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Metabolism of Tranylcypromine-C^{14} and *dl*-Amphetamine-C^{14} in the Rat

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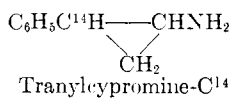
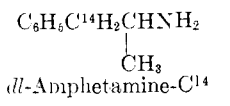
About 4% of injected tranylcypromine-C^{14} (label adjoining benzene ring) was excreted unchanged, and about 12% was excreted as hippuric acid-C^{14} in the 24-hr. urine, which contained about 72% of the injected C^{14}. Corresponding values for *dl*-amphetamine-C^{14} were 15, 2, and 70%. Four additional urine C^{14} metabolites of tranylcypromine-C^{14} and two of *dl*-amphetamine-C^{14} were demonstrated in paper chromatograms but were not identified.

trans-2-Phenylcyclopropylamine (tranylcypromine[2]) was synthesized by Burger and Yost[3] with the intention of incorporating into a single molecule the anesthetic, cyclopropane, and the central stimulant, amphetamine. The new compound was found to be a potent inhibitor of monoamine oxidase[4] and an effective antidepressant in man.[5] In the present investigation the metabolism of tranylcypromine was studied with particular interest centered on the fate of the cyclopropane ring, since such knowledge appears limited to cyclopropane and its methyl, ethyl, and vinyl ethers, all of which are believed to be metabolically inert and are excreted unchanged.[6] The present results indicate that tranylcypromine is metabolized extensively in the rat by pathways including cleavage of the cyclopropyl ring. Because of the close structural similarity of tranylcypromine and amphetamine, a comparative metabolic study was made using the C^{14} form of each compound, labeled as follows[7]



Tranylcypromine-C^{14}



dl-Amphetamine-C^{14}

Methods

Purity of C^{14} Compounds.—Tranylcypromine-C^{14} sulfate behaved identically with an authentic standard throughout ultraviolet spectroscopy, paper-strip chromatography employing ninhydrin, and C^{14} analysis after paper-strip chromatography. C^{14}-Purity was over 99.9%, and the specific activity was 3.81 μc./mg. *dl*-Amphetamine-C^{14} sulfate was analyzed by the third method only. C^{14}-Purity was over 99.9% and the specific activity was 2.73 μc./mg.

Recovery of C^{14} in Urine, Feces, and CO_2.—The C^{14} compounds were dissolved in water and were either injected subcutaneously (s.c.) between the scapulae into male albino rats (205–215 g.) or were given orally by stomach tube to similar rats (240–262 g.). Aliquots of urine plus cage washings, collected daily, were dissolved in "aqueous" phosphor[8] and were counted in a Packard liquid scintillation spectrometer. Corrections for quench were made by recounting each sample with internal standard.

Fecal pellets were dried for 2–3 days *in vacuo* over KOH, weighed, powdered in a Waring blendor, pressed into planchets, and counted at infinite thickness in a gas-flow geiger counter. For comparison, aliquots of dose solutions were added to unlabeled fresh rat feces which were then treated similarly. Direct proportion was obtained between added and measured activity.

Expired CO_2 was analyzed for C^{14} in two male albino rats (229 g.) given tranylcypromine-C^{14}. Immediately after s.c. injection, each rat was placed in a plastic chamber through which CO_2-free, humidified air was drawn and bubbled successively through two columns in series containing 10% NaOH. Carbon dioxide was precipitated as $BaCO_3$ which was suspended in "non-aqueous" phosphor[9] and counted. Urine was collected quantitatively and analyzed for C^{14}.

Paper Chromatography of Urine.—Ten male albino rats (300–330 g.) were each given a single s.c. injection

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(7) Tranylcypromine-C^{14} and *dl*-amphetamine-C^{14} sulfates were synthesized by Drs. D. W. Blackburn and J. W. Wilson, respectively, at Smith Kline & French Laboratories, Philadelphia, Pa.

(8) 2,5-Diphenyloxazole (10 g.) plus 160 g. of naphthalene dissolved to 2 l. with a solution of toluene–1,4-dioxane–ethanol (1:1:0.6 v./v.).

(9) 2,5-Diphenyloxazole (0.3%) plus 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene.

