Monensin Absorption and Metabolism in Calves and Chickens

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[^{14}C]Monensin was given to bile-cannulated chickens and calves to determine whether or not monensin was absorbed, metabolized, and eliminated through bile. Eleven to thirty-one percent of the ^{14}C was absorbed by the chickens, and 36–40% of the ^{14}C was absorbed by the calves. Monensin was isolated from chicken bile but not from calf bile. Four metabolites were isolated from chicken bile and tentatively identified by mass spectral interpretation. One metabolite from calf bile and three metabolites from calf feces were isolated and tentatively identified. Monensin accounted for 50% or more of the ^{14}C in the calf feces.

Monensin is an antibiotic used to control coccidiosis in chickens and to improve efficiency of feed conversion in cattle. Monensin also increases the rate of body weight gain in cattle under some conditions.

Excretion and tissue distribution of [^{3}H]monensin have been described in chickens (Herberg and Van Duyn, 1969), and more recently excretion and tissue distribution of [^{14}C]monensin have been described in chickens (Donoho et al., 1982). Excretion and tissue distribution of [^{14}C]-monensin have also been described in cattle (Herberg et al., 1978), and metabolism of [^{14}C]monensin by cattle and rats has been reported (Donoho et al., 1978).

Conclusions drawn from the studies with [^{3}H]monensin in chickens may not be valid because of possible tritium exchange. [^{14}C]Monensin was synthesized after the [^{3}H]monensin studies were done. At the time that [^{14}C]monensin metabolism was investigated in cattle and rats, monensin was thought to be either not absorbed or absorbed in very small quantitites because most of the ^{14}C was found in the feces and only very small amounts of ^{14}C were found in tissues and urine. However, absorbed ^{14}C could have been returned to the intestine in bile and then eliminated in feces.

In objectives of our investigations were to determine whether or not monensin was absorbed, metabolized, and eliminated through bile of calves and chickens.

MATERIALS AND METHODS

Chickens. Four- to five-month-old White Leghorn roosters and 1-year-old White Leghorn hens were used. Monensin was given orally as a single dose in gelatin capsules. Dosages varied from 2.6 to 100 mg of monensin and from 1 to 4.5 μCi of [^{14}C]monensin, specific activity 0.362 μCi/mg (Table I). Feed and water were available ad libitum.

Total bile was collected from bile-cannulated roosters and total urine and feces were collected from colostomized roosters from the time of dosing until the chickens were killed 3 days after dosing. The feathers were removed when the chickens were killed, various tissues were sampled, and the remainder of the carcass was ground and sampled. Bile and urine were either frozen or stored at 3 °C. Feces, eggs, tissues, and carcass material were lyophilized and stored at room temperature.

Calves. Three-month-old bile-cannulated calves, one male (Shorthorn) and one female (Angus), were used. Each calf was given, in gelatin capsules orally, 10 mg of monensin/kg of body weight and ca. 80 μCi of [^{14}C]monensin, specific activity 0.164 μCi/mg. Feed and water were available ad libitum.

Table I. Sex, Dose, and Surgical Modifications of Chickens

chicken i.d.	sex	dose, mg	μCi	surgical modification
A	M	100	4.60	bile cannula
C	M	100	4.49	bile cannula
D	M	100	4.71	bile cannula
E	M	12	4.21	bile cannula
G	M	12	4.35	bile cannula
H	M	12	4.39	bile cannula
I[a]	M	13	4.71	bile cannula
14	M	2.6	0.94	colostomized
15	M	2.3	0.83	colostomized
16	M	2.8	1.01	colostomized
17[a]	F	3.0	1.09	none
18[a]	F	2.7	0.98	none

[a] Placed in a respiration chamber to determine the respiratory loss of ^{14}C.

Total bile was collected following dosing. Bile collected immediately following surgery from the respective calves was refrigerated and later reinfused into the duodenum through a latex cannula to replace bile collected during the monensin metabolism studies. Feces from the female calf was collected separately from the urine by means of a screen. Urine from the male calf was collected entirely free of feces by means of a urinal. The calves were killed 4 days after dosing. Various tissues were sampled, and then the remainder of the carcasses were ground and sampled. Bile, urine, and feces were either stored frozen or refrigerated at 3 °C. Tissues and carcass material were lyophilized and stored at room temperature.

