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

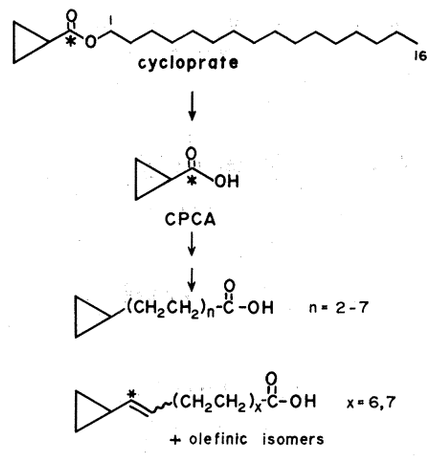

Fig. 1. Structural formulas of ω-cyclopropyl fatty acid metabolites of CPCA and cycloprate. Asterisk denotes the site of the ^{14}C label. Structural abbreviations are modified from standard lipid nomenclature: 13-cyclopropyltridecanoic acid (n = 6), 16(13cPr) : 0; 15-cyclopropylpentadecenoic acid (x = 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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10. Contribution 60 from Zoecon Research Laboratories.

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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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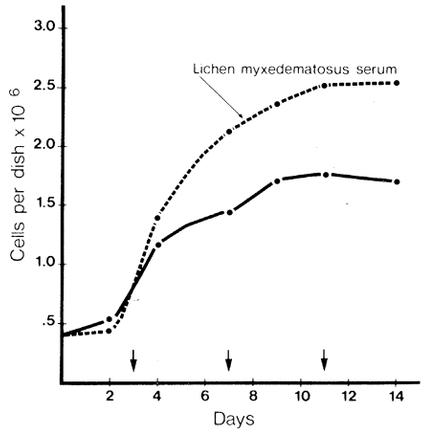

Fig. 1. Effect of lichen myxedematosus serum on the growth of normal human skin fibroblasts. Cells were plated into 100-mm Falcon culture dishes at approximately 4×10^5 cells per dish. Control dishes (solid line) contained culture fluid plus 10 percent (final concentration) normal human serum. Test dishes (dashed line) received culture fluid and 10 percent lichen myxedematosus serum. At the time indicated, duplicate dishes were removed and the cell numbers were determined with a hemocytometer. At the times indicated by the arrows, the medium in each set of cultures was removed and fresh culture fluid plus the appropriate serum (10 percent) was added.

occurred, and where autopsies were performed mucin deposits were found in the heart, brain, and kidney (*2*), indicating that the disease may be systemic rather than solely cutaneous. In the serum of most patients with lichen myxedematosus there appears an immunoglobulin G (IgG) paraprotein similar to the long-acting thyroid stimulator, a 7*S* gamma globulin (IgG) present in patients with hyperthyroidism (*3*). However, no abnormalities of thyroid function are present in lichen myxedematosus. In the majority of lichen myxedematosus cases reported, the IgG paraprotein contains lambda-type light chains. This particular globulin has been purified and biochemically characterized (*4*). However, a few cases of the disease have been reported in which the IgG paraprotein contained kappa-type light chains (*5*), and in at least one reported case the serum contained no IgG paraprotein (*6*). The relationship, if any, between this abnormal serum globulin and the cause of the disease is unknown.

Experiments were performed to determine whether the serum from patients with lichen myxedematosus contained a factor (or factors) which could stimulate normal skin fibroblast proliferation. Whole-thickness skin was obtained at surgery from a 45-year-old black female (experiment I in Table 1), a 48-year-old white male (experiment II), and a 50-year-old white male (experiment III). The upper (papillary) dermis was mechanically removed and explanted onto the bottom of a 60-mm Falcon culture dish, using a plasma clot. To the dishes were added 6.0 ml of culture fluid (Eagle's minimum essential medium containing 10 percent fetal calf serum and 100 units of penicillin and streptomycin and 50 units of mycostatin per milliliter). Cells were allowed to propagate for 30 days in primary culture maintained at 37°C in a high-humidity incubator containing a mixture of 5 percent CO_2 in air. At the end of 30 days the cells were trypsinized (0.25 percent trypsin) and passed into 100-mm Falcon dishes. Cells were trypsinized every 7 days thereafter, with 400,000 cells seeded per dish. All experiments were carried out with 48- to 50-day-old cultures which represented three passages with trypsin and approximately 13 to 15 doublings in vitro.

