Pharmacokinetic and Metabolic Fate of Potassium Canrenoate (SC-14266) in Man

A. KARIM*, R. E. RANNEY*, and H. I. MAIBACH†

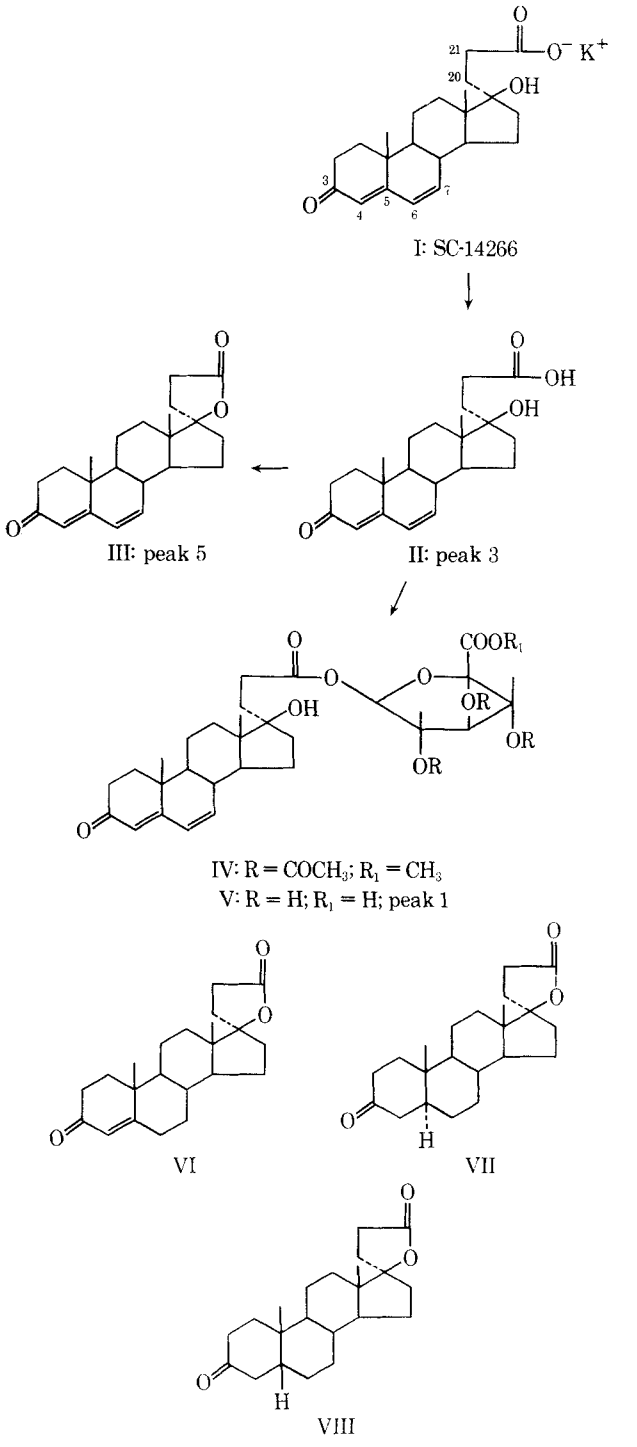
Abstract □ The metabolism of intravenously administered radioactive potassium canrenoate (SC-14266-^{3}H) was studied in three men. Within 5 days, 47.45 ± 4.17% of the administered radioactivity was recovered in urine and 14.36 ± 0.12% in the feces. In the urine, canrenone was identified as the major metabolite in the free fraction, and the major water-soluble metabolite was identified as the glucuronic acid ester conjugate of the hydroxy acid analog of SC-14266. The elimination of the total radioactivity in the plasma occurred in three phases, their half-lives being 0.073, 0.85, and 43.31 hr., respectively. The disappearance of the total label during the first and second phases was predominantly due to the excretion of the hydroxy acid derivative and canrenone. The third phase was a function of the excretion of the conjugate. Three hours after administration of the drug, the plasma levels of the hydroxy acid and canrenone were similar and declined, with a half-life of 8.25 hr.

Keyphrases □ Canrenoate-^{3}H, potassium (SC-14266)—pharmacokinetic, metabolic fate, human □ Pharmacokinetic parameters—potassium canrenoate-^{3}H in human □ Metabolites, potassium canrenoate-^{3}H—identification □ TLC—analysis □ Scintillometry—analysis □ GLC—analysis □ Spectrophotometry, IR, UV—analysis

SC-14266 [potassium canrenoate, potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl) propionate, I][1] is a water-soluble steroidal diuretic (1). It is structurally related to spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone][2], a useful aldosterone antagonist. The metabolism of spironolactone in humans has been studied by a number of investigators (2–5), but so far only one metabolite, canrenone [aldadiene, 3-(3-oxo-17β-hydroxy-4,6-androstadiene-17α-yl) propionic acid γ-lactone, III], has been fully characterized. Tentative characterization of the metabolites of SC-14266 was attempted by Wagner *et al.* (6), who reported the presence of seven metabolites in human urine. All of the work reported in the literature on the metabolism of spironolactone and its congeners in humans has been carried out using the unlabeled compound. With the availability of specifically tritiated SC-14266 (7), the present investigation was undertaken to identify and quantitate the major metabolites in the plasma and urine of human subjects and to study their kinetics of distribution and elimination after intravenous administration of the drug.

MATERIALS AND METHODS

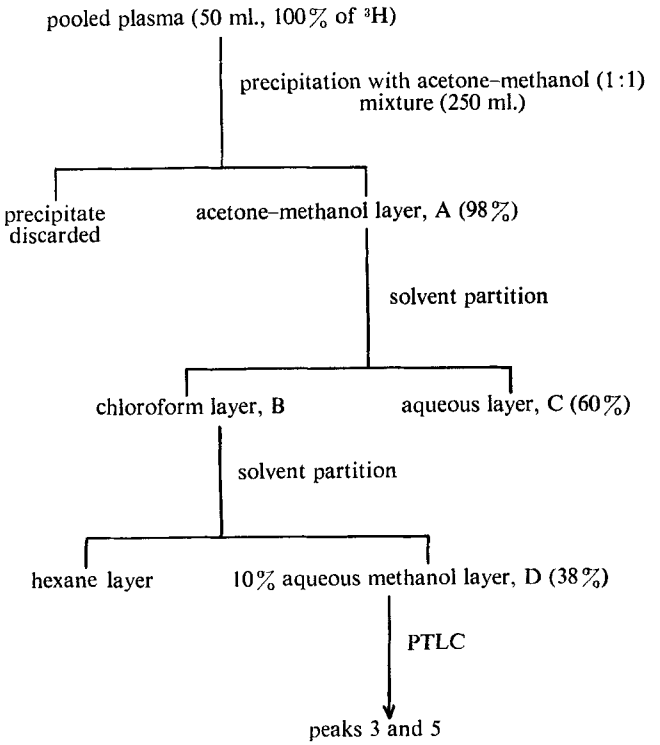
Labeled SC-14266 (I)—The studies on the metabolism of SC-14266 (Scheme I) were carried out using the ^{3}H-labeled compound, with a specific activity of 647 μc./mg.; the tritium label in I was present in the side chain of the steroid on C-20. The compound migrated as a single spot, and its radiochemical purity was 92% as determined by a TLC system.