Monensin and [^{14}C]Monensin. Monensin and [^{14}C]-monensin were the sodium salts of monensin factor A. [^{14}C]Monensin was prepared by fermentation from propionate-2-^{14}C (Donoho et al., 1982). The purity of Monensin was >99% and radiochemical purity of [^{14}C]monensin was >99%, as stated by the manufacturer and as confirmed by us; a single sharp radioactive band was detected by reverse phase high performance liquid chromatography (HPLC) using methanol and water on an analytical C_{18} column, and only one area was detected by thin-layer chromatography (TLC) using two solvent systems (Donoho et al., 1978).

Carbon-14 Analysis. Bile (0.5 g), chicken urine (0.5 g), and lyophilized materials (0.1–0.3 g) were combusted in a Packard Model 306 oxidizer and assayed as described by Davison et al. (1982). Calf urine (1 mL) was counted in Insta-Gel (Packard Instrument Co., Inc.). Carbon-14 in respired air was determined as described by Davison (1976), except that Carbosorb (Packard) and methanol were used as the trapping agents.

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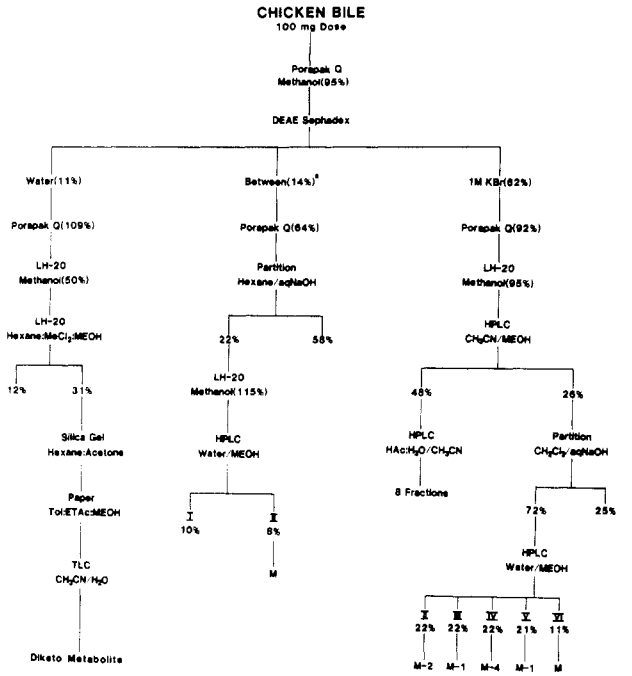

Figure 1. Flow diagram of the isolation of metabolites from chicken bile. M is monensin. M-1, M-2, and M-4 are metabolites 1, 2, and 4 (Donoho et al., 1978). Percentages represent the recovery based on the amount of ^{14}C material applied at each step in the separation. [a]*Poorly* defined radioactive peaks eluted with water and 1 M KBr. All radioactive material between these peaks was pooled for the "between" fraction.

Apparatus. The HPLC unit was an Altex Model 332 gradient liquid chromatograph system (Altex Scientific Inc.) attached to a Packard Model A7500 radioactive flow monitor. The HPLC columns were Waters Radial Pak C_{18} (10 μm, 8 mm × 10 cm, Waters Associates, Inc.), Waters μBondapak C_{18} (3 mm × 30 cm), and Whatman Partisil 10 ODS (3 mm × 25 cm; Whatman, Ltd.). Thin-layer plates were Analtech precoated silica gel G-250 μm thick (Analtech, Inc.). Electron impact mass spectra were obtained with a Varian 112S equipped with a solid sample probe and a Varian SS-200 data system.

