COMMUNICATIONS

Metabolism of 2-Chloro-4, 6-bis(isopropylamino)-*s*-triazine (Propazine-^{14}C) in the Milk Goat and Sheep

Balance Study and Urinary Metabolite Separation

Propazine-^{14}C, ring-labeled [2-chloro-4,6-bis(isopropylamino)-*s*-triazine], was administered to lactating goats in doses of 8.8 and 90.9 mg. per kg. Milk, urine, and feces were assayed for radioactivity during the following 72 hours after dosing. Ion-exchange chromatography of the goat urine separated at least 16 metabolites. Less than 2% of the radioactivity in the urine consisted of Propazine. Propazine-^{14}C (isopropyl-labeled) was given to a sheep. Twenty-four per cent of the radioactivity was collected as $^{14}CO_2$ from the ^{14}C-isopropyl groups.

The metabolism of 2-chloro-4,6-bis(isopropylamino)-*s*-triazine (Propazine) has not been studied in ruminants. A study with 2-chloro-4,6-bis(ethylamino)-*s*-triazine (Simazine) in the dairy cow indicated that the urine contained 1% of the intact herbicide (St. John *et al.*, 1965). Simazine was not detected in the milk samples. Since the study was done with unlabeled Simazine, the metabolic pattern of the triazine was not determined. Bakke *et al.* (1967) studied the metabolism of ring-labeled Propazine-^{14}C and Prometone-^{14}C. Ion-exchange chromatography of the urine separated 18 radioactive fractions from Propazine and 11 metabolites from Prometone. Rats exhaled 50% of the radioactivity as $^{14}CO_2$ within 72 hours after a single oral dose of ^{14}C-isopropyl-labeled Propazine. No $^{14}CO_2$ was detected in expired air from rats receiving the ring-^{14}C-labeled compounds.

This study was designed to determine the extent of absorption from the gastrointestinal tract, the routes and rates of excretion, and the deposition in various tissues of the metabolites after a single oral dose of Propazine. Isolation and characterization of urinary metabolites were initiated.

MATERIALS AND METHODS

Chemicals. Propazine-^{14}C (ring- and side chain-labeled) used in this study, including synthesis, specific activity, and radiochemical purity, has been reported (Bakke *et al.*, 1967).

Apparatus and Design. The metabolism unit used to confine the animals for $^{14}CO_2$ and excreta collection has been described (Robbins and Bakke, 1967).

Radioanalysis. Methods for liquid scintillation counting of samples and their preparation, including combustion analyses, have been reported (Bakke *et al.*, 1967).

Ion-Exchange Chromatography of Urinary Metabolites. Methanol extracts of the freeze-dried urine were taken to dryness with a flash evaporator, redissolved in 200 ml. of distilled water, and placed on a column (46 × 2.2 cm.) of a strong cation exchange resin (AG 50W-X8, Calbiochem, Richmond, Calif.) in the hydrogen ion form. Four to 6% of the radioactivity was recovered from the column in the water wash. Five hundred milliliters of 2*N* NH_4OH was used to elute the remaining radioactivity. The eluate was taken to dryness with a flash evaporator and dissolved in 50 ml. of distilled water. The material was then placed on a strong anion exchange resin column (22 × 1.2 cm.; AG1-X2, Calbiochem, Richmond, Calif.) in the hydroxide ion form. Two hundred milliliters of water was used to wash the column. The water wash contained 7 to 10% of the radioactivity. Elution with 300 ml. of 1*N* HCl removed 90 to 95% of the radioactivity. The water and HCl washings were then combined and taken to dryness on a flash evaporator. A portion of the yellow pigments remained on the anion exchange column.

The precleaned material was then dissolved in 0.1*N* HCl for further ion-exchange chromatography, using the gradient elution technique, with the methodology and equipment used for amino acid analysis (Bakke *et al.*, 1967).

Chromatography of Fecal Extracts. Five grams of the freeze-dried feces from each goat was extracted with methanol. The methanol was reduced to dryness and the residue dissolved in 2 ml. of chloroform. One-milliliter portions of the chloroform were placed on a Florisil column (20 × 1.2 cm.) poured in chloroform. The radioactivity eluted with chloroform in approximately 2 volumes of the column. The radioactive material was then subjected to TLC, GLC, and infrared analysis according to Shimabukuro *et al.* (1966).

Animal Treatment. Two lactating goats weighing 57 and 55 kg. were used in this study (goat 51 and goat 52). During the experimental period (72 hours) they were placed in a metabolism crate, and a catheter was placed in the urinary bladder one hour prior to treatment. Feces, urine, and milk were collected at the time intervals shown in Table I.

Goat 51 and goat 52 were given 500 mg. (56.0 μc.; 8.8 mg. per kg.) and 5 grams (55.6 μc.; 90.9 mg. per kg.) of ring-labeled Propazine-^{14}C, respectively. The labeled and unlabeled amounts of the compound were weighed into gelatin capsules, and balling gun was used to administer the dose.