Scheme I

Treatment of Subjects—Three adult male subjects were each given single intravenous injections of SC-14266 (200 mg. containing 182 μc. of ^{3}H). The sterile dry powder was dissolved in 10 ml. of sterile saline immediately before intravenous injection. An aliquot was

[1] Soldactone, G. D. Searle and Co.
[2] Aldactone, G. D. Searle and Co.



Scheme II—Isolation scheme for separation of metabolites from pooled plasma of Subject A

taken for radioactivity measurement to determine the actual amount of radioactivity injected. Blood samples were withdrawn at various intervals into heparinized vacutainers. Urine and fecal samples were taken for 5 days. Plasma samples and excreta were stored at −20° until analyzed.

Measurement of Radioactivity—All samples were counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer. For counting aqueous solutions (plasma and urine) and extracts containing polar compounds, Bray's scintillation solution (8) was used; for samples scraped from thin-layer chromatograms, Snyder's scintillation mixture (9) was employed. For counting radioactivity in the feces, samples (10 g.) were freeze-dried and the dry powder was exhaustively extracted with methanol in a continuous extraction apparatus. The methanol extract was diluted to 100 ml. and 100 μl. counted in Bray's solution. Chemical quenching was corrected by a channels-ratio method, and color quenching was done by the addition of an internal standard (toluene-³H). Tritiated water in plasma and urine was determined by the method of Werbin *et al.* (10).

TLC—Analytical TLC was carried out on silica gel HF_{254}[3] coated (250-μ thickness) glass plates using the saturated chamber technique. The solvent system was composed of methanol–glacial acetic acid–chloroform (5:1:94). Compounds were visualized under UV and by spraying with either a 10% ethanolic solution of phosphomolybdic acid or a 5% solution of ceric sulfate in 10% (v/v) aqueous sulfuric acid. The sprayed plates were heated at 110° for 10 min. Preparatory TLC (PTLC) was run on 20 × 20-cm. glass plates coated with 1 mm. of silica gel HF_{254}.

For detecting radioactive compounds on a thin-layer plate, 3-mm. bands of silica gel were scraped from the plate, using a Snyder-Kimble zonal scraper[4] (11); the radioactivity in each zone was determined by liquid scintillation spectrometry. The radioactivity distribution pattern of the chromatogram was then plotted by a computer-plotter which was programmed to give the total radioactivity eluted from the plate, the number of radioactive peaks, their R_f values, and the percentage of radioactivity in each peak.

Separation of Metabolites from Plasma and Urine—Metabolites from plasma and urine were isolated according to the schemes outlined in Schemes II and III.

[3] E. Merck, Darmstadt, West Germany.
[4] Analabs Inc.

GLC—GLC studies were made using a Varian Aerograph model 2100 instrument having a flame-ionization detector with a 1.5-m. (5-ft.) long, 2-mm. i.d., silanized glass U-tube packed with 1.3% OV-17[5] on 80–100 mesh Chromosorb W-H.P.[6]. Operating conditions were as follows: column temperature, 250°; detector and injection port temperature, 275°; carrier gas helium flow rate, 27 ml./min.; hydrogen flow rate, 30.4 ml./min.; and air flow rate, 300 ml./min. Peak areas and retention times were obtained using a Varian Aerograph model 481 electronic digital integrator.

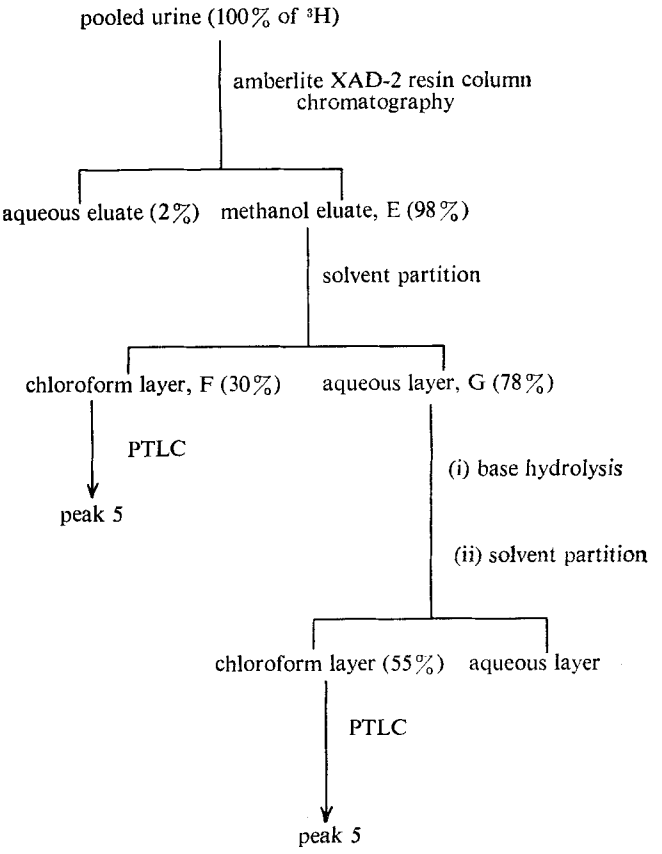
The smallest quantity of reference Compounds III, VI, VII, and VIII that could be accurately measured using these conditions was 0.05 mcg. To test the recoveries of canrenone (III) and hydroxy acid (II) from plasma and urine, known amounts of these compounds were added to the zero-hour urine and plasma samples, and the isolation procedures indicated in Schemes II and III were repeated. The recoveries of II and III from plasma ranged between 75 and 80%, while the recovery of III from urine was between 90 and 95%.

Spectroscopic Analysis—IR spectra were measured in chloroform using an ultramicro cell in a Beckman IR-12 instrument. UV absorption spectra were determined in methanol in a Beckman DK-2A ratio recording spectrometer.

Hydrolysis of Water-Soluble Conjugated Fraction—For base hydrolysis, potassium hydroxide (1.5 g.) was dissolved in an aliquot of Extract G (50 ml., 2 × 10^5 d.p.m.), which was left at room temperature for 12 hr. The extract was acidified (pH 3) with dilute hydrochloric acid, and the free steroids were extracted with chloroform. This procedure was repeated on another aliquot of Extract G without the addition of base.

Acidic hydrolysis was done by refluxing an aliquot of Extract G (4 × 10^6 d.p.m.) in 2 *N* hydrochloric acid (20 ml.) for 1 hr. The released aglycone was extracted with chloroform.

