Disposition of Ronidazole by Swine. 1. Radiocarbon Content of Tissues

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The antiparasitic and antibacterial compound, ronidazole ((1-methyl-5-nitroimidazol-2-yl)methyl carbamate), was rapidly absorbed and excreted by the pig after oral administration of $^{14}CH_3$-labeled drug. Of the total excreted, less than half of the radioactivity was present in the urine. Radioactive residues were ubiquitously distributed in all tissues analyzed. Radioactive residues were present in edible tissues 42 days after dosing and were associated with tissue proteins. The relative depletion rates of different tissues is suggestive of the incorporation of endogeneous metabolites. Liberation of labeled methylamine by acid hydrolysis indicated that as much as 20–30% of the persistent residues retain some chemical relationship to the drug. As the fraction of the persistent residues which liberate methylamine declined with time, at least two general types of residues are present. Further studies using nitric acid oxidation of the residues showed that the fraction containing an intact 1-methyl-5-nitroimidazole nucleus was about 1% of the total residue.

Ronidazole [(1-methyl-5-nitroimidazol-2-yl)methyl carbamate] is a nitroimidazole useful in animal husbandry. Metabolic studies in the turkey have been reported (Rosenblum et al., 1972). These studies showed extensive metabolic degradation with resultant persistent radioactivity in various tissues, especially muscle. Since evolution of $^{14}CO_2$ was reported as 2.5% when the label was at the 2-ring atom and only 0.8% when the CH_3 was labeled, $^{14}CH_3$-labeled ronidazole was used in the current studies except that one animal was dosed with 2-ring-labeled ronidazole. The current studies were designed to determine the distribution of drug and metabolites in edible tissues of the pig, the depletion of radioactive residues with time, and the macromolecular nature of the tissue residue due to ronidazole. Chemical degradation studies designed to determine if the radioactive residues were due exclusively to metabolic degradation to single carbon fragments were carried out.

MATERIALS AND METHODS

Chemicals. Radiolabeled ronidazole was synthesized in the Merck Sharp & Dohme Research Laboratories. All lots were analyzed for radiochemical purity by paper or thin-layer chromatography and had a radiochemical purity of greater than 98% when used. No single impurity was present in a quantity greater than 0.5%. The specific activity was adjusted for each experiment to a suitable level by dissolving unlabeled and labeled material and either dosing the resultant solution or crystallizing the product before use.

$^{14}CH_3$-Labeled ronidazole was synthesized from $^{14}CH_3OH$ by treatment with methanesulfonic acid anhydride. The methyl methanesulfonate reacted with 4-nitroimidazole to yield predominantly [*methyl*-^{14}C]-1-methyl-5-nitroimidazole. Reaction with formaldehyde followed by transesterification with methylcarbamate yielded [*methyl*-^{14}C] ronidazole.

Ring 2-labeled ronidazole was synthesized from [2-^{14}C]imidazole obtained by reaction of ethylenediamine and [^{14}C]formic acid at 500 °C via the usual steps of methylation, nitration, condensation with formaldehyde, and transesterification to yield the carbamate.

Radioactivity Measurements. *Total Radioactivity Determination.* All radioactivity determinations were carried out by liquid scintillation using spectrometers optimized for ^{14}C.

Tissue, plasma, blood, fecal, and some urine samples were combusted for analysis for total radioactivity. The $^{14}CO_2$ was trapped in a suitable reagent mixed with phosphor and counted. Sample blanks were prepared and run simultaneously. In the early study quantitation was by comparison with standard samples prepared in the same matrix and counted at the same time in the same spectrometer. Recent samples were converted to dpm by using standard quench curves and external standards.

Samples of urine were run in 70:30 toluene–ethanol phosphor by using appropriate standards and blanks. Quenching corrections utilized the external standard method except in the early study when internal standards were used.

