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RESEARCH ARTICLES

Pharmacokinetics of the β-Adrenergic Blocker Sotalol in Dogs

KURT SCHNELLE and EDWARD R. GARRETT▲

Abstract □ The time course of absorption, distribution, and excretion of the β-blocker sotalol was studied in three unanesthetized dogs at three dosage levels of 1, 2, and 4 mg./kg. i.v. and 2, 4, and 8 mg./kg. p.o. The drug was assayed in body fluids and excreta by a spectrophotofluorometric method and by counting of tritiated drug. Unchanged sotalol is excreted up to 90% (±12%) in the urine. There was no protein binding, and the partition coefficient between plasma and red blood cells was unity. The data obtained in the studies were fitted graphically and by analog computer techniques and demonstrated no dose dependence. The graphical fit of the plasma levels following intravenous administration in accordance with a two-compartment open body model revealed a rapid distribution phase with a $t_{1/2}$ of 3.2 ± 1.1 min., which was followed by a disposition phase with a $t_{1/2}$ of 4.8 ± 1.03 hr. The analog computer fittings of the plasma and urine data according to the two-compartment model gave constants similar to those obtained from the graphical fits. The addition of a third tissue compartment that was in slow equilibrium with the central compartment proved necessary for a better fit of the data obtained at the high dosage level and in the ^{3}H-sotalol assays. The 75–90% absorption of sotalol in solution following oral administration was rapid ($t_{1/2}$ = 11–17 min.).

Keyphrases □ Sotalol pharmacokinetics—absorption, distribution, and excretion after intravenous and oral administration to dogs, protein binding and plasma/red blood cell partition studies □ Pharmacokinetics, sotalol—absorption, distribution, and excretion following intravenous and oral administration, dogs □ Absorption, sotalol—following intravenous and oral administration, dogs

Sotalol hydrochloride[1], 4′-[1-hydroxy-2-(isopropylamino)ethyl]methanesulfonanilide monohydrochloride, is a specific and potent β-adrenergic blocking drug. Several studies in both animals and man have demonstrated that the compound reverses isoproterenol- and epinephrine-induced tachycardia (1–3) and reverses the increase in blood levels of free fatty acids, lactic acid, and glucose that follows isoproterenol and epinephrine infusion (4, 5). Since the drug possesses less "quinidine-like" activity, it has been suggested that sotalol therapy may be preferred over other β-blockers with pronounced negative inotropic side effects (6).

It has been indicated that the plasma levels of tritium-labeled drug are related to the drug's pharmacological

[1] MJ 1999.

Table I—Rate Constants[a] (in min.$^{-1}$) and Apparent Half-Lives ($t_{1/2}$) after Intravenous and Oral Administration of Sotalol in Dogs

Dog Number	Weight, kg.	Dose, mg./kg.	α	$10^2\beta$	10^2k_a	$10^2k_{P,T}$	$10^2k_{T,P}$	$10^2k_{P,U}$	$10^2k_{P,T'}$	$10^2k_{T',P}$	$t_{1/2}$[b], hr.
1[c]	18.1	2 (i.v.)	0.273	2.50	—	22.1	3.26	2.08	—	—	4.62
						(14.9)	(2.26)	(2.46)			(3.57)
	16.8	1 (i.v.)	0.196	2.40	—	14.3	1.26	3.70	—	—	4.81
						(9.0)	(1.08)	(2.85)			(3.79)
	17.2	4 (i.v.)	0.416	2.60	—	35.1	2.76	3.91	—	—	4.44
						(23.8)	(2.72)	(2.79)	(6.98)	(2.30)	(5.29)
	20.4	8 (p.o.)		1.80	1.49	—	—	—	—	—	6.58
					(6.33)	(23.7)	(2.71)	(2.47)	(6.99)	(2.30)	(5.97)
	18.6	4 (p.o.)		2.10	6.2	—	—	—	—	—	5.48
					(4.16)	(23.7)	(2.71)	(2.52)	(6.99)	(2.30)	(5.84)
	20.2	2 (p.o.)		1.70	—	—	—	—	—	—	6.8
					(6.50)	(23.7)	(2.71)	(2.27)			(4.96)
2[d]	18.1	1 (i.v.)	0.266	3.20	—	21.0	2.45	3.47	—	—	3.57
	20.0	2 (i.v.)	0.174	1.93	—	14.0	1.65	2.05	—	—	5.99
	19.1	4 (i.v.)	0.125	2.03	—	9.45	1.33	1.90	—	—	5.69
						(12.1)	(1.50)	(1.61)	(7.32)	(5.93)	(7.41)
	21.8	2[e] (i.v.)	0.245	2.32	—	20.0	2.16	2.62	—	—	4.99
						(16.5)	(2.07)	(2.50)	(7.59)	(2.65)	(5.48)
	21.8	2[e] (i.v.)	0.213	1.91	—	17.4	1.65	2.46	—	—	6.05
						(16.5)	(2.07)	(2.50)	(7.54)	(2.65)	(5.48)
	18.6	1[f] (i.v.)	0.301	1.72	—	25.6	3.04	1.71	—	—	6.72
						(17.9)	(2.45)	(2.11)	(5.69)	(2.15)	(6.00)
	18.6	1[f] (i.v.)	0.259	2.86	—	20.4	3.88	1.91	—	—	4.04
						(17.9)	(2.45)	(2.11)	(5.69)	(2.15)	(6.00)
3[g]	19.1	4 (i.v.)	0.235	2.86	—	17.2	1.27	5.29	—	—	4.04
						(20.2)	(1.78)	(4.82)	(8.27)	(3.44)	(3.54)
	18.6	2 (i.v.)	0.145	2.90	—	10.2	3.28	1.28	—	—	3.98
						(15.5)	(2.13)	(2.71)			(3.53)
	18.6	1 (i.v.)	0.203	3.29	—	15.3	2.03	3.83	—	—	3.52
						(15.5)	(2.19)	(2.52)			(3.71)

[a] The values for a cited experiment were obtained from graphical analysis of the data; the parenthetical values were obtained from analog computer fitting of the data. [b] Values obtained from the graphically determined slope, $\beta = k_e$ of ln(plasma levels) against time. The parenthetical values were calculated in accordance with $t_{1/2} = \ln 2/k_e$ where (12):

$$k_e = \frac{k_{P,U}}{1 + \frac{k_{P,T}}{k_{T,P}} + \frac{k_{P,T'}}{k_{T',P}}}$$

and $k_{P,T'}$ and $k_{T',P}$ values were included when significant and needed for a good fit of the data with the analog computer. [c] The studies were conducted in the tabular sequence at 0, 1, 8, 9.5, 11, and 12.5 months from the initial experiment. [d] The studies were conducted in the tabular sequence at 0, 3.5, 6, 13, and 14 months from the initial experiment. [e] The sotalol concentrations in blood and urine were analyzed by spectrophotofluorometric and radioactive assays simultaneously in the same experiment. The upper listing is based on the radioactive assay. All other studies were conducted by spectrophotofluorometric assays. [f] The sotalol concentrations in blood and urine were analyzed by the spectrophotofluorometric and radioactive assays simultaneously in the same experiment. The upper listing is based on the radioactive assay. In this experiment, bile samples were obtained on a biliary-cannulated dog. All other studies were conducted by spectrophotofluorometric assays. [g] The studies were conducted in the tabular sequence at 0, 1.5, and 2.5 months from the initial experiment.

activity in dogs (2). No significant metabolism occurred in dogs where 90% of the unmetabolized drug was excreted in the urine (2). Therefore, knowledge of the pharmacokinetic profile of sotalol should allow the design of an optimum dosage regimen from the plasma levels. The availability of a reasonably sensitive cold assay for the drug (7) is convenient for such studies. This paper describes the pharmacokinetics in the dog at multiple dosage levels of sotalol administered intravenously and orally.

EXPERIMENTAL

Treatment of Animals—The dogs were conditioned to the metabolic cages and trained for laboratory routine prior to experimentation. They were fasted for 24 hr. before each experiment. On the day of the experiment, the animals were placed on a table in a standing position and restrained by two straps which were tied around the fore and hind legs and then fixed to a horizontal bar above the dog. A jugular vein was punctured with a 14-gauge needle, and a vinyl tubing [0.11-cm. (0.044-in.) i.d., 0.16-cm. (0.065-in.) o.d.] was inserted through the needle. After free blood flow through the tubing was established, the needle was removed and 0.9% saline solution was dripped continuously at a rate of 1 ml./min. This infusion was maintained throughout 24 hr. to compensate for blood loss and to establish an adequate urine flow.

The urinary bladder was catheterized routinely through the urethra by inserting a ureteral catheter[2], French size 6 or 8. This catheter was kept in place for 24 hr. for continuous urine collection.

In one dog, Dog 2, the common bile duct was cannulated 1 week prior to an experiment. A laparotomy was performed under pentobarbital anesthesia (30 mg./kg.). After the cystic duct was tied, two 3.8-cm. (1.5-in.) pieces of polyethylene tubing[3] were inserted into the proximal and distal portions of the common bile duct. The two pieces were then connected by a flexible silicone rubber tubing [0.15-cm. (0.059-in.) i.d., 0.35-cm. (0.138-in.) o.d.] of 1.3-m. length to shunt bile. After the abdomen was closed, the loop was pulled under the skin by a wire to an incision on the back of the neck. There the tubing was cut and reconnected through a 14-gauge metal adapter which could be separated at will. Free passage through the tubing was assured by daily flushing of the distal part leading to the duodenum.

Pharmacokinetic Studies—Three male mongrel dogs, approximately 2 years of age and in apparent good health, were used in the balanced study. Hematocrit, white blood cell count, and erythrocyte sedimentation rate were within normal ranges (8). No dirofilarial larvae in the blood were observed microscopically. Body weights and the sequence of the experiments are listed in Table I.

Intravenous Studies—Sotalol[4] was administered intravenously to each of three dogs at three dosage levels: 1, 2, and 4 mg./kg. The

[2] Bard Inc., Murray Hill, N. J.
[3] Intramedic PE 240.
[4] Mead Johnson & Co., Evansville, Ind.

