Metabolism and Distribution of [2,3-^{14}C]Acrolein in Lactating Goats

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The metabolism and distribution of [2,3-^{14}C]acrolein were studied in a lactating goat orally administered 0.82 mg/kg of body weight/day for 5 days. Milk, urine, feces, and expired air were collected. The goat was killed 12 h after the last dose, and edible tissues were collected. The nature of the radioactive residues was determined in milk and tissues. All of the identified metabolites were the result of the incorporation of acrolein into the normal, natural products of intermediary metabolism. There was evidence that the three-carbon unit of acrolein was incorporated intact into glucose, and subsequently lactose, and into glycerol. In the case of other natural products, the incorporation of radioactivity appeared to result from the metabolism of acrolein to smaller molecules followed by incorporation of these metabolites into the normal biosynthetic pathways.

Keywords: *Metabolism; acrolein; goat*

INTRODUCTION

Apart from being used extensively as a chemical intermediate, acrolein (propenal) has been utilized as an aquatic herbicide for some time (*1*−*4*). Because it is cytotoxic to plant tissue and decomposes rapidly in water (*2*, *5*, *6*), it is an effective herbicide that leaves virtually no residue that would be harmful to the environment (*7*−*9*). The objective of this study was to quantify and characterize the nature of the radioactive residues of acrolein in the milk and edible tissues of a lactating goat.

Although no complete study of acrolein metabolism in any species has been reported in the literature to date, some partial studies have been reported. Acrolein was shown to be metabolized to glycidaldehyde, glyceraldehyde, and acrylic acid in rat liver microsomes, although the metabolites were identified only by cochromatography (*10*). No other phase I metabolites of acrolein have been reported.

Although the acrolein-glutathione adduct, 3-oxopropyl-*S*-glutathione, also has not been isolated in vivo (*11*), mercapturic acids resulting from further metabolism of glutathione adducts have been reported for acrolein. 3-Hydroxypropylmercapturic acid [*S*-(3-hydroxypropyl)-*N*-acetylcysteine] has been reported to be present in the urine of rats treated with acrolein (*12*−*14*).

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Another mercapturic adduct that has been identified in the urine of acrolein-exposed rats is the carboxyethylmercapturic acid [*S*-(3-carboxyethyl)-*N*-acetylcysteine]. Draminski et al. (*15*) identified this metabolite by comparison of the gas chromatographic retention time and mass spectrum of the methyl ester to that of a synthetic standard. Linhart et al. (*14*) recently have described this metabolite in the urine of rats following both inhalation and intraperitoneal administration of acrolein.

We recently have reported an extensive study of the identification of the urinary and fecal metabolites of acrolein in Sprague−Dawley rats (*16*, *17*). To our knowledge, the current paper and the accompanying paper on the metabolism of acrolein in the laying hen (*18*) are the first reports of the metabolism of acrolein in livestock and poultry.

The demonstration of the metabolic incorporation of xenobiotics into natural products has been little discussed in the open literature other than the seminal work of Dorough and Ivie (*19*−*21*); however, demonstration of the disposition of nearly all the radioactivity is required for the regulatory approval of the use of agrochemicals. Additionally, studies of the incorporation of xenobiotic-derived radioactivity into products of normal intermediary metabolism are not readily accessible by literature searches because relevant techniques are scattered among studies of different compounds. We have reported the techniques used in our laboratory in detail in order that they might prove useful for others.

EXPERIMENTAL PROCEDURES

Test Materials. [2,3-^{14}C]Acrolein (specific activity = 7.1 mCi/mmol) was supplied as a solution in dimethylformamide with 0.25% hydroquinone as stabilizer with a stated radiochemical purity of >95% by Sigma Chemical Co., St. Louis, MO. The non-radiolabeled acrolein (stabilized with 0.25% hydroquinone) was used as supplied by Baker Petrolite Corp.

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Table 1. Total Recovery of Radioactivity in a Lactating Goat Administered [2,3-^{14}C]Acrolein (0.82 mg/kg of Body Weight/Day) by Gavage for Five Consecutive Days

	%
milk	22.9
urine	9.8
feces	14.9
tissues	8.0
gastrointestinal tract contents	6.6
carbon dioxide and organic volatiles	14.2
total	76.4

It contained 96.05% acrolein by UV analysis (the remainder was mostly water). The non-radiolabeled acrolein was dissolved in acetonitrile (ACN) containing 0.25% hydroquinone and mixed with the radiolabeled acrolein solution. Immediately before each dose, the required amounts of this solution were diluted in water to final ACN, dimethylformamide, and hydroquinone concentrations of 0.92, 0.08, and 0.0025%, respectively.

Animal Husbandry and Sample Collection. The facilities and staff of the Colorado State University Metabolic Laboratory were used for the housing and handling of the goat. The goat used was a 4-year-old nonpregnant lactating Nubian goat weighing 51.5 kg at dosing. The goat received 220 mL of the dose solution via a stomach tube on each of five consecutive days, resulting in a dose administered of 42.6 mg/day (0.82 mg/kg of body weight/day) and a final specific activity of 45745 dpm/μg acrolein. Doses were administered just after the morning milking and feeding.

Milk, urine, and feces were collected from the goat morning and evening and stored frozen until analysis. The goat was monitored for expired radioactivity by means of a modified indirect calorimetry chamber system. After the first dose of the second day, the goat was placed in a respiration chamber for 11 h. The air from the chamber was pumped sequentially through a flow meter to record total air flow, and then a scrub fraction (0.63%) was pumped through a second flow meter and then through two CO_2 scrubbers, each containing 1 M NaOH (200 mL). All samples were stored frozen at −20 °C until analysis.

The goat was killed by exsanguination after stunning with a captive bolt pistol, and blood, liver, kidneys, and samples of muscle and fat were collected. The contents of the gastrointestinal tract were collected also. All samples were immediately frozen on dry ice and were stored frozen at −20 °C until analysis.

