WTH

File

RETURN TO FC-95

METAB FILE

RECEIVED
MAR 19 1980
3M
TOXICOLOGY

Enhanced Elimination of FC-95-^{14}C and FC-143-^{14}C in Rats with Cholestyramine Treatment

March 5, 1980

Conducted at: Riker Laboratories, Inc.
Subsidiary of 3M
St. Paul, Minnesota 55101

During: November, 1979 to
January, 1980

Conducted by: S. J. Gibson and J. D. Johnson

Report by: James D. Johnson 3/17/80
James D. Johnson, MS Date
Senior Biochemical Pharmacologist

Reviewed by: R. E. Ober 3/14/80
Robert E. Ober, PhD Date
Manager, Drug Metabolism

Summary

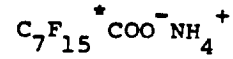
In male rats cholestyramine administered (4% w/w) in feed decreased the retention of carbon-14 in liver, plasma, and red blood cells and increased the elimination of carbon-14 via feces after iv dosing with either FC-95-^{14}C or FC-143-^{14}C. Groups of five rats were dosed iv with FC-95-^{14}C (mean dose, 3.4 mg/kg) or FC-143-^{14}C (mean dose, 13.3 mg/kg). Groups of five control rats were dosed similarly but were not treated with cholestyramine. The FC-95-^{14}C rats were sacrificed at 21 days and the FC-143-^{14}C rats at 14 days post dose. The mean liver, plasma, and red blood cell concentration as well as fecal and urinary excretion of carbon-14 for cholestyramine-treated rats were compared to mean control rat values. For FC-95-^{14}C, mean cholestyramine-treated rat carbon-14 concentrations in liver (9.4 ug/g), plasma (0.9 ug/ml), and red blood cells (0.3 ug/g) represent a decrease from mean control rat concentrations of 3.8, 7.7, and 6.0 fold, respectively; fecal elimination (75.9% with cholestyramine treatment) was increased 9.5 fold. For FC-143-^{14}C, mean cholestyramine-treated rat carbon-14 concentrations in liver (12.1 ug/g), plasma (5.1 ug/ml), and red blood cells (1.8 ug/g) represent a decrease from mean control concentrations of 2, 3, and 2 fold, respectively; fecal elimination (43.2% with cholestyramine treatment) was increased 9.8 fold. For both FC-95-^{14}C and FC-143-^{14}C, the extent of urinary cabon-14 elimination, as a result of the relatively high rate of fecal elimination of carbon-14, was lower in cholestyramine-treated rats. The extent of total elimination of carbon-14 (urine plus feces) was higher in the cholestyramine-treated rats. Since cholestyramine is approved for use in humans as a cholesterol lowering agent, these results in rats support the concept of testing cholestyramine in humans to promote excretion of FC-95 and FC-143.

Introduction

Cholestyramine, an agent approved for use in humans to lower cholesterol blood levels, is an anion exchange resin which has been administered orally to promote fecal excretion of the pesticide chlordecone (Kepone®) in rats (1) and man (2). Since both FC-95 and FC-143 are perfluorinated salts and exist as anions at physiologic pH, they would be expected to complex with cholestyramine. Non-ionic binding could also be involved. These two experiments in rats were designed to assess the effect of cholestyramine treatment on carbon-14 elimination and tissue retention after iv dosing with FC-95-^{14}C (FC-Experiment 8) and with FC-143-^{14}C (FC-Experiment 9).

Methods

Radiolabeled FC-143-^{14}C

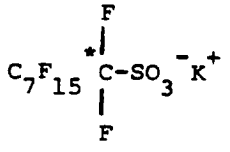


FC-143

*Denotes postion of carbon-14

The carbon-14 label is at the carbonyl carbon atom (see above structure). The specific activity of this lot of FC-143-^{14}C (Riker Isotope Inventory Number 459) is 0.51 uCi/mg. Details of specific activity, chemical characterization, and radiochemical purity have been reported separately (3); the FC-143-^{14}C was found suitable for metabolism studies.

Radiolabeled FC-95-^{14}C



*Denotes postion of carbon-14

The carbon-14 label is at the carbon atom α to the sulfur atom (see above structure). The specific activity of this lot of FC-95-^{14}C (Riker Isotope Inventory Number 442) is 0.46 uCi/mg. Details of specific activity, chemical characterization, and radiochemical purity have been reported separately (4); the FC-95-^{14}C was found suitable for metabolism studies.

Animals

FC-95-^{14}C (FC-Experiment 8)

Ten male Charles River[a] CD rats, twelve weeks old, were randomly divided into a control group of five (weight 304 to 331 g, mean 320 g) and a group of five to be treated with cholestyramine (weight 322 to 342 g, mean 330 g). For 24 hours prior to dosing, the rats were conditioned to individual metal metabolism cages and fasted with free access to water. The rats were allowed free access to Purina[b] Ground Chow (with or without added cholestyramine) and water immediately after dosing.

---

[a] Charles River Breeding Laboratories, Wilmington, Massachusetts.
[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.

FC-143-^{14}C (FC-Experiment 9)

Ten male Charles River[a] CD rats, twelve weeks old, were randomly divided into a control group of five (weight 300 to 341 g, mean 313 g) and a group of five to be treated with cholestyramine (weight 300 to 344 g, mean 317 g). For 24 hours prior to dosing, the rats were conditioned to individual metal metabolism cages and fasted with free access to water. The rats were allowed free access to Purina[b] Ground Chow (with or without added cholestyramine) and water immediately after dosing.

Dosing

FC-95-^{14}C (FC-Experiment 8)

Each rat was weighed, anesthetized lightly with diethyl ether, and then given a single iv dose (via tail vein) of FC-95-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 1.13 mg FC-95-^{14}C/2.0 ml. The average dose administered the cholestyramine-treated rats was 3.4 mg/kg and the average dose for control rats was 3.5 mg/kg. The dose was delivered with a 3.0 cc disposable plastic syringe (Monoject®[c]) fitted with a 26 gauge 1/2" needle. The dosing solution was prepared by adding ≒150 mg of FC-95-^{14}C to 0.9% NaCl, shaking for one hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 1).

FC-143-^{14}C (FC-Experiment 9)

Each rat was weighed, anesthetized lightly with diethyl ether, and then given a single iv dose (via tail vein) of FC-143-^{14}C. The dose was 2.0 ml of a 0.9% NaCl solution containing 4.21 mg of FC-143-^{14}C/2.0 ml. The average dose administered the cholestyramine-treated rats was 13.3 mg/kg and the average dose for control rats was 13.5 mg/kg. The dose was delivered with a 3.0 cc disposable plastic syringe (Monoject®[c]) fitted with a 26 gauge 1/2" needle. The dosing solution was prepared by dissolving 225 mg of FC-143-^{14}C in 0.9% NaCl. The carbon-14 content of the dosing solution was determined by direct counting (see Appendix 2).

---

[a] Charles River Breeding Laboratories, Wilmington, Massachusetts.
[b] Purina Lab Chow, Ralston Purina Company, St. Louis, Missouri.
[c] Sherwood Medical Industries, Inc., Deland, Florida 32720.

Cholestyramine Treatment

Cholestyramine[a] (dried and ground resin Z-620) was mixed 4% by weight with Purina Ground Chow. A weighed portion of the cholestyramine feed was prepared for each rat so that consumption could be individually monitored. Each of the cholestyramine-treated rats in both FC-Experiments 8 and 9 received only treated feed during the course of the studies. The actual mean dose of cholestyramine for the FC-95-^{14}C treated rats was 2.7±0.2 (SD) g/kg/day. The actual mean dose of cholestyramine for the FC-143-^{14}C treated rats was 3.0±0.2 (SD) g/kg/day.

