Excretion and Tissue Distribution of [14C]Monensin in Cattle

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[14C]Monensin administered orally to steers was excreted rapidly and quantitatively in the feces. None of the dose appeared in the urine. Liver tissue from a steer, conditioned for 2 days on [14C]monensin and sacrificed 12 h after the last dose, contained a residue of radioactivity equivalent to 0.59 ppm monensin. No other edible tissue contained a residue. Liver tissue from a heifer and two steers, conditioned for 5 days on [14C]monensin and sacrificed 12 h after the last dose, contained radioactivity equivalent to 0.214, 0.425, and 0.357 ppm, respectively. No other tissue contained radioactivity in excess of 0.021 ppm. Monensin assays on the livers from the latter three animals gave values of 0.015 ppm or less, indicating that most of the liver radioactivity was not monensin.

Monensin, a polyether monocarboxylic acid, is the principal factor of the antibiotic complex produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1968). The structure of monensin and some related minor components of the complex were described by Agtarap and Chamberlin, 1968; and Chamberlin and Agtarap, 1970. The sodium salt of monensin is an effective agent for increasing feed efficiency in cattle (Raun et al., 1976) and is an effective anticoccidial agent (Shumard and Callender, 1968).

The studies herein described were conducted to determine the excretion pattern and tissue distribution of [14C]monensin sodium in cattle. For convenience, throughout the rest of this paper the term monensin will be used to indicate monensin sodium.

EXPERIMENTAL SECTION

Labeled Compound. [14C]Monensin was prepared by fermentation as described by Day et al. (1973). [14C]-Monensin used in the balance-excretion studies and the first tissue residue study was synthesized from propionate-*1*-^{14}C and propionate-*2*-^{14}C. These substrates labeled the monensin in 14 positions, including the carboxyl side chain and every ring except ring C (Figure 1). [14C]-Monensin used in the second tissue residue study was synthesized from propionate-*1*-^{14}C and butyrate-*1*-^{14}C. These substrates labeled the monensin in eight positions, including the carboxyl carbon and every ring. Each lot was evaluated by thin-layer chromatography on silica gel plates developed in ethyl acetate. More than 97% of the radioactivity was associated with monensin or monensin factor B, a minor component of the antibiotic complex (Figure 1). Factor B represented approximately 8% of the radioactivity in the doses used in the balance-excretion studies. The [14C]monensin used in the tissue residue studies was purified by silica gel column chromatography and contained essentially no factor B.

Animal Feeding and Dosing. For the balance-excretion experiments, three Angus steers weighing 260–300 kg were housed in metabolism cages and fed a corn-based 65% concentrate ration. For approximately 2 weeks before the labeled dose was given, the steers received 300 mg of unlabeled monensin mixed with soybean meal as a top-dressing on the daily ration. Feeding of the unlabeled monensin was resumed the day following the labeled dose. The [14C]monensin was given orally as a single dose contained in a gelatin capsule. Steer 1 received 300 mg

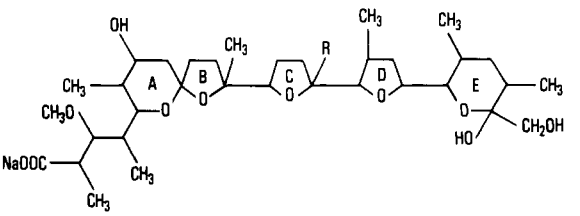

Figure 1. Structure of monensin sodium (R = C_2H_5) and factor B (R = CH_3).

(sp act. 0.027 μCi/mg). Steers 2 and 3 received 301 and 299 mg (sp act. 0.030 μCi/mg), respectively.

For the first tissue residue study, a Hereford steer, steer 4, weighing 365 kg was fed a corn-based 40% concentrate ration and was conditioned to 300 mg of unlabeled monensin per day as above. The steer was then given 300 mg of [14C]monensin (sp act. 0.283 μCi/mg) per day for 2 days. Half of each daily dose was given orally by gelatin capsule at 12-h intervals beginning at 8 a.m. on the first day. At approximately 8 a.m. on the third day (12 h after the last dose), steer 4 was sacrificed and muscle (round), liver, subcutaneous fat, kidney, heart, lung, spleen, and blood were taken for analysis.