Sample Preparation and Radioanalysis. Fluid samples were homogenized by mixing; muscle and fat were cut into pieces and ground in a meat grinder until homogeneous. Liver was ground in a meat grinder and then further homogenized in a food processor. Kidneys were homogenized in a food processor. Feces were mixed with deionized water and homogenized in a blender.

Milk, urine, and the CO_2 scubber solutions were assayed directly for total ^{14}C by liquid scintillation counting (LSC). All of the other matrices were oxidized, and the resulting CO_2 was analyzed by LSC. The values obtained were corrected for combustion efficiency using the values obtained from control matrices fortified with a ^{14}C reference solution.

The mass balance and tissue and milk concentrations of radioactivity are found in Tables 1−3.

High-Performance Liquid Chromatography (HPLC). HPLC analyses were performed with the following columns and solvent systems. Detection was by either ultraviolet (UV) absorbance or refractive index (RI) detection, as many of the standards used for comparison to the radioactive residues had no UV absorbance at wavelengths >210 nm. Radioactivity was detected either by using fraction collection followed by static LSC or by on-line detection using Radiomatic HPLC radioactivity detectors (Packard Instrument Co., Downers Grove, IL) and liquid scintillant.

System A (Reversed Phase): column, Phenomenex Spherex 5 μm C-18, 4.6 × 250 mm; temperature, ambient; gradient, 100% 5 mM KH_2PO_4, pH 3.0/ACN (95:5) to 100% ACN over 20 min, followed by 15 min at 100% ACN; detection, UV at 210 nm; flow rate, 0.8 mL/min; scintillant flow rate, 3.2 mL/min.

Table 2. Total ^{14}C Residues in Tissues of a Lactating Goat Administered [2,3-^{14}C]Acrolein (0.82 mg/kg of Body Weight/Day) by Gavage for Five Consecutive Days

	ppm[a]	% total dose administered
liver	9.14	3.6
kidney	1.71	0.1
muscle[b]	0.36	3.5
fatb	0.16	0.1
blood[b]	0.37	0.7

[a] Parts per million acrolein equivalents [b] The percentage of dose for muscle, blood, and fat is extrapolated using the following values for percentage of body weight: muscle, 40%; blood, 8%; fat, 2.5% (personal communication from Donald E. Johnson, Colorado StateUniversity Metabolic Laboratory).

Table 3. Total ^{14}C Residues in Whole Milk from a Lactating Goat Administered [2,3-^{14}C]Acrolein (0.82 mg/kg of Body Weight/Day) by Gavage for Five Consecutive Days

collection interval	ppm[a]	% total dose administered
day 1 p.m.	6.73	3.1
day 2 a.m.	1.67	1.1
day 2 p.m.	8.78	3.7
day 3 a.m.	3.39	2.1
day 3 p.m.	6.31	3.3
day 4 a.m.	1.92	0.7
day 4 p.m.	9.41	4.0
day 5 a.m.	1.44	0.9
day 5 p.m.	7.48	4.0
total		22.9

[a] Parts per million acrolein equivalents.

System B (Anion Exchange): column, Supelco Supelcogel C610H-SP anion exchange, 7.8 × 300 mm; temperature, 60 °C; mobile phase, isocratic 0.1% phosphoric acid; detection, RI or UV at 210 nm; flow rate, 0.5 mL/min; scintillant flow rate, 2.0 mL/min.

System C (Ion Exclusion): column, Phenomenex ORH-801 organic acids column, 6.5 × 300 mm; temperature, 35 °C; mobile phase, isocratic 1 mM sulfuric acid; detection, RI or UV at 210 nm; flow rate, 0.8 mL/min; scintillant flow rate, 3.2 mL/min.

System D (Reversed Phase): column, Phenomenex Spherex 5 μm NH_2, 4.6 × 250 mm; temperature, ambient; gradient, (A) 10 mM KH_2PO_4, pH 4.3; (B) ACN/water (88:12); (C) 50 mM KH_2PO_4, pH 4.3; 5:95 A/B for 5 min to 30:70 A/B at 20 min to 50:50 A/B at 27 min, followed by 23 min at 50:50 A/B, then to 50:50 A/C at 60 min to 10:90 A/C at 70 min; detection, UV at 210 nm; flow rate, 0.8 mL/min; scintillant flow rate, 3.2 mL/min.

System E (Reversed Phase): column, Alltech Nucleosil C_{18} 5 μm, 4.6 × 250 mm; temperature, 52 °C; gradient, (A) 50 mM ammonium acetate, pH 6.8/ACN (95:5); (B) 100 mM ammonium acetate, pH 6.8/methanol/ACN (46:10:44); 100% A to 85:15 A/B at 15 min to 50:50 A/B at 30 min to 100% B at 1834 min followed by 11 min at 100% B; detection, UV at 254 nm; flow rate, 1.0 mL/min; scintillant flow rate, 3.2 mL/min.

System F (Reversed Phase): column, Phenomenex Spherex 5 μm C-18, 4.6 × 250 mm; temperatue, 45 °C; gradient, isocratic ACN/2-propanol (50:50); detection, UV at 210 nm; flow rate, 0.5 mL/min; scintillant flow rate, 2.0 mL/min.

Lipid Fractionation and Fatty Acid Analysis. Lipid-containing extracts were fractionated on an aminopropyl bonded silica gel column (30−70 μm, 60 Å, Alltech Associates, Deerfield, IL). The column was eluted sequentially with 2-propanol/$CHCl_3$ (1:2), 2% acetic acid in ether, methanol, and $CHCl_3$/methanol/0.8 M sodium acetate (60:30:45) to deliver neutral lipid, fatty acid, other lipid, and phospholipid fractions, respectively (*22*).

