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Fate of Avermectin B_{1a} in Lactating Goats

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Lactating goats were administered [^{3}H]avermectin B_{1a} by 10 daily oral doses at one of three dose levels (0.005, 0.05, 1.0 mg/kg). Excreta and milk were collected daily and the total radiolabeled residues measured. After the fourth dose, maximal residue levels were observed in the excreta and milk. One day after the last dose, various goat tissues were obtained and the total radiolabeled residue was measured. Tissue residue levels were dose dependent and were less than 100 ppb for goats treated at the high dose (1 mg/day). Most of the dose (89–99%) was recovered in the feces, with <1% recovered in the urine or milk. The levels of avermectin B_{1a} in the edible tissues were mostly between 70 and 95% of the total residue for all goats. The major metabolite in the edible tissues and milk was identified as 24-(hydroxymethyl)avermectin B_{1a}. A minor metabolite, 3″-desmethylavermectin B_{1a}, was also identified in the edible tissues. Both metabolites were previously identified in the corresponding tissues of rats administered avermectin B_{1a}. The parent and these two metabolites accounted for 85–99% of the total residue in the edible tissues, milk, and feces of the goats. The extent of tritium exchange was determined to be <0.1% of the dose.

Avermectins are a new class of biological agents that contain a macrocyclic lactone and are produced by *Streptomyces avermitilis* (Burg et al., 1979). The structures have been elucidated (Albers-Schonberg et al., 1981), and some of the biological activities have been reported (Campbell et al., 1983). Abamectin is a commercial product of the avermectins that is being developed by Merck & Co. Inc. as an acaricide/insecticide. The major active ingredient in abamectin is avermectin B_{1a} (Figure 1). This report describes the findings from goat metabolism studies with avermectin B_{1a}. Some of these data have been previously presented (Maynard et al., 1985a).

In these studies, lactating Nubian goats (40–51 kg) were orally dosed daily for 10 days with 3H-labeled avermectin B_{1a} at three dose levels. The dosage rates of avermectin B_{1a} administered to lactating goats were 0.005, 0.05, and 1.0 mg/goat per day. The low dose was calculated on the basis of an anticipated total daily feed intake of range grass treated with abamectin fire ant bait (Affirm) at 50 mg/acre. The intermediate dose was calculated on the basis of an anticipated total daily feed intake of dried citrus pulps from fruit treated with abamectin miticide (Agrimec) (Maynard et al., 1989a). The high dose represents an exaggerated rate to better study the metabolism in lactating ruminants.

The objective of this study was to determine the fate of the total [^{3}H]avermectin B_{1a} residue in goat tissues, excreta, and milk. Furthermore, the level of avermectin B_{1a} and the identification of metabolites were deter-

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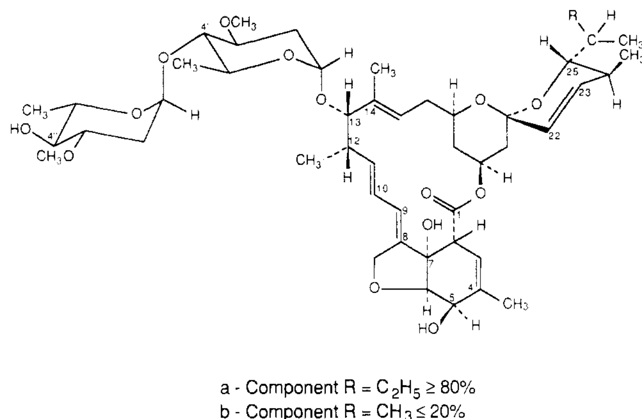

Figure 1. Structures of the two major components of abamectin.

mined in the edible tissues, milk, and feces. By understanding the level and the nature of the tissue and milk residues, we can better determine the safety of these residues with respect to human exposure. Lastly, the extent of exchange of the tritium label into biological water was investigated.

MATERIALS AND METHODS

Animal handling, dosing, tissue isolation, and total radioactive residue analyses were conducted by Borriston Laboratories, Inc., Temple Hills, MD. Determination of avermectin B_{1a} levels, isolation, and identification of the metabolites and other analyses were performed at Merck.

The [^{3}H]avermectin B_{1a} labeled at carbon 5 was obtained in-house from the Labeled Compound Synthesis Group, Department of Animal Drug Metabolism, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ. All other chemicals and solvents were reagent grade or better and were purchased commercially.

Animal Handling and Dosing. Seven lactating Nubian goats (40–51 kg) were acclimated to the test facilities for 1 week prior to the start of the study. Three groups (two goats per group) were dosed orally once per day (a.m.) for 10 days with 0.005, 0.05, or 1.0 mg of [^{3}H]avermectin B_{1a}/goat per day. One goat served as a control and received capsules containing only mannitol at the same dosing regimen. [^{3}H]Avermectin B_{1a}, specific activity 405 μCi/mg, was mixed with mannitol, and the appropriate dosage was placed in gelatin capsules (size 13). The daily ration for each goat consisted of 2400 g of roughage (alfalfa hay) and 1600 g of concentrate (farm ground ear corn, soybean meal, mineral mix) divided and fed twice daily. The daily ration was placed in a feed box. Water was available ad libitum. Forty-eight hours before the start of the study, each goat was housed individually in a steel metabolism cage designed to collect the urine and feces separately.