Sample Collection

FC-95-^{14}C (FC-Experiment 8)

Urine and feces were collected at intervals (see Tables 3 and 5) for each of the ten rats for 21 days. At 21 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. The rats were sacrificed by exsanguination, and liver was collected as the whole organ.

FC-143-^{14}C (FC-Experiment 9)

Urine and feces were collected at intervals (see Tables 4 and 6) for each of the ten rats for 14 days. At 14 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. The rats were sacrificed by exsanguination, and liver was collected as the whole organ.

Sample Analysis for Carbon-14

Feces and whole liver were prepared for carbon-14 analysis by homogenizing and aliquoting a sample of the homogenate into combustion cones[b]. Homogenizing was done in Waring blenders by adding nine parts of water (w/w) to one part of biological material. The homogenates were weighed into combustion cones in duplicate on a top-loading balance by taring the cone and adding 1.0 g of the homogenate. Red blood cells were measured into combustion cones by weight.

---

[a] Mead Johnson Research Center, Evansville, Indiana 47721.
[b] Packard Instrument Co., Inc., 220 Warrenville Road, Downers Grove, Illinois.

Homogenates and red blood cells were combusted with a Packard Model 306 Oxidizer[a]. Recovery of carbon-14 from biological samples was determined by combusting suitable blank homogenates (feces and liver) spiked with dilutions of the appropriate dosing solution at the beginning, middle, and end of the experimental sample sets. All mean recoveries were >95%; thus, no correction for recovery was made for either FC-95-^{14}C or FC-143-^{14}C samples. Good recovery from combustion analysis has previously been reported for FC-95-^{14}C (5) and FC-143-^{14}C (6). Urine collections were sampled before freezing and were counted directly; duplicate 1.0 ml aliquots of each sample were pipetted directly into scintillation vials and 15 ml Aquasol® was added. It has previously been reported that direct counting in Aquasol® is appropriate for FC-95-^{14}C urine samples (5) and FC-143-^{14}C urine samples (6). All samples were cooled to refrigerator temperature in the dark before counting. All radiometric analyses were done using a Packard Model 3385 Tri-Carb Liquid Scintillation Spectrometer [a]. Counting efficiency for each sample was determined by use of the AES (Automatic External Standardization) ratio method. To calibrate the external standard, internal standard was added to selected samples from the group of samples (three with low AES ratios and three with high ratios), and these samples were recounted along with a sealed standard. Data were collected on punch tape and processed by the CDC 1700 Computer System in the 3M Central Research Data Processing Laboratory. Data reduction to dpm was accomplished with the Biological Automatic External Standardization computer program.

Results and Discussion

The results of the liver, plasma, and red blood cell analyses for carbon-14 content after intravenous dosing with FC-95-^{14}C are shown in Table 1; the results after intravenous dosing with FC-143-^{14}C are shown in Table 2. Cholestyramine treatment caused a decrease in mean liver, plasma, and red blood cell carbon-14 content for both FC-95-^{14}C and FC-143-^{14}C dosed rats. For FC-95-^{14}C, mean cholestyramine-treated rat carbon-14 concentrations in liver (9.4 ug/g), plasma (0.9 ug/ml), and red blood cells (0.3 ug/g) represent a decrease from mean control rat ^{14}C concentrations of 3.8, 7.7, and 6.0 fold, respectively. For FC-143-^{14}C, mean cholestyramine-treated rat carbon-14 concentrations in liver (12.1 ug/g), plasma (5.1 ug/ml), and red blood cells (1.8 ug/g) represent a decrease from mean control concentrations of 2, 3, and 2 fold, respectively. Thus, oral cholestyramine treatment effectively reduced the liver, plasma, and red blood cell concentrations of these two fluorocarbons and/or their metabolites. The larger decrease in plasma concentration as compared to decrease in liver concentration for both compounds may indicate, however, that some of the liver fluorocarbon is not affected to the same extent as plasma fluorocarbon by cholestyramine treatment.

The results of carbon-14 fecal analyses for FC-95-^{14}C dosed rats are shown in Table 3 and in Figure 1. The mean cumulative percent of dose eliminated via feces by cholestyramine-treated rats (75.9%) is 9.5 fold the mean percent of dose eliminated via feces by control rats. For FC-143-^{14}C

---

[a]Packard Instrument Co., Inc., 220 Warrenville Road, Downers Grove, Illinois.

rats, the carbon-14 fecal analyses results are shown in Table 4 and in Figure 2. The mean cumulative percent of dose eliminated via feces by cholestyramine-treated rats (43.2%) is 9.8 fold the mean percent of dose eliminated via feces by control rats. Thus, the decrease of liver, plasma, and red blood cell carbon-14 concentrations after oral cholestyramine treatment of FC-95-^{14}C and FC-143-^{14}C dosed rats is accompanied by a large increase in the extent of fecal elimination of carbon-14.

The results of the urine analyses for FC-95-^{14}C dosed rats are shown in Table 5 and in Figure 3. The cumulative percent of dose eliminated in urine by the cholestyramine-treated rats (5.4%) is about one third of the percent of dose eliminated by the control rats. The results of the urine analyses for FC-143-^{14}C dosed rats are shown in Table 6 and Figure 4. The cumulative percent of dose eliminated in urine by cholestyramine-treated rats (41.1%) is about three fifths of the percent of dose eliminated by the control rats. Thus for both FC-95-^{14}C and FC-143-^{14}C dosed rats, the cholestyramine-treated rats eliminate less carbon-14 in urine than do the control rats. The lower urinary excretion by cholestyramine-treated rats is a result of the nearly 10 fold increase in fecal elimination of carbon-14, especially during the first few days post dosing; since in cholestyramine-treated rats much of the carbon-14 is eliminated via feces, it is not available for urinary elimination. Total elimination of carbon-14 (urine plus feces) is shown for FC-95-^{14}C dosed rats in Figure 5 and for FC-143-^{14}C dosed rats in Figure 6. For FC-95-^{14}C dosed rats, after 21 days of cholestyramine treatment, 81.2% of the dose has been eliminated versus only 25.6% of the dose for control rats. The mean percentages of dose remaining in the body, 19% for cholestyramine-treated rats and 74% for control rats, are in about the same proportion (1:4) as the differences in liver carbon-14 content (cholestyramine-treated, 9.4 ug/g; control, 35.6 ug/g). The total carbon-14 eliminated (urine plus feces) by FC-143-^{14}C dosed rats is 84% of the dose for cholestyramine-treated rats and 72% of the dose for control rats; the percentages of dose remaining in the body are 16% and 28%, respectively. Again as with FC-95-^{14}C, the mean percentages of dose remaining in the body are reflected in the relative mean liver concentrations (cholestyramine-treated, 12.1 ug/g; control, 22.3 ug/g).

The individual body weights at time of dosing and the individual body weights and liver weights at time of sacrifice are shown, along with corresponding control groups, in Table 7 for FC-95-^{14}C dosed rats and in Table 8 for FC-143-^{14}C dosed rats. Cholestyramine does not appear to have caused a change in weight gain or liver weight in either experiment.

Cholestyramine is approved for human use as a cholesterol lowering agent and has been used in humans to promote the excretion of xenobiotics (2). These results in rats with FC-95 and FC-143 support the concept of testing cholestyramine in humans to promote excretion of fluorocarbons.

References

(1) Boylan JJ, Egle JL, Guzelian PS: Cholestyramine: Use as a New Therapeutic Approach for Chlordecone (Kepone®) Poisoning. Science 199: 893-895, 1978.