Isolation of Metabolites. Metabolites were isolated from chicken bile according to the general flow diagram shown in Figure 1. Glass distilled water and reagent-grade solvents were used in all cases. Pooled bile for all time periods from roosters A, C, and D and for the 8–24-h time period from calves was applied in 20–40-mL fractions to Porapak Q columns (2 × 10 cm; 100–120 mesh; Waters Associates, Inc.) poured in water. The Porapak Q columns were washed with 75 mL of water and then eluted with methanol. The methanol eluates were pooled, dried under vacuum, dissolved in water and, in the case of chickens, applied to DEAE-Sephadex columns (1 × 23 cm; 40–120 μm; Pharmacia Fine Chemicals).

The DEAE-Sephadex columns were eluted first with about 250 mL of water and then with 1 M KBr in water until all radioactive material was recovered. KBr was removed by putting the sample back through Porapak Q.

LH-20 columns (1 × 50 cm; 25–100 μm; Pharmacia Fine Chemicals) were eluted at ca. 1 mL/min with methanol or a mixture of hexane–methylene chloride–methanol (3:2:1). Silica gel columns (1 × 23 cm; 60–200 mesh; J. T. Baker Co.) were eluted with a mixture of hexane–acetone (8:2). Only fractions of the most intense radioactive areas were taken to the next step of the cleanup. Paper chromatography (Whatman No. 1) and TLC (precoated silica gel 250 μm thick; Analtech, Inc.) developed with a mixture of toluene–ethyl acetate–methanol (90:100:10) and acetonitrile–water (1:1) provided final cleanup of a fraction from which a metabolite characterized as containing two carbonyl groups was isolated.

Some fractions were partitioned between hexane or methylene chloride and 0.01 M aqueous NaOH. Metabolites were eventually isolated from the organic phase of this partitioning. However, metabolites were never isolated from the aqueous phase of the partitioning despite several attempts.

For calf bile, DEAE-Sephadex was eliminated from the isolation procedure. Partitioning with dichloromethane and water was done prior to column chromatography on LH-20. HPLC followed the LH-20 chromatography.

For reverse-phase HPLC, solvent was pumped at 1 mL/min, beginning with water for 3 min, then with a linear gradient of water to methanol for 17 min, and finally with methanol for 15 min. Acetonitrile or a mixture of acetic acid–water (4:500) was substituted for water in HPLC, but these solvents did not improve the separation of metabolites.

For both chicken and calf bile, poorly resolved radioactive material usually eluted when 100% methanol was reached during HPLC. Fractions were taken from discernible peaks of radioactive material, and these fractions were again subjected to HPLC 1 or more times with the same solvent system. Usually, Radial Pak C_{18} columns were used for the first HPLC and analytical C_{18} columns were used for subsequent HPLC. In some cases, TLC using solvent systems I and II of Donoho et al. (1978) provided final cleanup.

Ten grams of feces from the 24–32-h collection period of each calf was pooled and extracted 3× with 500 mL of methanol. The residue was removed by filtering through fritted glass funnels. The filtrate was dried under vacuum, dissolved in water, and applied to Porapak Q columns. Cleanup involved Porapak Q, LH-20, and DEAE-Sephadex columns and HPLC in this order and developed as described previously.

Characterization of Metabolites. Tentative identifications were made based on interpretation of the mass spectra. Monensin was identified by comparative mass spectra with authentic material and, when appropriate, by cochromatography on HPLC and TLC. The sample was introduced into the mass spectrometer as a solid via probe. The voltage and temperature of the ion source were 70 eV and 270 °C, respectively.

RESULTS AND DISCUSSION

All chickens given the 100-mg dose showed signs of illness on the second day. They developed a general ruffled appearance, and droppings were scant and watery. Chicken D convulsed and died when caught for bile collection at the 48-h time period. Chickens A and C recovered by the third day. Chickens given 12 mg of monensin and the calves remained vigorous and healthy throughout the experiments.

Recovery of ^{14}C. Recovery of ^{14}C is shown in Table II. These data show that feces were the primary route for eliminating ^{14}C by both chickens and calves, that small amounts of ^{14}C were eliminated in urine by both species, and that in chickens small amounts of ^{14}C were eliminated through respiration. In chickens, elimination of ^{14}C through urine was about equal to elimination of ^{14}C through respiration. Cumulative elimination of ^{14}C in droppings and bile for the chickens and in feces, urine, and bile for the calves is shown in Figures 2 and 3.

