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THE URINARY OUTPUT OF *d*- AND *l*-AMPHETAMINE IN MAN

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Abstract—Racemic amphetamine or methylamphetamine was administered to four human subjects, and the urine was collected in a total of fourteen experiments. The proportion of the optical isomers present in the urine was determined by gas chromatography of the diastereosiomers formed when a urine extract was reacted with trifluoroacetyl-*l*-prolyl chloride. After administration of racemic amphetamine the optical isomers were not excreted at the same rate. The ratio of the *d*-isomer to the *l*-isomer in the urine decreased from 0·99 during the first 12 hr to 0·46 at 48–60 hr.

Although the total urinary excretion of amphetamine is pH-dependent, the differential excretion of the *d*- and *l*-isomers occurred whether the urine was acid or alkaline.

Racemic methylamphetamine was metabolized to amphetamine and the main isomer excreted in the urine was *d*-amphetamine, whereas *l*-amphetamine was formed to a lesser extent.

IN VARIOUS species of animals, including man, *d*-amphetamine is a potent central stimulant with sympathomimetic effects, whereas *l*-amphetamine has a lower activity, possibly none. Earlier studies of the urinary excretion of *d*- and *l*-amphetamine have been handicapped by a lack of methods for the simultaneous estimation of small amounts of both stereoisomers. Beckett and Rowland[1] gave *d*-amphetamine and *l*-amphetamine separately to three subjects. In two of the subjects they found evidence of a somewhat shorter biological half-life of the *d*-isomer. However, since the output of amphetamine is known to be pH-dependent,[2, 3] minor differences in the excretion rates of separately administered isomers may be difficult to interpret.

Recently a method has been described[4] for the gas chromatographic resolution of the optical isomers of amphetamine. This method allows for a simultaneous estimation of microgram amounts of *d*- and *l*-amphetamine in tissues and urine and makes it possible to study stereospecific mechanisms involved in the metabolism of this drug. In the present study it has been used to measure the urinary output of the two optical isomers after administration of racemic amphetamine or methylamphetamine in human subjects.

METHODS

Four healthy subjects (three male, one female) were given oral doses of racemic amphetamine ranging from 2·5 to 15 mg in a total of twelve experiments. In two additional experiments 10 mg of racemic methylamphetamine was administered.

In two of the amphetamine experiments the urine was kept alkaline by administration of sodium bicarbonate. After the excretion pattern had been determined for 48 hr,

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ammonium chloride was given for an additional 12 hr in order to enhance the amphetamine excretion to measurable levels on the third day. In another amphetamine experiment the urine was kept acid throughout by oral doses of ammonium chloride. The doses of ammonium chloride and sodium bicarbonate were 3–5 g 1 hr before the amphetamine administration followed by 1 g hourly, during waking hours, throughout the experiments.

In each experiment urine was first collected during a 12-hr control period. The drug was then administered and the urine collected, usually in 12-hr periods; in some experiments (when the expected amphetamine excretion was low) urine samples were collected instead during 24-hr periods. No preservatives were added, and the urine was stored at room temperature during the collection period.

Extraction and determination. Each urine sample was extracted in two separate portions, the first into benzene for quantitative determination of total amphetamine and the second into ethyl ether for estimation of the optical isomers.

In the gas chromatographic system employed, amphetamine was most easily detected by converting it quantitatively to its N-propionyl derivative. This is easily accomplished by drawing up propionic anhydride together with the amphetamine solution into the same syringe and injecting the mixture into the column. The propionyl derivative forms instantly and quantitatively in the hot vapors of the injection port. The minimal discernible amount by this method was 1 ng of amphetamine. The reaction was complete, with a total disappearance of the unreacted amphetamine peak, if the propionic anhydride was added in sufficient amounts. For estimation of a maximum of 5 μg amphetamine, 1 μl redistilled propionic anhydride was found to be adequate.

For the quantitative determination of amphetamine, a known amount of triphenylamine was added to the extract as an internal standard. Triphenylamine is a tertiary amine and does not react with propionic anhydride. The quantity of amphetamine in the extract was calculated from the ratio propionyl-amphetamine/triphenylamine. Added amounts of amphetamine to urine were recovered with adequate precision (92–102 per cent).