Sample Collection. Sample collection of blood, urine, feces, and milk was initiated 24 h prior to the first dose and terminated 24 h after the last dose. Urine and feces were collected daily just prior to the daily dosing. The total amount of each sample was measured, and all samples were frozen before analysis. Before freezing, 2 mL of toluene was added to each urine sample to prevent microbial growth. Milk was collected twice a day (a.m. and p.m.) and the volume recorded and frozen before analysis. The a.m. milk samples were taken just prior to the daily dosing. Blood (two vacutainers) was collected by jugular venipuncuture once a day just prior to the daily dosing and frozen before analysis.

All goats were sacrificed on day 11 (24 h after the last dose), and necropsy was performed. The whole organ or approximately 1 kg of tissue was collected, labeled, and frozen immediately before analysis. The tissues taken were omental fat, peripheral fat, brain, leg muscle, loin muscle, heart, kidneys, liver, mammary glands, and lungs.

Analysis of the Total Radioactive Residue. The radioactivity in the samples was determined by combustion fol-

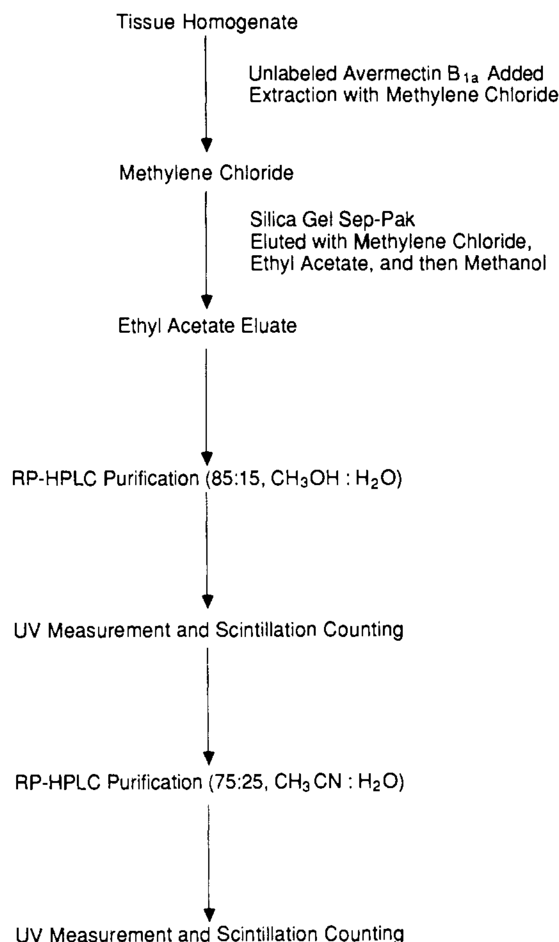

Figure 2. Fractionation and purification procedure for unchanged B_{1a} in goat tissues.

lowed by liquid scintillation counting (LSC) of the liberated tritiated water (HTO). All tissues and feces samples were homogenized with dry ice to a fine consistency, and duplicate subsamples (ca. 0.2 g) were combusted. Blood and urine samples were mixed by shaking, and duplicate subsamples (ca. 0.5 mL) were combusted. Milk samples were mixed by shaking, and duplicate subsamples were counted directly (2 mL) without combustion. Radioactivity quantitation was performed by LSC (Packard 460C instrument) using external standardization and automatic quench control. Sample combustion was performed with either a BMO Harvey or a B306 Packard instrument. Combustion efficiencies averaged >96%. The limits of quantitation (Currie, 1968) were 0.02 ppb for milk and 0.2 ppb for blood, tissues, urine, and feces. All residue levels below the limit of quantitation are reported as <0.02 ppb for milk and <0.2 ppb for all other samples.

Analysis of the Avermectin B_{1a}. To quantitate the level of unchanged avermectin B_{1a} in the edible tissues and milk, a reverse isotope dilution assay (RIDA) was developed. The procedure developed to isolate and quantitate avermectin B_{1a} is shown in Figure 2. Briefly, a tissue homogenate sample containing a known amount of radioactivity (10 000–100 000 dpm) was mixed with a known amount (ca. 100 μg) of unlabeled avermectin B_{1a}. Some samples containing less radioactivity than this were processed to obtain preliminary information and are reported as such. The homogenate was extracted several times with CH_2Cl_2 and the CH_2Cl_2 layers removed, combined, and concentrated. The CH_2Cl_2 concentrate was applied to a Silica Sep-Pak (Waters Associates) and eluted twice with 10 mL of the solvents shown. The ethyl acetate eluate containing the avermectin B_{1a} was concentrated, solubilized with mobile phase, and then injected on the first HPLC system. For the first and second HPLC systems, an ODS column (4.6 mm × 25 cm; Du Pont Zorbax) was used. The mobile phase for the first LC system was 85:15 CH_3OH/H_2O, at a flow rate of 1 mL/min. The peak corresponding to avermectin B_{1a} was collected, concen-

Table I. Residue Levels in Blood[a]

dose day	goat 1	goat 2	goat 3	goat 4	goat 5	goat 6
1	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
2	<0.2	<0.2	<0.2	<0.2	1.1	0.7
3	<0.2	<0.2	<0.2	0.2	1.6	1.0
4	<0.2	<0.2	<0.2	<0.2	1.0	0.6
7	<0.2	<0.2	<0.2	0.2	1.3	0.8
9	<0.2	<0.2	<0.2	0.3	1.8	1.1
11	<0.2	<0.2	<0.2	0.3	3.6	0.8

