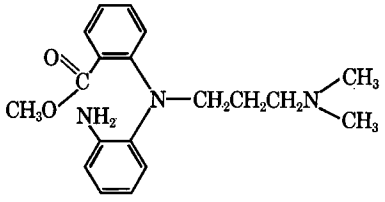
Metabolism and Excretion of Methyl *N*-(*o*-Aminophenyl)-*N*-(3-dimethylaminopropyl) Anthranilate in the Dog and Human

By DAVID L. SMITH, ALBERT L. PULLIAM, and HOWARD KO

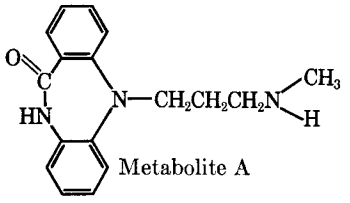
Four urinary metabolites of the title drug, excreted by both the dog and human, have been identified and quantitated. Metabolite A is 5-(3-methylaminopropyl)-5,10-dihydro-11H-dibenzo[*b*,*e*][1,4]diazepin-11-one, metabolite B is the methyl ester of *N*-(*o*-aminophenyl)-*N*-(3-methylaminopropyl)-anthranilic acid, metabolite C is 5-(3-dimethylaminopropyl)-5,10-dihydro-11H-dibenzo[*b*,*e*][1,4]diazepin-11-one, and metabolite D is 5-(3-aminopropyl)-5,10-dihydro-11H-dibenzo[*b*,*e*][1,4]diazepin-11-one. At least 46.3 ± 4.4 per cent (S.E.M.) of the dose was absorbed in the human, 8.6 ± 1.5 per cent was excreted as metabolite A, 30.5 ± 3.4 per cent as metabolite B, 2.4 ± 0.2 per cent as metabolite C, less than 0.2 per cent as metabolite D, and 4.8 ± 0.7 per cent as unchanged drug. The kinetics of excretion of the drug and metabolites indicates that absorption and metabolism take place rapidly. In the human, the average (±S.E.M.) half-lives of excretion of drug and metabolites A, B, and C were 3.0 ± 0.2, 6.5 ± 0.6, 6.2 ± 0.7, and 7.7 ± 1.2 hr., respectively. A comparison of urinary excretion after 10-mg./Kg. oral and intravenous doses of the drug to the same dog indicates efficient absorption of the oral dose. In the dog, the half-lives (±S.D.) for urinary excretion of the drug and metabolites A, B, and C were 5.5 ± 0.8, 6.6 ± 0.5, 4.3 ± 0.5, and 7.2 ± 0.6 hr., respectively. Biliary excretion of the drug and/or metabolites or the presence of undetected metabolites may be indicated from the low recovery after intravenous administration.

THIS STUDY was undertaken to determine the absorption, half-life, and metabolic fate of methyl *N*-(*o*-aminophenyl)-*N*-(3-dimethylaminopropyl) anthranilate (I), an orally active diuretic agent in the rat and dog (1). This report describes the quantitative determination of the drug and its metabolites in dog and human urine samples. Qualitative aspects of the metabolism of this drug in the dog and human have already been reported (2).

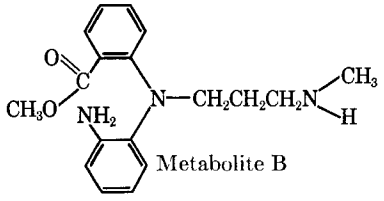


I

Thin-layer chromatography of chloroform extracts of dog and human urine revealed a zone corresponding to unchanged drug and four other drug-related zones (designated metabolites A, B, C, and D). Thin-layer chromatography in conjunction with the bromcresol purple procedure of Woods *et al.* (3) was used to determine individually the drug and three of its metabolites. Two of these metabolites (A and C) were synthesized to yield samples for analytical calibration purposes. Since standards of metabolites B and D were not available for calibration purposes, the calibration curves for the drug and metabolites A and C were averaged and used for these metabolites.



Metabolite A



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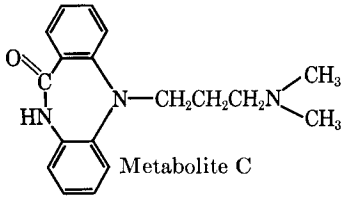
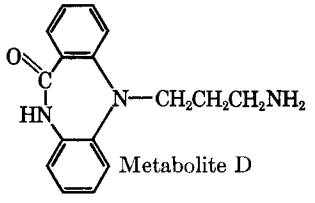


Metabolite C



Metabolite D

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TABLE I—THIN-LAYER CHROMATOGRAPHIC R_f VALUES[a]

Compd.	System A	System B	System C
Drug	0.61	0.96	0.20
Metabolite A	0.24	0.48	0.02
Metabolite B	0.30	0.79	0.04
Metabolite C	0.49	0.88	0.20
Metabolite D	0.24	0.48	0.45

[a] The zones used for analysis are underlined.

