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RESEARCH ARTICLES

In Vivo and *In Vitro* Pharmacokinetics and Fate of Furaltadone in Meat- and Milk-Producing Animals

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Abstract □ The metabolism of furaltadone was examined by an *in vitro* hepatic study in cows and goats and an *in vivo* study in goats using ^{14}C-labeled and unlabeled drug. The half-life of furaltadone was 13 min in the homogenates of caprine and bovine liver and 35 min in the *in vivo* study of the goat. Less than 2% of the parent drug was present in the urine of animals dosed either intravenously or intramammarily. No furaltadone was detected in the milk after 24 h. Overall, the parent compound was rapidly absorbed, distributed, and widely degraded in the lactacting goat. The compound, labeled at the 2-formyl carbon of the furan ring, had a radioactivity recovery of 81% in the feces and urine. Of the total radioactivity, 99.4% infused into the udder had been absorbed after 72 h. Tissue distributions of radioactivity in decreasing order of abundance were: kidney, udder, liver, duodenum, muscular tissue, adipose tissue, and bile.

Keyphrases □ Furaltadone—*in vitro* and *in vivo* metabolism, fate in cattle and goats, pharmacokinetics □ Metabolism—furaltadone, *in vitro* and *in vivo*, fate in cattle and goats, pharmacokinetics □ Pharmacokinetics—furaltadone, *in vitro* and *in vivo* metabolism, fate in cattle and goats

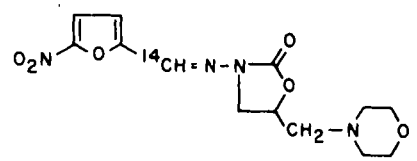
The nitrofuran furaltadone, (±)-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone (I), had been commonly administered intramammarily to lactating and dry cows for treatment against the micrococci in mastitis (1, 2). In 1973, Cohen *et al.* (3) reported that this nitrofuran had a strong carcinogenic response, and soon after, this compound was withdrawn from the market because of a lack of information on residues of the compound in animals. Paul *et al.* (4) demonstrated the presence of furaltadone in the bile of the chicken and dog and in the urine, but not the feces, of the rat. No furaltadone was found in animal tissues after oral administration, with sensitivity to 1 ppm. Other investigators were unable to detect the parent drug in milk 48 h after treatment using methods with detection limits at 10 ppb (5) and 5 ppm (6). Although these studies showed that furaltadone is biodegradable in mammals, an evaluation of its metabolsim and excretion in livestock and of residues in the blood, milk, and tissues has never been reported.

In the present study, the hepatic metabolism of furaltadone in goats and cattle are evaluated. Data from a pharmacokinetic study in lactating goats, as a model for the cow, are also presented. The distribution and metabolic fate of furaltadone, labeled at the 2-formyl carbon of the furan ring, and unlabeled drug administered intramammarily are compared.

EXPERIMENTAL

Materials—Unlabeled furaltadone was obtained from Norwich Pharmaceutical Co., Norwich, N.Y. [^{14}C]Furaltadone (0.106 μCi/mg) was synthesized in our laboratory[1]. The chemical purity of the compound was determined by TLC in acetone–chloroform (3:1, v/v) on a plate[2]. The spot corresponding to furaltadone in reference to a standard was cut from the plate and counted by liquid scintillation counting (LSC). Purity was >99%. Nonradioactive furaltadone was synthesized by the same preparative procedure. This product was compared with the commercial standard using melting point; elemental analysis; IR, UV, ^{1}H-NMR, ^{13}C-NMR, and MS spectrometry to support the identity of the [^{14}C]-furaltadone.

***In Vitro* Metabolism**—Fresh goat and cattle livers of known weights (~6 g) were homogenized[3] in 0.05 M sodium phosphate buffer, pH 7.4. The 125-mg/mL homogenate was centrifuged[4] at 5°C and 4500 × *g* (7000 rpm) for 10 min and then at 14,000 × *g* (12,500 rpm) for 30 min using a



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[1] Personal communications from Norwich Pharmaceutical Co., Norwich, N.Y.
[2] SAF; Gelman, Ann Arbor, Mich.
[3] Potter-Elvehjem Homogenizer.
[4] Ti-50 8-in. rotor, Model L2-65, Class D; Beckmann, Fullerton, Calif.