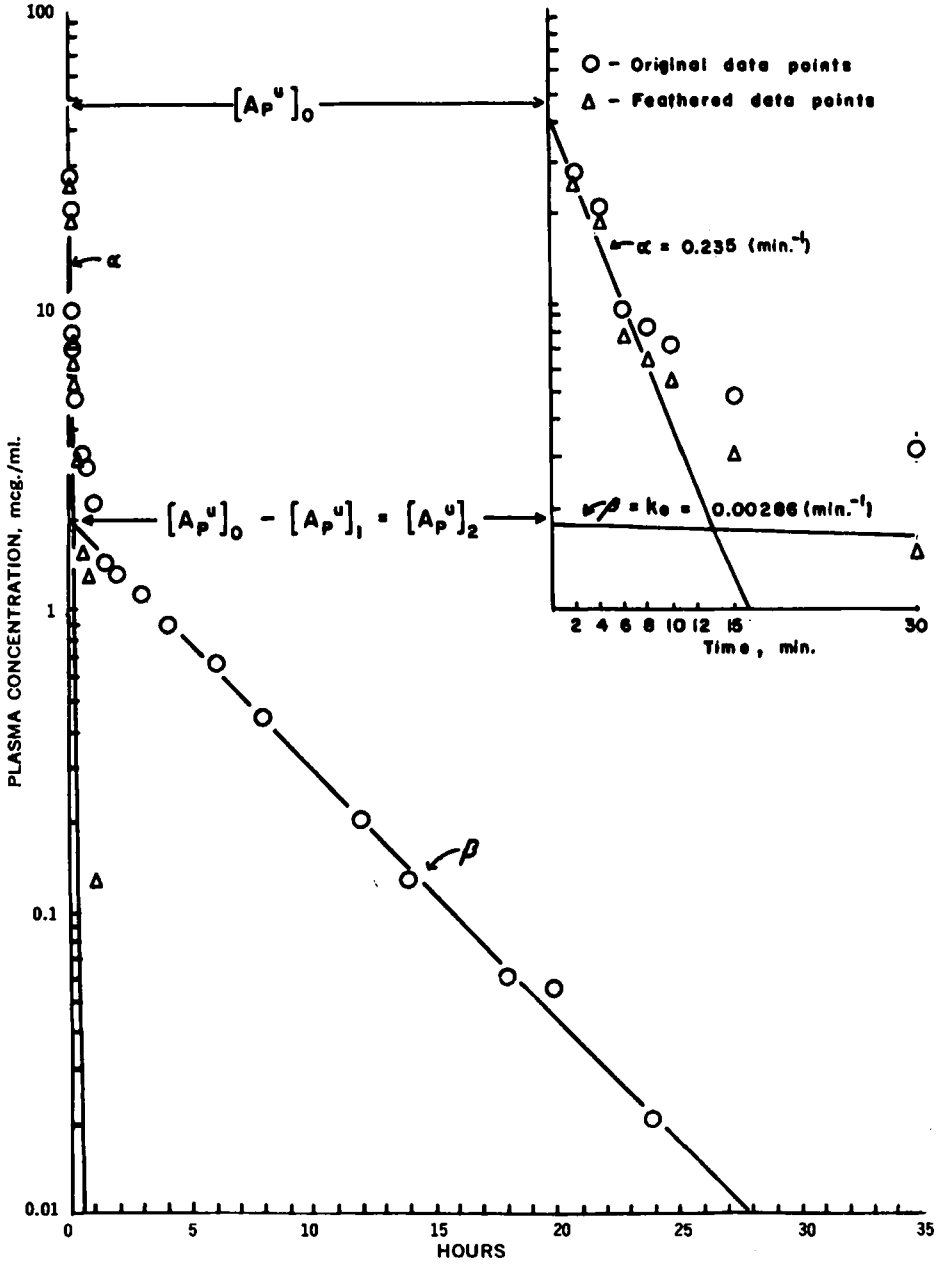


Figure 1—*Semilogarithmic plot of plasma levels following intravenous administration of 4 mg./kg. sotalol to 19.1-kg. Dog 3. The antilogarithmic values of the linear terminal portion with slope β were subtracted from the initial data points, and these feathered points were fitted to a straight line of slope α. $[A_P^u]_0$ is the intercept of the extrapolated line with slope α, i.e., the initial drug concentration in the central compartment, and $[A_P^u]_2$ at zero time is the drug concentration in the equilibrated tissues from the extrapolation of the linear terminal portion of the curve with slope β = k_e in accordance with a two-compartment open body model (Scheme I). The inset is for an expanded time scale. Key: O, original data points; and Δ, feathered values.*

time intervals between experiments in one animal were at least 4 weeks. The drug was injected as its hydrochloride salt in sterile solution containing 10 mg./ml. of the drug through a threeway stopcock into the jugular tubing. The duration of the injection was kept constant at 10 sec. The catheter was flushed with isotonic saline solution after the injection. A distinct reaction due to the rapid administration was only observed after the 4-mg./kg. dose as tachycardia, dyspnea, and increased salivation. Blood samples were withdrawn from the jugular vein at 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, and 30 min. after completion of the injection; then in 15-min. intervals up to 120 min.; in 30-min. intervals up to 4 hr.; hourly up to 12 hr.; and every 2 hr. up to 24 hr. Urine was collected every 30 min. up to 4 hr., hourly up to 12 hr., and every 2 hr. up to 24 hr. The dogs were then transferred to a metabolic cage, and blood and urine were collected at 6-hr. intervals up to 48 hr. and every 12 hr. up to 72 hr. Completeness of urine collection was assured by catheterization. The blood volume withdrawn for each sample was 7 ml. from 0 to 12 hr. and 16 ml. thereafter.

Intravenous Studies with ^{3}H-Sotalol—The tritiated sotalol[4] was prepared as described elsewhere (2). The sterile solution contained 0.5 mg./ml. of the drug with a specific activity of 70 μc./ml. Sterile solutions of cold drug (10 mg./ml.) and labeled material were mixed in a sterile beaker to obtain a drug concentration that was adequate to yield dosages of 1 and 2 mg./kg. of total drug administered to Dog 2. The total activity administered was 1046 μc. at the 2-mg./kg. dose and 1123 μc. at the 1-mg./kg. dose. The schedule for blood and urine sampling was the same as described previously for up to 72 hr. Additional samples were collected every 24 hr. up to 144 hr. Over the same period of time, the feces were collected every 24 hr. in both experiments. Bile samples were obtained in one experiment on Dog 2 (1 mg./kg.) at 2.5, 5, 8, 10, 15, 20, 23, and 30 hr. after injection.

Oral Studies—The same sotalol solution (10 mg./ml.) used in the intravenous study was administered through stomach tubing in doses of 8, 4, and 2 mg./kg. to Dog 1. Blood samples were withdrawn at 2.5, 5, 7.5, 10, and 15 min. after drug administration and at the schedule given previously in the intravenous studies up to 4 hr., every 2 hr. up to 24 hr., and then again as described for the intravenous study. Urine was collected every 30 min. up to 4 hr. and thereafter at the same schedule given for blood samples.

Treatment of Samples—Blood was withdrawn with a disposable syringe and transferred to centrifuge tubes containing 30 USP units of sodium heparin[5] in a volume of 0.1 ml. distilled water to prevent clotting. Blood and urine samples were centrifuged at 3000 r.p.m.

[5] The Upjohn Co., Kalamazoo, Mich.

Table II—Values for First-Order Elimination Rate Constants, k_e (in min.$^{-1}$) as Obtained from Urinary Excretion Data; Estimates of Apparent Volumes of Distribution in Liters Determined by Different Methods for Intravenous and Oral Studies

Dog Number	Dose, mg./kg.	$10^3 k_e$ ln $(\Delta U/\Delta t)$ *versus* Time	$10^3 k_e$ ln $(U_\infty - U)$ *versus* Time	Renal Clearance, ml./min.	Recovery in Urine, % of Dose	V_{P_e}[a]	$V_{P_{true}}$[b]	$V_P{}^u$[c]	V_T[d]	$V_{T'}$[e]	$V_{tot} = (V_P{}^u + V_T + V_{T'})$	$V_{DP}{}^u$[f] from Clearance	$V_{DP}{}^u$[g] from Extrapolated β-Phase
1	2 i.v.	1.70	2.60	50.0	116	2.63	1.04	1.91	12.6	—	14.5	20.0	26.8
	1 i.v.[h]	—	—	—	—	2.53	1.01	1.85	15.4	—	17.2	—	29.9
	4 i.v.	2.04	1.83	45.6	87	2.58	1.03	1.89	16.5	4.3	22.7	17.5	33.8
	8 p.o.	2.31	2.47	56.7	78	—	1.22	1.67	14.6	5.0	21.3	32.2	39.7
	4 p.o.	2.28	2.43	80.0	102	—	1.12	1.97	17.3	5.9	25.2	38.1	34.4
	2 p.o.	1.72	1.63	51.3	88	—	1.21	1.79	15.6	—	17.4	32.0	40.7
2	1 i.v.[i]	2.31	2.98	37.5	108	2.36	1.04	1.77	15.2	—	17.0	11.7	26.1
	2 i.v.[i]	2.42	2.22	67.5	98	3.99	1.20	3.34	28.3	—	31.6	34.9	42.7
	4 i.v.	1.86	1.36	62.0	76	4.48	1.15	3.86	31.1	4.8	39.8	30.5	46.4
	2 i.v.[i]	1.54	1.62	75.0	94	3.29	1.31	2.58	20.6	7.4	30.6	32.4	46.4
	2 i.v.[i]	1.59	1.56	54.4	72	4.41	1.31	3.70	29.5	10.5	43.2	28.5	47.9
	1 i.v.[i]	1.79	2.00	66.7	91	2.90	1.12	2.30	16.8	6.1	25.2	38.8	40.3
	1 i.v.[i]	2.25	1.99	70.0	79	3.20	1.12	2.60	19.0	6.9	28.5	24.5	39.4
3	4 i.v.	2.72	2.87	103.0	97	1.79	1.15	1.03	11.7	2.5	15.2	36.0	39.6
	2 i.v.	2.58	2.64	93.3	82	4.71	1.12	3.97	28.9	—	32.9	32.2	37.0
	1 i.v.	1.92	2.25	145.0	87	4.62	1.12	3.88	27.5	—	31.4	44.1	37.6

[a] V_{P_e} calculated from the quotient, $A_0/[A_P]_0$, of the dose administered intravenously, A_0, and the initial concentration of total drug in the plasma, $[A_P]_0$, obtained from the intercept of the $[A_P]$ plot of the best computer fits against time with zero time. [b] $V_{P_{true}} = (1 - H)V_{B_{true}}$, where $V_{B_{true}}$ = dog weight in kg. × 100 ml./kg. (17), where the hematocrit, H, is taken as 0.40, 0.35, and 0.40 for Dogs 1, 2, and 3, respectively. [c] Calculated in accordance with $V_P{}^u = V_{P_e}{}^u - HV_{P_{true}}D/(1 - H)$ (Eq. 6). The volume of the central compartment, $V_P{}^u$, in the oral studies was calculated in accordance with Eq. 14 after solving for $V_P{}^u = (\gamma A_0/\text{I.C.}) - V_{RBC}$. When there is no protein binding, as in this case, $V_P{}^u$, the volume of the central compartment referenced to the concentration of total drug in the plasma, is the same as V_P, the volume of the central compartment referenced to the concentration of total drug in the plasma. [d] Calculated in accordance with $V_T = V_P{}^u(k_{P,T}/k_{T,P})$ (Eq. 16); the rate constants were taken as the values obtained from the best analog computer fits. [e] Calculated in accordance with $V_{T'} = V_P{}^u(k_{P,T'}/k_{T',P})$ (Eq. 17). [f] Calculated in accordance with the clearance equation (12), where the renal clearance of sotalol in milliliters per minute was divided by k_e in min.$^{-1}$ to yield $V_{DP}{}^u$ in milliliters. The fraction F of drug renally excreted becomes 1 when, as in this case, virtually all drug is excreted through the kidneys. [g] Calculated in accordance with $V_{DP}{}^u = V_{DP_e}{}^u - HDV_{P_{true}}/(1 - H)$ (Eq. 9), where the $V_{DP_e}{}^u$ was obtained in accordance with $V_{DP_e}{}^u$ = dose/$[A_P{}^u]_0$ (Eq. 7), where $[A_P{}^u]_0$ is the intercept of the extrapolated slope of k_e to zero time. [h] No urine data were obtained in this experiment. [i] The values for V_{P_e}, $V_P{}^u$, and V_T were obtained from values derived from the graphical fitting in accordance with the two-compartment body model (Scheme I). [j] The sotalol concentrations in blood and urine were analyzed by the spectrophotofluorometric and radioactive assays simultaneously in the same experiment. The upper listing is based on the radioactive assay.

for 10 min., and the supernatant plasma and urine were then refrigerated. The samples obtained in the radioactive study were split; one portion was deep frozen and sent away for the counting and chromatographic studies[6], and one portion was kept for the chemical assay.

Assay of Samples—The analyses were based on a modified spectrophotofluorometric method which was reported previously (7). The sensitivity was increased from 0.1 to 0.02 mcg./ml. of the drug by extracting from a larger volume (5 ml.) of deproteinized plasma. The lower limit of detection in the urine was 2.5 mcg./ml. Spiked plasma and urine samples were carried through the analytical procedures and assayed at the same time as the samples of unknown concentration. Radioactivity measurements[6] were conducted with a liquid scintillation spectrometer[7] corrected for background and quenching. Duplicate samples of 0.5 ml. plasma were mixed with 1 ml. of a solubilizer[8] and 10 ml. of a counting solution consisting of 5 g. 2,4-diphenyloxazole[9] and 0.5 g. *p*-bis-(*o*-methylstyrylbenzene)[9]/l. of toluene. Duplicate samples of 0.2 or 0.1 ml. of urine and 0.1 ml. of bile were added to 0.6 ml. of water and 15 ml. of solution of 5 g. 2,4-diphenyloxazole, 0.3 g. *p*-bis-(*o*-methylstyrylbenzene), and 120 g. naphthalene/l. of dioxane. Fecal samples were homogenized with nine volumes of water, and aliquots (100–200 mg.) of the homogenates were mixed with 0.6 ml. water and 15 ml. of the same counting solution used for urine.