[a] Levels are expressed as ppb avermectin B_{1a} equivalents. Detection limit of radioactivity in blood was 0.2 ppb.

trated, and injected on the second HPLC system. After purification by the second LC system using same column as above with a mobile phase of 75:25 CH_3CN/H_2O at a flow rate of 1 mL/min, the radioactivity (LSC) and the mass (UV spectrophotometer) were determined to yield a final specific activity. The final specific activity divided by the initial specific activity (total dpm in tissue homogenate sample divided by the mass of the total unlabeled avermectin B_{1a} added) times 100 gave the RIDA value. This value represented the amount of avermectin B_{1a} as a percent of the total tissue residue. From the RIDA value of avermectin B_{1a} multiplied by the tissue residue level, the level of avermectin B_{1a} as nanograms per gram of tissue was determined.

Metabolite Identification. The ethyl acetate eluate obtained from the isolation procedure of avermectin B_{1a} for the RIDA analysis were injected on an HPLC system (Figure 2). To characterize the total tissue and milk residue, 1-min fractions of the column effluent were collected and a radioactivity profile was generated. Since the direct isolation of these metabolites from the tissue for structural analyses was not possible due to the low levels, two cochromatographies with a standard were necessary for identification.

An incubation of [^{14}C]avermectin B_{1a} with control rat liver microsomes was conducted to generate metabolite standards. The incubation and isolation procedures were similar to a previous report (Miwa et al., 1982). The [^{14}C]-24-(hydroxymethyl)avermectin B_{1a} (24-OHMe-B_{1a}) metabolite isolated from the microsomal incubation was identified by NMR and mass spectrometry (Miwa et al., 1982). The major metabolite (metabolite A) isolated by HPLC from goat 6 liver homogenate was cochromatographed with [^{14}C]-24-OHMe-B_{1a} standard on a different RP-HPLC system and a NP-HPLC system. Furthermore, the major metabolite in milk corresponding to metabolite A in the edible tissues was isolated and cochromatographed with [^{14}C]-24-OHMe-B_{1a}.

A minor metabolite (metabolite B) was observed in the ethyl acetate eluate of the edible tissues from goats, yet its levels were too low for direct isolation and structure identification. Since this metabolite had the same retention time as the major metabolite found in the edible tissues from rats administered [^{3}H]avermectin B_{1a} (Maynard et al., 1985a), the metabolite was isolated by HPLC from rat liver and purified for NMR and MS analyses. The identity of this rat metabolite was determined to be 3″-desmethylavermectin B_{1a} (3″-DM-B_{1a}) (Maynard et al., 1985a). This standard was mixed with metabolite B isolated by HPLC from goat 5 liver and cochromatographed by successive NP- and RP-HPLC systems.

Another minor metabolite (metabolite C) was observed in the ethyl acetate eluate of the edible tissues from goats. Since direct isolation was not possible and a standard compound with a similar retention time was not available, the identification of this metabolite is unknown. No other metabolites greater than 2% of the total residue were observed.

Characterization of Radioactive Residues in Goat Feces. To investigate the radioactivity recovered in the feces, the 7- and 9-day fecal samples from goat 6 were combined. The combined fecal sample was extracted with CH_2Cl_2, concentrated to dryness, taken up in CH_3OH/H_2O (2:1), and then partitioned with hexane. The hexane was concentrated to dryness, taken up in CH_2Cl_2, applied to a silica Sep-Pak, and then eluted with ethyl acetate. The ethyl acetate eluate was injected on the first HPLC system shown in Figure 2. A radioactivity profile of the residue in feces was obtained and compared to the tissue radioactivity profiles. Metabolite B was isolated by HPLC from this fecal sample, mixed with 3″-DM-B_{1a} standard, and cochromatographed on successive NP- and RP-HPLC systems.

Tritium Exchange. Since the dosage rates were very low for this study, high specific activity radiolabeled avermectin B_{1a} was necessary to measure low tissue residue levels. The specific activity of the [^{3}H]avermectin B_{1a} used in this study was 25 times greater than the highest available [^{14}C]avermectin B_{1a} material. However, due to the possible lability of the ^{3}H label, the amount of exchangeable ^{3}H from avermectin B_{1a} or its metabolites to water (HTO) was investigated. Since exchanged ^{3}H would be excreted as HTO in the urine and milk, these samples were investigated. The procedure involved heating an aliquot of the 2- and 11-day urine and milk samples in an enclosed plastic Petri dish. A beaker containing crushed ice was placed on top of the lid, and water from the heated aqueous sample was condensed inside the lid. A known volume of the condensate (ca. 0.5 mL) was counted by LSC and the total HTO for the sample determined and expressed as a percent of the daily dose.

RESULTS

The radioactivity levels in blood after various doses are shown in Table I. At the lowest dose of [^{3}H]-avermectin B_{1a}, goats 1 and 2, no detectable residues (<0.2 ppb) were observed from day 1 to 11. Most of the blood samples from the animals administered the intermediate dose, goat 3 and 4, were also below the detection limit except for a few which were 0.2–0.3 ppb. The goats administered the highest dose had blood residue levels between 0.7 to 3.6 ppb. The residue levels in blood for an individual goat were similar from 2 to 11 days except goat 5 at day 11.

