kg. Urine was collected for varying intervals and extracted for hydroxyamphetamine by a modification of the method of Axelrod.[10] After incubation with a commercially available glucuronidase at 37° for 48 hr, the urine was washed with ethyl ether at pH 4·5 until the ether phase was clear, then saturated with sodium chloride and

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adjusted to pH 9·5 with solid sodium carbonate. The ethyl ether extract made at pH 9·5 was evaporated to about 1 ml, dried over sodium sulfate, and diluted with 1 ml absolute methanol. Hydroxyamphetamine in the extract was quantitatively converted to its *O*-methyl ether by allowing it to react overnight at room temperature with 2 ml freshly prepared diazomethane solution.[14]

Gas chromatography was used to resolve and identify the optical isomers of *p*-methoxyamphetamine according to a method described for amphetamine by Gordis.[15] The methylated extract was evaporated to about 100 μl and injected into the gas chromatograph together with trifluoroacetyl-*l*-prolyl chloride (TPC). The diastereoisomers formed from *d*, *l*-methoxyamphetamine and TPC are completely resolved by gas chromatography. Unmethylated hydroxyamphetamine is not efficiently resolved by this method.

The columns used for resolution were U-shaped tubes 10 ft by 5 mm i.d. The stationary phase was SE-30 on a Gas-Chrom P support (Applied Science Laboratories), prepared according to the method of Fales and Pisano.[16] The carrier gas was argon, delivered at a pressure of 16 lb/in^2 and a temperature of 190–200°. Compounds present in the effluent gas were detected with a strontium-90 ionization detector.

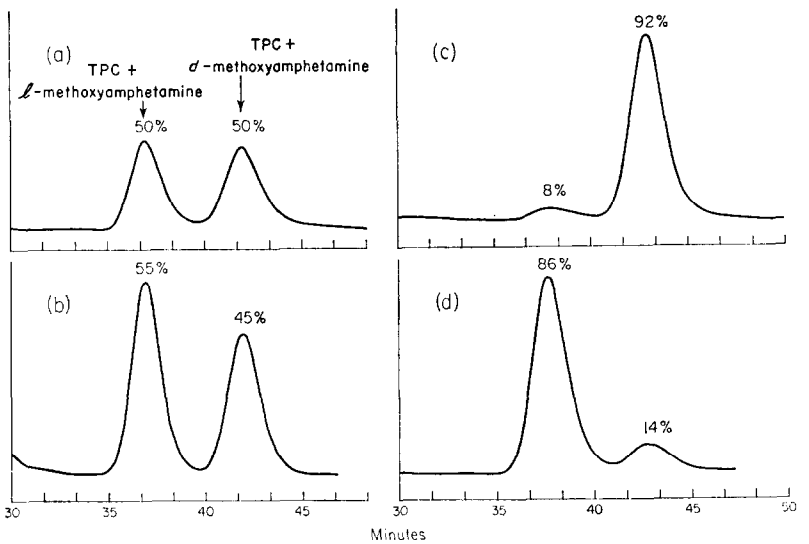

FIG. 1. Gas chromatograms of the resolution of diastereoisomers formed by reacting trifluoroacetyl-*l*-prolyl chloride (TPC) with (a) racemic (*d*, *l*) methoxyamphetamine, (b) methylated extract of rat urine collected for 4 hr after administration of *d*, *l*-amphetamine, (c) methylated extract of rat urine collected for 24 hr after administration of *d*-amphetamine, (d) methylated extract of rat urine collected for 24 hr after administration of *l*-amphetamine.

A mixture of TPC and *d*, *l*-methoxyamphetamine produced two well-separated peaks in the chromatogram (Fig. 1(a)). The second peak was identified as *d*-methoxyamphetamine by treating a sample of *d*-hydroxyamphetamine with diazomethane and injecting it along with TPC. A single peak, which proved to be identical with the second peak, appeared in the chromatogram. When TPC was injected along with a racemic mixture of methoxyamphetamine isomers, the ratio of the area under the second peak to the area under the first peak (*d*/*l* ratio) was 1·00 (Fig. 1(a)). Any column producing a *d*/*l* ratio for racemic methoxyamphetamine of more than 1·02 or less than 0·98 was discarded.

