Environmental Degradation of the Miticide Cycloprate (Hexadecyl Cyclopropanecarboxylate). 4. Beagle Dog Metabolism

Gary B. Quistad,* Luana E. Staiger, and David A. Schooley

When beagle dogs were given a single oral dose at 0.7 mg/kg of [*carboxyl*-^{14}C]cycloprate (hexadecyl cyclopropanecarboxylate) only 25 and 2% of the applied dose was excreted after 4 days in urine and feces, respectively. Thus, 73–77% of the administered radiolabel was retained mainly in kidney (7%), liver (2%), adipose tissue (6%), hide (6%), and muscle (56%). About two-thirds of the ^{14}C in adipose tissue and hide was characterized as ω-cyclopropyl fatty acids (as triacylglycerols) with 13-cyclopropyltridecanoic acid being the major component. These ω-cyclopropyl fatty acids also comprised the main ^{14}C residue in liver and kidney where 28 and 67% existed as [^{14}C]phospholipid. About half of the applied radiolabel was retained in muscle as a unique conjugated pesticide metabolite, *O*-(cyclopropylcarbonyl)carnitine.

As part of a continuing investigation of the metabolic fate of cycloprate [hexadecyl cyclopropanecarboxylate, trademark ZARDEX, ZR-856, Henrick and Staal (1975)] we report its biodegradation in beagle dogs.

EXPERIMENTAL SECTION

Dosage. Two male beagle dogs (9–10 kg) were given a single oral dose of [*carboxyl*-^{14}C]cycloprate (0.68 mg/kg, 54.1 mCi/mmol, 99.5% radiochemical purity, for radiosynthesis see Quistad et al., 1978). Periodically samples of blood, urine, and feces were taken for 4 days after dosage at which time the dogs were sacrificed with tissue removal for subsequent quantitation of ^{14}C residues. The entire dosage, handling, sacrifice, and preliminary analysis of tissues, blood, and excrement were performed under contract by International Research and Development Corporation (Mattawan, Mich.). Tissues and excrement were shipped in dry ice to Zoecon for further analysis.

Radioassay and Chromatography. Radioactivity was quantitated by liquid scintillation counting (Packard Model 3330 or 2425) and total combustion (Oxymat-Teledyne Intertechnique or Packard Model 305 oxidizer). More detailed procedures are given elsewhere (Quistad et al., 1974). Silica gel GF plates (Analtech) were used for thin-layer chromatography (TLC) while the techniques of high-resolution liquid chromatography (HRLC) have been described (Quistad et al., 1978). Gel permeation chromatography (GPC) utilized a pump (Waters M6000), glass column (Chromatronix, LC-1/2), Sephadex LH-20 (Pharmacia, 1.27 × 93 cm), and methanol for elution (1.5 mL/min, 50 psi). Authentic ω-cyclopropyl fatty acids were provided by the Chemical Research Department at Zoecon. The preparation of *O*-(cyclopropylcarbonyl)-DL-carnitine (CPCA-carnitine) and *N*-(cyclopropylcarbonyl)glycine (CPCA-gly) have been described (Guilbert and Chung, 1974, and Quistad et al., 1978, respectively).

Urine. An aliquot of urine (12–24 h maximum) was separated into two radioactive zones by GPC chromatography on Sephadex LH-20. The faster eluting zone (elution volume/void volume, V_e/V_o = 1.8; 62% total urinary ^{14}C) was saponified and all radiolabel was converted to cyclopropanecarboxylic acid (CPCA). The labeled conjugate in this zone was characterized as CPCA-carnitine by HRLC on μBondapak C_{18} (Waters Associates, 30 × 0.4 cm, eluted with 100% water). The slower eluting zone (V_e/V_o = 3.3) from Sephadex LH-20 (33% urinary ^{14}C) was derivatized with α-bromo-*p*-phenylacetophenone, and the *p*-phenylphenacyl esters of CPCA and CPCA-gly were separated by TLC (ether) for subsequent verification of structural assignment by HRLC (μBondapak C_{18}, methanol–water, 70:30 and 55:45, respectively).