For enzyme hydrolysis, aliquots of conjugated Extract C or G (6 × 10^5 d.p.m.) in acetate buffer at pH 5.1 (25 ml.) were incubated at 37° for 48 hr. with 1 ml. (5000 Fishman units) of Ketodase[7]; the



Scheme III—Isolation scheme for separation of metabolites from pooled urine

[5] Applied Science.
[6] Johns-Manville.
[7] Warner-Chilcott.

Table I—Excretion of Radioactivity (Percent Administered ^3H) in the Urine and Feces of Men after Intravenous Administration of SC-14266-^3H

	Subjects					
	A		B		C	
Day	Urine	Feces	Urine	Feces	Urine	Feces
1	44.49	0.05	34.50	0.80	34.93	0.24
2	7.35	0.60	5.17	2.01	4.74	5.82
3	2.15	3.77	2.90	9.54	1.47	6.24
4	0.81	9.20	1.21	0.01	0.58	1.05
5	0.86	0.98	0.83	2.82	0.37	0.96
Total	55.66	14.60	44.61	14.18	42.09	14.31

major free steroid liberated was extracted with chloroform and purified by PTLC.

Preparation of Methyl Ester Acetate of the Conjugated Metabolite (Peak 1)—An aliquot of urinary Extract G (2×10^6 d.p.m.) in methanol (10 ml.) was treated with an excess of an ethereal solution of diazomethane at 0°. After 30 min., the solvent and the excess of diazomethane were removed in a stream of nitrogen, and the residue was dissolved in 20 ml. of pyridine–acetic anhydride (1:3) mixture and left at room temperature for 12 hr. The reagents were removed in a stream of nitrogen; the residue, dissolved in benzene (2 ml.), was passed through a 7.5-g. column (12×1 cm.) of silica gel packed in benzene. The column was first eluted with benzene (40 ml.) followed by chloroform (40 ml.) and then 5% methanol in chloroform (40 ml.). The last eluate, which contained 71% of the radioactivity applied to the column, was further purified twice by PTLC (solvent system of 2.5% methanol in chloroform). The UV-absorbing radioactive zone (R_f 0.4–0.6) was eluted with a methanol–methylene chloride (20:80) mixture, and the residue was subjected to spectral examination.

Calculation of Pharmacokinetic Parameters—The nonlinear plasma radioactivity curve obtained after an intravenous administration of SC-14266-^3H was analyzed by both biexponential and triexponential decay functions. The mathematical expressions for these relationships are given in Eqs. 1 and 2:

$$\text{biexponential decay:} \quad C_p = h_1 e^{-\alpha t} + h_2 e^{-\beta t} \qquad \text{(Eq. 1)}$$

$$\text{triexponential decay:} \quad C_p = h_1 e^{-\alpha t} + h_2 e^{-\beta t} + h_3 e^{-\gamma t} \qquad \text{(Eq. 2)}$$

where C_p = plasma radioactivity at time t; h_1, h_2, and h_3 = extrapolated zero-time intercepts of three phases; and α, β, and γ = hybrid rate constants corresponding to these three phases. The best values of constants h_1, h_2, h_3, α, β, and γ were obtained by the iterative least-squares method with a digital computer. This method gave values of the constants that were independent of personal error in drawing the semilogarithmic plots of plasma radioactivity concentration *versus* time.

The volume of the central compartment (V_p) in the case of the triexponential decay curve was evaluated by use of Eq. 3 given by Loo *et al.* (12):

$$V_p = \frac{\text{dose}}{h_1 + h_2 + h_3} \qquad \text{(Eq. 3)}$$

The plasma clearance was evaluated by use of Eq. 4:

$$\text{plasma clearance} = \frac{\text{dose}}{\text{plasma area}} \qquad \text{(Eq. 4)}$$

where area under blood level curve is from time = 0 to time = ∞.

RESULTS AND DISCUSSION

Distillation of water from plasma and urine samples showed that less than 1% of the ^3H in these samples was present as tritiated water. This finding suggests that the label on the steroid was not appreciably displaced during metabolic transformations. Since SC-14266 was labeled in the side chain, only those compounds that retained the side chain were detected.

The urinary and fecal excretions of the total radioactivity at different time intervals are listed in Table I. Within 5 days, 47.45 ± 4.17% of the administered radioactivity was excreted in the urine and 14.36 ± 0.12% in the feces; 37.97 ± 3.26% of the urinary radioactivity was excreted in the first 24 hr. The time course of the excretion of the radioactivity in the feces was very erratic. No attempt was made to investigate the fecal metabolic composition. Complete recovery of the label was not achieved, probably because of the short period for fecal collection and the relatively long half-life of the conjugated metabolite.

Plasma Metabolic Composition—The TLC distribution pattern of the total plasma metabolites 1 hr. after an intravenous administration of the drug to Subject A is shown in Fig. 1A. The R_f values of peak 5 (16.5%, R_f 0.76) and peak 3 (42.2%, R_f 0.29) were identical to canrenone (III) and the hydroxy acid (II), respectively. Since the parent drug (I) is a potassium salt of II, the conversion of I to II would be expected to take place instantaneously. Thus, the biotransformation and pharmacokinetic profile of the administered drug I would be that of the acid II. A large percentage of the radioactivity (31.6%) was associated with the polar metabolite (peak 1) which