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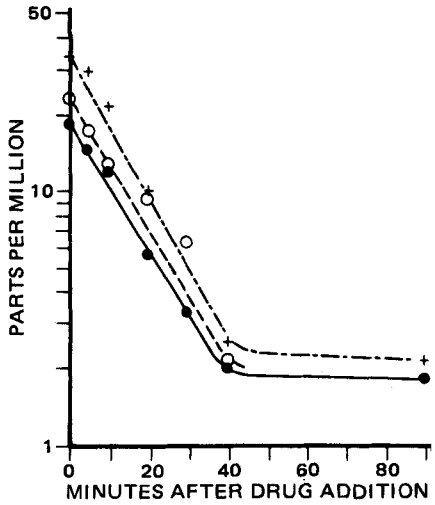

Figure 1—*Comparison of the metabolism of furaltadone in cattle and a goat. Key: (—) bovine liver, ^{14}C-labeled; (- - -) bovine liver, unlabeled; (-·-) caprine liver, unlabeled.*

fixed-angle rotor. The pellet containing the nuclei and mitochondria was discarded and the supernatant was saved.

In vitro experiments were carried out by adding the following ingredients in sequence into test tubes immersed in ice:

1. One milliliter of ^{14}C-labeled or unlabeled furaltadone (~2 μmol) and magnesium chloride (25 μmol) in water,
2. One milliliter of a tissue blank. For the control without furaltadone, magnesium chloride (25 μmol) in water was added.
3. Cofactor buffer mixture consisting of 0.65 μmol of NADP monosodium[5], 10.0 μmol of glucose-6-phosphate disodium[5], and 50.0 μmol of nicotinamide[5] dissolved in 3 mL of 0.5 M sodium phosphate buffer, pH 7.4.
4. Two milliliters of enzymes, containing microsomes equivalent to 250 mg of liver added while the tube was in the incubation bath. The resulting mixture in each test tube was incubated aerobically at 37°C with magnetic stirring in a water bath[6]. Samples in duplicate were removed from the water bath at 0, 5, 10, 20, 30, 40, and 90 min. The control containing no nitrofuran was incubated for 90 min.

***In Vivo* Metabolism with Unlabeled Furaltadone**—Two lactating goats, acclimatized in individual metabolism cages, were fitted with catheters[7] the evening before dosing, and control samples of urine and blood (10 mL) from the jugular vein were collected. A balanced diet consisting of alfalfa hay and water was provided *ad libitum* and a 12% concentrate[8] was given in limited amounts. The animals were milked once a day.

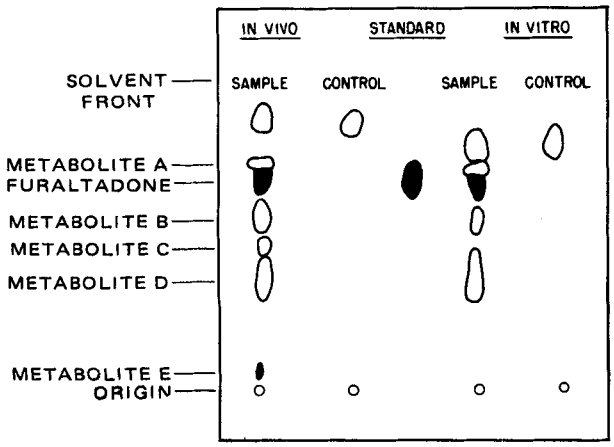

Figure 2—*Comparison of extracts of* in vivo *and* in vitro *metabolites of furaltadone by TLC in acetone–methanol–glacial acetic acid (50:50:1).*

[5] Calbiochem, San Diego, Calif.
[6] Landa MGW WB-20/R, West Germany.
[7] 16 FR Bardex Foley, Murray Hill, N.J.
[8] Illinois Calf 12, Ill.