Animal Handling. *Preliminary Experiment.* Three barrow pigs were dosed with labeled ronidazole in the feed at a concentration of 0.006% for 4 days. The animals were fed at 8 a.m. and 4 p.m. One pig was dosed with [2-*ring*-^{14}C]ronidazole, sp act. 0.125 μCi/mg, and killed 16 h after the last feeding. The remaining pigs were dosed with [*methyl*-^{14}C]ronidazole, sp act. 0.134 μCi/mg, and killed 5 and 10 days after dose. Plasma samples were taken and feces collected daily for all animals. Terminal samples were stomach and GI tract contents, bile, brain, fat, heart, kidneys, large and small intestine, liver, lungs, muscle, pancreas, skin, spleen, and stomach.

Balance Studies. Two experiments were carried out each by using two barrow pigs. Ronidazole was administered once daily for 3 days in a feed slurry. The dosage varied from 6.6 to 12 mg/kg, sp act. 8–9 μCi/mg. In each experiment one animal was killed at 6 and 72 h after the last dose. Urine and feces were collected for the duration of the experiment and the edible tissues fat, muscle, liver, and kidney were collected for analysis.

Long-Term Depletion Study. For each withdrawal time two barrow pigs and one gilt pig, weight 20-30 kg, were dosed with $^{14}CH_3$-labeled ronidazole administered once daily for 3 days in a feed slurry. The dose was 7 mg/kg and is similar to the dose expected in field use when ronidazole is administered in the feed at 0.006%. The feed slurry was generally consumed within 30 min. The specific activity of the ronidazole was adjusted to 3.0 μCi/mg for the animals killed at 3 days after the last dose, 5.5 μCi/mg for animals killed at 7 and 14 days and 7.0 μCi/mg for animals killed 6 h, 28 days, and 42 days after the last dose. Samples of muscle, kidney, liver, and fat were ground and

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Table I. Total Radioactivity Analysis of Plasma and Excretion Samples of Swine Dosed with ^{14}C-Labeled Ronidazole[a]

animal no.:	697			698			696		
wt, kg:	13.6			17.7			20.4		
total drug consumed, mg:	195			192			192		
label site:	2-ring			CH_3			CH_3		
	urine, mg	feces, mg	plasma, μg/mL	urine, mg	feces, mg	plasma, μg/mL	urine, mg	feces, mg	plasma, μg/mL
			On Drug						
7 h			0.61			0.77			0.62
day 1	5.5	4.5	1.28	6.15	7.0	0.97	6.88	4.0	0.90
day 2	10.45	13.6	1.07	9.25	24.3	1.09	10.10	31.6	0.95
day 3	12.43	19.2	1.41	7.65	23.5	1.44	9.93	21.4	1.10
day 4	17.30	30.6	1.77	9.93	23.2	0.56	9.83	24.4	0.50
			Off Drug						
day 1				3.49	13.3	0.39	2.82	14.4	0.30
day 2				0.66	5.37		0.66	8.6	
day 3				0.30	1.04	0.21	0.19	0.66	0.19
day 4				0.19	0.44	0.14	0.19	0.20	0.17
day 5				0.17	0.15	0.2	0.14	0.11	0.17
day 6							0.16	0.17	0.12
day 7							0.10	0.15	0.08
day 8							0.08	0.09	0.08
day 9							0.08	0.05	0.09
day 10							0.07	0.07	0.06
total mg	45.68	67.9[b]		37.79	98.3		41.04	105.73	
% of dose	23.4	34.8		19.7	51.2		21.5	55.2	

[a] Feed was administered at 8 a.m. and 4 p.m. daily, containing 0.006% ronidazole, sp act. 0.125 and 0.134 μCi/mg for ring and CH_3 label. [b] Contents of stomach and intestinal tract were estimated to contain 2.5 and 0.8 mg, respectively.

blended in a meat grinder and packaged in polyethylene containers. Plasma was separated by centrifugation. All samples were stored at −30 °C until used.

Tissue Fractionation. Protein was separated by the salt fractionation procedure (Rosenblum et al., 1972). Tissue homogenates were prepared by homogenizing 25 g of tissue with 75 g of water. After 10 g of sodium chloride was added, the mixture was heated at 100 °C for 30 min in a boiling water bath. The suspension was centrifuged for 30 min at 10 000 rpm, the solids were suspended with 100 mL of 10% sodium chloride solution, and the heating and centrifuge steps were repeated. The solid product was suspended in 100 mL of acetone and filtered on a sintered glass funnel and air-dried. The dry product, amounting to 6–6.5 g, was analyzed for total radioactivity by combustion as described above.