The residue levels in milk after various doses are shown in Table II. The residue levels were higher in the p.m. milk sample than the a.m. sample for all goats. The residue levels in milk after the fourth dose from animals administered the lowest (goats 1 and 2), intermediate (goats 3 and 4), and highest (goats 5 and 6) doses were <0.02–0.03, 0.20–0.48, and 1.18–5.05 ppb, respectively. The residue levels in milk were observed to depend on the dose of [^{3}H]avermectin B_{1a}. For all animals, the residue levels in milk for each individual goat increased to day 4 and then remained constant to the end of the study.

The amounts of HTO measured in the 2- and 11-day urine and milk samples for goats 5 and 6 are presented in Table VI. The total amount of exchanged ^{3}H was <0.1% of the daily dose.

The residue levels in the urine and feces after various doses are shown in Table III. For all goats, the amount of dose recovered in the urine was less than detection limit to 0.6% of the daily dose. Almost all of the daily dose was recovered in the feces. The total recovery of the daily dose in the excreta increased up to 4 days and then remained constant to the end of the study. Total recovery of the administered doses (total divided by 10 doses) was between 80 and 99%, with five of the six goats being between 89 and 99%. Goat 5 was off feed with low water consumption for days 9–11, which may explain its low recovery of dose (e.g., 63 and 68% for days 10 and 11). When expressed as a percentage of the dose, no difference in the levels of residue in the urine and feces were observed among the three doses administered.

The residue levels in the various tissues and organs after 10 daily doses are shown in Table IV. At the lowest dose, goats 1 and 2, all tissue residue levels were below the detection limit except for liver and kidney tissue, which were between 0.2 and 0.6 ppb. The residue levels in the

Table II. Residue Levels in Milk[a]

dose	goat 1		goat 2		goat 3		goat 4		goat 5		goat 6	
day	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
1	<0.02	<0.02	<0.02	<0.02	<0.02	0.08	<0.02	1.0	<0.02	0.45	<0.02	0.84
2	<0.02	<0.02	<0.02	0.02	0.17	0.26	0.13	0.36	1.11	1.80	0.70	1.33
3	<0.02	<0.02	<0.02	0.02	0.23	0.33	0.29	0.45	2.03	3.00	1.10	1.87
4	<0.02	<0.02	<0.02	0.02	0.34	0.35	0.28	0.40	3.40	4.26	1.31	1.64
5	<0.02	<0.02	<0.02	0.03	0.26	0.30	0.31	0.38	3.40	4.48	1.38	1.87
6	0.02	0.02	<0.02	0.03	0.23	0.36	0.32	0.48	3.29	4.48	1.18	2.16
7	<0.02	0.02	<0.02	0.03	0.23	0.38	0.28	0.47	3.11	4.71	1.31	2.33
8	<0.02	0.02	0.02	0.03	0.20	0.29	0.31	0.44	3.19	4.25	1.31	2.06
9	<0.02	<0.02	<0.02	0.03	0.21	0.29	0.29	0.41	3.60	3.71[b]	1.30	1.93
10	<0.02	<0.02	<0.02	<0.02	0.22	0.34	0.34	0.41	3.05[b]	4.70[b]	1.36	2.26
11	<0.02	S	0.02	S	0.25	S	0.29	S	5.05[b]	S	1.62	S

[a] Levels are expressed as ppb avermectin B_{1a} equivalents. Detection limits of radioactivity in milk are 0.02 ppb. S = sacrifice after a.m. milking. [b] Animal off feed, low water consumption. All further clinical observations were normal.

Table III. Radioactivity Recovered in Urine and Feces Expressed as a Percentage of the Total Dose

dose	goat 1			goat 2			goat 3			goat 4			goat 5[d]			goat 6		
day	urine	feces	total	urine	feces	total	urine	feces	total	urine	feces	total	urine	feces	total	urine	feces	total
1	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	25.7	25.7	ND	30.9	30.9	ND	18.1	18.1	ND	23.3	23.3	0.1	6.3	6.4	0.2	33.5	33.7
3	ND	77.3	77.3	ND	86.0	86.0	NEd	64.5	64.5	ND	67.5	67.5	0.1	54.7	54.8	0.3	77.2	77.5
4	ND	90.5	90.5	ND	102.8	102.8	ND	82.5	82.5	0.2	83.5	83.7	0.1	76.1	76.2	0.6	99.1	99.7
5	ND	113.5	113.5	ND	94.0	94.0	ND	102.3	102.3	0.2	107.2	107.4	0.1	97.0	97.1	0.5	85.5	90.0
6	ND	112.2	112.2	ND	107.8	107.8	ND	104.5	104.5	0.3	108.4	108.7	0.1	126.8	126.9	0.5	104.8	105.3
7	ND	89.1	89.1	ND	85.5	85.5	ND	98.2	98.2	0.2	92.4	92.6	0.1	100.1	100.2	0.4	109.7	110.1
8	ND	101.8	101.8	ND	98.5	98.5	ND	82.1	82.1	0.3	77.3	77.6	0.1	93.9	94.0	0.4	90.8	91.2
9	ND	117.0	117.0	ND	94.8	94.8	ND	94.1	94.1	0.5	88.1	88.6	0.1	111.4	111.5	0.4	119.3	119.7
10	ND	95.6	95.6	ND	90.8	90.8	ND	110.1	110.1	0.3	104.6	104.9	0.2	63.2	63.4	0.4	100.5	100.9
11	ND	132.9[b]	132.9	ND	125.5[b]	125.5	ND	130.6[b]	130.6	0.4	133.7[b]	134.1	0.2	68.3[b]	68.5	0.4	157.4[b]	157.8
			95.6[c]			91.7[c]			88.7[c]			88.8[c]			79.9[c]			98.6[c]

