Absorption, Tissue Distribution, and Excretion of Tritium-Labeled Ivermectin in Cattle, Sheep, and Rat

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Tritium-labeled ivermectin was studied in cattle, sheep, and rat for absorption, tissue residue distribution, and excretion at doses of 0.3 mg/kg of body weight. The drug was absorbed by various dosing routes. By intraruminal and subcutaneous dosing routes, highest tissue residues were present in fat and liver of cattle, with half-lives of 6-8 and 4-5 days, respectively. Shorter half-lives (1-2 days) were observed in sheep and rat. The tissue residue distribution pattern was essentially the same for all species studied and similar in male and female rats. With doses of tritium-labeled avermectin B_{1a} ranging from 0.06 to 7.5 mg/kg of body weight, plasma and tissue residue concentrations increased proportionally with the dose. When ivermectin was administered by various routes (ip, sc, iv, oral, and intraruminal), blood residue levels converged to 20-50 ppb 4 h after dosing and then depleted at a similar rate regardless of the dosing route. Ivermectin was excreted primarily in the feces, with only less than 2% of the doses being eliminated in the urine in all three species studied.

Ivermectin is the 22,23-dihydro derivative of avermectin B_1, a macrocyclic lactone produced by an actinomycetes, *Streptomyces avermitilis* (Chabala et al., 1980; Burg et al., 1979; Miller et al., 1979; Egerton et al., 1979). It is active at extremely low dosage against a wide variety of nematode and arthropod parasites. It is widely used for the treatment and control of parasites in cattle, horses, sheep, swine, and dogs (Campbell et al., 1983). Ivermectin consists of two closely related homologues containing no less than 80% 22,23-dihydroavermectin B_{1a} (H_2B_{1a}) and no more than 20% 22,23-dihydroavermectin B_{1b} (H_2B_{1b}) as shown in Figure 1. In vivo metabolism and in vitro metabolism of ivermectin have been studied previously in cattle, sheep and rat (Chiu et al., 1986, 1988) and by hepatic microsomes from cattle and rat (Miwa et al., 1982). A similar in vitro study was also carried out with swine hepatic microsomes (Chiu et al., 1984, 1987). Pharmacokinetics of ivermectin using various formulations have also been reported in various species, e.g., swine, dog, sheep, and cattle (Lo et al., 1985; Wilkinson et al., 1985; Prichard et al., 1985; Fink and Porras, 1989). The biological half-lives ($t_{1/2}$) of the drug increase among these species in the same order, ranging from 0.5 day (swine) to 1.8 (dog), 2.7 (sheep), and 2.8 days (cattle). The studies herein described were carried out with the radiolabeled drug in target animals of drug use (cattle, sheep) as well as the laboratory animal (rat) mainly for tissue residue levels, distribution, and excretion of the radioactive dose. Absorption of the radioactive dose was also studied for comparison with tissue residue levels.

MATERIALS AND METHODS

Radiolabeled Chemicals. [22,23-³H]Ivermectin consisting of [22,23-³H]H_2B_{1a} and [22,23-³H]H_2B_{1b} (4:1) was prepared by reduction of avermectins B_{1a} and B_{1b} separately with tritium in the presence of Wilkinson's catalyst [Ph₃P]₃RhCl (Chabala et al., 1980). The two compounds were then mixed into the required proportion and formulated appropriately for each study (Jacob et al., 1983). [5-³H]H_2B_{1a} was prepared as reported for [5-³H]B_{1a} (Chabala et al., 1981). Radiopurity of [22,23-³H]-H_2B_{1a} and [22,23-³H]H_2B_{1b} was 99% and that of [5-³H]H_2B_{1a}
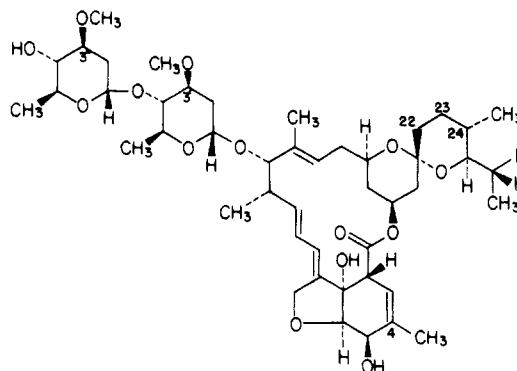
Figure 1. Structure of ivermectin. H_2B_{1a}, R = CH_2CH_3 (≥80%); H_2B_{1b}, R = CH_3 (≤20%).

was 98.8% by HPLC analysis. Specific activity of stock [22,23-³H]H_2B_{1a} and -H_2B_{1b} ranged between 12.6 and 60 mCi/mg, and that of [5-³H]H_2B_{1a} was 2.45 mCi/mg. The radioactive compounds were stored at -65 °C in ethanol solution and analyzed periodically. Analyses were by reverse-phase HPLC using a C_{18} Chromegabond column developed with CH_3CN/methanol/water (53:35:10). Fractions were collected and scintillation was counted for radioactivity distribution.