Methylamine Generation. Samples were hydrolyzed with 6 N hydrochloric acid in a sealed tube heated at 125–130 °C for 16 h. Crystalline methylamine *p*-toluenesulfonate was added as a carrier prior to the hydrolysis. The hydrolysis mixture was made strongly alkaline with sodium hydroxide and the methylamine distilled by using a nitrogen gas sweep. The gas stream was passed through a scrubber containing ethanol and *p*-toluenesulfonic acid. Methylamine *p*-toluenesulfonate was isolated from the scrubbing solution by evaporating to dryness and crystallizing from acetonitrile. The specific activity was determined by using aliquots of the crystalline salt.

1-Methyl-5-nitroimidazole Generation. Tissue samples were refluxed for 18 h with concentrated nitric acid, 20 mL/g of tissue. Ronidazole carrier and 10–12 mg of ammonium vanadate were added to the mixture before refluxing. The reaction mixture was evaporated to dryness by using a rotary evaporator. A second evaporation using an equal volume of water was carried out to eliminate excess nitric acid. The residue was dissolved in 100 mL of 10% sodium carbonate and extracted 3 times with 100 mL of ethyl ether. After 1 mL of 12 N hydrochloric acid was added, the ether was evaporated to dryness. Ten milliliters of 2-propanol was added and again evaporated. The residue was recrystallized from 2-propanol.

Table II. Recovery of Radioactivity from Pigs Dosed with [*methyl*-^{14}C]Ronidazole[a]

	animal no.:	19	17	21	22
	wt/kg:	21.4	22.3	19.8	20.5
sample	dose, mg:	430	615.5	712.8	738
muscle: ppb		5.0	0.5	8.6	1.1
%		7.9	0.6	9.6	1.2
liver: ppb		7.8	1.6	12.3	2.4
%		1.1	0.16	1.0	0.2
kidney: ppb		7.9	1.1	11.9	2.5
%		0.2	0.02	0.15	0.03
fat: ppb		2.5	0.4	1.3	0.2
%		1.2	0.13	0.36	0.06
plasma: ppb		5.4	0.47	9.4	0.8
%		2.0	0.12	2.1	0.18
urine,[b] %		27	35	30	23.3
feces,[b] %		20	47	13.9	42.6
GI tract, %		10	0.1	15.7	
total recovery		69.4	83.1	72.8	67.6
slaughter time, h[c]		6	72	6	72

[a] Barrow pigs, dosed 3 days; animal no. 17, 9.2 mg/kg; animal no. 19, 6.7 mg/kg, animals no. 21 and 22, 12 mg/kg. [b] Total collection over entire experiment. [c] After last feeding.

RESULTS

The total recovery in the excreta increased with time (Tables I and II). Under the conditions employed the total recovered after 10 days was about 77% of the dose. During the dosing interval the ratio of total radioactivity in the feces was 2–3 times greater than that in urine after day 1. Although this could reflect poor absorption of the compound, analysis of the bile (Table III) indicates that considerable biliary excretion occurs. The concentration in the bile in the short-term animal was about 2 times the plasma level whereas in later samples the ratio is less than 1.

Plasma levels show the presence of radioactive components with rapid clearance at early times and components with slow elimination rate at later times. At later times the fraction excreted in the feces is not greater than that

Table III. Total Radioactivity Analyses of Tissues and Fluids of Pigs Dosed with ^{14}C-Labeled Ronidazole[a] (Micrograms per Gram Expressed as Drug)

