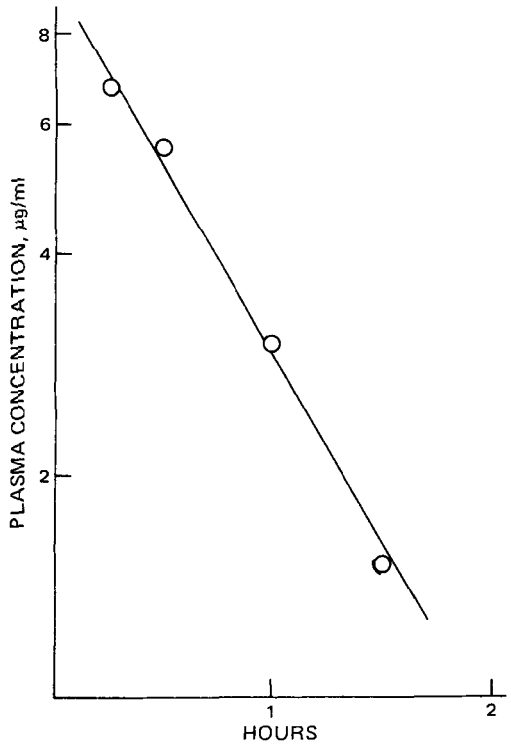

Figure 2—*Plasma concentration profile of chloramphenicol in a rabbit after 20 mg/kg iv.*

The low limit of accurate quantitative measurement of chloramphenicol was about 2.5 μg/ml in plasma when the detector sensitivity was set at 0.004 absorbance unit for full-scale deflection by use of the attenuator. Chloramphenicol at the 0.5-μg/ml level could be detected by the enhancement of the sensitivity to 0.0029 absorbance unit for full-scale deflection. Sample preparation by solvent extraction can improve the low limit of accurate measurement greatly. Sensitivity by solvent extraction depends on the sample size and the volume fraction of the reconstituted solution of the extract injected. For example, by extracting 0.2 ml of plasma with 2.5 ml of ethyl acetate and injecting 20 μl of the 50-μl reconstituted solution of residue from a 2.0-ml aliquot of the organic extract, the low limit of accurate quantitation could be as low as 0.5 μg/ml.

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Perfluorooctyl Bromide Concentration in Plasma and Tissues of Beagle Dogs

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Abstract □ Plasma levels were determined frequently after single doses of perfluorooctyl bromide were administered to beagle dogs at doses of either 30.8 g/kg po or 3.9 g/kg intratracheally. The apparent first-order half-life during the terminal elimination phase was about 1 day after oral medication and about 7 days after intratracheal administration. Analysis of tissues revealed the highest concentrations of the compound in abdominal fat of dogs autopsied 4 weeks later.

Keyphrases □ Perfluorooctyl bromide—tissue distribution in dogs after oral and intratracheal administration □ Distribution, tissue—perfluorooctyl bromide in dogs after oral and intratracheal administration □ Fluorocarbons—perfluorooctyl bromide, tissue distribution in dogs after oral and intratracheal administration

Liquid fluorocarbon studies provided the stimulus for the development of radiopaque perfluorocarbon compounds (1). The most effective compound tested was perfluorooctyl bromide (I), $C_8F_{17}Br$ (2). Toxicity and efficacy studies indicated a low toxicity and satisfactory radiographic density (3). Concentrated oil-in-water emulsions were found useful for bronchographic examinations in humans and animals (4). The use of I as a diagnostic contrast medium for gastroenterography of laboratory animals was reported (5). Recently, I was reported as a potential antiobesity agent (6).

Some data on the disposition of I after intratracheal administration to rats, dogs, and human subjects were reported (7); the sensitivity of the method did not allow quantitation of I in blood. This report describes studies on the plasma and tissue I concentrations in beagle dogs after oral and intratracheal administrations.