For the second tissue residue study, one Hereford heifer, heifer 5, weighing 393 kg and two Hereford steers, steers 6 and 7, weighing 464 and 461 kg, respectively, served as the treated animals and a third steer, steer 8, served as the control. All four animals were conditioned on a corn-based 40% concentrate ration containing unlabeled monensin at a level of 33 mg/kg (30 g/ton). Three days before they were dosed with [14C]monensin, all four animals were changed to a ration containing no monensin. The heifer received 9.1 kg and the steers 10 kg of ration daily. Each of the three treated animals received a gelatin capsule containing [14C]monensin, 0.25 μCi/mg, each morning and evening for 5 days. The heifer received 150 mg and the two steers received 165 mg in each dose for a total daily dose equivalent to 33 ppm in the feed.

Twelve hours after the last capsule dose the treated animals were slaughtered and muscle (round), kidney fat, subcutaneous fat, kidney, liver, and heart were taken for analysis. Equal quantities of kidney fat and subcutaneous fat were combined to give a composite fat sample from each animal. Tissues from the control steer were collected for determination of baseline radioactivity.

Sample Preparation and Analysis. Urine and feces were collected daily in the balance-excretion experiments. The feces samples were diluted with 0.5 g of water/g of feces and blended to give a uniform homogenate. Six independent determinations of radioactivity were made

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Table I. Daily Recovery of Radioactivity in Feces and Urine of Steers Given Single Oral Doses of [^{14}C]Monensin

days after dosing	radioactivity[a] as % of dose					
	steer 1		steer 2		steer 3	
	feces	urine	feces	urine	feces	urine
1	22	0	2	0	3	0
2	41	0	49	0	45	0
3	18	0	24	0	27	0
4	7	0	8	0	13	0
5	3	0	3	0	6	0
6	2	0	1	0	4	0
7-11	1	0	1	0	4	0
totals	94	none	88	none	102	none

[a] Each value is the mean of at least six observations. The experimental data are net values above the predose mean.

for each blended feces sample. Weighed 1-g aliquots of feces homogenate were placed in quartz boats and combusted to $^{14}CO_2$ and H_2O in quartz tubes in three-section electrically heated furnaces (Lindberg Division of Sola Basic Industries, Watertown, Wis.). Combustion products were absorbed in 10 mL of 30% ethanolamine in 2-methoxyethanol. This solution was combined with 10 mL of a scintillator solution of the following composition: toluene, 100 mL; dioxane, 300 mL; 2-methoxyethanol, 300 mL; PPO (2,5-diphenyloxazole), 7 g; dimethyl POPOP (1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene), 350 mg; and naphthalene, 56 g. Recovery efficiency for the combustion process was determined regularly and was always greater than 92%. Observed rates were adjusted to 100% recovery. Six 2-mL portions of urine were combined with 15 mL of Aquasol (New England Nuclear Corporation) in dim incandescent light preparatory to liquid scintillation counting. All samples were counted in polyethylene vials (Amersham/Searle No. 3327). Samples were counted for 10 min with a Packard, Model 3380 or 3385, Tri-Carb liquid scintillation counter. Count rates were adjusted to 100% counting efficiency by a factor obtained by internal standardization of each solution with [^{14}C]toluene.

Tissues were ground and frozen until assayed. Approximate 0.2-g portions of blood and fat and 0.5-g portions of other tissues were assayed for radioactivity by the combustion procedures described above. In the first tissue residue study ten independent determinations were made on each tissue. Muscle, liver, fat, and kidney from four control steers were assayed similarly to establish baseline radioactivity levels for the primary edible tissues. In the second tissue residue study eight independent determinations were made on each tissue and each sample was counted twice for 10 mins. Tissues from a control steer were assayed similarly.

Determination of Criteria of Residue and of Sensitivity. In the first tissue residue experiment the means of the ten determinations for each tissue from each of the four control animals were derived. From the four mean values for each tissue a mean and an associated 95% upper confidence limit (UCL) were calculated for that tissue from the formula 95% UCL = $\bar{x} + t_{n-1,\alpha/2}s/\sqrt{n}$, where $\bar{x}$ is the mean of the four control animal means, s is the standard deviation of the four control animal means, and t (Student's t) was taken as 3.182. This 95% UCL was chosen as the concentration value that a treated tissue must exceed to represent a radioactivity residue. The sensitivity of detection of radioactivity was taken as the difference between the 95% UCL and the mean. It represents the concentration by which a treated mean must differ from the control mean to enable one to say, according to the residue criterion mentioned, that the control and treated means differ.