		no. 697	T/P[b]	no. 698	T/P	no. 696	T/P
tissue/fluid:	bile	3.49	1.97	0.07	0.4	0[c]	
	brain	1.25	0.7	0.1	0.5	0.11	1.8
	fat	1.04	0.6	0.19	1.0	0.16	2.7
	heart	1.53	0.9	0.24	1.2	0.19	3.2
	kidney	3.47	2.0	0.54	2.7	0.25	4.2
	large intestine	3.45	2.0	0.30	1.5	0.14	2.3
	small intestine	1.44	0.8	0.41	2.1	0.18	3.0
	liver	2.55	1.4	0.57	2.9	0.30	5.0
	lung	1.72	1.0	0.39	2.0	0.24	4.0
	muscle	1.40	0.8	0.30	1.5	0.28	4.7
	pancreas	1.67	0.9	0.51	2.6	0.34	5.7
	skin	1.32	0.7	0.34	1.7	0	
	spleen	1.87	1.1	0.59	3.0	0.28	4.7
	stomach	2.12	1.2	0.26	1.3	0.19	3.2
	plasma	1.77		0.20		0.06	
GI tract contents:	stomach	3.63		0		0	
	large intestine	6.24		0.03		0	
	small intestine	0.76		0.07		0	

[a] See Table I for details. [b] Ratio of concentration in tissue to plasma. [c] Detection limit about 0.05–0.1 μg/g.

found in the urine. (Tables I and IV).

The total radioactivity is rather evenly distributed among the tissues analyzed (Table III) with liver and kidney containing the highest concentrations at early times.

For the "on-drug" animals the tissue to plasma ratio varied from about 0.6 to 1.4 (excluding kidney). Thus, in the on-drug animals the radioactivity appears to diffuse readily into all tissues. During the first 3 days after drug withdrawal a rapid decrease in the residue level is observed. The residues remaining in the tissues do not appear to diffuse readily as the tissue:plasma ratio of all tissues increases (Tables III and IV).

The total radioactivity levels in edible tissues after dosing for 3 days are contained in Table IV. By 3 days after dosing levels have declined to $^1/_{12}$ to $^1/_4$ of the initial level, but the rate of decline for the interval from day 3 to day 7 is much less. By 28 days all residues are 0.1 ppm or lower except muscle, which had not reached a level of 0.1 ppm by 42 days.

A greater fraction of the total radioactivity is contained in the "protein" fraction of both liver and muscle tissue of the animals slaughtered 7 days after the last dose compared to those slaughtered at earlier times (Figure 1).

Analyses for methylamine liberated on vigorous chemical hydrolysis of tissues by the reverse isotope dilution method shows that the fraction of radioactivity which liberates methylamine declines with time (Table V). By this method the yield of methylamine is nearly quantitative when ronidazole is added to tissue samples from untreated animals.

Only a small portion of the residue yields 1-methyl-5-nitroimidazole on treatment with nitric acid (Table VI).

DISCUSSION

The data presented indicate complex metabolism and distribution. The changing nature of the substances remaining in the animal is clearly illustrated by the shifting excretion ratio between urine and feces during the 10-day collection interval. The corresponding decrease in the ratio of concentration in the bile and plasma indicates that biliary excretion serves as an excretory route during the initial period and becomes less important later. The high fraction of the dose found in feces is unusual for a nonpolar drug of this low (200) molecular weight and may be the result of reductive activation of the drug and trapping of the reactive intermediate by anaerobic organisms in the intestinal tract. Most of the excretion occurs during the

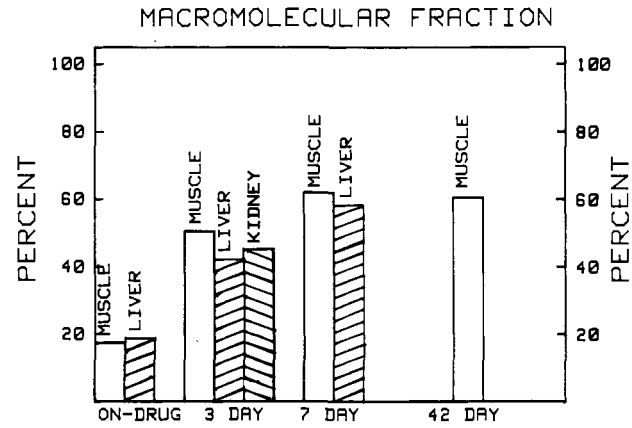


Figure 1. Macromolecular fraction obtained by hot salt precipitation as described under Materials and Methods. Pooled samples from three animals from long-term depletion study.

first 24 h after dosing, indicating that the absorption and excretion processes for the drug and most of its metabolites are rapid (Table I).