EXPERIMENTAL

Materials—Bromcresol purple (5′,5″-dibromo-*o*-cresolsulfonphthalein indicator) was obtained from Eastman Organic Chemicals. The bromcresol purple reagent was prepared by dissolving 50 mg. of the solid in 100 ml. of analytical reagent grade $CHCl_3$. The synthesis of the drug and metabolite C have been described previously (4). Metabolite A was prepared by Dr. R. S. P. Hsi, The Upjohn Co., using the procedures described by Hanze *et al.* (4).

Thin-Layer Chromatography (TLC)—Silica Gel G plates of 250 μ thickness, impregnated with $ZnSiO_3$ and $(ZnCd)_2S$ phosphors, were employed with the following three solvent systems: system *A*, C_6H_6:CH_3OH:NH_4OH (50:50:1); system *B*, EtOAc:DMF:NH_4OH (50:50:5); system *C*, CH_3OH:$(CH_3)_2C{=}O$:C_6H_6:7 *M* NaOH (50:50:10:1). The zones were located with a mercury lamp (254 mμ). System *A* was used for the isolation of I and metabolite C, system *B* for metabolites A and B, and system *C* for metabolite D. The R_f values are presented in Table I.

Procedure for Determination of Drug and Metabolites—To 5.0 ml. of urine or serum in a 35-ml. centrifuge tube was added 3.0 ml. of pH 10 buffer (20.0 Gm./L. Na_2CO_3 + 12.4 Gm./L. $Na_2B_4O_7$), followed by 25.0 ml. of $CHCl_3$. The mixture was shaken vigorously. After 30 min. of standing at room temperature, the phases were separated by centrifugation (2000 r.p.m. for 10 min.) and the aqueous layer removed by aspiration. A 20.0-ml. aliquot of the $CHCl_3$ was transferred to a 50-ml. open-mouth centrifuge tube and evaporated to dryness under a stream of nitrogen. The tube was washed down successively with 5, 3, and 1 ml. of $CHCl_3$, and evaporated to dryness each time. The residue was dissolved in a few drops of $CHCl_3$, and quantitatively transferred to a thin-layer plate. Standards were spotted and the plate was developed with the appropriate system (Table I). The drug and metabolite zones were individually scraped from the chromatogram and transferred to 15-ml. centrifuge tubes. Five milliliters of pH 10 buffer was added to each tube and, after shaking, the mixtures were centrifuged. Four-milliliter aliquots of the supernates were transferred to another set of 15-ml. centrifuge tubes, 5.0 ml. of $CHCl_3$ added, and the mixtures were shaken vigorously and centrifuged at 2000 r.p.m. for 5 min. The aqueous phases were aspirated and 3.0 ml. of the $CHCl_3$ phases transferred to 10-ml. volumetric flasks containing 0.3 ml. of bromcresol purple reagent. The absorbances were measured at 410 mμ with a Cary spectrophotometer, employing $CHCl_3$ in the reference cell. To correct for background, a zero-hour urine sample was subjected to the same procedure. The concentrations of drug and metabolites were calculated from calibration curves having the following slopes (mcg. drug equivalents/ml./absorbance unit): drug, 32.9; metabolite A, 30.7; metabolite C, 28.1. Since samples of metabolites B and D were not available for calibration, an estimated slope of 30.0 was used.

Dog Studies—To test the extent of absorption of the drug, dog *1* (10.0-Kg. female) was given a 105-mg.[1] oral dose on day 1, no drug on days 2–7, and a 105-mg. intravenous dose on day 8. To test the effect of pretreatment, dog *2* (10.5-Kg. male) was given a 50-mg. oral dose on both days 1 and 2 and dog *3* (9.5-Kg. male) was given a 50-mg. oral dose on day 1, and a 250-mg. oral dose on day 2. In the case of dog *1*, a single 0–24-hr. urine sample was collected and 10 ml. of blood was withdrawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hr. after each dose. In the case of dogs *2* and *3*, urine was collected quantitatively during the intervals 0–1, 1–3, 3–5, 5–8, 8–12, 2–16, 16–24, 24–25, 25–27, 27–32, 32–36, 36–40, 40–49, 49–56, 56–74, and 74–80 hr. after the first dose of drug.

Human Studies—A single dose of 350 mg.[1] of the drug was administered to each of five male volunteer subjects after an overnight fast. Food was allowed 1 hr. after drug administration. Water intake was limited to 8 fl. oz. at the following times relative to the time of dosing: −1, 0, 1.5, 4, 6.5, 10, 14, 23, 24, 25.5, 28, and 30.5 hr. Urine was collected quantitatively during the intervals 0–1, 1–3, 3–5, 5–8, 8–12, 12–16, and 16–32 hr. after drug administration. Blood (10–12 ml.) was withdrawn from each subject at 0, 0.5, 1.5, 4, 6.5, 10, 14, and 24 hr.