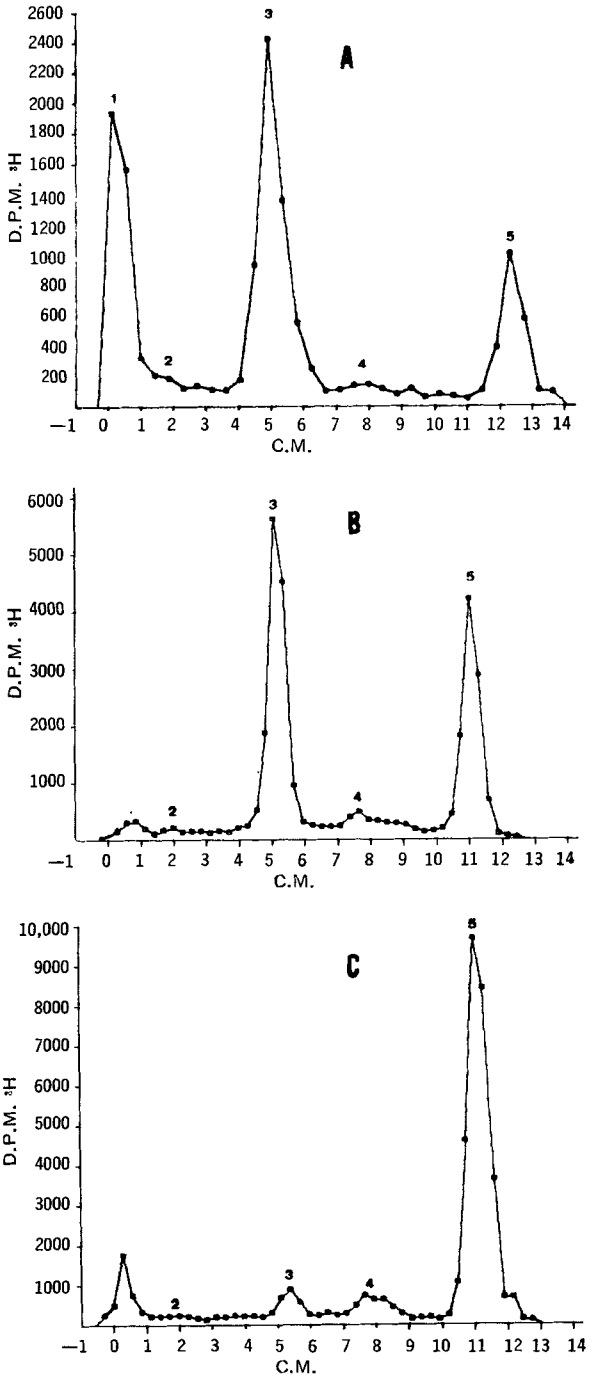


Figure 1—*Radiochromatograms of plasma metabolites. For identities of peaks, see text. Key: A, Extract A, free plus conjugated metabolites; B, Extract D, free metabolites; and C, free metabolites after enzymatic hydrolysis of Extract C.*

remained at the origin. This polar compound was also isolated from urine, and its structure is discussed later.

To characterize the plasma metabolites further, all plasma samples of Subject A were pooled and the total radioactivity was fractionated between the free fraction (Extract B) and the conjugated fraction (Extract C). To remove the plasma lipids, chloroform Extract B was fractionated between hexane and 10% aqueous methanol. The latter fraction contained 38% of the plasma radioactivity, while 60% was present as water-soluble metabolites in Extract C. The metabolic composition of the free fraction (Extract D) is given in Fig. 1B, which shows that 28.2% of the radioactivity was present in peak 5 whose mobility coincided with canrenone. This chromatographic system, however, did not resolve canrenone

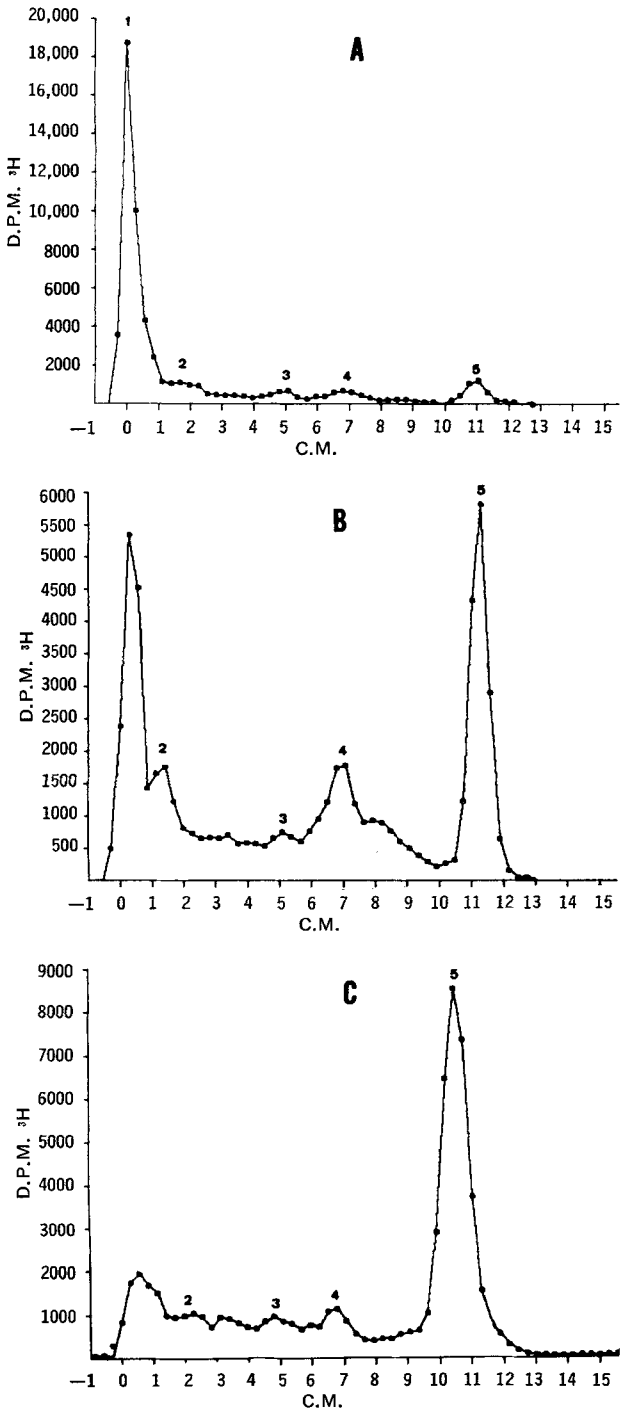


Figure 2—*Radiochromatograms of urinary metabolites. For identities of peaks, see text. Key: A, Extract E, free plus conjugated metabolites; B, Extract F, free metabolites; and C, free metabolites after enzymatic hydrolysis of Extract G.*

Table II—Total Radioactivity in Plasma after Intravenous Administration of SC-14266-³H in Subjects A, B, and C

Time	Percent ³H per Liter Plasma — Subject A	B	C	Mean	*SEM*, ±
1 min.	12.8	24.1	20.66	19.19	3.34
3 min.	10.9	18.9	13.41	14.40	2.36
5 min.	9.81	19.5	11.76	13.69	2.96
10 min.	7.10	10.9	9.73	9.24	1.12
15 min.	5.51	9.28	6.95	7.25	1.10
30 min.	4.02	6.67	4.90	5.20	0.78
45 min.	2.84	6.71	3.85	4.47	1.16
1 hr.	2.27	5.63	3.77	3.89	0.97
1.5 hr.	2.02	4.31	2.46	2.93	0.70
2.0 hr.	1.67	3.74	2.16	2.52	0.62
2.5 hr.	1.47	2.88	2.07	2.14	0.41
3.5 hr.	1.24	1.94	1.58	1.59	0.20
4.0 hr.	1.13	1.86	—	1.50	0.37
6.0 hr.	1.28	1.20	1.79	1.42	0.18
8.0 hr.	1.04	1.35	1.40	1.26	0.11
10.5 hr.	0.93	1.10	1.42	1.15	0.14
11.8 hr.	1.06	0.95	1.44	1.15	0.15
18.0 hr.	0.81	0.83	1.29	0.98	0.16
24.0 hr.	0.99	0.81	—	0.90	0.13

from its dihydro and tetrahydro derivatives VI, VII, and VIII. These compounds may be regarded as potential metabolites. An excellent separation of canrenone and its reduced derivatives was obtained by GLC, using an OV-17 column. The retention times of Compounds III, VI, VII, and VIII were 24.8, 22.7, 17.5, and 14.6 min., respectively. GLC analysis indicated that peak 5 was canrenone, and there was no evidence of Compound VI, VII, or VIII. On mild acid treatment of peak 3, a change in its polarity from R_f 0.31 to 0.70 was observed. The less polar product was identified as canrenone by GLC. This change in the polarity can be explained as lactonization of the hydroxy acid (II) to the γ-lactone (III).