TABLE I
EXCRETION OF C^{14} AFTER A SINGLE SUBCUTANEOUS INJECTION OF TRANYLCYPROMINE-C^{14} OR *dl*-AMPHETAMINE-C^{14}

Dose = 5 mg./kg.		Percentage of injected C^{14} recovered[a]				
		Day 1	Day 2	Day 3	Days 4–8	Total
Tranylcypromine-C^{14}	Urine	71.5 **5.7**	12.6 **2.6**	2.1 **0.5**	2.7 **2.0**	88.9 **1.8**
	Feces	2.1 **1.8**	2.7 **1.1**	0.2 **0.1**	0.6 **0.2**	5.6 **0.5**
	Total	73.6 **7.1**	15.3 **3.5**	2.3 **0.5**	3.3 **2.2**	94.5 **2.1**
dl-Amphetamine-C^{14}	Urine	69.5 **1.8**	9.8 **1.7**	2.1 **0.3**	1.6 **0.4**	83.3 **1.4**
	Feces	2.1 **1.4**	1.3 **0.8**	0.1 **0.0**	0.4 **0.1**	3.9 **1.0**
	Total	71.6 **2.0**	11.1 **2.4**	2.5 **0.3**	2.0 **0.5**	87.2 **1.2**

[a] Values are averages of four rats per compound with standard deviations in **italics**.

TABLE II
EXCRETION OF C^{14} AFTER A SINGLE ORAL DOSE OF TRANYLCYPROMINE-C^{14} OR *dl*-AMPHETAMINE-C^{14}

		Percentage of orally administered C^{14} recovered[a]								
	Dose,	Day 1		Day 2		Day 3		Total		
	mg./kg.	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Total
Tranylcypromine-C^{14}	0.073	74.2	7.9	3.9	...	1.8	...	79.9	7.9	87.8
		66.4	7.2	4.9	...	1.3	...	72.9	7.2	80.1
	0.73	77.9	8.6	2.7	0.6	0.8	0.3	81.4	9.5	90.9
		73.9	9.6	3.7	0.3	1.0	1.1	78.6	11.0	89.6
	1.83	78.2	10.1	2.5	2.2	0.6	0.2	81.3	12.5	93.8
		71.5	8.9	1.8	1.8	0.6	0.3	73.9	11.0	84.9
	3.66	67.0	6.9	5.8	3.5	0.6	0.6	73.4	11.0	84.4
		77.2	5.6	2.5	1.5	0.5	0.3	80.2	7.4	87.6
		67.5	7.7	2.6	1.4	0.3	0.2	70.4	9.3	79.7
	5.00	77.2	1.5	7.0	3.9	1.5	3.1	85.7	8.5	94.2
		69.0	3.1	12.8	1.0	1.4	0.7	83.2	4.8	88.0
dl-Amphetamine-C^{14}	0.073	85.7	6.1	4.2	1.9	0.9	...	90.8	8.0	98.8
		82.0	4.2	5.1	1.3	1.6	...	89.0	5.3	94.3
	0.73	85.8	6.9	3.4	1.2	0.9	0.3	90.1	8.4	98.5
		84.4	4.2	4.3	0.7	2.2	0.2	90.9	5.1	96.0
	1.83	80.0	7.7	5.2	0.9	1.1	0.3	86.3	8.9	95.2
		79.6	5.6	4.9	1.0	1.2	0.3	85.8	6.9	92.7
	3.66	85.4	2.0	4.6	0.8	0.8	0.2	90.8	3.0	93.8
		75.1	6.6	4.0	0.9	0.8	0.3	79.9	7.8	87.7

[a] Individual rat values.

of tranylcypromine-C^{14} equal to 5 mg. of free base per kg. of body weight (5 mg./kg.). They were housed in pairs in metabolism cages. 2-Propanol (1 ml.) was added initially to each urine jar to minimize microbial growth. The 24-hr. urine samples were pooled. This procedure was followed with four male albino rats (250–270 g.) given *dl*-amphetamine-C^{14}. Homogeneous urine aliquots including sediment were applied to strips of Whatman No. 1 filter paper. These were chromatographed downward in solvents I and II.[10] Separate strips containing authentic tranylcypromine-C^{14}, *dl*-amphetamine-C^{14}, hippuric acid, benzoic acid, phenylacetic acid, and hydrocinnamic acid (suspected metabolites) were run concurrently. After development the C^{14} zones were located with a strip scanner. Unlabeled acids in standard strips were located under ultraviolet light and by acid reaction (red) to phenol red solution.

