The Absorption and Metabolism of Orally Administered Tritium Labeled Sodium Stearyl Fumarate in the Rat and Dog

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Sodium stearyl fumarate labeled with tritium at carbon atom 1 of the stearyl alcohol moiety was administered by stomach tube to rats and dogs. Examination of excreta and body fluids indicated that in the rat approximately 80% of the dose was absorbed. The major portion of absorbed sodium stearyl fumarate was metabolized within 2 hr following administration, and was completely metabolized in less than 8 hr. Tritium water was the source of the only significant radioactivity found in body fluids. The sodium stearyl fumarate that was not absorbed, approximately 20% of the administered dose, was excreted in the feces as a mixture of stearyl fumarate and stearyl alcohol. When the experiment was repeated with rats which had received 300 mg/kg unlabeled sodium stearyl fumarate daily for 90 days (stressed rats), the absorption and metabolism of sodium stearyl fumarate was indistinguishable from results obtained with control untreated rats. In the dog, approximately 35% of the administered dose of sodium stearyl fumarate was absorbed and rapidly metabolized. Tritium water was the only source of significant radioactivity found in body fluids within 8 hr after administration. Sodium stearyl fumarate not absorbed, approximately 65% of the dose, was excreted unchanged in the feces within the first 24 hr. The metabolism of sodium stearyl fumarate is qualitatively the same in the rat and dog.

The proposed use of sodium stearyl fumarate (SSF) [PRUV is the registered trade name of Chas. Pfizer & Co. for sodium stearyl fumarate] as a food additive and particularly as a dough improver in bakery products (Geminder *et al.*, 1965; Thomas *et al.*, 1966) required that feeding studies be carried out to determine the metabolic fate of SSF in mammals. Constituent parts of SSF, stearyl alcohol, and fumaric acid individually are regarded as safe for human consumption. Fumaric acid is approved as a food additive and is currently used primarily as a food and beverage acidulant (Federal Register, 1964). Stearyl alcohol is a naturally-occurring substance and is Generally Recognized As Safe (GRAS). It has been isolated from such widely divergent sources as herring and goose, and also occurs in hydrogenated cottonseed, corn, and sunflower oils (Encyclopedia of Chemical Technology, 1951). However, the ester of these components, monostearyl fumarate, is regarded as a new substance and demonstration of its suitability as a food additive is desirable.

Normal rats and dogs were given a single oral dose of tritium labeled sodium stearyl fumarate and absorption and metabolism were assayed by the appearance of tritium water in body fluids.

In addition to acute metabolism experiments it was also necessary to ascertain whether prolonged feeding of sodium stearyl fumarate at a high dose affected the rat's subsequent ability to metabolize SSF. A group of normal rats of both sexes was stressed by consuming regular rat chow to which unlabeled SSF was added, equivalent to a daily dose of 300 mg/kg. At the end of 90 days the absorption, metabolism, and excretion of tritium-labeled SSF was compared in the stressed group and in an untreated control group.

METHODS

Synthesis of Tritium Labeled Sodium Stearyl Fumarate. Stearyl alcohol labeled with tritium at carbon atom 1 was prepared by the reduction of stearic acid with lithium aluminum hydride-^{3}H (50 mCi, New England Nuclear Corp.) according to Nystrom and Brown (1947). The resulting ^{3}H labeled alcohol was used to prepare sodium stearyl fumarate as described by Thomas (1967).

Radiometric Methods. All radioactive samples were measured in a Packard Model 314 or Nuclear Chicago Model 725 liquid scintillation spectrometer. Quenching corrections were made by the internal standard procedure employing tritiated toluene. Nonaqueous samples were assayed in a scintillator consisting of 0.3% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in toluene. Aqueous samples were counted in a similar scintillator in which the solvent was 30% ethanol in toluene (30/70 Scintillator). A maximum of 0.2 ml of aqueous solution was assayed in 15 ml of scintillator solution. Plasma was assayed by dissolving 0.2 ml plasma in 0.5 ml Hydroxide of Hyamine (Packard Instrument Co., Downers Grove, Ill.), followed by the addition of 15 ml 30/70 Scintillator solution.

