Pharmacokinetics of Intravenous and Oral 1,2-*O*-Isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose Hydrochloride in the Dog as a Function of Dose and Characterization of Metabolites

EDWARD R. GARRETT ˣ **and ACHIEL VAN PEER**

Received April 16, 1982, from *The Beehive, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.* Accepted for publication August 6, 1982.

Abstract □ The pharmacokinetics of 1,2-*O*-isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose hydrochloride (I) was studied in dogs at intravenous and oral doses of 1–50 mg/kg. There was no significant difference between the electron-capture GLC of the heptafluorobutyric derivative of I and the radiochemical assay of chloroform extracts of plasma and urine for 1- to 20-mg/kg doses. Urinary amounts of I measured by GLC were 20% lower than radioassays of chloroform extracts at the 50-mg/kg dose. The pharmacokinetics of intravenous I was described by a two-compartment body model with sequential plasma half-lives of 7.5 ± 0.7 and 136 ± 6 min. No apparent dose-dependent pharmacokinetics for I was observed on intravenous or oral administration. The apparent volume of distribution of the central compartment, 13.1 ± 0.7 liters, is approximately the volume of the total body water in a 20-kg dog. The apparent overall volume of distribution of 40.0 ± 1.5 liters exceeds the total body water, indicative of sequestration of I in tissues. Total and renal clearances were 205 ± 5 and 155 ± 5 ml/min, respectively. The high renal clearance of I indicated an excess of tubular secretion. Renal clearance of I was not dependent on urine flow nor urine pH. Recovery of radioactivity in the feces after I was intravenously administered was <1%. Plasma protein binding of I was <5%, and the erythrocyte–plasma water partition coefficient was approximately unity. Compounds excreted in urine were separated into chloroform-extractable (pH 12), ethyl acetate-extractable (pH 2), and unextractable fractions which were further characterized by TLC. A multiple-extraction system was developed to estimate relative amounts and intrinsic partition coefficients of these extractable compounds from radioactivity counts of scraped plates and was applied to the assay of these compounds in the urine after intravenous administration of I. There was a readily chloroform-extractable metabolite with an apparent partition coefficient of 3.3 and R_f 0.43 on TLC in the systems used. This apparent major metabolite could account for 8% of the administered radioactivity. Minor chloroform-extractable metabolites (0.8–3.3%) had lower apparent partition coefficients (0.26) but R_f values of 0.28 and 0.44. Ethyl acetate-extractable compounds (1.3–2.7%) had an apparent partition coefficient of 0.81 with R_f values of 0.52 and 0.68. Three unextractable compounds had R_f values of 0.20, 0.50, and 0.62 and accounted for 0.16, 2.8, and 0.9% of the administered radioactivity.

Keyphrases □ 1,2-*O*- Isopropylidene-3- *O*-3′- (*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose—pharmacokinetics, intravenous and oral doses, dogs □ Metabolites—1,2-*O*-isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose, multiple extraction, radiochemical analysis in urine, dogs □ Pharmacokinetics—1,2-*O*-isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose and metabolites, dogs, intravenous and oral doses

The properties, stability, assay, and preliminary pharmacokinetics in a dog of the immunomodulatory 1,2-*O*-isopropylidene-3-*O*-3′ - (*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose hydrochloride (I) were described previously (1). This initial pharmacokinetic study in a dog showed that 60–80% of I was renally excreted unchanged and 20–40% as unidentified metabolites at doses of 2 and 10 mg/kg iv. This contrasted with the urinary recovery of the GLC-assayed I in humans of 93 ± 2% in preliminary pharmacokinetic studies, indicative that only a small fraction was probably metabolized (2). Assays of chloroform-extracted radioactivity at pH 11 from plasma and urine appeared to be synonomous to electron-capture GLC assays of the heptafluorobutyric derivative of I, indicating the greater polarity of the unextracted metabolites.

This paper presents detailed studies on the pharmacokinetics of I in dogs after intravenous and oral administration as a function of dose (1–50 mg/kg). A fractionation and multiple-extraction system is presented that permits the quantitative assays of unidentified metabolites and the estimations of the partition coefficients of extractable metabolites from radioassays of sequential organic extracts.

EXPERIMENTAL

Materials—The following analytical grade materials were used: ammonium hydroxide[1], volumetric concentrations of sodium hydroxide[2] and hydrochloric acid[2], chloroform suitable for GC[3], ethyl acetate[3], isopropyl alcohol[3], methanol[3], *n*-propyl alcohol[4], 30% hydrogen peroxide[4], and a toluene-based scintillation cocktail[4]. 1,2-*O*-Isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose hydrochloride[5] (I) and radiolabeled I (randomly ^{14}C-labeled in the glucose component[6], 324.0 mCi/mmole) were used to prepare doses for the pharmacokinetic studies. The TLC plates used were silica gel G-coated glass plates[7].

Purity of ^{14}C-Labeled I—Radiolabeled [^{14}C]I applied on TLC and developed with *n*-propyl alcohol–ethyl acetate–water–ammonium hydroxide (6:1:4:1) had a 98% recovery of the administered radioactivity at the R_f (0.67) of I. No significant radioactivity over background was observed for its hydrolysis product, 3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-D-glucofuranose hydrochloride, at R_f 0.30. In addition no significant radioactivity was observed over background at any other R_f value.

Plasma Protein Binding of I by Ultrafiltration—Fresh dog plasma was spiked with 0.1–100 μg/ml ^{14}C-labeled I (47,000 dpm). An aliquot (0.1 ml) was taken prior to filtration to determine total radioactivity. Spiked plasma (2.00 ml) was centrifuged through ultrafiltration cones[8] at 3000 rpm for 10 min for no more than 30% (usually 10%) filtration of the plasma. Aliquots (0.1 ml) of the ultrafiltrates were assayed by liquid scintillation counting (2). Membrane binding (3–29%) of ^{14}C-labeled I was found by ultrafiltration of spiked plasma water samples and subtracted from the studies of spiked plasma.

Red Blood Cell–Plasma Water Partitioning of I—Red blood cells obtained from centrifugation of fresh, heparinized dog blood were washed five times with isotonic saline[9], recentrifuged, and resuspended in plasma water obtained by ultrafiltration to give a hematocrit of 46. Aliquots (2.00 ml) of the suspensions were spiked with 0.01–100 μg/ml of ^{14}C-labeled I (47,000 dpm), incubated at 25° for 30 min, and centrifuged at 3000 rpm for 10 min. Aliquots (0.1 ml) of the original suspension and the separated

[1] Mallinckrodt, Saint Louis, MO 63147.
[2] Ricca Chemical Co., Arlington, TX 76012.
[3] Burdick and Jackson Laboratories, Muskegon, MI 49442.
[4] Scinti-Verse, Fisher Scientific Co., Fair Lawn, NJ 07410.
[5] Lots 3646 and 13553; Greenwich Pharmaceuticals, Greenwich, CT 06830.
[6] Lot 1141-220; New England Nuclear, Boston, MA 02118.
[7] Analtech, Newark, DE 19711.
[8] Centriflo ultrafiltration membrane cones, CF50; Amicon Corp., Lexington, MA 02173.
[9] Sodium Chloride Injection USP; McGaw Laboratories, Irvine, CA 92714.

0022-3549/83/0900-1045$01.00/0

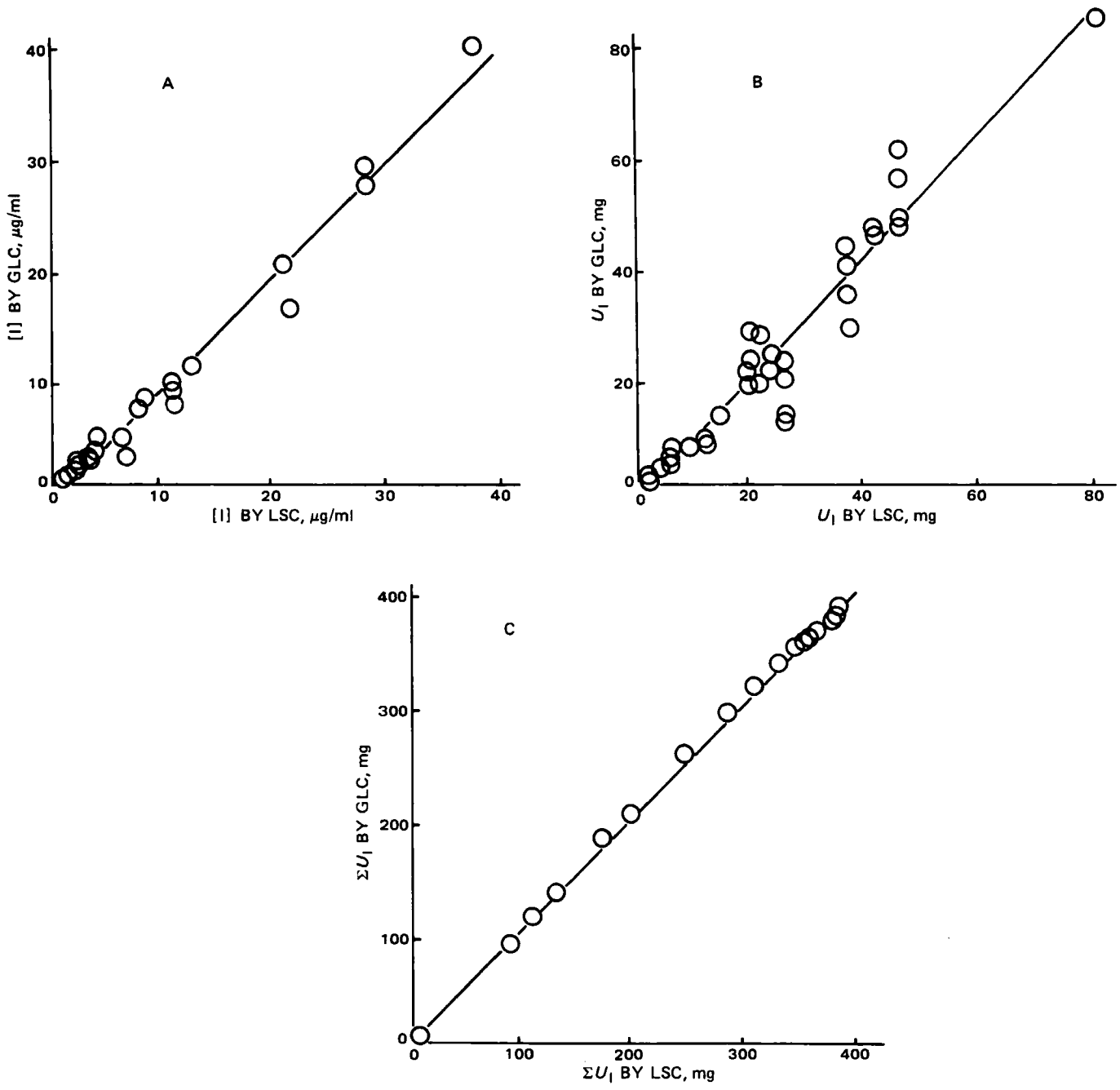


Figure 1—*Linear regressions of (A) concentrations of I ([I]) in plasma, (B) amounts of I (U_I) excreted in the urine in a given time interval, and (C) cumulative amounts of I (ΣU_I) excreted in the urine at a given time assayed by electron-capture GLC on the radioassay (LSC) of chloroform extracts. Dog A was administered 24.0 mg/kg iv of I.*

plasma water were assayed for radioactivity after digestion with 1.00 ml of a mixture of solubilizer[10]–isopropyl alcohol (1:1 v/v) and 0.5 ml of 30% hydrogen peroxide.

The partition coefficient, D, was calculated from the equation cited in the literature (3) where the fraction of bound I was taken as zero. Partition coefficients between red blood cells and plasma water were also determined as a function of time for a 1-μg/ml spiked red blood cell suspension.

Analysis of ^{14}C-Labeled I in Plasma and Urine—The electron-capture GLC assay of the heptafluorobutyric derivative of I and the radioassays after chloroform extraction of plasma and urine were described previously (1).