The presence of possible metabolites of ^{3}H-sotalol was evaluated by paper chromatography of urine, plasma, and bile samples using the following systems: (*a*) strips of 2.54-cm. (1-in.) Whatman No. 1 paper and a solvent mixture of *n*-butanol–acetic acid–water (30:7.5:12.5), and (*b*) Whatman DE20 paper strips and 0.02 *N* phosphate buffer of pH 7.4 as the developing solvent. In each case, 50 µl. of sample was used. The strips were dried, cut into 1.27-cm. (0.5-in.) segments, and placed in counting vials with 0.6 ml. of water and 15 ml. of the dioxane counting solution already described.

Protein Binding Study—Binding of sotalol to plasma proteins was studied by an ultrafiltration method (9). The cone membranes used in these experiments[10] are retentive for plasma protein and permit only passage of ions and compounds of low molecular weight. Thus, only free diffusable drug was found in the filtrate. Possible binding to the membranes is cancelled out when equally spiked buffer–drug and plasma–drug solutions are carried through the entire procedure.

The binding to plasma protein can be estimated by:

$$100f(\%) = \frac{R_B - R_P}{R_B} \times 100 \qquad \text{(Eq. 1)}$$

where f is the fraction bound to plasma protein, R_B is the fluorescence reading of buffer filtrates, and R_P is the reading of plasma filtrates that were spiked with equal concentrations of drug.

Heparinized dog plasma and isotonic phosphate buffer solutions of pH 7.45 were spiked with sotalol hydrochloride to achieve drug concentrations of 0.3, 0.6, 1.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 1000.0 mcg./ml. Aliquots (6.00 ml.) of these solutions and of plasma and buffer blanks were transferred to cone membranes which were placed in plastic supports on top of 40-ml. centrifuge tubes. The solutions were then centrifuged at 1700 r.p.m. The time needed to obtain approximately half-filtration was 2 min. for the buffer solutions and 25 min. for the plasma solutions. The filtrates were carried through the analytical procedure and assayed spectrophotofluorometrically.

Red Blood Cell Partition—The partition of sotalol into the red blood cells was studied as functions of drug concentration and time. Freshly drawn heparinized whole blood and plasma were transferred into 125-ml. conical flasks which were placed in a slowly shaking water bath at 37.5°. The whole blood was then spiked with sotalol to achieve concentrations of 0.5, 1.0, 10.0, and 100.0 mcg./ml. of the drug. Plasma spiked with 1.0 mcg./ml. and whole blood without drug were processed by the experimental procedure and

[6] Performed by Mead Johnson & Co., Evansville, Ind.
[7] Model 3375, Packard Instrument Co., Palo Alto, Calif.
[8] Bio-Solv BBS-3, Beckman Instruments Inc., Palo Alto, Calif.
[9] Eastman Kodak Co., Rochester, NY 14650

[10] Centriflo CF50 membrane filter cone with CSTL support, Amicon Co., Lexington, Mass.

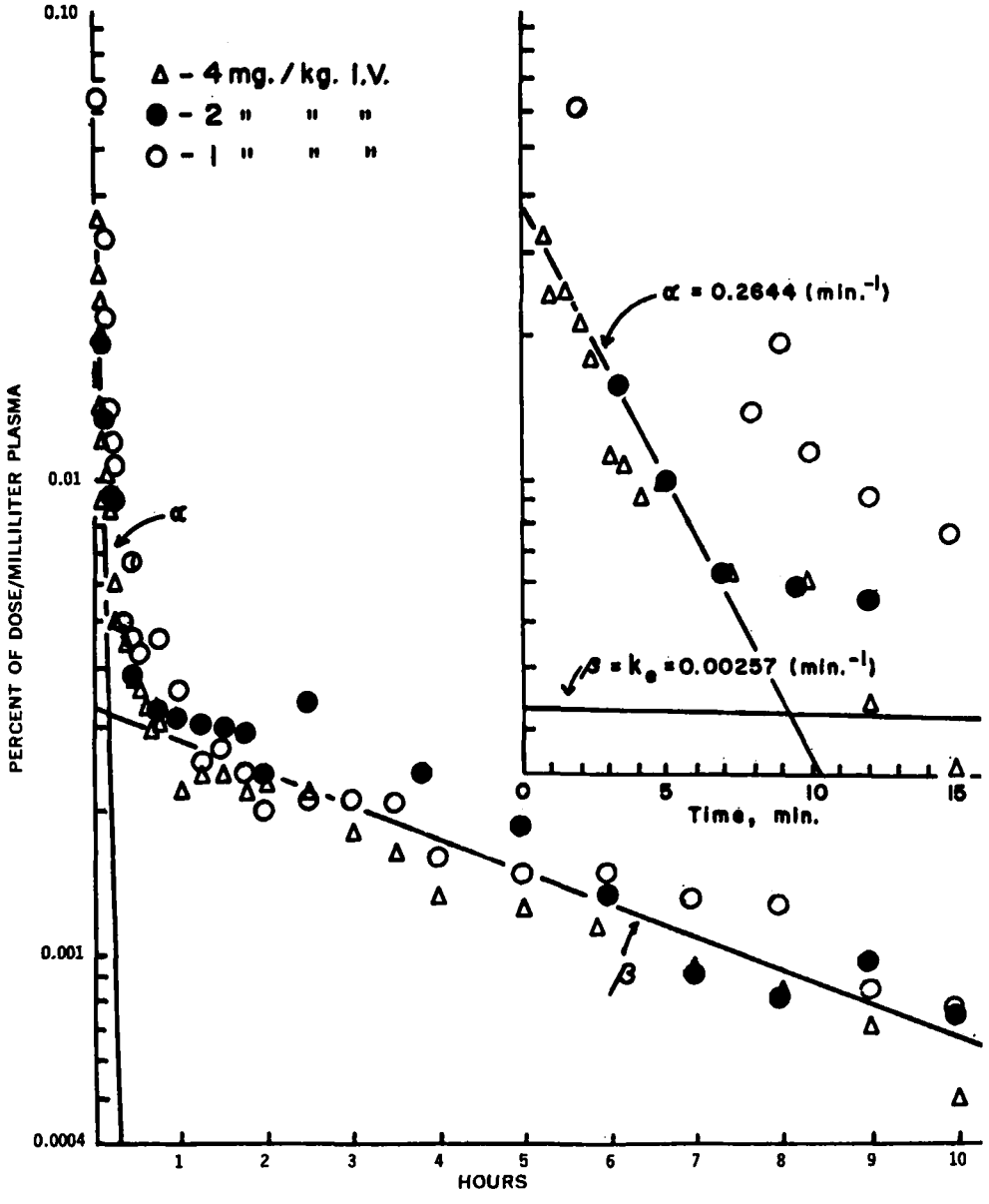


Figure 2—*Semilogarithmic plots of the plasma levels in terms of percent of dose per milliliter plasma against time following intravenous administration of 4 (Δ), 2 (●), and 1 (○) mg./kg. of sotalol to 19-kg. Dog 1. The data points obtained at the three dose levels could be graphically fitted with one common curve in accordance with a two-compartment open body model (Scheme I). The individual values for α and β are given in Table I. The inset is for an expanded time scale.*

the obtained results served as reference standard and blank, respectively. Samples of the blank blood and of the whole blood containing 1.0 mcg./ml. of the drug were withdrawn at 1, 3, 7, 10, 30, 60, and 120 min. after spiking. Samples of the plasma solution of 1.0 mcg./ml. were taken at 1, 30, and 120 min. Samples were withdrawn at 30 min. from all whole blood solutions of the various drug concentrations. Small specimens for the hematocrit determination were taken immediately after sampling, and the blood was centrifuged for 5 min. at 3000 r.p.m. The plasma was stored at 4° until the assay was performed. The fluorescence readings of all samples were compared to a calibration curve in plasma spiked with 0.1, 0.3, 0.6, and 1.0 mcg./ml. sotalol and to the readings of the plasma standard of 1.0 mcg./ml. that was carried through the procedure. The red blood cell/plasma unbound drug partition coefficient could be estimated by (10, 11):

$$D = [A_{RBC}]/[A_P^u] = \{[A_B]/[A_P](1 - f)(1 - H) - [f/(1 - f)] - 1\}(1 - H)/H \quad \text{(Eq. 2)}$$

where $[A_{RBC}]$, $[A_B]$, and $[A_P]$ are the drug concentrations in the red blood cells, whole blood, and plasma, respectively; $[A_P^u]$ is the drug concentration in plasma unbound to proteins; H is the hematocrit; and f is the fraction of drug in plasma bound to plasma proteins. If, as in this specific case, there is no significant protein binding, $f = 0$ and then $[A_P^u] = [A_P]$, which is the total concentration of drug in the plasma, and Eq. 2 reduces to:

$$D = \{[A_B]/[A_P^u](1 - H) - 1\}(1 - H)/H \quad \text{(Eq. 3)}$$

CALCULATIONS AND TREATMENT OF DATA

Plasma—The raw data were plotted in terms of micrograms per milliliter of plasma *versus* time on semilogarithmic paper. In the great majority of cases, the concentrations of unbound drug in the plasma, $[A_P^u]$, on intravenous administration exhibited a biexponential decay of the plasma levels as a function of time (Fig. 1), which can be described as:

$$[A_P^u] = [A_P^u]_1 e^{-\alpha t} + [A_P^u]_2 e^{-\beta t} \quad \text{(Eq. 4)}$$

The value for β was obtained from the slope of the linear terminal portion of the logarithm of the plasma level–time curve where excretion was the rate-determining process. The value for the slope of the rapid distribution phase, α, was estimated by subtracting the corresponding antilogarithmic values of the line of slope β extrapolated to zero time from the antilogarithms of the initial points of the log plasma concentration–time curve (12). Hence, a two-compartment open body model (Scheme I) was assumed, where P_u is amount of drug in the central compartment that is associated with plasma and referenced to the concentration of unbound drug in the

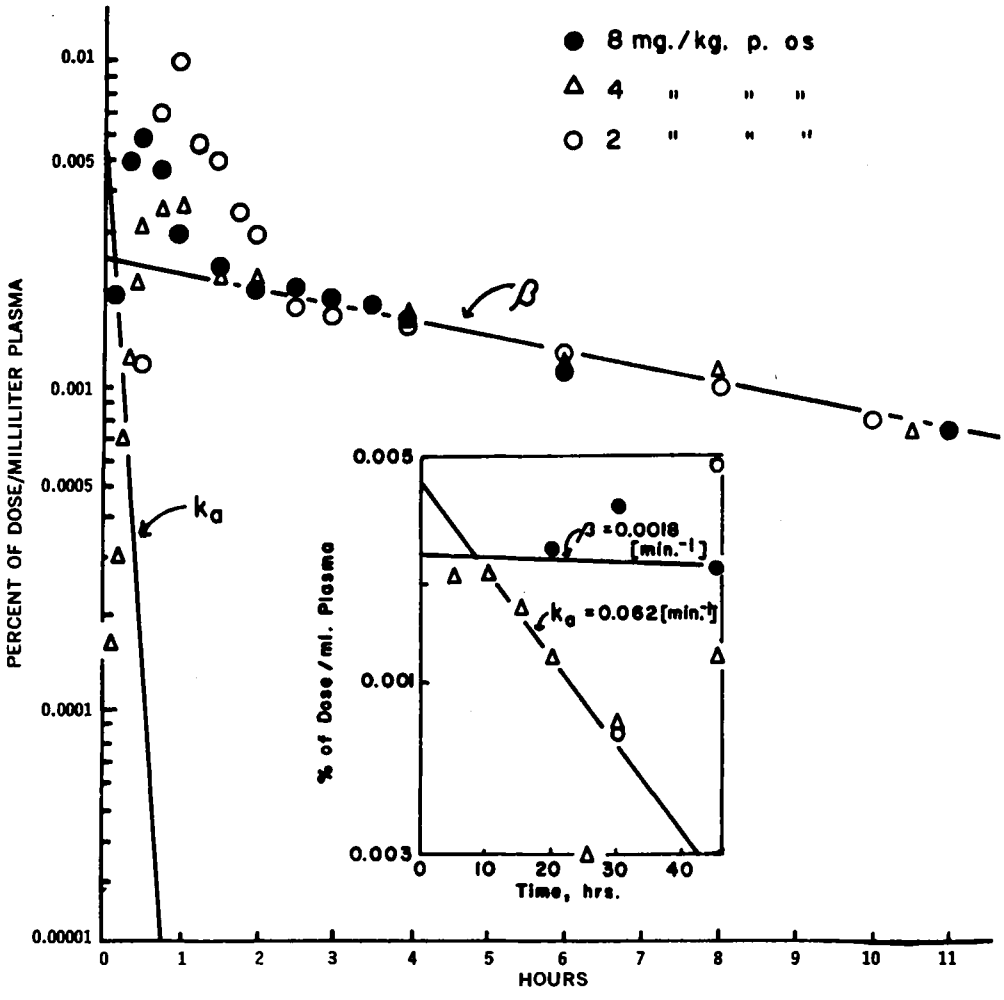


Figure 3—*Semilogarithmic plots of plasma levels in percent of dose per milliliter plasma following oral administration of 8 (●), 4 (△), and 2 (○) mg./kg. of sotalol to 19-kg. Dog 1; the plots show an apparent independence of dose. The extrapolation of the linear terminal portion of the curve cuts off the top of the absorption phase. Therefore, it was not possible to obtain an accurate estimate of k_a by the feathering technique. The individual values for $\beta = k_e$ and the absorption rate constants k_a estimated by graphical and analog computer methods are given in Table I.*

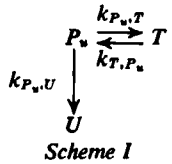
plasma (11, 13), T is the amount of drug in a peripheral tissue that equilibrates readily with P_u, and U is the amount eliminated from the body.