Feces were assayed by homogenizing in a Waring Blendor with an equal weight of water and lyophilizing the resulting slurry. Samples of the dried powdered feces weighing approximately 50 mg were burned in an atmosphere of oxygen in a Schöniger Combustion Flask containing 10 ml of methanol (Buyske *et al.*, 1962; Kalberer and Rutschmann, 1961). Five ml of the methanol was added to 10 ml of a concentrated toluene scintillator solution and the resulting solution was equivalent to 30/70 Scintillator described above.

Papergrams and thin-layer plates were developed in the systems described. That portion of the papergram containing the controls was sprayed with 0.001% Rhodamine in water, while that portion containing the radioactive sample was scanned with an Atomic Accessories Model RSC-180 4Pi Continuous Papergram Strip Scanner. Nonlabeled sections of thin-layer plates were sprayed with sulfuric acid or Tinopal. Radioactive portions of thin-layer plates were removed by scraping, followed by extraction (see Fecal Work-up, below).

ABSORPTION AND METABOLISM EXPERIMENTS

In each experiment described, SSF was administered by stomach tube as an aqueous suspension in an isotonic vehicle

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Table I. SSF Metabolism in Normal Rats
(Percent of Administered Radioactivity)

Cage No.	Dose Activity μCi/kg	Dose Mass mg/kg	0–24 Hr Excretion Water Urine	0–24 Hr Excretion Water Other[a]	0–24 Hr Feces (non-volatile)	0–24 Hr Total (0–24 hr)	24–48 Hr Excretion Water Urine	24–48 Hr Excretion Water Other[a]	24–48 Hr Feces (non-volatile)	24–48 Hr Total (24–48 hr)	Body Water[b] 48 hr	0–48 hr Total Account[c,d]
2	250	30	5.5	9.9	21.8	37.2	3.9	7.0	1.5	12.4	28.9	78.5
3	250	30	7.9	14.2	25.8	47.9	4.2	7.6	2.9	14.7	35.1	97.7
4	80	30	6.9	12.4	10.7	30.0	4.7	8.5	2.4	15.6	33.8	79.4
5	80	30	3.4	6.1	10.1	19.6	3.0	5.4	3.9	12.3	28.7	60.6
6	80	30	5.8	10.4	8.2	24.4	4.5	8.1	2.8	15.4	37.3	77.1

[a] Estimated from accepted water metabolism of the rat. For each ml of water lost as urine per 100 g of body weight, the rat loses 1.8 ml of water through respiration, feces water, and tissue incorporation (Spector, Handbook of Biological Data, p. 339). [b] "Body Water" is an estimate of the radioactivity remaining in the rat as water at 48 hr. It is the product of plasma specific activity and total body water. Rat body water is taken as 65% of body weight (Spector, Handbook of Biological Data, p. 340). [c] 0–48 Hr, Total Account, is the sum of radioactivity excreted as water by all routes (see Note *a*) and feces for the intervals 0–24 and 24–48 hr, plus the radioactivity remaining in the rat as body water. [d] It should be noted that the total accountable radioactivity is considerably less than 100%. This is due to the evaporation of urine (which contains tritium water) from the metabolism cages. This loss was measured by noting the loss of radioactivity from an equivalent amount of tritium water in a metabolism cage under conditions similar to the metabolic experiment. When the urine radioactivity (and subsequently "other" water excreted) is corrected for loss due to evaporation, the total 0–48 hour Account averages 96.6% for the five cages reported.