Analysis of Kidney. Metabolites were extracted from kidney (38 g) with methanol (3 × 100 mL) and a mixture of $CHCl_3$–methanol (2:1, 3 × 100 mL) using a Virtis homogenizer (apparatus also used for other tissues). Cycloprate and triacylglycerols were isolated from the combined extract by TLC (hexane–ethyl acetate–acetic acid, 60:20:1). The radiolabel comigrating with cycloprate on TLC was analyzed further for structure confirmation on μBondapak C_{18} (methanol–water, 90:10). Free CPCA was quantitated by derivatization of an aliquot of the extract to the *p*-phenylphenacyl ester which was then analyzed by HRLC (μBondapak C_{18}, methanol–water, 70:30). Free ω-cyclopropyl fatty acids were determined by treating an aliquot of the extract with CH_2N_2, purifying the resultant esters by TLC (hexane–ethyl acetate, 38:2), and comparing the esters to authentic standards by HRLC (μBondapak C_{18}, methanol–water, 90:10).

The presence of [^{14}C]phosphoglyceride was suspected from the preponderance of polar radiolabeled residues upon TLC (R_f 0 in hexane–ethyl acetate–acetic acid, 60:20:1). Indeed, when a portion of the extract was subjected to GPC, radioactivity eluted in a zone near dipalmitoyl L-α-phosphatidylcholine synthetic standard, suggesting that the ^{14}C residue was higher in molecular weight than most expected primary metabolites. The polar lipid metabolites were freed from nonpolar materials by adsorbing a portion of the extract onto silica gel and rinsing with ether, then polar lipid was eluted from the silica with methanol. This polar eluate was used in subsequent characterization of [^{14}C]phosphoglycerides.

Treatment of the polar eluate with phospholipase C (*C. welchii*, Sigma; for conditions of this and subsequent enzymatic reactions, see Christie, 1973) gave [^{14}C]diacylglycerol (73% yield, determined by TLC). This [^{14}C]diacylglycerol could be acetylated quantitatively to [^{14}C]triacylglycerol (acetic anhydride/pyridine). TLC of the resultant [^{14}C]triacylglycerol (hexane–ethyl acetate, 100:15) indicated two partially overlapping zones of radioactivity which would be expected for acetylated diacylglycerols in which certain molecules contain long-chain ω-cyclopropyl fatty acids and others contain more polar CPCA in place of a longer chain acid. Phospholipase C from *B. cereus* also produced [^{14}C]diacylglycerol from the polar eluate, but in reduced (16%) yield. Phospholipase

Zoecon Corporation Research Laboratory, Palo Alto, California 94304.

0021-8561/78/1426-0080$01.00/0

A_2 (bee venom, Sigma) produced free acids from the 2 position of [^{14}C]phosphoglycerides (38% ^{14}C cleavage). Pancreatic lipase (hog mucosa, Sigma) produced free acids, presumably from the 1 position of the [^{14}C]phospholipids (27% ^{14}C release). In all cases blank incubations lacking enzyme gave negligible free acid formation. Released CPCA was analyzed by conversion to its *p*-phenylphenacyl ester, followed by HRLC while ω-cyclopropyl fatty acids were converted to methyl esters for structure authentication by HRLC.

Analysis of Muscle. Samples of muscle from the thigh (5 g) and dorsal lumbar area (5 g) were pooled, then extracted with $CHCl_3$ and methanol. Examination of the extract by TLC revealed that about 90% of the ^{14}C residue was quite polar (R_f 0.12, $CHCl_3$–methanol–58% NH_4OH, 50:30:8). Saponification gave only CPCA.

