Environmental Degradation of the Miticide Cycloprate (Hexadecyl Cyclopropanecarboxylate). 3. Bovine Metabolism

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When a lactating cow was given a single oral dose (0.3 mg/kg) of [*carboxyl*-^{14}C]hexadecyl cyclopropanecarboxylate, 89, 5, and 6% of the applied dose was excreted in urine, feces, and milk, respectively. After 7 days 65 and 7% of the applied dose was eliminated as urinary *N*-(cyclopropylcarbonyl)glycine and free cyclopropanecarboxylic acid. While trace amounts of cycloprate (0.01 ppm) were found in milk, the majority of the ^{14}C residue in milk (52–76%) was characterized as triacylglycerols. Analysis of the latter showed the radiolabel was contained in cyclopropanecarboxylic acid and a homologous series of C_8–C_{18} saturated ω-cyclopropyl fatty acids, with 13-cyclopropyltridecanoic acid representing about half of the ^{14}C acids. *O*-(Cyclopropylcarbonyl)carnitine contributed 11–39% of the total ^{14}C in milk and was also the primary metabolite retained in trace quantities in muscle (0.04–0.06 ppm).

As part of our continued study of the environmental fate of cycloprate [hexadecyl cyclopropanecarboxylate, ZR-856, trademark ZARDEX, Henrick and Staal (1975)], we now report the metabolic degradation of this miticide in a lactating dairy cow.

EXPERIMENTAL SECTION

Radioassay and Chromatography. Thin-layer chromatography (TLC) utilized silica gel GF (0.5 mm, Analtech). The methods of high-resolution liquid chromatography (HRLC) have been presented (Quistad et al., 1978a). The techniques of radiolabel location and quantitation by both TLC and HRLC have been described previously (Quistad et al., 1974). Synthetic ω-cyclopropyl fatty acid standards were provided by the Chemical Research Department at Zoecon (Henrick et al., 1976).

In Vitro Rumen Metabolism. Cycloprate (177 μg, 4.9 mCi/mmol) was absorbed onto cellulose (300 mg) and incubated anaerobically with ca. 40 mL of rumen fluid from a fistulated steer by Dr. Richard Hedde (Syntex Corporation). The rumen contained an active microbial population as evidenced by (1) total gas production, (2) evolution of $^{14}CO_2$ from controls spiked with [^{14}C]urea, and (3) total cellulose digestion.

After incubation for 24 h, the rumen was extracted with ether. The resultant extract was purified by TLC to give unchanged cycloprate and cyclopropanecarboxylic acid (CPCA) which were quantitated by liquid scintillation counting and structurally verified by HRLC. The aqueous phase was saponified to yield only CPCA.

Dosage. An emulsion was prepared with the following composition: [*carboxyl*-^{14}C]cycloprate (54.1 mCi/mmol, 17.4 mCi, 99.8 mg, 99.5% radiochemical purity by μBondapak C_{18} HRLC), Igepal emulsifier (55 mg, GAF Corp), Kelzan thickening agent (173 mg, Kelco Corp), and water (50 mL). The emulsion was administered directly into the rumen of a lactating Holstein-Jersey cow (344 kg) by syringe through Tygon tubing by Dr. Allen Braemer, Syntex Corporation. The animal was maintained in a metabolism stall with periodic collection of blood, milk, urine (by catheter), and feces. In order to collect $^{14}CO_2$, the cow was fitted with a face mask for 1-h intervals beginning 4 and 7 h posttreatment. A measured fraction of the expired air was drawn from the mask into two traps containing 2-methoxyethanol and ethanolamine (2:1). Aliquots of the trapping solution were quantitated by liquid scintillation counting (Insta-Gel, Packard). After 7 days the cow was sacrificed (barbituate overdose) for removal of tissues which were frozen (−18 °C) for subsequent analysis.