However, a small fraction of the dose is slowly excreted. By the 10th day this amounted to less than 0.2%/day. The rate of excretion appears little changed between days 7 and 10. The depletion rate of the tissue residues is similar to that observed in pigs dosed with $NaH^{14}CO_3$ (Schach von Wittenau, 1967). Thus, the rate of depletion of the total radioactivity in liver and fat for $^{14}CO_2$- and ronidazole-dosed animals is identical during the same period (6–18 and 7–14 days after the last dose) whereas the rate of depletion of muscle and kidney from ronidazole-dosed animals is more rapid during this time interval but the terminal (28–42 days) rate is similar to that observed with $^{14}CO_2$-dosed animals during the 6–18 day interval.

The quantity of total radioactivity in the tissue at 3 days after dose (Table IV) and the ubiquitous distribution among all the tissues analyzed (Table III) indicate that as much as 1–2% of the dose could have been converted to persistent metabolites associated with cellular macromolecules. Confirmation of the macromolecular nature of the residues was obtained by the protein analysis which showed that about 60% of the radioactivity was present in this fraction of muscle at 7 days. This fraction did not change appreciable by 42 days after dose.

The mode of formation and the chemical nature of these persistent residues are of interest. The N-$^{14}CH_3$ group used

Table IV. Total Radiocarbon Content of Tissue and Plasma of Swine Dosed with ^{14}C-Labeled Ronidazole[a] (Drug Equivalents, ppm)

		tissue/fluid															
		muscle				liver				kidney			fat			plasma	
expt	days post third dose	gilt[b]	av[c]	SD[d]	T/P[e]	gilt	av	SD	T/P	gilt	av	SD	T/P	gilt	av	SD	
RN 163	on drug[f]	6.47	6.32	0.16	0.85	11.4	10.63	0.8	1.43	9.50	9.37	0.38	1.26	1.2	1.46	0.21	0.2	7.6	7.44	0.31
	3	0.40	0.49	0.08	1.11	1.45	1.53	0.06	1.2	1.0	1.22	0.18	2.77	0.26	0.30	0.04	0.7	0.39	0.44	0.04
	7	0.51	0.52	0.01	1.58	1.02	1.15	0.19	3.48	0.82	0.85	0.10	2.58	0.23	0.25	0.04	0.76	0.28	0.33	0.05
	14	0.32	0.35	0.05	2.92	0.40	0.44	0.08	3.67	0.26	0.27	0.02	2.25	0.12	0.15	0.02	1.25	0.11	0.12	0.02
	28	0.17	0.18	0.02	6.0	0.09	0.10	0.01	3.33	0.08	0.09	0.01	3.0	0.054	0.06	0.005	2.0	0.024	0.03	0.004
	42	0.11	0.13	0.02	13.0	0.056	0.06	0.004	6.0	0.045	0.05	0.004	5.0	0.050	0.050	0.007	5.0	0.009	0.01	0.002

[a] Animals were dosed as described under Materials and Methods. The dose was 7 mg kg^{-1} day^{-1} for 3 days. [b] Each group consisted of one gilt and two barrow pigs. Value for gilt only. [c] Average of all three animals. [d] Standard deviation of group. [e] Ratio of total radioactivity in tissue to plasma. [f] Six hours after last dose.

Table V. Methylamine Liberation from Tissues of Swine Dosed with $^{14}CH_3$-Labeled Ronidazole[a]

sample time	tissue	% of total radioact	ppb
	whole tissue homogenate		
3 days	muscle	28	137
	fat	33	99
7 days	muscle	32.9	171
14 days	liver	35.5	156
	muscle	32.9	116
28 days	liver	24.1	24
	muscle	22.8	41
42 days	liver	20.9	12.5
	muscle	18.8	24
	protein fraction only		
on drug	liver	53	1050
	muscle	91	1000
3 days	liver	47.2	303
	muscle	43.0	106
	kidney	42.0	231
7 days	liver	38.0	254
	muscle	35.8	117
	salt wash		
on drug	liver	77	6600
	muscle	91	4540
	acetone wash		
on drug	liver	68	50
	muscle	58	102

[a] Composite samples were treated as described under Materials and Methods. Addition of ronidazole to tissue samples from control animals gave values for liberated methylamine of 95% from muscle homogenate and 98% from fat.