(2) Cohn WJ, Boylan JJ, Blanke RV, Fariss MW, Howell JR, Guzelian PS: Treatment of Chlordecone (Kepone®) Toxicity with Cholestyramine. New Engl J Med 298: 243-278, 1978.

(3) Behr FE, Johnson JD: Synthesis and Characterization of FC-143-^{14}C (Report) December 28, 1979.

(4) Johnson JD, Behr FE: Synthesis and Characterization of FC-95-^{14}C (Report) November 2, 1979.

(5) Johnson JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single IV Dose of FC-95-^{14}C (Report) December 28, 1979.

(6) Johnson JD: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Male and Female Rats after a Single IV Dose of FC-143-^{14}C (Report) January 30, 1979.

Acknowledgement

The preparation of the 4% w/w cholestyramine in Purina Ground Chow by L. J. Sibinski of Pathology-Toxicology is gratefully aknowledged.

List of Tables and Figures

Table 1: Liver, Plasma, and Red Blood Cell Concentration of Carbon-14 in Rats at 21 Days after a Single IV Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) NB 53102 P 25-27.

Table 2: Liver, Plasma, and Red Blood Cell Concentration of Carbon-14 in Rats at 14 Days after a Single IV Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) NB 53102 P 38,39.

Table 3: Excretion of Total Carbon-14 in Feces in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) NB 53102 P 20,21.

Table 4: Excretion of Total Carbon-14 in Feces in Rats after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) NB 53102 P 30,32.

Table 5: Excretion of Total Carbon-14 in Urine in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine Treated, 3.4 mg/kg; Control, 3.5 mg/kg) NB 53102 P 23,24.

Table 6: Excretion of Total Carbon-14 in Urine in Rats after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) NB 53102 P 35,36.

Table 7: Body Weights at Dosing and Body and Liver Weights at Sacrifice for Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) NB 53102 P 26

Table 8: Body Weights at Dosing and Body and Liver Weights at Sacrifice for Rats after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) NB 53102 P 38.

Figure 1: Cumulative Excretion of Total Carbon-14 in Feces after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) Mean of Five Rats NB 53102 P 28.

List of Tables and Figures
(continued)

Figure 2: Cumulative Excretion of Total Carbon-14 in Feces after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) Mean of Five Rats NB 53102 P 40.

Figure 3: Cumulative Excretion of Total Carbon-14 in Urine after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) Mean of Five Rats NB 53102 P 28.

Figure 4: Cumulative Excretion of Total Carbon-14 in Urine after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) Mean of Five Rats NB 53102 P 40.

Figure 5: Cumulative Excretion (Urine + Feces) of Total Carbon-14 after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg) Mean of Five Rats NB 53102 P 28.

Figure 6: Cumulative Excretion (Urine + Feces) of Total Carbon-14 after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg) Mean of Five Rats NB 53102 P 40.

Appendix 1: Determination of Carbon-14 Content of FC-95-^{14}C Dosing Solution NB 51807 P 41,44.

Appendix Table 1 : Carbon-14 Content of FC-95-^{14}C Dosing Solution NB 51807 P 44.

Appendix 2: Determination of Carbon-14 Content of FC-143-^{14}C Dosing Solution NB 51807 P 41-45.

Appendix Table 2 : Carbon-14 Content of FC-143-^{14}C Dosing Solution NB 51807 P 45.

Table 1

Liver, Plasma, and Red Blood Cell Concentration[a] of Carbon-14 in Rats at 21 Days after a Single IV Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg

	Cholestyramine-Treated					
	Rat Number					Mean ± SD
	1	2	3	4	5	
Liver	9.8	11.5	9.7	7.1	8.8	9.4 + 1.6
Plasma	0.9	1.1	0.8	0.7	0.9	0.9 ± 0.1
Red Blood Cells	0.2	0.3	0.3	0.2	0.3	0.3 ± 0.1

	Control					
	Rat Number					Mean ± SD
	1	2	3	4	5	
Liver	30.8	33.2	40.6	30.7	42.5	35.6 ± 5.6
Plasma	7.0	7.4	6.2	6.4	7.5	6.9 ± 0.6
Red Blood Cells	2.1	2.2	1.6	1.7	1.3	1.8 ± 0.4

[a] Total carbon-14 concentration for liver and red blood cells is expressed as ug equivalents of FC-95-^{14}C/g; plasma carbon-14 concentration is expressed as ug FC-95-^{14}C equivalents/ml.

Table 2

Liver, Plasma, and Red Blood Cell Concentration[a] of Carbon-14 in Rats at 21 Days after a Single IV Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg

	Cholestyramine-Treated					
	Rat Number					Mean ± SD
	1	2	3	4	5	
Liver	14.6	9.4	13.5	11.9	10.9	12.1 ± 2.1
Plasma	7.1	3.0	6.1	5.5	3.9	5.1 ± 1.7
Red Blood Cells	2.7	1.0	2.4	2.0	1.2	1.8 ± 0.7

	Control					
	Rat Number					Mean ± SD
	1	2	3	4	5	
Liver	25.4	31.4	21.0	17.5	16.3	22.3 ± 6.2
Plasma	19.9	23.9	10.1	8.8	10.7	14.7 ± 6.8
Red Blood Cells	6.7	7.0	2.3	2.6	2.4	4.2 ± 2.4

[a]Total carbon-14 concentration for liver and red blood cells is expressed as ug equivalents of FC-143-^{14}C/g; plasma carbon-14 concentration is expressed as ug FC-143-^{14}C equivalents/ml.

Table 3

Excretion of Total Carbon-14 in Feces[a] in Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg)

Collection Period (Days)	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
0-0.5	0.08	2.90	1.97	2.07	0.0[b]	1.40±1.30	0.35	0.60	0.40	0.52	0.0[b]	0.37±0.23
0.5-1	1.49	7.94	11.30	7.58	3.97	6.46±3.80	0.64	0.46	0.72	0.58	0.54	0.59±0.10
1-2	6.75	14.27	15.63	17.59	16.47	14.14±4.31	0.59	0.86	0.53	0.70	0.92	0.72±0.17
2-3	15.34	11.03	12.68	12.03	14.19	13.05±1.72	0.57	1.11	0.50	0.65	0.62	0.69±0.24
3-4	11.85	7.24	8.42	9.96	8.71	9.24±1.75	0.41	0.55	0.48	0.45	0.51	0.48±0.05
4-5	8.54	5.67	5.77	6.02	8.20	6.84±1.41	0.40	0.35	0.39	0.45	0.29	0.38±0.06
5-6	5.26	3.81	4.42	4.61	5.12	4.64±0.58	0.35	0.37	0.43	0.52	0.36	0.41±0.07
6-7	4.54	2.69	3.28	3.67	5.13	3.86±0.98	0.32	0.40	0.31	0.36	0.44	0.37±0.05
7-9	5.07	4.48	4.17	4.80	5.30	4.76±0.45	0.58	0.69	0.69	0.77	1.01	0.75±0.16
9-11	3.65	3.38	3.37	3.96	3.90	3.65±0.28	0.56	0.64	0.69	0.80	0.75	0.69±0.09
11-15	4.36	4.57	4.08	4.76	5.27	4.61±0.45	1.01	1.25	0.95	1.17	1.18	1.11±0.13
15-18	2.48	2.50	2.40	2.63	2.54	2.51±0.08	0.64	0.54	0.65	0.91	1.00	0.75±0.20
18-21	0.48	0.58	0.64	0.77	0.98	0.69±0.19	0.48	0.58	0.64	0.77	0.98	0.69±0.19
Total	69.89	71.06	78.13	80.45	79.78	75.86±5.01	6.90	8.40	7.38	8.65	8.60	7.99±0.80

[a] Data are expressed as percent of dose excreted during collection period.
[b] No feces excreted.