In order to investigate further the chemical nature of this very polar metabolite, a third dog (10.5 kg) was given an exaggerated dose (572 mg/kg) of [*carboxyl*-^{14}C]-cycloprate (0.05 mCi/mmol) to acquire sufficient mass for spectral analysis. The actual dosage, maintenance, and sacrifice were performed by Brian Rice (Syntex Corp). A 489-g sample of muscle was obtained 4 days after the single oral dose and was extracted with $CHCl_3$–methanol. The extract (2.4×10^7 dpm) was evaporated to dryness, rinsed with ether (to remove nonpolar lipid), and dried to yield 12 g of biomass. Dissolution in CH_2Cl_2–methanol (3:1, 50 mL), followed by addition of Celite (3 g) and low-temperature (−70 °C) precipitation of nonradioactive impurities reduced the mass to 3 g. A portion of the sample (0.6 g) was purified by gradient-elution column chromatography on DEAE–cellulose (Cellex D, Bio-Rad, 15 g, 2 × 25 cm column; for column preparation, see Christie, 1973). The unknown polar metabolite eluted as a single radiolabeled component in $CHCl_3$–methanol (5:1, 180 mL), and the total mass was reduced to 56 mg. Gel permeation chromatography (Sephadex LH-20, Pharmacia, 1.27 × 93 cm, methanol) reduced the mass only to 43 mg. For the final purification 18 mg was divided into three equal fractions for HRLC on μBondapak C_{18} (^{14}C elution with $k' = 5$ in 100% water). The ^{1}H NMR spectrum was taken of the purified metabolite (2.4 mg): Varian T-60, 60 MHz (D_2O, internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate), δ 1.02 (d, 4 H, J = 6 Hz, cyclopropyl CH_2), 1.72 (m, 1 H, cyclopropyl CH), 2.56 (d, 2 H, J = 6 Hz, CH_2CO_2), 3.12 (s, 9 H, $(CH_3)_3N^+$), 3.82 (d, 2 H, J = 9 Hz, N-CH_2), 5.68 (m, 1 H) C-CH-C. The isolated muscle metabolite had an identical NMR spectrum with an authentic synthetic standard of CPCA-DL-carnitine. A satisfactory ^{1}H NMR comparison of metabolite and synthetic standard was also obtained at higher sensitivity and resolution (Bruker WH-90, 90 MHz, Fourier Transform) by Dr. M. Maddox and Dr. L. Tökes (Syntex Corp). Attempted mass spectral analysis (Hewlett Packard Model 5984A GLC/MS/data system) of the muscle metabolite or synthetic standard resulted in apparent decomposition of sample and inconclusive spectra. Although attempts were not made to be completely quantitative, the overall recovery of ^{14}C for the purification of CPCA-carnitine from dog muscle (Figure 2) was 46%.

Analysis of Adipose, Hide, and Liver. Each tissue (2–5 g) was extracted with $CHCl_3$–methanol and the resultant extract was subjected to TLC (hexane–ether, 6:1) for separation into radioactive metabolite zones. The verification of structural assignments proceeded as already described (vide supra).

A previously unknown radioactive TLC zone was found in hide extracts. Radiolabel was associated with biomass

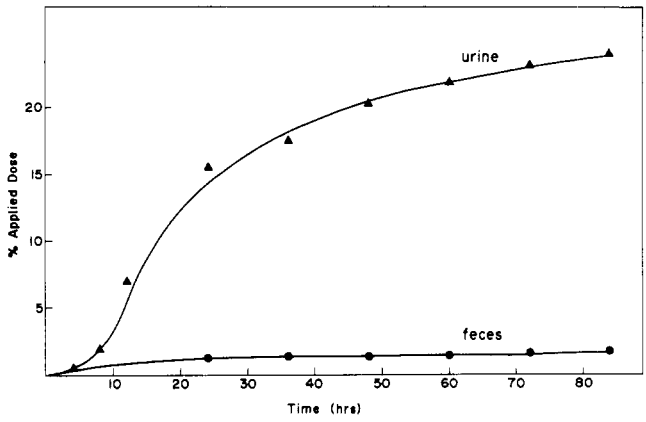

Figure 1. Excretion of radiolabel by dogs given a single oral dose (0.7 mg/kg) of [*carboxyl*-^{14}C]cycloprate.

Table I. Metabolites in Urine and Muscle of Dogs Given a Single Oral Dose of [*carboxyl*-^{14}C]Cycloprate

	Urine (12–24 h max)	Muscle	
	0.7 mg/kg	0.7 mg/kg	572 mg/kg
Extractable ($CHCl_3$–CH_3OH)	100	98	96
CPCA (free)	5.3	8.9	≤7
CPCA-gly	24	<1	<1
CPCA-carnitine	58	86	89
Residual solids	0	2	4

with polarity similar to cycloprate. The ^{1}H NMR spectrum of TLC-purified material suggested it to be natural triacylglycerols, probably containing some methyl-branched fatty acids. Most (56%) of these natural fatty acids in this zone were high in molecular weight (C_{19}–C_{26}) as determined by GLC–mass spectrometry. Transesterification (0.5 M methanolic sodium methoxide, 50 °C, 10 min) of the zone gave 14(11cPr):0 [1.4% total hide ^{14}C]; 16(13cPr):0 [4.3%]; 18(15cPr):0 [12.2%]; 20(17cPr):0 [2.0%]; and >20(17cPr):0 [2.5%]. Abbreviations for the above ω-cyclopropyl acids are explained in Table III.