EXPERIMENTAL

Animal Procedure—Following an overnight fast, two groups of beagle dogs of both sexes (Tables I and II) received either a 30.8-g/kg po dose

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Table I—Tissue I Concentrations (Micrograms per Gram) in Beagle Dogs that Received 30.8 g/kg po

Dog (Sex; Weight in kg)	Lung	Abdominal Fat	Mesenteric Lymph	Thoracic Lymph	Adrenal	Ovary	Testes
			1 Week				
1 (M; 10.9)	0.04	5.44	<0.08	<0.18	<0.10	—	<0.05
2 (M; 8.4)	0.03	9.24	0.24	<0.18	<0.10	—	<0.05
3 (M; 9.5)	0.09	5.60	<0.08	1.57	<0.10	—	<0.05
4 (F; 8.4)	0.12	23.9	0,29	<0.18	<0.10	<0.04	—
5 (F; 10.2)	2.4	19.4	0.38	5.06	<0.10	<0.04	—
6 (F; 8.2)	0.06	16.7	0.36	<0.18	<0.10	<0.04	—
Mean ± *SE*[a]	0.46 ± 0.39	13.4 ± 3.1	0.22 ± 0.06	1.16 ± 0.82	<0.10	<0.04	<0.05
			4 Weeks				
7 (M; 7.5)	<0.02	<0.72	<0.08	<0.18	<0.10	—	0.46
8 (M; 9.1)	<0.02	<0.72	<0.08	<0.18	<0.10	—	<0.05
9 (M; 8.4)	0.04	20.6	2.2	2.13	<0.10	—	<0.05
10 (M; 10.3)	0.10	4.67	0.24	<0.18	<0.10	—	<0.05
11 (F; 9.4)	0.06	1.46	0.18	<0.18	<0.10	<0.04	—
12 (F; 12.2)	0.04	<0.72	0.24	<0.18	<0.10	<0.04	—
13 (F; 11.2)	0.07	14.8	1.72	<0.18	<0.10	<0.04	—
14 (F; 8.4)	0.05	<0.72	0.30	<0.18	<0.10	0.54	—
Mean ± *SE*[a]	0.05 ± 0.01	5.37 ± 2.79	0.62 ± 0.30	<0.26	<0.10	<0.13	<0.11

[a] Half of minimum quantifiable level used in calculating mean.

or a 3.9-g/kg intratracheal dose of I[1]. The oral dose was administered *via* a gastric tube. Intratracheal administration was carried out under light thiopental[2] anesthesia (35 mg/kg iv), and a gastric tube was passed into the trachea, reaching a point a few centimeters above the bifurcation of the left and right bronchi.

At intervals, 5 ml of blood was withdrawn from the femoral vein. The blood was collected with potassium oxalate as the anticoagulant and centrifuged promptly at 2500 rpm for 10 min. The plasma was separated, frozen, and stored at −4° until assay.

Three dogs of each sex of the orally dosed group were anesthetized with pentobarbital sodium[2] (45 mg/kg iv) and sacrificed after 7 days; the remaining animals were sacrificed after 28 days. Of the intratracheally dosed animals, two dogs of each sex were sacrificed at 7, 14, and 28 days. At sacrifice, samples of the abdominal fat, gonads, adrenals, mesenteric lymph nodes, thoracic lymph nodes, and lungs were removed and stored at −4° pending assay. Earlier studies demonstrated that the highest levels of I were found in these tissues after tracheal administration (7).

Extraction Procedure—To 1 ml of plasma, 1 ml of *n*-hexane containing either 1.6 μg, for analysis of plasma from orally dosed animals, or 0.41 μg, for analysis of plasma from intratracheally dosed animals, of the internal standard, tetrachlorodifluoroethane[3], was added. The mixture was mechanically shaken for 10 min. After centrifugation at 2500 rpm for 10 min, 2 μl of the organic phase was analyzed by GLC.

From 0.5 to 50 g of tissue sample was homogenized with 0.8–9 parts by weight of water. Homogenates equivalent to 0.1–1 g of tissue samples were extracted with 1 ml of *n*-hexane containing 0.41 μg of the internal standard, and 2 μl of the organic phase was analyzed.

GLC Determination—All assays were performed using a gas chromatograph equipped with a ^{63}Ni-electron-capture detector[4], an automatic

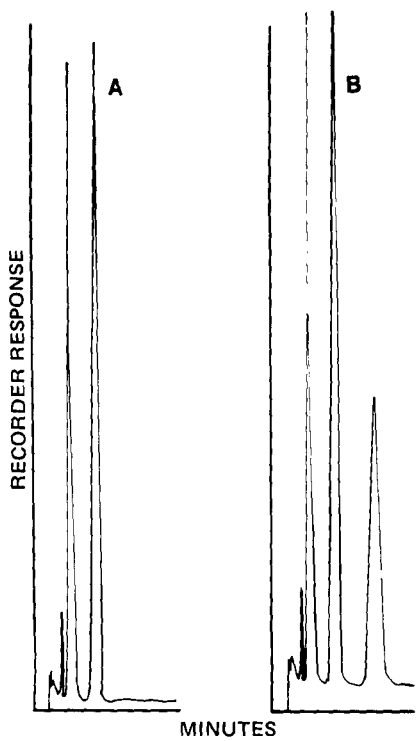

Figure 1—*Chromatogram of extracted dog plasma containing the internal standard (A) and the same sample containing 1.5 μg of I/ml (B).*