The separation of *d*- and *l*-amphetamine was performed according to the method of Gordis,[4] with trifluoroacetyl-*l*-prolyl chloride (TPC) as the resolving agent. TPC reacts with *dl*-amphetamine to form the diastereoisomeric pair N-(*l*-trifluoroacetyl-prolyl)-*d,l*-amphetamine. The diastereoisomers can be completely resolved by gas chromatography. The reaction between the resolving agent and amphetamine can be performed directly on the column by mixing the two in the same syringe before injection. Since TPC moves more slowly on the column than does amphetamine, it has to be taken up into the syringe after (and expelled before) the sample.

The proportion of the isomers in a standard solution of racemic amphetamine was determined at the start of each experiment and ranged from 0·98 to 1·02. It was found that repeated overloads of the column tended to produce asymmetric peaks, resulting in a more than 2 per cent deviation from the ideal *d*/*l* ratio. In such instances the columns were replaced, even after a limited number of injections. The peaks were identified by comparison of their retention times with those of standard solutions of the optical isomers.

Experimental. Quantitative estimations of amphetamine were carried out in seven experiments. The pH of the urine was adjusted to 5 and an aliquot, about 10 per cent

of the volume, was repeatedly shaken with benzene in order to remove emulsifiers. When the benzene layer remained clear, a volume corresponding to 4 per cent of the total urine was taken for extraction and 50 μg triphenylamine, dissolved in benzene, was added. The pH was adjusted to 12 with 2 N NaOH and extraction was made into benzene. The extract was evaporated to a volume of about 50–100 μl before assay on the gas chromatograph.

The remaining 96 per cent of each urine sample was shaken with 250 ml ethyl ether four or five times, until the ether phase remained clear. The remaining urine was then extracted with 50 ml ether three times at pH 12. The combined extracts were dried with sodium sulfate and evaporated to a volume of about 200 μl. The viscous material obtained was re-extracted into 2 ml of 0·1 N HCl, centrifuged, and the water phase transferred to a clean tube. After addition of 1 ml of 2 N NaOH, the amines were extracted into petroleum ether and the sample was evaporated to 50–100 μl.

Gas chromatography was performed essentially according to Fales and Pisano.[5] A 10-ft $\times$ 4 mm (I.D.) glass column was employed, packed with 4% SE 30 siloxane polymer on Gaschrom P. Argon, delivered at 15 psi, was the carrier gas. The column temperature was 195°. Effluents were detected with a strontium-90 ionization detector.

RESULTS

Figure 1 illustrates the gas chromatographic resolution of the diastereoisomers formed by the reaction of TPC with amphetamine: (a) standard solution of racemic amphetamine; (b) chromatogram of control urine reacted with TPC; (c), (d), (e), and (f) chromatograms of urine extracts reacted with TPC obtained 0–12, 12–24, 24–36, and 36–38 hr after the administration of 10 mg *dl*-amphetamine. The *d*/*l* ratios of the four amphetamine-containing urine samples were: 0·91, 0·80, 0·70, and 0·60 respectively. In this experiment the urine had been kept acid with oral doses of ammonium chloride.

The specificity of the method seemed to be sufficient, since in no instance did the pre-trial sample (Fig. 1b) yield peaks with the same retention time as amphetamine. When the amphetamine content was low, at the end of a test period, the loads had to be increased to obtain sufficiently high peaks. These injections diminished the resolution to some extent, and additional unidentified peaks were sometimes seen in the vicinity of amphetamine (Fig. 1f).

After administration of *dl*-amphetamine, all subjects excreted approximately equal amounts of both isomers during the first 12 hr. Urines collected after 12 hr contained a continually decreasing proportion of the *d*-isomer (Fig. 2). The initial *d*/*l* ratio seemed to vary between individuals and to a lesser extent in the same individual from one experiment to another. Thus in one subject the initial *d*/*l* value was above 1·0 in three experiments. The mean 0–12-hr *d*/*l* ratio was 0·99. In the three experiments that were carried out for more than 48 hr, the mean *d*/*l* ratio had decreased to 0·46.

There was no apparent effect on the *d*/*l* ratio when the urine was acidified by administration of ammonium chloride or alkalinized by sodium bicarbonate, although the total excretion of amphetamine was greatly influenced (Table 1). The chromatograms obtained during administration of sodium bicarbonate contained a series of unidentified peaks.