The apparent first-order rates $k_{P_u,T}$, k_{T,P_u}, and $k_{P_u,U}$ were calculated from the α- and β-values obtained (14). Estimates of the half-lives ($t_{1/2}$) of the distribution and disposition–excretion phases were obtained by dividing α and β into ln 2.

The extrapolation of the α-phase to zero time represented the initial concentration, $[A_P{}^u]_0$, of drug in the central compartment that was in the plasma and unbound, where instantaneous mixing of the drug with the plasma and its associated fluids occurred (12). Thus, the apparent volume of distribution of the central compartment, $V_P{}^u$, referenced to the concentration of unbound drug in the plasma would be estimated (11) from:

$$V_P{}^u = \{A_0 - (A_{RBC})_0 - (A_P{}^b)_0\}/[A_P{}^u]_0 = V_{P_s}/(1-f) - HV_{P_{true}}D/(1-H) - V_{P_{true}}f/(1-f) \quad \text{(Eq. 5)}$$

where A_0 is the total amount of drug administered; and $(A_{RBC})_0$ and $(A_P{}^b)_0$ are the initial amounts of drug in the red blood cells and in plasma bound to plasma proteins, respectively. The V_{P_s} is the pseudo-apparent volume of distribution of the central compartment referenced to total drug concentration in plasma, $A_0/[A_P]_0$, where $[A_P]_0$ is the total concentration of drug in the plasma at zero time; D, H, and f are the red blood cell/plasma partition coefficient, the hematocrit, and the fraction of drug in the plasma bound to plasma proteins, respectively. The $V_{P_{true}}$ is the true plasma volume of the animal (11).



Scheme I

If there is no protein binding, $f = 0$ and $(A_P{}^b)_0 = 0$, which is so in this specific case; then $V_P{}^u = V_P$, which is the apparent volume of distribution referenced to total drug concentration in the plasma. Similarly, $V_{P_s}{}^u = V_{P_s}$. Thus, Eq. 5 reduces (11) to:

$$V_P{}^u = V_{P_s}{}^u - HV_{P_{true}}D/(1-H) \quad \text{(Eq. 6)}$$

The sum of the pseudoapparent volumes of distribution of the central compartment and of the readily perfused tissue, $V_{D_{P_s}}{}^u$, referenced to the concentration of unbound drug in the plasma can be estimated (11) from:

$$V_{D_{P_s}}{}^u = V_{P_s}{}^u + V_{T_{P_s}}{}^u = \text{dose}/[A_P{}^u]_2 = A_0/([A_P{}^u]_0 - [A_P{}^u]_1) \quad \text{(Eq. 7)}$$

where the divisor of the last two terms is the concentration of unbound drug in the equilibrated fluids after the rapid equilibration of the α-phase on the assumption of insignificant drug excretion during the time interval of this equilibration.

It has been shown (11) that the sum of the apparent volumes of distribution of the central compartment and of the readily perfused tissues, $V_{D_P}{}^u$, referenced to the total unbound drug concentration in the plasma is actually:

$$V_{D_P}{}^u = V_P{}^u + V_{T_P}{}^u = \frac{\text{dose} - (\text{amounts in red blood cells and bound to plasma protein})}{[A_P{}^u]_2} = V_{D_{P_s}}/(1-f) - HDV_{P_{true}}/(1-H) - V_{P_{true}}f/(1-f) \quad \text{(Eq. 8)}$$

and can be calculated from the equivalent pseudovolume of dis-

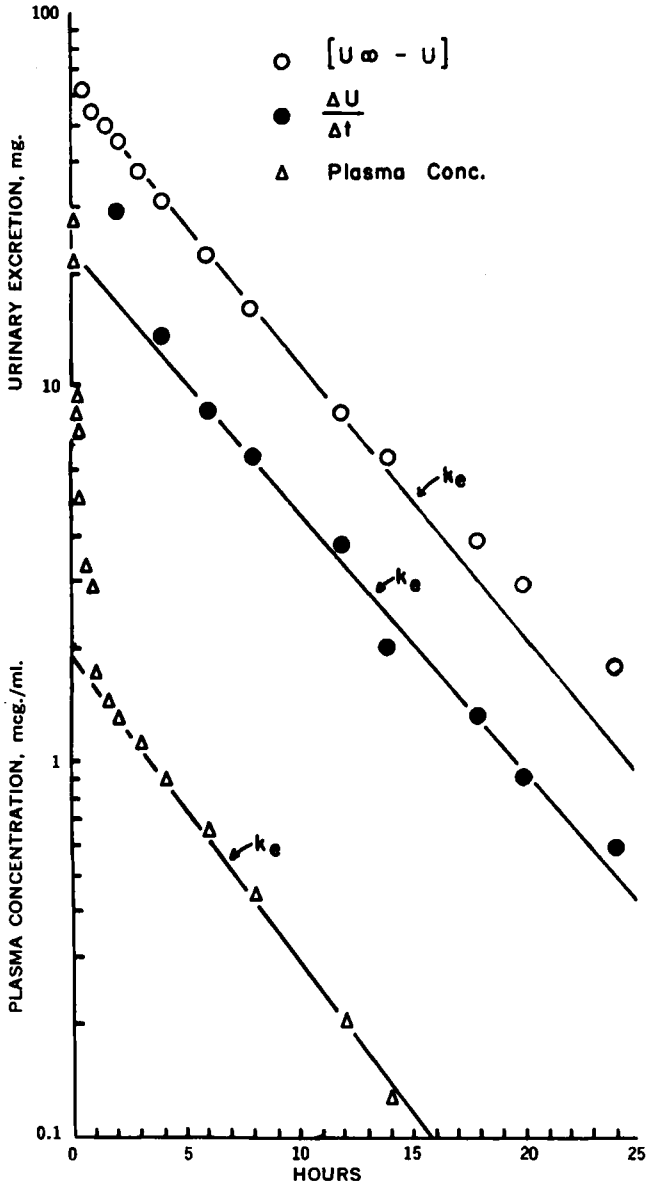


Figure 4—*Semilogarithmic plots of plasma concentrations in micrograms per milliliter (Δ), of the rate of urinary excretion per 2 hr., ΔU/Δt, in milligrams (●), and of the differences between the amount of drug recovered in the urine, U_∞, and the amounts excreted at time t, U, in milligrams (○). Data were obtained following the intravenous administration of 4 mg./kg. sotalol to 19.1-kg. Dog 3.*

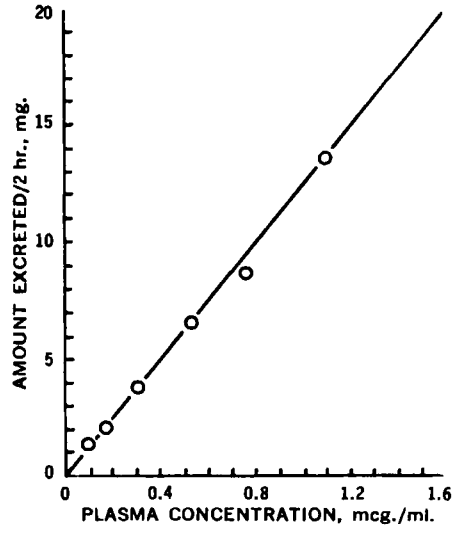


Figure 5—*Linear plot of the rate of urinary excretion per 2 hr., ΔU/Δt, against the plasma concentrations at midpoint of the periods of urine collection. The resulting points were fitted to a straight line through the origin whose slope is the renal clearance in accordance with* $\Delta U/\Delta t = k_{P,U}A_P^u = k_e F V_{D_P}^u [A_P^u]$ *(Eq. 11), where* k_e *is the overall elimination constant,* F *is the drug concentration in the plasma cleared by the kidneys,* $V_{D_P}^u$ *is the apparent volume of distribution, and* $[A_P^u]$ *is the drug concentration at the midpoint of urine collection. Data were obtained following the intravenous administration of 4 mg./kg. sotalol to 19.1-kg. Dog 3.*

tribution (Eq. 7) estimated from the linearly extrapolated intercept, $[A_P^u]_2$, of the β-phase if negligible excretion is assumed in the α-phase.

If $f = 0$, as in this case, the last term of Eq. 8 vanishes and:

$$V_{D_P} = V_{D_P}^u = V_{D_{P_2}}^u - HDV_{P_{true}}/(1 - H) \qquad \text{(Eq. 9)}$$

These estimates of $[A_P^u]_2$ and thus $V_{D_{P_2}}^u$ were obtained from plots such as the typical one given in Fig. 1. The rate constants and the other parameters obtained by graphical procedures are summarized in Tables I and II.

The plasma levels were plotted as percent of dose administered per milliliter of plasma against time for the several doses in the same dog. If those peaks were superimposable, the possibility of dose-dependent pharmacokinetics could be rejected. Such a typical set of plots is given for the intravenous studies in Fig. 2 and for the oral studies in Fig. 3. The β-values, $V_{D_P}^u$, and $t_{1/2}$, in the oral studies were obtained from the linear terminal portion of the semilogarithmic plasma concentration–time plots. A preliminary estimate of the absorption rate constant, k_a, was obtained from feathering the plasma level–time data (15) in accordance with:

$$\ln [A_P^u] = -k_a t + \ln [A_P^u]_0' \qquad \text{(Eq. 10)}$$

where $[A_P^u]_0'$ is an initial estimate of the concentration of the drug in the rapidly equilibrated tissues. It is realized that the validity of this method of k_a and $[A_P^u]_0'$ estimation is based on the premise of negligible excretion during the absorption phase. Since the line extrapolated from the semilogarithmic plot of the terminal data that represented the disposition phase with the slope of β was considerably lower than the actual plasma level data at its maximum values in all three oral experiments (Fig. 3), this feathering procedure could not yield the real k_a. This phenomenon is a consequence of relatively rapid absorption where equilibration of drug among the available tissues is not readily effected during the absorption phase.

Urine—The concentrations determined in the urine were multiplied by the volumes of urine excreted, and these amounts were plotted cumulatively against time to obtain the total recovery of drug, U_∞. Estimates of the overall elimination rate constant, k_e (*i.e.*, β), were also obtained (12) from semilogarithmic plots of the amounts not yet excreted in the urine, $\ln (U_\infty - U)$, *versus* time and the rate of excretion, $\ln \Delta U/\Delta t$, *versus* time (Fig. 4 and Table II). The renal clearance of sotalol was determined by a plot of the rate of excretion, $\Delta U/\Delta t$, against the plasma concentrations taken at the midtime of the interval of the urine collections, where ΔU is the amount of drug excreted in the urine in the time interval, Δt (Fig. 5 and Table II). The slope of this plot, in accordance (12) with:

$$\Delta U/\Delta t = k_{P,U}A_P^u = k_e F V_{D_P}^u [A_P^u] \qquad \text{(Eq. 11)}$$

is the renal clearance, where k_e is the disposition constant β, F is the fraction of the drug in the body that is cleared by the kidneys, $V_{D_P}^u$ is the apparent volume of distribution of the equilibrated tissue referenced to the concentration of unbound drug in the plasma, and $[A_P^u]$ is the concentration of drug in the plasma at the midtime of the interval of urine collection. Since the k_e values are known from other treatments of the data (Figs. 1–4) and F is the ratio of drug renally excreted to the dose administered, this slope also permits the determination of the apparent volume of distribution of the readily equilibrated tissues, $V_{D_P}^u$, referenced to the plasma concentration of unbound drug (Table II).