The data show that 11–31% absorption of ^{14}C occurred in the chickens and that 36–40% absorption occurred in

Table II. Recovery of ^{14}C from Chickens and Calves Given [^{14}C]Monensin

animal i.d.	sex	dose, mg	dose, μCi	% of dose recovered: feces	urine	droppings	bile	gallbladder	GI tract	liver ×10⁻³	kidneys ×10⁻³	carcass	respiratory	total
Chickens														
A	M	100	4.60			60.8	17.5	0.13	11.4	26	4	0.35		90.4
C	M	100	4.49			69.0	17.2	0.81	9.4	23	4	0.23		96.8
D	M	100	4.71			48.0	10.9	0.02	33.6	39	6	0.52		93.3
mean ± SEM						59.3 ± 6.1	15.2 ± 2.2	0.32 ± 0.25	18.1 ± 7.8	29 ± 5	5 ± 0.7	0.37 ± 0.08		93.5 ± 1.9
E	M	12	4.21			75.0	13.3	0.004	0.28	14	2	0.16		88.7
G	M	12	4.35			80.4	15.0	1.59	0.29	23	3	0.053		97.4
H	M	12	4.39			79.0	12.3	0.062	0.11	9	3	0.11		91.5
mean ± SEM						78.1 ± 1.6	13.5 ± 0.8	0.55 ± 0.52	0.23 ± 0.06	15 ± 4	3 ± 0.3	0.11 ± 0.03		92.5 ± 2.6
I		13	4.71			59.9	30.7	0.09	0.74	19	2	0.22	1.6	93.3
14	M	2.6	0.94	106.3	1.59			0.01	0.83	ND[a]		ND		109.1
15	M	2.3	0.83	95.2	1.44			0.02	4.48	ND		ND		101.3
16	M	2.8	1.01	87.4	0.91			0.009	1.03	ND		ND		89.4
mean ± SEM				96.3 ± 5.5	1.31 ± 0.21			0.01 ± 0.004	2.1 ± 1.2					99.9 ± 5.7
17	F	3.0	1.09			93.7			1.17	ND	ND	ND	1.4	97.1
18	F	2.7	0.98			99.8			0.66	ND	ND	*b*	1.8	102.3
Calves														
63	F	502	73.79	50.9	3.4		37.3	0.001	1.64	180	10	0.77		94.9
64	M	550	80.85	64.8	1.0		34.6	0.003	1.60	110	4	0.62		102.7

[a] ND = not detectable. About 10 counts per minute (cpm) above background was detectable. Where detectability was uncertain, the gross cpm resulting from oxidations of six samples of a given tissue from treated and untreated chickens were compared by a one-sided *t* test, $p = 0.05$. [b] Enough ^{14}C was found in the sample of carcass to extrapolate to 22% of the dose; this was attributed to accidental contamination of the carcass because liver, kidneys, and adipose tissue contained insignificant amounts of ^{14}C and total recovery was complete.

Table III. Monensin Equivalents in Selected Tissues from Chickens and Calves Given [^{14}C]Monensin

animal i.d.	sex	dose, mg	dose, μCi	μg/g of tissue dry matter: skin	muscle	abdominal fat	heart	liver	kidneys	carcass
Chickens										
A	M	100	4.60		4.70	0.81		3.83	2.27	1.34
C	M	100	4.49		0.49	1.14		8.06	2.57	0.91
D	M	100	4.71		0.41	1.22		5.90	3.46	1.91
mean ± SEM					1.87 ± 1.42	1.06 ± 0.13		5.93 ± 1.22	2.76 ± 0.36	1.39 ± 0.29
E	M	12	4.21		0.04	0.02		0.21	0.10	0.05
G	M	12	4.35		0.03	0.04		0.38	0.18	0.02
H	M	12	4.39		0.07	0.06		0.17	0.20	0.04
mean ± SEM					0.05 ± 0.01	0.04 ± 0.01		0.25 ± 0.06	0.16 ± 0.03	0.04 ± 0.01
Calves										
63	F	502	73.79	0.63	0.41	0.58	0.42	3.46	1.23	0.41
64	M	550	80.85	0.32	0.20	0.44	0.18	2.04	0.33	0.21