Pharmacokinetic Studies of I in Dogs—One male dog (A) received intravenous doses of 1.9, 2.0, 10.0, 24.0, and 48.4 mg/kg and oral doses of 5.1 and 20.0 mg/kg of ^{14}C-labeled I. Except for the 2.0 and 10.0 mg/kg iv studies, effected respectively 1 year and 6 months before the others (1), the studies had intervals of 3 weeks. Four other male dogs (B, C, D, and E) were intravenously injected with ~1, 5, and 10 mg/kg at ~3-week intervals. Intravenous doses of known specific activity were injected into the jugular catheter[11] by a three-way stopcock[12] over 10 sec. The dosing stopcock and catheter were flushed with 10.0 ml of isotonic saline. In the oral studies, a tube was placed into the stomach of dog A to administer 10.0 ml of the solution of ^{14}C-labeled I. The stomach tube was flushed twice with 50.0 ml of water before it was removed.

Blood samples after oral administration were withdrawn at 2 and 5 min, every 5 min up to 60 min, every 10 min up to 90 min, and then at the same times as previously reported for the intravenous studies (1). Urine samples in oral studies were collected at the same times as for intravenous studies (1). Further details of the treatment of the dogs, before and on the days of the experiments, the collection times of plasma and urine for

[10] Bio-Solv solubilizer; Beckman Instruments, Fullerton, CA 92634.

[11] Intracath, intravenous placement unit, catheter size 16 gauge, catheter length 12 inch, needle size 14 gauge; Deseret Pharmaceutical Co., Sandy, UT 84070.

[12] Pharmaseal Inc., Toa Alta, PR 00758.

Table I—Comparison of GLC (y) and LSC (x) Assays of Chloroform Extracts of Plasma and Urine With Time by Regression Analysis[a] of the Plasma Concentration of I after Intravenous and Oral Doses to Dog A

Assay	Dose, mg/kg	n	Range	$m \pm s_m$	$b \pm s_b$	$s_{y \cdot x}$	r
[I], μg/ml	24 iv	26	1–40	1.006 ± 0.032	−1.11 ± 0.43	1.52	0.988
	48.4 iv	39	0.5–120	0.724 ± 0.033	0.91 ± 1.29	6.13	0.963
		38[b]	0.5–85	0.941 ± 0.029	−2.36 ± 0.77	3.26	0.984
	20.0 po	30	0.3–15	1.161 ± 0.070	0.03 ± 0.46	1.40	0.952
U_I, mg	24 iv	51	0.4–85	1.074 ± 0.040	−1.89 ± 1.09	4.69	0.968
	48.4 iv	25	0.1–130	0.820 ± 0.013	−1.19 ± 0.72	2.62	0.997
	20.0 po	35	2–55	0.927 ± 0.094	1.23 ± 2.10	6.23	0.863
ΣU_I, mg	24 iv	18	5–380	0.992 ± 0.009	6.27 ± 2.61	4.46	0.999
	48.4 iv	25	81–920	0.795 ± 0.007	−4.11 ± 5.38	9.20	0.999
	20 iv	19	5–365	1.019 ± 0.008	−3.11 ± 1.91	4.06	0.999

[a] $y \pm s_{y \cdot x} = m (\pm s_m)_x + b (\pm s_b)$, where the constants m and b are the slope and intercept, respectively, of the regression of GLC assays of I (y) on LSC (x) assays of chloroform extracts of plasma and urine, and $s_{y \cdot x}$, s_m, and s_b are the respective standard errors of estimate; r is the correlation coefficient (4). [b] The 1-min plasma sample was excluded from the regression analysis.

the intravenous studies, and the handling of the samples were given previously (1).

Treatment of Feces—Feces were collected at 24-hr intervals and homogenized with a 10-fold volume of distilled water. An aliquot (0.2 ml) was transferred to a scintillation vial[13]; 0.5 ml of solubilizer[10] was added and the mixture was digested at 50° for 1 hr in a water bath. After cooling, 1 ml of 30% H_2O_2 was added dropwise, and the mixture was gently swirled for 30 min. After air bubbles were eliminated, 15 ml of liquid scintillation fluid was added. Samples were dark-adapted overnight before counting.

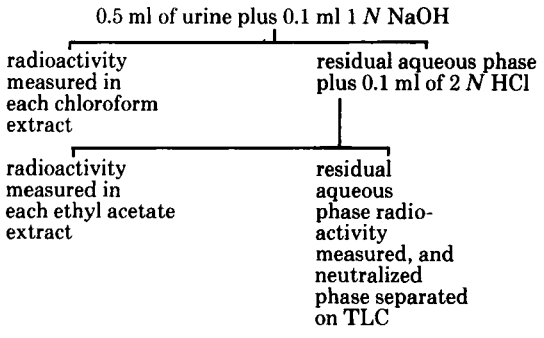
Extraction of Urine with Various Organic Solvents and at Different pH Values—The strategy of extractions is given in Scheme I. Aliquots of urine (0.5 ml), before and at various times after intravenous administration of 1017 and 43.1 mg of [^{14}C]I (48.4 and 1.9 mg/kg), from dog A were alkalinized to pH 12 with 0.1 ml of 1 N NaOH. Radioactivity before extraction was counted after addition of 10 ml of liquid scintillation fluid to 0.02 ml of the alkalinized urine. Alkalinized samples were then extracted sequentially 15 times with 5.0 ml of water-saturated chloroform. Each chloroform extract was transferred to a scintillation vial and evaporated under a nitrogen stream. The dried residues were each reconstituted in 10 ml of liquid scintillation fluid, and the radioactivity was counted.

The aqueous phase, previously extracted 15 times with chloroform, was acidified with 2 N HCl to pH 2, and then extracted seven times sequentially with 5.0 ml of water-saturated ethyl acetate. Each ethyl acetate extract was transferred to a scintillation vial and evaporated under a nitrogen stream. The dried residues were each reconstituted in 10 ml of liquid scintillation fluid to count radioactivity.

After all chloroform and ethyl acetate extractions, 10.0 ml of liquid scintillation fluid was added to 0.05 ml of the aqueous phase to count unextractable radioactivity. The remaining aqueous phase was neutralized with 2 N NaOH to pH ~7, and an aliquot (0.05 ml) was applied on a silica gel-coated TLC plate. Aliquots of solutions of the administered radiolabeled drug, 585 and 1056 ng in 0.6 ml of blank urine and distilled water, respectively, were treated similarly (Scheme I).

TLC Separation of Chloroform- and Ethyl Acetate-Extractable and Unextractable Radioactivity—An aliquot (0.05 ml) of the final aqueous phase, previously multiple extracted by chloroform and ethyl acetate, was neutralized, spotted on a silica gel G-coated glass plate, and developed with n-propyl alcohol–ethyl acetate–water–ammonium hydroxide (6:1:4:1). The TLC plate was scraped in 0.5-cm sections after development to 15 cm and being dried in air. The scrapings were transferred to scintillation vials and dissolved in 3.0 ml of water. Liquid scintillation fluid (10.0 ml) was added, and the vials were counted for radioactivity after overnight dark adapting.

Samples of repetitive chloroform and ethyl acetate extracts of a 20.0-ml combined sample of 120-, 180-, and 240-min urine and of 20.0 ml of 665-min urine were evaporated, reconstituted in 50–100 μl of solvent, spotted on TLC, and developed similarly. Similar studies were effected for the spiked blank urine solutions of radiolabeled I.



Scheme I—Multiple extractions of urine taken at various times after intravenous administration of 48.4 mg/kg of ^{14}C-labeled I to dog A.

[13] Kimble, Division of Owens-Illinois, Toledo, OH 43668.

RESULTS AND DISCUSSION

Plasma Protein Binding and Red Blood Cell–Plasma Water Partitioning of I—Plasma protein binding of I by ultrafiltration was 3.4 ± 2 (*SEM*)%, n = 4, when corrected for membrane binding and was independent of the spike concentration (0.1–100 μg/ml). This was similar to the plasma protein binding, 1.5 ± 1.9%, in humans (2). The red blood cell–plasma water partition coefficient, D, was not concentration dependent (0.01–100 μg/ml of red blood cell suspension), and was approximately unity [0.99 ± 0.08 (*SEM*), n = 5], close to the 0.95 ± 0.04 of human erythrocytes (2). There was also no time-dependent distribution of I in the erythrocytes, measured between 5 and 60 min at 1 μg/ml.

Recovery of Radioactivity in Feces—Negligible amounts (0.3 and 0.1%) of the administered radioactivity were found in the feces of dog A after intravenous administrations of 40 (0.3%) and 200 mg (0.1%) of radiolabeled I.

Comparison of Radiolabeled and GLC Assays of I—Plasma sampled from dog A as a function of time (1–600 min) after intravenous administration of 24.0 and 48.4 mg/kg and after oral administration of 20.0 mg/kg of ^{14}C-labeled I were analyzed for I by electron-capture GLC and by the radioassay of the single 5-ml chloroform extracts of 500 μl of solution containing 0.5–500 μl of plasma. The assays were linearly related (Fig. 1A). The regression coefficients, m, were not significantly different than unity, nor were the intercepts, b, different than zero (Table I) for the 24- (iv) and 20- (po) mg/kg doses. There is a possibly greater assay value by radioassay than GLC at the 48.4-mg/kg dose, indicating a small amount of chloroform-extractable metabolite, although elimination of the 1-min plasma sample increased the regression coefficient to 0.94 ± 0.03.

Similarly, the amount of I excreted in the urine, U_I and the amounts cumulatively excreted in the urine, ΣU_I, with time (15–900 min) were the same by the GLC assay and the radioactivity counting of the single 5-ml chloroform extracts of 500 μl of a solution containing 0.02–500 μl of urine (Fig. 1B and C) at 24.0-mg/kg iv and 20.0-mg/kg po doses. However, GLC assays of urinary I at the 48.4-mg/kg iv dose were 20% lower than radioassays of the chloroform extracts, as ascertained by regression analysis (Table I), indicating the presence of significant amounts of chloroform-extractable metabolites at this dose.

The good correlation between the GLC assays of I and the radioassays of chloroform extracts of plasma and urine at the 2- and 10-mg/kg iv doses in dog A had been reported previously (1). It can be concluded that any radiolabeled metabolite extracted in a single chloroform extraction of

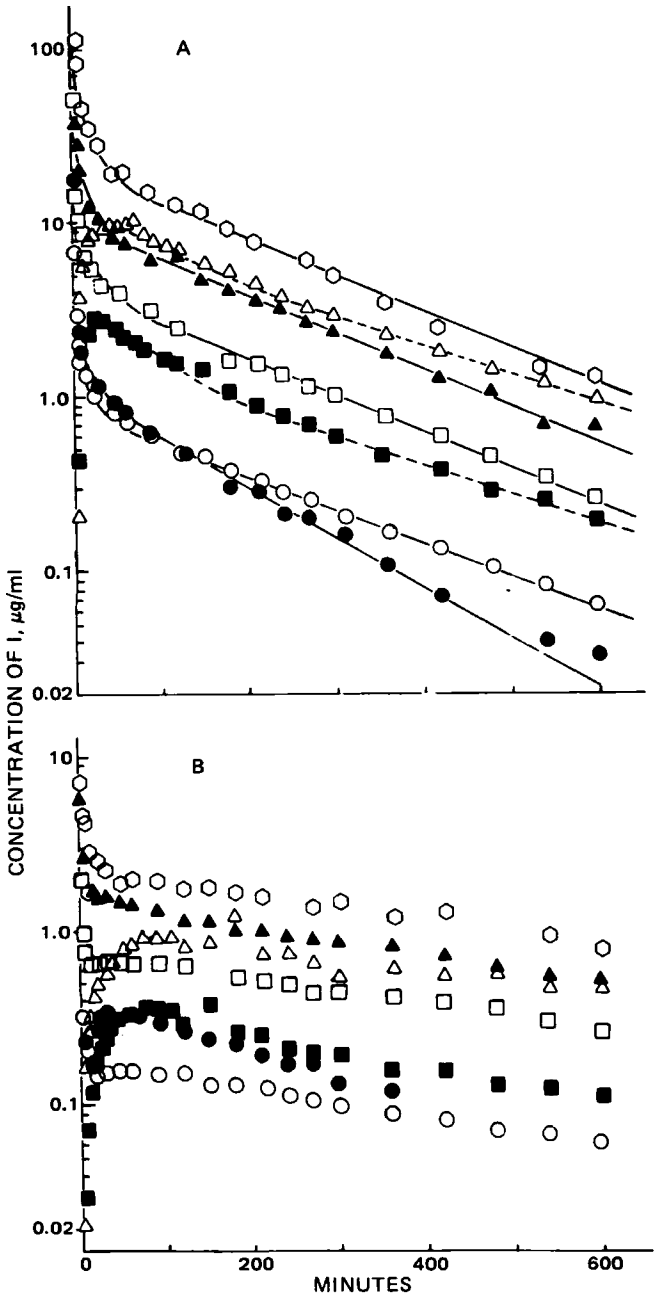


Figure 2—*Semilogarithmic plasma concentration* versus *time plots of (A) I determined by radioassay (LSC) of chloroform extracts and (B) total metabolites determined by direct radioassay of chloroform-extracted aqueous phases after administration of ^{14}C-labeled I to dog A at mg/kg doses of (○) 1.9 iv, (●) 2.0 iv, (□) 10.0 iv, (▲) 24.0 iv, (○) 48.4 iv, (■) 5.1 po, and (△) 20.0 po. Solid lines were drawn in accordance with [I] = $Ae^{-\alpha t} + Be^{-\beta t}$ where parameters are given in Table II.*

plasma or urine at pH 12 for low doses of I (2 to 24 mg/kg) did not disturb assays of I by LSC, although some radiolabeled metabolite(s) were extracted with chloroform at the high dose of 48 mg/kg iv.

