seeded at high initial population densities (10^5 cell/cm^2). Seventeen percent of the cells attached, but only 5 percent remained by day 5. The alkaline phosphatase activity was low (28.3 ± 5.3 μmole per hour per culture) in comparison with that of cell cultures prepared from subperiosteal bone. Fibroblasts obtained by collagenase digestion of fetal rat skin died over the initial 2- to 3-day period in culture.

Growth and apparent differentiation at a high, but not at a low, initial population density may reflect the continuing action of tissue or serum growth factors, or both, that adhered to the cells during preparation. Alternate explanations include (i) the elaboration by cultured cells of their own growth factors or essential nutrients that are absent from the medium (*19*) and (ii) inactivation by the larger cell mass of toxic substances in the medium (*20*). The latter possibility would explain not only the failure of cells to proliferate when cultured at low density but also the low plating efficiency. Although there was appreciable cell death during the first 24 hours in culture, stimulation of proliferation cannot be attributed easily to growth-promoting factors released into the incubation medium by the dying cells. The incubation medium was replaced completely at 24 hours (Fig. 1), whereas proliferation was evident long afterward. Moreover, fibroblasts and periosteal cells did not proliferate, despite greater initial cell attrition. The system described herein should permit clarification of the mechanism of density-dependent proliferation and an examination of the effects of growth factors on the proliferation and differentiation of bone cells in vitro in the absence of complex and ill-defined serum additives.

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Cyclopropanecarboxylic Acid: Chain Elongation to ω-Cyclopropyl Fatty Acids by Mammals and Plants

Abstract. *Rats dosed orally with* [carboxyl-^{14}C]*cyclopropanecarboxylic acid (or its hexadecyl ester) retain radioactivity in tissue as novel triacylglycerols. The most abundant* ^{14}*C-labeled metabolites were identified by gas-liquid chromatography–mass spectrometry as 13-cyclopropyltridecanoic and 15-cyclopropylpentadecanoic acids. Similar ω-cyclopropyl fatty acids are produced by beagle dogs and a lactating cow, as well as by apple and orange trees.*

Certain esters of cyclopropanecarboxylic acid (CPCA) are selectively toxic to phytophagous Acarina (spider mites) without significant insecticidal activity (*1, 2*). We have studied the metabolic fate of the hexadecyl ester of CPCA (cycloprate), which is currently under commercial development as a miticide. We have identified unusual metabolites arising from the apparent entry of CPCA into pathways of fatty acid anabolism, with net additions of acetate to give a homologous series of ω-cyclopropyl fatty acids.

Duncombe and Aising (*3*) studied the metabolism of ^{14}C-labeled CPCA in rat tissue in vitro and postulated the formation of unsaturated ω-cyclopropyl fatty acids, but were unable to identify these products. Linscott *et al.* (*4, 5*) have shown that plants can elongate the carbon chains of 2,4-dichlorophenoxyalkanoic [for example, -acetic (2,4-D) and -butyric] acids by insertion of pairs of methylene groups. The major metabolites of these acids were identified as resulting from addition of one to three acetate units (that is, two to six CH_2 groups), whereas we find addition of up to eight acetates to CPCA. We know of

Table 1. Quantitative abundance of ω-cyclopropyl fatty acids, as a percent of the administered dose of [^{14}C]cycloprate, in portions of various organisms that were analyzed (*6*).

ω-Cyclopropyl fatty acids*	Rat carcass	Cow milk	Dog carcass	Apple fruit	Orange fruit
8(5cPr) : 0		0.2			
10(7cPr) : 0		0.3			
12(9cPr) : 0		0.3			
14(11cPr) : 0	1.0	0.7	0.4		
16(13cPr) : 0	9.9	1.6	6.5		
18(15cPr) : 0	2.5	0.2	2.7	4.5	7.6
18(15cPr) : 1				1.9	3.7
20(17cPr) : 1					0.3

*Structural abbreviations are explained in the legend to Fig. 1.

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no other precedents for such metabolism of a xenobiotic by mammals or higher plants. This unusual chain-elongation results in an apparent persistence of residues from an alicyclic acid which a priori would seem readily amenable to excretion.

We first identified conclusively such metabolites from tissue of a rat dosed orally with [*carboxyl*-^{14}C]cycloprate (738 mg/kg, 0.5 mCi). Extracts of tissues (4 days after treatment) with organic solvents contained ^{14}C associated with triacylglycerol (up to 80 percent of tissue ^{14}C, or 14 percent of the applied dose) as determined by thin-layer chromatography (TLC). Transesterification of this lipophilic, neutral fraction gave ^{14}C-labeled fatty acid methyl esters, which were purified on $AgNO_3$-impregnated TLC plates (developed with a mixture of hexane and ether, 95 : 5), followed by reversed-phase high-resolution liquid chromatography (HRLC) (μBondapak-C_{18}; eluted with 85 percent methanol in water) and normal-phase HRLC (Zorbax-SIL eluted with 0.5 percent ether in hexane). The two ^{14}C-labeled esters thus obtained were analyzed separately by gas-liquid chromatography–mass spectrometry and found to be identical to synthetic standards (*2*) of the methyl esters of 13-cyclopropyltridecanoic acid [16(13cPr) : 0] and 15-cyclopropylpentadecanoic acid [18(15cPr) : 0] (Fig. 1). In addition, smaller amounts of a C_{14} homolog [that is, 14(11cPr) : 0] were identified by reversed-phase HRLC analysis. The same acids in similar quantities are formed by rat metabolism of [*carboxyl*-^{14}C]CPCA itself, indicating that CPCA is an obligatory precursor in those experiments where cycloprate was used. In contrast to the suggestion of unsaturated acids by Duncombe and Rising (*3*), only saturated ω-cyclopropyl fatty acids are produced by rats in vivo.