Identification of Hippuric Acid-C^{14}.—After removal of the aliquots for chromatographic analysis, the urine was extracted four times with ethyl ether, and the combined extracts were evaporated to dryness. The residues were dissolved in dilute ammonium hydroxide and chromatographed downward on sheets of Whatman No. 3 filter paper in solvents I–IV,[10] hippuric acid-C^{14} being eluted and reapplied to a new sheet for each development. Unlabeled hippuric acid carrier was dissolved in the final eluates, and the solutions were acidified with HCl. Fine crystals formed slowly and were collected by filtration. The specific activity (c.p.m./mg.) of the powdered crystals was determined, using "aqueous" phosphor, after each of three consecutive precipitations from acidified ammonia solution; after suspension in petroleum ether; after formation of the anilide derivative.

Hippuric anilide was prepared as follows: 30 mg. of the final ether-washed precipitate and 0.2 ml. of aniline were heated in a capped 20-ml. glass vial for 10 min. on a hot plate. The material was suspended in ethyl ether to remove excess reactants, dissolved in 5 ml. of ethanol, diluted to 20 ml. with water, and precipitated at 4°. Melting points of starting material (hippuric acid) and end product (hippuric anilide) were identical with known values. The acidity of the starting material was absent from the end product.

Another portion of the final ether-washed hippuric acid was dissolved in dilute ammonia, applied to strips of Whatman No. 1 filter paper, and developed downward in solvents I–IV.[10] R_f values of C^{14} and hippuric acid carrier were compared.

Results

Excretion of C^{14}.—Following single s.c. or oral doses of tranylcypromine-C^{14} or *dl*-amphetamine-C^{14}, most of the administered C^{14} was recovered in the 24-hr. urine. However, small amounts of C^{14} were excreted in feces and urine for at least four days after s.c. injection

(10) Solvent I: isoamyl alcohol–*t*-amyl alcohol–water–formic acid (5:5:10:2 v./v.); II: 1-butanol saturated with NH_3-$(NH_4)_2CO_3$ buffer (1.5 *N* each); III: 1-butanol–ethanol–buffer of II (40:11:19 v./v.); IV: 1-butanol–benzene–buffer of II (80:5:15 v./v.). Organic phase = moving (descending) phase. Solvent systems II–IV were described by M. E. Fewster and D. A. Hall, *Nature*, **168**, 78 (1951).

(Tables I and II). Expired CO_2 collected for periods of 4 and 6 hr. after a single 5 mg./kg. injection of tranylcypromine-C^{14} contained only traces of C^{14}, amounting to less than 0.1% of the dose. On the other hand, the amounts excreted in urine during these periods were 16.3 and 42.9%, respectively.

Urine Chromatograms.—Six C^{14} peaks were obtained in the solvent I chromatogram of pooled urine from 10 rats given tranylcypromine-C^{14} (Fig. 1). The major peak comprising 47% of the total strip C^{14} had an R_f (0.88) similar to those of standard hippuric, benzoic, phenylacetic, and hydrocinnamic acids. A second peak comprising 6% of the total strip C^{14} had an R_f (0.67) similar to that of standard tranylcypromine-C^{14} (0.60; corresponding values from preliminary runs were 0.63 and 0.64, 0.63 and 0.57, 0.59 and 0.57). Further characterization of this peak was based on the volatility of free tranylcypromine. A chromatogram of 3-hr. urine was developed in solvent I and was scanned before and after exposure to a chamber saturated with NH_4OH. Before exposure the chromatogram was similar to the 24-hr. chromatogram of Fig. 1. After exposure the suspected tranylcypromine-C^{14} peak with R_f 0.63 had been reduced in area by 70% while the other five peaks were unaffected. A standard strip containing tranylcypromine-C^{14} was run concurrently and the peak at R_f 0.64 had also been reduced by 70%.

Chromatographic development of the 24-hr. urine in solvent II revealed six C^{14} peaks of which four had R_f values (0.48, 0.57, 0.64, 0.75) corresponding to those of hippuric (0.50), benzoic (0.62), phenylacetic (0.62), and hydrocinnamic (0.78) acids (Fig. 2). This chromatogram was further analyzed by cutting out these four C^{14} zones, soaking the paper segments in dilute ammonia, dissolving each acid as carrier in a separate aliquot, and determining the specific activity of each carrier acid after consecutive precipitations from acidified ammonia solution (Table III). By this method it was found that 79.3% of the C^{14} material with R_f 0.48 was due to hippuric acid-C^{14} and, with less certainty, 4.2% of the material with R_f 0.64 was due to benzoic acid-C^{14}. Hydrocinnamic and phenylacetic C^{14}-acids were not identified in any of the four extracts.