RESULTS AND DISCUSSION

Identification of Metabolites—The isolation procedure developed for the drug employed chloroform extraction of pH 10 serum or urine followed by thin-layer chromatography (TLC). When this method was subsequently applied to the determination of the drug in dog and human urine samples, TLC revealed not only a zone corresponding to drug but also at least two other drug-related zones. Two additional TLC solvent systems (Table I) revealed that four metabolites were actually present. The metabolites were isolated from the urine and identified (2) as metabolites A, B, C, and D.

Both the dog and human convert the drug to these metabolites, but, as described below, the relative amounts and rates of excretion of the metabolites differed in the two species.

Quantitation of Drug and Metabolites—The drug and metabolites could be quantitatively extracted from pH 10 serum or urine with 5 vol. of chloroform. The diazotized drug did not react with *N*-1-naphthylethylenediamine, and since its spectral properties were not suitable for direct spectrophotometric determinations at microgram levels in serum or urine, the bromcresol purple procedure of Woods *et al.* (3) was investigated. Both the drug and its metabolites responded in this sensitive procedure. Thin-layer chromatography

[1] In all cases the drug was administered as the dihydrochloride.

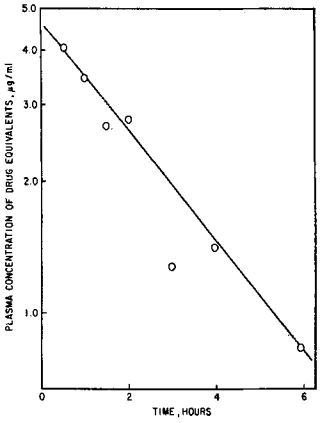


Fig. 1—Plasma levels of drug equivalents after a 10-mg./Kg. intravenous dose to dog 1.

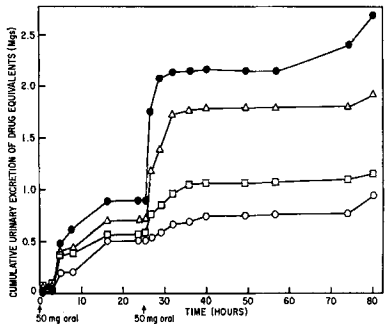


Fig. 2—Cumulative excretion of drug and metabolites after two 50-mg. oral doses to dog 2. *Key:* ●, *drug;* ○, *metabolite A;* △, *metabolite B;* □, *metabolite C.*

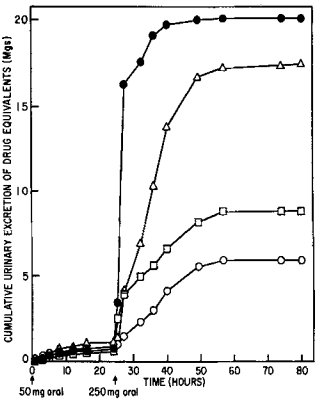


Fig. 3—Cumulative excretion of drug and metabolites after 50- and 250-mg. oral doses to dog 3. *Key the same as Fig. 2.*

was added to the procedure, since the bromcresol purple procedure lacks specificity. The final procedure, therefore, included the following steps. The serum or urine was adjusted to pH 10 and extracted with chloroform. A measured volume of the chloroform extract was evaporated to a smaller volume and quantitatively transferred to a silica gel thin-layer plate. The compounds were eluted from the silica gel support with pH 10 buffer, which was then extracted with chloroform to yield solutions for colorimetric measurement. Three thin-layer systems were required to resolve completely the drug and its four metabolites.

Serum Levels—*Dog Studies*—At a dose of 10 mg./Kg. to the dog, the serum levels after both oral and intravenous administration approached the lower limits of the analytical method; consequently, the half-life for the disappearance of drug-related material from the serum can only be estimated. Because of the extremely low serum concentrations, no attempt was made to quantitate the drug and metabolites individually. After the intravenous dose, the drug-related material disappears from the serum with a half-life of about 3 hr. (Fig. 1). Although the serum concentrations after oral administration were of the same order of magnitude as those after intravenous administration, the points were too scattered to yield a half-life estimate.

Human Studies—The concentrations of the drug and metabolites in the human serum samples were below detectable limits (< 1 mcg./ml.). Since an intravenous dose of 10 mg./Kg. of the drug to the dog had given only barely detectable serum levels, the observation of low serum levels in man was not surprising, and certainly did not prove poor absorption.

Urinary Excretion of Drug and Metabolites—*Dog Studies*—The distribution of drug and metabolites A, B, and C and the per cent of the dose recovered in the urine of each dog are presented in Table II. Since metabolite D was present only in trace amounts, no attempt was made to quantitate it.

The cumulative urinary excretion of drug and metabolites in the dogs which received the drug on 2 successive days is presented in Figs. 2 and 3.