Pharmacokinetics of Intravenously Administered I—The semilogarithmic plots of the plasma levels of I with time at doses of 1.9, 24.0, and 48.4 mg/kg iv and at the previously reported 2.0 and 10.0 mg/kg iv doses in dog A (Fig. 2A) and at doses of 1, 5, and 10 mg/kg iv in dogs B, C, D, and E showed primarily biphasic curves, indicative of a two-compartment body model for I. The lines drawn through the experimental points were calculated from the general equation $[I] = Ae^{-\alpha t} + Be^{-\beta t}$ in μg/ml of plasma where the parameters A, B, α, and β are listed in Tables II and III for the intravenous data. Similar curves were obtained for the studies in the other dogs.

In all intravenous studies the first 1-min data point after administration was omitted to fit the experimental plasma values to a sum of two

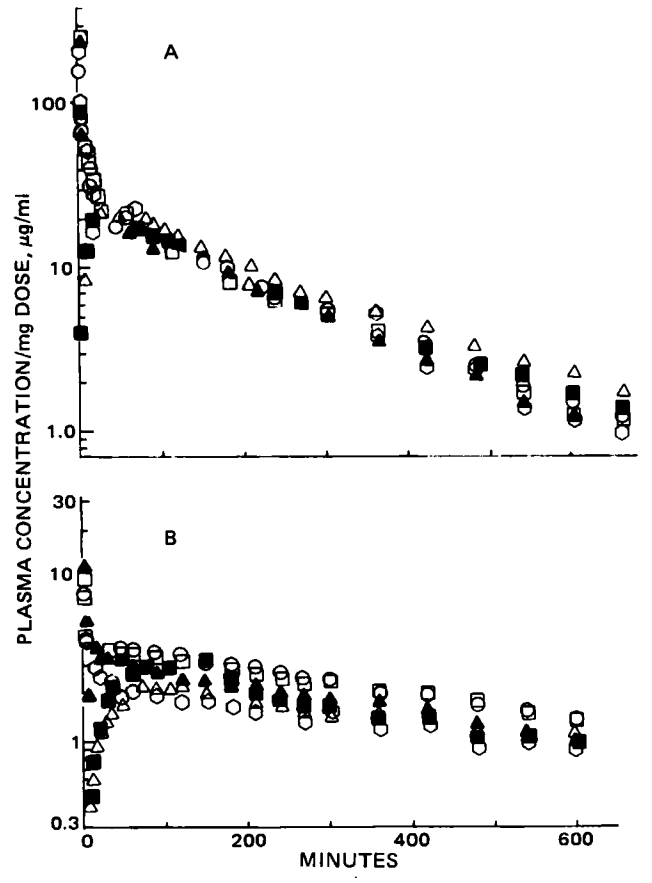


Figure 3—*Semilogarithmic plots of plasma concentrations with time of (A) I per milligram administered dose determined by radioassay (LSC) of chloroform extracts and (B) total metabolites, in equivalents of I per milligram administered dose, determined by direct radioassay of chloroform-extracted aqueous phases after administration to dog A of ^{14}C-labeled I in mg/kg doses of (○) 1.9 iv, (□) 10.0 iv, (▲) 24.0 iv, (○) 48.4 iv, (■) 5.1 po, and (△) 20.0 po. Drawn curves through iv data were in accordance with $[I]/D_0 = (A/D_0)e^{-\alpha t} + (B/D_0)e^{-\beta t}$.*

exponentials. This 1-min plasma concentration was significantly higher than the concentration calculated from this sum in all studies and indicated a possibly faster initial distribution phase with an apparent half-life of 0.5–1.0 min. It could not be explained by the first samples being contaminated with residual ^{14}C-labeled I, which was administered into the jugular catheter, since plasma levels of I obtained simultaneously from the jugular catheter and a leg vein catheter were identical in both the intravenous 48.4-mg/kg and 1.0-mg/kg doses of I administered to dogs A and C, respectively. Subsequent samples up to 1 hr from both sites also showed coincident plasma levels.

The average plasma half-lives of the distribution and elimination phases for all intravenous studies in dogs A, B, C, D, and E were, respectively, 7.5 ± 0.7 (*SEM*) and 136 ± 6 (*SEM*) min and were independent of dose in the range of 1–50 mg/kg (Tables II and III). The plasma levels calculated per milligram unit dose, $[I]/D_0$ with time were reasonably superimposable for all intravenous doses in dog A (Fig. 3A). This was also true for the different dose studies in the other dogs. These facts indicated dose-independent pharmacokinetics of I on intravenous administration.

The plasma levels of total metabolites as equivalents of I were estimated from the radioactivity in the aqueous phase after chloroform extraction of the plasma. The radioactivity was corrected for unextracted I (1), and the resultant curves of apparent total metabolite concentrations in plasma are given directly in Fig. 2B and per milligram of administered dose of I in Fig. 3B. The apparent metabolite plasma levels per milligram of dose (Fig. 3B) decreased slightly, but systematically, with increasing intravenous doses, indicating a possible dose dependence in metabolite formation in contrast to the plasma levels of I. This was also observed for other dog studies at different doses. Since metabolic clearance is only 13% of the total clearance, this apparently minor dose-dependent metabolism would not be expected to significantly affect the observed dose-independent overall pharmacokinetics of I.

Table II—Pharmacokinetic Parameters of I Administered Intravenously to Dog A

Parameter						Mean ± *SD*(*SEM*)
Dose (D_0), mg	40[a]	43.1	200[a]	480	1017	
Dose, mg/kg	2.0	1.9	10.0	24.0	48.4	
Specific activity, dpm/μg	50304	40898	9995	21707	7180	
A^b, μg/ml	3.4	2.6	11.5	28.9	87.4	
B^b	1.19	0.86	5.30	10.73	21.9	
α^b, min^{-1} ($t_{1/2}$, min)	0.195(3.6)	0.119(5.8)	0.160(4.3)	0.101(6.9)	0.082(8.4)	0.131 ± 0.046(0.020) [5.8 ± 1.9(0.9)]
$10^3\ \beta^b$, ($t_{1/2}$, min)	6.68(104)	4.44(156)	5.44(127)	4.98(139)	4.74(146)	5.26 ± 0.88(0.44) [134 ± 20(9)]
Clearances, ml/min						
CL^I_{tot}[c]	204	200	191	197	179	194 ± 10(4)
CL^I_{ren}[d]	135	152[o]	163[o]	162[o]	177[o], 141[n]	158 ± 16(7)[o]
Apparent volumes of distribution, liters						
V_c[e]	8.7	12.4	11.9	12.1	9.3	10.9 ± 1.7(0.8)
V^I_d[f]	30.6	45.0	35.1	39.5	37.8	37.6 ± 5.3(2.4)
$V^I_{dextrap}$[g]	33.6	50.1	37.7	44.7	46.5	42.5 ± 6.7(3.0)
Disposition, fraction of dose[h]						
ΣU^I_∞/Dose	0.60[n], 0.64[o]	0.796[o]	0.82[n], 0.85[o]	0.82[n], 0.82[o]	0.71[n], 0.90[o]	0.80 ± 0.10(0.04)[o]
ΣU^M_∞/Dose[i]	0.18	0.13	0.15	0.09	0.07	0.12 ± 0.04(0.02)
ΣU^{tot}_∞/Dose[j]	0.980	1.00	1.02	0.98	0.97	0.99 ± 0.02(0.01)
k^k_{TB}, min^{-1}	0.0555	0.0329	0.0542	0.0310	0.0202	0.0388 ± 0.0155(0.0069)
k^l_{BE}	0.0235	0.0161	0.0161	0.0162	0.0192	0.0182 ± 0.0032(0.0014)
k^m_{BT}	0.1227	0.0744	0.0951	0.0588	0.0473	0.0797 ± 0.0300(0.0134)

[a] Pharmacokinetic parameters of 2.0- and 10.0-mg/kg studies were reported previously (1). [b] Parameters estimated from best fit of LSC assay of I in plasma against time (Fig. 2) in accordance with $[I] = Ae^{-\alpha t} + Be^{-\beta t}$; LSC and GLC assays were coincident. The 1-min plasma level datum was omitted from consideration. [c] Total clearance of I, D_0/AUC_∞, where AUC_∞ is the total area under the I plasma level-time plot. [d] Renal clearance of I consistent with the $\Sigma U = CL_{ren}AUC_t$ (Figs. 4 and 6), where AUC_t is the area under the plasma level of I-time plot for the time when the cumulative amount of I in the urine, ΣU, was measured. At the highest dose of 48.4 mg/kg iv, GLC and LSC of extracts were not coincident. [e] Apparent volume of central compartment for I, $Dose/(A + B)$. [f] Apparent overall volume of distribution for I, CL^I_{tot}/β. [g] Apparent extrapolated volume of distribution for I on presumption of one-compartment body model, $Dose/B$. [h] Estimated from asymptotes of ΣU *versus* time plots of Fig. 6. [i] Based on LSC assay of radioactivity in aqueous phase after chloroform extraction of urine. [j] Based on LSC assay of total radioactivity in urine (1). [k] Transfer rate constant from the tissue compartment to the central compartment, $k_{TB} = (A\beta + B\alpha)/(A + B)$ (7). [l] Elimination rate constant from the central compartment, $k_{BE} = \alpha\beta/k_{TB}$ (7). [m] Transfer rate constant from the central compartment to the tissue compartment $k_{BT} = \alpha + \beta - k_{TB} - k_{BE}$ (7). [n] Based on GLC assay of urine. [o] Based on LSC assay of chloroform extract of urine.

Apparent Volumes of Distribution of I—The apparent volumes of distribution (Tables II and III) were referenced to the plasma concentration of I. The average apparent volume of distribution of the central compartment was 13.1 ± 0.7 (*SEM*) liters, $V_c = D_0/(A + B)$, which is equivalent to the total body water in a 20-kg dog (12–14 liters) (5). The average apparent overall volume of distribution, $V_d = CL_{tot}/\beta$, was 40.0 ± 1.5 (*SEM*) liters, which exceeded total body water and indicated tissue sequestration.

Clearances of I—The renal clearances (Tables II and III) were estimated from plots of the urinary excretion rates of I against the plasma concentrations at the midpoint time of each urine collection interval and from plots of the amounts of I cumulatively excreted in the urine at time t (ΣU_t) against the area under the plasma level–time curve at time t (AUC_t), according to the equation $\Sigma U_t = CL_{ren}AUC_t$ (Fig. 4). The presence of a possible initial distribution phase of 0.5–1-min half-life was supported by the fact that the positive intercepts frequently observed for renal clearance plots of ΣU_t against AUC_t when the initial 1-min datum was ignored did not exist when the additional area given by its inclusion was considered. Then the intercept was not significantly different from zero.