Table II. Tritium Water Content of Plasma and Urine Following the Oral Administration of SSF (30 mg/kg, 41 μc/kg) to Normal Rats

Time Hours Post Dose	Plasma Percent of Total Plasma Radioactivity as: Water[a]	Plasma Percent of Total Plasma Radioactivity as: Residue[a]	Plasma Residue Radioactivity as Percent of Administered Dose	Collection Interval Hours Post Dose	Urine Percent of Total Urine Radioactivity as: Water[a]	Urine Percent of Total Urine Radioactivity as: Residue[a]	Urine Residue Radioactivity as Percent of Administered Dose
2	77.6	25.3	0.36	0–2	5.5	94.1	0.09
4	79.0	11.6	0.24	2–4	38.5	52.9	0.59
8	108.5	1.8	0.03	4–8	80.0	11.8	0.14
24	112.6	3.9	0.06	8–24	83.2	2.3	0.08
48	107.0	1.8	0.02	24–48	99.7	1.0	0.01

[a] Plasma or urine was lyophilized, reconstituted with water, and lyophilized again. Water radioactivity is the sum of radioactivity obtained from the two lyophilizations. Residue radioactivity is the radioactivity remaining in the residue after the two lyophilizations.

containing Tween, carboxymethyl cellulose, methocel and methyl, and propyl-*p*-hydroxybenzoate.

No. 1. Absorption and Excretion of SSF in Normal Rats. Ten male Charles River C-D rats weighing approximately 100 g each were maintained in metabolism cages, two rats to a cage. The animals had free access to food and water during the experiment. Tritium labeled SSF was administered to two groups of rats at 30 mg/kg. Each rat received the dose in 1 ml of suspension by stomach tube and was then placed in the metabolism cage. The data for urine and feces are the results of pooled excreta for two rats in each cage. Urine and feces were collected at the end of 24 and 48 hr. The fecal radioactivity recorded in Table I is activity remaining in fecal residues after water had been removed by lyophilization. The chemical identity of fecal radioactivity was established by extraction, addition of carrier SSF and stearyl alcohol, and chromatography over a silica gel column designed to separate SSF and stearyl alcohol (see Extraction and Identification of Fecal Radioactivity below). Both fractions were recrystallized to constant specific activity. At 48 hr the rats were sacrificed and plasma was obtained. The results are recorded in Table I and are reported as percent of administered radioactivity found in each sample.

No. 2. Time Course of Metabolism of SSF in Normal Rats. Fifteen male Charles River C-D rats weighing approximately 135 g received 30 mg/kg tritium labeled SSF. The rats were maintained as groups of three in metabolism cages with free access to food and water. Urine was collected for the following intervals: 0–2, 2–4, 4–8, 8–24, 24–48 hr. At the end of each collection period one group of three rats was sacrificed, and plasma was obtained and pooled. Plasma and urine were processed in the same fashion; after counting, an aliquot of plasma or urine was lyophilized, reconstituted with water, lyophilized again, and reconstituted once more. The sum of radioactivity accumulated in the water removed during the two lyophilizations is regarded as "water radioactivity"; the label found in the last reconstituted sample is taken as "residue radioactivity." The results of the plasma and urine work-up are recorded in Table II.

No. 3. Metabolism of SSF in Stressed and Nonstressed Rats. A group of eight Charles River Weanling rats evenly divided by sex, and weighing approximately 50 g each were fed a normal diet to which sodium stearyl fumarate was added to achieve a dose level of 300 mg/kg. These rats are designated SSF Stressed Rats. A similar group, untreated, served as a control. After 90 days each rat from the treated and control groups received 30 mg/kg tritium labeled sodium stearyl fumarate by stomach tube. Two rats were placed in each metabolism cage, and had free access to normal rat chow and water during the course of the experiment. Urine and feces were collected at the end of 24 and 48 hr. At 48 hr, the rats were sacrificed, bled, and the plasma assayed for radioactivity. The results of the experiment are summarized in Table III. Data are recorded as percent of administered radioactivity found in each sample.