In the second tissue residue study the mean and 95% UCL of the eight determinations were calculated for each control tissue, and the criteria for determination of residue and sensitivity were the same as for the first experiment. In addition, a direct determination of sensitivity was made by fortifying control tissues with various levels of 0.25 μCi/mg of [^{14}C]monensin. A chloroform solution of [^{14}C]monensin equivalent to a specific residue level in 0.2 g of fat or 0.5 g of other tissue was pipetted into a quartz combustion boat. After the solvent had evaporated, control tissue was weighed into the boat. These fortified tissues were assayed as described for tissues from treated animals. Control and fortified control tissue means were compared by means of t tests to determine at what fortified level they were no longer significantly different. The calculated dpm/g values for each tissue (control and treated) and dpm values for each fortified tissue were checked for outlying values as described by Li (1964). A few values were discarded by the criterion of the reference.

Monensin Assay in Cattle Liver. Fifty grams of liver were extracted with methanol, and the monensin was partitioned into carbon tetrachloride as described by Donoho and Kline (1968). The sample was evaporated and partitioned between hexane and methanol–10% aqueous sodium chloride, 80:20. The monensin was recovered from the aqueous methanol by extraction with hexane–carbon tetrachloride, 1:1, and the sample was purified by silica gel column chromatography and assayed by bioautography as described by Donoho and Kline (1968).

RESULTS AND DISCUSSION

Balance-Excretion Studies. Steers excreted all of the recovered radioactivity in the feces. Radioactivity in the urine did not exceed predose levels. The rate and extent of excretion of [^{14}C]monensin are shown in Table I. Total

Table II. Radioactivity in Tissues of One [^{14}C]Monensin-Treated Steer and Four Control Steers

tissue	treated, dpm/g	control dpm/g ± SD[c]	95% UCL,[a] dpm/g	assay sensitivity,[b] ppm	net residue, ppm
liver	371.3	5.5 ± 1.7	8.2	0.004	0.59
muscle	5.9	17.3 ± 19.6	48.5	0.050	none
fat (subcutaneous)	32.9	26.5 ± 11.3	44.5	0.029	none
(kidney)	27.4				none
kidney	19.2	11.2 ± 9.6	26.5	0.024	none
heart	6.2				none[d]
lungs	20.0				none[d]
spleen	9.5				none[d]
blood	23.6 (dpm/mL)				none[d]

[a] 95% upper confidence limit. [b] Control tissue (95% UCL-$\bar{x}$) as ppm. [c] Standard deviation. [d] No residue when compared to control data from the four primary tissues.

Table III. Net Radioactivity (ppm Monensin Equivalent) in [^{14}C]Monensin-Treated Cattle Tissues and Gross Radioactivity in Control and Treated Cattle Tissues

	treated			control	
	net ppm, dpm/g ± SE			steer 8	
tissue	heifer 5	steer 6	steer 7	dpm/g	95% UCL
liver	0.214	0.425	0.357		
	128.3 ± 4.0[a,b]	245.3 ± 2.6[a]	207.5 ± 4.5[a]	9.5 ± 1.8	13.8
muscle	0.016	0.008	0.011		
	12.6 ± 1.7[a]	8.2 ± 2.3[a]	10.0 ± 1.7[a]	3.7 ± 0.2	4.2
fat	none	none	0.021		
	21.9 ± 3.5	25.1 ± 3.6	35.2 ± 2.3[a]	23.8 ± 2.7	30.3
kidney	0.008	0.014	0.013		
	14.6 ± 1.4[a]	18.3 ± 2.2[a]	17.8 ± 1.9[a]	10.3 ± 1.4	13.8
heart	0.007	0.009	none		
	11.9 ± 1.1[a]	12.9 ± 1.1[a]	10.5 ± 0.8	8.0 ± 1.3	11.0

[a] Exceed 95% upper confidence limit (UCL) of control mean. [b] Number of determinations (n) was 8 for all tissues except steer 8 muscle (n = 6) and heifer 5 kidney, and steer 7 fat and heart (n = 7).