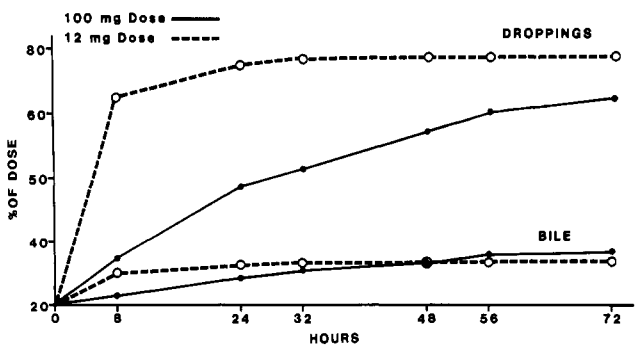

Figure 2. Recovery of ^{14}C in droppings and bile from chickens given a single dose of either 12 or 100 mg of [^{14}C]monensin. Each point represents an average from three animals, except for the 56- and 72-h time periods for chickens given 100 mg of monensin where only two animals survived.

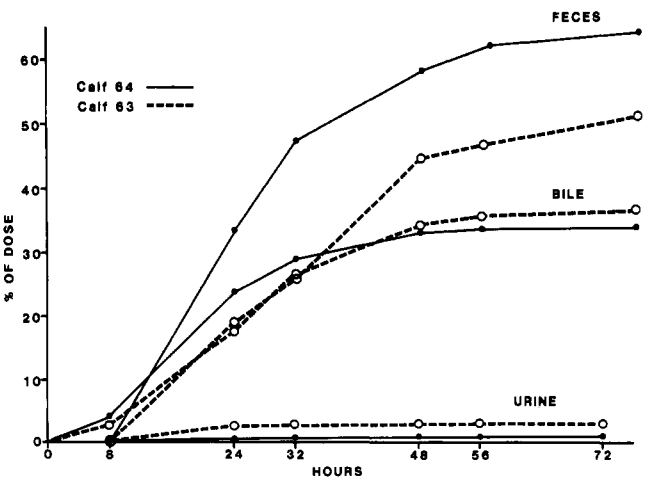

Figure 3. Recovery of ^{14}C in feces, bile, and urine from calves given a single dose, 10 mg of [^{14}C]monensin/kg of body weight.

the calves. Carbon-14 present in tissues (exclusive of the gastrointestinal tract), bile, urine, and respired air are all indicative of absorption. Most of the absorbed ^{14}C was recovered in the bile, and recovery of ^{14}C in bile was lower and more variable in the chickens than in the calves (Table II).

A higher percentage of ^{14}C was recovered in the gastrointestinal tract, liver, kidneys, and carcasses of chickens given 100 mg of monensin than in like tissues from chickens given 12 mg of monensin. It is possible that the