No. 4. Metabolism of SSF in Normal Dogs. Four normal mongrel dogs, two males and two females weighing approximately 10 kg each, received a single dose of 30 mg/kg tritium labeled sodium stearyl fumarate as an aqueous suspension by stomach tube. The dose was contained in 30 ml of vehicle described above. The radioactivity dose was approximately 250 μCi/kg. Dogs were placed in individual

Table III. Summary of Sodium Stearyl Fumarate Metabolism in SSF Stressed and Control Rats[a] Following 30 mg/kg (230 μc/kg) SSF

Percent of Administered Radioactivity

Group	Protocol	0–24 Hr Excretion: Water: Urine	0–24 Hr Excretion: Water: Other[b]	0–24 Hr Excretion: Feces (nonvolatile)	0–24 Hr Excretion: Total (0–24 hr)	24–48 Hr Excretion: Water: Urine	24–48 Hr Excretion: Water: Other[b]	24–48 Hr Excretion: Feces (nonvolatile)	24–48 Hr Excretion: Total (24–48 hr)	Percent of Admin. Dose as Body Water at 48 hr[c]	0–48 Total Account[d]
Males	SSF Stressed	8.0	14.4	7.1	29.5	1.2	2.3	5.1	8.6	44.1	82.2
	Controls	7.6	13.7	9.6	30.9	3.0	5.3	2.3	10.5	43.9	85.3
Females	SSF Stressed	12.4	22.2	6.7	41.3	2.9	5.2	8.0	16.1	54.4	111.8
	Controls	12.6	22.7	0	35.3	3.9	6.9	9.6	20.4	57.3	113.0

[a] The data for each group listed is the average obtained from four rats in the group. [b] Other Water is an estimate of water radioactivity lost by other metabolic processes, and is estimated from the volume of urine excreted. The rat loses 1.8 ml of water via respiration, perspiration, feces water, and tissue incorporation for each ml of urine excreted (Spector, Handbook of Biological Data, p. 339). [c] Body Water is an estimate of total tritium radioactivity (as water) remaining in the rat at 48 hr post dose. It is the product of plasma specific activity at 48 hr and total body water. The choice of what fraction of body weight constitutes total body water in these rats is probably arbitrary. Taking into consideration the size, age, and background, we regard them as obese rats, and subsequently have followed the recommendation of Spector (Handbook of Biological Data, p. 240). Rat body water is (males) 56% of body weight, and (females) 59% of body weight. [d] 0–48 Hr Total Account is the sum of radioactivity excreted for the period 0–48 hr (*i.e.*, urine, other water, and feces) and radioactivity remaining in the rat as body water at 48 hr after the dose.

Table IV. Metabolism of Sodium Stearyl Fumarate in the Normal Dog

Percent of Administered Radioactivity

Dog Number	Dose	0–24 Hr Excretion: Water: Urine	0–24 Hr Excretion: Water: Other[a]	0–24 Hr Excretion: Feces (nonvolatile)	0–24 Hr Excretion: Total (0–24 hr)	24–48 Hr Excretion: Water: Urine	24–48 Hr Excretion: Water: Other[a]	24–48 Hr Excretion: Feces (nonvolatile)	24–48 Hr Excretion: Total (24–48 hr)	Per Cent of Admin. Dose as Body Water at 48 hr[b]	0–48 Hr Total Account[c]
1 Male	Mass	0.5	1.2	51.7	53.4	0.5	1.2	10.9	12.6	13.7	79.7
2 Female	30 mg/kg	0.3	0.5	74.3	75.1	0.2	0.4	0.2	0.8	6.8	82.7
3 Female	Label	0.3	0.6	59.4	60.3	0.4	0.9	2.4	3.7	12.0	76.0
4 Male	250 μc/kg	0.2	0.5	60.2	60.9	0.4	0.9	0.5	1.8	15.6	78.3
Av. of 4 dogs		0.3	0.7	61.4	62.4	0.4	0.9	3.5	4.8	12.0	79.2

[a] Estimated from accepted water metabolism of the dog. For each ml of water excreted as urine, the dog "loses" 2.16 ml of water through respiration, perspiration, feces water, and tissue incorporation (Spector, Handbook of Biological Data, p. 339). [b] Body water is an estimate of the radioactivity remaining in the dog as water at 48 hr after the dose. It is the product of plasma specific activity at 48 hr, and total body water. Dog body water is accepted at 68% of body weight (Spector, Handbook of Biological Data, p. 340). [c] 0–48 Hours Total Account is the sum of the radioactivity excreted for the period 0–48 hr (*i.e.*, urine, other water, and nonvolatile feces), and radioactivity remaining in the dog as body water at 48 hr after the dose.