These acids are also formed at lower dose rates, although the abundance (as a percent of ingested dose) decreases with lowered dose (*6*). Excretion studies for 4 days and for 60 days in rats showed that ω-cyclopropyl fatty acids are ultimately excreted, with a turnover time quite similar to that of natural fatty acids (*6*). Dogs and a lactating cow metabolize [^{14}C]cycloprate to similar acids (*6*), with cow milk containing lower homologs (Table 1).

In contrast to mammals, foliage or fruit of apple and orange trees metabolize [*carboxyl*-^{14}C]cycloprate to homologous saturated and unsaturated ω-cyclopropyl fatty acids (*6*) (Table 1). Both CPCA and chain-extended metabolites are present in foliage and fruit cuticle

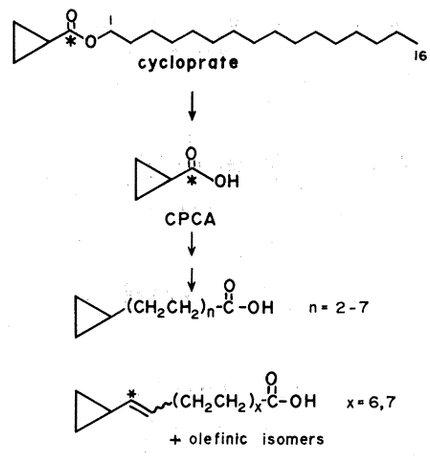

Fig. 1. Structural formulas of ω-cyclopropyl fatty acid metabolites of CPCA and cycloprate. Asterisk denotes the site of the ^{14}C label. Structural abbreviations are modified from standard lipid nomenclature: 13-cyclopropyltridecanoic acid (n = 6), 16(13cPr) : 0; 15-cyclopropylpentadecenoic acid (x = 6), 18(15cPr) : 1.

predominantly as polar conjugates that are cleaved by saponification. Analysis of the methyl esters of plant ω-cyclopropyl fatty acids by $AgNO_3$-TLC showed that unsaturated plant acids are mainly monoenoic, with apparently some trienoic, but no dienoic acids. Analysis of monoenoic methyl esters by reversed-phase HRLC and ozonolysis revealed several double bond isomers of 15-cyclopropylpentadecenoic acids, and smaller amounts of 17-cyclopropylheptadecenoic acid.

The biosynthesis of these novel ω-cyclopropyl fatty acids by two different plants (*6*), three mammalian species (*6*), and bacteria (*7*) suggests a fairly general metabolic pathway for CPCA. In contrast, the generality of the chain-elongation for other alicyclic acids has not been demonstrated. For example, (2-cyclopentenyl)carboxylic acid is converted to longer-chain products by an alga (*8*), but not by higher plants except for those few species that contain the analogous ω-alicyclic fatty acids as natural products (*9*).

Our results assume additional practical relevance since functionality that should generate CPCA on catabolism is present in xenobiotics other than cycloprate, such as cyproquinate (a coccidiostat), cypromid (a herbicide), prazepam (a tranquilizer) and several narcotic antagonists (naltrexone, cyclazocine, oxilorphan, diprenorphine, and buprenorphine). Possibly this metabolic reaction occurs with acidic metabolites of xenobiotics other than CPCA, but may have escaped detection because of lower quantitative importance or difficulty of isolation of tissue metabolites (or both).

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Lichen Myxedematosus Serum Stimulates Human Skin Fibroblast Proliferation

Abstract. *Serum from patients with lichen myxedematosus, when added to exponentially growing normal human skin fibroblasts, stimulates DNA synthesis and cell proliferation. The degree of response in vitro is correlated with the extent of the disease in vivo and is specific for fibroblasts. The results suggest that there is a systemic factor (or factors) which may play a role in the etiology of diseases affecting the connective tissue.*

Lichen myxedematosus, a rare but well-defined clinical entity of unknown etiology, is a connective tissue disorder appearing in the skin which is characterized by proliferation of fibroblasts with a concomitant increase in acid mucopolysaccharides (*1*). The disease, at least in the initial stages, is confined to the upper dermis (papillary). The disease may consist of localized papules (papular mucinosis) or may be generalized, involving the whole integument (scleromyxedema). In reported cases of generalized lichen myxedematosus death commonly

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