Dose Formulation. For the cattle studies, tritium-labeled ivermectin was diluted with a mixture of unlabeled H_2B_{1a} and H_2B_{1b} (4:1) to give a final specific activity of 0.1 mCi/mg. The mixture was dissolved in sterile formulation A [propylene glycol/glycerol formal, 60:40 v/v, containing 5% poly(vinylpyrrolidone)] provided by the Department of Animal Formulation Development, MSDRL, for subcutaneous (sc) dosing. Concentration of the drug was 7.21 mg/g of solvent. For the intraruminal (ir) dose given to cattle and sheep, the drug was dissolved in propylene glycol at 0.967 mg/g of solvent. Drug concentrations in all the doses were verified by Animal Formulation Development by HPLC. For rat studies, [5-³H]H_2B_{1a}, [22,23-³H]H_2B_{1a}, or [22,23-³H]H_2B_{1b} was used. Subcutaneous and intramuscular (im) doses were prepared in formulation A; oral doses were prepared either in formulation A, propylene glycol, or sesame oil at 60 μg/0.5 mL. Interperitoneal (ip) doses were prepared by dissolving the radiolabeled drug in ethanol followed by dilution with 0.9% saline to a final concentration of ethanol at 2.5% (v/v). Intravenous (iv) doses were prepared either in ethanol/saline as for the ip dose or in rat plasma. To prepare a dose in plasma, two 2-mL fresh rat plasma samples

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Table I. Volatile Radioactivity[a] in Plasma and Excreta from Cattle Dosed Subcutaneously with [22,23-^{3}H]Ivermectin at 0.3 mg/kg of Body Weight

day postdose	plasma			urine			feces		
	total residue, ppb	volatile fraction, ppb	% volatile fraction	total residue, ppb	volatile fraction, ppb	% volatile fraction	total residue, ppb	volatile fraction, ppb	% volatile fraction
1	157	1.5	1.0	29	1.1	3.8	292	0.8	0.3
2	105	1.9	1.8	37	1.6	4.3	806	1.7	0.2
3	90	2.3	2.6	30	2.1	7.0	597	2.0	0.3
4	74	2.6	3.5	28	2.6	9.3	513	2.2	0.4
5	60	2.7	4.5	22	1.3	5.9	301	1.9	0.6
6	55	2.8	5.1	17	2.6	15.3	333	1.8	0.5
7	45	2.3	5.1	12	2.6	21.7	273	2.6	1.0

[a] Volatile radioactivity was determined from condensate collected from plasma, urine, and feces as described under Materials and Methods.

Table II. Volatile Radioactivity in Samples of Control Bovine Urine Mixed with either (A) [22,23-^{3}H]H$_2$B$_{1a}$ or (B) ^{3}H$_2$O

sample	total radioact,[a] ppb	condensate radioact % of total radioact
	(A) Urine plus [22,23-^{3}H]H$_2$B$_{1a}$	
1	102	0.4
2	29	1.2
3	8	4.8
4	2	4.3
	(B) Urine plus ^{3}H$_2$O	
1	3302	95
2	1021	93
3	339	96
4	112	97
5	36	96
6	12	97
7	3	96
8	1	8

[a] Calculated for comparison with tissue residue levels based on specific activity of 180 000 dpm/μg, assuming tissue sample weight of 0.125 g.

were each equilibrated with 2 mL of isooctane containing 1 mg/200 μCi [5-^{3}H]H$_2$B$_{1a}$. From each equilibrated plasma sample, 1.5 mL was removed, combined, and mixed with 4 mL of plasma diluent to give 7 mL at 118.5 μg/mL and 24 μCi/mL, i.e., a specific activity of 0.202 mCi/mg.

Animal Handling. Angus steers weighing between 240 and 290 kg were used for cattle studies. The animals were weighed and then maintained in metabolism crates during the study. They were fed alfalfa hay cubes plus a ration made up of ground alfalfa and grain. Water was supplied ad libitum. Three steers were randomly assigned to each of four withdrawal times (7, 14, 21, and 28 days postdose), at which time they were slaughtered. Muscle, fat, liver, and kidney were collected from all steers at this time. From the sc dosed animals, muscle from beneath the injection site (500 g) was also collected. In addition, from one randomly selected steer in each withdrawal group, the following biological samples were collected: abomasum, adrenals, bone marrow (sternum), brain, cecum, colon, heart, intestine (small), lung, lymph gland (mediastinal), pancreas, rumen (anterior sac), rumen fluid, spleen, thymus, thyroid, and tongue. Bile was collected directly from the gallbladder at sacrifice. All materials were homogenized (where appropriate) and frozen in preparation for assay of tritium radioactivity. Unmedicated steers supplied control muscle, liver, kidney, and fat. Plasma was collected for assay from all medicated animals at 0, 1, 2, 4, 8, 12, and 24 h postdose and daily through the first 15 days; thereafter it was collected weekly. Daily collection of urine and feces was made from selected steers (7-day withdrawal group) during the first week postdose. Urine and feces (feces plus water) were weighed, and the feces and water were homogenized. Samples of both urine and homogenized feces were frozen in preparation for assay. The daily intake of feed, daily weight of urine and feces, and individual weight of liver and pair of kidneys were recorded. The single sc or ir dose for each animal (0.3 mg/kg of body weight) was calculated on the basis of its mean body weight determined from weight collected on two consecutive days just prior to dosing.