The water-soluble conjugates in the plasma were present in Extract C. After enzymatic hydrolysis, 80% of the radioactivity was extractable by chloroform. The thin-layer radiochromatogram of the free fraction thus released is shown in Fig. 1C. The major aglycone (peak 5, 75%) was identified as canrenone (III) by TLC and GLC analyses.

Urinary Metabolic Composition—The Amberlite XAD-2 resin[8] (13) chromatography of the pooled urine removed large amounts of the nonradioactive materials in the aqueous eluate. The methanol eluate (Extract E) contained 98% of the radioactivity applied to the column. The metabolic composition of this extract, which represented the total urinary radioactive metabolites, is shown in Fig. 2A. The major urinary metabolite was a polar compound (peak 1, 70.8%) which remained at the origin of the TLC plate. The mobilities of peak 5 (6.7%, R_f 0.68) and peak 3 (4.8%, R_f 0.31) were similar to canrenone (III) and hydroxy acid (II). Peak 4 (6.1%, R_f 0.41) and peak 2 (9.6%, R_f 0.09) were due to unknown metabolites.

On further fractionation of Extract E, 30% of the radioactivity was extracted with chloroform (Extract F) while the remainder was present as water-soluble material (Extract G). The metabolic composition of the chloroform-soluble Extract F is shown in Fig. 2B. In this radiochromatogram, 27.6% ofthe radioactivity was associated with canrenone (peak 5, III), 23% with unknown metabolite peak 4 (R_f 0.45), and 12% with polar peak 2; 25% remained at the origin of the plate.

Structure Elucidation of the Conjugated Metabolite (Peak 1)—In both plasma and urine, the major metabolite was the water-soluble conjugate. To investigate the nature of the conjugated functionality, the urinary conjugate (Extract G) was subjected to various hydrolytic procedures. Chloroform extraction of the conjugated fraction at pH 3 gave 30% of the radioactivity in the organic phase. After acid hydrolysis, 83% of the radioactivity was extracted by chloroform. TLC analysis of the aglycone released after acid hydrolysis indicated the presence of a highly fluorescent compound with an R_f value of 0.64. This compound showed a maximum UV absorption at 355 nm. No further attempt was made to characterize this material. Riegelman *et al.* (14) found that spironolactones possessing a 4,6-dien-3-one grouping gave highly fluorescent com-

[8] Rohm & Haas, Philadelphia, Pa.

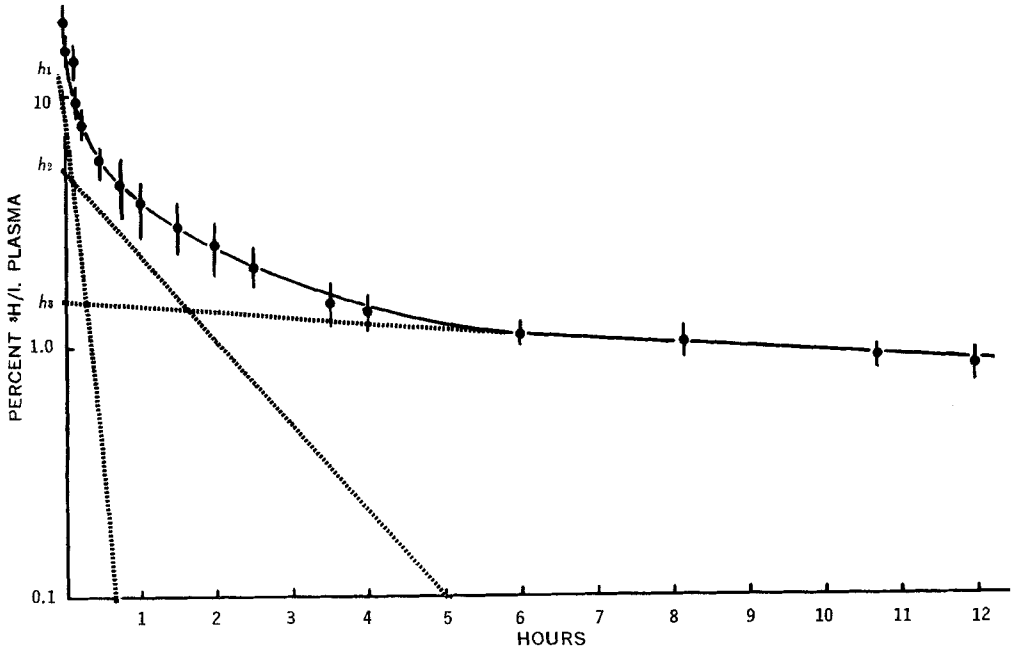


Figure 3—*Semilogarithmic plot of the mean plasma total radioactivity after intravenous administration of SC-14266-³H to Subjects A, B, and C. Vertical bars represent standard errors of the mean.*

pounds on treatment with an acid. The formation of the fluorescent compound with acid is also the basis for the estimation of canrenone in biological fluids (15). After mild base hydrolysis, 70% of the radioactivity was extracted by chloroform, giving an aglycone with TLC and GLC behavior identical to canrenone. Release of the free steroid on mild base hydrolysis suggested the presence of an ester linkage in the conjugate. After enzymatic hydrolysis, 60% of the radioactivity was extracted by chloroform; the major aglycone thus released (55%, peak 5, Fig. 2C) was identified as canrenone by TLC and GLC analyses.

To provide spectral evidences for the structure of the conjugate, the urinary water-soluble Extract G was methylated with diazomethane and the product acetylated with acetic anhydride–pyridine mixture at room temperature.. The acetylated methyl ester thus obtained was purified by silica gel column chromatography followed by PTLC. The IR absorption spectrum of this derivative gave strong absorption bands at 1745, 1245, and 1040 cm.$^{-1}$, which were characteristic of four ester groups of the sugar moiety (16). The absorption bands at 1670 and 1620 cm.$^{-1}$ and UV maximum at 282 nm. were indicative of the steroidal 4,6-dien-3-one chromophore. The IR spectrum also showed a weak broad absorption at 3480 cm.$^{-1}$, suggesting the presence of an unacetylated hydroxyl group. These spectroscopic data were compatible with Structure IV for the acetylated methyl ester derivative of the conjugate; thus, Structure V was proposed for the parent conjugate (peak 1).