Table I—*In Vivo* Metabolites of Furaltadone by TLC in Two Discrete Systems

Compound	Appearance Under UV Light	R_f Values: Acetone–Acetic Acid (100:1)	R_f Values: Acetone–Methanol–Acetic Acid (50:50:1)
Metabolite A	Bright yellow fluorescent	0.47	0.75
Furaltadone	Dark quenched	0.50	0.66
Metabolite B	Purple fluorescent	0.19	0.55
Metabolite C	Blue fluorescent	0.06	0.46
Metabolite D	Bright yellow fluorescent	0.00	0.42
Metabolite E	Dark quenched	0.00	0.05

Intravenous Dosing—Furaltadone dissolved in acidified water just before dosing was injected into the jugular vein utilizing an intravenous catheter[9] at a dose of 10 mg/kg of body weight immediately after the morning milking. Blood and urine samples were collected at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, and 210 min.

Intramammary Dosing—The two goats were administered furaltadone equivalent to 37.5 mg/kg of body weight. The nitrofuran was suspended in pure peanut oil[10] using an ultrasonicator[11]. Blood and urine samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 19 and 23 h.

***In Vivo* Metabolism with ^{14}C-Labeled Furaltadone**—A 31-kg milking goat was accommodated and fed under similar conditions as the goats in the unlabeled study. One day prior to treatment, control samples of feces, urine, milk, and blood were collected. The goat was dosed with 777 mg (82.6 μCi) of [^{14}C]furaltadone dispersed in an equal amount of pure peanut oil into the udders immediately after the morning milking. The goat was milked twice a day. Blood and urine were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, 14, 24, 30, 36, 48, 54, 60, and 72 h. The goat was milked every 12 h after infusion for a total of 72 h. Feces were collected every 6 h for the first 12 h and every 12 h for the remainder of the experiment. Aliquots of urine were frozen at −40°C for later analysis. The goat was slaughtered 72 h after treatment. Selected tissue samples and organs were collected and frozen for subsequent analysis.

Analytical Methods—The *in vitro* incubation mixtures were analyzed for furaltadone by direct UV measurement[12] at 360 nm as well as by HPLC. An incubated control served as the background. No metabolite was detectable by HPLC at the concentrations used. All the *in vivo* samples were quantitated in duplicate on a reverse-phase column[13] by HPLC[14] (7) at 360 nm.

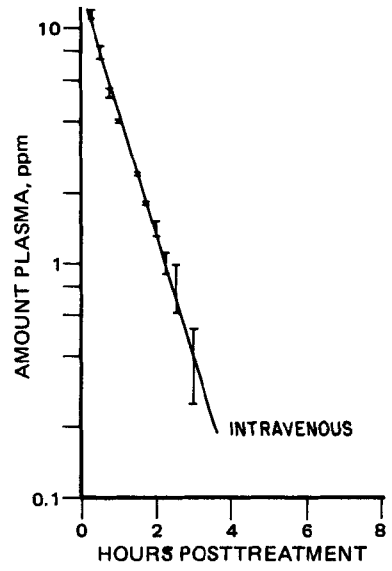

Figure 3—*Concentration of furaltadone in plasma in two intravenously dosed goats: $K_e = 1.17\ h^{-1}$; $t_{1/2} = 0.59\ h$; r = 0.996.*

[9] 16-Gauge, 8-in. Radiopaque Teflon Intravenous Placement; Sandy, Utah.
[10] Planters, Div. of Standard Brands, New York, N.Y.
[11] Branson Sonic Power Co., Danbury, Conn.
[12] Cary Model 11M UV Spectrophotometer, Pasadena, Calif.
[13] μ-Bondapak C_{18}, Waters Associates, Milford, Mass.
[14] Tracor, Austin, Tex.

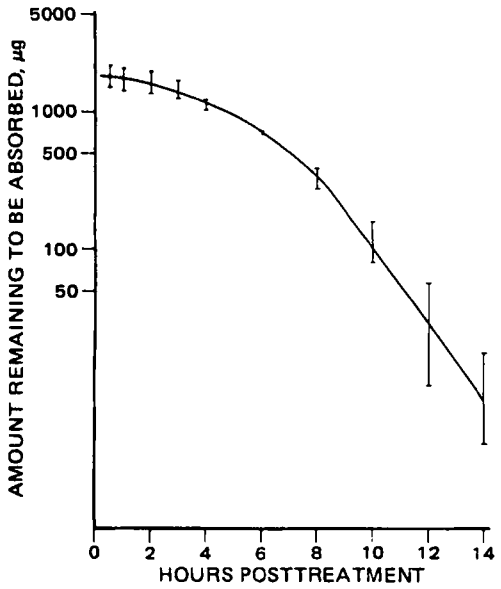

Figure 4—*Amount of furaltadone remaining to be absorbed at various times following its intramammary administration to two goats:* $K_a = 0.53\ h^{-1}$; $t_{1/2} = 1.31\ h$.