[a] ND = not detected at >70 dpm/mL (urine) or >175 dpm/g (feces) based on 35 dpm/aliquot counted as significant. All control samples were ND. [b] Include cage scrape down at sacrifice. [c] Percent of total dose = sum of all days values divided by the 10 daily doses value. [d] Animal off feed with low water consumption for days 9–11; otherwise normal clinical signs.

Table IV. Residual Levels in Tissues and Organs[a]

matrix	goat 1	goat 2	goat 3	goat 4	goat 5[b]	goat 6
liver	0.2	0.6	2.1	3.5	98.0	16.4
kidney	0.3	0.3	0.9	1.2	22.7	4.8
lung	<0.2	<0.2	0.3	0.7	11.9	2.5
peripheral fat	<0.2	<0.2	1.3	2.2	50.0	7.6
omental fat	<0.2	<0.2	1.4	2.2	49.3	6.8
leg muscle	<0.2	<0.2	0.3	0.4	7.6	1.7
loin muscle	<0.2	<0.2	0.3	0.3	9.9	1.2
mammary gland	<0.2	<0.2	0.4	0.6	13.3	3.6
brain	<0.2	<0.2	<0.2	<0.2	1.0	0.3
heart	<0.2	<0.2	0.4	0.8	20.6	2.6

[a] Levels are expressed as ppb avermectin B_{1a} equivalents. All control tissues were <0.2 ppb, which is the detection limit. [b] Animal off feed with a low water consumption for days 9–11; otherwise normal clinical signs.

Table V. Avermectin B_{1a} in Goat Tissue Residue as a Percentage of the Total Tissue Residue[a]

goat	liver	kidney	leg muscle	lion muscle	fat	milk[b]
1	76[c]					
2	77[c]					
3	95 (92)	97		96[c]	97	
4	87	92			99	
5	95	94 (89)	91 (88)	84	99	95 (98)
6	41 (40)	40 (37)	68	73	86	70 (79)

[a] Values reported are the result of a single determination. Except in the case of milk samples, the parentheses indicate repeat determinations. [b] Milk samples are 7-day samples. Values in parentheses for milk take into account the amount of HTO present. [c] Tissue residue levels were very low (0.2–0.6 ppb) so the RIDA value should be considered as an estimate.

tissues were observed to depend on the dose administered. For the edible tissues, the residue levels followed the order muscle < kidney < fat < liver. The residue levels were similar between the two types of fat and muscle tissue samples for the same goat. The two goats dosed at the highest level, goats 5 and 6, had the greatest variability in the residue level for the corresponding tissue; goat 6 had lower residue levels than goat 5.

The quantitation of avermectin B_{1a} in the edible tissues and milk was conducted by a reverse isotope dilution assay (RIDA) procedure shown in Figure 2. The level of avermectin B_{1a} as a percentage of the total tissue residue is shown in Table V. In most samples, the level of avermectin B_{1a} was 87–99% of the tissue residue. For goats 3–5, the level of avermectin B_{1a} was similar among the different tissues. The RIDA values for goats 1 and 2 liver should be considered as an estimate since the residue levels were extremely low (i.e., 0.2–0.6 ppb). In all tissues the amount of avermectin B_{1a}, as a percentage of the total tissue residue, was lower for goat 6 than goat 5. The levels of avermectin B_{1a} as a percent of the total residue for the milk were similar to the corresponding edible tissues. The RIDA value for avermectin B_{1a} in milk was also calculated by correcting for the amount of HTO in the samples (0.02–0.04% of the dose). The resulting RIDA values were slightly higher and are shown in parentheses in Table V.

The HPLC radioactivity profiles for the ethyl acetate eluate from the edible tissue and milk homogenates were observed to be qualitatively similar. In general, the amount of avermectin B_{1a} as a percent of the radioactivity profile was similar to the corresponding RIDA value. A representative profile (goat 6 kidney) is shown in Figure 3 where avermectin B_{1a} elutes at fractions 22–27 but was removed for RIDA analysis. The radioactivity, not including avermectin B_{1a}, was recovered in three peaks that were observed in most samples analyzed. The minor peaks (fractions 7–9, metabolite C; fraction 18, metabolite B)

Table VI. Extent of Tritiated Water as a Percent of a Daily Dose[a]

goat	day	urine	milk	total
5	2	0.004	0.012	0.016
	11	0.014	0.030	0.044
6	2	0.003	0.014	0.017
	11	0.025	0.076	0.101

[a] Tritiated water was determined as described in Materials and Methods.