TABLE II—URINARY EXCRETION OF DRUG AND METABOLITES IN THE DOG

Treatment Day	Dog	Rt.	Dose, mg.	% of Dose Excreted: Metabolite A[a]	Metabolite B	Metabolite C	Drug	Total
1	*1*	Oral	105	2.51	5.50	2.94	4.51	15.46
1[b]	*1*	i.v.	105	3.67	6.62	3.51	5.53	19.33
1	*2*	Oral	50	1.02	1.40	1.10	1.76	5.28
2[c]	*2*	Oral	50	0.86	2.42	1.18	3.58	8.04
1	*3*	Oral	50	1.72	2.18	1.10	1.60	6.60
2[c]	*3*	Oral	250	2.05	6.56	3.32	8.16	20.09

[a] Includes a small amount of metabolite D (<10% of the values given). [b] Received the 105-mg. oral dose 1 week prior to the i.v. dose. [c] Received the 50-mg. oral dose on the previous day.

TABLE III—CUMULATIVE EXCRETION OF DRUG AND METABOLITES IN THE HUMAN (DOSE = 350 mg.)

Subject	Time, hr.	Drug Equivalents Excreted, mg. Metab. A[a]	Metab. B	Metab. C	Drug	Total
1	1	1.5	6.7	0.6	2.1	10.9
	3	11.0	29.9	2.8	9.5	53.2
	5	17.9	48.0	4.3	13.2	83.4
	8	26.1	71.5	5.7	15.4	118.7
	12	34.1	103.7	7.4	17.3	162.6
	16	35.1	107.9	7.7	17.4	168.1
	32	42.3	129.3	9.8	18.5	199.9
2	1	0.2	1.6	0.1	0.6	2.5
	3	3.0	17.6	1.7	9.9	32.2
	5	5.3	27.7	2.9	14.7	50.5
	8	8.0	41.3	3.9	19.4	72.6
	12	12.0	54.9	5.5	22.1	94.5
	16	14.1	60.4	6.2	22.7	103.4
	32	18.9	76.7	8.3	23.8	127.7
3	1	1.0	7.2	1.0	2.5	11.7
	3	4.5	27.3	3.0	7.3	42.1
	5	9.2	41.7	4.5	10.5	65.9
	8	12.2	58.9	6.3	13.1	90.6
	12	17.1	72.8	8.1	14.3	112.3
	16	18.0	76.1	9.2	14.8	118.0
	32	21.8	85.6	11.1	15.4	133.9
4	1	0.4	2.0	0.1	0.5	2.9
	3	0.4	2.0	0.1	0.5	2.9
	5	7.7	32.7	1.4	6.1	47.9
	8	21.1	50.8	2.2	7.3	81.4
	12	27.2	74.4	3.1	8.2	112.9
	16	30.4	96.1	3.8	8.6	138.9
	32	42.4	138.3	6.6	9.4	196.8
5	1	0.5	4.1	0.3	1.1	6.0
	3	3.9	27.9	1.5	7.8	41.1
	5	7.8	41.9	2.5	11.3	63.4
	8	14.3	66.2	3.6	14.4	98.5
	12	18.8	85.3	4.8	15.4	124.3
	16	21.5	93.4	5.4	15.7	135.9
	32	25.3	103.6	6.6	16.0	151.6

[a] Includes a small amount of metabolite D (<2% of the values given).

The urinary excretion of the first dose seems to be essentially complete prior to the administration of the second one. In the dog which received the two 50-mg. doses, the urinary excretion of drug-related material was greater after the second dose than after the first. Also, in the dog which received the 50-mg. dose on the first day and the 250-mg. dose on the second day, the urinary excretion of drug and metabolites after the second dose—both individually and collectively—was greater than five times the excretion after the first dose. Therefore, in both dogs that received doses on successive days, the percentage of the first dose excreted in the urine was smaller than that of the second. These data illustrate the importance of crossover experiments when comparing different formulations of the same drug.

The intact drug accounts for a greater percentage of the total recovered in the urine on the second day of treatment than on the first, while metabolite A accounts for a much smaller percentage of the total on the second day. These data indicate either a decrease in metabolism or a change in excretion pathways with chronic administration, *e.g.*, the ratio of biliary to urinary excretion of metabolite A may vary with chronic administration in a different manner from that of the intact drug.

The distribution of drug and metabolites in the urine was essentially identical after oral and intravenous administration. An estimate of the oral efficiency can be obtained by comparing the total urinary excretion of drug and metabolites after

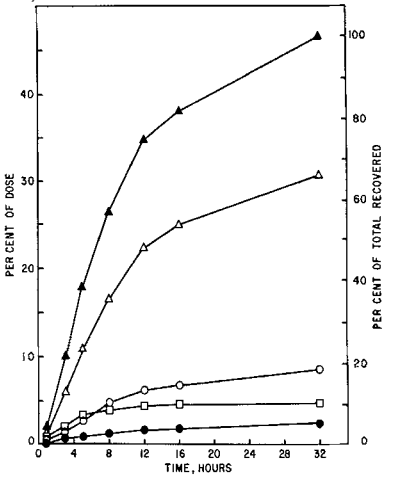


Fig. 4—Average cumulative excretion of drug and metabolites in five humans. Key: ▲, total excretion; ○, metabolite A; △, metabolite B; ●, metabolite C; □, drug.

oral and intravenous administration of the same dose. When the oral and intravenous doses were given on successive weeks to the same dog, the 24-hr. urinary excretion after the oral dose (first week) was 80% of the 24-hr. excretion after the intravenous dose. Assuming the first dose had no effect on the amount excreted after the second dose, the oral efficiency in this dog was about 80%. Actually, it is impossible to determine from these data whether the oral dose had any effect on the amount excreted in the urine after the intravenous dose 1 week later. However, since pretreatment tends to increase the percentage excreted in the urine, the 80% value is very likely a minimum estimate.