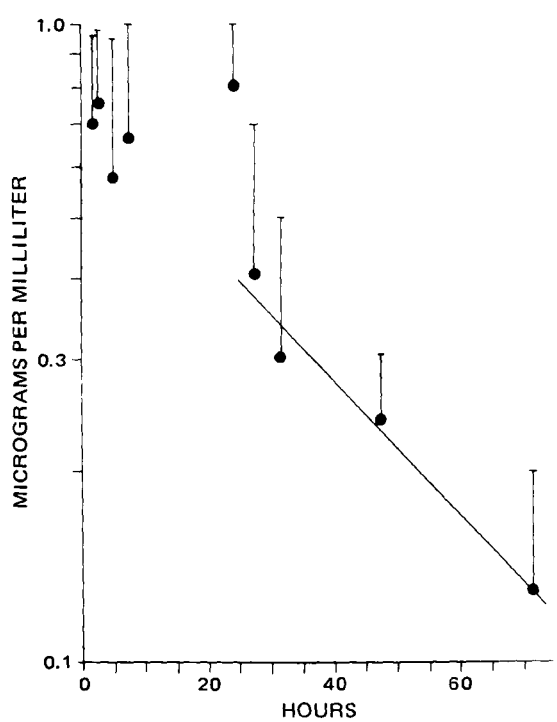

Figure 2—*Plasma concentrations in beagle dogs after oral administration of 30.8 g of I/kg;* n = *14. The vertical bar represents 1* SE.

[1] E. I. du Pont de Nemours, Wilmington, Del.
[2] Abbott, North Chicago, Ill.
[3] Freon 112, E. I. du Pont de Nemours, Wilmington, Del.
[4] Model 5710A, Hewlett-Packard, Avondale, Pa.

Table II—Tissue I Concentrations (Micrograms per Gram) in Beagle Dogs that Received 3.9 g/kg Intratracheally

Dog (Sex; Weight in kg)	Lung	Abdominal Fat	Mesenteric Lymph	Thoracic Lymph	Adrenal	Ovary	Testes
			1 Week				
15 (M; 8.9)	894	613	15.8	58.3	11.1	—	4.10
16 (M; 8.3)	947	286	5.17	13.0	21.1	—	4.84
17 (F; 8.2)	107	250	10.6	<0.18	19.8	3.57	—
18 (F; 7.5)	201	242	4.98	87.6	2.84	3.66	—
Mean ± *SE*[a]	537 ± 222	348 ± 89	9.14 ± 2.57	39.7 ± 20.2	13.7 ± 4.2	3.62 ± 0.04	4.47 ± 0.37
			2 Weeks				
19 (M; 8.6)	12.3	151	0.44	<0.18	<0.10	—	1.64
20 (F; 7.7)	53.4	357	4.25	11.2	1.28	38.9	—
21 (M; 8.4)	12.8	197	1.91	6.63	3.39	—	1.28
22 (F; 7.4)	7.72	189	4.66	6.13	<0.10	<0.04	—
Mean ± *SE*[a]	21.6 ± 10.7	224 ± 46	2.82 ± 1.00	6.01 ± 2.28	1.19 ± 0.79	19.5 ± 19.4	1.46 ± 0.18
			4 Weeks				
23 (M; 8.7)	8.58	151	0.31	4.33	1.39	—	<0.05
24 (M; 8.2)	2.13	49.9	0.58	<0.18	3.78	—	<0.05
25 (F; 7.4)	3.73	61.9	3.50	3.98	<0.10	<0.04	—
26 (F; 6.9)	11.0	58.8	12.4	14.8	1.56	<0.04	—
Mean ± *SE*[a]	6.36 ± 2.07	80.4 ± 23.7	4.20 ± 2.83	5.80 ± 3.15	1.70 ± 0.77	—	—

[a] Half of minimum quantifiable level used in calculating mean.

sampler, and a 1-mv recorder. A 1.54-m × 3-mm presilanized glass column was packed with porous polymer beads[5], 80–100 mesh. The column temperature was 220°, the injection port temperature was 250°, and the temperature of the electron-capture detector was 300°. The carrier gas was a mixture of 7% methane in argon[6], with a flow rate of 40 ml/min. When using these conditions, the retention times of I and tetrachlorodifluoroethane were 6 and 3 min, respectively.