Analysis of Excrement. Aliquots (50 μL–1 mL) of urine were evaporated to dryness then treated with α-bromo-*p*-phenylacetophenone (15 mg) in dimethylformamide (2 mL) containing $KHCO_3$ (~70 mg) to form the *p*-phenylphenacyl esters of expected metabolites. After addition of water the products were extracted into ether and separated by TLC (silica gel GF, ether). The structural assignments of bands presumed to be the *p*-phenylphenacyl esters of CPCA and *N*-(cyclopropylcarbonyl)glycine (CPCA-gly) were further authenticated by HRLC on μBondapak C_{18} (Waters Associates, 30 × 0.4 cm, eluted with 70:30 and 55:45, methanol–water, respectively). In addition, the *p*-phenylphenacyl ester of CPCA-gly coeluted with authentic standard on normal phase HRLC using a Zorbax-SIL column (du Pont, 22 × 0.46 cm, eluted with ether–pentane, 70:30).

An aliquot (1–2 mL from ca. 800 mL) of the methanolic extract of feces was evaporated and derivatized as in the urine analysis. Cycloprate and the *p*-phenylphenacyl ester of CPCA were separated by TLC (hexane–ether–58% ammonium hydroxide, 50:50:2). Structural assignments were confirmed by HRLC on μBondapak C_{18}.

Analysis of Milk. An aliquot (10 mL) of milk was shaken with Celite (2.5 g) and acetonitrile (20 mL) and then filtered. The filter cake was washed with ether and water (25 mL each). The combined filtrate was extracted with ether (3 × 20 mL) to provide organic and aqueous phases, which were examined separately.

Fractionation of the organic extract by TLC (Rhodamine impregnated silica gel, eluted with hexane–ether, 6:1) gave radioactive zones containing cycloprate, ω-cyclopropyl fatty acids (as triacylglycerols), and CPCA (as triacylglycerol). The identity of cycloprate was confirmed by HRLC (μBondapak C_{18}, methanol–water, 90:10) while the more abundant triglyceride fraction was transesterified (0.5 M methanolic sodium methoxide, 50 °C, 10 min) to the methyl esters of a homologous series of ω-cyclopropyl fatty acids. These methyl esters were resolved for quantitation by HRLC on μBondapak C_{18} (methanol–water, 80:20 for C_8–C_{14} and 90:10 for C_{14}–C_{18} acids) with co-injected authentic 5-, 7-, 9-, 11-, 13-, and 15-cyclopropylalkanoic acid esters.

Triacylglycerols containing CPCA were characterized from the following considerations: (1) the radioactive zone was resolvable from but similar in polarity to triacylglycerol

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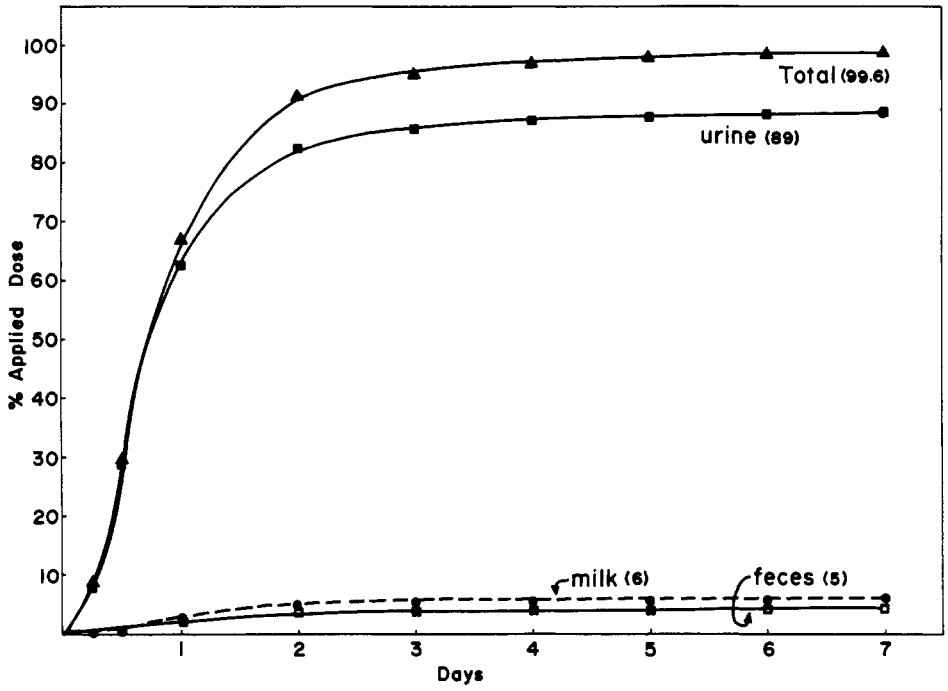