Not only does the percentage of the dose excreted in the urine increase with repeated administration, it also increases with increasing dose. The dogs given 50-mg. oral dose excreted an average of 6% of the dose in the urine, while the dog given the 105-mg. oral dose excreted 15.5%. These data support hypotheses such as (*a*) sufficiently high concentrations of the drug or its metabolites may inhibit nonurinary (*e.g.*, biliary) excretion, (*b*) saturation of binding sites of drug and metabolites, or (*c*) stimulation of urinary transport mechanisms.

The low recovery of the drug-related material in the urine after intravenous administration probably results from extensive biliary excretion. Other possibilities, considered to be less likely, include urinary excretion of undetected metabolites or binding of drug and/or metabolites by body constituents.

Human Studies—The cumulative excretion data for the drug and metabolites A, B, and C for each of the five subjects are presented in Table III. The average cumulative excretion is plotted in Fig. 4. Since the cumulative levels of metabolites do not appear to have reached their asymptotic values, excretion may not be complete in 32 hr. The average cumulative urinary excretion of intact drug and metabolites at each time interval, expressed as per cent of dose, is presented in Table IV. The distribution of drug and metabolites in the 0–32 hr. urine of each subject is presented in Table V.

Of the 350-mg. dose, at least 162.0 ± 15.3 mg. (S.E.M.), *i.e.*, 46.3 ± 4.4% (range: 36.5–57.1%), was absorbed. Expressed as drug equivalents, an average (±S.E.M.) of 30.2 ± 5.1 mg. was excreted as metabolite A, 106.7 ± 11.9 mg. as metabolite B, 8.5 ± 0.9 mg. as metabolite C, and 16.7 ± 2.3 mg. as unchanged drug. The percentage of drug-related compounds in each urine collection present as unchanged drug decreases from about 20% in the 0–1-hr. sample to about 3% in the 16–32-hr. sample, whereas beyond 1 hr., the percentage of the total as each metabolite increases only slightly.

Rate of Excretion of Drug and Metabolites—*Dog Studies*—The cumulative excretion data were used to estimate the over-all half-lives for the appearance in the urine of the drug and its metabolites. The half-lives were estimated by plotting the data in first-order fashion (Fig. 5). In the case

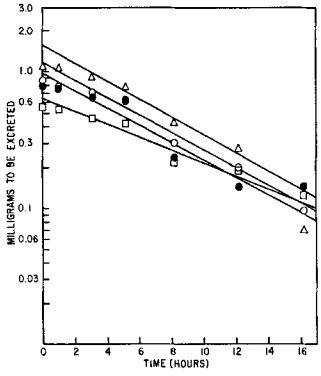


Fig. 5—First-order plots of drug and metabolite excretion after a 50-mg. oral dose to dog 3. Key the same as Fig. 2.

TABLE IV—AVERAGE CUMULATIVE URINARY EXCRETION OF DRUG AND ITS METABOLITE IN FIVE HUMANS

Time, hr.	% of Dose				
	Metabolite A	Metabolite B	Metabolite C	Drug	Total
1	0.20	1.23	0.12	0.39	1.94
3	1.31	5.97	0.52	2.00	9.80
5	2.74	10.97	0.89	3.19	17.79
8	4.67	16.51	1.23	3.98	26.39
12	6.24	22.35	1.65	4.42	34.66
16	6.80	24.79	1.85	4.52	37.96
32	8.62	30.48	2.42	4.75	46.27

TABLE V—DISTRIBUTION OF DRUG AND METABOLITES IN THE 0–32 hr. URINE OF EACH HUMAN (DOSE = 350 mg.)

Subject	mg. Drug Equivalents					% of Dose
	Metabolite A	Metabolite B	Metabolic C	Drug	Total	
1	42.3	129.3	9.8	18.5	199.9	57.1
2	19.0	76.7	8.3	23.9	127.9	36.5
3	21.8	85.6	11.1	15.5	134.0	38.3
4	42.4	138.2	6.6	9.4	196.6	56.2
5	25.3	103.6	6.6	16.1	151.6	43.3
Mean	30.2	106.7	8.5	16.7	162.0	46.3
S.E.M.	±5.1	±11.9	±0.9	±2.3	±15.3	±4.4

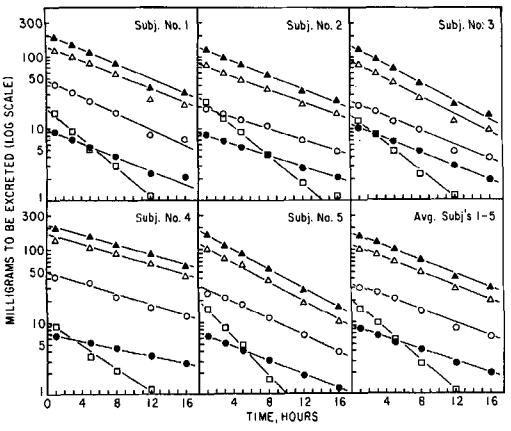


Fig. 6—First-order plots of drug and metabolite excretion after a 350-mg. oral dose of the drug (as the dihydrochloride) to five humans. Key same as Fig. 4.