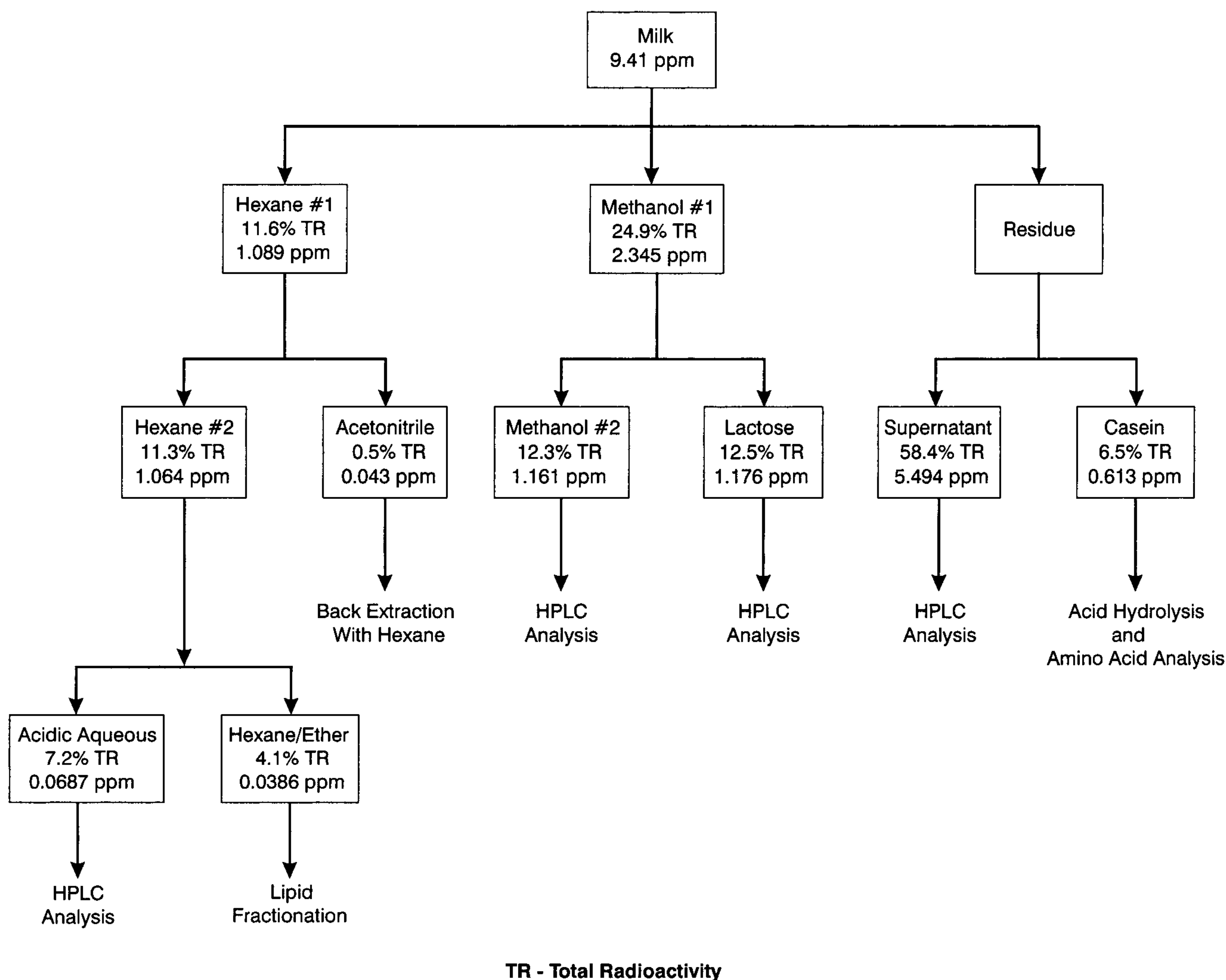

Figure 1. Fractionation scheme for milk.

Free fatty acid fractions were derivatized with phenacyl bromide and analyzed by HPLC using a modification of the method of Hanis et al. (*23*).

Amino Acid Analysis. Amino acids were derivatized with phenyl isothiocyanate using the method of Heinrickson and Meredith (*24*). The phenylthiocarbamoyl amino acids were resolved on HPLC system E.

Liquid Chromatography—Mass Spectrometry (LC-MS). Mass spectra were obtained in either negative or positive electrospray mode on a Fisons model VG-Quattro triple quadrapole mass spectrometer (Fisons Instruments, Altrincham, U.K.). LC-MS used either direct flow injection or a 4.6 mm × 250 mm Spherex 5 μm C-18 column at a flow rate of 0.5 mL/min with both using a mobile phase consisting of a 1:1 mixture of deionized water adjusted to pH 3 and methanol.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Proton and ^{13}C broad band decoupled and proton coupled NMR analysis was performed with a Bruker model AM500 instrument at the National Magnetic Resonance Laboratory, University of Wisconsin—Madison.

Thin-Layer Chromatography (TLC). The extracts were applied to a 0.25 mm thickness, 20 cm × 20 cm Merck Kieselgel 60 F254 glass plate. Plates were developed to a height of 16 cm in a preequilibrated tank using *n*-butanol/acetic acid/water (4:2:1). Following development, the plates were allowed to dry and then analyzed for radioactivity on an Ambis Radioanalytical Imaging System.