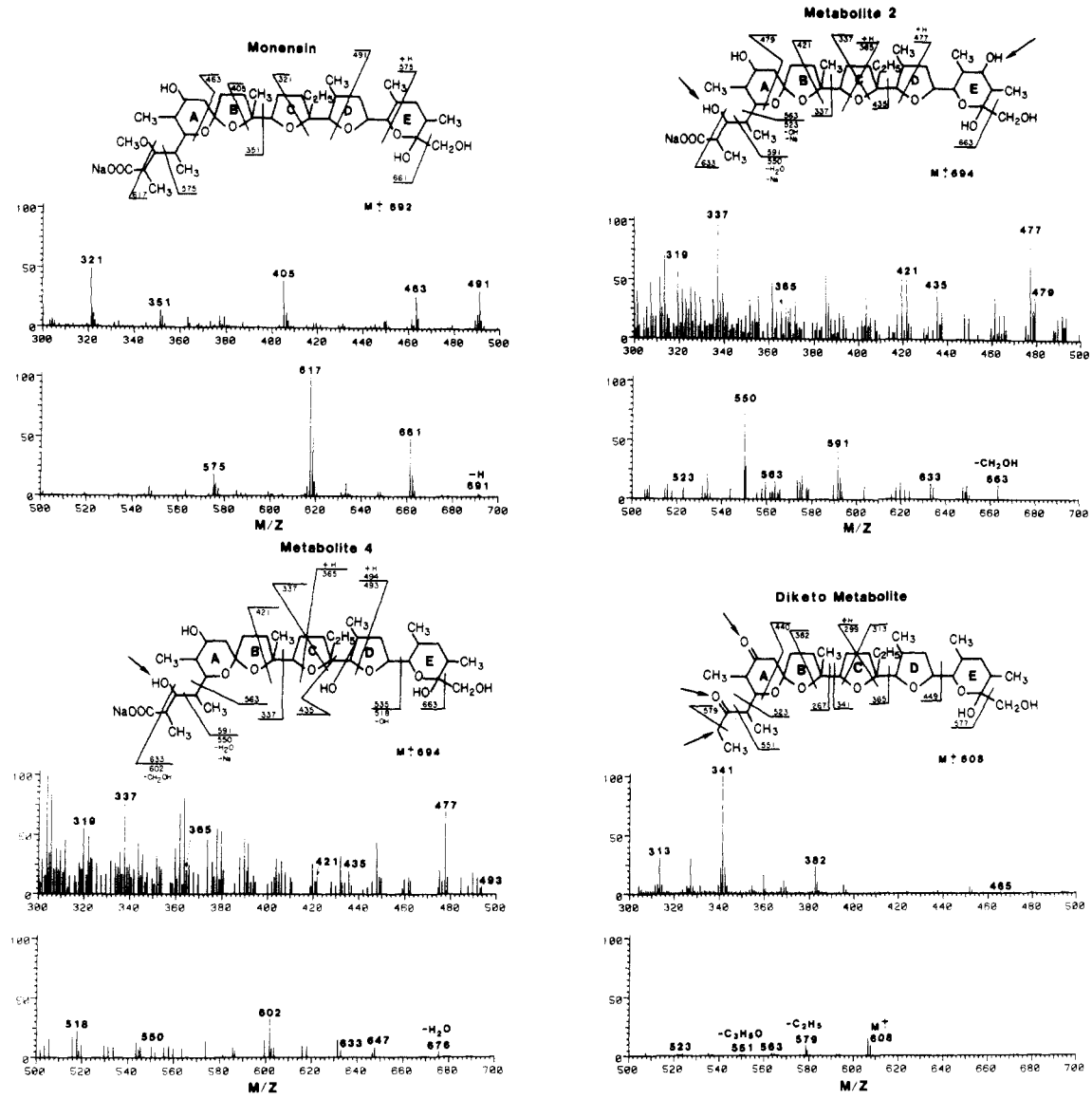

Figure 4. Electron impact mass spectra of monensin, M-2, M-4, and a proposed diketo metabolite. Possible fragmentation is shown on the molecular structure, and sodium is retained with the fragment unless indicated as lost.

illness of the chickens given 100 mg of monensin contributed to their greater retention of ^{14}C by slowing the rate of elimination of the ^{14}C. Residues of ^{14}C in livers, kidneys, and carcasses, however, were quite low in all chickens and calves.

Carbon-14 was not detectable in six eggs collected from the two hens dosed with about 3 mg of [^{14}C]monensin.

Residues in terms of monensin equivalents in selected tissues are given in Table III. Chickens given the higher dose of monensin had higher residues in their tissues. The residue levels for the chickens given 12 mg of monensin are consistent with those recently published by Donoho et al. (1982), where a similar level of monensin was studied.

Identification of Metabolites. The spectra shown (Figure 4) were recorded between m/z 300 and m/z 700, and the ion intensities were normalized to a base ion in this mass range. When the lower masses were included, the data system became saturated and the more interpretable low-intensity high-mass fragments were not recorded. The abundant low-mass ions were probably due to sample contamination and/or metabolite fragmentation. When necessary for interpretation, the MS was repeated to obtain lower masses.