Table V. Tritium Water Content of Plasma and Urine Following the Oral Administration of SSF (30 mg/kg, 250 μc/kg) to a Dog (No. 2, Female)

Time Hours Post Dose	Plasma: Percent of Total Plasma Radioactivity as: Water[a]	Plasma: Percent of Total Plasma Radioactivity as: Residue[a]	Plasma: Plasma Residue Radioactivity as Percent of Administered Dose	Collection Interval Hours Post Dose	Urine: Percent of Total Urine Radioactivity as: Water[a]	Urine: Percent of Total Urine Radioactivity as: Residue[a]	Urine: Urine Residue Radioactivity as Percent of Administered Dose
2	73.3	30.6	0.17	0–2	68.4	38.7	0.01
4	79.9	15.3	0.09	2–4[b]	...	...	...
8	79.2	19.3	0.12	4–8	56.9	42.2	0.02
24	83.9	14.7	0.09	8–24	81.9	15.4	0.03
48	91.4	7.6	0.04	24–48	81.6	3.2	<0.01

[a] See footnote Table II. [b] No urine obtained during this interval.

metabolism cages, and urine, when available, was collected at 2, 4, 8, 24, and 48 hr post dose. Feces were collected at the end of 24 and 48 hr. Blood samples were drawn from all dogs at 2, 4, 8, 24, and 48 hr after the administration of sodium stearyl fumarate. Plasma, urine, and feces were processed as described in the rat experiment.

A summary of the dog experiments is presented in Table IV and the data are recorded as percent of administered radioactivity found in each sample. The time course of metabolism, which was virtually identical for all four dogs, is shown in Table V for one dog only, No. 2, a female.

Extraction and Identification of Fecal Radioactivity. Fecal samples were homogenized in a Waring Blendor with an equal weight of water and then lyophilized. The dried powder was slurried in 0.1*N* hydrochloric acid and extracted with ether by mechanical shaking overnight. The ether was dried over sodium sulfate and concentrated *in vacuo*. By this method it was possible to routinely recover 80 to 97% of the radioactivity present in feces, based on the original combustion assay.

Aliquots of the concentrated ether extract were applied to thin-layer plates (90 ethyl acetate/15 diethylamine/5 water also 5% acetic acid in isooctane) with known controls. Following development of the plates, the areas corresponding to stearyl alcohol and stearyl fumarate were scraped off the glass plate and placed in 15 ml centrifuge tubes. The scrapings

were extracted with ethanol by mechanical shaking for 1 hr, after which aliquots were removed for radioactive assay. Total recovery of radioactivity applied to the plates ranged between 88 and 100%.

Identification of Rat Fecal Radioactivity. To a portion of the above fecal ether extract was added 97 mg stearyl fumarate and 49 mg stearyl alcohol. The solution was taken to dryness, the residue was dissolved in a small volume of benzene, and applied to a column of 15 g silica gel (28–200 mesh). Stearyl alcohol was eluted with benzene, and subsequently stearyl fumarate was eluted with ether. The fractions corresponding to stearyl alcohol were combined and recrystallized to constant specific activity from acetone. The stearyl fumarate was recrystallized twice from hexane and twice from ether to constant specific activity.

Identification of Dog Fecal Radioactivity. The chemical identity of dog fecal radioactivity matter was established as stearyl fumarate and stearyl alcohol by the following method. Ether extraction of acidified homogenized dog feces (1 male and 1 female) resulted in the recovery of approximately 80% of the radioactivity present in the feces. The extract was dissolved in acetone, carrier stearyl fumarate added, and the solution treated with excess 1*N* sodium hydroxide. The precipitated sodium stearyl fumarate was removed by centrifugation and then converted back to the free acid by dissolving in 10% acetic acid in chloroform. The chloroform was washed with water, dried over sodium sulfate, concentrated to dryness, and the residues analyzed by paper chromatography followed by strip scanning. (Papers were impregnated with 15% phenylcellosolve and 3% glacial acetic acid in acetone, and developed in hexane saturated with phenylcellosolve.)