The renal clearances [155 ± 5 (*SEM*) ml/min] were independent of dose, urine flow, and urinary pH and indicated an excess of tubular secretion of the negligibly plasma protein-bound I in addition to glomerular filtration, which is in the range of the inulin clearance (85 ± 35 ml/min) in a 20-kg dog (6). Thus, at doses <24 mg/kg, dose-dependent renal clearances cannot be concluded. However, the possibility of lowered renal clearance at higher doses is still open, as the one study at 48.4 mg/kg did not show coincident LSC and GLC assays of extracts of urine, and the renal clearances from the latter assay appeared to be significantly lower (Table II).

Bioavailability on Oral Administration—The fact that no dose dependency was observed on intravenous administration of 1–48.4 mg/kg (Fig. 3A, Tables II and III) demonstrated that that systemic distribution and disposition of I followed first-order linear pharmacokinetics and permits us to estimate the absolute bioavailability of oral solutions of I with respect to intravenous administration by the ratio of areas under the plasma level–time curves. The areas under the plasma level–time curves (Table IV) were calculated by integration to time infinity according to $AUC_\infty = A/\alpha + B/\beta$ for the intravenous doses. They were calculated for the oral doses by the trapezoidal rule to time 900 min and by adding the terminal area estimated by dividing the plasma level at 900 min by the disposition rate constant. The area per milligram oral dose of 5.1 μg·min/ml at the 5.1-mg/kg dose was virtually identical to the averaged area of 5.2 μg·min/ml for the intravenous doses of 1.9–48.4 mg/kg. However, the area per milligram dose of 6.1 μg·min/ml at the 20.0-mg/kg oral dose was 10% higher than the highest area of 5.58 μg·min/ml found for the 48.4-mg/kg intravenous dose. This finding could indicate a possible saturable first-pass metabolism of I at high oral doses. This was also indicated by the fact that the plasma concentrations per unit dose were slightly higher for the higher oral dose (Fig. 3A).

Absorption Rates of Orally Administered I—The rates of absorption of I were determined by the Loo–Riegelman deconvolution method (7). The values of the apparent volume of distribution of the central compartment (V_c) and the parameters α, β, and A and B per milligram intravenous dose necessary for estimates of the microscopic rate constants between the central and tissue compartments, k_{BT} and k_{TB}, and the

Table III—Pharmacokinetic Parameters[a] of I Administered Intravenously to Dogs B, C, D, and E

Parameters	B			C			D		E		Mean ± *SD*(*SEM*)	Overall Mean ± *SD*(*SEM*) for Dogs A–E
Dose (D_0), mg	19.9	116.1	231.2	22.2	84.6	203.3	24.8	130.6	113.1	220.3	—	—
Dose, mg/kg	0.97	5.05	10.05	1.00	4.15	10.30	1.00	5.10	4.98	9.40	—	—
Specific activity dpm/μg	95691	14381	7531	78618	21797	7779	78746	12617	15229	7206	—	—
A^b, μg/ml	0.734	5.25	11.35	0.857	5.16	11.99	1.10	5.27	11.43	10.86	—	—
B^b	0.423	2.66	5.27	0.515	1.96	5.09	0.412	2.51	2.35	4.49	—	—
α^b, min^{-1} ($t_{1/2}$, min)	0.046(15)	0.087(8.0)	0.092(7.5)	0.074(9.4)	0.086(8.1)	0.094(7.3)	0.100(6.9)	0.094(7.4)	0.112(6.2)	0.084(8.3)	0.087 ± 0.013(0.005) [8.4 ± 2.5(0.8)]	0.102 ± 0.036(0.009) [7.54 ± 2.57(0.66)]
$10^3\ \beta^b$, ($t_{1/2}$, min)	5.24(132)	4.84(143)	5.24(132)	6.47(107)	5.96(116)	5.69(122)	3.83(181)	5.40(128)	5.05(137)	4.08(170)	5.18 ± 0.80(0.25) [137 ± 23(7)]	5.20 ± 0.79(0.20) [136 ± 21(6)]
Clearances, ml/min												
CL^{I}_{tot}[c]	205	190	205	243	218	203	209	251	200	179	210 ± 22(7)	205 ± 20(5)
CL^{I}_{ren}[d]	124	128	161	168	149	184	145	196	154	154	154 ± 20(6)	155 ± 19(5)
Apparent volumes of distribution, liters												
V^{I}_{c}[e]	17.2	14.7	13.9	16.1	11.9	12.1	16.3	16.8	8.2	14.4	14.2 ± 2.8(0.9)	13.1 ± 2.9(0.7)
V^{I}_{d}[f]	39.1	39.3	39.0	37.5	36.6	35.7	54.5	46.5	39.5	43.9	41.2 ± 5.7(1.8)	40.0 ± 5.7(1.5)
$V^{I}_{dextrap}$[g]	47.0	43.6	43.8	42.9	43.2	40.7	60.1	52.0	48.2	49.0	47.0 ± 5.7(1.8)	45.5 ± 6.2(1.6)
Disposition, fraction of dose[h]												
ΣU^{I}_{∞}/ Dose	0.660	0.795	0.831	0.751	0.741	0.810	0.789	0.799	0.839	0.888	0.79 ± 0.06(0.02)	0.79 ± 0.07(0.02)
ΣU^{M}_{∞}/ Dose[i]	0.233	0.161	0.115	0.193	0.181	0.154	0.160	0.117	0.169	0.103	0.16 ± 0.04(0.01)	0.15 ± 0.04(0.01)
ΣU^{tot}_{∞}/ Dose[i]	0.994	0.995	1.00	1.04	0.979	1.00	0.920	0.971	1.00	1.01	0.99 ± 0.03(0.01)	0.99 ± 0.03(0.01)

[a] Pharmacokinetic parameters were based on LSC. Refer to Table II for footnotes *b*–*j*.

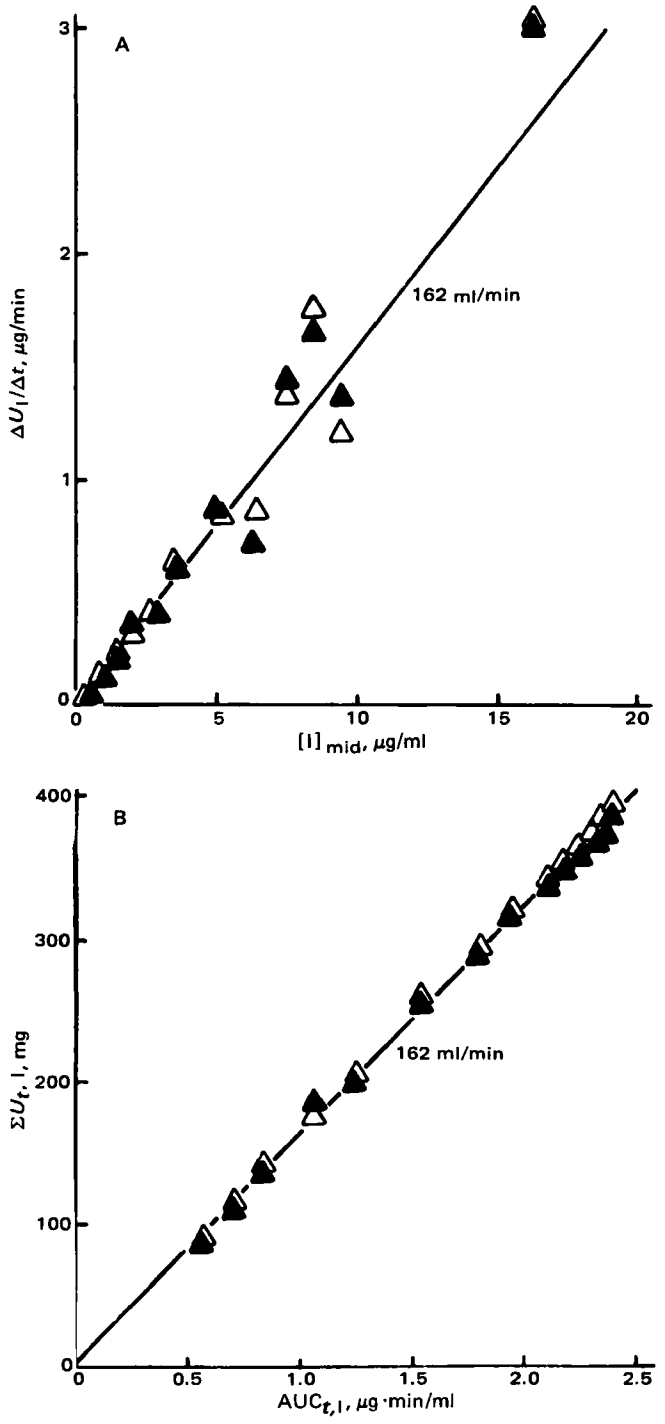


Figure 4—*Typical renal clearance plots for I assayed by GLC (▲) and LSC (△) of extracts, (A) plotting the urinary excretion rates* ($\Delta U/\Delta t$) versus *the plasma levels of I at the midpoint of the urine collection,* ($[I]_{mid}$) *and (B) the cumulative amounts of I excreted in the urine at time* t (ΣU_t) versus *the area under the plasma level–time curve to time* t (AUC_t) *after intravenous administration of 24.0 mg/kg of I to dog A.*

elimination rate constant from the central compartment, k_{BE}, were obtained from the average values of those parameters in the intravenous studies in dog A. The semilogarithmic plots of the percent unabsorbed I with time for the oral doses administered to dog A are given in Fig. 5 and were linear, indicating first-order absorption. First-order rate constants of absorption were 0.017 min^{-1} (absorption $t_{1/2}$ of 41 min) for the 5.1-mg/kg dose and 0.042 min^{-1} (absorption $t_{1/2}$ of 16 min) for the 20.0-mg/kg dose after negligible lag times of 5 and 2 min, respectively. The ratio of the first-order absorption rate constants to the dose was reasonably constant and indicates a possible dose dependence.

Table IV—Areas Under the Plasma Concentration–Time Curves (AUC_∞) of I for Various Intravenous and Oral Doses in Dog A

Dose, mg	AUC_∞, μg·min/ml	AUC_∞/Dose, μg·min/ml·mg
	Intravenous Studies	
40	196	4.90
43.1	215	5.00
200	1047	5.24
480	2442	5.09
1017	5677	5.58
		5.16 ± 0.26(0.12)[a]
	Oral Studies	
112.5	578	5.14
444	2708	6.10
		5.62 ± 0.68(0.48)[a]
		($p < 0.01$)[b]

[a] Mean ± *SD*(*SEM*) are given. [b] *t* Test for two means.

Urinary Recoveries of Intravenously and Orally Administered I—Unchanged I was recovered in the urine for 79 ± 2 (*SEM*)% of the intravenous doses (1–50 mg/kg). Chloroform-unextracted metabolites accounted for 15 ± 1% (Tables II and III, Fig. 6). There were no significant differences between renally excreted total radioactivity and the sum of the cumulative amounts of extracted and unextracted radioactivities (Tables II and III).

The total recovery of radioactivity from the cumulative total urine was 106 and 103% of the administered radiolabeled I for the 5.1- and 20.0-mg/kg oral doses, respectively, in dog A, implying complete absorption. The plots of the cumulative amounts of extracted and unextracted activities, expressed as the percentage of the orally administered 5.1- and 20.0-mg/kg doses in dog A, with time are also given in Fig. 6. The cumulative plots of I were fitted for constant renal clearances of 158 and 141 ml/min for the respective 5.1- and 20.0-mg/kg oral doses; these renal clearances were consistent with the renal clearances for the intravenous doses (2–48.4 mg/kg) in dog A (Table II). The urinary recoveries after 5.1- and 20.0-mg/kg oral doses in dog A were 84.5 and 87.2%, respectively, of the dose for extracted radioactivity and 11.5 and 8.1% for the unextracted radioactivity. The slightly lower yield of urinary metabolites (8.1%) at the 20.0-mg/kg oral dose than at the 5.1-mg/kg oral dose (11.5%) and a slightly higher area under the plasma level–time curve of unchanged I at the 20.0-mg/kg oral dose (6.10 μg·min/ml) than the area for the highest intravenous dose of 48.4 mg/kg (5.58 μg·min/ml) could indicate the possibility of saturable first-pass metabolism of I. However, these differences in metabolite recoveries in the urine and in area under the plasma level–time curves of I were small.