RESULTS AND DISCUSSION

Since subacute toxicology studies with cycloprate suggested that the beagle dog is a substantially more sensitive species than the rat, we began an investigation of the metabolic fate of [*carboxyl*-^{14}C]cycloprate in the dog with the hope of rationalizing this differential toxicity on a biochemical basis.

Excreted Metabolites. The dissimilarity of rat metabolism of cycloprate (Quistad et al., 1978) compared to that of the dog was quickly evident in the greatly reduced excretion of radiolabel by dogs. After 4 days only 25 and 2% of the applied dose was excreted in urine and feces, respectively (Figure 1). Also in striking contrast to rats, the urinary ^{14}C (Table I) was predominantly (58%) *O*-(cyclopropylcarbonyl)carnitine (CPCA-carnitine) instead of *N*-(cyclopropylcarbonyl)glycine (CPCA-gly). However, CPCA-gly was still a major urinary metabolite (24% urine ^{14}C) and a lesser amount of free cyclopropanecarboxylic acid (CPCA) was also found (5% urine ^{14}C).

Muscle. Since about three-fourths of the applied radiolabel was not excreted 4 days posttreatment, various tissues were examined in order to localize the site of the ^{14}C. Using the ^{14}C residue data (from sample combustion) and estimates of the mass for various tissues in a beagle dog (e.g., Anderson and Goldman, 1970), we calculated the expected tissue distribution of ^{14}C (Table II). The main

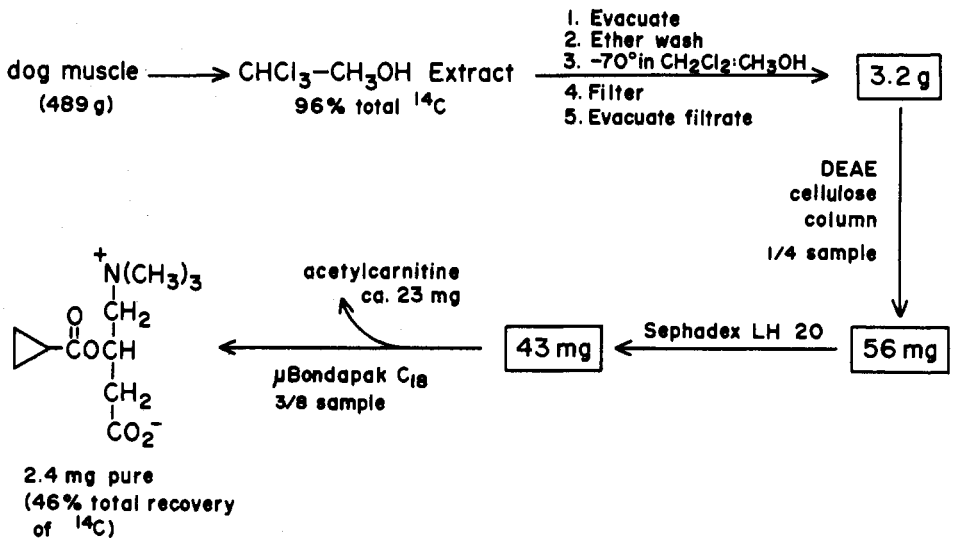

Figure 2. Purification scheme for *O*-(cyclopropylcarbonyl)carnitine (CPCA-carnitine) from dog muscle.

Table II. Balance of Radioactivity and Tissue ^{14}C Residues for Dogs Given Single Oral Dose (0.7 mg/kg) of [^{14}C]Cycloprate

	ppm equivalent	% applied dose
Spleen	0.32	0.14
Muscle (thigh)	0.86	ca. 56[a]
Muscle (dorsal lumbar)	0.79	
Heart	0.20	0.24
Lung	0.37	0.55
Kidney	9.37	6.8
Liver	0.27	1.56
Testes	0.21	0.05
Thymus	0.21	0.03
Brain	0.16	0.19
Gall bladder	0.17	
Bile	0.41	
Adipose (subcutaneous)	0.38	ca. 5.5[b]
Adipose (perirenal)	0.96	
Bone marrow	0.42	
Hide	0.30	ca. 5.6[c]
Total calculated in tissues		ca. 77
Total expected in tissues (from excretion data)		73
Urine		25.2
Feces		1.8
Total recovery		ca. 104

[a] Assuming 46% wet weight as muscle, Anderson (1970). [b] Assuming 10% wet weight as adipose. [c] Assuming 9-17% wet weight as hide, Anderson (1970) and Davis et al. (1975).

reservoir of radiolabel was muscle tissue (ca. 56% applied dose), which received intense perusal.