The *in vivo* radioactive urine samples were analyzed by TLC. Quantitation was as described for the chemical purity evaluation. Control urine served as the background.

Total Radioactivity Quantitation—Fecal pellets were blended with water (1:8, w/v) in a blender[15]. An aliquot of slurry, in triplicate, equivalent to ~50 mg of feces and 1.5 mL of solubilizer[16] was heated at 52°C for 90 min, cooled, and 0.5 mL of hydrogen peroxide added for decolorization. Radioactivity was determined by LSC[17] after the addition of 10 mL of a fluor[18] and 0.8 mL of 1 M HCl. Whole blood (0.2 mL) was swirled with 0.5 mL of solubilizer[16]–ethanol (1:2) at 60°C. After cooling, 0.5 mL of hydrogen peroxide was added. Whole milk was similarly treated with the elimination of the hydrogen peroxide step. Both milk and blood samples, each in triplicate, were mixed with 10 mL of the fluor and 0.5 mL of 0.5 M HCl and counted by LSC after dark adaptation. Radioactivity in the urine was determined by counting of a mixture containing 0.2 mL of urine, 0.2 mL of water, and 10 mL of fluor. These determinations were carried out at each time period. The level of radioactivity in

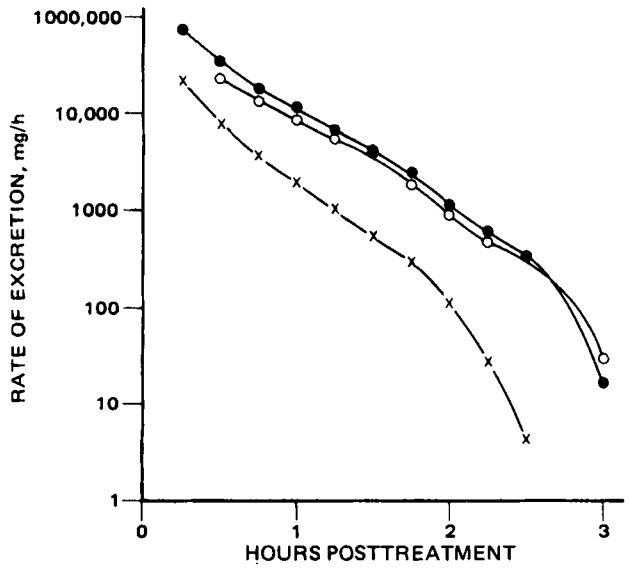

Figure 5—*Average rate of urinary excretion of furaltadone (X) and metabolites following intravenous administration to two goats. Values for metabolites A (●) and E (○) are as furaltadone equivalent.*

[15] Waring, Hartford, Conn.
[16] Protosol; New England Nuclear, Boston, Mass.
[17] Tricarb Model 3320; Packard, Downers Grove, Ill.
[18] Aquasol; New England Nuclear, Boston, Mass.

Table II—Radioactivity Distribution in a Goat Dosed Intramammarily with [^{14}C]Furaltadone [a]

Source	Recovery After 72 h, % of dose
Blood	0.093
Milk	0.76
Feces	25.11
Urine	56.01
Kidney	0.13
Liver	0.51
Total	82.61

[a] Dose: 82.57 µCi/776.97 mg of furaltadone.

the bile was quantitated by counting 0.2 mL of bile with 10 mL of fluor. Tissues, cut in small pieces, were quick-frozen and ground to a powder. An equivalent of 100 mg of tissue powder was mixed with 0.4 mL of water and 2 mL of solubilizer[16] and warmed at 52°C until dissolved. The total radioactivity was quantitated by LSC after the addition of 10 mL of the fluor and 0.8 mL of 1 M HCl and dark adaptation. Each tissue sample was counted in quadruplicate. The internal standard method with [^{14}C]toluene was employed for all samples. Counting efficiencies were 65–86% for urine, 49–64% for feces, 71–82% for blood, 84–88% for milk, and 73–85% for tissues.