Figure 1. Radioactivity balance for cow dosed at 0.3 mg/kg with [*carboxyl*-^{14}C]cycloprate.

containing longer-chain ω-cyclopropyl fatty acids; (2) saponification gave only CPCA; and (3) a synthetic standard of 2-cyclopropylcarbonyl-1,3-dioleoylglycerol (from 1,3-dioleoylglycerol and cyclopropanecarbonyl chloride/pyridine) showed similar TLC behavior and had approximately the same molecular weight as determined by chromatography on Sephadex LH-20 (methanol). The upper limit of the free CPCA concentration in milk was determined by mild acidification, followed by steam distillation which afforded volatile fatty acids in the distillate.

O-(Cyclopropylcarbonyl)carnitine (CPCA-carnitine) was characterized from the aqueous phase after extraction of milk with ether. Lyophilization gave a methanol-soluble ^{14}C residue which was coincident with authentic standard by TLC on silica gel ($CHCl_3$–methanol–58% NH_4OH, 50:30:8) and by HRLC (μBondapak C_{18}, eluted with 100% water). A complete identification of CPCA-carnitine as a cycloprate metabolite is presented elsewhere (Quistad et al., 1978b).

Analysis of Muscle. Samples (100 g) of leg and shoulder muscle were separately extracted with $CHCl_3$–methanol (2:1). Less than 1% of the extractable ^{14}C residue was ether-soluble when partitioned with water after acidification. TLC analysis on silica gel ($CHCl_3$–methanol–58% NH_4OH, 50:30:8) revealed a single radioactive peak which upon saponification gave only CPCA. Gel permeation chromatography on Sephadex LH-20 also evidenced a single ^{14}C component which was identical with the polar metabolite found in milk. The muscle polar metabolite was characterized as CPCA-carnitine by HRLC (μBondapak C_{18}, 100% water).

RESULTS AND DISCUSSION

Although previous metabolism studies with rats (Quistad et al., 1978a) had shown that only negligible amounts of $^{14}CO_2$ were produced from [*carboxyl*-^{14}C]cycloprate, we tested the likelihood of ^{14}C volatiles from bovine metabolism by in vitro incubation of cycloprate with rumen from a fistulated steer. As expected less than 0.5% of the cycloprate was converted to $^{14}CO_2$ (Table I), but approximately half of the applied cycloprate was cleaved to cyclopropanecarboxylic acid (CPCA, Table II) which was recovered as free acid (4% applied dose) and as aqueous polar conjugates (31%). Potential in vivo evolution of $^{14}CO_2$ was monitored for 1 h intervals beginning at 4 and 7 h after dosing a cow at which times only 0.006 and 0.004% of the applied dose were expired as ^{14}C volatiles. Hence, production of $^{14}CO_2$ from [*carboxyl*-^{14}C]cycloprate by bovine metabolism is minimal.

Table I. Bovine Rumen Metabolism of Cycloprate in Vitro

	% applied dose
$^{14}CO_2$	<0.5[a]
Cycloprate	50
CPCA (free)	4
CPCA (polar conjugates)	31