In each papergram, the only radioactivity present aligned with known stearyl fumarate controls. One sample of a chloroform residue was added to cold carrier stearyl fumarate and recrystallized three times to constant specific activity.

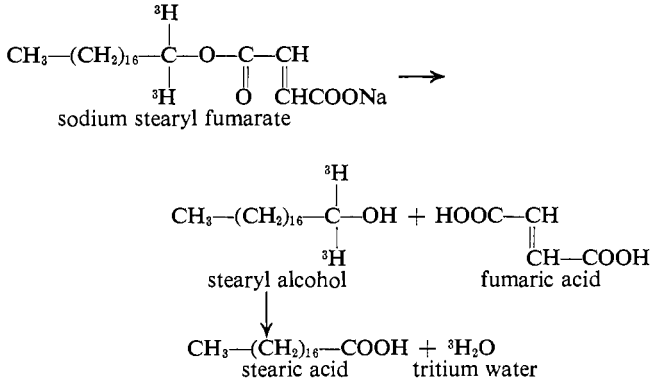
The supernatant solutions remaining after the precipitation of sodium stearyl fumarate (above) were concentrated to dryness, chromatographed over neutral alumina, and fractions containing radioactivity were assayed by paper chromatography (phenylcellosolve system) followed by radioscanning. The single peak of radioactivity on each papergram aligned with known stearyl alcohol controls.

RESULTS AND DISCUSSION

In designing the experiments described in this report, it was necessary to distinguish between three possibilities that: (1) SSF is not absorbed; (2) SSF is absorbed but is *not* metabolized; (3) SSF is absorbed *and* metabolized.

SSF labeled with tritium at carbon 1 of stearyl alcohol was prepared in order to differentiate between various possibilities of absorption and metabolism. Nonabsorbed SSF should be recoverable as fecal residue radioactivity, whereas metabolized SSF would be determined as body water radioactivity. This specific label was selected so that the appearance of tritium water in body fluids following administration of tritium labeled SSF may be taken as evidence of absorption and metabolism. As long as the ester linkage remains intact, tritium is expected to be stable at carbon 1 of stearyl alcohol, and no radioactivity is anticipated in body water. Upon hydrolysis of the ester, tritium will appear in body water at a rate directly proportional to the rate of oxidation of stearyl alcohol to stearic acid. In the rat, cetyl and stearyl alcohols are efficiently absorbed and metabolized to the corresponding fatty acid (Blomstrand and Rumpf, 1954; Stetten and Schoenheimer, 1940).

Since tritium water is rapidly distributed throughout the body, and the isotopic composition of water can be assumed to be constant through the body (Foy and Schmieden, 1960), measurement of water radioactivity in body fluids serves as a direct measure of absorption and metabolism of SSF.



Based on the rationale for tritium labeling, the following observations may be anticipated.

Absorption of SSF	Expected Results
(1) Not absorbed	No radioactivity in body fluids. Label recovered in fecal residues.
(2) Absorbed but *not* metabolized	Radioactivity in body fluids. Label associated with residues and *not* with water.
(3) Absorbed *and* metabolized	Radioactivity in body fluids. Label found *only* in water fraction.

The finding that, following SSF administration, rat and dog body fluids contained a significant portion of the administered radioactivity in the form of tritium water is taken as evidence that absorption and hydrolysis of SSF occurred.

Experiment No. 1. ORAL ABSORPTION AND EXCRETION OF SSF IN NORMAL RATS. During the first 24 hr of Experiment 1, 3 to 8% of the administered radioactivity was excerted in urine and 8 to 26% in feces (Table I). Additional tritium water was lost via respiration and other routes and is estimated at 6 to 14% (see footnote *a* in Table I). For the period 24 to 48 hr, urine accounts for 3–5% and feces 2–4% of the administered radioactivity. The remaining radioactivity may be obtained by extrapolating the activity found in plasma to total body water. After 48 hr the radioactivity remaining in rats as tritium water accounted for one-third of the administered dose. The total radioactivity found or accounted for at 48 hr ranged from 61–98% of the administered dose (see footnote *d* in Table I).