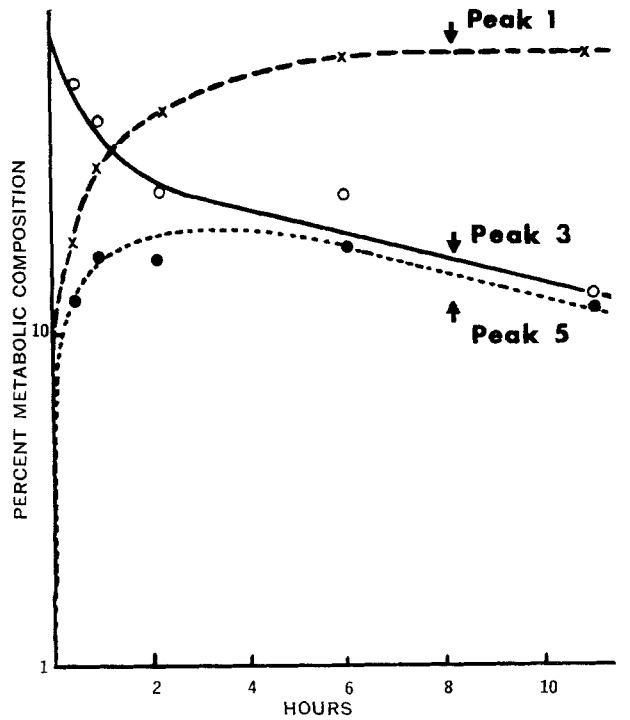


Figure 4—*Time course composition of peaks 1, 3, and 5 in plasma of Subject B. For identities of peaks, see text.*

Pharmacokinetic Analysis of Plasma Radioactivity—The plasma radioactivity (total) expressed as percent ³H per liter of plasma in Subjects A, B, and C is given in Table II. Figure 3 illustrates the semilogarithmic plot of the mean plasma levels. The curve in this figure is clearly nonlinear; by the method of residual analysis, it could be resolved into three distinct phases. The best values for the slope of each phase and their corresponding zero-ordinate concentration intercepts were derived by digital computer and are given in Table III. Immediately after injection of SC-14266-³H, there was a rapid phase of disappearance of the total label, with a half-life of only 0.073 hr. After 1 hr., a second phase, with a half-life of 0.849 hr., was observed; from 6 hr. onward, the total label was eliminated very slowly with a half-life of 43.31 hr.

To study the kinetics of elimination of individual plasma metabolites, the nature of compounds present in the plasma samples of Subject B were analyzed by TLC. The radioactivity in the plasma was attributed to three major compounds. These compounds were identified as already described as canrenone (peak 5), its hydroxy acid analog (peak 3), and the glucuronic acid ester conjugate of the hydroxy acid (peak 1). The time course composition of these plasma metabolites in Subject B is illustrated in Fig. 4. The percentage composition in the plasma due to the hydroxy acid (peak 3) decreased rapidly from 0 to 3 hr., while the percentage composition due to canrenone (peak 5) increased rapidly during this period. After 3 hr., the percentage composition of both hydroxy acid and canrenone remained about the same and decreased with a half-life of 8 hr. These results suggest that after 3 hr., an equilibrium between the hydroxy acid and its corresponding γ-lactone was established. The percentage composition of the conjugate (peak 1) increased very sharply; after 6 hr., 60% of the plasma radioactivity was associated with this compound.

For evaluation of pharmacokinetic parameters of the hydroxy acid (peak 3), the radioactivity in each plasma sample of Subject C was separated by TLC. The semilogarithmic plot of peaks 1, 3, and 5 is given in Fig. 5. The results were essentially similar to those obtained with Subject B. The plasma radioactivities due to the hydroxy acid and to canrenone were similar after 3 hr., and their levels declined with a half-life of 8.25 hr. The plasma level due to the conjugate (peak 1) decreased sharply initially, showed a gradual rise between 1 and 4 hr., and then declined very slowly with a half-life of 40

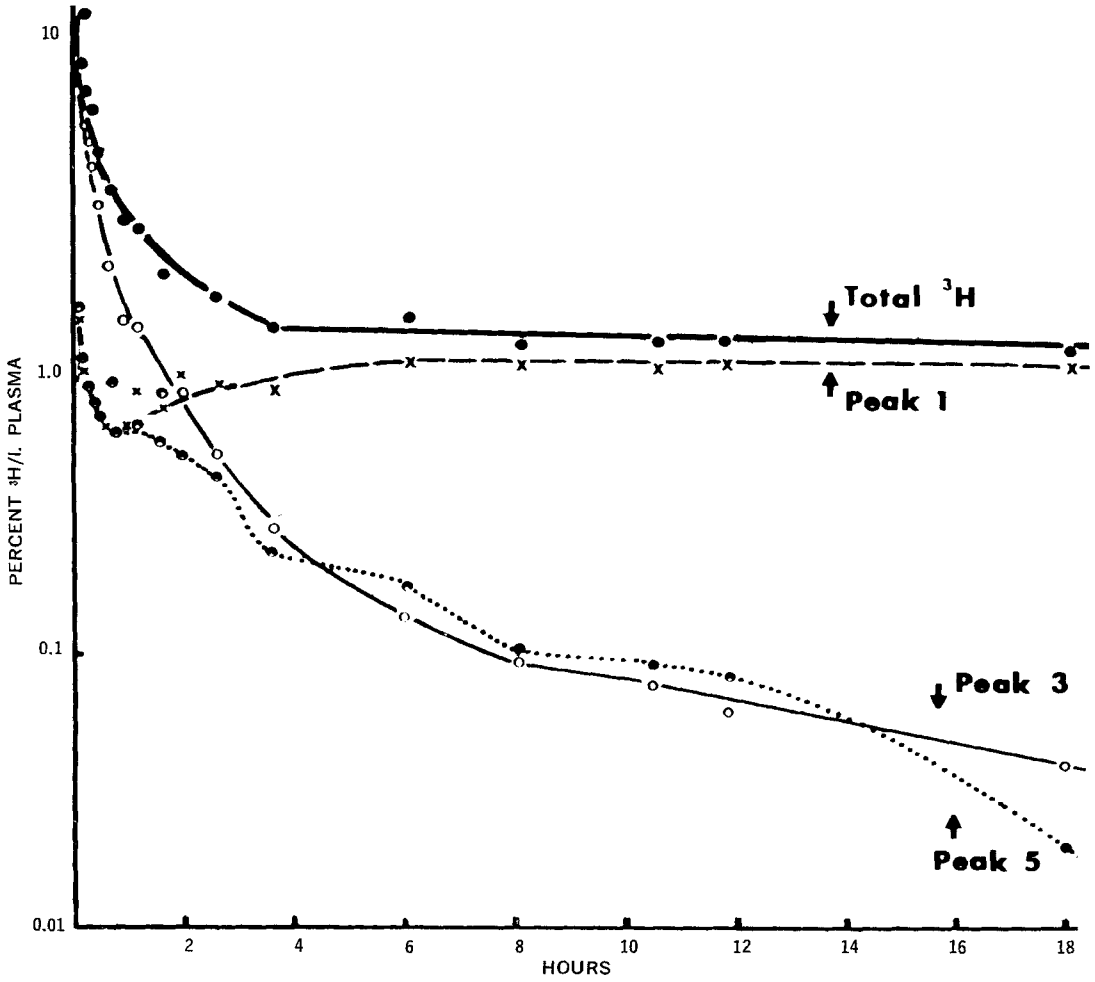


Figure 5—*Semilogarithmic plot of peaks 1, 3, and 5 in plasma of Subject C. For identities of peaks, see text.*