Goat 51 was placed in the CO_2 collection unit during 0 to

4 and 8 to 12 hours after the compound was administered.

Seventy-two hours after treatment, the animals were slaughtered and appropriate tissues collected.

A wether sheep weighing 45 kg. was dosed by capsule with 65 mg. (13.3 μc.) of 2-chloro-4,6-bis(isopropyl-2-^{14}C-amino)-*s*-triazine and placed in the CO_2 collection unit for 72 hours to determine the amount of expired $^{14}CO_2$.

RESULTS AND DISCUSSION

Elimination of radioactivity in the feces and urine is shown in Table I. Goat 51 excreted a total of 84.5% of the radioactive dose (41.5% in the feces and 43.0% in the urine). A total of 72.7% of the radioactive dose (33.8% in the feces and 38.9% in the urine) was excreted by goat 52. However, fecal excretion during the first two days was markedly different for the two animals, probably because of the rate of passage of the material through the gastrointestinal tract, since goat 52 did not eat the first day of the experiment. This is not unexpected in animal experiments. A log plot of the per cent of the dose not excreted in the urine against time was linear up to 48 hours. The actual amount of radioactivity (calculated on a Propazine equivalent basis) excreted in the urine in 24 hours was 590 mg. for goat 52 and 150 mg. for goat 51. These rates of excretion show that an animal has considerable ability to convert Propazine to water-soluble compounds which are rapidly excreted. This rapid rate of renal clearance of Propazine metabolites probably accounts for the low toxicity of the compound, even though a large amount of the compound is absorbed from the gastrointestinal tract (Radeleff, 1964).

Samples of urine from the two goats were partitioned with chloroform to determine if Propazine or hydroxypropazine was present. Less than 2% of the radioactivity was found to partition into chloroform at the highest periods of excretion of radioactivity.

Less than 2% of the chloroform extracts from the feces partitioned into water. The chloroform-soluble extract gave a single radioactive peak in TLC and GLC. Infrared spectra obtained by trapping of the radioactive effluent from the gas chromatograph were identical to infrared spectra of Propazine. Therefore, Propazine is principally excreted unchanged in the feces.

Tissue residues of radioactivity, 72 hours after treatment, are presented in Table II. These residues as Propazine-^{14}C equivalents averaged 0.88 p.p.m. for goat 51 and 17.37 p.p.m. for goat 52. Tissues lowest in radioactivity in goat 51 and goat 52 were blood, heart, and muscle. Since the radioactivity was rapidly excreted, tissues with abundant blood supply (liver, brain, and kidney) would be expected to contain the higher levels of radioactivity. No radioactivity was detectable in the fat samples.

Propazine-^{14}C equivalents appearing in the milk are given in Table I. Milk from goat 51 reached a maximum of metabolite content (1.5 p.p.m.) in the 8- to 16-hour sample and declined to 0.17 p.p.m. in the 64- to 72-hour sample, while a rather steady excretion occurred in the milk of goat 52 from 16 to 56 hours. The levels of ^{14}C activity in the milk and urine showed a similar trend. Goat 52 received a 5-gram dose of Propazine to provide an adequate quantity of the metabolites for use in identification. Since a large difference in milk residues existed between the two animals (reflective of dosage), a lower oral dose should result in lowered milk residues.

Methods of chromatography other than ion-exchange chromatography were not applicable for separation of the metabolites in the urine. The ion-exchange chromatography of the radioactive fractions and the per cent of the radioactivity contained in each are presented in Figure 1.

Sixteen radioactive fractions were found in the 16- to 24-hour urine sample of goat 52. Seven of these fractions (4, 8, 9, 10, 11, 14, and 15) each contained more than 5% of the radioactivity. Three fractions (8, 9, and 10) contained 54% of the radioactivity. Similar results were obtained from the urine of goat 51. Valine-^{14}C (eluted at 240 to 250 ml.) and arginine-^{14}C (eluted at 640 to 650 ml.) were selected for interval standards so that various analyses could be compared. Other separation procedures must be used to isolate and determine the purity of each fraction.

Dealkylation of the isopropyl-labeled Propazine occurred in the sheep (Table III). Twenty-four per cent of

Table I. Radioactivity of Urine, Feces, and Milk of Goat after a Single Oral Dose of Propazine-^{14}C (Ring-Labeled)

Collection Interval, Hr.	Accumulated % of ^{14}C Dose in Urine and Feces				Milk Residues, P.P.M. of Propazine-^{14}C Equivalents	
	Urine		Feces			
	51[a]	52	51	52	51	52
0–8	8.6	4.3			1.2[b]	5.5[c]
8–16	20.8	6.6			1.5	12.4
16–24	29.4	11.8	25.4[d]	3.8[d]	1.1	17.2
24–32	35.2	17.5			1.1	19.7
32–40	38.6	22.8			0.8	20.4
40–48	40.7	30.5	38.3	18.3	0.5	20.7
48–56	42.0	32.5			0.3	18.8
56–64	42.7	36.0			0.3	15.8
64–72	43.0	38.9	41.5	33.8	0.2	13.5

[a] Goat number.
[b] 207 d.p.m. per 1 μg. of Propazine-^{14}C equivalent.
[c] 25 d.p.m. per 1 μg. of Propazine-^{14}C equivalent.
[d] Fecal collections taken at 0–24, 24–48, and 48–72 hours.