TABLE VI—EXCRETION HALF-LIVES OF THE DRUG AND ITS METABOLITE IN EACH HUMAN

Subject	Half-Life, hr. Metabolite A	Metabolite B	Metabolite C	Drug
1	5.3	6.2	6.2	3.0
2	7.8	6.8	7.6	2.9
3	6.0	4.9	6.1	3.1
4	7.9	8.6	12.3	3.8
5	5.3	4.6	6.3	2.4
Mean	6.5	6.2	7.7	3.0
S.E.M.	0.6	0.7	1.2	0.2

of dog *3*, the half-lives (±S.D.) for the appearance in the urine of drug and metabolites A, B, and C after the first dose were 5.5 ± 0.8, 6.6 ± 0.5, 4.3 ± 0.5, and 7.2 ± 0.6 hr., respectively, and, after the second dose, 3.8 ± 0.4, 4.7 ± 0.5, 5.4 ± 0.6, and 4.6 ± 0.6 hr. The half-life for the appearance of total drug-related material was 5.0 ± 0.4 hr. after the first dose, and 5.3 ± 0.4 hr. after the second.

In the case of dog *2*, the scatter of the data does not justify the calculation of the individual half-lives. The appearance of total drug-related material in the urine after both doses also seemed to be consistent with a half-life of about 5 hr. Unlike that found in the human (*vide infra*), the rate constant for the appearance of drug in the urine is not significantly larger than for the metabolites. Therefore, the dog is probably not a satisfactory species for the study of the unusual excretion kinetics observed in the human.

Metabolite C was also administered to the dog. As expected, metabolites A and D were found in the urine. The average half-lives for the appearance of metabolites A, C, and D in the urine, after a single dose of metabolite C, were 4.1, 3.6, and 6.2 hr., respectively. These half-lives for metabolites A and C do not differ significantly from those obtained when I was administered, which indicates that, at least in the dog, the rate of excretion of these compounds is not limited by their rate of manufacture. Like the case of I, when the same dose of metabolite C was administered on 2 successive days to the same dog, the percentage of the second dose excreted in the urine was much more than twice that of the first dose. This was true even though cumulative urinary excretion plots indicated that excretion of the first dose was essentially complete prior to administration of the second dose.

TABLE VII—AVERAGE[a] FITTING PARAMETERS FOR URINARY EXCRETION OF DRUG AND ITS METABOLITES

Compd.	Method of Fitting[b]	Half-Life ($t_{1/2}$), hr.[c]	Total Excreted at t = ∞ hr. U_∞ (mg.)	Lag Time t_0 (hr.)
A	(*1*)	6.5	30.2	0
	(*2*)	9.1	33.7	0
	(*3*)	7.0	31.8	1.04
B	(*1*)	6.2	106.7	0
	(*2*)	8.2	116.3	0
	(*3*)	6.9	111.7	0.69
C	(*1*)	7.7	8.48	0
	(*2*)	8.7	9.2	0
	(*3*)	8.1	9.0	0.38
Drug	(*1*)	3.0	16.62	0
	(*2*)	3.6	16.9	0
	(*3*)	2.8	16.44	0.52
Total	(*1*)	6.1	162.0	0
	(*2*)	7.6	173.5	0
	(*3*)	6.3	167.2	0.73

[a] Average for five human adult males. [b] Methods: (*1*) fit of $U = U_{32}(1 - e^{-Kt})$; (*2*) fit of $U = U_\infty (1 - e^{-Kt})$; (*3*) fit of $U = U_\infty [1 - e^{-K(t-t_0)}]$ for $t > t_0$ hr. [c] $t_{1/2}$ (hr.) = $0.693/K$.

Human Studies—As a first approximation, in order to estimate the excretion half-lives, the assumption was made that the amount excreted in 32 hr. was equal to the total amount excreted, *i.e.*, $U_{32} = U_\infty$ [method (*1*) of Table VII]. The data for the individual subjects and the average of the five subjects are plotted in first-order fashion in Fig. 6. It is apparent from these plots that first-order excretion kinetics are followed for both intact drug and metabolites. The excretion half-lives over the whole collection period, obtained from the slopes of Fig. 6, are presented in Table VI. The average half-lives (±S.E.M.) for drug and metabolites A, B, and C are 3.0 ± 0.2, 6.5 ± 0.6, 6.2 ± 0.7, and 7.7 ± 1.2 hr., respectively.