Identification of 9% of the muscle ^{14}C (0.7 mg/kg dose, Table I) as CPCA was trivial. However, 86% of the remaining unknown radiolabel behaved as a *single polar component* on TLC and GPC on Sephadex LH-20. The extreme polarity of the unknown ^{14}C complicated its eventual structural elucidation since it was not extractable from water into common organic solvents. In fact, the unknown ^{14}C was as polar by TLC as the most hydrophilic complex lipids (e.g., sphingomyelin). Since attempts at chemical derivatization of the muscle ^{14}C (e.g., methylation and acetylation) were largely fruitless, another dog was given a rather massive dose (572 mg/kg) of [*carboxyl*-^{14}C]cycloprate in order to acquire a sufficient mass of metabolite for structural identification. The purification scheme of the major muscle metabolite is summarized in Figure 2. This substance was identified as *O*-(cyclopropylcarbonyl)carnitine by its ^{1}H NMR spectrum.

Role of Carnitine. Carnitine is a widely distributed biochemical which occurs in most tissues (Fraenkel, 1957; Bressler, 1970). It is an essential cofactor that mediates the transport of long-chain fatty acids (as acylcarnitines) from the cytoplasm across the inner mitochondrial membrane and into the mitochondrial matrix for subsequent β oxidation (Bressler, 1970). Although the inner mitochondrial membrane is impermeable to free long-chain acids (and their CoA thioesters), the enzyme carnitine palmityltransferase (EC 2.3.1.21) permits O-acylation of carnitine to form a mitochondrion-permeable product. After entering the mitochondrion, the acylcarnitine is converted into acyl CoA (for β oxidation) and free carnitine. Since free carnitine also cannot penetrate the mitochondrial membrane, carnitine is O-acetylated by carnitine acetyltransferase (EC 2.3.1.7) for recycling back into the cytoplasm and regeneration of the transport cycle. Short-chain fatty acids such as butyrate and octanoate circumvent the usage of carnitine as a cofactor by direct mitochondrial membrane penetration (Bressler, 1970). Thus, carnitine is vital for normal utilization of adipose depots (i.e., long-chain fatty acids) as energy sources and impairment of carnitine's role as a transport vehicle results in dysfunctional accumulation of triacylglycerol in carnitine-deficient tissues (Entman and Bressler, 1967; Bressler, 1970).

The relative concentration of carnitine in various tissues is known for the dog (Fritz, 1963), muscle being the single largest reservoir of tissue carnitine (56%). Therefore, it is not surprising that a metabolite conjugated with carnitine would also appear predominantly in muscle. Broekhuysen and Deltour (1961) reported a carnitine titer of 4.1 mg/g of dry weight for dog skeletal muscle. Using this value and the measured tissue metabolite levels, we calculate that the beagles given the low single oral dose (0.7 mg/kg) of [*carboxyl*-^{14}C]cycloprate had only 0.06% of the muscle carnitine bound with CPCA. However, at an exaggerated dose rate of 572 mg/kg, our data indicate that 12% of the available muscle carnitine was sequestered as the CPCA conjugate. The presence of CPCA-carnitine in dog muscle suggests that conversion of CPCA-CoA to the acylcarnitine (Figure 3) is not readily reversible, in contrast to the analogous reactions of natural fatty acids mediated by carnitine acyltransferases. Thus, sufficiently large doses of CPCA in the dog would be expected to

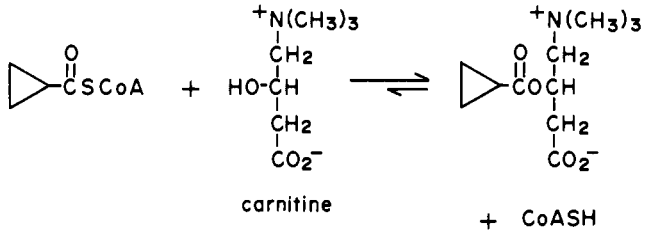

Figure 3. Enzymatic formation of *O*-(cyclopropylcarbonyl)-carnitine.