Milk and Tissue Extraction and Fractionation Procedures for Metabolite Identification. Milk (day 4 p.m. collection, 41 mL) was lyophilized, and the residue was extracted sequentially with hexane (3 × 60 mL) and methanol (6 × 60 mL). A flowchart outlining the fractionation scheme is found in Figure 1. The hexane (hexane 1), which contained 11.6% of the milk radioactivity, was partitioned with hexane-saturated acetonitrile (2 × 200 mL), a portion (40 mL) of the resulting hexane fraction (11.3% of the milk radioactivity) was evaporated to dryness under reduced pressure, and the residue saponified at reflux in 3 M KOH (30 mL) overnight. The mixture was acidified to pH 2 with concentrated HCl and extracted with hexane/ether 1:1 (3 × 250 mL). The hexane/ether fraction (4.1% of the milk radioactivity) was evaporated to dryness, dissolved in chloroform, and fractionated as described under Lipid Fractionation and Fatty Acid Analysis to obtain a free fatty acid fraction and another nonpolar lipid fraction (3.7 and 0.05% of the milk radioactivity, respectively). The free fatty acid fraction so obtained was derivatized and analyzed. The HPLC chromatograms indicated the presence of radioactivity in caprylic, capric, lauric, myristic, and palmitic acids, as well as some minor fatty acids that did not correlate with any standards. The ACN fraction (0.5% of the milk radioactivity) was back extracted with hexane to give hexane and ACN fractions containing 0.37 and 0.02% of the radioactivity in milk, respectively. The hexane was saponified to give an aqueous (0.21% of the milk radioactivity) and a fatty acid fraction (0.12% of the milk radioactivity), indicating that this fraction also contained triglycerides.

The aqueous fraction from the saponification (7.2% of the milk radioactivity) was lyophilized, and the resulting solids were extracted with absolute ethanol (2 × 50 mL). The ethanol was evaporated to dryness and the residue reconstituted in deionized water for analysis in HPLC systems B and C. The

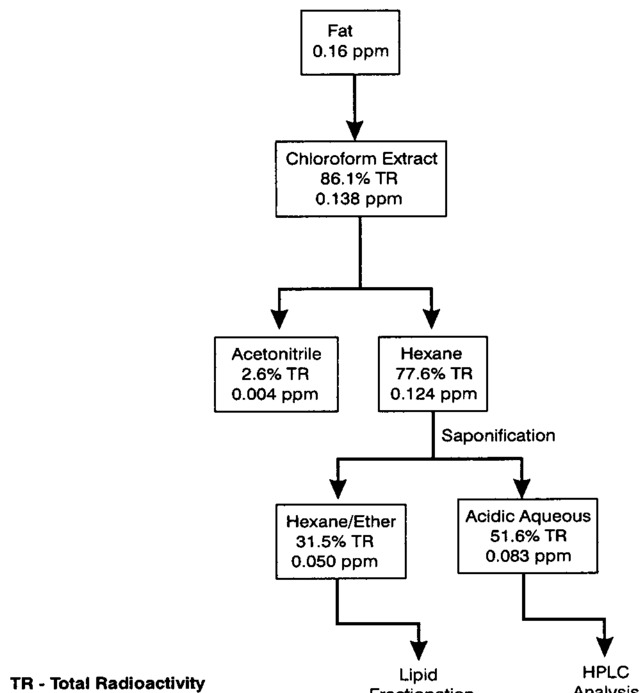

Figure 2. Fractionation scheme for fat.

HPLC chromatograms indicated the presence of glycerol (6.4% of the milk radioactivity).

The methanol fractions (24.9% of the milk radioactivity) were concentrated under reduced pressure and stored in a freezer at −20 °C to allow the crystallization of lactose. After filtration, the mother liquor was further concentrated and cooled to isolate additional lactose. The lactose was recrystallized from water/ethanol to a constant specific activity of 7.15 $\times$ 10^6 dpm/g. The lactose did not depress the melting point of standard lactose upon admixture. The mother liquor was evaporated to dryness, reconstituted in water, and analyzed on HPLC systems B and C. A peak corresponding to lactose was the only radioactive peak observed. TLC analysis also confirmed the presence of lactose.

The residual solids remaining after the hexane and methanol extraction were dissolved in 0.2 M NaOH (40 mL). Phosphoric acid was added with stirring to pH 4.5 to precipitate a casein/protein fraction (6.5% of the milk radioactivity). The solution−precipitation steps were repeated until a constant specific activity of 8.52 $\times$ 10^5 dpm/g was achieved. A portion of the supernatant from the first precipitation (58.4% of the milk radioactivity) was lyophilized, dissolved in a minimum volume of water, and analyzed on HPLC systems B and C. The only radioactive peak coeluted with lactose.

The purified casein fraction was hydrolyzed with 6 M HCl at 110 °C for 24 h. The sample was repeatedly dissolved in water and evaporated under reduced pressure to remove residual HCl and then dissolved in a minimum volume of water and purified on a cation exchange column (Bio-Rad AG 50W-X8, 200−400 mesh, 4.5 $\times$ 10 cm) in its hydrogen form by eluting sequentially with water (500 mL) and 1 M NH_4OH (500 mL). LSC indicated that the radioactivity was in the NH_4OH fraction. This was concentrated under reduced pressure to a minimum volume, and the pH was adjusted to 1.5 with 1 M HCl. The column was then converted to its ammonium form, the radioactivity reapplied, and the column eluted sequentially with deionized water (500 mL) and 1 M NH_4OH (500 mL). The two eluates were collected and lyophilized, and the phenyl thiocarbamoyl derivatives were formed and analyzed by HPLC in system E. Incorporation of radioactivity into amino acids was seen with alanine (1.3% of the milk radioactivity) and glutamate (0.9% of the milk radioactivity).

Fat. Fat homogenate was extracted with chloroform (Figure 2 shows the fractionation scheme for fat.) The chloroform fraction was evaporated to dryness and dissolved in hexane, and the hexane was partitioned with ACN. The resultant hexane and ACN extracts contained 77.6 and 2.6% of the radioactivity in fat, respectively. The hexane fraction was evaporated to dryness under reduced pressure and the residue saponified with 3 M KOH. The saponified sample was acidified to pH 1.5 with concentrated HCl and extracted with hexane/ether (1:1, 3 $\times$ 250 mL). The resulting acidic aqueous and hexane/ether fractions contained 51.6 and 31.5% of the radioactivity in fat, respectively. Approximately one-third of this extract was evaporated to dryness and fractionated as outlined under Lipid Fractionation and Fatty Acid Analysis, which separated it into fatty acids (37.2% of the fat radioactivity) and other lipids (0.8% of the fat radioactivity). The analysis of the individual fatty acids by radiochemical HPLC was not possible due to a specific activity of only 3 dpm/mg.