The half-lives for the appearance of drug and metabolites in the urine of the humans were also estimated by fitting the data by the method of least squares [methods (*2*) and (*3*) of Table VII] to the following first-order equations:

$$U = U_\infty [1 - e^{-Kt}] \qquad \text{(Eq. 1)}$$

$$U = U_\infty [1 - e^{-K(t-t_0)}] \qquad \text{(Eq. 2)}$$

where U is the milligrams of drug equivalents excreted to time t, U_∞ is the amount excreted at infinite time, K is the observed rate constant for excretion ($t_{1/2} = 0.693/K$), and t_0 in Eq. 2 is a "lag time" which corrects for delays caused by absorption, metabolism, and distribution. Employing these equations, the least squares fit of the data yields estimates of U_∞ as well as the half-life (Table VII). All of the average half-lives obtained from Eq. 2 are within 0.5 hr. of the values obtained from the slopes of the $U_{32} - U$ *versus* t plots of Fig. 6. The average half-lives indicate that the excretion of

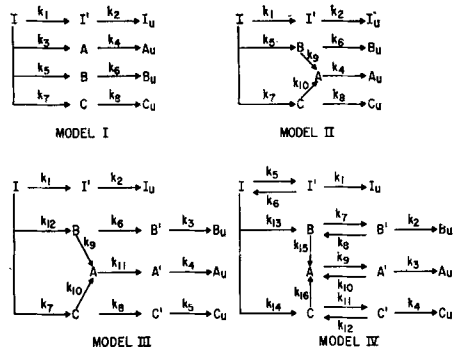


Fig. 7—Schematic models. Drug and metabolites in plasma: I, A, B, C; drug and metabolites in "compartment": I', A', B', C'; drug and metabolites excreted in urine: I_u, A_u, B_u, and C_u.

each of the metabolites is at least 95% complete in 32 hr., thus justifying the original approximation that $U_{32} = U_\infty$.

Rate of Absorption and Metabolism—Since simple first-order excretion appears to be followed for both drug and metabolites (Fig. 6) beginning with the first hour after administration, the drug which is absorbed by the human must be absorbed and metabolized very rapidly. In general, these data indicate that absorption and metabolism are so rapid that excretion of the metabolites is not limited by their rate of formation or by the rate of absorption of the drug.

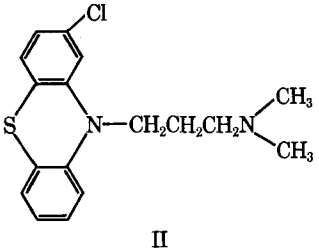
Kinetic Models for Urinary Excretion in the Human—An attempt was made to formulate a mathematical model or models consistent with all the available data. For simplicity, it was assumed that drug absorption had essentially ceased 1 hr. after oral administration and only the data after 1 hr. were considered.

The complexity of kinetic models and equations required to describe the metabolism and excretion of a drug is dependent upon the precision and discrimination of the data. In the present case, measurement by a nonspecific assay of total urinary drug and metabolites yields data which can be approximated by a single exponential function (plus a constant) and which suggest that a single rate constant is involved. However, the added information that three metabolites are formed and excreted at their individual rates requires a more complicated description.

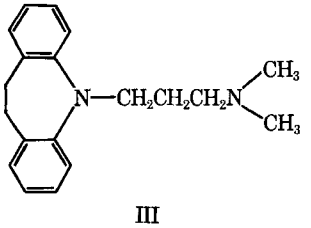
The increasing complexity of mathematical models resulting from increasing discrimination of analytical results is demonstrated by the evolution of models I–IV (Fig. 7). In order to account for the observed urinary excretion, model I postulates that the rate of metabolism of the drug is much faster than the excretion rates of the drug and its metabolites, that each of the metabolites is formed *directly* from the drug (*i.e.*, metabolites B and C are not necessary intermediates in the formation of metabolite A), and that there is a compartment in which the drug is stored and no longer metabolized prior to excretion. Assumption of such a compartment would be consistent with the incomplete metabolism of the drug, even though its excretion rate is postulated to be relatively slow compared to its metabolism.

Once the chemical structures of the metabolites were known, however, model II was constructed to take into account the possibility that metabolites B and C may be chemical intermediates in the formation of metabolite A. In this model, the assumptions are made that the rate of metabolism of the drug is much faster than the excretion rates of the drug or its metabolites (B and C), or the formation rates of the metabolite A, that metabolite A is formed from B and C, and that there is a compartment in which the drug is no longer metabolized prior to excretion.

Since the drug and metabolites have similar chemical structures, it is likely that they enter into the same physiological compartments in which solubility probably plays the main selective role. Hence, in addition to the assumptions of model II, model III postulates a compartment in which the drug *and* its metabolites are no longer metabolized prior to excretion. Model IV is similar to model III except that equilibria are postulated. At present, model IV appears to be the most reasonable description of the chemical and physiological data but none of the models have been completely excluded. A mathematical discussion of these models will be the subject of a future communication.