Table III. Total Radioactivity (Equivalents in Parts per Billion) in Plasma of Cattle and Sheep Dosed Intraruminally or Subcutaneously with [22,23-^{3}H]Ivermectin at 0.30 mg/kg of Body Weight[a]

time postdose	total radioact, ng equiv ppb		
	cattle		sheep
	intraruminal	subcutaneous	intraruminal
1 h	0.1 ± 0.3	9.1 ± 7.5	0
2 h	0.6 ± 1.2	23.3 ± 24.4	0.5 ± 0.9
4 h	2.3 ± 2.0	59.7 ± 36.4	3.9 ± 3.4
8 h	7.9 ± 4.1	103.4 ± 62.5	10.3 ± 4.7
12 h	16.3 ± 5.6	121.6 ± 57.7	12.2 ± 4.5
1 day	29.2 ± 5.4	133.2 ± 45.8	12.5 ± 4.4
2 days	28.8 ± 4.8	106.4 ± 26.9	8.8 ± 3.8 (n = 9)
3 days	23.5 ± 2.6	81.9 ± 19.5	6.3 ± 3.1 (n = 9)
4 days	18.0 ± 4.4	62.8 ± 12.1	4.3 ± 2.7 (n = 6)
5 days	13.7 ± 2.3	49.5 ± 10.0	3.3 ± 2.3 (n = 6)
6 days	10.7 ± 2.0	40.5 ± 9.9	3.7 ± 2.5 (n = 3)
7 days	8.3 ± 1.7	31.9 ± 8.4	3.3 ± 2.1 (n = 3)
14 days	2.2 ± 0.7 (n = 9)	9.7 ± 3.0 (n = 9)	
21 days	0.8 ± 0.4 (n = 6)	4.8 ± 1.0 (n = 6)	
28 days		2.3 ± 0.6 (n = 3)	

[a] n = 12 for all entries except those designated in parentheses.

For rat experiments, male or female Sprague-Dawley rats (from Charles River, Wilmington, MA) were used. The animals were randomly selected for grouping and dosed at 0.3 mg/kg of body weight on the basis of the mean body weight of all animals in a particular study. In all studies, a single dose was administered. Three rats were housed in one metabolism cage for daily collection of urine and feces. At sacrifice (by heart puncture), blood, tissues, and organs were collected. Animals were given free access to food (Ralston Purina Rodent Chow) and water.

Crossbred wether lambs weighing between 22.5 and 32.6 kg were used in two sheep studies. The single ir dose for each animal (0.3 mg/kg of body weight) was calculated on the basis of its mean body weight determined from weights collected on two consecutive days just prior to dosing. The lambs were maintained in individual metabolism cages, and they were fed a ration consisting of ground alfalfa and grain. Water was provided ad libitum. In one study three lambs were randomly assigned to each of four withdrawal times (1, 3, 5, and 7 days postdose), at which time they were slaughtered and muscle, liver, kidneys, and fat collected. In the other study, the four withdrawal times were 7, 14, 21, and 28 days postdose. From one randomly selected sheep in each withdrawal group of the latter study 18 tissue and body fluid samples in addition to liver, kidney, muscle, and fat as described for the cattle study were collected. All tissues were homogenized and frozen in preparation for assay of tritium radioactivity. Unmedicated lambs provided the control tissues. Plasma was prepared from blood collected at 0, 1, 2, 4, 8, 12, and 24 h postdose and then daily until slaughter. The daily intake of feed and the individual weight of liver and pair of kidneys were recorded.

Volatile Radioactivity in Body Fluid and Feces. One milliliter of bovine plasma sample was added to 1.0 mL of distilled water in a plastic Petri dish and the covered dish placed on a warm surface (50–60 °C) until at least 1.0 mL of condensate was collected on the Petri dish cover. Half milliliter of con-

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Table IV. Total Radioactive Residues (as Nanogram Equivalents/Gram, or Parts per Billion) in Tissues and Body Fluids from Cattle Dosed Subcutaneously or Intraruminally with [22,23-³H]Ivermectin at 0.3 mg/kg of Body Weight[a]