Table II. Radioactive Residues Detected in Tissue Samples of Goats at Sacrifice (72 Hours) after Single Oral Dose of Propazine-^{14}C (Ring-Labeled)

Tissue and Organs	Tissue Residues, P.P.M. of Propazine-^{14}C Equivalents	
	Goat 51 (500-mg. dose)[a]	Goat 52 (5-g. dose)[b]
Blood	0.68	14.96
Brain	1.49	22.36
Heart	0.51	8.96
Kidney	1.52	28.48
Liver	1.53	24.88
Lung	0.63	13.96
Muscle	0.51	10.84
Spleen	0.79	14.56
Udder	0.34	...[c]
Omental fat	...[d]	...[d]
Kidney fat	...[d]	...[d]

[a] 207 d.p.m. per 1 μg. of Propazine-^{14}C equivalent.
[b] 25 d.p.m. per 1 μg. of Propazine-^{14}C equivalent.
[c] No determination made.
[d] None detected.

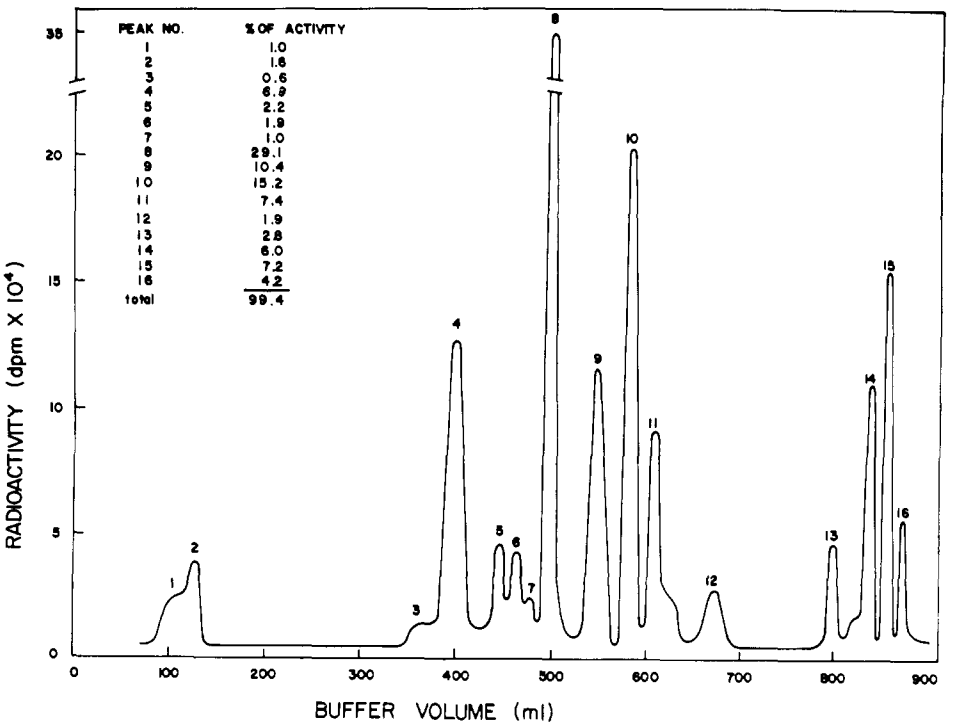

Figure 1. Ion-exchange chromatography of urinary metabolites of ring-labeled ^{14}C-Propazine

Table III. Radioactivity Recovered as $^{14}CO_2$ from a Sheep Dosed with Propazine-^{14}C (Side Chain-Labeled)

Collection Interval, Hr.	Accumulated % of ^{14}C Dose
1–8	2.5
8–16	11.2
16–24	17.8
24–32	22.1
32–72	24.1

the radioactivity was collected as $^{14}CO_2$ within 72 hours after treatment. Isopropyl-labeled Propazine was dealkylated by rats at a faster rate (40% appeared as $^{14}CO_2$ within 24 hours) (Bakke *et al.*, 1967). Dealkylation of triazines also occurs in plants. Shimabukuro *et al.* (1966) found that mature pea plants dealkylated Atrazine to 2-chloro-4-amino-6-isopropylamino-*s*-triazine. The degree of dealkylation of Propazine in these studies has not been determined.

Since no $^{14}CO_2$ was found in expired air from goats receiving ring-labeled Propazine, it is probable that all of the metabolites contain the triazine ring.

A comparison of retention volumes of metabolites found in goat urine with metabolites present in rat urine indicated seven to nine similar components. However, metabolism of Propazine in ruminants is as complex as in the rat. This suggests that several detoxication mechanisms are involved in the metabolism of Propazine by ruminants, and that they result in several compounds which are water-soluble and capable of urinary excretion by the animal. The identification of the metabolites is in progress.

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