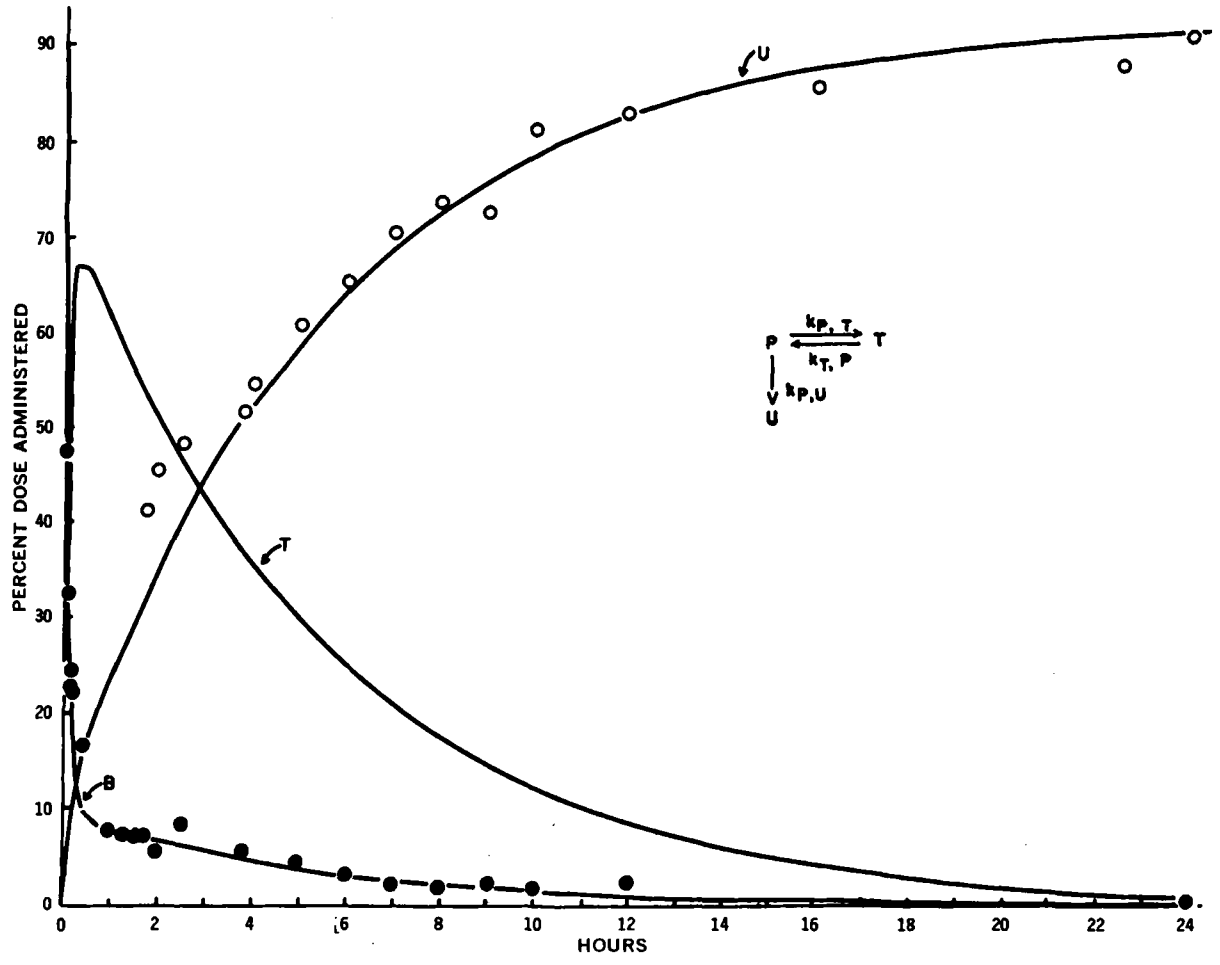


Figure 6—*Analog computer fitting of plasma* (●) *and urine* (○) *data in accordance with a two-compartment body model (Scheme I) following the intravenous administration of 2 mg./kg. to 18.1-kg. Dog. 1.* T *is the generated curve for the tissue compartment.*

Analog Computer Fitting: Programming—The program used in the analog computer[11] fitting of the pharmacokinetic data for a three-compartment body model was given previously (16). The initial conditions that represented the administered dose were fed into the central compartment *P* (plasma) for the data of the intravenous studies and into the GI compartment (gastrointestinal tract) for the data of the oral studies. The time scaling was set with a "ramp function" in such a manner that 0.5 in. on the graph paper equaled 1 hr. of the total 30 hr. of data and consumed 4 sec. of machine time. The recorder speed for the 3- and 6-hr. fits was increased by a factor of 10 and 5, respectively, through different settings of the *x*-axis range switch on the *x*–*y* recorder[12]. The range of the *y*-axis was increased 10-fold when the later portion of the plasma curves was fitted.

Intravenous Administration—The initial drug concentration in the central compartment was obtained from the best analog computer fit of the linear plot of the plasma concentrations against time in accordance with the two-compartment body model (Scheme I) and was considered as 100% of the dose in the plasma and associated fluids. Instantaneous mixing of the drug in the blood was assumed. The other data were represented as fractions of this 100%. The cumulative urinary excretion was plotted in terms of percent of dose administered. The urinary data were plotted over 30 hr.; the plasma data were plotted on a 30-hr. and expanded 6- and 3-hr. time scales and over 30 hr. on a 10 times enlarged ordinate covering 0–10% of dose administered. In this manner, an excellent fitting of the early as well as the later phases of the plasma level–time curves was possible.

As a first approach, the two-compartment body model (Scheme I) was assumed and the rate constants obtained from the graphical fit were used as first estimates for the potentiometer settings. A good fit was obtained with this model for the lower dosages of 1 and 2 mg./kg., and the curves for the latter are shown in Fig. 6. At the high dosage level of 4 mg./kg. and in the radioactive studies where the assay sensitivity was greater, positive deviations of the urinary excretion and terminal plasma data from the best fit with time were observed. A typical fit of such data by the two-compartment body model is given in Fig. 7.

The plateau observed in the terminal plasma data could be assigned to the return of the drug from a deeper compartment. Therefore, to obtain a better fit in these instances, a tissue compartment *T′* with comparatively slow equilibration rate constants, $k_{P,T'}$ and $k_{T',P}$, was added, and the same data were fitted according to the resultant three-compartment body model (Fig. 8). The three-compartment body model fitted the data following administration of lower doses equally well (Fig. 9). The rate constants obtained from the best fits of plasma and urine data following intravenous administration are summarized in Table I.

Oral Administration—The plasma levels after oral administration were plotted in micrograms per milliliter as a function of time. The initial conditions (I.C.) given to the analog computer were placed in the GI compartment. They were estimated on the presumption that they were equal to the initial concentrations, $[A_P^u]_0$, that would appear in the plasma and be unbound to plasma proteins if the fraction γA_0 of the dose were absorbed instantaneously. Such initial conditions, I.C., can be calculated from:

$$\text{I.C.} = [A_P^u]_0 = \frac{\gamma A_0 - (V_{RBC}D + V_{P_{true}})[f/(1-f)][A_P^u]_0}{V_{P^u}} \quad \text{(Eq. 12)}$$

since $[A_{RBC}]_0 = D[A_P^u]_0$ from Eq. 2, and $[A_P^b]_0 = [f/(1-f)]\,[A_P^u]_0$ (11). When there is no protein binding and $D = 1$, as in this case,

[11] Model TR 48, Electronics Associates, Inc., West Long Branch, N. J.

[12] Model 12D2, Hewlett-Packard/Moseley Division, Pasadena, Calif.

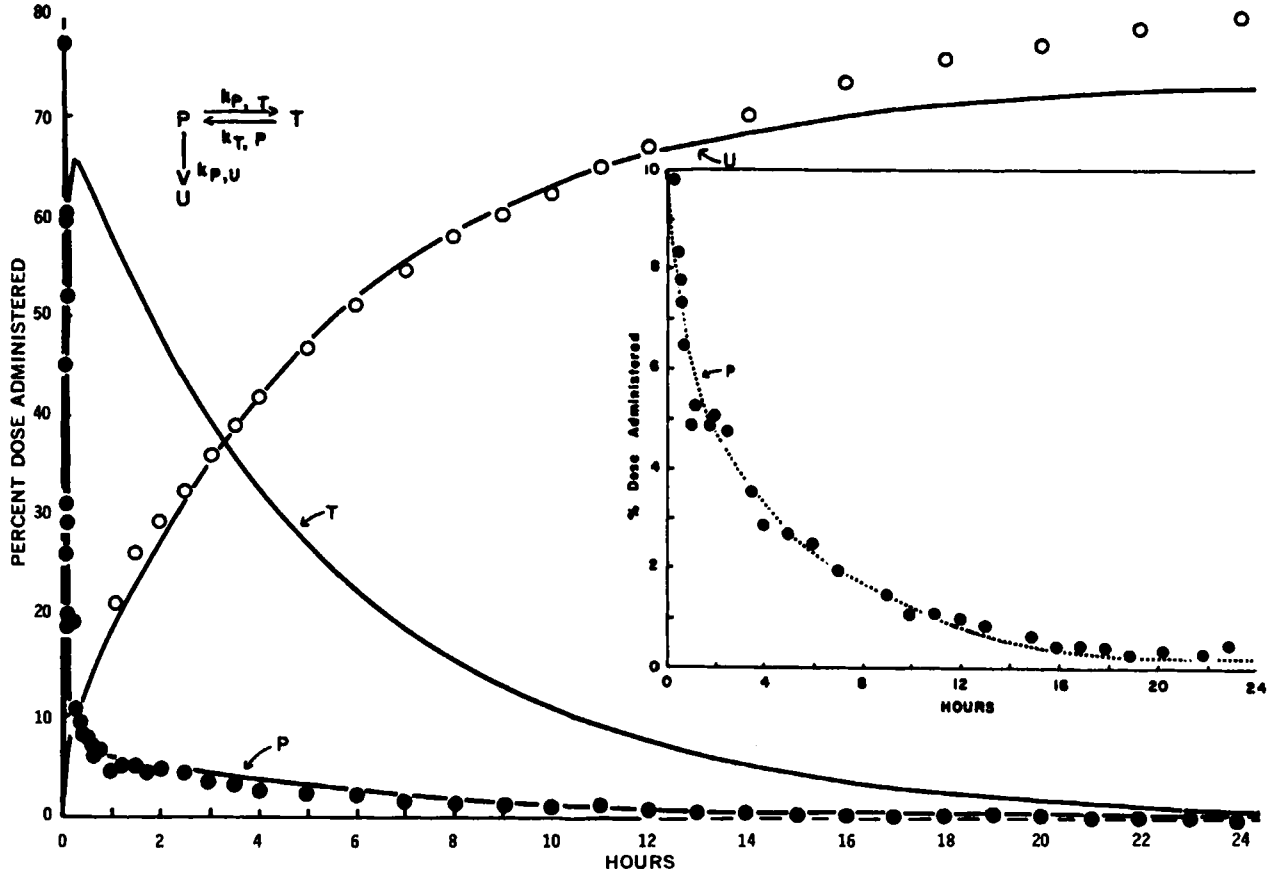


Figure 7—*Analog computer fitting of plasma (●) and urine (○) data following the intravenous administration of 4 mg./kg. sotalol to 17.2-kg. Dog 1 in accordance with a two-compartment body model. The computer curve for urine does not fit the data points in the later time period, indicating a "dribbling out" of drug from a deeper compartment. The inset is for an expanded ordinate scale.*