| | total radioact residue, ppb | | | | | | | |
| | intraruminal[b] | | | | subcutaneous[c] | | | |
days postdose:	7	14	21	28	7	14	21	28
abomasum	18	4	1	1	44	17	10	1
adrenals	12	2	1	1	29	6	7	2
bile	105	24	3	1	273	54	22	1
bone marrow	31	10	3	3	92	21	23	9
brain	1	0	0	0	4	1	0	0
cecum	12	3	1	0	33	9	3	0
colon	14	2	1	1	44	11	9	0
fat	84 (26)	26 (5.5)	10 (2.1)	7.7 (3.1)	220 (58)	88 (6.4)	45 (21)	34 (9)
heart	15	2	1	0	41	8	3	0
intestine (small)	11	2	1	0	22	5	6	1
kidney	19 (5.7)	4.7 (0.6)	1 (0)	0	55 (18)	12 (1.5)	5.3 (1.5)	3.7 (1.5)
liver	119 (38)	49 (29)	14 (0)	3.7 (1.2)	622 (223)	104 (43)	48 (17)	32 (19)
lung	29	4	2	1	66	12	4	1
lymph gland	15	3	1	1	41	13	20	6
muscle	7.7 (2.9)	2.7 (2.1)	0.3 (0.6)	0	18 (6.8)	4 (1.7)	2.3 (1.5)	1 (0)
pancreas	38	6	1	0	83	16	9	1
plasma	8.3 (2.5)	2.7 (2.10)	1	0	45	11	6	3
rumen	20	2	1 (0)	1	34	10	10	2
rumen fluid	32	1	0	0	7	1	0	0
spleen	16	3	1	0	38	8	5	3
thymus	19	6	2	1	64	21	9	1
thyroid	19	3	1	1	58	16	8	6
tongue	10	2	1	0	27	4	4	0

[a] Each entry was from one animal except for kidney, liver, fat, and muscle, where values were the mean from three animals; SD values are in parentheses. All assays were carried out in duplicate and values were averaged. Deviations between duplicates were <1 ppb in all samples. [b] Values below detection limits were reported as zero. Detection limits (ppb) were as follows: liver, 0.8–1.4; fat, 0.8–1.3; kidney, 1.4–1.6; muscle, 0.7–1.5; and plasma, 0.1–0.3. [c] Values below detection limits were reported as zero. Detection limits (ppb) were as follows: liver, 0.3–3.1; fat, 1.6–3.3; kidney, 0.6–1.1; muscle, 0.4–0.7; and plasma, 0.1–0.4.

densate was added to 19.5 mL of 70/30 phosphor (toluene/ethanol/Omnifluor) (Packard, Downers Grove, IL) in a vial and the sample counted by the liquid scintillation method. The same method was applied for urine and feces samples, using 2 mL of urine (undiluted) and 4 mL of feces homogenate (1:4) in water.

Volatility of [22,23-³H]Ivermectin. To examine the intrinsic volatility of tritium in the radiolabeled compound, 0.2 mL of [22,23-³H]ivermectin was added to 15 mL of control steer urine. A 3-fold serial dilution was carried out (5 mL of solution plus 10 mL of urine). Samples from each of the dilutions were counted by liquid scintillation, and aliquots were removed for warming (50–60 °C) in a covered Petri dish; the condensate was assayed for radioactivity as directed above. For a control experiment, 1.0 mL of tritium water (19 400 cpm/mL) was added to 9.0 mL of control bovine urine. A 3-fold serial dilution was carried out and dish condensate assayed as described above.

Radioanalysis. For blood, plasma, and urine samples, approximately 0.5-mL aliquots were weighed and combusted directly. For brain, lung, bone, spleen, and heart, the tissue from each animal was weighed and combusted directly. For all remaining tissues, approximately 0.5-mL samples of 1:4 water homogenate were weighed and combusted directly. The samples were combusted in a Packard 306 sample oxidizer with released ³H₂O trapped in 18 mL of Monophase 40 (Packard). All radioactivity measurements were made in a Packard scintillation spectrometer (Model 3255) with quenching corrections based on the external standard method. All samples were combusted in duplicate aliquots except for brain, lung, bone, spleen, and heart. These tissues from each animal were weighed and combusted directly. Samples were reassayed if duplicates differed by more than 3σ and ±5% from their mean and more than 1 ppb from each other as described in previous studies from this laboratory (Chiu et al., 1989).

RESULTS AND DISCUSSION

Stability of Tritium Label in Dose. The tritium labels at $C_{22,23}$ and C_5 positions were proven to be stable biologically in several cattle and sheep experiments. As shown in Table I, only insignificant loss of volatile

radioactivity was observed (1–5%) in plasma, urine, and feces until the total residue levels were below 50 ppb. In all samples, regardless of the amount of initial total radioactivity in the sample or the biological matrix, the recovered volatile radioactivity was 1–3 ppb, which amounted to 2–6 times the background radioactivity. This amount, when calculated relative to total radioactivity in samples of low concentrations (<50 ppb), gave rise to increasing percentages of volatile fractions, as shown in Table I by urine samples of later time points. This may reflect the limitation of the method employed in detecting low-level radioactivity rather than a real increase of volatility with time. As expected, volatile fractions were the lowest in fecal samples as they contained the highest levels of radioactivity among samples studied. On the basis of these results, we concluded that the tritium labels at $C_{22,23}$ were stable in vivo. Even if there were an increase of volatile fraction in urine at later time points, as data in Table I could be interpreted to show if the analytical limitation discussed above were disregarded, this portion of the volatile radioactivity would be insignificant since usually <2% of the dose was excreted in urine with the rest being in the feces. The volatility of the radiolabeled parent drug added to control urine was also examined. As shown in Table II, the percent of volatile radioactivity was <1% until total added radioactivity approached a calculated concentration of 29 ppb. By use of the same method, tritiated water added to control urine was recovered at ~95% as condensate, thus verifying the method in these studies.