Table VI. Isotope Dilution Analysis for 1-Methyl-5-nitroimidazole Generated from Muscle Tissue of Swine Dosed with $^{14}CH_3$-Labeled Ronidazole[a]

	crystallization			
days postdosing	I, dpm/mg	II, dpm/mg	% of total	ppb
7	13.1	12.7	1.19	6.2
14	11.8	11.8	1.57	5.5
28	5.0	5.0	1.03	1.9
42	3.0	3.0	0.8	1.1
control muscle + ronidazole	756 653	758 522	100 70	

[a] Muscle tissue from animals, experiment RN-163, using an equal weight aliquot from each animal.

in this study can yield a single carbon fragment by either oxidative or reductive metabolic reactions. Oxidative N-demethylation mediated by cytochrome P-450 is known to yield labeled formaldehyde and carbon dioxide from a variety of substrates. These substances in turn are incorporated into endogeneous metabolites.

Reduction of the nitro group can yield the hydroxylamine or amino derivative. In vivo the reduction of nitro groups is known to be carried out by soluble enzymes such as xanthine oxidase and by the cytochrome P-450 system. Chemical studies showed that the amino compounds derived from 5-nitro-1-alkylimidazoles are unstable substances. Reduction of 1-methyl-5-nitroimidazole with stannous chloride and hydrochloric acid yields methylamine, glycine, and ammonia (Bhagwat and Pyman, 1925). Catalytic reduction of ronidazole at neutral pH also yields methylamine (Buhs et al., 1979). The reduction in vivo and ring cleavage of metronidazole, a related 5-nitroimidazole, by rats to form acetamide and (2-hydroxyethyl)oxamic acid has been reported by Koch and Gold-

man (1979). Both ronidazole and metronidazole were observed to form the nitroanion radical, the one electron reduction product, when exposed to liver microsomes under anaerobic conditions (Perez-Reyes et al., 1980). Methylamine was isolated from the liver tissue of turkeys dosed with $^{14}CH_3$-labeled ronidazole, and ^{14}C-labeled acetamide was isolated from the urine of pig no. 697 dosed with 2-ring-labeled ronidazole (*Rosenblum* et al., 1972). These studies show that the nitro group of ronidazole is reduced in vivo and that methylamine or a metabolite readily converted to methylamine is present in vivo. It is known that the $^{14}CH_3$ moeity of methylamine, 1-naphthyl *N*-methylcarbamate (carbaryl), and [*p*-(isopropylcarbamoyl)benzyl]methylhydrazine (Natulen) is incorporated into rat tissues (Krishna and Casida, 1966; Schwartz, 1966).

Thus, two metabolite routes are available which yield a single carbon fragment known to be incorporated into tissue as endogenous substances. Both pathways could take place simultaneously in vivo, and the major route could vary from species to species and from tissue to tissue. In the turkey it appears that the reductive route predominates. As imidazoles, and especially 5-nitroimidazoles, are stable to oxidation, the formation of $^{14}CO_2$ from 2-ring-labeled ronidazole must be a result of reductive, not oxidative, metabolism. Thus, the observation that 2.5% of the dose is converted to $^{14}CO_2$ when turkeys are dosed 2-ring-labeled ronidazole compared to conversion of 0.8% of the dose to $^{14}CO_2$ when $^{14}CH_3$-labeled ronidazole is dosed indicates a predominance of the reductive pathway. In fact, the oxidative pathway may be nonexistent as the $^{14}CO_2$ produced from the $^{14}CH_3$ label could also arise from the reductive pathway via methylamine.