The molecular weight of sodium monensin is 692. But, in the MS of monensin, the most intense ion in the cluster near the molecular weight is 691 (Chamberlin and Agtarap, 1970). Apparently, a hydrogen is easily lost during MS, and hydrogen rearrangement also occurs during the fragmentation process. We assumed that hydrogen rearrangement occurred during MS of the metabolites as well.

Because of the difficulty of isolating metabolites, I did not attempt to isolate metabolites from bile of chickens given 12 mg of monensin. Monensin-Na was isolated from bile of chickens given 100 mg of monensin (Figure 4). This material gave a mass spectrum identical to that of the dosing material and cochromatographed with monensin. It was present in amounts >2% of the dose.

Monensin-Na was not isolated from calf bile but was isolated from calf feces. [^{14}C]Monensin accounted for about 50% of the ^{14}C in the calf feces. To further verify these observations, samples of calf bile and feces were analyzed for monensin by the Food and Drug Administration Laboratory, Denver, CO, using a microbiological procedure (Okada et al., 1980). According to this assay, monensin could account for 68% of the ^{14}C in the feces. Stated sensitivity of the assay was 1.0 $\mu g/g$ (Bond, 1983). Microbiological activity was not detected in bile. We concluded that monensin either was not present at all in calf bile or was present in very small amounts that could not be determined with present technology.

Donoho et al. (1978) found monensin and six metabolites in feces of intact calves and rats. Their metabolite numbering scheme and the proposed metabolite changes were as follows: M-1, O-demethylation; M-2, O-demethylation and an oxidation on ring E (Figure 4); M-3, an epimer of M-2; M-4, O-demethylation and an oxidation on ring D; M-5, an oxidation on ring D; M-6, decarboxylation with accompanying loss of sodium and O-demethylation with oxidation of the resultant hydroxyl to a ketone. The EI mass spectra of M-1 and M-6 were published, but only a table of important ions was published for the MS of M-2 through M-5.

M-1 was isolated from bile of both chickens and calves in amounts >4% of the ^{14}C in the bile. These mass spectra were identical with that published by Donoho et al. (1978). Metabolites M-2 (or M-3) and M-4 were isolated from chicken bile, but not calf bile, in amounts >2% of the ^{14}C in the bile. The mass spectra of M-2 and M-4 are shown in Figure 4. There is no way to distinguish M-2 from M-3 by MS or TLC. Both M-2 and M-4 were present as the sodium salts, and both retained the sodium in some fragments during MS. This retention of sodium during MS is well documented for monensin and some of its metabolites (Chamberlin and Agtarap, 1970; Donoho et al., 1978).

A metabolite tentatively identified as a diketone (Figure 4) was isolated from chicken bile, but not calf bile, in amounts >2% of the ^{14}C in the bile. Metabolic changes included decarboxylation with accompanying loss of sodium, O-demethylation, and oxidation of two hydroxyls to carbonyls. One carbonyl group is on the side chain and one is on ring A. Evidence for the diketo metabolite (Figure 4) includes the molecular ion at 608, ions at 579 and 551 suggesting losses of CH_3CH_2- and CH_3CH_2CO- and that a carbonyl group might be on the side chain, ions at 440, 382, and 341 suggesting that rings B, C, D, and E are present, and ions at 523 and 440 suggesting that ring A was present with two hydrogens missing, thus a carbonyl group might be on ring A. Additionally, when the MS was examined between m/z 200 and m/z 300, an ion at 299 and two relatively intense ions at 267 and 239 were observed. Possible structures from the 299 and 267 ions are shown on Figure 4; a 239 ion could occur if CH_3CH_2- was fragmented from the 267 ion. Other fragments indicated are consistent with this structure.

M-1, M-2 (or M-3), M-4, and the diketo metabolite were also isolated from calf feces. We conclude that monensin is absorbed and metabolized extensively by both species tested and that metabolites that could not be isolated are present. Further investigation of the metabolic fate of this compound awaits new developments in separation science and in the ability to synthesize possible metabolites for their positive identification.

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Registry No. Monensin, 17090-79-8; metabolite 2, 92009-89-7; metabolite 4, 92009-90-0; diketo metabolite, 92009-91-1; metabolite 1, 92096-16-7.

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