After the administration of *dl*-methylamphetamine, both methylamphetamine and

amphetamine appeared in the urine. Urine specimens contained a continually decreasing proportion of *d*-methylamphetamine to l-methylamphetamine. At the same time, however, more *d*-amphetamine than *l*-amphetamine was found in the urine (Fig. 3). The chromatographic system used in these experiments, designed for the

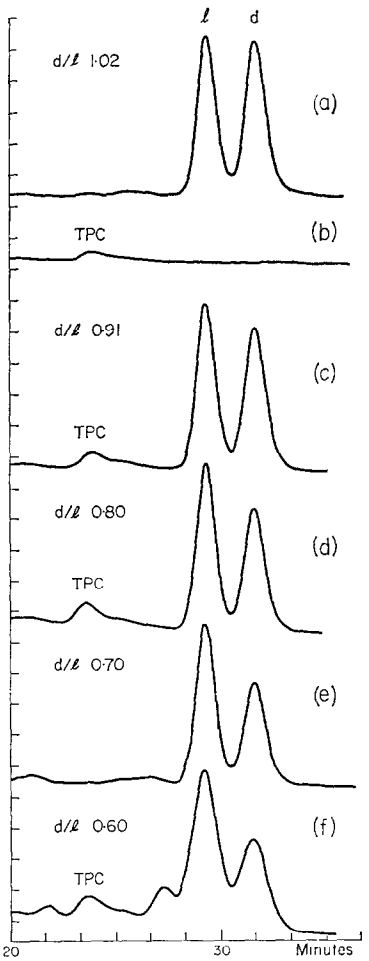

FIG. 1. Six chromatograms showing the diastereoisomers formed by reaction of TPC with *d*- and *l*-amphetamine: (a) 5 μg racemic amphetamine; (b) urine extract before administration of amphetamine; (c) urine extract 0–12 hr after administration of 10 mg *dl*-amphetamine; (d) urine extract 12–24 hr after; (e) urine extract 24–36 hr after; (f) urine extract 36–48 hr after. The portions of the chromatograms recorded during the first 20 min have been omitted.

resolution of the optical isomers of amphetamine, resolved the isomers of methylamphetamine incompletely. For this reason the areas of the methylamphetamine peaks could not be accurately measured.

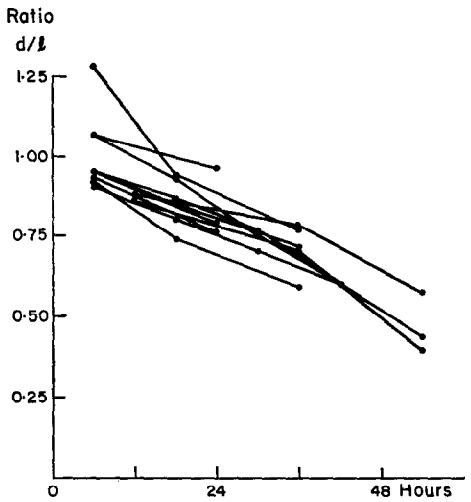

FIG. 2. Decreasing ratio of *d*-amphetamine to *l*-amphetamine in urine collected up to 60 hr after the administration of 2·5 – 15 mg racemic amphetamine in twelve experiments.

TABLE 1. THE URINARY EXCRETION OF AMPHETAMINE AFTER THE INGESTION OF *dl*-AMPHETAMINE

Additional treatment	Amphetamine dose (mg)		Hours after amphetamine administration 0–12	12–24	24–36	36–48	48–60
None	2·5	*d*/*l* ratio	0·93	— 0·76 —			
		% excr.	44	— 15 —			
		pH	5·4	— 5·6 —			
	5	*d*/*l* ratio	0·95	0·85	— 0·72 —		
		% excr.	16	7·2	— 9·4 —		
		pH	5·7	5·8	— 5·9 —		
	5	*d*/*l* ratio	1·28	0·94	— 0·77 —		
		% excr.	10	12	— 9·8 —		
		pH	6·8	6·7	— 6·0 —		
	10	*d*/*l* ratio	1·07	0·93	0·75	0·60	
		% excr.	19	11	2·7	1·4	
		pH	5·5	5·2	5·6	5·6	
Ammonium chloride	10	*d*/*l* ratio	0·91	0·80	0·70	0·60	
		% excr.	43	13	3·1	0·8	
		pH	5·2	5·1	5·1	5·0	
Sodium bicarbonate (0–48 hr) ammonium chloride (48–60 hr)	10	*d*/*l* ratio	— 0·88 —		— 0·78 —		0·57
		% excr.	— 1·3 —		— 0·5 —		1·1
		pH	— 7·6 —		— 8·1 —		6·7
	15	*d*/*l* ratio	— 0·87 —		— 0·71 —		0·39
		% excr.	— 0·4 —		— 0·1 —		1·1
		pH	— 8·2 —		— 8·5 —		7·2

Urinary *d*/*l* ratio, amount excreted in per cent of dose (% excr.) and urinary pH at various time intervals after an oral dose.