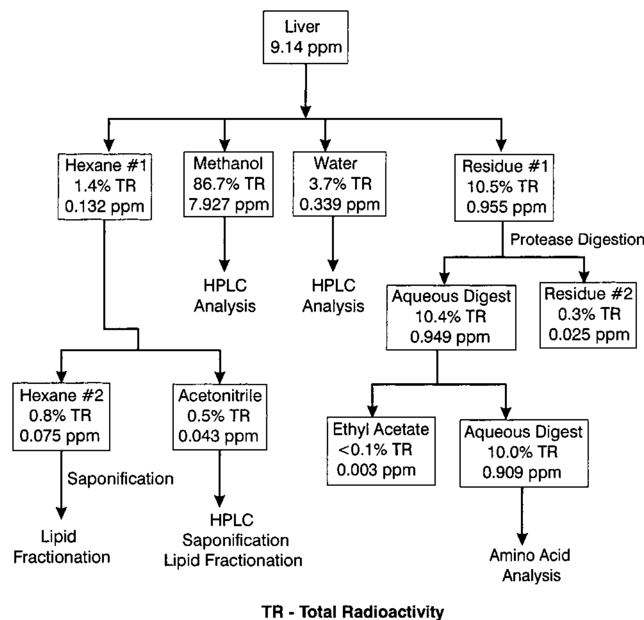

Figure 3. Fractionation scheme for liver.

The acidic aqueous fraction was lypophilized and the residue extracted with absolute ethanol. The residue and the ethanol contained 5.6 and 46.0% of the fat radioactivity, respectively. The ethanol fraction was dissolved in water and analyzed by HPLC on systems B and C. Glycerol (42.0% of the fat radioactivity) was the predominant radioactive peak in the chromatograms.

Liver. Liver homogenate was extracted sequentially with hexane, methanol, and water (3 $\times$ 100 mL each) using a probe-type homogenizer, and the supernatants for each solvent extraction were combined. See Figure 3 for the fractionation scheme for liver. The hexane, methanol, water, and residue contained 1.4, 86.7, 3.7, and 10.5% of the liver radioactivity, respectively. Portions of the methanol and water extracts were evaporated under reduced pressure, reconstituted in water, and analyzed in HPLC systems A−D. The majority of the radioactivity was incorporated into glucose (a total of 56.7% of the liver radioactivity in the methanol and water extracts combined). Another 11.3% of the liver radioactivity had the same retention times as glucose-1- and 6-phosphates and glycogen, which were not resolved in these systems. To confirm these metabolites, a portion of the water extract was hydrolyzed with 3 M HCl and analyzed in HPLC system B. The peak at the retention time of glycogen and glucose-1- and 6-phosphates disappeared, and the glucose peak increased, indicating that the radioactivity was hydrolyzable to glucose. Lactic acid (3.6% of the liver radioactivity) also was found in the methanol and water extracts of the liver.

The hexane extract was concentrated to ∼100 mL and then partitioned against hexane-saturated ACN (3 $\times$ 100 mL). The hexane (0.8% of the liver radioactivity) was saponified and worked up as described under Lipid Fractionation and Fatty

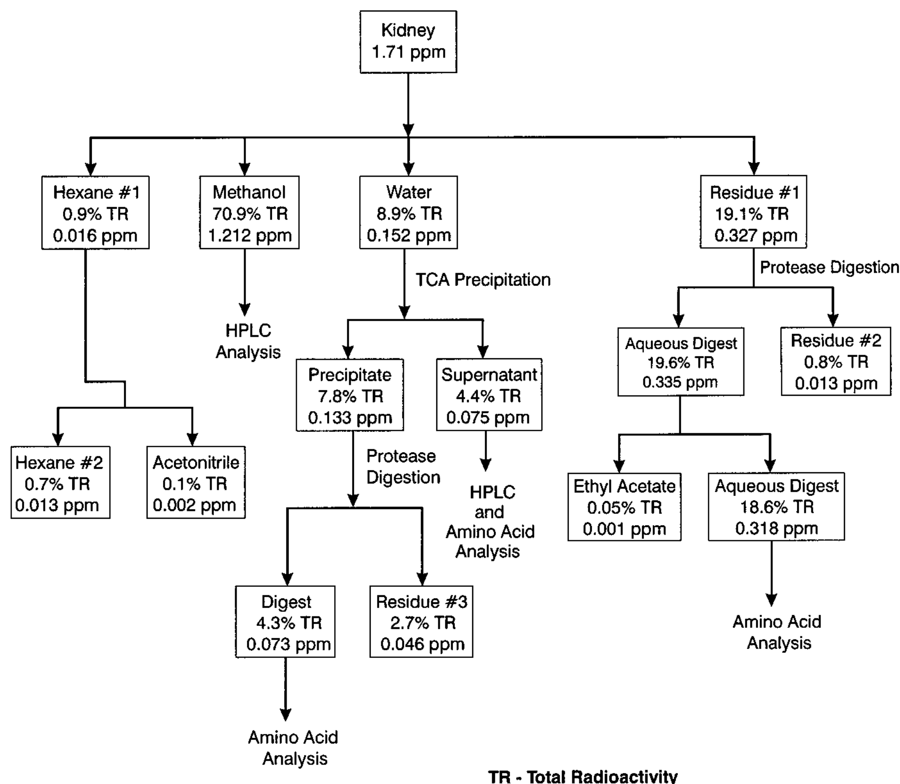

Figure 4. Fractionation scheme for kidney.

Acid Analysis, and the resultant hexane/ether fraction separated as described above. Radioactivity was found in the nonpolar neutral lipids, fatty acids, and neutral phospholipids and glycolipids fractions. The fatty acid fraction was derivatized and analyzed as described above.