Similar stability was also observed with ivermectin tritium labeled at C_5 in studies with plasma, urine, and feces as discussed for [22,23-³H]ivermectin. The best supporting data are from a tissue residue study with rats administered a single dose containing both [5-³H]avermectin and [3,7,11,13,23-¹⁴C]avermectin (Maynard et al., 1990). The results from this study show that total tissue radioactive

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Table V. Total Radioactive Residues (as Nanogram Equivalents/Gram, or Parts per Billion) in Tissues and Body Fluids from Sheep Dosed Intraruminally with [22,23-³H]Ivermectin at 0.3 mg/kg of Body Weight[a]

days postdose:	total radioact residue, ppb			
	7[b]	14	21	28
abomasum	4	4	2	0
adrenals	5	9	2	1
bile	31	24	3	1
bone marrow	11	12	9	7
brain	1	1	0	0
cecum	2	3	1	1
colon	4	7	3	1
fat	32 (7.6)	24 (12)	13 (8.7)	9.7 (1.5)
heart	5	4	1	1
intestine (small)	3	3	2	1
kidney	43 (1.5)	2.3 (2.3)	0.3 (0.6)	2.0 (0)
liver	11 (3)	5.0 (4.4)	0.7 (0.6)	2.0 (1.0)
lung	4	5	2	0
lymph gland	7	16	4	3
muscle	1.3 (0.6)	1.3 (0.6)	0	1.3 (0.6)
pancreas	5	4	1	1
plasma	1.3 (0.6)	0.3 (0.6)	0	0
rumen	1	2	1	0
rumen fluid	1	0	0	0
spleen	3	4	1	0
thymus	3	4	2	1
thyroid	5	5	1	0
tongue	4	6	2	1

[a] Each entry was from one animal except for kidney, liver, fat, and muscle, where values were the mean from three animals; SD values are in parentheses. All assays were carried out in duplicate and values averaged. Deviations between duplicates were <1 ppb in all samples. Values below detection limits were designated as zero. Detection limits (ppb) were as follows: liver, 0.7–0.8; fat, 1.3–1.9; kidney, 0.9–1.2; muscle, 1.4–1.7 and plasma, 0.4–0.7. [b] In a separate study, animals were slaughtered at 1, 3, 5, and 7 days after dosing. Mean tissue residue levels (ppb) 7 days post dose were higher: liver, 43.7 ± 30.9; fat, 72.7 ± 35.2; kidney, 12.7 ± 8.7; muscle, 9.7 ± 5.9; and plasma, 3.3 ± 2.1.

residue and the HPLC profiles of radioactivity levels were similar regardless of whether the measurements were made with ^{14}C or ^{3}H. This indicates that the 5-^{3}H label of avermectin is stable in vivo. On the basis of the similar chemistry of the C_5 positions in avermectin and ivermectin, stability is also expected for ivermectin.

Absorption of [22,23-³H]Ivermectin in Cattle and Sheep. The absorption of the radiolabeled dose was determined solely for the purpose of comparing the value with tissue residue levels and distribution. The plasma levels of drug-related radioactivity (calculated as radioactivity nanogram equivalents/milliliter or parts per billion) from steers dosed sc or ir and sheep dosed ir are shown in Table III. The plasma levels peaked at about 1 day after dosing in both species. Comparison of the area under the plasma concentration curves (AUC) of the ir [3963 ± 539.3 (μg·h)/mL] and sc [14 986 ± 2922.6 (μg·h)/mL] doses up to 21 days showed that absorption of radioactivity by the sc dosing route is 3.8 times of that of the ir route. On the other hand, absorption of the radioactive dose was comparable by the cattle and sheep via the ir dosing route based on AUC calculated up to 7 days after dosing. The elimination half-lives were 3.7, 4.3 and 2.4 days for ir and sc dosing in steers and ir dosing in sheep, respectively. Similar half-life (2.7 days) was reported by Wilkinson et al. (1985) for the parent drug in cattle dosed iv at the same dosage, and the data were analyzed with a three-compartment model. For the purpose of comparing the target animals with a laboratory animal species, a study was carried out with rats in which animals were dosed orally with either [22,23-³H]H₂B₁ₐ or

[22,23-³H]H₂B₁ᵦ in formulation A. Absorption based on areas under the plasma concentration time curves (not shown) for the two components between 0.5 h and 4 days was estimated to be 1570 and 874.4 (μg·h)/mL for H₂B₁ₐ and H₂B₁ᵦ, respectively. The lower AUC value for H₂B₁ᵦ-related radioactivity is probably due to relatively more rapid metabolism of H₂B₁ᵦ, as had been reported previously in metabolism studies (Chiu et al., 1986), rather than better absorption of the H₂B₁ₐ.