Samples of urine collected at 24 and 48 hr and plasma collected at 48 hr were lyophilized and all significant radioactivity could be accounted for in the water lyophylate. Thin-layer chromatography of 0–24 hr fecal extracts from material collected from combined excreta of cages 3 and 4 indicated that approximately 80% of the radioactivity applied to the plate aligned with known stearyl fumarate controls, and approximately 10% of the applied radioactivity was identified with stearyl alcohol.

These results indicate that following an oral dose of tritium labeled SSF at 30 mg/kg, approximately 80% of the dose is absorbed and metabolized, and that approximately 20% of the administered dose is recovered in the feces as unchanged stearyl fumarate and free stearyl alcohol. The major portion of fecal radioactivity is associated with stearyl fumarate.

Experiment No. 2. TIME COURSE OF METABOLISM OF SSF IN NORMAL RATS. Results of the second experiment in which the time course of plasma and urine radioactivity were studied are recorded in Table II. In a sample of plasma obtained 2 hr after the oral administration of tritium labeled SSF, approximately 77% of the plasma radioactivity was due to tritium water and 23% was found in plasma residues. It should be pointed out, however, that the actual amount of radioactivity in the residue was quite small. Two hours after the administration of SSF the amount of radioactivity found in the plasma residue of a rat, after all water has been removed, was approximately 0.3% of the administered dose.

The amount of label found in plasma residue after 2 hr diminished rapidly. At 8 hr and later periods that were examined, no significant activity was found in the residue, and all of the radioactivity of plasma could be recovered in the water portion of the plasma.

The radioactivity found in urine and urine residues follows a pattern similar to that observed in plasma. During the intervals 0–2, 2–4, and 4–8 hr following the administration of SSF, urine residues contained a significant portion of the radioactivity found in urine. As shown in Table II, urine residue radioactivity decreases with time, and for the intervals 8–24 and 24–48 hr, no significant radioactivity was found in the residue portion. As in the case of plasma, the actual quantity of radioactivity remaining in urine residues after water has been removed is small. During the interval 0–8 hr, the total amount of radioactivity found in urine residues was less than 1% of the administered dose of SSF.

Detailed examination of plasma at intervals immediately following the administration of SSF suggests that within the first 2 hr, almost all of the absorbed SSF has been hydrolyzed and the resulting labeled stearyl alcohol has been oxidized to yield tritium water. The data also indicate that the process of absorption, hydrolysis, and oxidation is complete in less than 8 hr.

The completeness of metabolism is also indicated by the finding that approximately 1% of the administered dose of SSF is excreted in urine, in the residue portion, within the first 8 hr. After 8 hr tritium water is the sole source of radioactivity in urine.

Experiment No. 3. METABOLISM OF SSF IN STRESSED RATS. The purpose of this experiment was to determine whether prolonged feeding of a large dose of SSF will affect the ability of the rat to absorb and metabolize sodium stearyl fumarate. At the end of 90 days feeding of unlabeled SSF at 300 mg/kg, the stressed and control group of rats (non-stressed) received identical doses of tritium labeled SSF.

A summary of Experiment 3 is tabulated in Table III. The data for each group are the average obtained from the four rats in that group. It may be seen from Table III that male rats, SSF stressed and controls, excreted approximately 30% of the dose of label in the first 24 hr, excreted approximately 10% of the dose during the period 24 to 48 hr, and that plasma radioactivity accounted for 44% of the administered dose of labeled SSF at 48 hr. These data indicate that the rats absorbed approximately 90% of the dose, and excreted approximately 10% of the dose in the feces. It is clear that the pattern of excretion of label is identical for the SSF stressed group and the controls. The results obtained with female rats are quite similar. At the end of 24 hr, SSF stressed rats excreted 41.3% of the dose of label and control rats excreted 35.3%. The difference in excretion between the two groups is due to the fact that female control rats did not defecate during the first 24 hr. During the period 24 to 48 hr, SSF stressed rats excreted 16.1% of the dose and control rats excreted 20.4% of the dose. At 48 hr after the administration of labeled SSF, the stressed rats were found to contain 54.4% of the administered dose as plasma radioactivity and the control rats 57.3%, a difference which is not significant.