Freshly prepared samples of normal, untreated plasma and tissue containing known amounts of I were used as standards. They were extracted and assayed as described.

RESULTS AND DISCUSSION

Typical chromatograms of plasma extracts are shown in Fig. 1. The relationship between the relative peak size ratio and the I concentration was linear up to 1.5 μg/ml or g; the standard line was computer estimated by a least-squares fit. There was some day-to-day variation in the slopes and intercepts, as can be seen from the standard errors in the equation for the plasma standards line:

$$\text{(peak size ratio)} = (0.0038 \pm 0.008) \times [\text{plasma concentration}] + (0.1294 \pm 0.0362) \quad \text{(Eq. 1)}$$

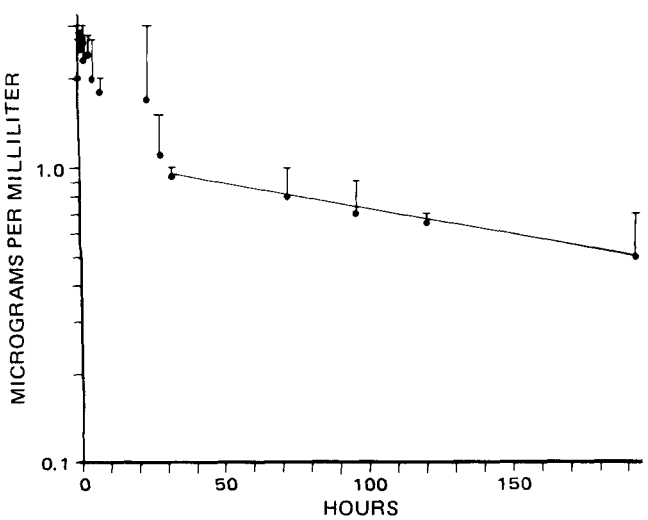

Figure 3—*Plasma concentrations in beagle dogs after intratracheal administration of 3.9 g of I/kg;* n = *12. The vertical bar represents 1* SE.

[5] Porapak QS, Waters, Framingham, Mass.
[6] Linde (Union Carbide), New York, N.Y.

The assay precision within groups of samples augmented with I ranged from 2.9 to 11.1% *SE*, and the accuracy ranged from 10.9% low to 8.5% high. The concentration in the unknowns was calculated from the extracted standards line, which was run on the same day as the samples. The mean minimum quantifiable level, defined as the value whose 80% confidence limit just encompasses zero, was 0.0909 ± 0.0127 μg/ml for 11 different sets of plasma standards. Tissue standards behaved similarly; the minimum quantifiable level value for the tissues was 0.08 ± 0.02 μg/sample analyzed. The recovery of extracted standards, relative to direct standards, was 53.1 ± 4.1%.

The plasma I concentrations in beagle dogs following oral and intratracheal administrations of the liquid are summarized in Figs. 2 and 3, respectively. The apparent first-order terminal phase elimination half-lives of the compound were about 1 day after oral administration and about 7 days after intratracheal administration, as determined by least-squares regression on the data points of the respective terminal phases. At 24 hr after the oral dose and at 1.5 hr after the endotracheal dose, the mean quantities of drug in plasma were 0.34 mg (1.2×10^{-4}% of the administered dose, assuming 45 ml of plasma/kg) and 9.49 mg (3.4×10^{-3}% of the administered dose), respectively.

Some tissue I concentrations after oral and intratracheal routes are summarized in Tables I and II, respectively. After 1 month, the highest concentrations of I administered by both routes were measured in abdominal fat, with mean values of 5.4 μg/g after the oral dose and 80.4 μg/g after the intratracheal dose. The apparent elimination half-life of the intratracheally administered compound in fat was 9.8 days, determined from three data points at 1, 2, and 4 weeks (Table II). In the other tissues, only two data points were available; no half-life estimates were made.

In summary, the developed GLC assay for the determination of I concentrations permitted estimation of apparent first-order terminal elimination half-lives in plasma and selected tissues of beagle dogs that received I by either the oral or intratracheal route.

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