Table 4

Excretion of Total Carbon-14 in Feces[a] in Rats after a Single Intravenous Dose of FC-143 -^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg)

Collection Period (Days)	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
0-0.5	0.04	0.13	0.01	3.15	1.78	1.02±1.40	0.0[b]	0.09	0.21	0.37	0.07	0.15±0.15
0.5-1	5.06	3.72	1.73	5.68	4.63	4.16±1.54	0.44	0.54	0.44	0.33	0.0[b]	0.35±0.21
1-2	5.50	7.21	7.20	8.11	7.81	7.17±1.01	0.32	0.51	0.69	0.74	0.71	0.59±0.18
2-3	6.85	7.21	7.74	7.52	5.50	6.96±0.88	0.34	0.54	0.52	0.44	0.62	0.49±0.11
3-4	4.96	4.45	5.91	5.15	3.84	4.86±0.78	0.28	0.33	0.51	0.75	0.48	0.47±0.18
4-5	4.54	2.79	5.14	3.57	3.22	3.85±0.97	0.31	0.33	0.42	0.22	0.26	0.31±0.08
5-6	3.58	2.34	4.09	3.35	2.41	3.15±0.76	0.21	0.54	0.65	0.20	0.25	0.37±0.21
6-7	2.54	2.04	3.26	2.56	1.98	2.48±0.52	0.16	0.25	0.22	0.25	0.32	0.24±0.06
7-9	4.87	2.73	5.04	3.74	2.91	3.86±1.07	0.33	0.97	0.69	0.40	0.73	0.62±0.26
9-11	3.66	1.83	3.87	2.61	2.21	2.84±0.89	0.41	0.48	0.54	0.35	0.38	0.43±0.08
11-14	4.01	1.94	3.61	2.82	2.04	2.88±0.92	0.27	0.59	0.61	0.27	0.33	0.41±0.17
Total	45.61	36.39	47.60	48.26	38.33	43.23±5.50	3.07	5.17	5.50	4.32	4.15	4.43±0.95

[a]Data are expressed as percent of dose excreted during collection period.
[b]No feces excreted.

Table 5

Excretion of Total Carbon-14 in Urine[a] in Rats after a Single Intravenous Dose of FC-95-^{14}C
(Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg)

Collection Period (Days)	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
0-0.5	1.58	1.53	1.75	1.91	1.53	1.66±0.17	1.98	1.61	1.83	1.02	1.60	1.61±0.37
0.5-1	0.49	0.48	0.50	0.30	0.26	0.41±0.12	1.06	1.00	0.90	0.87	0.92	0.95±0.08
1-2	0.69	0.61	0.58	0.83	0.61	0.66±0.10	1.14	1.30	0.94	1.07	1.41	1.17±0.19
2-3	0.67	0.61	0.52	0.50	0.62	0.58±0.07	1.45	1.31	1.11	1.19	1.55	1.32±0.18
3-4	0.59	0.33	0.39	0.48	0.42	0.44±0.10	1.13	1.17	0.97	1.09	1.30	1.13±0.12
4-5	0.26	0.20	0.22	0.23	0.32	0.25±0.05	0.84	0.87	0.88	0.89	0.88	0.87±0.02
5-6	0.23	0.16	0.19	0.21	0.25	0.21±0.03	0.96	0.92	0.75	0.91	1.23	0.95±0.17
6-7	0.22	0.13	0.11	0.15	0.16	0.15±0.04	0.76	0.68	0.71	0.72	1.08	0.79±0.16
7-9	0.25	0.19	0.18	0.21	0.24	0.21±0.03	1.55	1.62	1.26	1.40	1.94	1.55±0.26
9-11	0.20	0.17	0.18	0.21	0.19	0.19±0.02	1.51	1.41	1.30	0.97	1.56	1.35±0.23
11-15	0.28	0.23	0.25	0.26	0.35	0.27±0.05	2.62	2.51	2.18	2.25	2.69	2.45±0.23
15-18	0.22	0.17	0.23	0.22	0.22	0.21±0.02	1.79	1.76	1.63	1.62	2.21	1.80±0.24
18-21	0.15	0.11	0.14	0.14	0.12	0.13±0.02	1.72	1.59	1.50	1.34	2.04	1.64±0.26
Total	5.83	4.92	5.24	5.65	5.29	5.37±0.36	18.51	17.75	15.96	15.34	20.41	17.59±2.03

[a]Data are expressed as percent of dose excreted during collection period.

Table 6

Excretion of Total Carbon-14 in Urine[a] in Rats after a Single Intravenous Dose of FC-143 -^{14}C
(Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg)

Collection Period (Days)	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
0-0.5	4.72	8.60	5.21	8.79	12.17	7.90±3.04	3.85	5.06	4.11	15.09	4.37	6.50±4.83
0.5-1	2.46	5.11	2.23	2.97	5.00	3.55±1.40	3.48	4.15	7.88	5.49	5.07	5.21±1.68
1-2	4.15	9.05	4.09	5.00	8.09	6.08±2.33	6.07	7.21	8.72	11.64	10.66	8.86±2.32
2-3	3.50	8.68	3.95	4.73	5.75	5.32±2.06	6.35	5.54	6.68	9.00	9.60	7.43±1.77
3-4	2.87	5.74	4.19	3.18	4.18	4.03±1.12	6.10	4.76	6.67	7.14	9.48	6.83±1.73
4-5	2.27	3.34	2.65	2.57	2.90	2.75±0.40	5.22	4.20	6.32	4.88	7.35	5.59±1.25
5-6	2.44	2.99	2.67	1.88	2.36	2.47±0.41	4.83	3.69	5.08	4.45	5.37	4.68±0.65
6-7	1.87	2.15	2.11	1.41	1.66	1.84±0.31	4.34	3.40	4.14	3.99	5.58	4.29±0.80
7-9	3.55	3.22	3.48	2.21	3.23	3.14±0.54	7.21	6.37	6.37	6.24	6.69	6.58±0.39
9-11	2.38	2.19	2.05	1.54	1.98	2.03±0.31	6.09	5.55	5.62	5.24	5.37	5.57±0.32
11-14	2.69	1.98	1.85	1.48	1.78	1.96±0.45	6.95	6.98	5.57	4.48	5.21	5.84±1.10
Total	32.90	53.05	34.48	35.76	49.10	41.07±9.31	60.49	56.91	67.16	77.64	74.75	67.38±8.90

[a] Data are expressed as percent of dose excreted during collection period.