Table III—Kinetic Parameters[a] Calculated from Plasma Radioactivity Data Obtained after Intravenous Injection of SC-14266-³H

Subject	Radioactivity	h_1, % ³H/l.	α, hr.$^{-1}$	$t_{1/2}$, hr.	h_2, % ³H/l.	β, hr.$^{-1}$	$t_{1/2}$, hr.	h_3, % ³H/l.	γ, hr.$^{-1}$	$t_{1/2}$, hr.	Area, % ³H·hr./l.	Clearance, l./day
Mean of A, B, and C	Total ³H	13.86	9.461	0.073	5.661	0.816	0.849	1.390	0.016	43.31	95.28	25.19
C	Total ³H	10.26	5.903	0.117	4.224	0.931	0.744	1.727	0.018	38.5	102.22	23.48
C	Peak 3 ³H	8.028	4.721	0.147	2.422	0.712	0.973	0.193	0.084	8.25	9.98	240.48

[a] See text for definition of symbols.

Table IV—Analysis of the Radioactivity[a] in the Urine after Intravenous Administration of SC-14266-³H to Subjects A, B, and C

	Total ³H					"Free Fraction" ³H					Percent Free Fraction				
	Subject				*SEM*,	Subject				*SEM*,	Subject				*SEM*,
Hours	A	B	C	Mean	±	A	B	C	Mean	±	A	B	C	Mean	±
0–1	7.71	4.36	6.35	6.14	0.97	4.28	2.53	3.54	3.45	0.51	55.5	58.0	55.7	56.4	0.80
1–2	5.82	5.01	2.86	4.56	0.88	1.94	1.92	0.97	1.61	0.32	33.3	38.3	33.9	35.17	1.58
2–3	5.04	4.94	5.59	5.19	0.20	1.42	1.61	1.46	1.50	0.06	28.2	32.6	26.1	28.97	1.92
3–4	4.03	2.46	3.18	3.22	0.45	1.14	0.63	0.96	0.91	0.15	28.3	25.6	30.2	28.03	5.33
4–8	8.90	7.23	6.02	7.38	0.83	2.45	1.76	1.86	2.02	0.22	27.5	24.3	30.9	27.57	1.91
8–12	4.18	3.61	4.34	4.04	0.22	1.11	0.87	1.25	1.08	0.11	26.5	24.1	28.8	26.47	1.36
12–24	8.81	6.89	6.57	7.42	0.70	2.18	1.53	1.93	1.88	0.19	24.7	22.2	29.4	25.43	2.11
24–36	6.39	3.87	3.23	4.50	0.96	1.60	0.99	0.99	1.19	0.20	25.0	25.6	30.6	27.07	1.78
36–48	0.96	1.30	1.51	1.26	0.16	0.25	0.32	0.48	0.35	0.07	26.0	24.6	31.8	27.47	2.20
48–72	2.15	2.90	1.47	2.17	0.41	0.61	0.71	0.54	0.62	0.05	28.4	24.5	36.7	29.87	3.60
72–96	0.81	1.21	0.58	0.87	0.18	0.25	0.34	0.19	0.26	0.04	30.9	28.1	32.7	30.57	1.34
96–120	0.86	0.83	0.37	0.69	0.16	0.23	0.24	0.14	0.20	0.03	26.7	28.9	37.8	31.13	3.39
Total	55.66	44.61	42.07	47.45	4.17	17.46	13.45	14.31	15.07	2.11					

[a] Expressed as percent administered ³H.

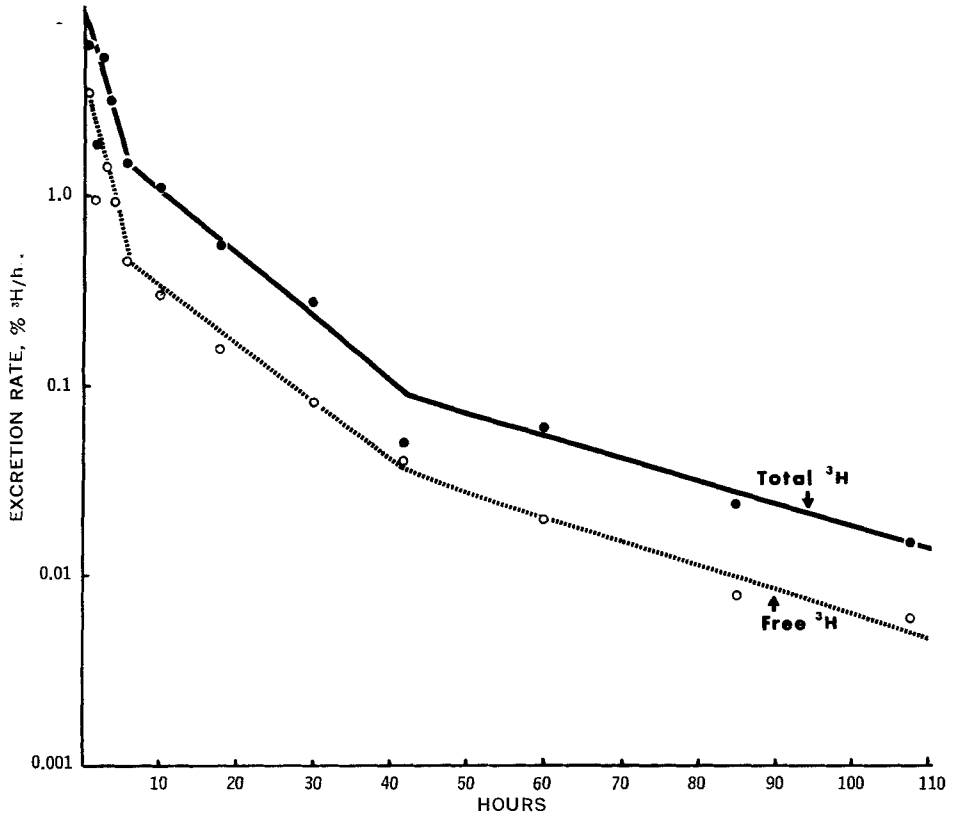


Figure 6—*Urinary excretion rate of total label and urinary free fraction in Subject C.*

hr. The rapid initial fall in the plasma level of the conjugate seen in Fig. 5 is difficult to rationalize. One explanation for this may be that the rapid decline was an artifact and not due to the conjugate. This view is supported by the facts that the injected SC-14266-^{3}H had 8% of a polar impurity and the radioactivity due to the polar compound 1 min. after intravenous administration was 9.2% of the total plasma radioactivity.