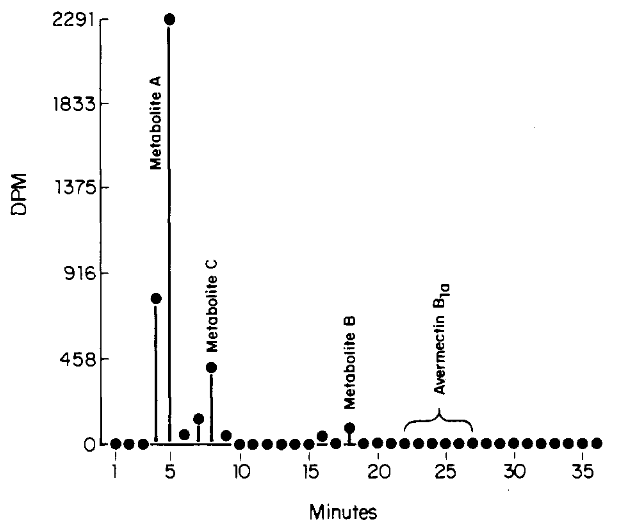

Figure 3. RP-HPLC radioactivity of the ethyl acetate eluate from goat 6 kidney tissue. RP-HPLC conditions: Zorbax ODS column (0.46 × 25 cm) with an 85:15 CH_3OH/H_2O mobile phase at 1 mL/min.

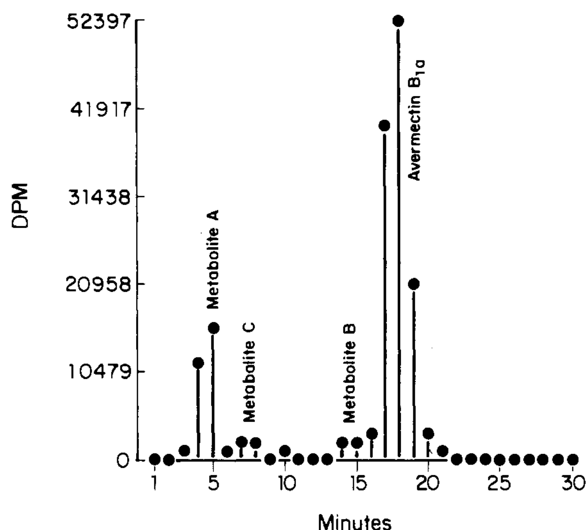

Figure 4. RP-HPLC radioactivity profile of the residue extracted from goat 6 feces. Same conditions as Figure 3.

were only a few percent of the radioactive profile while the major peak (fractions 4–6, metabolite A) was between 1 and 43% of the total radioactive profile with most values between 3 and 10%. The sum of metabolite A and avermectin B_{1a} was 85–99% of the total radioactive profile for all tissue and milk samples. The radioactivity profile for goat 6 feces (Figure 4) demonstrated the presence of the same three metabolites and avermectin B_{1a}, which was 71% of the total feces residue.

To identify metabolite A in vivo, a cochromatography with a standard was conducted. [^{14}C]-24-OHMe-B_{1a} was purified from a microsomal incubation of [^{14}C]avermectin B_{1a} and identified by NMR and mass spectrom-

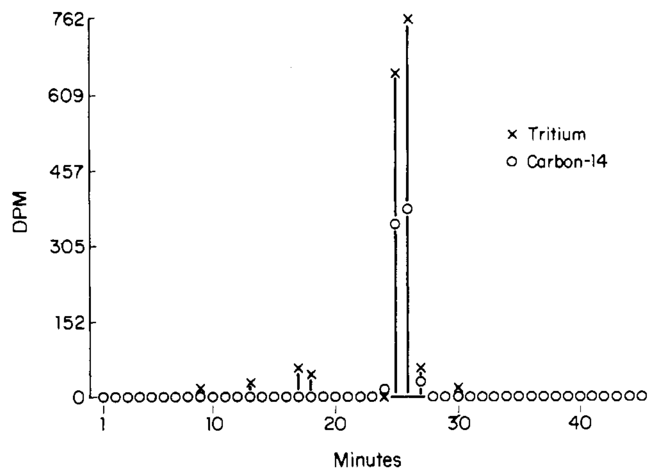

Figure 5. RP-HPLC radioactivity profile of metabolite A isolated from goat 6 liver and [^{14}C]-24-OHMe-B_{1a} standard. Same column as Figure 3 with a mobile phase of 32.5:35:32.5 $CH_3CN/H_2O/CH_3OH$ at 1 mL/min.

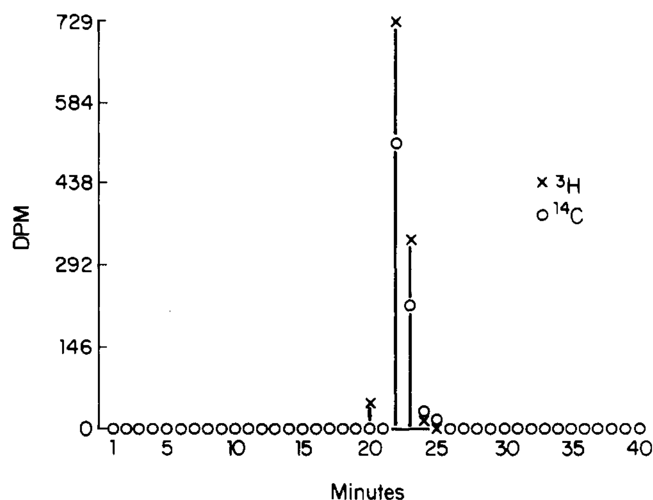

Figure 6. NP-HPLC radioactivity profile of the mixture of metabolite A and [^{14}C]-24-OHMe-B_{1a} collected from chromatography (Figure 5). NP-HPLC conditions: Zorbax silica column (0.46 × 25 cm) with an 85:15 isooctane/ethanol mobile phase at 1 mL/min.