Table 7

Body Weights at Dosing and Body and Liver Weights at Sacrifice for Rats after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine-Treated, 3.4 mg/kg; Control, 3.5 mg/kg)

	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
Body Weight at Dosing (g)	324	329	322	342	331	330±8	316	304	331	321	328	320±11
Body Weight at Sacrifice (g)	400	422	403	410	402	407±9	393	409	394	440	388	405±21
Percent Change in Body Weight	23.5	28.3	25.2	19.9	21.5	23.7±3.3	24.4	34.5	19.0	37.1	18.3	26.7±8.7
Liver Weight (at Sacrifice) (g)	14.0	15.0	13.9	13.1	11.8	13.6±1.2	14.6	13.3	11.7	14.9	10.8	13.1±1.8
Percent Liver Weight of Body Weight (at Sacrifice)	3.5	3.6	3.4	3.2	2.9	3.3±0.3	3.7	3.3	3.0	3.4	2.8	3.2±0.4

Table 8

Body Weights at Dosing and Body and Liver Weights at Sacrifice for Rats after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine-Treated, 13.3 mg/kg; Control, 13.5 mg/kg)

	Cholestyramine-Treated						Control					
	Rat Number					Mean±SD	Rat Number					Mean±SD
	1	2	3	4	5		1	2	3	4	5	
Body Weight at Dosing (g)	344	330	302	307	300	317±19	341	312	301	300	310	313±17
Body Weight at Sacrifice (g)	414	382	354	347	389	377±27	384	356	383	358	381	372±14
Percent Change in Body Weight	20.4	15.8	17.2	13.0	29.7	19.2±6.4	12.6	14.1	27.2	19.3	22.9	19.2±6.1
Liver Weight (at Sacrifice) (g)	17.0	14.1	11.2	11.8	14.9	13.8±2.4	15.5	14.7	15.0	10.6	14.9	14.1±2.0
Percent Liver Weight of Body Weight (at Sacrifice)	4.1	3.7	3.2	3.4	3.8	3.6±0.4	4.0	4.1	3.9	3.0	3.9	3.8±0.4

Figure 1

Cumulative Excretion of Total Carbon-14 in Feces after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine Treated, 3.4 mg/kg; Control, 3.5 mg/kg)

Mean of 5 Rats

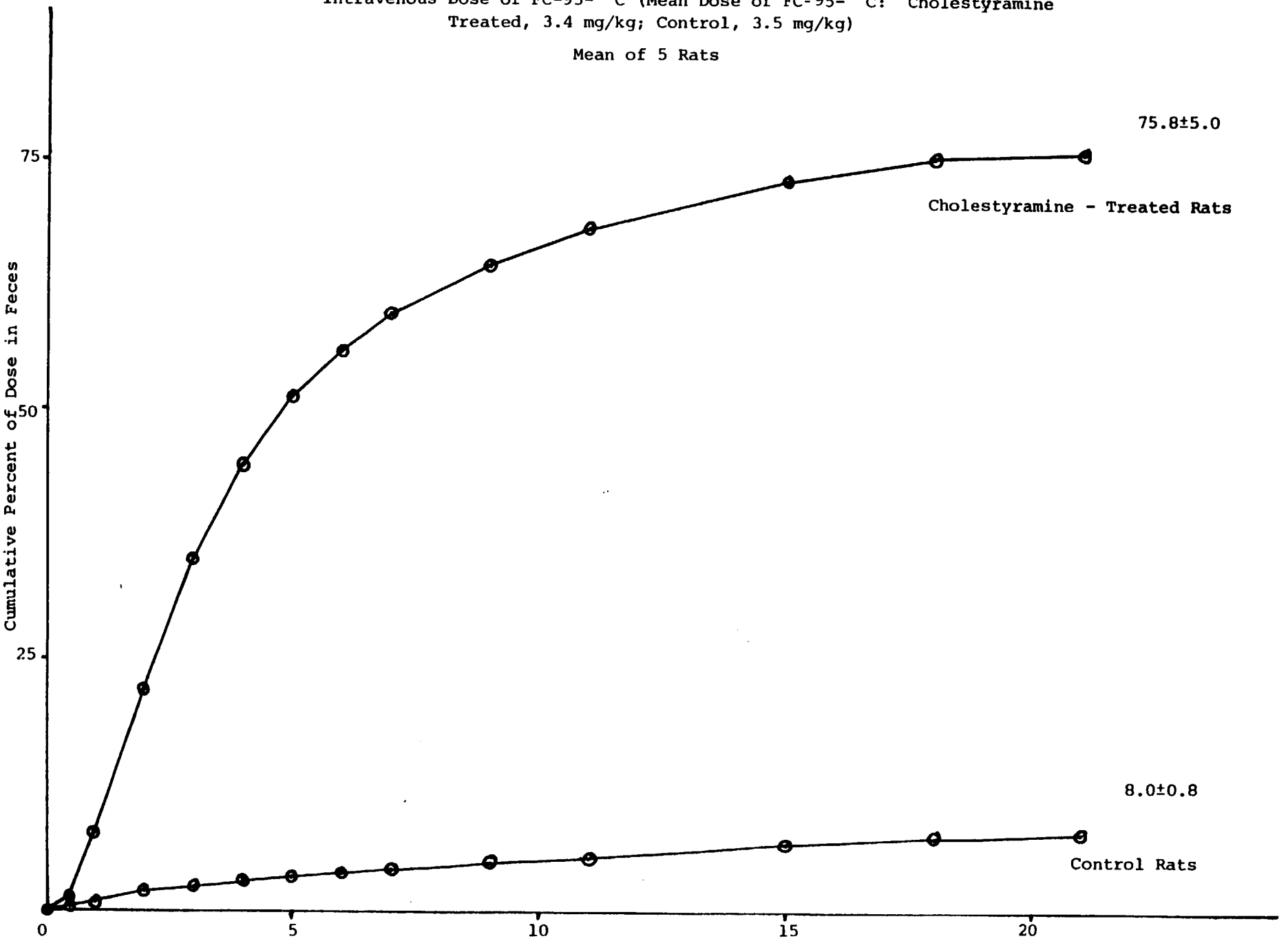


Figure 2

Cumulative Excretion of Total Carbon-14 in Feces after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine Treated, 13.3 mg/kg: Control, 13.5 mg/kg)