Determination of Bound and Free Radioactivity in the Tissues—The powdered adipose tissue was extracted directly with chloroform (1:7, w/v). The fluor[18] was added to 1- and 2-mL aliquots of chloroform extracts from LSC. The liver, kidney, udder, duodenum, and muscular tissue powders were homogenized with water (1:5, w/v) and extracted with dichloromethane–isopropyl alcohol–water (75:25:2). Each tissue extraction was duplicated and subsequently counted as three phases: organic, aqueous, and a residue pellet. The tissue, expressed in gram-equivalent of protein, was determined by a modified Folin–Lowry assay (8).

Metabolites—A homogenate mixture incubated for 3 h at 37°C was extracted by chloroform and by dichloromethane–isopropyl alcohol–water (75:25:2). Urine from the *in vivo* experiment was extracted by the same solvents. Furaltadone and its metabolites were separated by TLC on silica gel G[19] and developed in discrete systems of acetone–methanol–glacial acetic acid (50:50:1) and acetone–acetic acid (100:1).

RESULTS AND DISCUSSIONS

The first-order disappearance of furaltadone and [^{14}C]furaltadone in supernatants obtained from cattle and goat livers is illustrated in Fig. 1. The half-life of furaltadone disappearance was 13 min during the first 40 min of incubation. Three metabolites (A, B, and D) of furaltadone were

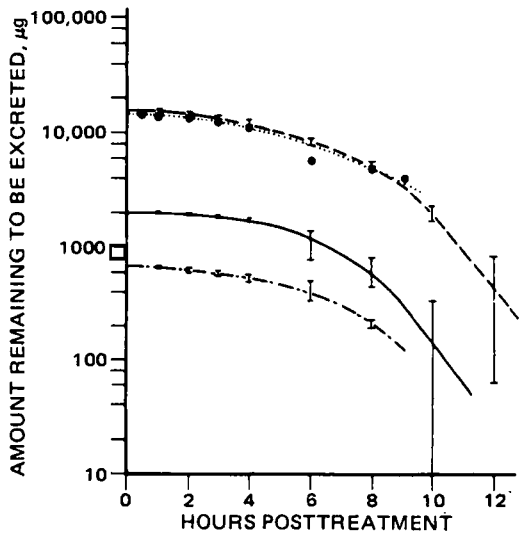

Figure 6—*Amount of furaltadone (– –) and metabolites remaining to be excreted at various times following intramammary administration to two goats. Values for metabolites A (—) and E (-·-) are as furaltadone equivalent. Key: (·····) [^{14}C]furaltadone.*

[19] Mallinckrodt, St. Louis, Mo.

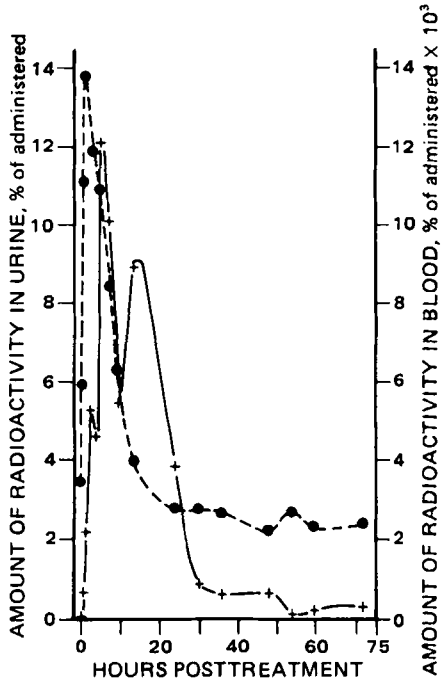

Figure 7—*Radioactive materials found in the urine and blood of a goat intramammarily infused with [¹⁴C]furaltadone. Key: (+) urine; (●) blood.*

isolated from the liver supernatant using TLC. The metabolites obtained from cattle and goats were similar. The metabolites obtained from the liver supernatant preparations were compared with those in the urine of animals dosed intravenously with furaltadone (Fig. 2 and Table I). Urine from furaltadone-treated animals contained two metabolites (C and E) not isolated from the *in vitro* liver preparations.