Theoretical Basis for Estimation of Number of Metabolites and Their Partition Coefficients from Multiple Extractions of Urine—The apparent partition coefficient (k) of any single radiolabeled compound can be defined as the ratio of the radioactivity (disintegrations per minute, dpm) in the organic phase (dpm_{org}) over the radioactivity in the aqueous phase (dpm_{aq}):

$$k = \frac{dpm_{org}}{dpm_{aq}} = \frac{dpm_{tot} - dpm_{aq}}{dpm_{aq}} \quad \text{(Eq. 1)}$$

where dpm_{tot} is the total radioactivity in the system and equal to the sum of the radioactivity in the aqueous and organic phases. On rearrangement of Eq. 1, the fraction of unextracted radioactivity after a single extraction can be expressed as:

$$\frac{dpm_{aq}}{dpm_{tot}} = \frac{1}{1+k} \quad \text{(Eq. 2)}$$

Thus, the radioactivity remaining in the aqueous phase after the first extraction ($dpm_{aq,1}$) is:

$$dpm_{aq,1} = \frac{dpm_{tot}}{1+k} \quad \text{(Eq. 3)}$$

and the solution can be extracted with organic solvent for a second time to give:

$$dpm_{aq,2} = \frac{dpm_{aq,1}}{1+k} \quad \text{(Eq. 4)}$$

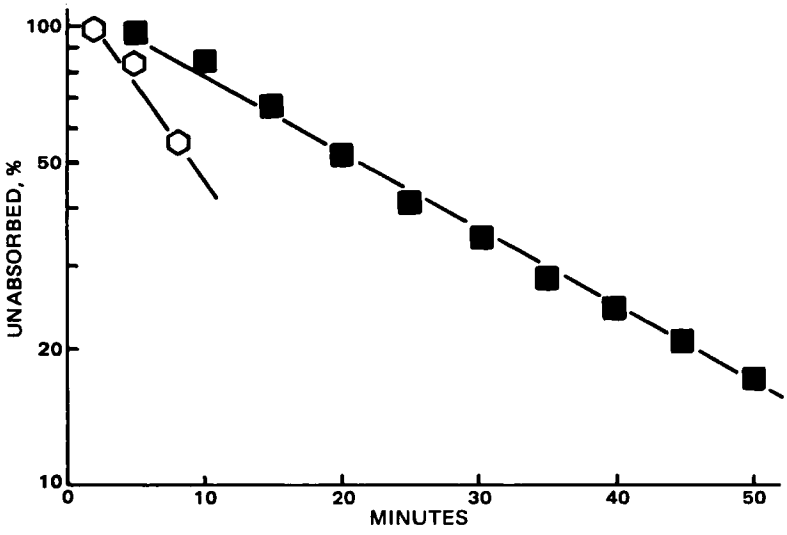


Figure 5—*Semilogarithmic plot of the percent unabsorbed I with time for oral doses of 5.1 (■) and 20.0 (○) mg/kg administered to dog A. Percent unabsorbed I was calculated by the Loo-Riegelman deconvolution method (7).*

Substitution of Eq. 3 into Eq. 4 gives:

$$\mathrm{dpm}_{aq,2} = \frac{\mathrm{dpm}_{tot}}{(1+k)^2} \qquad \text{(Eq. 5)}$$

Thus, the radioactivity left in the aqueous phase after n repetitive extractions is:

$$\mathrm{dpm}_{aq,n} = \frac{\mathrm{dpm}_{tot}}{(1+k)^n} \qquad \text{(Eq. 6)}$$

and

$$\log \mathrm{dpm}_{aq,n} = \log \mathrm{dpm}_{tot} - n\log(1+k) \qquad \text{(Eq. 7)}$$

and a plot of the logarithm of the radioactivity left in the aqueous phase and unextracted ($\mathrm{dpm}_{aq,n}$) *versus* the number of sequential extractions, n, will be linear. The intercept would be the logarithm of the total radioactivity (dpm_{tot}) of the compound before any extraction.

The linearity of the semilogarithmic plot of $\mathrm{dpm}_{aq,n}$ against n (Fig. 7, curves A and A′) implies the possibility of one extractable compound (or several with the same partition coefficient) on ethyl acetate extraction of the acidified chloroform pre-extracted urine samples obtained after intravenous administration of I (curve A) or on spiking blank urine with [^{14}C]I (Curve A′). The total radioactivity (dpm_{tot}) before any ethyl acetate extraction can be estimated from the intercept. This was 1.9% of the total radioactivity in the original urine collection of Fig. 7a, and corresponded to 1.04 mg of the total amount of material as I equivalents in this 370-min urine sample. The apparent partition coefficient of the compound, k, determined from the slope of the line, $\log(1+k)$, in Eq. 7 was 0.815.

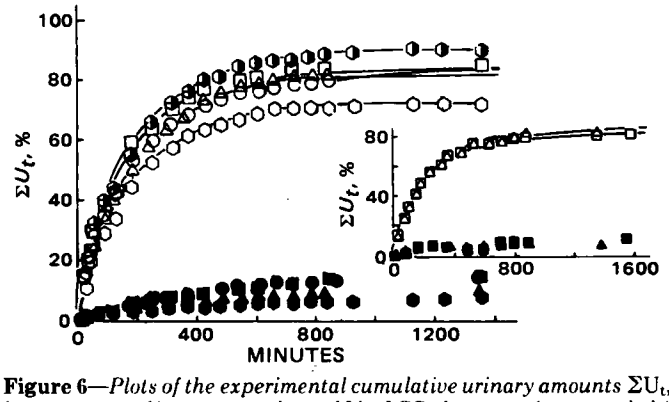


Figure 6—*Plots of the experimental cumulative urinary amounts ΣU_t, in percentage of intravenous dose, of I by LSC of extracts (open symbols) and of total metabolites by LSC of chloroform-extracted aqueous phase (solid symbols), for the 1.9- (○), 10.0- (□), and 24-mg/kg (△) doses of I administered to dog A, against time, where the GLC and LSC assays of urine extracts were coincident (Table I). The different values of the cumulative urinary amounts of I, by GLC (○) and LSC (◑) assays of chloroform extracts of urine after a 24.0-mg/kg dose to dog A, against time are both presented (Table I). Similar plots of the experimental cumulative urinary amounts of I, by LSC assay of extracts, which were coincident with the GLC assays (Table I) for the orally administered doses of 5.1 (□) and 20.0 (△) mg/kg to dog A, against time are given in the insert. Curves through the experimental points of I were fitted according to $\Sigma U = CL_{ren}AUC$ for the constant renal clearances summarized in Tables II and III.*

If there were two extractable compounds with significantly different partition coefficients, the radioactivity remaining in the aqueous phase after n extractions, $(\mathrm{dpm}_{aq,n})_{sum}$, would be the sum of the two radioactivities from each of compounds 1 and 2, $(\mathrm{dpm}_{aq,n})_1$ and $(\mathrm{dpm}_{aq,n})_2$, where each remaining activity could be defined by an equation of the form of Eq. 6, where $(\mathrm{dpm}_{tot})_1$, $(\mathrm{dpm}_{tot})_2$, and dpm_{tot} are the total radioactivities of compound 1, compound 2, and their sum, respectively, in the aqueous system before any extractions were made. Thus:

$$(\mathrm{dpm}_{aq,n})_{sum} = (\mathrm{dpm}_{aq,n})_1 + (\mathrm{dpm}_{aq,n})_2 = \frac{(\mathrm{dpm}_{tot})_1}{(1+k_1)^n} + \frac{(\mathrm{dpm}_{tot})_2}{(1+k_2)^n} \qquad \text{(Eq. 8)}$$

If compound 1 were more readily extracted than compound 2 as n increases to large numbers, there would be eventually no more of the former to be extracted and $(\mathrm{dpm}_{aq,n})_1$ would be equal to zero so that:

$$\lim_{n \to \infty} (\mathrm{dpm}_{aq,n})_{sum} = (\mathrm{dpm}_{aq,n})_2 = \frac{(\mathrm{dpm}_{tot})_2}{(1+k_2)^n} \qquad \text{(Eq. 9)}$$

and the logarithm of both sides would give an equation similar to Eq. 7. This can be seen in the examples of curves B and B′ in Fig. 7 for 15 sequential chloroform extractions at pH 12 of urine sampled 370 min after intravenous administration of ^{14}C-labeled I to dog A and for the [^{14}C]I spiked urine, respectively, where possibly two chloroform-extractable compounds may exist in the urine. The apparent partition coefficients (k_2) of this least readily extracted compound, calculated from the slopes of the terminal data, were 0.29 (curve B) and 0.24 (curve B′). The intercept of these extrapolated linear terminal data permitted the calculation of the radioactivity, $(\mathrm{dpm}_{tot})_2$ due to this compound that contributed to the total radioactivity in the original urine sample:

$$\lim_{n \to \infty} \log(\mathrm{dpm}_{aq,n})_{sum} = \log(\mathrm{dpm}_{aq,n})_2 = \log(\mathrm{dpm}_{tot})_2 - n\log(1+k_2) \qquad \text{(Eq. 10)}$$

Thus, the apparent metabolite 2 accounted for 2.24 mg (as I equivalents) or 4.1% of the total radioactivity in the urine collection of the example of curve B and 0.59% in the example of curve B′.

Since $(\mathrm{dpm}_{aq,n})_2$ can be calculated for any n by Eq. 9 from the estimated k_2 and $(\mathrm{dpm}_{tot})_2$, the logarithm of the difference between this value and the experimentally determined $(\mathrm{dpm}_{aq,n})_{sum}$ can be plotted against n (curve C in Fig. 7) to estimate k_1 and $(\mathrm{dpm}_{tot})_1$ (Eq. 8) from the straight line obtained in accordance with:

$$\log[(\mathrm{dpm}_{aq,n})_{sum} - (\mathrm{dpm}_{aq,n})_2] = \log(\mathrm{dpm}_{aq,n})_1 = \log(\mathrm{dpm}_{tot})_1 - n\log(1+k_1) \qquad \text{(Eq. 11)}$$

In the given example (Fig. 7a, curve C), the readily extractable compound, on the assumption that there was only one, had an apparent partition coefficient of 12.8 and accounted for 47.7 mg (as I equivalents) or 87.3% of the original radioactivity in the 370-min urine collection. For Fig. 7b, curve C′, the readily extractable compound had an apparent partition coefficient of 17.4 and accounted for 97.5% of the total radioactivity of

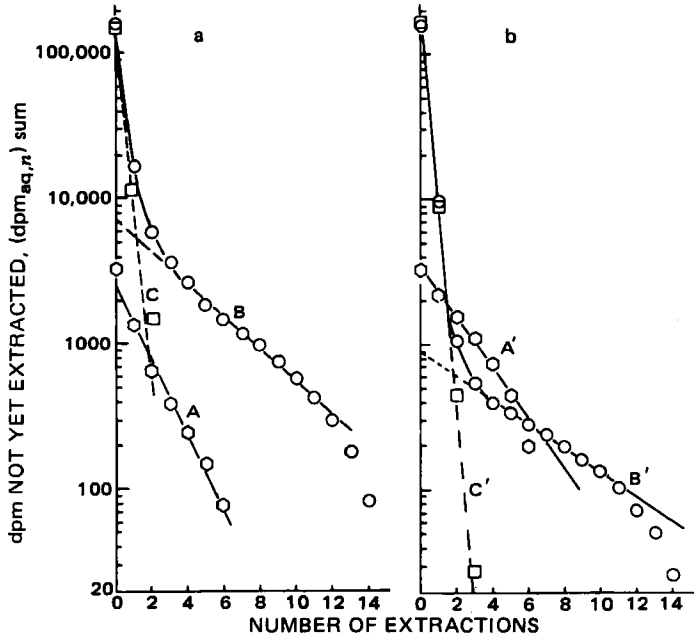


Figure 7—*Typical examples of semilogarithmic plots of not-yet-extracted urine radioactivity against the number of extractions for (a) the 310–370-min urine collection containing a total of 0.177 × 10⁶ dpm after administration of 1017 mg iv of [¹⁴C]I to dog A and for (b) the spiked blank urine containing 1.22 × 10⁶ dpm of standard [¹⁴C]I (585 ng). The ordinate values in (b) are scaled to 0.177/1.22 of their measured values for better comparison with (a). Curves A and A′ are for the 5-ml repetitive ethyl acetate extraction of the 0.6-ml acidified and previously exhaustively chloroform-extracted urines. Curves B and B′ are for the 5-ml repetitive chloroform extractions of the 0.6 ml of urine adjusted to pH 12. Curves C and C′ are the feathered lines obtained from the apparently biphasic curves B and B′.*

the standard [¹⁴C]I. It is evident that the $(dpm_{tot})_i$ and k_i values for each of a mixture of i different compounds of significantly different partition coefficients can be estimated by a series of such deconvolutions using this method, which is similar to the method of residuals used to estimate the parameters of a sum of exponentials.