etry (Miwa et al., 1982). Metabolite A, isolated from goat 6 liver homogenate, was cochromatographed with the [^{14}C]-24-OHMe-B_{1a} standard utilizing a different HPLC system, and the radioactivity profile is shown in Figure 5. Both the ^{3}H from liver tissue metabolite A and the ^{14}C from the 24-OHMe-B_{1a} standard eluted identically with the exact mixed proportion of ^{3}H and ^{14}C, radioactivity (2:1 dpm ratio). The composite peak from the RP-HPLC cochromatography was collected and injected on a NP-HPLC system. From the NP-HPLC system (Figure 6) the radioactivity attributed to metabolite A from goat liver and the 24-OHMe-B_{1a} standard also cochromatographed as a single peak.

The cochromatography of metabolite A isolated from goat 6 milk and the [^{14}C]-24-OHMe-B_{1a} standard was conducted. Both ^{14}C and ^{3}H eluted as a single peak on a RP-HPLC system in the same manner as shown for liver tissue (Figure 4) (data not shown). The cochromatography of metabolite B isolated from goat 5 liver and the 3″-desmethylavermectin B_{1a} (3″-DM-B_{1a}) standard was conducted. Since both the standard and metabolite B were ^{3}H-labeled, the sum of the added radioactivity and the peak shape were used as criteria for cochromatography. The radioactivity from both metabolite B

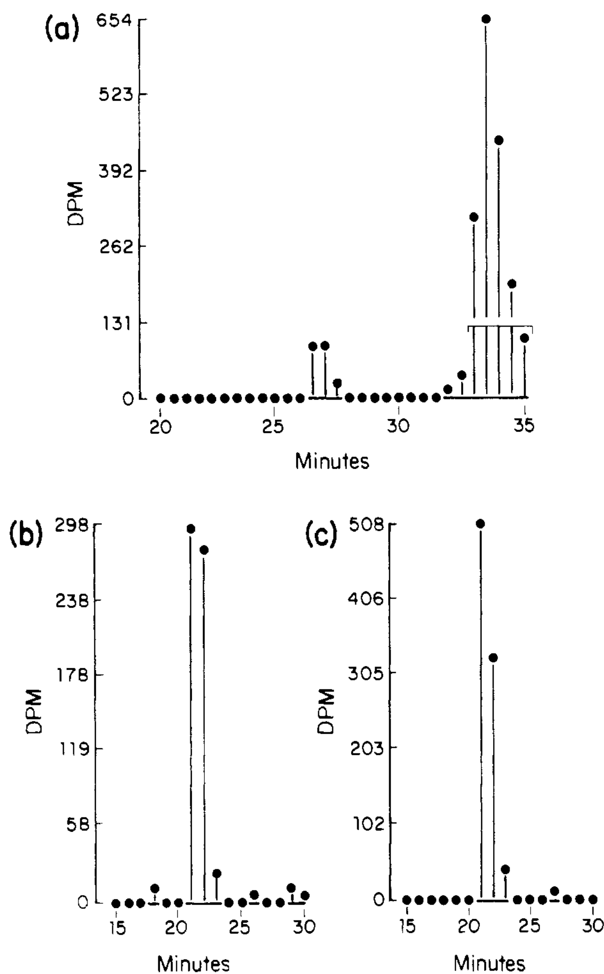

Figure 7. HPLC radiochromatography profiles of cochromatography of metabolite B and 3″-DM-B_{1a} standard. (a) Metabolite B from goat liver and 3″-DM-B1a by NP-HPLC. Same column as Figure 6 with 91:9 isooctane/ethanol at 1 mL/min; 0.5-min fractions collected. (b) Metabolite B from goat liver and 3″-DM-B1a by RP-HPLC. Same column as in Figure 3 with 40:40:20 $CH_3OH/CH_3CN/H_2O$ at 1 mL/min; 1-min fractions collected. (c) Metabolite B from goat feces and 3″-DM-B1a by RP-HPLC. Same column and conditions as in (b).

and the 3″-DM-B_{1a} standard eluted as a single peak on a NP-HPLC system (Figure 7a). This composite peak from the NP-HPLC cochromatography was collected and injected on a RP-HPLC system, and the cochromatography was again observed (Figure 7b). The successive NP-HPLC then RP-HPLC cochromatography of 3″-DM-B_{1a} standard and metabolite B isolated from feces was conducted, and cochromatography was observed (Figure 7c).

DISCUSSION

The administration of 10 daily doses of avermectin B_{1a} at 0.005, 0.05, or 1.0 mg/kg produced low ppb tissue residue levels that were dependent upon dose. The edible tissues were shown to contain the highest residue levels (1–98 ppb) and followed the order muscle < kidney < fat < liver. No differences in the residue levels between peripheral and omental fat or leg and loin muscle were observed. All goats dosed at the same rate had similar tissue residue levels except for goat 5, which were higher than for goat 6. This was probably due to reduced clearance the last few days before sacrifice for goat 5 and lower than expected residue levels for goat 6. The residue levels in blood and milk were less than 5 ppb for all goats, with the maximum levels attained after the third or fourth dose.