The metabolites of furaltadone and furaltadone isolated from liver supernatants failed to account for all the radioactivity present. An incubation mixture was fortified with 3.95 μg/mL of [¹⁴C]furaltadone. Following incubation the mixture contained 0.98 μg/mL. The postincubation supernatant was deproteinized and filtered. The filter cake contained 24.7% of the total radioactivity, the filtrate 72.6%. The presence

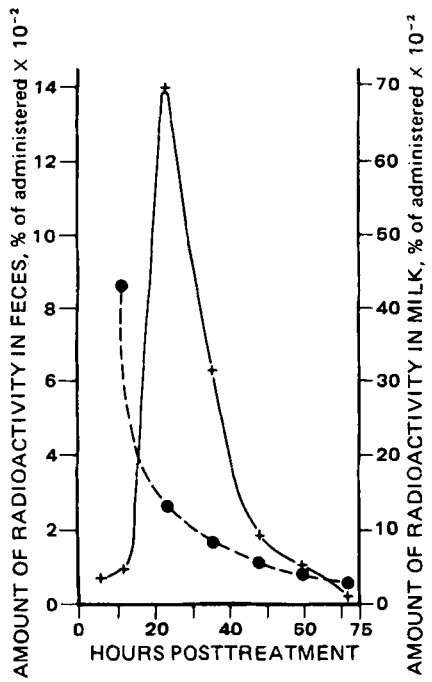

Figure 8—*Radioactive materials found in the feces and milk of a goat intramammarily dosed with [¹⁴C]furaltadone. Key: (+) feces; (●) milk.*

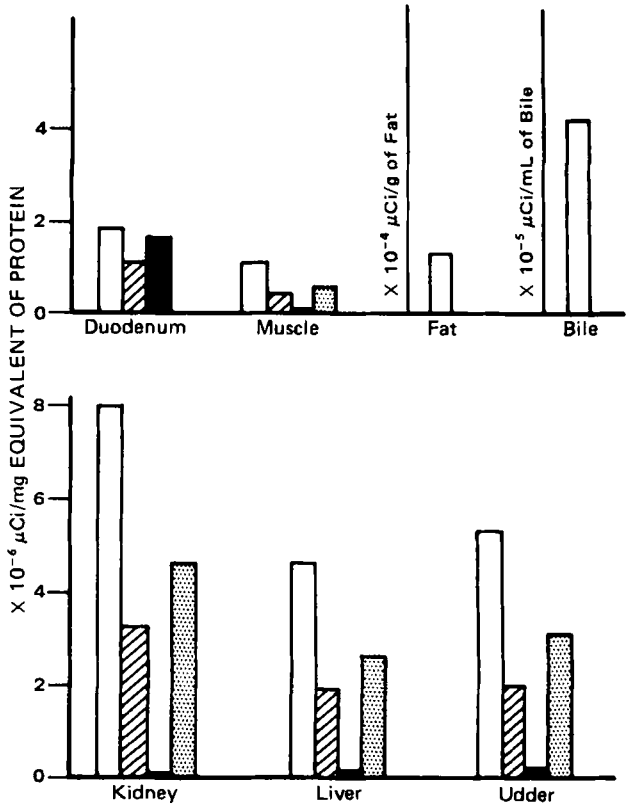

Figure 9—*Distribution of bound and free radioactivity in selected caprine tissues. Key: (□) before extraction; (▨) aqueous phase; (■) organic phase; (▩) residue.*

of carbon-14 in the filter cake indicated that nonextractable radioactivity from [¹⁴C]furaltadone was present. The bound material probably accounted for the persistence of the furaltadone in bovine and caprine liver during the 40–90-min incubation period, illustrated in Fig. 1. When the filtrate was extracted with dichloromethane–isopropyl alcohol–water the ratio of radioactivity in the organic extract to the aqueous filtrate was 1:1, which implied that polar metabolites were present. Similarly, in the *in vivo* study, chloroform and dichloromethane–isopropyl alcohol–water each extracted 13% of the radioactivity in urine.