Application and Limitations of the Method of Multiple Extractions of Urine on Dosing of Radiolabeled I—The semilogarithmic plots of $(dpm_{aq,n})_{sum}$ against the number (n) of sequential chloroform extractions appeared to be reasonably biphasic for the urine samples taken between 15 and 784 min after intravenous administrations of 1017-mg (Fig. 7, curves B and C) and 43-mg ¹⁴C-labeled I. Feathering of the semilogarithmic plots showed at least two radiolabeled chloroform-extractable compounds.

Possible Least Chloroform-Extractable Compound (II) as Metabolite—The least extractable compound (II), resolved from the terminal data, had an average apparent partition coefficient, k_{II}, of 0.264 ± 0.016 (0.004), n = 17, for the 1017-mg dose (Table V) and 0.268 ± 0.038 (0.009), n = 19, for the 43-mg dose (Table VI). It comprised increasing percentages of the urinary radioactivity with time, from 0.4% in the urine collected between 0 and 15 min to 6.4% in the urine collected between 665 and 725 min for the 1017-mg dose and from 0.9% in the urine collected between 0 and 15 min to 16.8% in the urine collected between 790 and 850 min for the 43-mg dose. This implies that II, relative to I, is formed later in the body and/or is eliminated more slowly.

When 0.6 ml of blank urine adjusted to pH 12 containing 1.22 × 10⁶ dpm of [¹⁴C]I[6] (585 ng of specific activity 324 mCi/mmole) was sequentially extracted 15 times, each time with 5 ml of fresh water-saturated chloroform, a semilogarithmic plot of amount radioactivity remaining in the aqueous phase against the number of extractions (n) was remarkably similar in its biphasic form (Fig. 7, curve B′) to that from the urines (Fig. 7, curve B). The slope of the terminal points paralleled that of the terminal points in the urine studies (Fig. 7, curve B) and gave a similar calculated apparent partition coefficient of 0.24 (Eq. 10) as compared with the average of 0.26 ± 0.02 (0.004) and 0.27 ± 0.04 (0.01) for the urine samples listed in Tables V and VI, respectively. A similar study for 950,000 dpm of [¹⁴C]I (0.56 ng of specific activity 137 mCi/mmole) in 0.6 ml of distilled water adjusted to pH 12 gave an apparent partition coefficient of 0.303.

Since the apparent partition coefficients for II in urine and in administered drug were similar, this could imply that this least chloroform-extractable compound II in the urine was a radiolabeled impurity in the original dose of intravenously administered I. The extrapolated intercept of this terminal phase (Fig. 7, curve B′) which permitted the estimation of the original amount of radioactivity of compound II that might contaminate the administered radiolabeled I, gave a value that could be interpreted as accounting for 0.59% of the total radioactivity of I for a radiolabeled purity of the administered I of 99.4%. In the similar aqueous study it accounted for 2.4% for a radiolabeled purity of the administered I of 97.6%, in agreement with the estimated chromatographic purity of I determined before the pharmacokinetic studies. The 2.4% was similar to an estimated 2.7% of II cumulatively excreted at infinite time (2.4% at 784 min) in the urine for the dog administered 1017 mg (Table V), but significantly less than a similar estimate of 8.0% of II for the administered 43-mg dose, (Table VI, 7.4% at 1378 min). This latter fact implies that a significantly greater amount of material of similar properties as the impurity II might result as a renally excreted biotransformation product in the dog.

TLC were made of the repetitive chloroform extracts of the mixed urine samples collected between 120 and 240 min and the urine collected at 605–665 min when the 1017-mg dose was administered intravenously. Definitive radioactivity was observed at R_f values of 0.29, 0.44, and 0.63 in all of the sequential extracts. However, sequential chloroform extracts of the spiked blank urine containing a solution of the administered dose showed radioactivity only at R_f 0.63, coincident with the R_f value of I but not necessarily I. This implied that compounds with R_f 0.33 and 0.47 can result from metabolic processes when the [¹⁴C]I is administered.

The fraction of radioactivity, F, extracted in each of n repetitive extractions of a single compound can be expressed as:

$$F = \frac{\text{dpm in } CHCl_3}{\text{Initial dpm in aqueous phase}} = \frac{k}{(1+k)^n} \qquad \text{(Eq. 12)}$$

Thus:

$$\log(\text{dpm extracted in } CHCl_3) = \log k(\text{initial dpm in aqueous phase}) - n\log(1+k) \qquad \text{(Eq. 13)}$$

The amount extracted into chloroform that appears at a given R_f value can be calculated from the product of the fraction of the total radioactivity applied on the TLC ($dpm_{R_f}/dpm_{applied}$) and the total dpm_{CHCl_3} in a given chloroform extract. This should be linear when plotted semilogarithmically against the number of extractions (n). When such plots were made for amounts at the several observed R_f values (Fig. 8) obtained from the 605–665-min urine sample from dog A after intravenous administration of 1017 mg of [¹⁴C]I, the materials at the three TLC spots all had terminal phases which indicated compounds with approximately equivalent partition values R_f, k: 0.29, 0.204; 0.44, 0.38; 0.63, 0.183. The percentage of these least extractable compounds in that particular urine sample can be estimated from the intercepts of the linearly extrapolated terminal data (Fig. 8, Eq. 13) and were R_f, percent of chloroform-extractable radioactivity, percent of radioactivity in total urine: 0.29, 0.96, 0.83; 0.44, 1.17, 1.02; 0.63, 3.33, 2.89. The 0.83 and 1.02% of total estimates of radioactivity in total urine at the given urine collection (R_f 0.29 and 0.44, respectively) do indicate small amounts of chloroform-extractable compounds produced by metabolism that cannot be assigned to the apparent radiolabeled impurity, II, in the administered radiolabeled dose of I. It is, of course, possible that the compounds at these R_f values are not metabolites of I, but of the apparent radiolabeled impurity, II, in the radiolabeled dose of I.

Possible Readily Chloroform-Extractable Compounds as Metabolites—The apparent partition coefficient of the more readily extractable chloroform compounds, k_{res}, systematically decreased from 19.8 in the 0–15-min urine to 10.9 in the 728–784-min urine for the 1017-mg study (Table V) and from 19.85 in the 15–30-min urine to 6.7 in the 850–1738-min urine for the 43.1-mg study (Table VI). These apparent k_{res} partition coefficients were estimated from the slopes based on first and second repetitive partitions of material from 0.6 ml of urine into 5 ml of $CHCl_3$ (Fig. 7, curve C). The apparent k_{res} partition coefficients for the radiolabeled dose added to urine (curve C′) and water, respectively, were 18.0 and 20.0. Inspection of curve C′ (Fig. 7b) shows no indication of changing partition coefficients for this residual line with increasing n. This strongly implies that not only I is readily chloroform extractable from the urine of dogs administered I, but also a compound, I′, with a slightly lower partition coefficient, and that I′, relative to I, is formed later in the body and/or is eliminated more slowly.

The TLC distribution of sequential chloroform extracts of the 605–

Table V—Data and Constants Estimated from Multiple Extractions of Collected Urines after Intravenous Administration of 1017 mg of I to Dog A[a]

Min[b]	U_{tot}, mg[c]	$U^{tot}_{CHCl_3}$, mg[d]	k_{II}[e]	U^{tot}_{II}, mg[f]	k_{res}[g]	U^{tot}_{res}, mg[h]	k_{ETAC}[i]	U^{tot}_{ETAC}, mg[j]	U^{tot}_{unext}, mg[k]	$U^{R_f\,0.20}_{unext}$, mg[l]	$U^{R_f\,0.50}_{unext}$, mg[l]	$U^{R_f\,0.62}_{unext}$, mg[l]
15	102.5	100.4	0.26	0.41	19.8	100.0	0.81	0.51	1.53	0.308	0.84	0.41
33	117.9	115.1	0.23	1.30	18.3	113.8	0.90	0.71	2.12	0.354	1.30	0.47
47	57.8	56.2	0.25	0.75	18.7	55.4	0.99	0.46	1.16	0.139	0.75	0.23
62	59.2	57.4	0.28	1.07	16.2	56.3	0.69	0.53	1.30	0.000	0.89	0.41
91	78.4	75.5	0.28	1.65	20.1	73.8	0.73	0.94	1.96	0.063	1.25	0.63
122	46.6	44.7	0.28	1.35	12.9	43.3	0.76	0.61	1.40	0.047	0.91	0.47
180	138.5	122.1	0.28	3.74	17.4	128.4	0.80	1.94	4.43	0.125	3.05	1.25
255	116.8	110.2	0.25	2.92	16.2	107.3	0.73	1.64	4.91	0.222	3.39	1.28
310	60.8	56.5	0.25	1.82	15.6	54.7	0.72	0.97	3.34	0.103	2.43	0.79
370	54.7	49.9	0.29	2.24	12.8	47.7	0.82	1.04	3.72	0.087	2.90	0.77
423	38.2	34.3	0.28	1.53	12.8	32.8	0.84	0.73	3.13	0.000	2.66	0.50
484	34.1	30.0	0.25	1.40	10.3	28.6	1.15	0.82	3.34	0.051	2.73	0.58
546	18.9	16.4	0.28	0.96	12.1	15.4	0.81	0.51	2.00	0.053	1.64	0.30
605	26.2	22.7	0.27	1.21	9.8	21.5	1.21	0.66	2.83	0.065	2.25	0.52
665	15.6	13.6	0.25	0.76	13.0	12.8	0.62	0.52	1.54	0.017	1.25	0.28
728	10.7	9.2	0.26	0.69	12.9	8.5	0.74	0.33	1.17	—[m]	—	—
784	5.9	5.0	0.26	0.36	10.9	4.6	0.70	0.21	0.71	—	—	—
Total, mg	982.8	929.2		24.15		904.9		13.12	40.59	1.67	28.24	8.89
Percent of ΣU_{tot}	100	94.5		2.46		92.1		1.33	4.13	0.17	2.87	0.90
Percent of Dose	96.6	91.4		2.37		89.0		1.29	3.99	0.16	2.78	0.87
Mean ± *SD* (*SEM*)			0.26 ± 0.016 (0.004)				0.82 ± 0.16 (0.04)					