deplete carnitine reserves resulting in toxicity. It has already been shown that several alkenoic acids (4-pentenoic, acrylic, and methylenecyclopropylacetic) deplete free carnitine levels in vivo in mice by a similar mechanism with a concomitant increase in acylcarnitine levels (Corredor et al., 1967). Presumably the acylcarnitines of these alkenoic acids and CPCA represent metabolic dead ends. Since about 90% of the total muscle ^{14}C in the dog was CPCA-carnitine and this product was even excreted in urine, it appears that CPCA-carnitine is fairly stable biochemically in this species. Reconversion of CPCA-carnitine to CPCA-CoA (Figure 3) is probably required for subsequent formation of CPCA-gly (cf. Yang, 1976), the principal excretory product of CPCA in other mammalian species (Quistad et al., 1978).

Pharmacology of CPCA. Since 4-pentenoic, methylenecyclopropylacetic, and CPCA acids are all presumed to elicit a toxic response by sequestering carnitine, it is of interest to compare their known pharmacological and biochemical effects. However, interpretation of various reports is confused by species and tissue differences, as well as occasionally conflicting data.

Species-specific hypoglycemic effects of these acids were noted for several mammals. For example, administration of 4-pentenoic and methylenecyclopropylacetic acids to mice resulted in hypoglycemia (Corredor et al., 1967), whereas CPCA was only weakly hypoglycemic in mice, rats, rabbits, and man, but elicited a stronger response in the guinea pig and monkey (Stewart, 1962). When kidney slices from the rat and guinea pig were incubated with CPCA, gluconeogenesis from pyruvate was much more strongly inhibited in the latter species than in the former, apparently partly explaining the differential hypoglycemia (Duncombe and Rising, 1972a). The hypoglycemic effect of methylenecyclopropylacetic acid (from hypoglycin) has been explained as a relative increase in glucose utilization in face of the impairment of fatty acid oxidation (von Holt et al., 1966).

As already discussed, inhibition of fatty acid oxidation is a major symptom of toxicity attributable to depletion of free carnitine. Not only is triacylglycerol degradation decreased, but there is actually a net increased synthesis of triacylglycerol as evidenced by lipid deposition in various tissues such as the heart (Bressler, 1970). CPCA (0.2 mM) inhibited the oxidation of [1-^{14}C]palmitate and [1-^{14}C]-acetate by liver mitochondria of the rat and guinea pig in vitro (Duncombe and Rising, 1972b). However, there are conflicting reports concerning the effects on ketone body levels. Senior et al. (1968) found that CPCA strongly inhibited acetoacetate *formation* (hence, inhibitor of ketosis) from medium-chain fatty acids in rat liver mitochondria, but their incubation medium contained DL-carnitine. CPCA and hypoglycin (precursor of methylenecyclopropylacetic acid) caused ketosis in rats in vivo and an increase in the acetoacetate/β-hydroxybutyrate ratio (Williamson and Wilson, 1965; similar data for CPCA and 4-pentenoic acid, Senior and Sherratt, 1969). The ketosis induced by CPCA (and hypoglycin) was rationalized as largely the result of inhibition of acetoacetate *utilization*.

Table III. Analysis of Kidney from Dogs Treated with [*carboxyl*-^{14}C]Cycloprate

	% total ^{14}C in kidney
Extractable ($CHCl_3$-CH_3OH)	95
Cycloprate	0.1
Triacylglycerol	2-5
ω-Cyclopropyl fatty acids (free)	13
16(13cPr):0[a]	6
18(15cPr):0	3
CPCA (free)	7
Phospholipid	67
Phosphoglyceride	49
1 position acids (lipase liberated)	19
16(13cPr):0	7
18(15cPr):0	2
CPCA	3
2 position acids (phospholipase A_2 liberated)	27
16(13cPr):0	12
18(15cPr):0	3
CPCA	3
Residual solids	5

[a] For structures of ω-cyclopropyl fatty acids see Quistad et al., 1978. Full names of such acids are abbreviated as follows: 13-cyclopropyltridecanoic acid, 16(13cPr):0 (= 16 carbon atoms, no unsaturation).