An *in vivo* absorption, distribution, and elimination study was carried out with unlabeled furaltadone in two milking goats. The linear disappearance of furaltadone from plasma on a semilogarithmic plot of concentration *versus* time (Fig. 3) indicated that the data could be described by a one-compartment open kinetic model. The estimated half-life of furaltadone disappearance in goats was 35.4 min; this was comparable with 33 min in the rat and 37 min in the dog (9). At time zero, the plasma concentration was 14 mg/L. If the plasma occupied 5% of the body weight, the calculated concentration in plasma would have been 200 mg/L based on a dose of 10 mg/kg of body weight. This discrepancy between the observed and calculated concentrations of furaltadone in plasma indicated that furaltadone was rapidly distributed to and sequestered at an extracellular or intracellular site. Similar findings in rats were reported by Buzard and Conklin (9).

The amount of furaltadone remaining to be absorbed was calculated by the method reported previously by Wagner and Nelson (10). The calculated amount absorbed at each time period was plotted against time following drug administration (Fig. 4). The value of K used for calculation was the K_e determined in the intravenous study (1.17 h^{-1}). The half-life of absorption was determined from the linear segment of the curve and used for calculation of the absorption rate constant (10). A comparison of the doses administered and the calculated amounts of drug absorbed during a 14-h postdose period following intramammary drug infusion indicated that an average of 112.3% of the administered dose was absorbed. Although the calculated amount absorbed exceeded 100% and was therefore somewhat in error, the magnitude of the value indicated that furaltadone was almost totally absorbed from the udder. Further support for the almost complete absorption from the udder comes from results obtained following the intramammary infusion of [¹⁴C]furaltadone, where 99.4% of the infused dose was absorbed.

An examination of the rate of excretion of furaltadone and its metabolites in urine following intravenous administration (Fig. 5) indicated that the metabolites were excreted faster than the parent nitrofuran. Less than 2% of the furaltadone administered was recovered as such following intravenous and intramammary administration (Fig. 6).

The two metabolites that were quantitated accounted for <3%, expressed as furaltadone equivalent of the total administered dose. Similar recovery of furaltadone was observed following intramammary [^{14}C]-furaltadone administration to a lactating goat. The 5% recovered as unchanged drug and two metabolites is minor compared with the 56% radioactivity in the urine. Hence, the remainder of the radioactivity must be attributed to the presence of metabolites.

Of the 82.6% radioactivity accounted for in the [^{14}C]furaltadone study, 81% was present in the feces and urine (Table II). Within 48 h after administration, 99% of the radioactivity had been excreted in the urine, (Fig. 7), 95% in the feces, and only 0.06% of the radioactive dose was detectable in the milk (Fig. 8). Furaltadone was rapidly absorbed from the udder, as less than half the radioactivity was detected in the milk from the 12-h to the 24-h milkings. This was also supported in the unlabeled study by the absence of detectable furaltadone in milk 24 h after intramammary dosing.

Radioactive materials were present in decreasing levels of radioactivity in these tissues: kidney, udder, liver, duodenum, muscularis, and adipose tissues (Fig. 9). Of the total radioactivity administered, 1.16 ppm (expressed as furaltadone equivalent) was present per gram of fat, 2.1 ppm per gram of muscle, and 0.5 ppm per milliliter of bile. In the adipose tissue, no radioactivity was extractable with chloroform, which implied the absence of free furaltadone. In the organic solvent extract of the muscle, the equivalent of 0.19 ppm of the original radioactivity was recovered. The remainder of the radioactivity was bound to the tissue residue or was in the aqueous phase. Since furaltadone is lipophilic, and if the parent compound migrated to the tissues in its unchanged form, more radioactivity would be expected in the fatty tissues than in the lean meat. Labeling of normal body constituents as a result of metabolic degradation of furaltadone may explain why more radioactivity was present in the aqueous phase of the muscular tissue. The normal sites of absorption, metabolism, and excretion of furaltadone contained a relatively high amount of radioactivity. However, most of the radioactivity was retained in the aqueous and residue pellet of the udder, liver, and kidney tissue. In the duodenum, on the other hand, more radioactivity was present in the organic extract.

Radioactivity was found in the udder after 72 h, but since the majority of the activity was present in the unextractable residue and in the aqueous phase, it was presumed that furaltadone as such had disappeared from the udder. Of the total radioactivity. 99.4% infused into the udder had been absorbed. From the results obtained, it would appear that the absorption, distribution, and biotransformation of furaltadone in the goat is rapid and extensive.

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