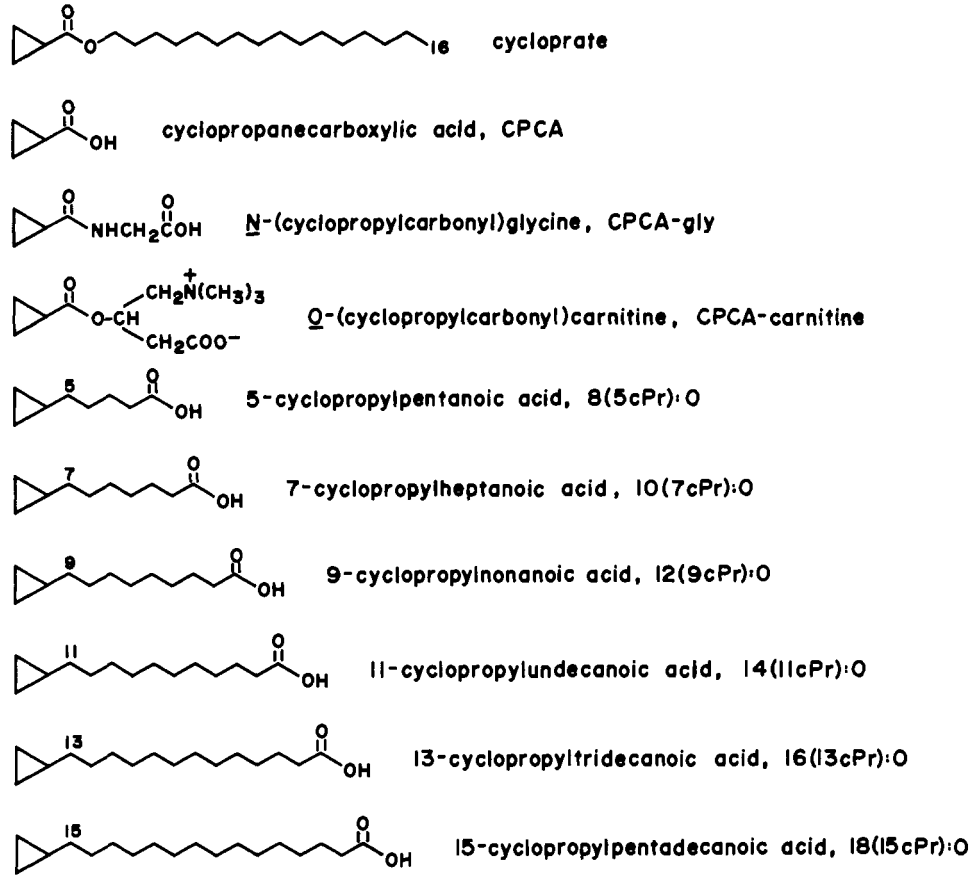
[a] $^{14}CO_2$ determined by Dr. R. Hedde, Syntex Corp., Palo Alto, Calif.

The distribution of radiolabel after administration of [^{14}C]cycloprate to a lactating cow is depicted in Figure 1. After 1 week virtually the entire applied dose had been eliminated in urine (89%), feces (5%), and milk (6%). The pharmacodynamics of ^{14}C residue elimination are given in Figure 2 where maximum levels of radioactivity in urine, blood serum, and milk occurred at 12, 12, and 30 h after dosage.

Urinary and Fecal Analysis. An examination of all urine samples taken within the first 3 days after dosage showed that 86% of the applied dose was excreted in urine with CPCA and *N*-(cyclopropylcarbonyl)glycine (CPCA-gly) representing 7 and 65% of the applied radiolabel (Figure 3). Thus, 84% of the total ^{14}C residue in urine could be characterized as these two products for 0–3 day samples.

A more exhaustive analysis of 12-h urine (maximum ^{14}C concentration) was undertaken in order to examine possible minor metabolites. Since saponification of this urine gave *only CPCA*, ω-cyclopropyl fatty acids are excreted neither as free acids nor glycine conjugates, but must be β oxidized prior to elimination from the animal (in urine). Treatment with diazomethane rendered 96% of the 12-h urinary metabolites ether soluble. Although several minor