The acetonitrile fraction (0.5% of the liver radioactivity) was evaporated to dryness under reduced pressure, dissolved in ACN/2-propanol (1:1), and analyzed using HPLC system F. Because it appeared to consist of triglycerides, it was saponified and extracted as described before to give a hexane/ether and acidic aqueous fraction.

The major metabolite in the methanol fraction was purified by evaporating a portion of the methanol extract to dryness, dissolving in water, and chromatographing the extract on a Sephadex P-200 gel permeation column (2.5 cm × 15 cm) eluted with water. The radioactive fractions were pooled and purified by repeated injections on HPLC system C and then further purified using the anion exchange column in HPLC system B using 0.5% formic acid as the mobile phase. The material was then analyzed by mass spectrometry and NMR and determined to be glucose by comparison with the spectra from authentic glucose.

A second portion of the methanol extract was evaporated to dryness, reconstituted in water, and eluted through a C_{18} Bond Elut solid-phase extraction cartridge. The filtrate was derivatized with pentafluorophenylhydrazine, purified by reversed phase HPLC, and analyzed by mass spectrometry. It had an $(M - H)^-$ of 359 in negative electrospray mode and an $(M + H)^+$ of 361 in positive electrospray mode consistent with the pentafluorophenyl hydrazone of glucose.

The residue after extraction was digested with a protease (type XIV, Sigma Chemical Co., St. Louis, MO) dissolved in Tris-HCl buffer, pH 7.0, in a shaking water bath at 37 °C overnight. Following the protease digestion the sample was centrifuged, the precipitate was washed with deionized water (5 mL) and centrifuged again, and the supernatants were combined. The supernatant was extracted with ethyl acetate (2 × 150 mL), which contained 0.03% of the liver radioactivity. The digest was purified by cation exchange chromatography in a similar manner as the casein hydrolysate. The aqueous eluate from the column in the hydrogen form and both the aqueous and ammonium hydroxide eluates from the column in the ammonium form were derivatized and analyzed for amino acids as described above. Incorporation of radioactivity into amino acids was observed with the greatest incorporation in glutamine and glycine (1.3% and 1.1% of the liver radioactivity, respectively).

Kidney. Kidney was extracted with hexane, methanol, and water in the same manner as the liver. See Figure 4 for the kidney fractionation scheme. The hexane, methanol, and water extracts contained 0.9, 70.9, and 8.9% of the kidney radioactivity, respectively. The residue (19.1% of the kidney radioactivity) was digested with protease as described for the liver. The hexane was partitioned with ACN into a hexane fraction and an ACN fraction, which contained 0.7 and 0.1% of the tissue radioactivity, respectively. The methanol extract was lyophilized to dryness, reconstituted in water, and injected on HPLC systems A−E (after derivatization with phenyl isothiocyanate as described above for system E). The amino acids that contained the largest amounts of radioactivity were aspartate and glutamate (16.9 and 20.5% of the kidney radioactivity, respectively). Radioactivity was also incorporated into hydantoin/allantoin, creatinine, creatine, and uric acid. They contained 4.5, 2.3, 3.9, and 2.2% of the kidney radioactivity, respectively. Another portion of the water extract was precipitated with trichloroacetic acid. The precipitate contained 7.8% of the kidney radioactivity. The supernatant (4.4% of the kidney radioactivity) was extracted with ether. No radioactivity was found in the ether. The aqueous fraction was analyzed on HPLC system D and then derivatized and injected on HPLC system E. Incorporation into amino acids was observed. The trichloroacetic acid precipitate was digested with protease. The digest contained 4.3% of the kidney radioactivity, whereas the insoluble residue contained 2.7% of the kidney radioactivity. The supernatant was derivatized and analyzed on HPLC system E. The protease digest of the original residue (19.6% of the kidney radioactivity) was purified by cation exchange chromatography and analyzed for amino acids as described above for the casein hydrolysate. Both of the protease digests showed incorporation into amino acids.

Muscle. Muscle was extracted with hexane, methanol, and water in the same manner as the liver. (See Figure 5 for a

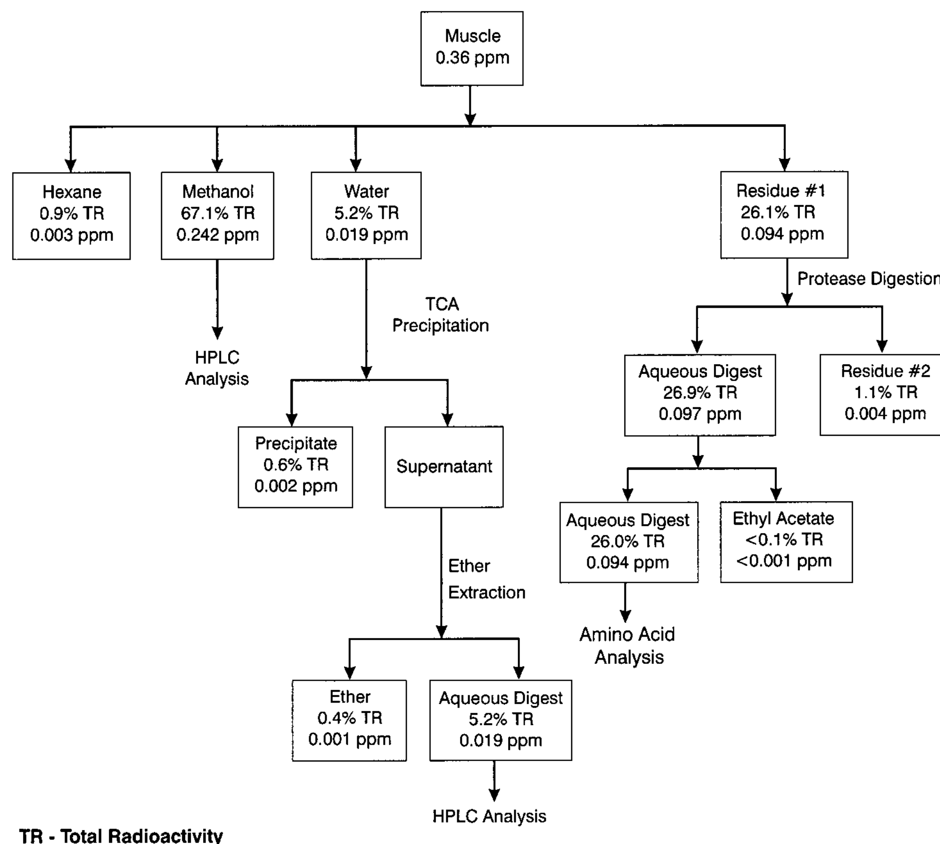

Figure 5. Fractionation scheme for muscle.