When racemic amphetamine was given to rats, less *d*- than *l*-hydroxyamphetamine appeared in the urine (Fig. 1(b)). This difference in the excretion of the optical isomers was highly significant (Table 1). It was most marked in the early hours after amphetamine administration and diminished with time, but there were too few trials to establish the statistical significance of this time trend.

After the administration of *d*- and *l*-amphetamine to rats, no significant racemization during the metabolism of amphetamine to hydroxyamphetamine was detected (Fig. 1(c), (d)). The lack of optical purity of the chromatographed metabolites is best explained by a lack of optical purity of the commercial amphetamine preparations used. The dextro-amphetamine sulfate injection administered contained 7–8 per cent *l*-amphetamine, and 8 per cent of its hydroxyamphetamine metabolite

TABLE 1. PROPORTIONS OF *d*- AND *l*-HYDROXYAMPHETAMINE IN RAT URINE COLLECTED FOR VARYING INTERVALS AFTER ADMINISTRATION OF RACEMIC (*d*, *l*) AMPHETAMINE

Time interval (hr after injection)	d/l ratio
0–2	0·84
0–4	0·82
2–5	0·91
4–24	0·91
5–24	0·95
0–24	0·92

Each value represents a single determination on urine pooled from four rats for the time interval shown. The mean *d*/*l* ratio of 0·89 is less than 1·00 ($P < 0·01$).

proved to be the *l*-form. Of the *l*-amphetamine sulfate solution which was prepared, 12–13 per cent was found to be *d*-amphetamine; 14 per cent of the hydroxyamphetamine produced from that solution was the *d*-form.

During the first 4 hr after *d*, *l*-amphetamine administration, 18 per cent less *d*- than *l*-hydroxyamphetamine was excreted by rats. Several explanations for this finding are possible, of which differential metabolism is one. The results are consistent with the observation that only the *d*-isomer of *p*-hydroxyamphetamine is β-hydroxylated. Other sites at which stereoselectivity may play a role in producing a *d*/*l* ratio of less than unity for hydroxyamphetamine are the uptake of amphetamine by the liver, the differential storage of amphetamine or hydroxyamphetamine isomers, and active secretion or absorption in the kidney tubules.

If the metabolism of *d*-hydroxyamphetamine to hydroxynorephedrine is considered an adequate explanation of the urinary *d*,*l* discrepancy, then the increase of the *d*/*l* ratio with time may be due to a large difference between the activities of liver and neuronal hydroxylases. The capacity of the neuronal (β-) hydroxylating system is likely to be far less than the capacity of the liver (*p*-) hydroxylating system. Relatively small amounts of *p*-hydroxyamphetamine would saturate the β-hydroxylase. With the continued production of relatively large amounts of hydroxyamphetamine by the liver, the percentage of the *d*-isomer that is further metabolized to hydroxynorephedrine would be expected to decline.

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The influence of 1-methyl-D-lysergic acid butanolamide on gastrointestinal serotonin in the Sprague–Dawley rat*

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D-LYSERGIC acid diethylamide (LSD-25) antagonizes the effect of serotonin on the rat uterus.[1] Sankar *et al.*[2, 3] have reported increased levels of serotonin in several brain areas and visceral organs in the rabbit (they omitted the gastrointestinal tract), after LSD-25. Similarly, Freedman,[4] and Freedman and Giarman[5] have reported small but definite elevations of cerebral serotonin after LSD-25 pretreatment. Of the many derivatives of LSD-25, 1-methyl-*d*-lysergic acid butanolamide (UML 491: Sansert, Sandoz Pharmaceuticals, Inc.), has been shown to be a more potent serotonin antagonist than LSD-25 in several tissues.[6, 7] Consequently, the effect of this drug on the bowel serotonin concentration in rats has been investigated.

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