Mean of 5 Rats

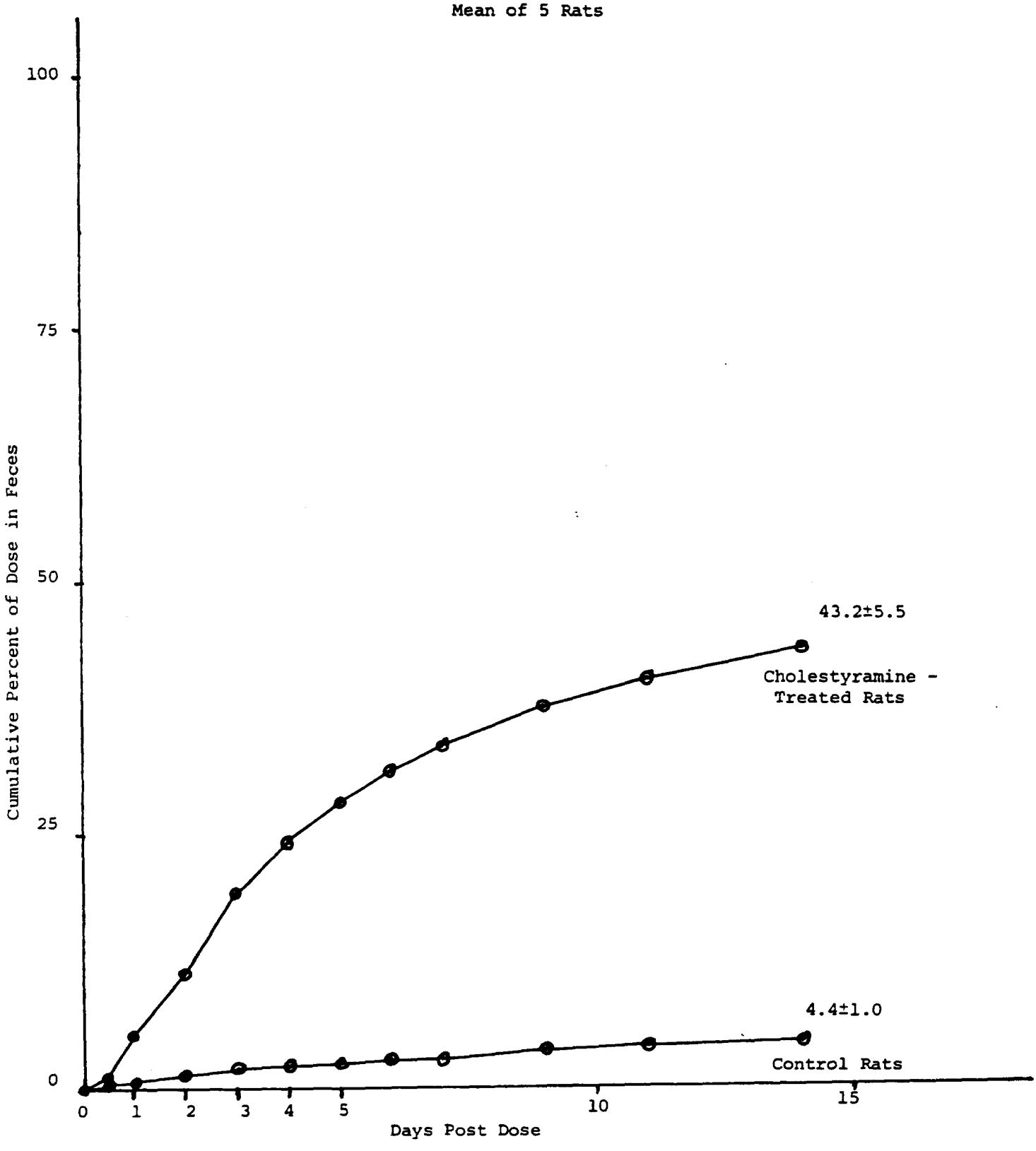


Figure 3

Cumulative Excretion of Total Carbon-14 in Urine After a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine Treated, 3.4 mg/kg; Control, 3.5 mg/kg)

Mean of 5 Rats

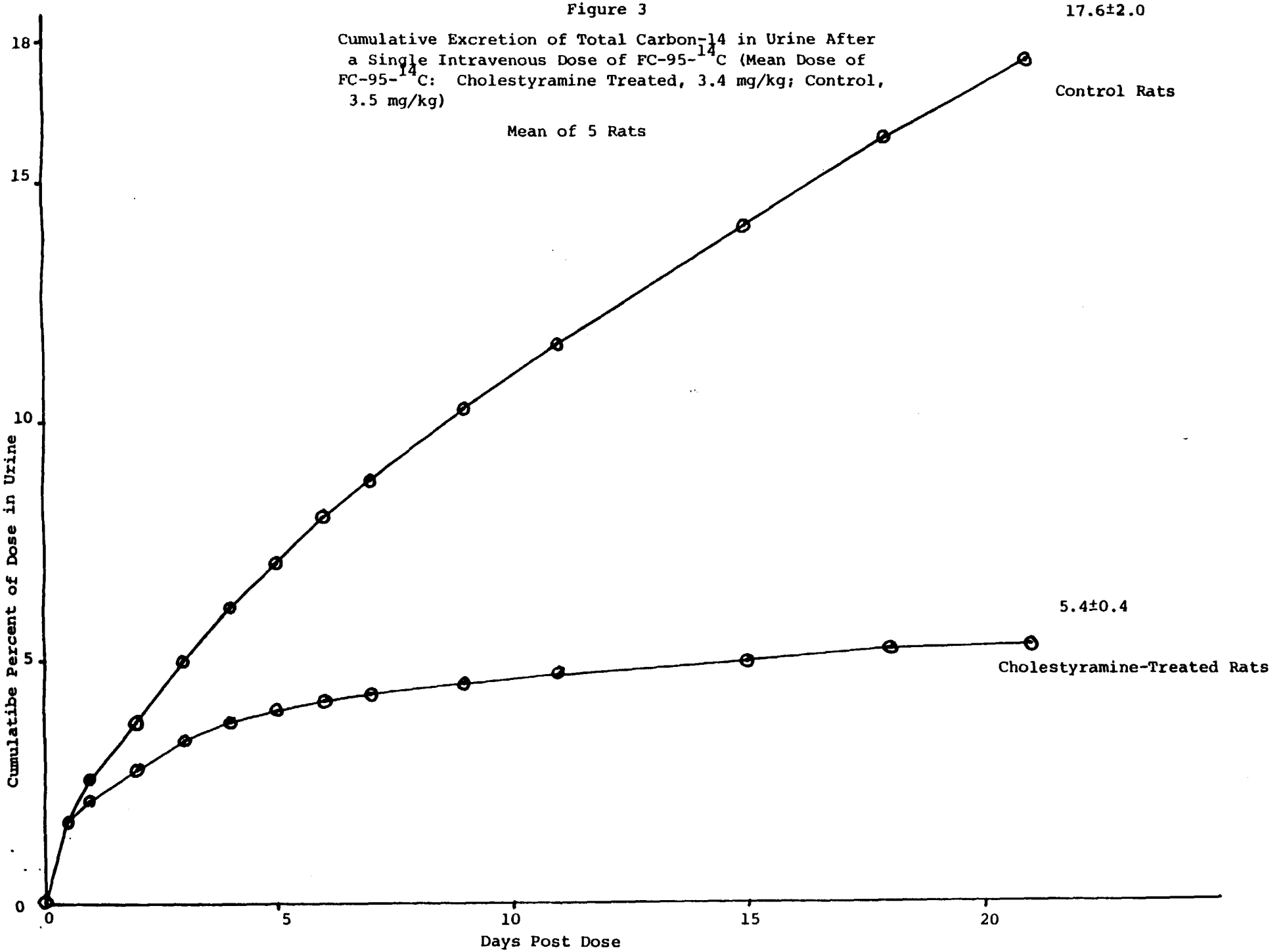


Figure 4

Cumulative Excretion of Total Carbon-14 in Urine after a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143: Cholestyramine Treated, 13.3 mg/kg; Control, 13.5 mg/kg)