Table 4. Summary of the Metabolites Identified in the Milk and Tissues of a Lactating Goat Administered [^{14}C]Acrolein (0.82 mg/kg of Body Weight) by Gavage for Five Consecutive Days[a,b]

	milk	fat	liver	kidney	muscle
glucose			56.7% 5.186 ppm		
metabolites hydrolyzable to glucose			11.3% 1.034 ppm		
amino acids	4.8% 0.450 ppm		4.3% 0.392 ppm	60.4% 1.034 ppm	19.8% 0.070 ppm
fatty acids	3.0% 0.285 ppm	37.2% 0.060 ppm	0.12% 0.011 ppm		
lactate			3.6% 0.329 ppm		39.3% 0.142 ppm
lactose	79:3% 7.461 ppm				
glycerol	6.4% 0.598 ppm	42.0% 0.067 ppm	0.21% 0.019 ppm		
glyceric acid					2.9% 0.011 ppm
oxalic acid					0.22% 0.001 ppm
creatinine				2.3% 0.039 ppm	
creatine				3.9% 0.066 ppm	13.5% 0.048 ppm
uric acid				2.2% 0.037 ppm	
hydantoin/allantoin				4.5% 0.076 ppm	

[a] Percentages in the table represent the percentage of radioactivity in tissue or milk. [b] Parts per million values in the table represent acrolein equivalents.

flowchart.) The hexane, methanol, and water extracts contained 0.9, 67.1, and 5.2% of the muscle radioactivity, respectively. The residue (26.1% of the muscle radioactivity) was digested with protease as described for the liver. The methanol extract was concentrated under reduced pressure and analyzed by HPLC on systems A–D. The major metabolite was lactic acid (36.6% of the muscle radioactivity). Radioactivity was also observed in glyceric acid, arginine, alanine, and creatine (1.9, 0.2, 6.0, and 12.3% of the muscle radioactivity, respectively). A portion of the water extract was lyophilized to a small volume and precipitated with trichloroacetic acid. The precipitate contained 0.6% of the muscle radioactivity. The supernatant was extracted with ether to give 0.4% of the muscle radioactivity in the ether. The aqueous fraction, which contained 5.2% of the muscle radioactivity, was analyzed by HPLC in systems B–D. It contained the same metabolites as