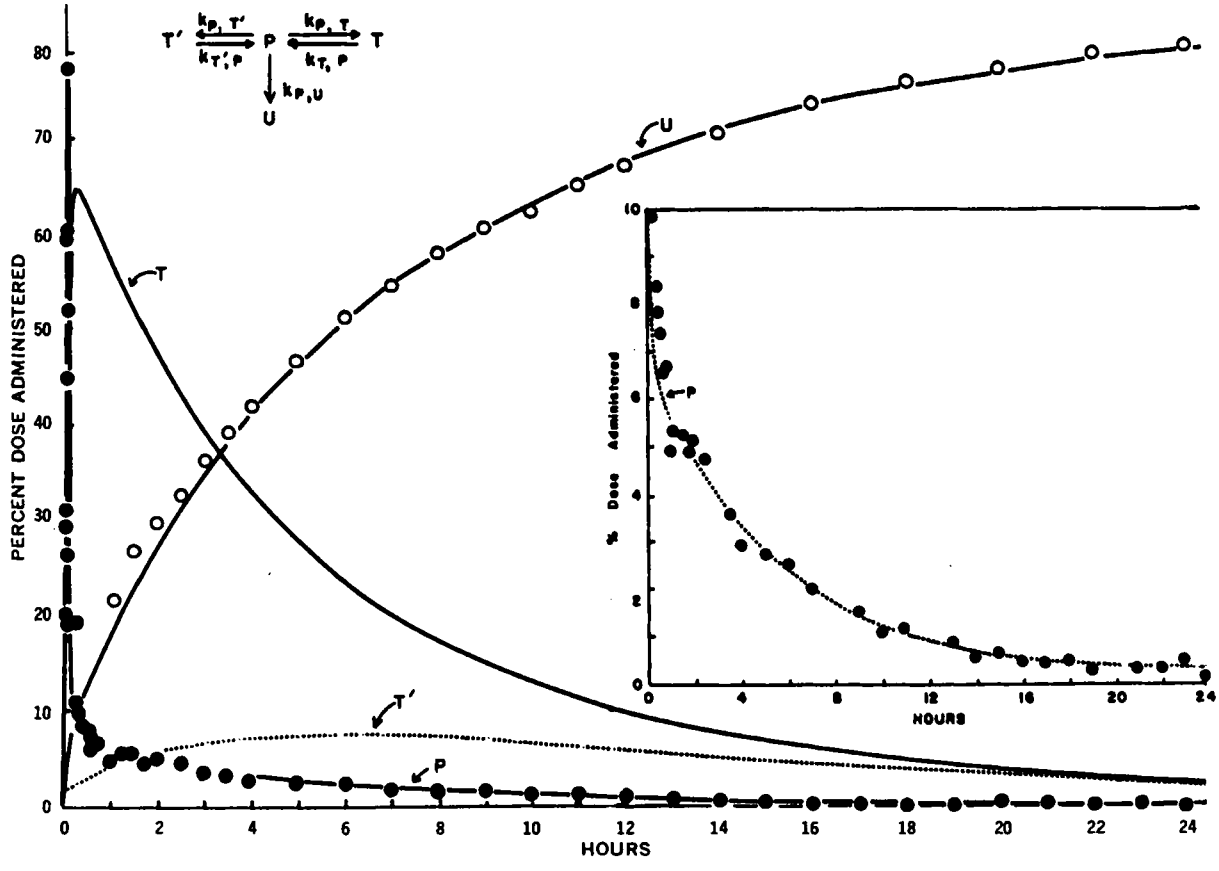


Figure 8—*Analog computer fitting of the same plasma (●) and urine (○) data as in Fig. 7 in accordance with a three-compartment body model. The addition of the tissue compartment, T', represented by the dashed line, that is in slow equilibrium with the central compartment, P, allows good fitting of the terminal portion of the cumulative urinary excretion and the 10-fold enhanced terminal plasma curve (inset).*

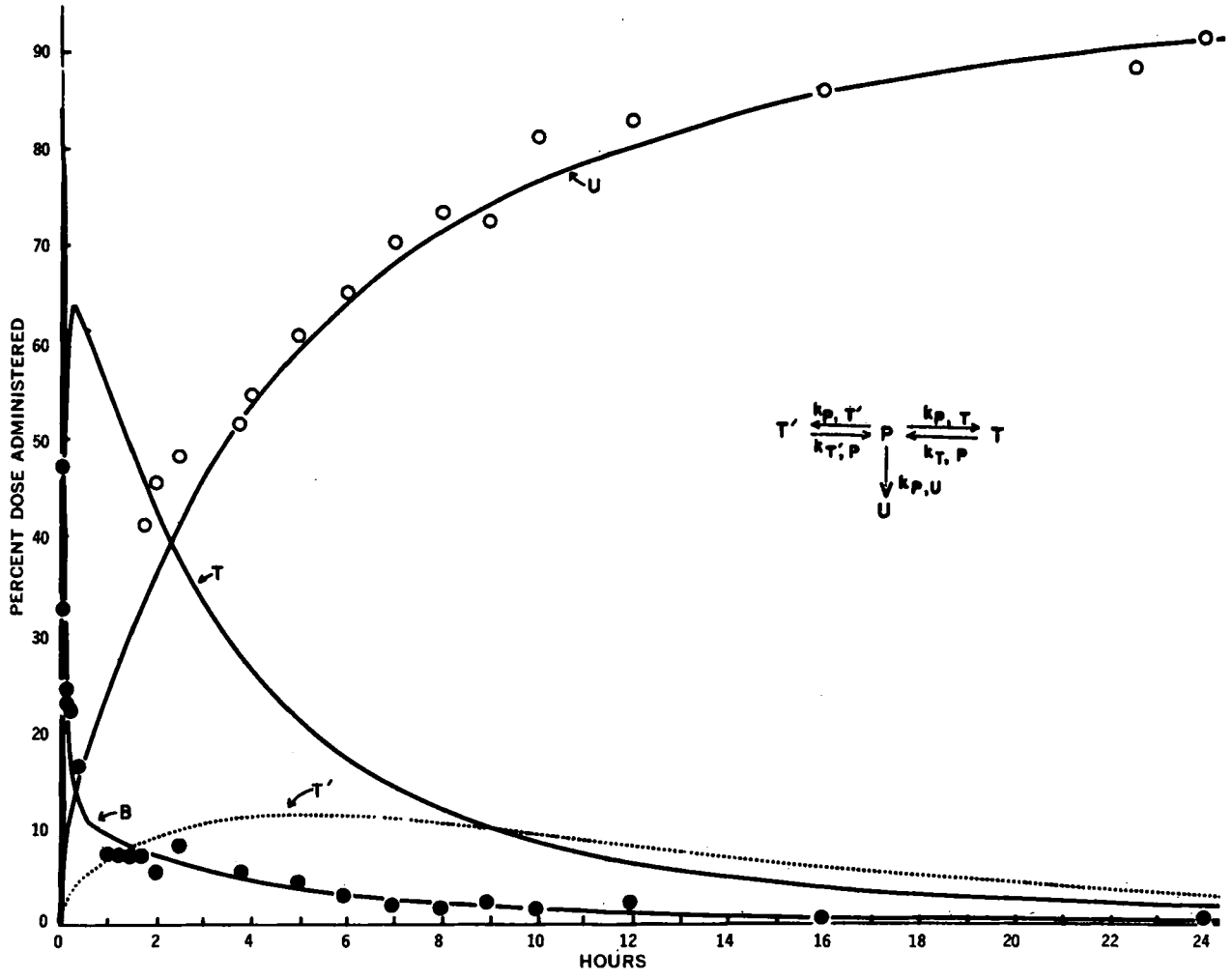


Figure 9—*Analog computer fitting of the same plasma (●) and urine (○) data as in Fig. 6 in accordance with a three-compartment body model (shown in figure). The dashed line represents the generated curve for* T'.

Eq. 12 simplifies to:

$$[A_P{}^u]_0 = \frac{\gamma A_0 - V_{RBC}[A_P{}^u]_0}{V_P{}^u} \qquad \text{(Eq. 13)}$$

Explicit solution of this equation results in:

$$[A_P{}^u]_0 = [A_P]_0 = \text{I.C.} = \frac{\gamma A_0}{V_P{}^u + V_{RBC}} \text{ [mcg./ml.]} \qquad \text{(Eq. 14)}$$

The fraction absorbed, γA_0, may be obtained from the ratio of the areas under the plasma curves of oral to intravenous administration of the same dose in the same dog (15). It may also be estimated from the ratio of the cumulative amounts excreted in the urine on oral administration to the amounts on intravenous administration of equal doses in the same dog. The volumes of distribution of the central compartment, $V_P{}^u$, referenced to unbound drug concentration in plasma were initially postulated to be the same as the corresponding intravenous values (Table II) for the first estimates of the initial conditions, I.C. The volume of the red blood cells was calculated for a blood volume, $V_{B_{true}}$, of 100 ml./kg. (17) and a hematocrit of 40% in this dog as $0.4 \times V_{B_{true}}$.

These I.C. values and the rate constants derived from the best fit of the data obtained from intravenous administration of 4 mg./kg. to the same dog (Table I) were used in the first fittings of the three sets of oral data. The potentiometers representing $k_{P,T'}$ and $k_{T',P}$ were turned to zero, and the data were first simulated according to a two-compartment model (Scheme I) by adjusting only the rate constant for absorption, k_a.

A good fit was obtained with this model for the data of the 2-mg./kg. dose (Fig. 10). The tissue, T', and the pertinent rate constants, $k_{P,T'}$ and $k_{T',P}$, were included for the 4- and 8-mg./kg. dose, and the fit of the oral dose of 8 mg./kg. is given in Fig. 11 as an example in accordance with the three-compartment body model. A lag time for absorption (15–45 min.) had to be assumed to fit the first portion of the data, and a slight decrease in the $k_{P,U}$ setting was needed to give the best fits of the plasma concentrations following oral administration (Table I). Curves for the amounts of drug in terms of the arbitrary microgram per milliliter concentrations for urine and the tissues were generated by the computer from the best fits of the plasma data in accordance with the two-compartment and three-compartment body models.

Since these curves were generated from drug concentrations that were distributed in the volume of the central compartment, $V_P{}^u$, they were all in terms of this $V_P{}^u$, *i.e.*, $T/V_P{}^u$, $T'/V_P{}^u$, and $U/V_P{}^u$, where T, T', and U are the amounts in the tissues and urine, respectively. The capacity of the $V_P{}^u$ in milliliters, *i.e.*, $V^u_{P(est)}$, can be estimated when the experimentally observed amounts excreted in the urine, U in micrograms, are divided by the generated urine curves, $U/V^u_{P(est)}$ in micrograms per milliliter, at the times of urine collection and is related to the true $V_P{}^u$ by:

$$V^u_{P(est)} = V_P{}^u \frac{\gamma A_0}{U_\infty} \qquad \text{(Eq. 15)}$$

If all of the drug absorbed were excreted in the urine as unchanged material, $V_P{}^u = V^u_{P(est)}$. In this case, there was no apparent difference in the percent of the dose recovered in the urine between intravenous and oral studies (Table II). Thus, a plot of U in micrograms divided by $V_{P(est)}$ in milliliters gave the cumulative excretion consistent with the actual data. These plots were only slightly off the computer-generated curves for urine. Small adjustments of the I.C. and of $k_{P,U}$ were sufficient.

The adjusted initial conditions, I.C., *i.e.*, $[A_P{}^u]_0$, were then inserted into Eq. 14, which was solved for the best estimates of $V_P{}^u$ based on the combined fittings of plasma and urine data (Table II).