Table II. Cycloprate and Principal Metabolites



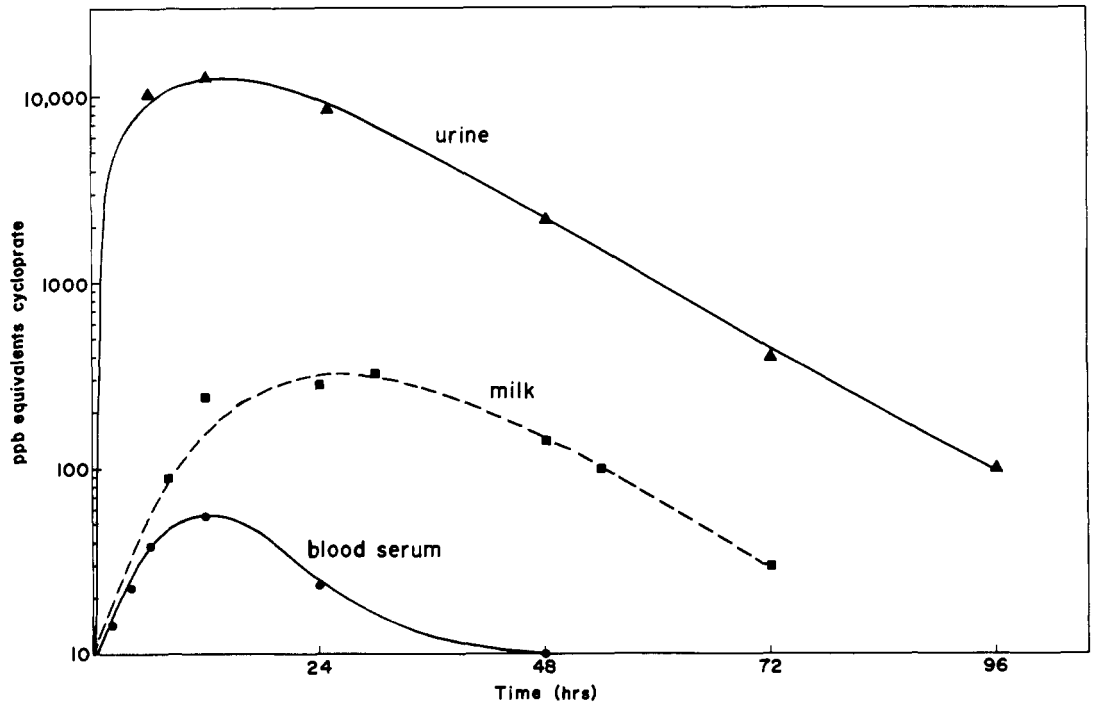


Figure 2. Profile of ^{14}C residues in urine, milk, and blood for a cow dosed at 0.3 mg/kg with [^{14}C]cycloprate.

products were observed, no metabolite other than free CPCA or CPCA-gly contributed more than 1–2% of the total ^{14}C label in this ether-soluble fraction. In particular, we actively sought, but could not find, conjugates with glucuronic acid, glutamic acid, and phenylalanine, none of which represented >1% of the total ^{14}C in 12-h urine. Also 4-hydroxybutyric acid was absent (<1%), indicating that the cyclopropane ring was stable to scission in contrast to metabolism by *Fusarium* fungus (Schiller and Chung, 1970).

Fecal ^{14}C residues consisted mainly of free CPCA and unaltered cycloprate which represented 1.5 and 1.3%, respectively, of the applied dose after 2 days (Table III). A small amount of CPCA-gly was found also which probably came from contamination via the urinary catheter.

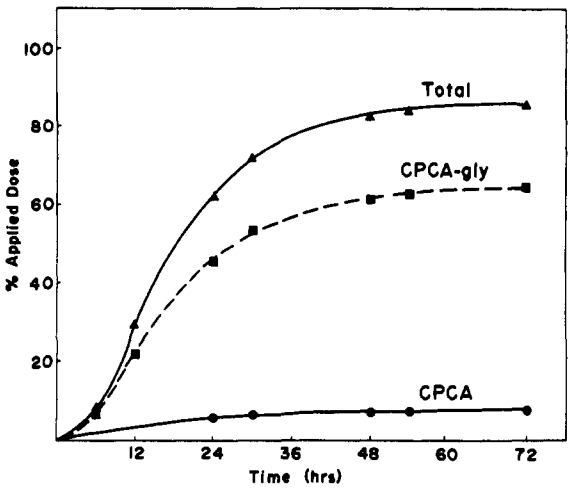


Figure 3. Excretion and distribution of urinary metabolites of [^{14}C]cycloprate.

Milk. Triacylglycerols contained 52–76% of the radiolabel in milk (Table IV). Saponification of these triacylglycerols gave a homologous series of ω-cyclopropyl fatty acids beginning with 8(5cPr):0 and ending with 15-cyclopropylpentadecanoic acid [18(15cPr):0]. The principal acidic metabolites were CPCA and 13-cyclopropyltridecanoic acids, which together represented 60% of the ^{14}C-acyl moieties in the triacylglycerols.

Cycloprate and free CPCA were minor components of milk. Cycloprate exceeded neither 0.01 ppm nor a total of 0.1% of the applied dose after 3 days (Figure 4). Free CPCA was always less than 0.004 ppm.