A comparison of the radioactivity that was recovered from male *vs.* female rats suggests that females may have absorbed a greater portion of the dose than the males. It should be noted that the total recovery of administered radioactivity averaged approximately 84% for male rats and 112% for female rats (see the last column in Table III). In SSF Metabolism Experiment No. 1, the average recovery of radioactivity from 10 male rats was 80% (Table I, last column and footnote *d*) in good agreement with the present male data. The recovery of radioactivity from dogs treated with SSF (last column, Table IV) showed no sex difference and averaged 79.2% for the four dogs. All this suggests that factors obtained from the literature for estimating metabolic water and total body water are estimates at best, and probably require refinements tailored to the specific experiment.

Experiment No. 4. ORAL ABSORPTION, METABOLISM, AND EXCRETION IN NORMAL DOGS. A summary of the results obtained in four normal dogs following the administration of tritium labeled sodium stearyl fumarate is presented in Table IV. Within the first 24 hr, urinary excretion and other body water losses (see Table IV, footnote *a*) account for 1% of the administered dose. The major portion of the administered radioactivity is found in the feces, and averaged 61.4% for the four dogs (Range: 51.7% to 74.3%).

During the period 24 to 48 hr, total water excretion averaged 1.3%, and fecal excretion of radioactivity, with the exception of Dog No. 1, was almost negligible. At the end of 48 hr, 12% of the administered dose could be accounted for as body water.

A more detailed pattern of the distribution of radioactivity found in dog plasma and urine is presented in Table V. The data from one dog only are recorded. However, these results are typical of the pattern observed in the three additional dogs. The level of radioactivity in plasma did not change appreciably through the 0 to 48 hr period. There is, however, a tendency to reach a peak at 8 hr and then drop off. At 2 hr, the first period examined, approximately two-thirds of the radioactivity in plasma is associated with water and one-third with the residue. The total amount of label in the residue, however, is very small and averages less than 0.3% of the administered dose for the four dogs. At subsequent periods examined, plasma residue radioactivity declined rapidly and was negligible within 24 hr.

The time course and distribution of radioactivity in dog plasma is virtually identical with the pattern observed in rats in the previous experiments.

Dog urinary excretion of label (Table V) is quite similar to previously obtained rat data (Table II). Urine collected within the first 24 hr contains approximately 40% of the excreted radioactivity as residue (Table V). As with plasma, residue falls off with time and urine collected over the interval 24 to 48 hr contains practically no radioactivity associated with the residue. The total amount of radioactivity excreted in urine as residue is extremely small and was approximately 0.06% of the administered dose for the 48 hr period of observation.

A detailed workup of dog feces, as described in the experimental section, indicated that approximately 75% of the labeled material found in dog feces is stearyl fumarate, and that the remainder is stearyl alcohol.

It may be concluded that the metabolism of SSF is qualitatively identical in the rat and the dog. That portion of tritium labeled SSF which is absorbed is rapidly hydrolyzed and metabolized to tritium water. That fraction of the dose not absorbed is rapidly excreted in the feces, mostly as unchanged stearyl fumarate. Normal young adult rats (100–150 g) absorb approximately 80% of a dose of 30 mg/kg; adult rats (300–500 g) absorb 90% or more of a similar dose. The dog absorbs approximately 35% of a 30 mg/kg dose. Rats of either sex, which have been stressed by feeding 300 mg/kg/day of SSF for 90 days, show no difference from control rats in the absorption and metabolism of administered tritium labeled SSF.

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