For evaluating the best model for the plasma disappearance of the hydroxy acid (peak 3), its plasma levels were tested for the best fit using both biexponential and triexponential functions. In the former case, the best values of the parameters describing the curve were

$$C_p = 8.243e^{-4.566t} + 2.367e^{-0.562t} \quad \text{(Eq. 5)}$$

For the triexponential equation, the best values of the parameters were

$$C_p = 8.028e^{-4.721t} + 2.422e^{-0.711t} + 0.193e^{-0.084t} \quad \text{(Eq. 6)}$$

Comparison of the theoretical curves obtained by use of these equations with experimental points showed that the best fit was obtained with the triexponential decay function. It is interesting to compare the half-lives of the three phases of elimination in Subject C due to total radioactivity and that due to the hydroxy acid. The half-lives and their corresponding zero time intercepts of the total label for the first and second phases were similar to that of the hydroxy acid, suggesting that, in these two phases, the elimination of the total label was predominantly a measure of the elimination of the hydroxy acid. However, the half-life of the third phase of the total plasma tritium was very long compared to that of the hydroxy acid, indicating that, in this phase, the elimination of the total label was predominantly a measure of the disappearance of the conjugated metabolite.

The volume of the central compartment (V_p) of the hydroxy acid in Subject C was evaluated as 9.4 l. and the plasma clearance of the total label in this subject was 23.4 l./day (Table III). This clearance value was one-tenth the value of the clearance of hydroxy acid.

Pharmacokinetic Analysis of Urinary Radioactivity—The total label excreted in the urine after intravenous administration of SC-14266-^{3}H to Subjects A, B, and C is given in Table IV. Within 5 days, 47.45 ± 4.17% of the administered radioactivity was recovered in the urine. The metabolic composition in the urine has already been described. Chloroform extraction of the pooled urine sample gave 30% of the urinary radioactivity as the free fraction in which canrenone (peak 5) was identified as the major metabolite. Seventy percent of the urinary radioactivity was present as the water-soluble metabolite which was identified as the glucuronic acid ester conjugate (peak 1) of the hydroxy acid (peak 3).

To study the kinetics of the urinary excretion of these metabolites, each urine sample of Subjects A, B, and C was extracted with chloroform to separate free and conjugated metabolites. Table IV shows the values of the free fraction present in the urine collected at different intervals. Except for the first urinary collection (0–1 hr.), the percentage of the free fraction excreted in the urine remained essentially constant (24–37%) in all three subjects. This is in contrast to the ratios of free and conjugated metabolites in the plasma, where in both Subjects B and C the percentage of the conjugate increased sharply after administration of the drug; after 24 hr., greater than 90% of the plasma radioactivity was associated with the conjugate. These differences in the ratio of the conjugate between plasma and urine suggest that some hydrolysis of the conjugate must have taken place after disappearance from the plasma and prior to excretion in the urine.

The urinary excretion rate (17) of both total label and urinary free fraction for Subject C is illustrated in Fig. 6. In both cases, three distinct phases of elimination were seen. For the free fraction, the half-lives of the three phases were 1.7, 10, and 29 hr.

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Pharmacokinetics of Ampicillin Trihydrate, Sodium Ampicillin, and Sodium Dicloxacillin following Intramuscular Injection

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Abstract □ Serum levels of penicillin activity were determined following intramuscular administration to humans of sodium ampicillin solution, three ampicillin trihydrate suspensions, and sodium dicloxacillin solution. The rates of absorption of the drugs from the intramuscular injection sites were calculated using the method of Wagner and Nelson, with and without intravenously determined elimination constants, and the method of Loo and Riegelman. The ampicillin suspension and dicloxacillin solution data exemplified the interesting pharmacokinetic case in which the absorption process is slower than the elimination process. In this situation, the descending portion of the serum curve reflects the absorption process rather than the elimination process. Calculations confirmed that intravenous data were required to differentiate these processes under these conditions. Ampicillin solution data followed the usual pattern where the descending portion of the serum curve reflects the elimination process. Serum level curves were displayed by an analog computer programmed with a two-compartment open model, and the rate constants were calculated by the Loo–Riegelman method. The computer-generated lines agreed with the experimental data except for the early times following the ampicillin trihydrate suspensions. When the computer was programmed with a kinetic model that depicted absorption as two successive first-order steps, however, the computer lines agreed with the experimental data at all times.

Keyphrases □ Ampicillin, sodium and trihydrate—pharmacokinetics □ Dicloxacillin, sodium—pharmacokinetics □ Absorption rates, half-lives, intramuscular injection—penicillins □ Biological availability—penicillins

The absorption rates of various dosage forms can be quantitated, at least comparatively, without knowledge of the absolute amount of drug absorbed (1); but this amount must be known to estimate availability reliably. If biological availability is defined in terms of the amount of drug that appears in the blood, the only method of drug administration certain to provide 100% of the dose to the blood is intravenous injection. Loo and Riegelman (2) developed a method for calculating the rate and extent of absorption of a drug from an extravascular dosage form when intravenous data are available. Often it is not practical during early stages of drug product development to administer a new drug intravenously to humans, and comparisons must be made between the dosage form candidate and a "readily available" extravascular dosage form. However, the use of extravascular reference dosage forms has important limitations which must be recognized by the investigator. It is not unusual to find that some drugs are not completely absorbed even from oral solutions. On the other hand, it is generally considered safe to assume that an intramuscular injection is completely absorbed. Intramuscular injections of penicillins do not appear to be completely absorbed; this paper deals with the hazards of interpreting penicillin serum data pharmacokinetically, especially with regard to biological availability, on the basis of intramuscular data.

The intramuscular route of administration is traditional for the penicillins, and many methods have been used to prolong their release from intramuscular injection sites. For example, suspensions of relatively insoluble penicillin salts have been used for this purpose. Ampicillin trihydrate is a relatively water-insoluble, chemically stable powder, and its potential use in formulating a sustained-release intramuscular dosage form of ampicillin was investigated. A dosage form that produces slow absorption of a drug which is rapidly eliminated presents an interesting pharmacokinetic case. If the kinetic model is assumed to be:

$$\text{dosage form} \xrightarrow{k_a} \text{blood} \xrightarrow{k_e} \text{eliminated drug}$$

Scheme I

and both processes are first order, it can be shown (3) that the rate constant corresponding to the descending curve of a semilogarithmic plot of blood level *versus* time is not necessarily k_e. In fact, such a procedure yields the smaller of the two rate constants whether it be k_a or k_e. In this paper, penicillin serum level data will be presented which demonstrate this point, and methods for handling such data will be discussed.

The purposes of these studies were: (*a*) to compare the rates of release from intramuscular injection sites