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K. J. Friedman
New Jersey Medical School

D. Glick
New Jersey Medical School

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Abstract

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Friedman, K. J. and D. Glick.

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Several analyses (Friedman 1977 J. Membr. Biol. 32: 33; Kushwaha and Kates 1976 Lipids 1: 778) of the fatty acid composition of Neurospora indicate that a small number of fatty acids serve as the alkyl moieties of Neurospora phospholipids. Using the *cel* mutant (FGSC #165), and fatty-acid supplemented media, it has been possible to change the proportions of the fatty acids present in the phorpholipid profile and to incorporate a branched-chain fatty acid (phytanic acid) normally not present in Neurospora (Brody and Allen 1972 J. Supramolec. Struct. 1: 125). In efforts to radically alter the fatty acid profile, we attempted to incorporate lignoceric acid (C24) into the phospholipids of the *cel* mutant.

Our results (Table 1) suggest that lignoceric acid is degraded mainly into C_{18} and, to a lesser extent, into C_{16} chain lengths prior to incorporation into Neurospora phorpholipids.

Our experimental protocol was similar to that previously employed (Friedman 1977). Lignoceric acid was added to Vogel's minimal medium (containing 20 gms. sucrose/liter) as the Tween detergent (240 mg/liter). Tween-lignoceric acid was synthesized by transesterifying Tween 40 with the methyl ester of lignoceric acid. 1 *cel* cultures were grown on solidified medium at 31° C and

harvested around mid-day. Freeze-dried hyphae were extracted for lipids using chloroform-methanol and a Folch washing procedure. The lipids were then separated into neutral and phorpholipidr via silicic acid column chromatography. Fatty acid methyl esters were mode via a transesterification process and analyzed both quantitatively and qualitatively by gas chromatography. As shown in Table 1, lignoceric acid is a minor constituent of the phospholipid alkyl chains present in hyphoe grown in this manner.

TABLE 1

Fatty acid composition of Neurospora phorpholipids.
(mole per cent)

Fatty acid present	15:0[a]	16:0	16:1	18:0	18:1	18:2	18:3	24:0
cel palmitic acid supplement	----	39.3	1.7	2.3	10.2	35.0	11.5	----
cel lignoceric acid supplement	2.24	15.3	.64	1.05	4.6	57.1	18.6	0.4
wild-type (no supplement)	----	19.4	0.1	.7	4.2	48.3	27.3	----

[a]Identified by retention time.

We have considered several possible explanations of there results. Since the cel mutant requires fatty acid supplementation for growth, we assume that the Tween-lignoceric acid satisfied this nutritional requirement. Since the Tween-lignoceric acid supplement contained 60% of the Tween conjugated to lignoceric acid, it is conceivable that the lignoceric acid was excluded from significant uptake or incorporation, and that the remaining 40% of nonreacted Tween 40 detergent supplied the growth requirements of the cel cultures. If this were the case, however, the fatty acid profile seen for cel growth with Tween-lignoceric acid supplementation would be similar to the profile seen for cel growth with Tween 40 (palmitic acid) supplementation. That this is not the case is shown in Table 1 which indicates that the fatty acid profile of cel grown on Tween-lignoceric acid more closely resembles the profile of wild-type Neurospora than that of cel grown on Tween 40.

Alternative explanations for the fatty acid profile observed for growth with lignoceric acid supplementation all involve some mechanism of fatty acid degradation. While we hove no direct experimental evidence to support any particular degradative mechanism, the literature (reviewed by Weete 1974 Fungal Lipid Biochemistry) favors α oxidation as the probable mechanism of lignoceric acid degradation in Neurospora. Such a system has been found in yeast and is active on fatty acid chain lengths between C_{18} and C_{26} (Fulco 1967 J. Biol. Chem. 242: 3608). β oxidation seems a less likely pathway for lignoceric acid degradation since it is believed (Weete 1974) that the enzymes responsible for β oxidation utilize fatty acid chain lengths between C_4 and C_{12}, or C_8 and C_{18}.

Although we have yet to achieve radical alteration of the fatty acid profile of Neurospora, our data indicate the existence of a metabolic mechanism which will degrade long chain fatty acids, when necessary, to maintain a wild-type-like fatty acid profile in the cel mutant. (Supported by Notional Institute of General Medical Science Grant GM24017.) [1]We are grateful to Dr. Josephine Readio for the custom synthesis of Tween-lignoceric acid. - - - Department of Physiology, New Jersey Medical School, 100 Bergen Street, Newark, NJ 07103.