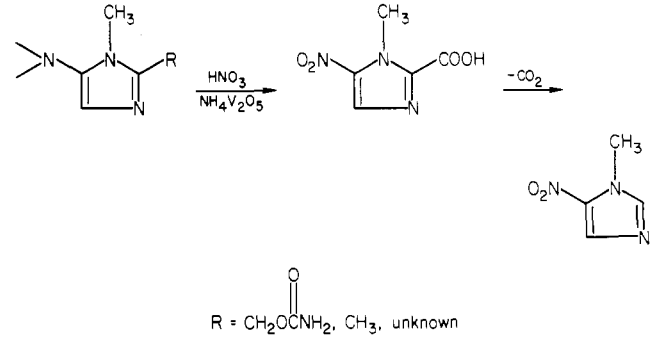
Persistent residues derived from single carbon metabolism do not represent a toxicity hazard. It is, therefore, important to ascertain if such residues account for the total persistent residues present in the pig tissue. Quantitative determinations of the specific substances which result from one-carbon metabolism seems fruitless. A few days after dosing it is unlikely that most of the residue would be present in only a few substances. Even if a substantial fraction of the total residues were due to a few compounds, it is unlikely that these would account for all of the radioactive residues and the radioactivity could be due to a number of diverse substances, each present in small quantity. Thus, determination of known metabolites would probably never account for all of the residue. All of the effort would have been expended determining substances known to be of no toxicological concern and nothing would be known concerning metabolites containing residual chemical features of the drug.

A much more fruitful approach would be to determine the fraction of the residue which is due to substances other than endogenous metabolites. Two such methods have been studied. The first is designed to include all derivatives of ronidazole derived from either reductive or oxidative metabolism which retain the N-CH_3 group. Unfortunately, it may include certain endogenous metabolites as well.

The second method is more specific and indicates only residues containing a 1-methyl-5-nitroimidazole moiety or substances converted to this moiety under the assay conditions. This analysis was carried out using an isotope dilution procedure. The chemical reaction is shown in Scheme I.

Radioactive methylamine was initially identified by ion-exchange chromatography of the strong acid hydrolysis products derived from tissue samples using ninhydrin

Scheme I



detection. Addition of [*methyl*-^{14}C]ronidazole to tissues of unmedicated animals and analysis of the hydrolysate by the isotope method showed that the yield of $^{14}CH_3NH_2$ was nearly quantitative. Lower yields, about 50%, were obtained in the absence of tissue. In our laboratory high yields of methylamine were also obtained from N-$^{14}CH_3$-labeled 1,2-dimethyl-5-nitroimidazole under the same conditions. Thus, the hydrolysis does not require a reactive group in the 2-position of the nitroimidazole. From these observations it seems likely that any *N*-methylimidazole would liberate methylamine under the assay conditions. Thus, all conceivable metabolite products which have not been degraded to a single carbon fragment would yield radioactive methylamine [including the unique fecal metabolite, 2,3-dihydro-2-(2-hydroxypropyl)-3-methyl-4-nitro-1*H*-imidazol-5-ol, recently isolated from ipronidazole by Weiss et al. (1981)].

From the methylamine liberation analyses in Table V it can be seen that for on-drug samples, about 90% of the radioactivity present in muscle and 70% of that in liver liberates radioactive methylamine. Three days after the last dose less than 30% of the muscle radioactivity liberated methylamine. At this time most of the methylamine-liberating residues are present in the protein fraction. As only about 8–10% of the total radioactivity present in the on-drug sample remains after 3 days and there is little change at 7 days after the last dose, it is clear that most of the methylamine-generating substances have been eliminated within 3 days after the last dose. From the interval 14 to 42 days after the last dose the fraction of radioactive residues which liberate methylamine diminishes for both liver and muscle. For both tissues the ratio of non-methylamine-liberating residues to methylamine-liberating residues changes from about 2 to over 5. Thus, the chemical analysis indicates that two distinct residue types are present, i.e., those residues which liberate methylamine and those which do not. The residue types are also different biochemically as indicated by differing rates of elimination.

From these data it is reasonable to conclude that the 70–80% of the residue which does not liberate methylamine represents endogenous substances. It is not clear, however, that the methylamine-liberating residues are necessarily "drug related". It is known that formaldehyde is converted to the methyl group of methionine and that 5-adenosylmethionine contributes the methyl group found in a variety of *N*-methyl-containing endogenous substances. Some of these substances, such as the *N*-methylarginines (Brostoff et al., 1972) methylated purines and the like, liberate methylamine on hydrolysis. In addition, there may be many other, as yet unidentified, natural substances which contain labile N-CH_3 groups derived from methionine. Thus, the liberated methylamine represents an upper limit of substances retaining some chemical structural features of the drug.