Tissue Distribution of Radioactive Residue. Of the edible tissues (Edible tissues refer to the major tissues/organs for the meat-consuming public, i.e., liver, kidney, muscle, fat/skin. These are also the edible tissues/organs specifically monitored under the drug residue monitoring program by the U.S. FDA on animal health drugs.) namely, liver, kidney, muscle, and fat, liver and fat tissue residue levels were the highest in cattle, with depletion half-lives of 4.8 and 7.6 days for the sc dose and 4.2 and 5.9 days for the ir dose, respectively (Table IV) (Chiu and Lu, 1989). Muscle contained the lowest residue for either sc or ir dosing route. A similar tissue residue distribution pattern existed in the sheep and rat, with levels in fat slightly higher than those in the liver at all time points studied (Tables V and VI). The residue depletion half-lives were much shorter in sheep and rat (1–2 days), reflecting probably more rapid depletion and metabolism in these animals. The proportion of the unchanged parent drug in the edible tissue of cattle, sheep, and rat has been reported previously from this laboratory (Chiu et al., 1986). The parent drug (H₂B₁ₐ and H₂B₁ᵦ) was found to account for at least 50% of the total radioactive residue in tissues of cattle up to 14 days after dosing, in sheep 5 days after dosing, and in swine and rats 7 and 3 days after dosing, respectively. The rest of the residue consisted of more polar metabolites. The half-lives of the parent drug and total tissue residue are in close agreement in liver tissue in cattle and sheep, suggesting equally efficient depletion of the parent drug and its metabolites.

Table VI shows the residue levels in "edible" tissue of rats dosed separately with either [22,23-³H]H₂B₁ₐ or [22,23-³H]H₂B₁ᵦ. In all tissues studied, the depletion half-lives for H₂B₁ₐ were about 1–1.5 days. The minor component H₂B₁ᵦ, however, appeared to deplete slightly faster with half-lives of ~0.4–0.8 days. This phenomenon was also observed in cattle but was not particularly noticeable in the sheep.

Effect of Dose Levels on Plasma and Tissue Residue. The therapeutic dose level of ivermectin in target animals is about 0.2–0.3 mg/kg of body weight. To establish the effect of dose on the plasma and tissue residue levels in animals, male rats were dosed by gavage with [5-³H]B₁ₐ (the structural analogue of H₂B₁ₐ) at levels of 0.06, 0.3, 1.5, and 7.5 mg/kg of body weight. The animals were sacrificed at 1, 3, or 7 days after dosing and samples of plasma and four major tissues assayed for total radioactivity. Results in Table VII show radioactivity levels in the plasma and major tissues 1 day after dosing. It should be noted that the dose levels are in the ratios of 1:5:25:125 to each other and that residue levels increased proportionally with the dose levels. Depletion half-lives of liver residue calculated by linear regression were nearly constant (0.8–1.0 day) for all dose levels studied.

Effect of Dose Route on Plasma and Tissue Residue Levels. Ivermectin is developed for use in target animals by several routes of administration. To establish the effect of the route of dosing on plasma and tissue levels in animals, male rats were dosed with [5-³H]H₂B₁ₐ at a level of 0.3 mg/kg of body weight by one of five routes: gav-

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Table VI. Total Radioactive Residues (Nanogram Equivalents/Gram, or Parts per Billion) in Blood and Tissues of Male Sprague-Dawley Rats Dosed Subcutaneously at 0.3 mg/kg with [22,23-³H]H₂B₁ₐ or [22,23-³H]H₂B₁ᵦ[a]

| postdose: tissues:	tissue residues, ppb						depletion $t_{1/2}$, days	
	1 day		2 days		4 days			
	H_2B_{1a}	H_2B_{1b}	H_2B_{1a}	H_2B_{1b}	H_2B_{1a}	H_2B_{1b}	H_2B_{1a}	H_2B_{1b}
liver	320 ± 6	229 ± 6	130 ± 4	82 ± 1	43 ± 3	10 ± 1	1.06	0.66
kidney	220 ± 21	185 ± 5	118 ± 2	68 ± 2	41 ± 1	9 ± 1	1.25	0.69
fat pad	493 ± 23	497 ± 2	274 ± 6	168 ± 2	110 ± 1	27 ± 1	1.40	0.72
muscle	109 ± 0	67 ± 3	44 ± 0	23 ± 1	17 ± 0	4 ± 0	1.16	0.75
lung	166 ± 4	108 ± 6	64 ± 11	41 ± 2	27 ± 1	4 ± 0	1.19	0.63
heart	126 ± 1	105 ± 6	73 ± 1	44 ± 1	27 ± 4	5 ± 1	1.36	0.68
blood	19 ± 1	13 ± 0	9 ± 0	4 ± 0	3 ± 0	0	1.14	0.42
plasma	23 ± 1	17 ± 0	12 ± 0	5 ± 0	4 ± 0	1 ± 1	1.20	0.75

[a] Three rats (200 g each) were dosed at each time point with radiolabeled drug at specific activity of 0.1 mCi/mg. Blood was collected by heart puncture. Fat pad was removed from the testicular areas of the animals. Tissue organ from the same time point of each compound was composited into one sample by homogenization, and duplicate homogenate aliquots were assayed. Blood was pooled by mixing. Values shown are averages of duplicate assays.