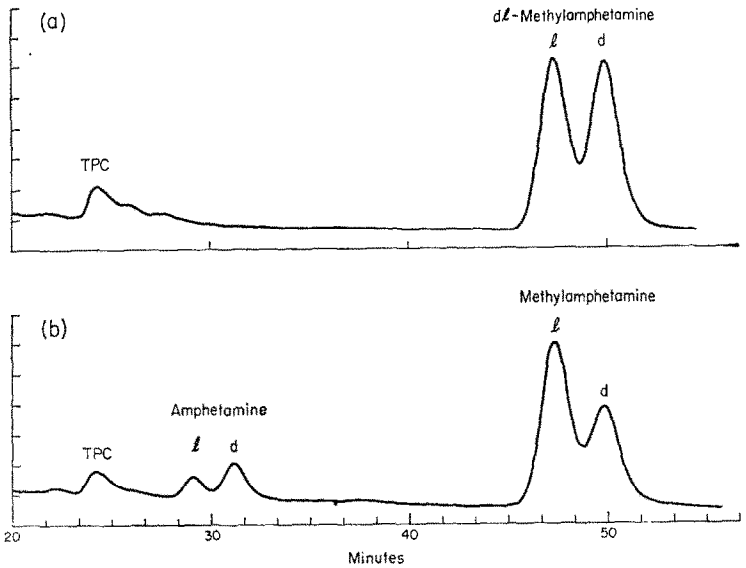

FIG. 3. Two chromatograms showing the diastereoisomers formed by reaction of TPC with (a) 10 μg racemic methylamphetamine, and (b) an extract of urine collected 24–36 hr after ingestion of 10 mg *dl*-methylamphetamine. The portions of the chromatograms recorded during the first 20 min have been omitted.

DISCUSSION

The experiments described above show that after the ingestion of *dl*-amphetamine, less of the *d*-isomer is excreted in the urine. Alles and Wisegarver[6] measured the optical activity of a single urine extract obtained in an amphetamine addict during intake of the very high dose of 600 mg daily of racemic amphetamine. After pooling "several large volumes" of urine, they found the extract to be levorotatory. Beckett and Rowland[1] reported that, after the separate administration of *d*-amphetamine and *l*-amphetamine, less *d*-amphetamine was excreted in a comparable collection period.

The present work confirms the observation that the fate of the two optical isomers of amphetamine in the body is not identical. How this is related to the difference in their pharmacological activity is not clear at present. Several mechanisms may account for differences in the excretion of the two isomers: there may be a selective uptake of the *d*-isomer from the blood into the tissues, combined with a stereospecific catabolism of the *d*-isomer, or a prolonged retention of the unmetabolized *d*-isomer, followed by a slower excretion at a later time. A stereospecific urinary excretion appears unlikely from the observed lack of effect of acid and alkaline loads. Great variations in the excretion of amphetamine failed to influence the *d*/*l* ratio in the present experiments.

Some data support the view of a stereospecific metabolism of amphetamine and methylamphetamine. Goldstein *et al.*[7] showed a β-hydroxylation of amphetamine *in vitro*, which was stereospecific for the *d*-isomer, but they failed to show a corresponding mechanism *in vivo* for the unmetabolized drug.[8] Axelrod[9] presented evidence *in vitro* of a stereospecific deamination of amphetamine by rabbit liver homogenate. However, in those experiments the enzyme showed a relative specificity for the levo-isomer.

Methylamphetamine is known to be metabolized to amphetamine by a demethylating enzyme.[10, 11] In the present study, *dl*-methylamphetamine was found to yield mainly urinary *d*-amphetamine. A specificity of the demethylating enzyme for the *d*-isomer seems to be the most likely explanation for this observation.

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