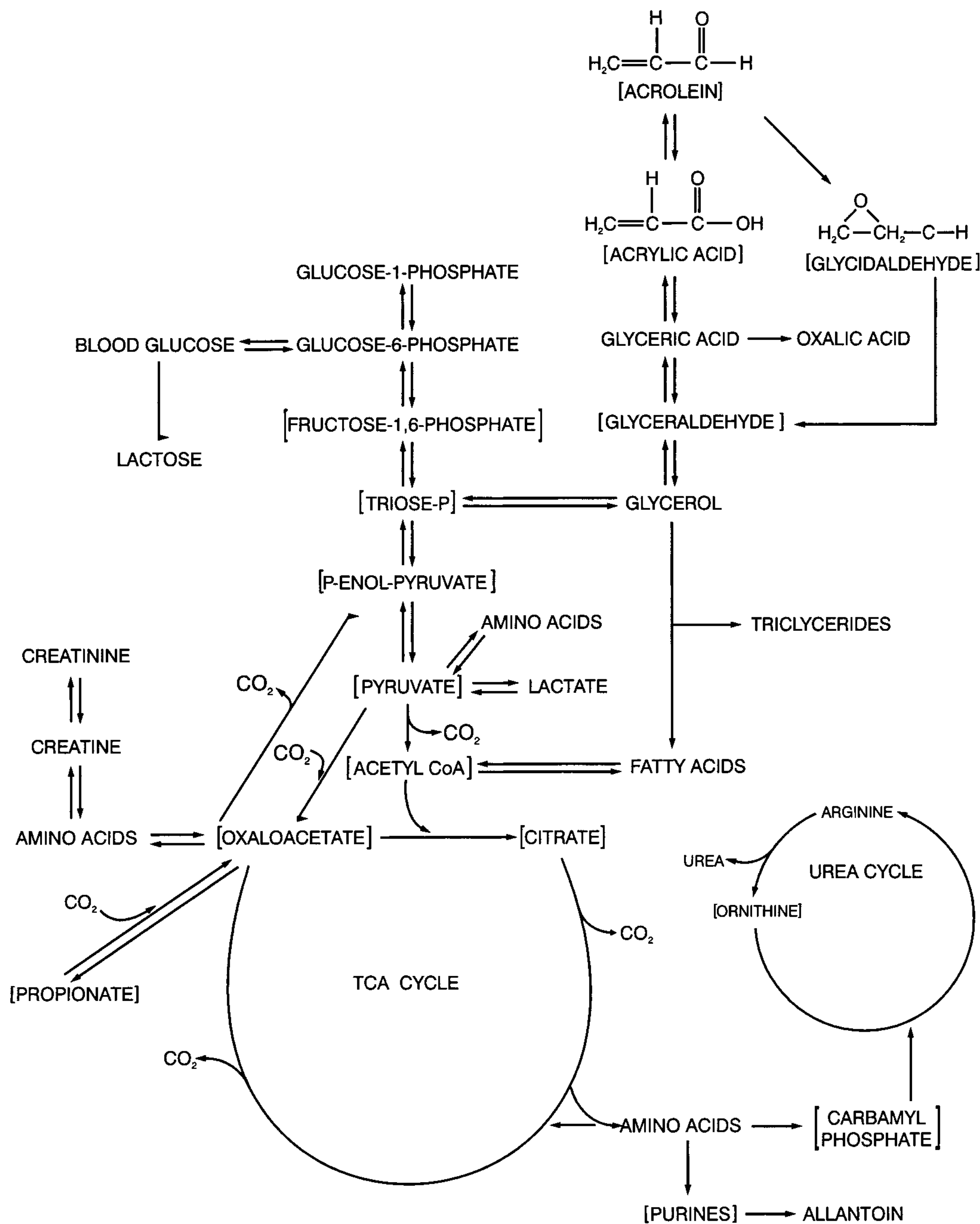

Figure 6. Proposed pathway for metabolism of acrolein in the goat.

seen in the methanol extract, with the exception of arginine, and the addition of a small amount of oxalic acid (0.2% of the muscle radioactivity). The protease digest was purified by ion exchange chromatography and analyzed for amino acids as described for the casein hydrolysate. Incorporation of radioactivity into several amino acids was observed. The amino acids that contained the most radioactivity were glutamate and glycine (4.3 and 3.2% of the kidney radioactivity, respectively).

RESULTS AND DISCUSSION

A summary of the incorporation of acrolein-derived radioactivity in the goat is found in Table 4.

Milk. Lactose was the primary radioactive component in milk, representing 79.3% of the milk radioactivity. Incorporation of radioactivity into fatty acids, amino acids, and glycerol represented 3.0, 4.8, and 6.4% of the milk radioactivity, respectively. The total accountability of radioactivity from all extracts, residues, and chromatograms was 104%. The fatty acids observed (capric, caprylic, lauric, myristic, and palmitic acids) are those found in the highest quantities in goat milk. Likewise, the amino acids (aspartate, glutamate, serine, glycine, alanine, methionine, cysteine, phenylalanine, and tyrosine) that have incorporated radioactivity are the same reported to be biosynthesized in rats, although this is not as clear-cut. Possibly bacterial biosynthesis in the ruminant digestive tract also produced some labeled amino acids. The greater incorporation of radioactivity into glycerol than into fatty acids on an absolute basis

(very much greater on a per carbon basis) and the extensive incorporation into lactose suggests that the three-carbon unit of acrolein is being directly incorporated into glycerol and lactose. Ruminants are known to utilize three-carbon units in biosynthesis (*25*). A logical pathway for direct incorporation is epoxidation of acrolein to glycidaldehyde followed by hydration of the epoxide to give glyceraldehyde, which can then be either reduced to form glycerol or used in glucose biosynthesis, ultimately forming lactose. This oxidation of acrolein to glyceraldehyde has been reported in rat liver microsomes (*10*). Alternatively, acrolein can be metabolized to CO_2, which can be incorporated by biosynthesis into the fatty acids.

Fat. Radioactivity in fat was found after saponification as glycerol and fatty acids, which represented 42.0 and 37.2% of the fat radioactivity, respectively. The specific activity of the fatty acids was too low to permit identification of incorporation into individual fatty acids. The total accountability of radioactivity from all extracts, residues, and chromatograms was 91.6%.

Liver. Glucose was the predominant radioactive metabolite in the liver, representing 56.7% of the liver radioactivity. An additional 11.3% of the liver radioactivity consisted of metabolites that could be hydrolyzed to glucose for a total of 68.0% of the liver radioactivity as glucose or hydrolyzable to glucose. Again, the relatively high incorporation into glucose is suggestive of the direct incorporation of the three-carbon unit of acrolein. Radioactivity also was incorporated into amino acids and lactic acid (4.3 and 3.6% of the liver radioactivity, respectively). The large amount of lactic acid was probably due to the anaerobic metabolism of glucose during the time required to harvest the tissues after sacrifice. Lesser amounts of radioactivity were incorporated into glycerol and fatty acids (0.2 and 0.1% of the liver radioactivity, respectively). The total accountability of radioactivity from all extracts, residues, and chromatograms was 90.4%.

Muscle. The muscle metabolite containing the greatest amount of radioactivity was lactic acid, which represented 39.3% of the muscle radioactivity. As mentioned with liver, the presence of lactate is probably due to anaerobic metabolism during tissue collection. Other important radioactive metabolites were amino acids and creatine (19.8 and 13.5% of the muscle radioactivity, respectively). Minor metabolites seen were glyceric and oxalic acids (2.9 and 0.2% of the muscle radioactivity, respectively). The total accountability of radioactivity from all extracts, residues, and chromatograms was 94.7%.

Kidney. The largest percentage of radioactivity in the kidney was incorporated into amino acids; they represented 60.4% of the kidney radioactivity. Other metabolites identified were hydantoin/allantoin, creatinine, creatine, and uric acid. They represented 4.5, 2.3, 3.9, and 2.2% of the kidney radioactivity, respectively. The total accountability of radioactivity from all extracts, residues, and chromatograms was 92.6%.

Conclusions. A proposed metabolic pathway for acrolein is outlined in Figure 6. All of the identified metabolites found in lactating goats after the administration of acrolein were the result of the incorporation of acrolein into the normal, natural products of intermediary metabolism. There was evidence that the three-carbon unit of acrolein was incorporated intact into glucose, and subsequently lactose, and into glycerol. In the case of other natural products, the incorporation of radioactivity appeared to result from the metabolism of acrolein to smaller molecules followed by incorporation of these metabolites into the normal biosynthetic pathways.

SAFETY

Acrolein is toxic and a powerful irritant, and neat acrolein should be handled in an efficient hood. The usual precautions for handling radioactive materials and disposal of radioactive waste were followed.

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