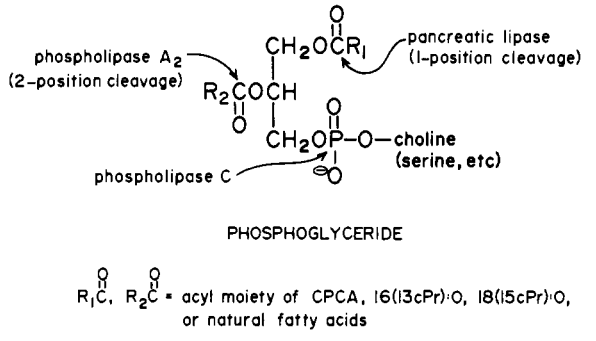

Figure 4. Phosphoglyceride and enzymatic cleavage reactions.

Kidney. In further contrast to rat and cow metabolism of cycloprate (Quistad et al., 1978), the dog retained considerable ^{14}C in the kidneys (7% applied dose) even after 4 days. Free CPCA and ω-cyclopropyl fatty acids represented 7 and 13% of the total kidney ^{14}C (Table III). Two-thirds of the kidney ^{14}C was characterized as a mixture of [^{14}C]phospholipids by treatment with pancreatic lipase and phospholipases A_2 and C (for enzyme specificities see Christie, 1973). Phospholipase C cleaved half of the total kidney ^{14}C to [^{14}C]diacylglycerols, and this result strongly implicates [^{14}C]phosphoglycerides as the major ^{14}C residues. The [^{14}C]phosphoglycerides could be resolved by TLC into two approximately equal zones, one of which had polarity similar to authentic dipalmitoyl L-α-phosphatidylcholine. Using phospholipase A_2 (cleaves only the 2 position of phosphoglyceride) and pancreatic lipase (cleaves only the 1 position) we determined that (1) CPCA was found in equal amounts bound to the 1 and 2 positions and (2) slightly more ω-cyclopropyl fatty acids were bound at position 2, but substantial amounts were found also at position 1 (Figure 4). In summary, most of the ^{14}C residue in kidney was characterized as CPCA and ω-cyclopropyl fatty acids which were present as free acids, but more abundantly as [^{14}C]phosphoglycerides.

Hide, Fat, and Liver. Both the hide and fat were calculated to contain 6% of the applied dose (Table IV).

Table IV. Distribution of ^{14}C Residues in Tissues of Dogs Treated at 0.7 mg/kg with [*carboxyl*-^{14}C]Cycloprate

	% total ^{14}C		
	Hide	Fat (subcut.)	Liver
Extractable ($CHCl_3$-CH_3OH)	97	99	67
Cholesterol esters	6	<1	<1
Cycloprate	4	25	<1
Triacylglycerol	67	62	2.3
14(11cPr):0	4.1	4.4	
16(13cPr):0	31	40	
18(15cPr):0	20	12	
20(17cPr):0	2.0	<1	
>20(17cPr):0	4.3		
Triacylglycerol with CPCA	3		
CPCA (free)			12
ω-Cyclopropyl fatty acids (free)			12
14(11cPr):0			0.5
16(13cPr):0			6.0
18(15cPr):0			3.8
Phosphoglyceride			28
Residual Solids	3	1	33

70% of the ^{14}C residue in hide was assigned to [^{14}C]triacylglycerols which could be separated into three radioactive zones, i.e., triacylglycerol containing (1) CPCA, (2) ω-cyclopropyl fatty acids plus common natural acids, and (3) ω-cyclopropyl fatty acids plus long and/or branched-chain natural acids. Mass spectrometry of the latter, least-polar class of natural fatty acids, showed that 56% of the acids had a molecular weight greater than stearic acid. The ^{14}C residue in subcutaneous fat consisted mainly of [^{14}C]triacylglycerol (62%), but one-fourth of the ^{14}C was contributed by cycloprate (Table IV). This is the highest relative concentration of cycloprate found in any mammalian tissue yet examined (cf. Quistad et al., 1978).

Liver contained 1.6% of the applied dose at the 4-day sacrifice. The radio-TLC profile of the ^{14}C residue strongly resembled that of the kidney with most of the ^{14}C present as CPCA and ω-cyclopropyl fatty acids (both free and conjugated as phosphoglyceride). Since carnitine is biosynthesized in the liver (Tyihak et al., 1977), we expected CPCA-carnitine, but were unable to find it (<0.1% applied dose).