[a] Dose of 7.30×10^9 dpm. $\Sigma U_{tot} = \Sigma U^{tot}_{CHCl_3}\ \Sigma U^{tot}_{ETAG} + \Sigma U^{tot}_{unext}$; $\Sigma U^{tot}_{CHCl_3} = \Sigma U^{tot}_{res} + \Sigma^{tot}_{II}$; $\Sigma U^{tot}_{unext} = \Sigma U^{R_f\,0.20}_{unext} + \Sigma U^{R_f\,0.50}_{unext} + \Sigma U^{R_f\,0.62}_{unext}$. [b] Final time of urine collection started at the time listed. [c] Total amount of excreted compound in urine collection, U_{tot}, in I equivalents as determined from total radioactivity in collection, $10^{-3}\,(V/v) \times \text{dpm}_{tot} \div$ specific activity of [^{14}C]I, where V is volume of urine collected, v is volume counted by LSC to obtain the dpm_{tot} values. The specific activity was 7180 dpm/μg. [d] Amount in each urine collection, $U^{tot}_{CHCl_3}$, that can be ultimately extracted into chloroform $10^{-3}\,(V/v) \times \text{dpm}_{CHCl_3} \div$ specific activity. dpm_{CHCl_3} is the total cumulative radioactivity extracted into all of the repetitive chloroform extracts. [e] Estimated from terminal slope, S, of log $(\text{dpm}_{aq,n})_{sum}$ *versus* n where $(\text{dpm}_{aq,n})_{sum}$ is the radioactivity not yet extracted into chloroform or log $(U^{tot}_{CHCl_3} - U^{n}_{CHCl_3})$ and where $U^{n}_{CHCl_3}$ is the cumulative amount extracted into chloroform from a given urine collection up to the nth extraction and k_{II} = antilog $(|S| - 1)$, in accordance with Eq. 10. [f] Estimated cumulative amounts of II that are chloroform extractable from a given urine collection and obtained from the intercept, dpm_{Int}, of the linear extrapolation of the plot of the terminal data as in footnote *e*; $U^{tot}_{II} = 10^{-3}\,(V/v) \times \text{dpm}_{Int} \div$ specific activity. [g] Obtained from slopes (S) of plots of log $[(\text{dpm}_{aq,n})_{sum} - (\text{dpm}_{aq,n})_{II}] = \log (\text{dpm}_{tot})_{res} - n\log (1 + k_{res})$, in accordance with Eq. 11 and calculated for the line through 0 and 1 values of n, where k_{res} = antilog $(|S| - 1)$. The $(\text{dpm}_{aq,n})_{II} = \text{dpm}_{Int}/(1 + k_{II})^n$ where dpm_{Int} is the total cumulative amount of II that is chloroform extractable from the given urine collection and was defined in footnote *f*. [h] Estimated amount of residual material that is not II and is more readily chloroform extractable obtained from the intercept $(\text{dpm}_{tot})_{res}$ of the plot described in footnote *g*, *i.e.*, $10^{-3}\,(V/v) \times (\text{dpm}_{tot})_{res} \div$ specific activity. [i] Estimated from slope, S, of log $(\text{dpm}_{aq,n})$ *versus* n where $(\text{dpm}_{aq,n})$ *versus* n where $(\text{dpm}_{aq,n})$ is the radioactivity not yet extracted into ethyl acetate or log $(U^{tot}_{ETAC} - U^{n}_{ETAC})$. U^{n}_{ETAC} is the cumulative amount extracted into ethyl acetate from a given chloroform pre-extracted urine collection up to the nth extraction and k_{ETAC} = antilog $(|S| - 1)$, in accordance with Eq. 7. [j] Estimated total ethyl acetate-extractable amounts, U^{tot}_{ETAC}, from a urine collection; obtained from the intercept, dpm_{Int}, of the linear plot of log U^{n}_{ETAC} *versus* n, as in footnote *i*. [k] Estimated amounts of unextractable compounds obtained from total radioactivity measurement (dpm_{unext}), in 50 μl of the aqueous phase previously sequentially chloroform and ethyl acetate extracted; $10^{-3}\,(V/v) \times \text{dpm}_{unext} \div$ specific activity. [l] Amounts that remained in urine collection after repetitive chloroform and ethyl acetate extractions and appearing at specified R_f values on TLC plates as determined by plate scraping and LSC counting where $U^{R_f}_{unext} = (\text{dpm}_{R_f}/\text{dpm}_{applied}) \times U^{tot}_{unext}$, where dpm_{R_f} and $\text{dpm}_{applied}$ are the counts measured at the R_f spot and the total counts applied on the plate respectively. [m] Not measured.

Table VI—Data and Constants Estimated from Multiple Extractions of Collected Urine after Intravenous Administration of 43.1 mg of I to Dog A[a]

Min[b]	U_{tot}, mg[c]	$U^{tot}_{CHCl_3}$, mg[d]	k_{II}[e]	U^{tot}_{II}, μg[f]	k_{res}[g]	U^{tot}_{res} mg[h]	k_{ETAC}[i]	U^{tot}_{ETAC}, μg[j]	U^{tot}_{unext}, μg[k]	$U^{R_f\,0.20}_{unext}$ μg[l]	$U^{R_f\,0.35}_{unext}$ μg[l,n]	$U^{R_f\,0.50}_{unext}$ μg[l,n]	$U^{R_f\,0.62}_{unext}$ μg[l]
15	6.30	6.17	0.34	0.57	25.30	6.11	0.52	31.5	101	0	0	101	0
30	3.69	3.56	0.35	137	19.85	3.42	0.65	33.2	100	0	0	38	63
45	2.32	2.23	0.33	125	17.02	2.10	0.73	32.5	67	0	0	38	30
60	2.14	2.03	0.29	124	14.91	1.91	0.81	34.2	73	0	14.1	30	28
90	3.56	3.35	0.27	224	13.92	3.13	0.86	71.2	139	0	21.0	72	46
120	2.85	2.50	0.27	217	15.04	2.28	0.83	57.0	291	3.7	0	157	100
180	5.02	4.64	0.25	377	14.50	4.26	0.84	130.5	251	0	47.7	121	105
250	3.97	3.64	0.24	361	14.59	3.28	0.87	119.1	202	0	47.6	127	64
310	2.74	2.47	0.26	296	15.10	2.17	0.78	95.9	184	0	43.8	104	36
370	1.98	1.77	0.25	218	16.70	1.55	0.83	69.3	139	0	27.7	87	24
430	1.56	1.37	0.23	156	15.37	1.21	0.83	60.8	134	0	23.4	83	28
490	1.52	1.30	0.31	170	13.91	1.13	0.83	65.4	150	0	31.1	102	17
550	1.04	0.877	0.24	121	13.00	0.756	0.84	47.8	115	3.4	0	92	21
610	0.77	0.611	0.24	96	12.26	0.515	0.83	40.8	118	4.5	0	91	22
670	0.59	0.443	0.24	89	9.75	0.354	0.81	36.0	111	6.9	0	84	20
725	0.57	0.415	0.25	76	11.08	0.339	0.82	37.6	118	6.3	0	89	23
790	0.40	0.285	0.24	53	7.47	0.232	0.80	27.2	84	5.0	0	64	16
850	0.37	0.264	0.27	62	8.81	0.202	0.82	26.6	80	4.1	0	61	14
1378	1.58	1.05	0.25	242	6.73	0.807	0.74	133.0	400	—[m]	—	—	—
Total, mg	42.97	38.98		3.20		35.8		1.15	2.86	0.034	0.26	1.54	0.66
Percent of ΣU_{tot}	100	90.7		7.45		83.2		2.68	6.66	0.08	0.61	3.59	1.54
Percent of Dose	99.7	90.4		7.42		82.9		2.67	6.64	0.08	0.60	3.57	1.53
Mean ± *SD* (*SEM*)			0.27 ± 0.04 (0.009)				0.79 ± 0.08 (0.02)						

[a] Dose of 1.76×10^9 dpm. $\Sigma U_{tot} = \Sigma U^{tot}_{CHCl_3} + \Sigma U^{tot}_{ETAC} + \Sigma U^{tot}_{unext}$; $\Sigma U^{tot}_{CHCl_3} = \Sigma U^{tot}_{res} + \Sigma U^{tot}_{II}$; $\Sigma U^{tot}_{unext} = \Sigma U^{R_f\,0.20}_{unext} + \Sigma U^{R_f\,0.35}_{unext} + \Sigma U^{R_f\,0.50}_{unext} + \Sigma U^{R_f\,0.62}_{unext}$. Refer to Table V for footnotes *b*–*m*. [n] These minor amounts of metabolites were found at different R_f values for different urine samples for unexplainable reasons.

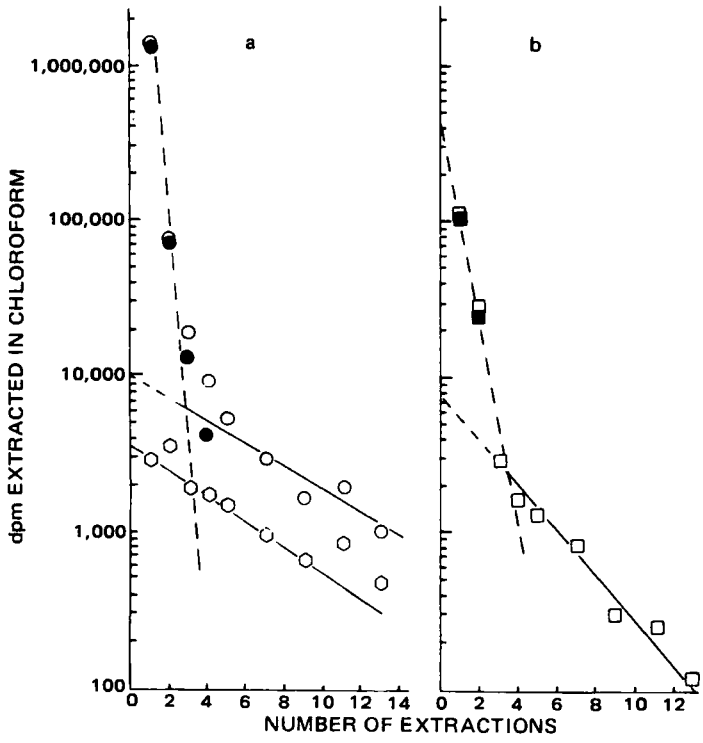


Figure 8—*Semilogarithmic plots against number of extractions (n) of radioactivities (dpm) of different TLC spots at* R_f (○) *0.60–0.69, (□) 0.40–0.47, and (○) 0.27–0.30 from 5.0-ml chloroform extracts of 0.6 ml of pH 12 urine. The urine was collected between 605 and 665 min when 1017 mg of [¹⁴C]I was intravenously administered to dog A. The amounts at each* R_f *value were calculated from* $dpm_{R_f}/dpm_{applied} \times dpm_{CHCl_3}$ *where* dpm_{R_f}, $dpm_{applied}$, *and* dpm_{CHCl_3} *are the radioactivities at the specified* R_f *values, total applied to the TLC plate, and the radioactivity extracted at the* n^{th} *chloroform extraction. The solid symbols are the feathered data.*

665-min urine collection from dog A after administration of 1017 mg of [¹⁴C]I gave the data for Fig. 8 with biphasic semilogarithmic plots. The data from the spot at R_f 0.63, when feathered, permitted the estimation of a partition coefficient of 18.0, consistent with I (Fig. 7, curve C'). The intercept of the feathered line permitted an estimation of 86.4% in the total urine collection that is chloroform extractable (75% of total radioactivity in urine samples) and can be assigned to I. The data from the spot at R_f 0.43, when feathered (Fig. 8) permitted the estimation of a partition coefficient of 3.3. The intercept of the feathered line permitted an estimation of 8.1% of this readily chloroform-extractable R_f 0.43 material of $k = 3.3$ in the total urine collection that is chloroform extractable (7.0% of total radioactivity in urine sample). This would be the possible metabolite I', that accounts for the greatest portion of the discrepancy between GLC and total radioactivity assays of I in urine at the 48.4-mg/kg iv dose (Table I).

When material found at R_f 0.43 was eluted, incubated at pH 12 or 7 for 2 hr at 50°, and rechromatographed on TLC, the radioactivity appeared at the same R_f value. When this material was incubated similarly at pH 2, the rechromatographed material appeared at R_f 0.10–0.23. This shift to much lower R_f values with acid hydrolysis is indicative of the loss of an isopropylidene group (1). This fact plus its ready alkaline chloroform extractability indicate that a possible structure assignment to I' is that it is a monodealkylated product of I.