Table VII. Comparison of Tissue and Plasma Residues 1 Day after Dosing in Rats Dosed Orally with [5-³H]B₁ₐ at 0.06-7.5 mg/kg of Body Weight[a]

dose level		radioactive residue									
		plasma		liver		fat		kidney		muscle	
mg/kg	ratio	ppb	ratio	ppb	ratio	ppb	ratio	ppb	ratio	ppb	ratio
0.06	1	3	1.2	24	0.6	188	2.6	50	1	7	0.7
0.3	5	15	6	166	4	356	5	269	5	43	5
1.5	25	78	31	753	19	1912	26	1051	21	220	24
7.5	125	313	125	5059	125	9079	125	6287	125	1168	125

[a] [5-³H]B₁ₐ was prepared as described for [5-³H]H₂B₁ₐ under Materials and Methods. Radiopurity was 98.5% by HPLC analysis, and specific activity was 2.6 mCi/mg. All rats were dosed with 0.5 mL of propylene glycol solutions containing the appropriate doses. The specific activities of the dosing solutions were 0.191, 0.188, 0.179, and 0.179 mCi/mg for 0.06, 0.3, 1.5, and 7.5 mg/kg dose levels, respectively. All residue ratios were calculated by assigning the value of 125 to the highest residue levels.
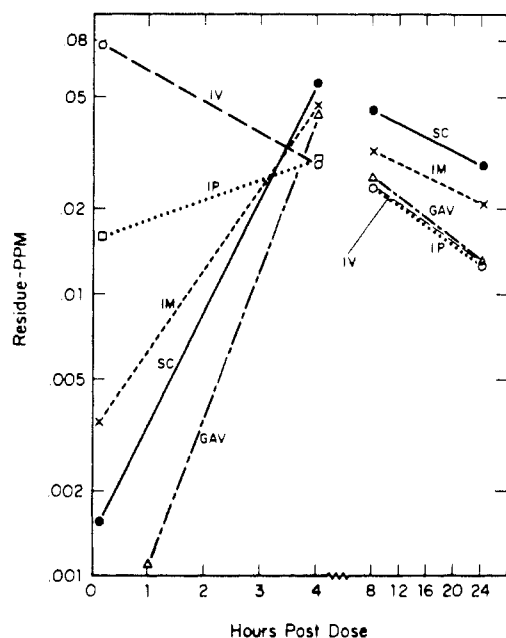
Figure 2. Total radioactive residues of [5-³H]H₂B₁ₐ in blood of male SD rats dosed at 0.3 mg/kg of body weight by various routes.

age (oral), sc, im, iv, or ip. Blood samples were taken by orbital bleeding at 10, 15, and 30 min and 1, 2, 4, 8, 12, 16, and 24 h. The rats were sacrificed at 1, 2, or 3 days after dosing and samples of plasma and tissues taken for assay of total radioactive residues.

Total radioactivity (expressed as milligram equivalents/gram or parts per million) in the orbital blood samples was plotted in Figure 2. The lines represent the best-fit straight lines from the least-squares regression equation. At 4 h postdose the blood residue levels are roughly the same for all routes of administration, even though the values from early times were quite different. The depletion rate during

8-24 h was almost identical for all routes. The residue levels in the plasma and liver were generally similar 3 days after dosing for all dosing routes.

Plasma and Tissue Residue Depletion Rates in Rats Dosed Intravenously. The intravenous administration of ivermectin was complicated by the insolubility of the drug in physiological saline. The drug, however, was soluble in plasma with K_D (isooctane/steer plasma) being ~0.5. Male rats were dosed iv with [5-³H]H₂B₁ₐ at a level of 0.3 mg/kg of body weight. The drug (0.060 mg/200 g rat) was dissolved in 0.5 mL of rat plasma for dosing. Orbital blood samples were taken from 0.5 through 40 min postdose, and the animals were sacrificed from 1 through 24 h postdose.

Total radioactivity in the blood showed a very rapid drop during the first 3 min ($t_{1/2}$ = 1.6 min) and a slower depletion from 3 to 15 min ($t_{1/2}$ = 6.3 min). Extrapolating the initial depletion curve to zero time gave a blood concentration of 1712 ppb. Radioactivity levels in the blood were among the lowest (except for brain) when compared to other tissues at all time points studied. Figure 3 shows the tissue residue levels in rats sacrificed from 1 to 24 h after dosing. The heart and lungs showed the highest residue levels during the first few hours. Interestingly, the fat residue increased slowly until about 12 h after dosing and then retained the highest level of residue in all tissues examined. The disposition and metabolism of ivermectin in fat tissue of steers, sheep, and rats have been reported previously (Chiu et al., 1988).