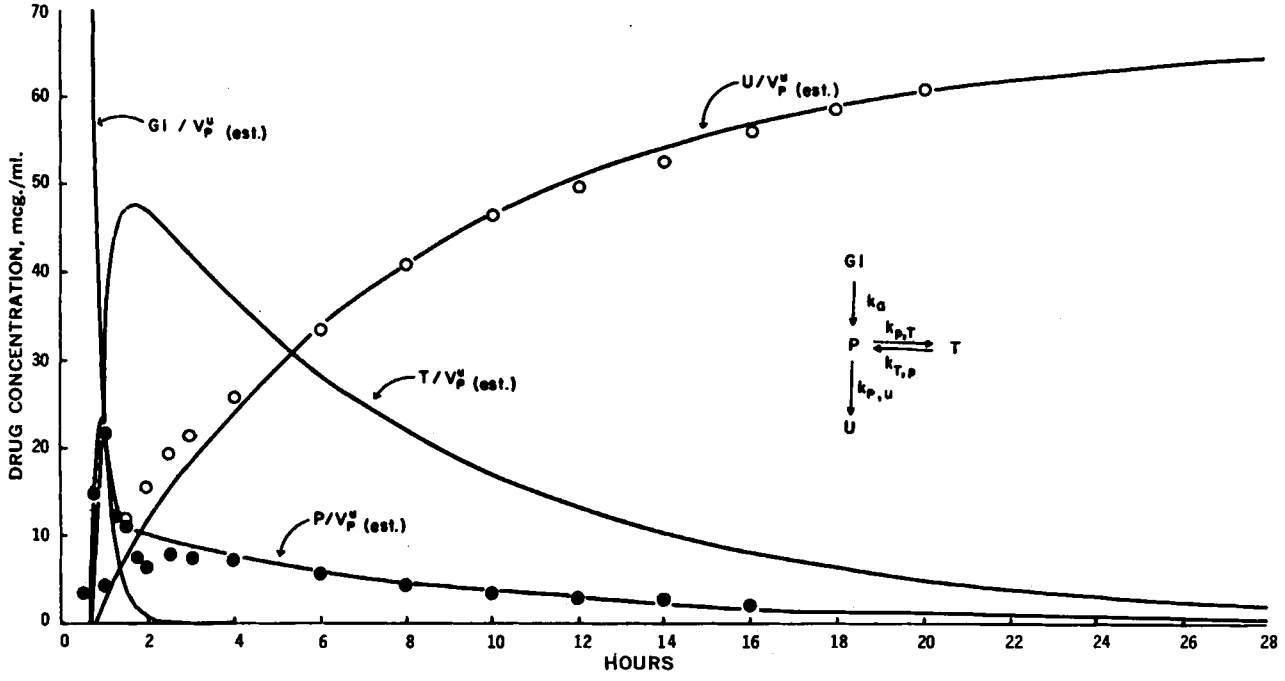


Figure 10—*Analog computer fitting of plasma* (●) *and urine* (○) *data following oral administration of 2 mg./kg. sotalol to 20.2-kg. Dog 1 in accordance with the two-compartment model and addition of a GI compartment. The plasma data in micrograms per milliliter were first fitted with rate constants identical to those of the 4-mg./kg. i.v. dose to the same dog. The I.C.'s were calculated according to Eq. 14 and only the* k_a *was varied. Curves for* $U/V^u_{P(est)}$ *and* $T/V^u_{P(est)}$ *were generated by the computer.* $V^u_{P(est)}$ *was obtained by dividing* U_{exp} *in micrograms by* $U/V^u_{P(est)}$ *in micrograms per milliliter. The final fit was made according to the plot of* $U_{exp}/V^u_{P(est)}$ *to yield the real* $V_P{}^u$ *in accordance with Eq. 14:*

$$V_P{}^u = \frac{\gamma A_0}{I.C.} - V_{RBC}$$

A lag time for gastric emptying of 45 min. had to be assumed to fit the early portion of the plasma curve.

Estimates of Absorption—The plasma concentrations following the intravenous administration of 1, 2, and 4 mg./kg. and oral administration of 2, 4, and 8 mg./kg. were plotted as functions of time, and the area under the curves was determined by a line follower[13] connected to the *x-y* recorder of the computer. The photocell followed the plasma concentration–time curves, and the voltages were accumulated by an integrator. Estimates of the percent of drug absorbed were obtained from the ratio of the area under the plasma level–time curve for oral absorption to that for intravenous administration for the same dosages (15). For the 8-mg./kg. oral dose, the average area under the curves for the three intravenous studies corrected for the different dose was taken as 100%.

RESULTS AND DISCUSSION

Metabolism of Sotalol—The chromatography of plasma and urine samples containing ^{3}H-sotalol revealed spots that were identical to those of the parent compound. Hence, it could be concluded that there was no significant metabolism of the drug. A scattering of spots assignable to radioactive material was observed in the bile. Since this route of excretion contributed less than 1% of the administered dose, it was of negligible significance.

Protein Binding Study—No binding of sotalol to plasma proteins was found in the 0.3–1000.0-mcg./ml. range of drug concentrations, *i.e.*, $f = 0$. The total drug concentrations in plasma assayed spectrophotofluorometrically and radiochemically were identical with the free diffusable drug. Thus, the rate constants and volumes of distribution were directly referenced to unbound drug concentrations in the plasma.

Red Blood Cell/Plasma Partitioning—The partition coefficient, D (Eq. 3), between plasma and red blood cells was found to be unity (1.16 ± 0.21). Equilibrium was achieved within 1 min. and did not change over a period of 2 hr. No drug concentration dependency of the 1:1 distribution between red blood cells and plasma could be detected in the range of 0.5–100.0 mcg. sotalol/ml. of blood.

Fit of Data from Intravenous Studies—The plasma data following intravenous administration were fitted graphically for all experiments in accordance with a two-compartment body model (Scheme I). The rapid initial drop of the drug concentration could be characterized by the α-slope of the first phase of the semilogarithmic plot of the data with time, which was equivalent to a $t_{1/2}$ of 3.2 ± 1.1 min. with extreme values of 1.6 and 5.5 min. and was followed by a comparatively slow disposition phase with the slope β or k_e of the terminal semilogarithmic plot that gave a $t_{1/2}$ of 4.8 ± 1.03 hr. with extreme values of 3.52 and 6.8 hr. (Table I and Figs. 1 and 2).

The k_e values obtained from the slopes of semilogarithmic plots of the rate of excretion, $\Delta U/\Delta t$, and the amounts of drug not yet excreted in the urine, $U_\infty - U$, against time were in good agreement with the estimates from the plasma data (Fig. 4 and Tables I and II). The microscopic rate constants derived from the graphical procedures and from the best analog computer fits were generally in good agreement (Table I). Whereas the plasma data could be fitted to a two-compartment body model by both methods in almost all cases (Figs. 1, 2, 6, and 10), it proved necessary to add a slowly equilibrating tissue compartment to fit the later portion of the urine curves at the high dosage level (Figs. 7 and 8) and in the case of the ^{3}H-sotalol data (Table I). The slow return from this "deep" compartment (12) may be held responsible for the prolonged enhancement of urinary excretion. A maximum of 5–12% of the dose was estimated to be in this compartment on acute administration.

The values for the individual rate constants were consistent within an animal for different doses as well as among the three animals. Dose-independent kinetics could also be demonstrated by the superimposable curves, where the plasma data were plotted in terms of percent of dose administered per milliliter plasma as a function of time (Fig. 2).

Rate Constants and Apparent Volumes of Distribution—The drug distributed instantaneously after bolus injection into the space associated with the central compartment, *i.e.*, $V_P{}^u$. The values in liters (Table II) corresponded generally to the volume of the extracellular space [190–350 ml./kg. (17)].

[13] Autograf Type F-3B, Hewlett-Packard/Moseley Division, Pasadena, Calif.

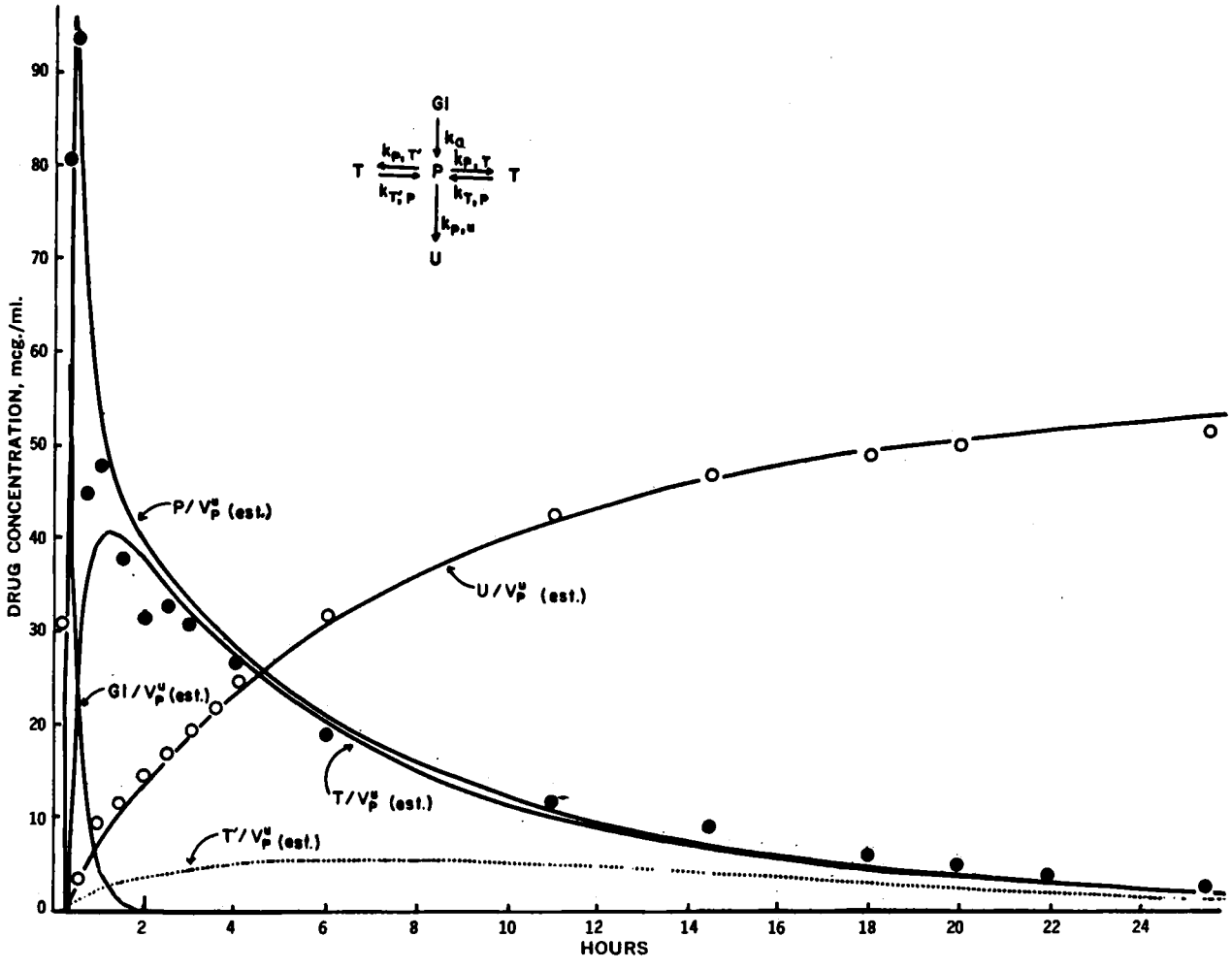


Figure 11—*Analog computer fitting of plasma and urine data following oral administration of 8 mg./kg. sotalol to Dog 1. Here, a lag time of 15 min. was assumed to fit the initial plasma data. For symbols, see Fig. 10.*

The drug diffuses rapidly from the central compartment into the readily equilibrating tissues, T. Since $k_{P,T}$ is 7–10 times greater than $k_{T,P}$, the capacity of this compartment is high. The apparent volume of T can be estimated by (11, 12):

$$V_T = V_P{}^u \frac{k_{P,T}}{k_{T,P}} \qquad \text{(Eq. 16)}$$

referenced to the concentration of unbound drug in the plasma. Similarly, for the apparent volume of the deep compartment, T':

$$V_{T'} = V_P{}^u \frac{k_{P,T'}}{k_{T',P}} \qquad \text{(Eq. 17)}$$

The apparent volumes, V_T, are reasonably consistent with a total body water of 600–800 ml./kg. (17). The fact that the V_T value exceeded this quantity in some cases indicated that the drug may be trapped or bound to the tissue, T, to some extent. It might be speculated that since sotalol is a zwitterion with a narrow pH range allowable for partitioning into fats (7), minute pH differences inside the cells would inhibit back-diffusion to the central compartment. The same phenomenon could rationalize the deeper compartment, T'.

Apparent Volumes of Distribution and Renal Clearance—The renal clearance of sotalol (Table II) was well within the range of a normal inulin clearance of 40–130 ml. for a 20-kg. dog (17). Also, an inulin clearance study in Dog 1 gave a value of 36 ml./min., as estimated from the plasma decay curve. Thus, sotalol can be assumed to be renally excreted essentially by glomerular filtration.