II



III

Metabolism of Structurally Related Drugs—The investigated drug, although noncyclic, has some structural similarity to the phenothiazine drugs *e.g.*, chlorpromazine (II) and imipramine (III). Both of these compounds are mono- and di-demethylated *in vivo* by several species (5).[2] However, both of these drugs are also ring-hydroxylated and then conjugated with glucuronic acid. Even after hydrolysis with β-glucuronidase, no evidence was found in the present study for ring-hydroxylated or conjugated metabolites. Although Fishman *et al.* (6) have reported the isolation of the *N*-oxides of chlorpromazine (0.7% of the dose) and imipramine (~2% of the dose), no evidence was found for *N*-oxide metabolites in the present study.

REFERENCES

(1) Graham, B. E., unpublished data.

[2] *Cf. Reference 5* for a review of phenothiazine metabolism. For imipramine, *cf.* Haflizer, F., and Burckhardt, V., in "Psychopharmacological Agents," vol. I, Gordon, M., ed. Academic Press Inc., New York, N. Y., 1964, chap. 3.

(2) Smith, D. L., and Grostic, M. F., *J. Med. Chem.*, presented to the Medicinal Division, American Chemical Society, September 1966, to be published.
(3) Woods, L. A., Cochin, J., Fornefeld, E. J., McMahon, F. G., and Seevers, M. H., *J. Pharmacol. Exptl. Therap.*, **101**, 188(1951).
(4) Hanze, A. R., Strube, R. E., and Greig, M. E., *J. Med. Chem.*, **6**, 767(1963).
(5) Emmerson, J. L., and Miya, T. S., *J. Pharm. Sci.*, **52**, 411(1963).
(6) Fishman, V., Heaton, A., and Goldenberg, H., *Proc. Soc. Exptl. Biol. Med.*, **109**, 548(1962); Fishman, V., and Goldenberg, H., *ibid.*, **110**, 187(1962).

Applications of Differential Scanning Calorimetry in Pharmaceutical Analysis

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The application of differential scanning calorimetry to several aspects of pharmaceutical analysis is presented. These include the utilization of this technique in the quantitative estimation of purity, in the relative determination of purity for a control procedure, and in the detection of polymorphism. The purities and heats of fusion of diallylbarbituric acid, naphthalene, glutethimide, dibucaine, and anthraquinone are given. A comparative analysis of several samples of methyl reserpate is presented and the detection of polymorphism in tripelennamine citrate is discussed.

THE AVAILABILITY of commercial instruments capable of performing various types of thermal analysis has led to an increasing number of publications of their application to organic compounds (1–4). One area of application which is of particular interest to a pharmaceutical analyst, and one which has not been extensively investigated, is the quantitative estimation of purity through thermal analysis. Phase solubility analysis is presently one of the most widely used techniques for purity determination (5, 6), since the equilibrium solubility of a pure compound is as characteristic a property as is the melting point. This method, however, suffers from certain disadvantages. Perhaps the most important of these is that the time required for equilibration may be several weeks. It was decided therefore to see if differential scanning calorimetry could overcome some of these disadvantages in determining the "absolute" purity of a compound. This determination is based on the other fundamental property of a compound, its melting point. This method would overcome some of the disadvantages of phase solubility in that the analysis could be completed in 1 day and since one determines the sum of the impurities, it would be possible to estimate purity to within $\pm 0.1\%$ when dealing with samples at the 99%+ level, even if errors of the order to 20% were introduced.

Two other areas of application were also investigated—namely, the utilization of this technique in the determination of relative purity and in the detection of polymorphism. It provides a rapid method for control procedures since one can compare the endotherm of the sample to endotherms of known purity without the necessity of a complete analysis. The presence of polymorphism in several samples of tripelennamine citrate is easily detected by comparing thermograms of different samples and this substantiated evidence that was obtained by spectroscopic methods.

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EXPERIMENTAL

A Perkin-Elmer model DSC-1B differential scanning calorimeter was used in this study (7). Aluminum sample pans and pan lids which fit the DSC-1B were used for all samples except the anthraquinone. Anthraquinone samples were sealed in a volatile sample sealer. Care was taken in sample handling and in covering the sample holders with the covers in the same relative position in order to minimize base line drift, which, as pointed out by Rogers and Morris (8), can be caused by differences and variations in thermal emissivity. All samples were run in a nitrogen atmosphere. The materials used were of the quality specified in the text.

A semimicro balance with a sensitivity of 0.01 mg. was used to weigh all samples. Areas were generally determined by planimeter, although in some cases areas were determined by cutting and weighing. In either case, the reproducibility of an area determination was to within 3%.

THEORY

Calorimetric methods for the determination of purity are well known (9, 10); hence, only a brief review of the theory will be given. The equation for the lowering of the freezing point in dilute solution is that developed by Van't Hoff:

$$\frac{dT}{dX_2} = \frac{RT^2}{\Delta H_f}(k/k' - 1) \qquad \text{(Eq. 1)}$$