CONCLUSIONS

Metabolism of [*carboxyl*-^{14}C]cycloprate by the dog was noticeably different from that in the lactating cow and rat in four major respects. First, a relatively high proportion of the ^{14}C in dog adipose tissue consisted of unmetabolized cycloprate. Secondly, much more radiolabel in the dog was retained in kidney (7% applied dose) and this ^{14}C consisted predominantly of a unique class of [^{14}C]phosphoglycerides. Thirdly, CPCA-carnitine was the major urinary metabolite in dogs (instead of CPCA-gly). Fourthly, and most importantly, 56% of the applied dose was found in dog muscle as CPCA-carnitine. It is this last difference which we think is responsible for the greater sensitivity of dogs vs. rats in subacute toxicology studies with cycloprate. To our knowledge, this work presents the first evidence of carnitine's role in pesticide metabolism. We also suggest that a careful perusal of data may reveal acylcarnitines in unknown polar fractions for other pesticides capable of generating acidic metabolites (e.g., pyrethroids and 2,4-D analogues).

ACKNOWLEDGMENT

The preliminary dosage, handling, sacrifice, and some ^{14}C quantitation were performed under contract to Zoecon by D. C. Jessup, W. P. Dean, and J. Thorstenson (International Research and Development Corp.). We thank B. Rice (Syntex Corp.) for dosage and sacifice of a third dog. We also thank M. A. Ratcliff for mass spectral analysis, M. Maddox and L. Tökes (Syntex Corp.) for ^1H NMR analysis, and T. L. Burkoth for useful discussions.

LITERATURE CITED

Anderson, A. C., Goldman, M., in "The Beagle as an Experimental Animal", Anderson, A. C., Ed., Iowa State University Press, Ames, Iowa, 1970, p 83.
Bressler, R., in "Lipid Metabolism", Wakil, S. J., Ed., Academic Press, New York, N.Y., 1970, p 49.
Broekhuysen, J., Deltour, G., *Ann. Biol. Clin. (Paris)* **19**, 549 (1961).
Christie, W. W., "Lipid Analysis", Pergamon Press, Oxford, 1973, p 261.
Corredor, C., Brendel, K., Bressler, R., *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2299 (1967).
Davis, C. N., Davis, L. E., Powers, T. E., *Am. J. Vet. Res.* **36**, 309 (1975).
Duncombe, W. G., Rising, T. J., *Biochem. Pharmacol.* **21**, 1089 (1972a).
Duncombe, W. G., Rising, T. J., *Biochem. Pharmacol.* **21**, 1075 (1972b).
Entman, M., Bressler, R., *Mol. Pharmacol.* **3**, 333 (1967).
Fraenkel, G., in "Vitamins and Hormones", Harris, R. S., Marian, G. F., Thimann, K. V., Ed., Academic Press, New York, N.Y., 1957, p 73.
Fritz, I. B., *Adv. Lipid Res.* **1**, 285 (1963).
Guilbert, C. C., Chung, A. E., *J. Biol. Chem.* **249**, 1026 (1974).
Henrick, C. A., Staal, G. B., U.S. Patent 3 925 460, Dec 9, 1975.
Holt, von C., Holt, von M., Böhm, H., *Biochim. Biophys. Acta* **125**, 11 (1966).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **22**, 582 (1974).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.*, preceding papers this issue, 1978.
Senior, A. E., Robson, B., Sherratt, H.S.A., *Biochem. J.* **110**, 511 (1968).
Senior, A. E., Sherratt, H. S. A., *J. Pharm. Pharmacol.* **21**, 85 (1969).
Stewart, G. A., *Dtsch.-Engl. Med. Rundsch.* **1**, 334 (1962).
Tyihak, E., Szende, B., Lapis, K., *Life Sci.* **20**, 385 (1977).
Williamson, D. H., Wilson, M. B., *Biochem. J.* **94**, 19C (1965).
Yang, R. S. H., in "Insecticide Biochemistry and Toxicology", Wilkinson, C. F., Plenum Press, New York, N.Y., 1976, p 177.

Received for review May 16, 1977. Accepted July 25, 1977. Contribution No. 59 Zoecon Research Laboratory.