Blood was obtained from four patients (1 to 4 in Table 1), who had clinically and histologically confirmed lichen myxedematosus, and allowed to clot at room temperature (~27°C) for 1 hour. Whole blood serum was removed and quickly frozen at −85°C until used. Normal human serum, used as the control, was purchased from Grand Island Biological Company, New York, and kept at −85°C until used. This serum represented donors of mixed sex and Rh specificity and was free of mycoplasm.

Results of the addition of lichen myxedematosus serum to exponentially growing normal human skin fibroblasts are shown in Table 1. In each case lichen myxedematosus serum stimulated [^{3}H]thymidine uptake. Because of lack of serum, experiments could not be repeated on normal fibroblasts derived from different donors, except in the case of patients 1 and 3. Although there was some variation in degree of response, the test values were always significantly higher than the control values. When lichen myxedematosus serum was added to primary cell cultures of normal human keratinocytes (epithelial cells), no increase in [^{3}H]thymidine uptake was observed. The results also indicate that the degree of [^{3}H]thymidine stimulation may be dependent on the severity of the disease. Patients 1 and 2 had disseminated disease in which the integument had acquired the typical hardened pachydermatous appearance, while the skin of patients 3 and 4 contained only isolated areas of papular eruptions.

To establish that lichen myxedematosus serum contains a factor that stimulates normal skin fibroblast proliferation and not just thymidine transport, a growth curve was obtained (Fig. 1),

Table 1. Effect of lichen myxedematosus serum on [^{3}H]thymidine uptake in normal human skin fibroblasts in vitro. All experiments were carried out in duplicate. Initially, 4×10^5 cells were seeded in 100-mm Falcon culture dishes. On the third day, the used culture fluid was removed and fresh culture fluid without serum was added along with 10 percent (final concentration) normal human serum to the control dishes and 10 percent lichen myxedematosus serum to the test dishes. At the end of 24 hours, all dishes were exposed to [^{3}H]thymidine for 1 hour (Amersham/Searle, 8.3 μCi/ml; specific activity, 20 Ci/mmole). At the end of the exposure, the culture fluid was immediately removed and the cells were washed three times with ice-cold Gey's balanced salt solution. The nucleic acids were then extracted from the cells by a modification of the method of Schmidt and Thannhauser (*9*). The DNA was quantitated by the diphenylamine reaction of Burton (*10*). All radioactivity was counted in a Searle Mark III liquid scintillation counter with a counting efficiency of 57 percent for tritium. Specific activity was calculated as counts per minute (cpm) per microgram of DNA as found in the fraction extracted with hot trichloroacetic acid. Data were statistically analyzed by Student's *t*-test for paired values.

Patient	Variant	Specific activity (cpm/μg)		Increase over control (%)
		Control	Test	
1	Scleromyxedema			
	Experiment I	38,575	97,913	154
	Experiment II	46,882	92,731	98
	Experiment III	33,504	65,667	97
	Mean ± S.D.	39,654 ± 6,754	85,437 ± 17,316*	
2	Scleromyxedema			
	Experiment I	38,575	79,907	107
3	Papular mucinosis			
	Experiment I	38,575	71,797	86
	Experiment II	46,882	73,710	57
	Mean ± S.D.	42,729 ± 5,874	72,754 ± 1,353†	
4	Papular mucinosis			
	Experiment I	38,575	63,363	64

*Significantly different from control at $P < .005$. †Significantly different from control at $P < .01$.

using the serum from patient 1 (Table 1). The results clearly show that addition of lichen myxedematosus serum to normal skin fibroblast cultures results in an increase in the number of cells at saturation density.

To determine whether the IgG paraprotein found in the serums of the majority of patients with lichen myxedematosus contributed to the in vitro results obtained in this study, the serums of the four patients were subjected to immunoelectrophoresis. Serum from patient 1 contained the IgG paraprotein with lambda-type light chains, serums from patients 2 and 4 contained the IgG paraprotein with kappa-type light chains, and serum from patient 3 did not show the abnormal globulin. Since serum from patient 3 did not contain the IgG paraprotein but stimulated fibroblast DNA synthesis, one might conclude that the abnormal globulin is not responsible for the results obtained in vitro. To further test this conclusion, lichen myxedematosus serum containing the IgG paraprotein was applied to a protein A–Sepharose CL-4B column. The IgG fraction was bound to the column and eluted with 3*M* potassium isothiocyanate. It was then dialyzed against isotonic saline and added to normal fibroblast cultures. The effect on [^{3}H]thymidine uptake was then measured as described in the legend of Table 1. No stimulatory activity was detected. This would seem to eliminate the possibility that the IgG paraprotein was responsible for the increased fibroblast proliferation.