O-(Cyclopropylcarbonyl)carnitine contributed 11–39% of the total ^{14}C residue in milk. After 3 days 1.4% of the applied dose had been secreted as this conjugate in milk. The presence of CPCA-carnitine is consistent with the known natural occurrence of carnitine and acetylcarnitine in milk (Abdel-Kader and Wolf, 1965; Erfle et al., 1970; Snoswell and Linzell, 1975). If the total carnitine in normal cow milk is 0.12 μmol/mL (Erfle et al., 1970), then CPCA had bound only 0.1–0.3% of the total naturally secreted carnitine.

Table III. Analysis of ^{14}C Residues in Cow Feces

	% total ^{14}C in feces	
	1 day (1.91% applied dose)	2 day (1.81% applied dose)
CPCA	46.3	33.0
Cycloprate	23.4	44.5
Total identified	69.7	77.5

Tissues. From the recovery of applied radiolabel (Figure 1) and data in Table V it is evident that after 3 days little radioactivity remained in the carcass of the cow. Relative to other tissues, muscle contained the highest residue level (an average of 0.085 ppm equivalents of cycloprate). The ^{14}C residue in muscle was highly polar, and 86–93% of the total muscle ^{14}C was identified as CPCA-carnitine (Table VI). Assuming a carnitine content of 0.029% in bovine muscle (Fraenkel, 1957), then only 0.02% of the natural muscle pool of carnitine would be conjugated as CPCA-carnitine. Assuming that 18% of the live weight of a cow is muscle (Morrison, 1961), as much as 2–5% of the applied dose probably remained in the total muscle after 1 week. Such a retention of radiolabel was not indicated by the recovery study (Figure 1), but is probably within the limits of experimental error associated with radiolabel quantitation.

The presence of CPCA-carnitine at these low residue levels in milk and muscle should be inconsequential in regards to bovine toxicology. Carnitine is biosynthesized in the ruminant liver (Snoswell and McIntosh, 1974) and a rather dynamic turnover rate is evidenced by the se-

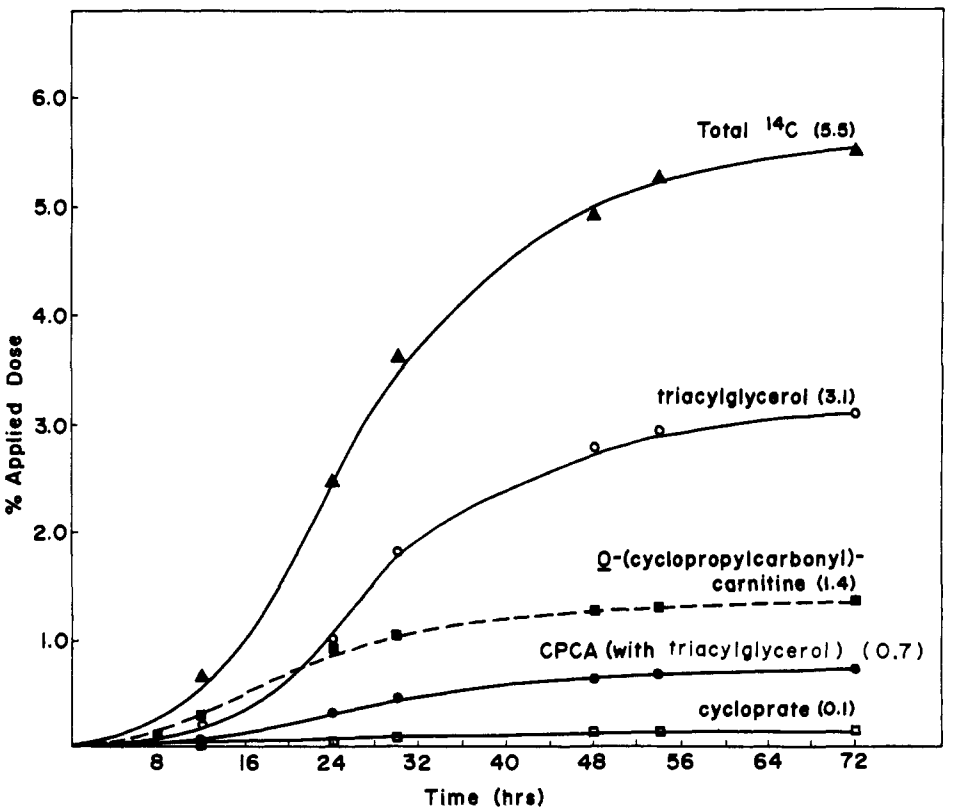


Figure 4. Distribution of radioactivity in milk from a dairy cow treated with [^{14}C]cycloprate.