The feces were shown to contain most (~99%) of the recovered drug residue, with <1% found in the urine. The residue levels in the feces were maximal after the fourth dose. Therefore, a steady state was achieved by the fourth dose for all goats in this study as indicated by maximal excretion in the milk, urine, and feces. The residue levels in blood are consistent with this finding.

The amount of dose excreted was between 80 and 99%, with five of the six goats excreting 89–99% of the dose. The goat with the lowest recovery (80%, goat 5) was off feed and water the last 2 days of the study. This may account for the higher tissue residue levels for goat 5 than goat 6, which was treated at the same rate as discussed above. Goat 5 was shown to have otherwise normal clinical signs. Furthermore, the extent of metabolism of avermectin B_{1a} by goat 5 was similar to goats 1–4 as indicated by the level of unchanged parent and the metabolites present as a percent of the total tissue residue.

The level of unchanged avermectin B_{1a} in the edible tissues and milk was 76–99% of the total tissue residue for goats 1–5. The percent of avermectin B_{1a} in the edible tissues and milk from goat 6 was lower than the other goats, while the percent of the major metabolite, 24-OHMe-B_{1a}, was higher. This suggests that goat 6 was a faster metabolizer than the other goats. The fate of avermectin B_{1a} in goat 6, however, was the same as for the other goats since the residue levels, distribution, excretion, and tissue metabolite profile were similar.

The metabolite profiles for all goat tissues, milk, and feces were qualitatively similar, indicating a similar fate of avermectin B_{1a} in all goats from this study. The two metabolites identified were the 24-OHMe-B_{1a} and 3″-DM-B_{1a} and were observed in all samples analyzed. In general, the levels of these metabolites were 3–10 and <1–5% of the total residue for the 24-OHMe-B_{1a} and 3″-DM-B_{1a}, respectively. These two metabolites and the unchanged avermectin B_{1a} accounted for 85–99% of the total residue found in the tissues, milk, and feces. The 24-OHMe-B_{1a} and 3″-DM-B_{1a} metabolites were previously identified in tissues from rats administered avermectin B_{1a} (Maynard et al., 1985a). These two corresponding metabolites were also observed in tissues from rats administered the 8,9-*Z* isomer of avermectin B_{1a} (Maynard et al., 1989b). In liver microsomal incubations, both metabolites were identified from rat liver microsomal (Maynard et al., 1985a) and steer liver microsomal (Kline et al., 1987) incubations of avermectin B_{1a}. Furthermore, these two corresponding metabolites were identified from rat liver microsomal incubations of the 8,9-*Z* isomer of avermectin B_{1a} (Maynard et al., 1989b). Last, the 24-OHMe metabolite formation was identified in tissues from rats, sheep, and steers administered ivermectin (22,23-dihydroavermectin B_{1a}) (Chiu et al., 1986).

The extent of ^3H exchange in the urine and milk was investigated since the HTO would be excreted in these samples. Since the half-life of HTO is much longer than that of the radioactive drug residue, differences would be observed over time. Thus, the amount of tritiated water would increase with time as a percentage of the total residue. This was observed. At 2 days, approximately 10% of the total residue in milk from goat 6 was HTO and at 11 days approximately 30%. Furthermore, since 79–98% of the residue in milk at 7 days was avermectin B_{1a}, and since it is known not to lose ^3H to any significant extent in vivo or in vitro, it would be incorrect to conclude that extensive ^3H exchange (up to 30%) of the ^3H-labeled material has occurred. Since HTO is excreted in the body water while avermectin B_{1a} and its

residues are not, and since HTO has a longer half-life, daily accumulation of gradual ^3H loss would result in an exaggeration of the level of HTO when expressed as a percentage of the total residue in milk. Indeed, it was found that only approximately 0.1% of the daily dose was accountable as tritiated water at day 11 in urine and milk. This may be a more meaningful way to express the results. Moreover, this value would be even smaller if calculated with the results from both 2 and 11 days since at 11 days less than 0.05% of the daily dose was excreted as HTO. Whenever ^3H-labeled compounds are used in animal studies, the exchange of ^3H should be considered. However, for this study, the ^3H-labeled avermectin B_{1a} was appropriate for goat metabolism studies. Furthermore, when the tissue residue levels, distribution, and parent levels in rats dosed with both [^3H]- and [^{14}C]avermectin B_{1a} are compared, the stability of the ^3H label of avermectin B_{1a} is demonstrated (Maynard et al., 1985b).

In conclusion, a steady state was achieved after the fourth daily dose and residue levels in tissues, milk, and urine were in the very low ppb range. Almost all (89–99%) of the dose was excreted in the feces. In general, the residues were qualitatively similar for all goats, edible tissues, milk, and feces. Most (85–99%) of the residue in edible tissues, milk, and feces was accounted for as avermectin B_{1a}, 24-OHMe-B_{1a}, and 3″-DM-B_{1a}. From the results of this study, the fate of avermectin B_{1a} administered to lactating goats was determined. The residues of avermectin B_{1a} in the edible tissues and milk, although present at low ppb levels, were characterized and represent minimal concern for humans exposed to these residues. The ^3H-labeled avermectin B_{1a} was apppropriate for animal studies.

Registry No. Avermectin B_{1a}, 65195-55-3; 24-(hydroxymethyl)avermectin B_{1a}, 82573-42-0; 3″-demethylavermectin B_{1a}, 99965-56-7.

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