Table IV. Gross Radioactivity in Control Steer Tissues with and without Added [^{14}C]Monensin

		fortified control			
		gross dpm[a] ± SE			
tissue	control	0.049 ppm	0.024 ppm	0.010 ppm	0.005 ppm
liver	5.1 ± 1.0	21.4 ± 1.5[b]	14.1 ± 1.0[b]	9.5 ± 0.7[b]	9.2 ± 0.6[b]
	(8)	(8,8)[c]	(8,8)	(8,6)	(8,8)
muscle	2.0 ± 0.1	17.4 ± 1.5[b]	12.5 ± 0.3[b]	10.1 ± 1.1[b]	11.5 ± 0.9[b]
	(6)	(6,8)	(6,7)	(6,8)	(6,8)
kidney	5.7 ± 0.8	18.0 ± 1.3[b]	15.4 ± 1.3[b]	8.8 ± 1.0	8.6 ± 0.6[b]
	(8)	(8,7)	(8,8)	(8,8)	(8,8)
	control	0.050 ppm	0.025 ppm	0.020 ppm	0.015 ppm
fat	5.5 ± 0.7	11.3 ± 0.7[b]	8.9 ± 0.4[b]	10.9 ± 0.8[b]	8.9 ± 0.7[b]
	(8)	(8,8)	(8,8)	(8,8)	(8,7)

[a] Dpm from nominal 0.2-g samples of fat or 0.5-g samples of other tissues. [b] Means differ significantly from control mean. [c] Numbers (n_1, n_2) indicate number of control and treated values used in t test comparisons of control and treated means.

recovery of ^{14}C averaged 95% for the three steers. The daily fecal excretion data show that at least 75% of the dose was excreted within 3 days.

First Tissue Residue Study. In this study, liver was the only edible tissue which contained a residue of radioactivity (see Table II). The liver level was equivalent to 0.59 ppm when calculated as monensin. Other studies (Donoho et al., 1978) have shown that only 2–3% of the total liver radioactivity was monensin. The calculated sensitivity for muscle, kidney, and fat was in the range of 0.02–0.05 ppm, which appears to be a practical sensitivity for this experiment. No control data were generated for heart, lung, spleen, and blood. However, application of the upper confidence limits given for the primary tissues showed that these other tissues contained little or no residue.

Second Tissue Residue Study. Results from the second tissue residue study (Table III) were similar to those from the first study. Liver contained the greatest levels of radioactivity, equivalent to 0.214, 0.425, and 0.357 ppm monensin for heifer 5, steer 6, and steer 7, respectively. No other residues exceeded 0.021 ppm. The fat tissues from steer 6 and heifer 5 and heart tissue from steer 7 contained no residue.

Monensin assays on the livers gave values of 0.005–0.010 ppm for heifer 5 and steer 7 and 0.010–0.015 ppm for steer 6. These results demonstrated that most of the liver radioactivity was not monensin.

Data from combustion of control and [^{14}C]monensin-fortified control tissues are presented in Table IV. The means of liver and muscle samples fortified at 0.005 ppm and above, and fat samples fortified at 0.015 ppm and above were significantly different from control tissue means. For kidney, the 0.010 ppm level mean was not significantly different from the control mean. Sensitivity values, defined as the difference between the mean dpm/g and the 95% UCL for control tissues, were 0.008, 0.001, 0.011, and 0.006 ppm for liver, muscle, fat, and kidney, respectively. Thus, these two methods of calculating sensitivity, the UCL treatment and the fortified control method, were comparable in this experiment. Comparison of the muscle, liver, fat, kidney, and heart control tissue means by Duncan's test showed that, except for fat, the means did not differ significantly for 95% confidence intervals.

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Metabolism of Monensin in the Steer and Rat

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The metabolism of [^{14}C]monensin was studied in both cattle and rats. Monensin was metabolized to many different compounds by both species. The metabolite pattern was qualitatively similar but quantitatively different in the two species. Six fecal metabolites were tentatively identified. Five resulted from O-demethylation and/or hydroxylation and one resulted from demethylation and decarboxylation. The radioactive residue in steer liver was characterized and the metabolite pattern was qualitatively similar to that of feces. The most abundant metabolite accounted for only 6% of the liver radioactivity.