Mean of 5 Rats

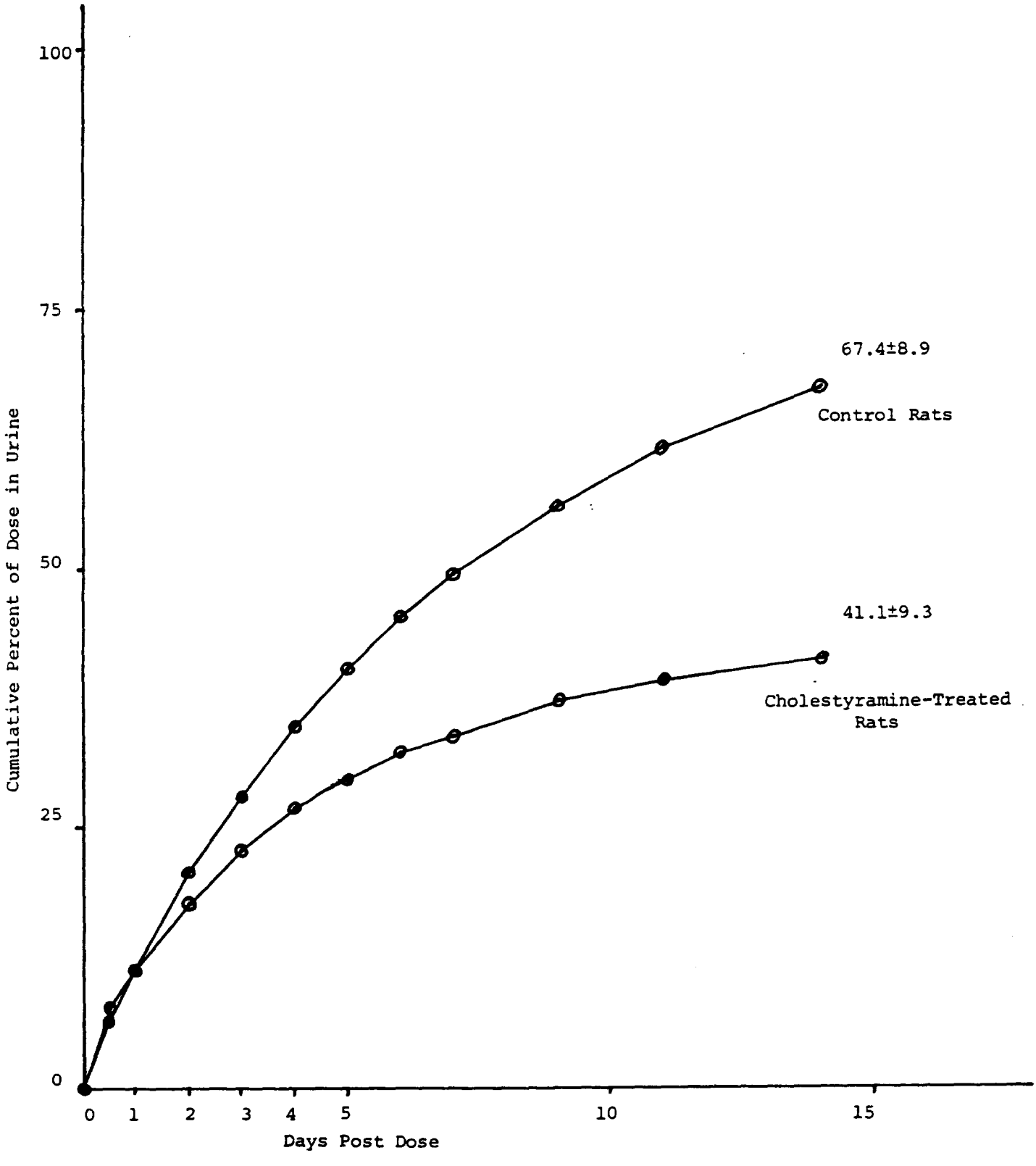


Figure 5

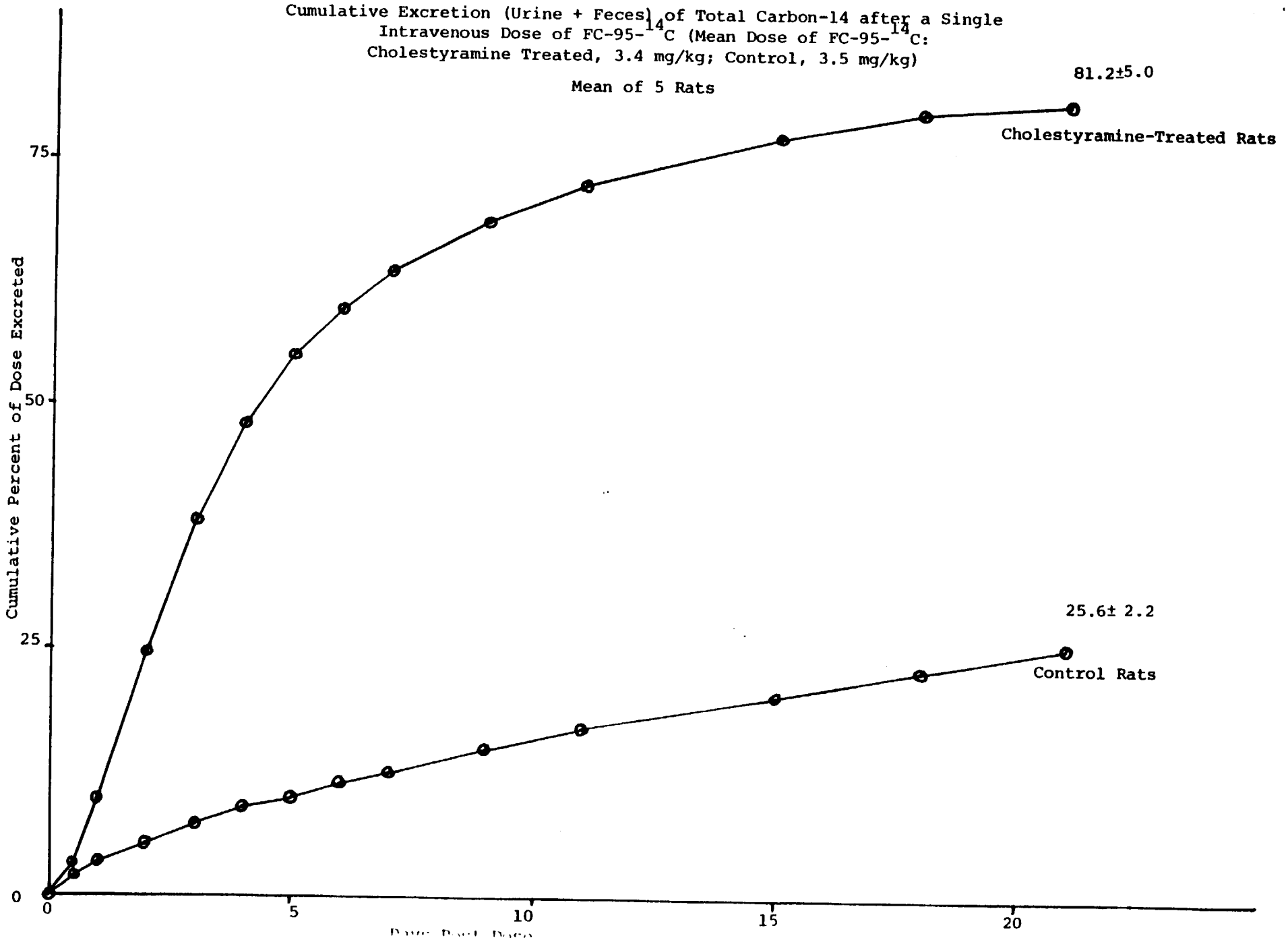
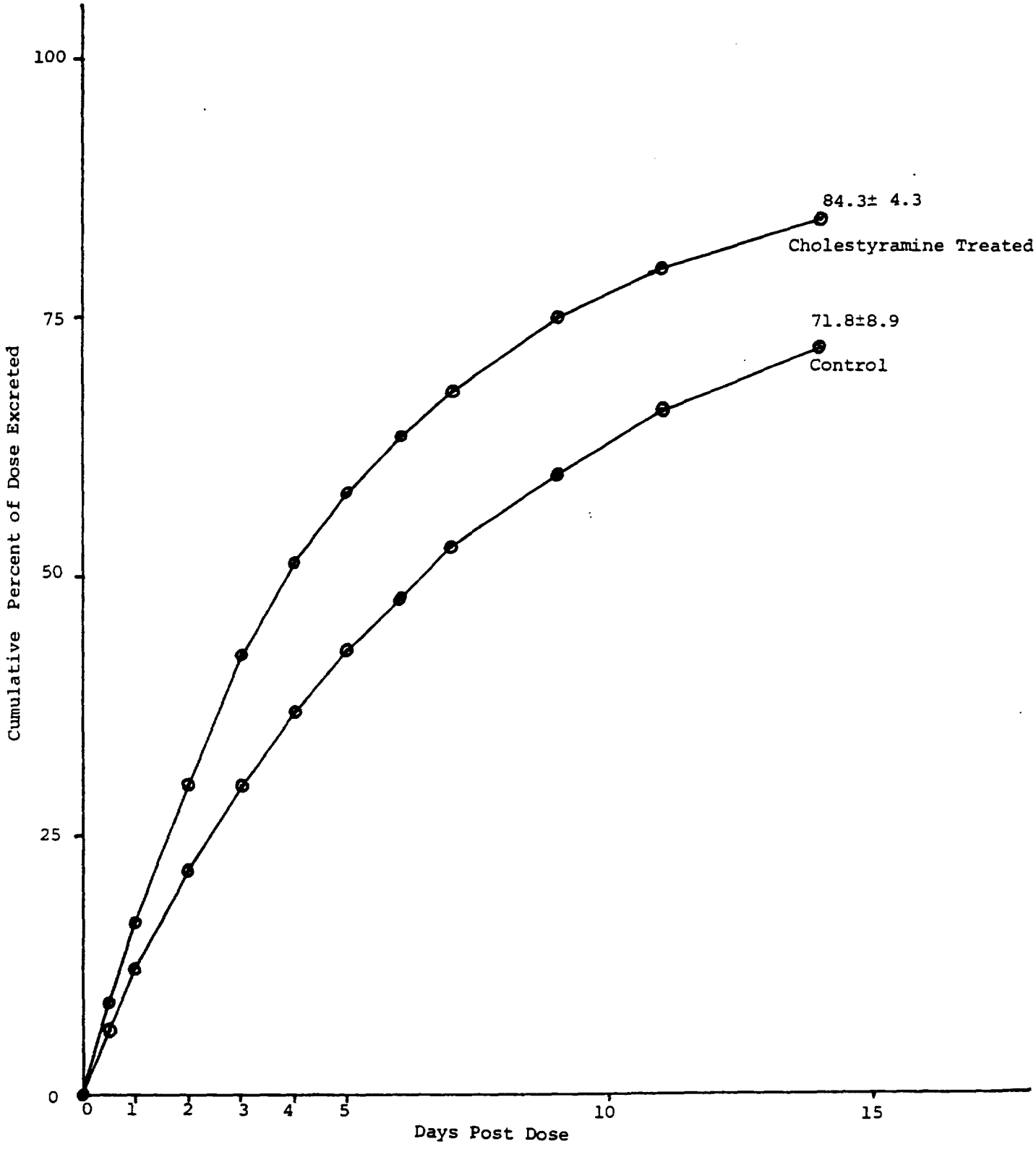