TABLE III

CARRIER PRECIPITATION ANALYSIS OF A TRANYLCYPROMINE-C^{14} URINE CHROMATOGRAM

R_f of extracted metabolite (Fig. 2)		0.48	0.57	0.64	0.75
Total c.p.m. extracted		17,700	3,620	11,200	4,220
C.p.m. added to 400 mg. of carrier acid		3,930	805	2,480	938
Carrier acid added	Precipitate	C.p.m./mg. of precipitate			
	1	8.63	0.14	0.15	0.14
	2	7.86	0	0	0
Hippuric	3	7.74			
R_f 0.50	4	7.63			
	Petroleum ether	7.93			
		7.79	Average excluding no. 1		
	Hippuric anilide	5.30	Theoretical = 5.49		
	1	0.28	0.23	0.52	0.27
Benzoic	2	0.06	0.07	0.34	0.10
R_f 0.62	3	0	0	0.30	0
	4			0.26	
Phenylacetic	1	0.26	0.22	0.16	0.09
R_f 0.62	2	0	0	0	0
Hydrocinnamic	1	0.92	0.21	0.33	1.17
R_f 0.78	2	0.05	0.09	0.11	0.32
	3	0	0	0	0

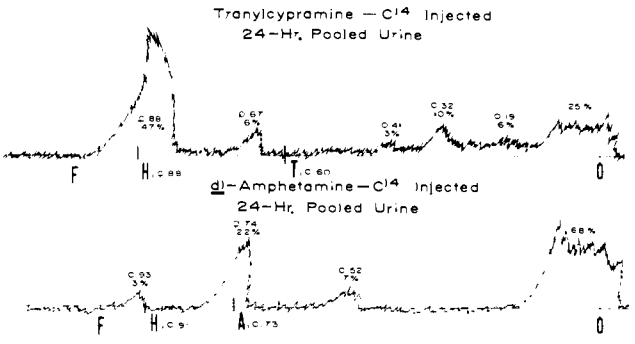

Fig. 1.—Paper chromatograms developed in solvent I.[10] The R_f and per cent of total strip C^{14} are shown above each peak. R_f values from separate standard strips, run concurrently, are shown at calculated positions in the urine chromatograms (abbreviations: O, origin; F, front; T, authentic tranylcypromine-C^{14}; A, authentic *dl*-amphetamine-C^{14}; H, authentic hippuric acid).

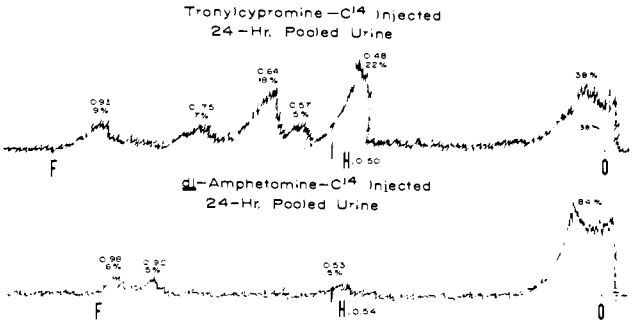

Fig. 2.—Paper chromatograms developed in solvent II.[10] The R_f and per cent of total strip C^{14} are shown above each peak. R_f values from separate standard strips, run concurrently, are shown at calculated positions in the urine chromatograms. Tranylcypromine-C^{14} and *dl*-amphetamine-C^{14} are volatile in this basic system and disappear from chromatograms during development (abbreviations: O, origin; F, front; H, authentic hippuric acid).

Four C^{14} peaks were obtained in chromatograms developed in solvent I of pooled 24-hr. urine from four rats given *dl*-amphetamine (Fig. 1). One peak comprising 22% of the total strip C^{14} had an R_f (0.74) similar to that of the parent compound (0.73). A second peak had an R_f (0.93) similar to that of hippuric acid (0.91). Four C^{14} peaks were also obtained in the chromatogram developed in solvent II, one of which comprised 5% of the total strip C^{14} and had an R_f (0.53) similar to that of hippuric acid (0.54).

Calculations using the figures reported above show that after a single 5 mg./kg. s.c. injection of tranylcypromine-C^{14} about 4% (71.5 × 6) of the dose was excreted in the 24-hr. urine as the parent compound and about 12% (71.5 × 22 × 79.3), as hippuric acid-C^{14}. Corresponding values for *dl*-amphetamine-C^{14} are 15% (69.5 × 22) and 2% (69.5 × 3).

Identification of Hippuric Acid-C^{14}.—The ether extracts of acidified urine from 10 rats given tranylcypromine-C^{14} and from four rats given *dl*-amphetamine-C^{14} contained a C^{14} metabolite which in the presence of carrier hippuric acid maintained a constant specific activity (c.p.m./μmole) throughout three precipitations from acidified ammonia solution; suspension in petroleum ether; formation of the anilide derivative (Table IV). The R_f values of the C^{14}-metabolite in solvents I–IV were almost identical with those of the carrier hippuric acid (Table V).