Herberg et al. (1978) have described the excretion and tissue distribution pattern when [^{14}C]monensin was fed to cattle. The radioactivity was excreted rapidly and quantitatively in the feces. Liver was the only edible tissue which contained a residue of radioactivity greater than 0.021 ppm in cattle sacrificed 12 h after either a 2- or 5-day [^{14}C]monensin dosing period. The mass spectrometry of four monensin metabolites from the rat was described by Donoho and Occolowitz (1975).

The studies described in this report were undertaken to characterize the radioactivity in feces and liver from [^{14}C]monensin-treated cattle and to compare the metabolic patterns in cattle and rats. The sodium salt of [^{14}C]-monensin was used throughout the studies. For convenience, the term monensin is used to indicate monensin sodium except where stated otherwise.

EXPERIMENTAL SECTION

Radiolabeled Compound. [^{14}C]Monensin was prepared by fermentation as described by Day et al. (1973), using as substrates propionate-*1*-^{14}*C* in combination with either propionate-*2*-^{14}*C* or butyrate-*1*-^{14}*C*. The propionate/propionate combination labeled the monensin in 14 positions including the carboxyl carbon and two carbons in each ring except ring C (see Day et al., 1973, for details). The propionate/butyrate combination labeled monensin in eight positions, including the carboxyl carbon and each of the five rings.

Experimental Samples. The cattle feces sample used for the comparative metabolite study was from a steer dosed orally for 5 days with 330 mg/day of [^{14}C]monensin, 0.25 μCi/mg, synthesized using the propionate/butyrate combination of substrates (steer 6 from the studies of Herberg et al., 1978). All other samples were derived from animals fed [^{14}C]monensin synthesized using the propionate/propionate combination.

Liver was obtained from a steer which was dosed orally for 2 days with 300 mg/day of [^{14}C]monensin, 0.283 μCi/mg, and sacrificed 12 h after the last dose (steer 4 from the studies of Herberg et al., 1978). Feces for isolation and identification of metabolites were obtained from steer 4 or one dosed in a similar manner.

Feces from Wistar-derived albino rats dosed orally ([^{14}C]monensin, sp act. 0.576 μCi/mg, 16 mg/kg) for 2 days were used for comparative studies.

Comparative Metabolite Study. Two grams of rat feces were macerated with added water to give a smooth paste and extracted with 200 mL of methanol by blending for 15 min. The sample was filtered, the extraction was repeated, and the combined filtrates were evaporated to 200 mL. The sample was diluted with 100 mL of 10% aqueous sodium chloride solution (aqueous NaCl) and extracted with $CHCl_3$–CCl_4 (1:1, 3 × 100 mL, then 3:1, 2 × 100 mL). The combined $CHCl_3$–CCl_4 fractions were evaporated, and the sample was reconstituted in 100 mL of methanol–aqueous NaCl (8:2). This sample was extracted with hexane (2 × 100 mL), and the combined hexanes were extracted with 100 mL of methanol–aqueous NaCl (8:2). The combined aqueous methanol fraction was diluted with 80 mL of aqueous NaCl and extracted with $CHCl_3$–CCl_4 (3:1, 3 × 200 mL). The extract was purified by gradient elution column chromatography on a 1 cm i.d. × 60 cm stainless steel column packed with Quantum LP-1 silica gel, 10–20 μ (Quantum Industries, Fairfield, N.J.). The sample was evaporated onto 8 mL of Woelm dry column silica gel, No. 202 (ICN Pharmaceuticals, Cleveland, Ohio), and placed in a 10-cm stainless steel extension on top of the column. The column was eluted at a flow rate of 9.9 mL/min using a Waters Model 6000 liquid chromatography pump. A nonlinear gradient was developed by pumping solvent from a 190-mL stirred reservoir filled with one solvent into which a more polar solvent was introduced as the elution progressed. The pump was situated between the reservoir and the column to avoid pressurization of the glass reservoir. The solvent sequence for elution is described in the Results and Discussion section. Fractions of 20 mL each were collected and aliquots were assayed for radioactivity by liquid scintillation counting (LSC). Specific fractions were pooled and aliquots of the pools were separated by thin-layer chromatography (TLC) on silica gel plates with a preadsorbent zone for sample application (Quantum LQ plates). Plates were developed in equilibrated chambers.

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