Cumulative Excretion (Urine + Feces) of Total Carbon-14 after a Single Intravenous Dose of FC-95-^{14}C (Mean Dose of FC-95-^{14}C: Cholestyramine Treated, 3.4 mg/kg; Control, 3.5 mg/kg)
Mean of 5 Rats
81.2±5.0
Cholestyramine-Treated Rats
25.6± 2.2
Control Rats
Cumulative Percent of Dose Excreted
0
25
50
75
0
5
10
15
20

Figure 6

Cumulative Excretion (Urine + Feces) of Total Carbon-14 After a Single Intravenous Dose of FC-143-^{14}C (Mean Dose of FC-143-^{14}C: Cholestyramine Treated, 13.3 mg/kg; Control, 13.5 mg/kg)

Mean of 5 Rats



APPENDIX 1

Determination of Carbon-14 Content of FC-95-^{14}C Dosing Solution

Just prior to dosing of rats, the FC-95-^{14}C dosing solution was sampled with calibrated micropipettors[a] directly into counting vials. Six 10 ul and six 50 ul aliquots were pipetted, and 1 ml of water and 15 ml of Aquasol®[b] were added to three of each of the 10 ul and 50 ul aliquots. The remaining three vials were prepared with 2.5 ml of methanol and 7.5 ml of MTSS[c,d]. Corrections were made for background and counting efficiency and, using the specific activity already determined[e], the uCi/2.0 ml was calculated. The data are shown in Appendix Table 1. The dose administered each rat is 2.0 ml of solution containing 0.519 uCi of FC-95-^{14}C which is 1.13 mg of FC-95-^{14}C. This is a mean dose of 3.4 mg/kg for the cholestyramine-treated rats and 3.5 mg/kg for the control rats.

---

[a] L/I Micropipettor, Lab Industries, Berkeley, California.

[b] New England Nuclear, Boston, Massachusetts.

[c] Modified TSS: 25.2 g PPO, 1.01 g Dimethyl POPOP 2nd 3.8 Liters Toluene.

[d] Aquasol® and MTSS were found to be suitable solvent-scintillents for FC-95-^{14}C during specific activity determination (4).

[e] Specific activity = 0.459 uCi/mg. Specific activity determination has been reported (4).

APPENDIX TABLE 1

Carbon-14 Content of FC-95-^{14}C Dosing Solution

10 ul Aliquot uCi/2.0 ml	50 ul Aliquot uCi/2.0 ml
0.5084[a]	0.5111[a]
0.5012[a]	0.5375[a]
0.5284[a]	0.5299[a]
0.5162[b]	0.5299[b]
0.5064[b]	0.5383[b]
0.5098[b]	0.5155[b]

Overall average = 0.5194±0.0128

$$\frac{0.519}{0.459 \text{ uCi/mg}} = 1.133 \text{ mg/2.0 ml}$$

[a] Sample prepared with Aquasol®.

[b] Sample prepared with MTSS.

APPENDIX 2

Determination of Carbon-14 Content of FC-143-^{14}C Dosing Solution

Just prior to dosing of rats, the FC-143-^{14}C dosing solution was sampled with calibrated micropipettors[a] directly into counting vials. Six 10 ul and six 50 ul aliquots were pipetted, and 1 ml of water and 15 ml of Aquasol®[b] were added to three of each of the 10 ul and 50 ul aliquots. The remaining vials were prepared with 2.5 ml of methanol and 7.5 ml of MTSS[c,d]. Corrections were made for background and counting efficiency and, using the specific activity already determined[e], the uCi/2.0 ml was calculated. The data are shown in Appendix Table 2. The dose administered each rat is 2.0 ml of solution containing 2.134 uCi of FC-143-^{14}C which is 4.21 mg of FC-143-^{14}C. This is a mean dose of 13.3 mg/kg for the cholestyramine-treated rats and 13.5 mg/kg for the control rats.

---

[a] L/I Micropipettor, Lab Industries, Berkeley, California.

[b] New England Nuclear, Boston, Massachusetts.

[c] Modified TSS: 25.2 g PPO, 1.01 g Dimethyl POPOP 2nd 3.8 Liters Toluene.

[d] Aquasol® and MTSS were found to be suitable solvent-scintillents for FC-143-^{14}C during specific activity determination (3).

[e] Specific activity = 0.507 uCi/mg. Specific activity determination has been reported (3).

APPENDIX TABLE 2

Carbon-14 Content of FC-143-^{14}C Dosing Solution

10 ul Aliquot uCi/2.0 ml	50 ul Aliquot uCi/2.0 ml
2.1312[a]	2.1212[a]
2.0693[a]	2.1867[a]
2.1588[a]	2.1509[a]
2.0884[b]	2.1012[b]
2.0838[b]	2.2108[b]
2.0985[b]	2.2119[b]

Overall average = 2.1344±0.0495

$$\frac{2.134}{0.507 \text{ uCi/mg}} = 4.21 \text{ mg/2.0 ml}$$

[a] Sample prepared with Aquasol®.

[b] Sample prepared with MTSS.