Table IV. Distribution of Metabolites from [^{14}C]Cycloprate in Milk

	% total ^{14}C in milk	
	12 h	30 h
Cycloprate	4.4 (0.0108 ppm)	2.5 (0.0083 ppm)
CPCA (free)	<1.65 (<0.004 ppm)	<0.4 (<0.0014 ppm)
ω-Cyclopropyl fatty acids (as triacylglycerols)	39	64
18(15cPr):0	2	3
16(13cPr):0	19	33
14(11cPr):0	7	14
12(9cPr):0	4	5
10(7cPr):0	4	6
8(5cPr):0	2	3
CPCA (as triacylglycerols)	13	12
CPCA-carnitine	39	11
Total	95	90

Table V. Distribution of ^{14}C Residues after 7 Days in Tissues of Lactating Cow Dosed with [*carboxyl*-^{14}C]Cycloprate

Tissue	ppm equiv as cycloprate	% applied dose in total tissue
Shoulder muscle	0.088	
Leg muscle	0.057	
Flank muscle	0.111	
Renal adipose	0.004	
Omental adipose	0.003	
Subcutaneous adipose	0.0019	
Lung	0.008	0.021
Heart	0.010	0.016
Kidney	0.008	0.006
Adrenal	0.012	0.0002
Spleen	0.002	0.001
Pancreas	0.021	0.008
Uterus	0.001	0.0007
Udder	0.007	
Tongue	0.006	0.005
Bladder	0.002	0.0003
Liver	0.005	0.024
Small intestine	0.003	0.019
Large intestine	0.002	0.008
Brain	0.030	0.013
Hide	0.026	
Stomach	0.001	0.022
Ovaries	0.003	0.00002
Thyroid	0.002	0.00005
Bone marrow	0.004	
Gall bladder	0.001	
Bile	<0.0001	
Blood	<0.001	

Table VI. Analysis of ^{14}C Residue in Cow Muscle 1 Week after Treatment with [^{14}C]Cycloprate

	% total ^{14}C in muscle
Leg	
Extractable ($CHCl_3$-CH_3OH)	94.3
Ether soluble	0.7
Water soluble	93.6
O-(Cyclopropylcarbonyl)carnitine	86.4
Residual solids	5.7
Shoulder	
Extractable ($CHCl_3$-CH_3OH)	94.7
Ether soluble	0.7
Water soluble	94.0
O-(Cyclopropylcarbonyl)carnitine	93.2
Residual solids	5.3

cretion of 0.5 g of carnitine/day in cow milk (Snoswell and Linzell, 1975). Thus, carnitine is a readily replaceable cofactor in ruminants.

Comparative Metabolism. When [*carboxyl*-^{14}C]-cycloprate was given as a single oral dose (0.3 mg/kg or 5× maximum expected residue on freshly sprayed fruit) to a lactating cow, nearly quantitative excretion of radiolabel occurred within 1 week. In contrast to rat metabolism of cycloprate (Quistad et al., 1978a) bovine carcass fat contained only small amounts of residual radiolabel. Like the dog (Quistad et al., 1978b), the lactating cow retained ^{14}C metabolites in carcass muscle mainly as CPCA-carnitine, but in contrast to dog metabolism of cycloprate, ^{14}C residues in bovine muscle were vastly lower.

ACKNOWLEDGMENT

We thank R. Hedde (Syntex Corp) for rumen incubations and $^{14}CO_2$ collection. We also thank A. Braemer, B. Garlow, and V. Buckley (Syntex Corp) for assistance in dosing and sample collection, and for helpful discussions we acknowledge R. Curtis and T. L. Burkoth.

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Received for review May 16, 1977. Accepted July 25, 1977. Contribution No. 58 from Zoecon Research Laboratory.