Clearance values of this magnitude affect the estimates of the apparent volumes of distribution that are based on the assumption of pseudosteady state (11) or, as it has also been called, the pseudo-distribution equilibrium (18). These estimated volumes can differ from those calculated for steady-state equilibrium in accordance with Eqs. 16 and 17 (13).

If excretion is significant during the rapid distribution phase, the initial decay in the plasma level of drug will be due to both distribution and excretion. Thus, the extrapolation of the β-phase from the semilogarithmic plots (Figs. 1–4) to zero time will estimate a value for the concentration of drug in the pseudoequilibrated tissues of distribution, $[A_P{}^u]_0$, which leads to values of $V_{D_P}{}^u$ (Eq. 7) and $V_{D_P}{}^u$ (Eq. 9) that are greater than the total apparent volumes of distribution of the central compartment and the rapidly equilibrating tissues T, which are derived from steady-state considerations (Eqs. 8, 16, and 17).

The $V_{D_P}{}^u$ calculated from the quotient of the renal clearance in milliliters per minute and k_e in min.$^{-1}$ (from plasma data, Table I) for the conditions when significant excretion occurs during the α-phase will also be higher than the sum of the steady-state volumes of distribution in Eq. 8 (13).

Estimates of the apparent volumes of distribution based upon these three methods are given in Table II, and it can be seen that the $V_{D_P}{}^u$ estimates based on the pseudosteady-state assumption and the renal clearance/k_e quotient tended to yield larger values than the sum of $V_P{}^u$ and steady-state volumes of the equilibrated tissues (Eq. 16), even when V_T (Eq. 17) was included.

Comparison of Chemical and Radioactive Assays—The semilogarithmic plots of the radioactive data in plasma showed a three-exponential decay and a slow terminal phase with a $t_{1/2}$ of approximately 12 days. This is consistent with the fact that deuterium oxide is cleared from plasma water with a $t_{1/2}$ of 9.3 ± 1.5 days (19). The terminal phase was, therefore, assigned to the clearance of tritiated water, which is a probable result of the exchange between the hydrogens of water and the tritium of labeled sotalol.

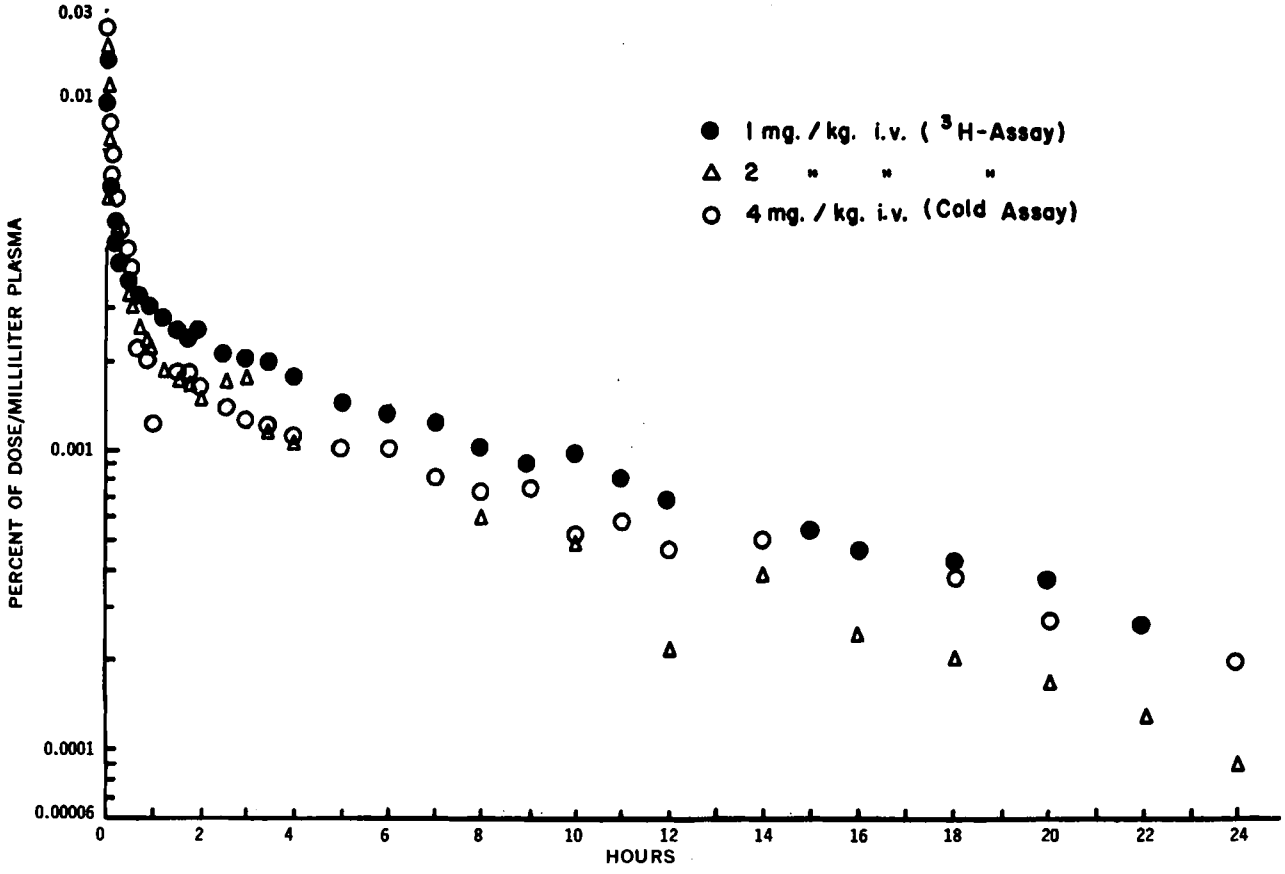


Figure 12—*Semilogarithmic plots of plasma levels in percent of dose per milliliter plasma following intravenous administration of 1 (●) and 2 (△) mg./kg. of ³H-sotalol, assayed by counting procedure, and 4 mg./kg. (○) of sotalol, assayed by spectrophotofluorometric analysis, to 20-kg. Dog 2. The radioactive data were corrected for the longer retention of tritiated water by prior feathering.*

The two assay methods were comparable after the terminal portion of the labeled plasma level–time curves was "feathered" (Fig. 12). The spectrophotofluorometric and radioactive data in plasma and urine could be fitted with identical rate constants in accordance with the three-compartment body model. The estimates for the apparent volumes of distribution of the central compartment, V_P^u, were slightly lower for the radioactive data since the initial concentrations of unbound drug in plasma, $[A_P^u]_0$, upon which these estimates are based (Eqs. 5 and 6) were slightly higher for the radioactive studies.

Unchanged ³H-sotalol, in the amounts of 91 and 93.6% of the dose, was found in the urine by radioactive assay as compared to 90.3 ± 12% (72–116%) from the spectrophotofluorometric data of the other experiments. The extreme overestimates of 108 and 116% recovery (Table II) were obtained in earlier experiments where the assay methodology was not as well established.

The fecal excretion of ³H-sotalol was 0.21 and 3.37% of the total dose in the two radioactive experiments.The former value was due to a constipated dog which permitted few fecal samples. The bile had 0.73% of the radioactive dose given to the biliary-cannulated dog.

Oral Studies—The graphical estimates of the absorption rate constants by feathering of the curves were not accurate since equilibration of drug among the available tissues was not readily effected during the absorption phase (Fig. 3). The $t_{1/2}$'s of absorption obtained from the best analog computer fits were (lag time for absorption in parentheses) 10.9 min. (15 min.) for 8 mg./kg., 16.7 min. (30 min.) for 4 mg./kg., and 10.7 min. (45 min.) for 2 mg./kg.

Such a lag time may be indicative of an intestinal absorption. The differences may be explained by variable gastric motilities in the fasted and achlorhydric dog.

Best fitting was achieved by applying the three-compartment body model, including a GI compartment, for the doses of 4 and 8 mg./kg. (Fig. 11). The data following administration of 2 mg./kg. could be fitted with both two- and three-compartment body models. The two-compartment fit is shown in Fig. 10.

Estimates of the percent of total dose absorbed were made from the total amounts excreted in the urine. They were 87% following a dose of 2 mg./kg., 101.7% for 4 mg./kg., and 78.2% for 8 mg./kg. The estimates from the ratio of the corresponding areas under the plasma level–time curves following intravenous and oral administration were 84.1, 83.0, and 90.5% absorption for the oral dosages of 2, 4, and 8 mg./kg., respectively. Hence, it can be concluded that sotalol is rapidly and rather completely absorbed from the GI tract (probably from the intestine) following oral administration of the drug in solution.

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GLC Determination and Urinary Recovery of Bumetanide in Healthy Volunteers

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Abstract □ A GLC determination of bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamylbenzoic acid), a potent "high ceiling" diuretic, was developed using flash-heater methylation. A mixture of tetramethylammonium hydroxide and trimethylanilinium hydroxide was advantageously used as the methylation reagent. The flash-heater methylation product proved to be methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate by spectroscopic comparison with authentic material achieved by a corresponding gram scale model experiment. The probable sequence of reactions involving methylation under simultaneous Smiles rearrangement is outlined. The GLC determination was found to be accurate at concentrations as low as 0.1 mcg./ml. human urine. In six healthy volunteers, the urinary recovery of bumetanide, the urinary excretion of sodium, potassium, and chloride ions, and the urine volume were determined after oral administration of 0.5 and 1 mg. of the drug. A parallelism between bumetanide excretion and saluretic action over the total period of response is shown.

Keyphrases □ Bumetanide—urinary excretion, man, relationship to saluretic action, GLC analysis after flash-heater methylation □ Urinary excretion, bumetanide—relationship to saluretic action, man □ Saluretic activity, bumetanide—relationship to urinary excretion, man □ GLC—analysis, bumetanide, after flash-heater methylation

Bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamylbenzoic acid) (I, Scheme I) was recently described as a new "high ceiling" diuretic in the experimental animal (1, 2) and in man (3, 4). In patients suffering from congestive heart failure, bumetanide has shown a diuretic profile and dose response comparable to that of furosemide, but at doses approximately one-fortieth of the latter drug (4). The aim of the present investigation was to develop a sensitive GLC assay for the determination of bumetanide in biological material. Special interests were to evaluate the urinary recovery after oral administration of the drug to healthy volunteers and to obtain information relating the renal drug excretion with the diuretic–saluretic activity.

Since functional groups of bumetanide make the drug unsuitable for direct GLC determination, it was necessary to find a suitable derivative. A relatively simple way of making derivatives of anionic compounds is by methylation in the injection port of the gas chromatograph, using a solution of tetramethylammonium hydroxide or trimethylanilinium hydroxide as the methylation reagent. The principle of flash-heater methylation was first utilized in preparation of methyl esters of carboxylic acid (5) with the aid of tetramethylammonium hydroxide. The advantage of using trimethylanilinium hydroxide was recently shown for barbiturates, phenolic alkaloids, and xanthine bases (6).

EXPERIMENTAL

GLC Determination—*Instruments and Conditions*—The GLC analyses were performed using a gas chromatograph[1] equipped with a flame-ionization detector and the following experimental conditions: column, 2 m. × 3.3-mm. o.d., stainless steel; packing, 1.5% OV-17 silicone[2] on 100-120-mesh diatomaceous earth[3]; column temperature, 270°; injection port temperature, 370°; detector temperature, 300°; carrier nitrogen flow, 30 ml./min.; and recorder chart speed, 0.3 cm. (0.1 in.)/min.

Reagents—For the methylation reagent, two parts of an approximately 1 *M* aqueous solution of trimethylanilinium hydroxide (6) were mixed with one part of a 10% methanolic solution of tetramethylammonium hydroxide. The latter was obtained by evaporating an aqueous tetramethylammonium hydroxide solution[4] in a rotating evaporator and redissolving the residue in the appropriate amount of methanol.

The internal reference solution consisted of 25 mcg. of 4-benzyl-3-*n*-butylamino-5-sulfamylbenzoic acid (7)/ml. in ether.

Procedure—Urine, 5–10 ml., was adjusted to pH 2 by addition of 1 *N* hydrochloric acid. Then 200 μl. of internal reference solution and 15 ml. of ether were added, and the mixture was shaken in a

[1] Perkin Elmer model 990.
[2] Applied Science.
[3] J.J.'s Chromatography Ltd., Diatomite CQ.
[4] Merck, Darmstadt, Germany.