Isotope dilution methodology was used to determine if the tissue residues contained a 1-methyl-5-nitroimidazole nucleus. Previous work in our laboratory had shown that the imidazole nucleus is resistant to oxidation by nitric acid (Baer et al., 1977). Studies with ronidazole showed that a high yield of 1-methyl-5-nitroimidazole resulted from the oxidation of ronidazole with nitric acid in the presence of ammonium vanadate catalyst. Hence, if the tissue residues contained the nitroimidazole functionality of ronidazole with a substituent on the 2-position, the residue should yield 1-methyl-5-nitroimidazole on similar treatment.

Studies with muscle samples showed that about 1% of the tissue residues are converted to 1-methyl-5-nitroimidazole on oxidation with nitric acid. By this analysis only a small fraction of the residue retains the biologically important structural features of the drug.

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Outdoor Dissipation of the Experimental Acaricide 2-Naphthylmethyl Cyclopropanecarboxylate on Apple Trees: Formation of Lipophilic Metabolites

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Fruit and foliage of an apple tree (Golden Delicious) were treated with the experimental acaricide 2-naphthylmethyl cyclopropanecarboxylate (Ro 12-0470; ^{14}C labeled) and samples analyzed over a period of 7 days. In apples the recovery of radioactivity was high throughout (88–96%) but on leaves fell from 89.5% to 28.5% during the trial period. The nature of the radioactivity washed from the surface of both commodities and extracted from the surface-washed commodities was investigated. The results indicate a half-life for Ro 12-0470 of ca. 12 h on apples and less than 6 h on foliage. Similar results were found in a trial with nonradioactive material under field conditions. Four lipophilic metabolites isolated from apples in the radioactive trial were esters of 2-naphthalenemethanol with naturally occurring C_{16}, C_{18}, C_{20}, and C_{22} saturated fatty acids. This therefore represents a further example of the rather unusual phenomenon of the formation of highly lipophilic metabolites between a xenobiotic alcohol and naturally occurring fatty acids.

The conversion of xenobiotic compounds into lipophilic metabolites in plants and mammals is a rather unusual phenomenon. In general, xenobiotics are modified by oxidation and/or conjugation, giving rise to more polar or water-soluble metabolites which are more readily excreted from the organism. Highly lipophilic metabolites have been formed between xenobiotic alcohols and naturally occurring fatty acids in mammals (Leighty et al., 1976, 1980). In addition, there are also reports of xenobiotic acids being converted to lipophilic metabolites (triglycerides) after esterification with endogenous alcohols (Quistad et al., 1976; Fears et al., 1978; Schooley et al., 1978; Crayford and Hutson, 1980). In their studies of the metabolism of the acaricide hexadecyl cyclopropanecarboxylate in mammals and plants (Schooley et al., 1978), the compound with a ^{14}C radiolabel in the carboxyl group was used. The fate of the cyclopropanecarboxylic acid moiety could thus be investigated in detail. As part of our studies of the environmental behavior of the experimental acaricide 2-naphthylmethyl cyclopropanecarboxylate (Ro 12-0470), the fate of the compound in apple fruits and foliage under outdoor conditions was investigated. The compound was available with a ^{14}C radiolabel in the naphthylmethyl group, and thus the fate of the 2-naphthylmethanol moiety could be elucidated.

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EXPERIMENTAL SECTION

Liquid Scintillation Counting (LSC). Triplicate aliquots of the sample solution were counted in 10 mL of Insta-Gel scintillation cocktail (Packard, Ltd., Zurich). The samples were counted twice in a Packard Model 3375 liquid scintillation spectrometer and the counts averaged; the automatic external standard ratio method was used to determine counting efficiency.

Thin-Layer Chromatography. Precoated Alox E (alumina) and Kieselgel 60 F 254 (silica) thin-layer chromatography (TLC) plates were obtained from E. Merck (Darmstadt, GFR). A Model LB 2723 radio-TLC scanner

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