TABLE IV

CONSTANT SPECIFIC ACTIVITY OF URINE METABOLITES OF TRANYLCYPROMINE-C^{14} AND *dl*-AMPHETAMINE-C^{14} IN HIPPURIC ACID CARRIER

		Cpm/μmole	
		Metabolite of tranylcypromine-C^{14}	Metabolite of *dl*-amphetamine-C^{14}
Consecutive	(1)	2610	82.3
Precipitations	(2)	2580	78.9
	(3)	2740	79.7
Suspension in petroleum ether		2670	76.8
Anilide derivative		2670	76.2

TABLE V

R_f OF URINE METABOLITES OF TRANYLCYPROMINE-C^{14} AND *dl*-AMPHETAMINE-C^{14} COMPARED WITH R_f OF CARRIER HIPPURIC ACID

Solvent[a]	Carrier hippuric acid	Metabolite of tranylcypromine-C^{14}	Carrier Hippuric acid	Metabolite of *dl*-amphetamine-C^{14}
I	0.82	0.85	0.84	0.85
II	0.50	0.51	0.50	0.52
III	0.65	0.65	0.62	0.64
IV	0.45	0.45	0.41	0.43

[a] See ref. 10.

Discussion

The identification of hippuric acid as a metabolite of tranylcypromine is the first demonstration that the cyclopropyl ring can be broken in the body. While the cleavage mechanism is unknown, it appears not to involve formation of amphetamine, since different patterns of peaks were observed in chromatograms of urine from rats given tranylcypromine-C^{14} and *dl*-amphetamine-C^{14} (Fig. 1 and 2). It is also unlikely that the first step is deamination by monoamine oxidase, since tranylcypromine has been recovered unchanged after incubation for 24 hr. in a rat-liver mitochondrial preparation containing the active enzyme.[11] This is in agreement with the view that monoamine oxidase is unable to oxidize α-alkylamines.[12] A liver microsomal enzyme system described by Axelrod[13] which deaminates such amines to ketones may be involved in the ring-splitting reaction. Another possibility is suggested from the propensity of tranylcypromine to decompose to ammonia and hydrocinnamaldehyde, upon standing in water solution for several weeks,[14] or upon distillation of the base.[15] In the body the aldehyde would be metabolized through hydrocinnamic, benzoic, and hippuric acids. Failure to identify hydrocinnamic acid in the present study would be explained by its rapid degradation to benzoic acid.[16] That an enzyme is involved in the cleavage reaction is suggested by the tested stability of tranylcypromine-C^{14} in water solutions buffered with phosphate at pH 7.8 and incubated at 37° for several days.

Identification of hippuric acid as a metabolite of amphetamine is evidence that the rat can degrade amphetamine, though this appears to be a minor pathway. The microsomal enzyme system described by Axelrod[13] deaminates amphetamine to phenylacetone, which is metabolized to hippuric acid in the body.[7] The small amount of hippuric acid-C^{14} recovered in the present study may be due to the low enzyme activities observed by Axelrod in rat liver as compared with rabbit liver. The present findings are in agreement with the view that the main metabolic pathway of amphetamine in the rat is not through deamination, as in the rabbit, but through ring hydroxylation with subsequent formation of the glucuronide.[13] The latter material probably accounts for the large peaks at the origins of the urine chromatograms shown in Fig. 1 and 2.

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Kinetics of the Formalin Inactivation of Poliovirus

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It is shown that the non-first-order course of the formalin inactivation of poliovirus is consistent with the kinetic requirements of a mechanism of the type, $N_n \rightleftharpoons nN \rightarrow nD$. In this formulation, N_n represents a non-infectious *n*-meric form of the virus, N is the infectious native form of the virus, and D is an antigenic but non-infectious species. The method used for testing the applicability of this mechanism is discussed in some detail. Several respects in which the postulated mechanism is related to previous proposals are also examined, especially the possible role of a superimposed thermal denaturation.

It is customary[1] to describe the time course of the denaturation of proteins, including viruses,[2,3] by a semi-logarithmic plot of protein concentration *vs.* time. Such denaturations are usually carried out in the presence of a large excess of denaturing agent, so the frequent linearity of plots of this type suggests a straightforward conversion of the protein to its denatured form with a pseudo-first-order mechanism for the rate-

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