Tissue Residue in Male and Female Rats. Table VIII shows the total residue levels in "edible" tissues, GI tract, and plasma of male and female rats dosed orally with [22,23-³H]ivermectin at 0.3 mg/kg of body weight. The same tissue distribution pattern existed in both sexes with similar residue levels at 1, 3, and 5 days postdosing.

Excretion of Radioactivity. Regardless of whether the drug was administered ir or sc to cattle, the major route

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Table VIII. Total Radioactive Residue (Nanogram Equivalents/Gram, or Parts per Billion) in Tissues and Plasma of Male and Female Rats Dosed Orally with [22,23-^{3}H]Ivermectin at 0.3 mg/kg of Body Weight[a]

days postdose	liver	kidney	muscle	fat	GI tract	plasma
			Male			
1	175.2 ± 6.5	130.2 ± 2.5	47.0 ± 0.0	254.1 ± 8.7	ND[b]	15.9 ± 0.1
3	30.9 ± 1.1	25.5 ± 0.1	10.1 ± 0.1	90.2 ± 3.3	ND	2.2 ± 0.0
5	9.4 ± 0.1	10.5 ± 0.7	4.8 ± 0.1	32.0 ± 1.1	10.5 ± 0.8	0.8 ± 0.1
			Female			
1	172.5 ± 1.3	128.5 ± 1.8	52.4 ± 0.1	252.5 ± 7.8	ND	13.9 ± 0.4
3	45.6 ± 0.4	38.9 ± 0.4	18.0 ± 0.2	146.5 ± 2.1	ND	4.5 ± 0.1
5	4.5 ± 0.0	8.1 ± 0.2	2.1 ± 0.0	31.8 ± 0.4	10.6 ± 0.7	0.5 ± 0.1

[a] Tissues were composited from three animals before analysis. All samples were assayed in duplicates. Detection limits were as follows: liver, 0.05–0.08; kidney, 0.09–0.1; muscle, 0.06–0.3; fat, 0.005–0.2; GI, 0.08–0.09; and plasma, 0.001–0.6. [b] ND, not determined.
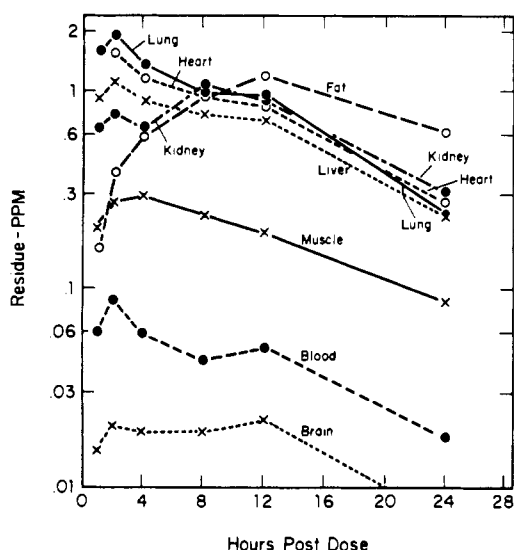

Figure 3. Depletion of total radioactive residues in tissues and blood of rats dosed intravenously with [5-^{3}H]H$_2$B$_{1a}$ at 0.3 mg/kg of body weight with steer plasma as the dosing vehicle.

of excretion was by feces. For sc dosed animals (n = 3) 1.51 ± 0.1% and 62 ± 9.7% of the dose were recovered in urine and feces, respectively, through 7 days post-dose. For ir dosed animals (n = 3), 0.46 ± 0.1% and 79.7 ± 4.8% of the dose were excreted in urine and feces, respectively (data not shown). Of the total excreted, >60% was eliminated in the first 3 days after dosing. This excretion pattern was also observed in the male and female rats. Only 0.3–0.4% of the radioactive dose was excreted via urine by both sexes, the rest (83.0–91.7%) were recovered in feces. By 5 days postdose, there was only 0.4% of the dose in the GI tract. The parent drug accounted for 39–78% of the fecal radioactivity in the cattle, sheep, and rat (Halley et al., 1989). The relatively high residue levels found in bile from cattle dosed sc or ir (Table V) suggest that biliary excretion is probably an important route of elimination for ivermectin.

In summary, we have studied the tissue residue distribution and excretion of tritium-labeled ivermectin in cattle, sheep, and rat at doses of 0.3 mg/kg of body weight. Absorption of the drug was also examined in these species. By various dosing routes, tissue residue levels were present at parts per billion levels with the highest levels in fat and liver tissues of all three species studied. Depletion half-lives were 4–8 days for liver and fat residues in cattle and 1–2 days in sheep and rat. Essentially the same tissue distribution pattern was observed in all three species and in male and female rats. With doses of tritium-labeled avermectin B$_{1a}$ ranging from 0.06 to 7.5 mg/kg of body weight, plasma and tissue residue concentrations increased proportionally with the dose. When ivermec-

tin was administered by various routes (ip, sc, iv, oral, and ir) to the rat, blood residue levels converged to 20–50 ppb 4 h after dosing and then depleted at a similar rate regardless of the dosing route. Fecal excretion was the major route of ivermectin elimination; only less than 2% of the dose was excreted in urine in all species studied.

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