Possible Ethyl Acetate-Extractable Compounds of Acidified Urines as Metabolites—The semilogarithmic plots of $(dpm_{aq,n})_{sum}$ against the number of sequential ethyl acetate extractions (n) of acidified chloroform-extracted urine indicated one radiolabeled extractable compound (Fig. 7, curve A) with an average apparent partition coefficient of 0.82 ± 0.16 (0.04), $n = 17$, for the 1017-mg dose (Table V) and 0.80 ± 0.08 (0.02), $n = 19$, for the 43-mg dose (Table VI). The cumulative amounts of such ethyl acetate-extractable material excreted in the total urine accounted for 1.29 and 2.67% of the administered doses, respectively. A similar analysis of ethyl acetate-extractable material for the [¹⁴C]I spiked into urine (Fig. 7, curve A') and water accounted for 0.6 and 0.7% of the administered compound, respectively, with respective apparent partition coefficients of 0.46 and 0.57 and a sole TLC spot at R_f 0.67. These facts imply that small, but additional, amounts of ethyl acetate-extractable material are formed in the animal. The ethyl acetate extracts of the 120–240-min urine from the 1017-mg dosed animal showed R_f values of 0.52 and 0.68, but only an R_f value of 0.68 in the 605–665-min urine indicates that the ethyl acetate-extractable material has the same R_f as I.

Possible Unextractable Materials as Metabolites—After the multiple extraction of urine with chloroform and ethyl acetate, 4.0% of the administered radioactivity from the 1017-mg dose of [¹⁴C]I was found in the accumulated urine (Table V), whereas 6.6% was found for the 43.1-mg study (Table VI). Only 1.4 and 1.2% of unextractable radioactivity was found in the [¹⁴C]I spiked urine and distilled water, respectively, implying that additional amounts of unextractable material were formed in the animal. The percentage of total unextractable radioactivity in the urine collections increased from 1.5% at 15 min to 12.1% at 784 min (Table V). The residual urine after multiple extractions with chloroform and ethyl acetate was applied to TLC plates, and sections were scraped for LSC counting. A major portion of the applied radioactivity (60–80%) appeared at R_f 0.47–0.53 with 15–30% at R_f 0.60–0.63 and a small amount (0.05%) at R_f 0.17–0.23. These comprised 2.8% (R_f 0.50), 0.87% (R_f 0.62), and 0.16% (R_f 0.20) of the total administered 1017-mg dose (Table V), and 3.6% (R_f 0.50), 1.5% (R_f 0.62), and 0.70% (R_f 0.20 and R_f 0.35) of the total administered 43.1-mg dose.

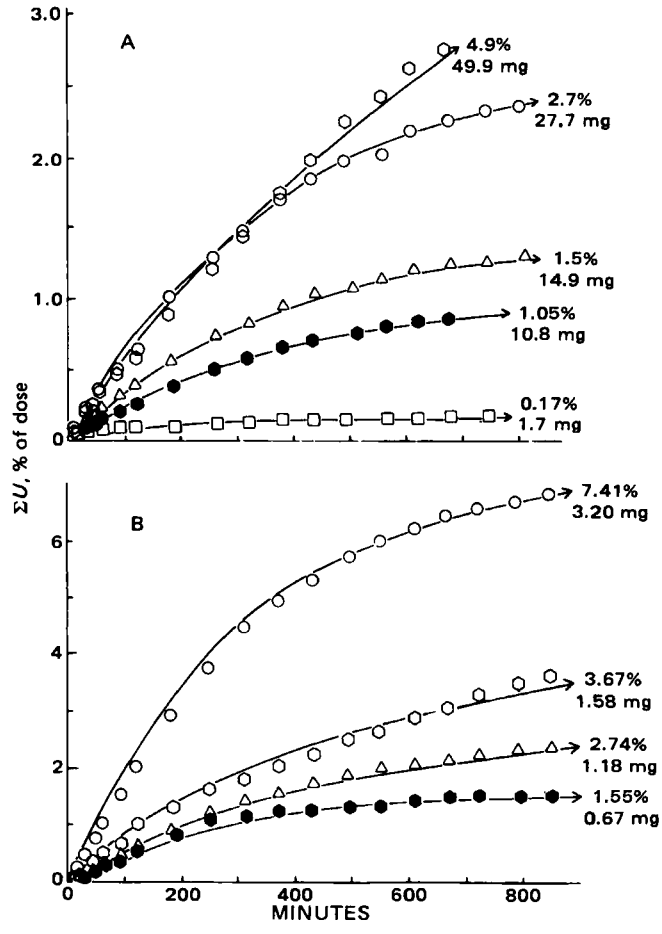


Figure 9—*Cumulative amounts (as percent of administered [¹⁴C]I dose) of variously characterized possible metabolites and/or impurities found in the urine of dog A intravenously administered (A) 1017 mg and (B) 43.1 mg of I. Key: (○) total amounts of II with a partition coefficient of ~0.24 transferred from pH 12 urine (0.6 ml) into repetitive 5-ml chloroform extractions; (△) total amounts of compound transferred from acidified and chloroform pre-extracted urine into repetitive 5-ml ethyl acetate extractions; and cumulative amounts of compounds at* R_f *0.50 (○),* R_f *0.62 (●), and* R_f *0.20 (□) that were unextracted by repetitive chloroform and ethyl acetate extractions. Each curve shows the total amounts of materials and percentage of administered dose estimated from their respective asymptotes. The lines drawn through the values for each curve were calculated from* $\Sigma U = \Sigma U_\infty (1 - e^{-\lambda t})$.

The cumulative amounts of non-I compounds (as percentage of administered radioactivity in the [^{14}C]I dose) are plotted against time in Fig. 9 for the 1017-mg (A) and 43.1-mg (B) doses. The lines drawn through the values were calculated from:

$$\Sigma U = \Sigma U_\infty (1 - e^{-\lambda t}) \qquad \text{(Eq. 13)}$$

The respective half-life values (0.693/λ) in min were: (A) ΣU_{II}, 266; ΣU_{ETAC}, 266; $\Sigma U_{Rf}^{0.2}$, 152; $\Sigma U_{Rf}^{0.5}$, 578; $\Sigma U_{Rf}^{0.62}$, 277; and (B) ΣU_{II}, 231; ΣU_{ETAC}, 300; $\Sigma U_{Rf}^{0.5}$, 278; and $\Sigma U_{Rf}^{0.62}$, 193. Most of these half-lives were approximately the same as I and may indicate the facile renal elimination of these compounds.

REFERENCES

(1) E. R. Garrett, A. Van Peer, H. Mahrous, and W. Schuermann, *J. Pharm. Sci.*, **71**, 387 (1982).

(2) E. R. Garrett, A. Van Peer, P. Altmayer, W. Schuermann, and P. Lücker, *J. Pharmacokinet. Biopharm*, **10**, 247 (1982).

(3) E. R. Garrett and H. J. Lambert, *J. Pharm. Sci.*, **62**, 550 (1973).

(4) Stat-1-22A "Linear Regression" HP-65 Stat Pac I, Hewlett-Packard, Cupertine, Calif., pp. 49–51.

(5) P. L. Altman and D. S. Dittmer, "Blood and Other Body Fluids," Biological Handbooks, Federation of American Societies for Experimental Biology, Washington, D.C., 1961, p. 352.

(6) H. M. Smith, "Principles of Renal Physiology," Oxford University Press, New York, N.Y., 1956, p. 32.

(7) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 55, 136–140.

ACKNOWLEDGMENTS

Supported in part by unrestricted grants from Greenwich Pharmaceuticals Inc., Greenwich, CT 06830 and Kali-Pharma Inc., Elizabeth, NJ 07207. A. Van Peer is grateful to the Belgian Foundation for Medicinal Scientific Research for their support.

The technical assistance of Kathy Eberst and Marjorie Rigby is gratefully acknowledged.

In Vitro Drug Release from Egg Albumin Microcapsules

TAKAFUMI ISHIZAKA ˣ and MASUMI KOISHI

Received February 12, 1982, from the *Faculty of Pharmaceutical Sciences and the Institute of Colloid and Interface Science, Science University of Tokyo, Shinjuku-ku, Tokyo, Japan.* Accepted for publication August 9, 1982.

Abstract □ The *in vitro* release of phenacetin from microcapsules prepared using egg albumin as the membrane material was investigated. It was shown by scanning electron microscopy that the albumin microcapsules have nonsmooth surfaces. The amount of phenacetin released is proportional to the square root of time up to 50–70% drug release. Increases in the albumin concentration and 1-vinyl-2-pyrrolidinone polymer content in the aqueous phases used in the microcapsule preparation have an effect on matrix porosity and channel tortuosity in the matrix of albumin microcapsules. The *in vitro* release rate was found to decrease with increasing albumin concentration and 1-vinyl-2-pyrrolidinone polymer content in the aqueous phases. The *in vitro* release rate per unit area also decreased with decreasing capsule size.

Keyphrases □ Phenacetin—albumin microcapsules, release rate, controlling factors □ Microcapsules, albumin—release rate of phenacetin, effects of albumin concentration, 1-vinyl-2-pyrrolidinone polymer content, capsule size □ Delivery systems—albumin microcapsules, release rate of phenacetin, effect of albumin concentration, 1-vinyl-2-pyrrolidinone polymer content, capsule size

Delivery of a chemotherapeutic agent to desired target sites with drug carriers could achieve effective local drug concentration and minimize systemic side effects by reducing the therapeutic dose of the chemotherapeutic agent. When a drug carrier, such as liposomes, microcapsules, and microspheres, is injected into the circulatory system, its distribution in the body is an important factor in drug delivery. The tissue distribution of albumin microspheres has been studied in detail (1–3). In addition, alteration in the tissue distribution of albumin microspheres was examined using magnetic guidance (4, 5). Albumin microspheres prepared using a water–oil emulsion have a hydrophilic matrix structure, consisting of albumin molecules, that is similar to that in albumin microcapsules (6).

However, despite many reports on the tissue distribution of albumin microspheres, the mechanism of drug release from minute drug carriers that have a hydrophilic matrix structure is not well known because there are few reports on drug release (7, 8). This paper describes *in vitro* drug release from albumin microcapsules having a hydrophilic matrix structure and some controlling factors.

EXPERIMENTAL

Materials—Isooctane, dibasic potassium phosphate, monobasic sodium phosphate, acetic acid, hydrochloric acid, and sodium acetate were reagent grade. Phenacetin powder (250–300 mesh) was used for microencapsulation as the core drug.

Egg albumin[1] solution was prepared as follows: Albumin was dissolved in buffer solution [0.033 *N* KH_2PO_4–0.033 *N* Na_2HPO_4, 1:16 (v/v); pH 8.0], and the solution was filtered after centrifugation at 16,000 × *g* for 30 min to remove the undissolved materials. The albumin concentration was either 10 or 20% (w/w). 1-Vinyl-2-pyrrolidinone polymer[2] was dissolved in the same buffer solution at 70°. The solution thus obtained (50% w/w) was stored in a refrigerator overnight and used for the preparation of aqueous albumin solutions containing the polymer.

Measurement of Viscosity—Viscosities of both the albumin and mixed polymer solutions [prepared from 20% (w/w) albumin solution and 50% (w/w) 1-vinyl-2-pyrrolidinone polymer solution] were measured at 25° with a cone-plate type viscometer[3] in a shear rate range of 50–3950 sec^{-1}. Viscosity values were calculated from the straight lines in rheograms.

Preparation of Microcapsules—Albumin microcapsules were prepared by a method similar to that described in a previous paper (6). Phenacetin powder (10% v/v) was previously dispersed in albumin solutions with and without the polymer. To 100 ml of isooctane containing 5.0% (v/v) sorbitan trioleate[4] as an emulsifier, in a three-necked flask, was added 15 ml of each of the aqueous dispersions, with stirring. After further stirring for 10 min, the flask was immersed in a water bath maintained at 85° for a given period to denature the egg albumin. The resultant dispersion was cooled to room temperature.

[1] Tokyo Kasei Kogyo Co., Tokyo, Japan.
[2] K-30; Tokyo Kasei Kogyo Co., Tokyo, Japan.
[3] Rheomat 30; Contraves AG, Zurich, Switzerland.
[4] Span 85; Nikko Chemicals Inc., Tokyo, Japan.

0022-3549/83/0900-1057$01.00/0