Lichen myxedematosus is one of a number of diseases affecting the connective tissue. Preliminary data from this laboratory indicate that the serums from patients with progressive systemic sclerosis (scleroderma) also stimulate normal fibroblast proliferation in vitro. This finding is especially interesting since the earliest histological findings in scleroderma include endothelial cell hyperplasia and an increase in mucopolysaccharides in the dermis (*7*). Also, Cheung *et al.* (*8*) recently reported a fibroblast-stimulating factor from the serums of patients with pretibial myxedema that stimulated normal human fibroblast mucopolysaccharide biosynthesis. Whether there is a common factor in the etiology of these diseases is unknown. Characterization of the serum factor from lichen myxedematosus should yield valuable information concerning its role in connective tissue diseases.

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Structure of Collagen in Cartilage of Intervertebral Disk

Abstract. *Small-angle x-ray and neutron diffraction patterns have been obtained from the annulus fibrosus of porcine intervertebral disk. These show that the collagen in this tissue is modified compared with that in tendon.*

Small-angle x-ray and neutron diffraction patterns have been obtained from the annulus fibrosus of intervertebral disk. These show that collagen fibrils are wound around the annulus fibrosus at a specific orientation to the axis of the vertebral column and that the one-dimensional structure of these fibrils is different from that of the collagen fibrils in rat tail tendon.

It has been shown by wide-angle x-ray diffraction that in normal articular cartilage, collagen occurs in a disoriented array (*1*) except at the cartilage surface. In certain pathological inclusions of intercostal cartilage, it has been demonstrated by the same techniques, that the collagen fibrils become oriented parallel to each other (*2*). To our knowledge, no small-angle x-ray diffraction patterns have been reported from normal cartilaginous tissue.

Intervertebral disk contains three anatomically distinct regions, the cartilaginous end plates, the hydrated gel-like nucleus pulposus, and the fibrocartilaginous annulus fibrosus. The latter structure connects the neighboring vertebrae and is comprised of 10 to 15 lamellae. When the vertebral column is subjected to axial load, much of this is taken up by deformation of the annulus fibrosus (*3–6*). The lamellae are concentric cylindrical sheets and polarization microscopy has shown that the collagen fibers are oriented at 40° to 70° to the vertebral axis with opposite directions of tilt occurring in adjacent lamellae (*6*, figure 10). The deformation caused by axial load results in the annulus fibrosus bulging in such a way that the angle between the axis of the collagen fibers and the vertebral axis is increased. It has been shown by electron microscopy that the

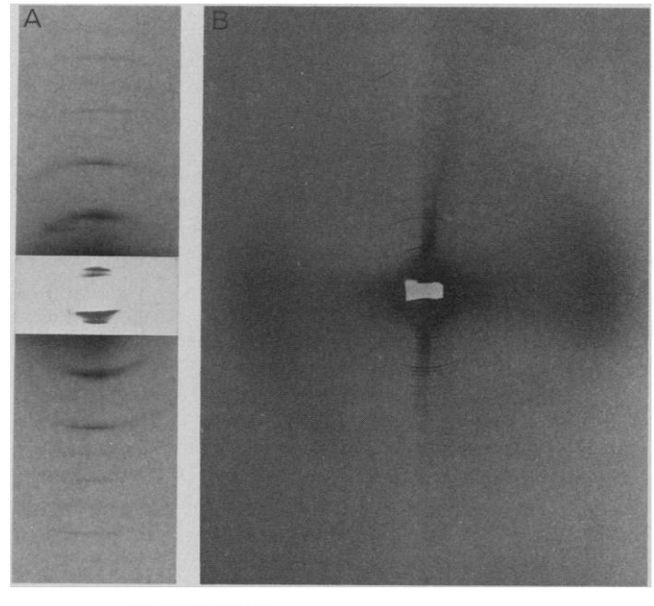

Fig. 1. (A) Low-angle x-ray diffraction pattern from structural fibers of porcine annulus fibrosus. X-rays were collimated by a mirror-monochromator camera (wavelength, 1.5405 Å). The specimen-film distance was 80 cm. Orders 1, 3, 5, and 7 to 10 of the 670 Å period from collagen are visible. Note the low exposure to show first order. (B) Medium-angle x-ray diffraction pattern from the same specimen. The specimen-film distance was 17.6 cm. Reflections up to order 22 from the 670 Å period of collagen may be seen.

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