FURTHER STUDIES ON THE BIOSYNTHESIS OF FATTY ACIDS IN THE CELLULAR SLIME MOLD

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In a previous communication (Davidoff and Korn, 1962,a) we reported the presence in D. discoideum of the unsaturated fatty acids 5,9-hexadecadienoic acid, 16:2(5,9)[1] and 5,11-octadecadienoic acid, 18:2(5,11), in addition to palmitoleic acid, 16:1(9), and vaccenic acid, 18:1(11). Oleic acid, 18:1(9) and 5,9-octadecadienoic acid 18:2(5,9) have also been observed in varying amounts. Preliminary data were reported on the synthesis of these acids from 1-C^{14}-octanoate, -decanoate and -stearate (Davidoff and Korn, 1962,b). Similar experiments have now been performed with 1-C^{14}-acetate, -hexanoate, -laurate, -myristate and -palmitate. The present results confirm the previous observations, but indicate the mechanism previously proposed for conversion of short chain fatty acids to long chain unsaturated fatty acids is unnecessary.

Hexanoate, octanoate and decanoate appeared to be degraded to two-carbon units and, therefore, metabolized in the same way as acetate which was incorporated into the carboxyl ends of the C-16 and C-18 unsaturated fatty acids. Labelled laurate, myristate and palmitate were incorporated directly into longer chain unsaturated fatty acids by elongation at the carboxyl end of the molecules.

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[1] The first number refers to the number of carbon atoms in the fatty acid chain, the second to the number of double bonds in the molecule, and the number in parenthesis to the position of the double bonds, counting from the carboxyl end.

METHODS

Cells of D. discoideum, aggregateless mutant 204, were grown on E. coli, previously extracted with methanol and ether, suspended and autoclaved in 0.04 M phosphate buffer, pH 6.0, to which was then added penicillin and Dow Antifoam AF. After 24 hours incubation with individual isotopic precursors, the cells were harvested, washed, and the lipids extracted as described previously (Davidoff and Korn, 1962,b). Lipids were transesterified at 65° in 9% H_2SO_4-methanol overnight. The distribution of radioactivity among the fatty acid methyl esters was determined by preparative gas-liquid chromatography (GLC) on Apiezon M. The effluent gas was divided by a stream-splitter, approximately 5% going to the detector cell, the remainder to the collecting outlet. The fractions were collected on anthracene coated with silicone oil, and counted directly in a scintillation spectrometer, according to the technique of Karmen et al. (1962). Recovery of radioactivity was 50-70%.

Individual fatty acid fractions were isolated and purified by silicic acid chromatography of their mercuric acetate adducts, followed by preparative GLC of the regenerated methyl esters. The distribution of radioactivity within each fatty acid was then determined by oxidative degradation by a modification of the technique of von Rudloff (1956) followed by analysis of the methyl esters of the oxidation products for mass and radioactivity by GLC as described above; recovery of radioactivity was approximately 35%. In addition, each intact acid was decarboxylated according to the technique of Brady et al. (1960), and the carbon dioxide collected quantitatively in Hyamine and counted.

RESULTS AND DISCUSSION

As determined by the pattern of incorporation of radioactivity into the fatty acid 18:2(5,11), the precursor fatty acids fell into three groups according to chain length (Table I).

TABLE I

Distribution of Radioactivity in Fatty Acid 18:2(5,11)

Precursor**	Carbons Counted* C C C C C C C = C C C C C C = C C C C COOH (COOH)			
	18 - 12	11 - 7	5 - 1	1
	Percent of Total Radioactivity			
Acetate	6	13	81	59
Hexanoate	10	12	78	53
Octanoate	6	10	84	57
Decanoate	0	15	85	53
Laurate	4	52	44	26
Myristate	2	3	95	13
Palmitate	1	2	97	5

* Carbon 1 was isolated as CO_2 following decarboxylation (Brady et al.,1960). Carbons 1-5, 6-11 and 12-18 were isolated as glutaric, adipic and heptanoic acids, respectively, following oxidative degradation (von Rudloff, 1956). The percent of total radioactivity was calculated from the specific radioactivities.

** All 1-C^{14}-labelled.

1) With 1-C^{14}-acetate, -hexanoate, -octanoate, and -decanoate as precursors, approximately 55% of the total radioactivity of 18:2(5,11) was in the carboxyl carbon. Carbons 1 through 5 contained 80% of the total radioactivity; the excess over that in the carboxyl group presumably occurred in carbons 3 and 5. Hence, it would appear that hexanoate, octanoate and decanoate were degraded to, and utilized as, two carbon units which were incorporated into long chain fatty acids by addition to the carboxyl end of pre-existing fatty acids. The sources of the unlabelled fatty acids of intermediate chain length appear to have been residual fatty acids in the extracted bacteria and in Dow Antifoam AF. The very small percentage of radioactivity in carbons 6 through 18 of 18:2(5,11) synthesized in the presence of 1-C^{14}-acetate indicates that under these conditions there was little de novo synthesis of long chain fatty acids from acetate.

2) The radioactivity in 18:2(5,11) derived from 1-C^{14}-laurate was divided approximately equally between carbons 1 through 5 and carbons 6 through 11. The carboxyl carbon accounted for the same proportion of the radioactivity in carbons 1 through 5 as in the experiments with the shorter chain fatty acids. Thus, laurate appeared to serve partially as a source of two carbon units and partially as an acceptor of two carbon units (accounting for the radioactivity in carbons 6 through 11).

3) In the experiments in which 1-C^{14}-myristate and 1-C^{14}-palmitate were the precursors, essentially all of the radioactivity was found in carbons 1 through 5, but only 13% and 5%, respectively, of the total radioactivity was in the carboxyl carbon. Myristate and palmitate, therefore, appeared to have been incorporated into 18:2(5,11) predominantly by addition of two carbon units to the carboxyl end of the molecules.

Some evidence for the stage of biosynthesis at which unsaturation was introduced can be gained from analysis of the radioactivity incorporated into the C-16 and C-18 unsaturated fatty acids (Table II). The efficient conversion of 1-C^{14}-palmitate to 16:1(9) and 16:2(5,9) suggests that the first step in its metabolism is unsaturation at the 9-10 position to form palmitoleate, 16:1(9) which could then be further unsaturated at the 5-6 position to form 16:2(5,9). Conversion of palmitate to vaccenate 18:1(11) might then occur by one of two alternative pathways: a) elongation of palmitate to stearate followed by unsaturation at the 11-12 position, or b) elongation of palmitoleate by addition of a 2-carbon unit to the carboxyl end of the molecule. As reported previously (Davidoff and Korn, 1962,b), 1-C^{14}-stearate was converted in growing cells almost quantitatively to oleate, 18:1(9). Therefore, stearate does not appear to be an intermediate and vaccenate is probably synthesized by elongation of palmitoleate. Vaccenate could then be further unsaturated at the 5-6

position to form 18:2(5,11). There would, thus, be enzymes present in the amebae which introduce double bonds specifically and sequentially at positions 9-10 and 5-6 of C-16 and C-18 fatty acids. In agreement with this proposed pathway, 18:2(5,9) was the only radioactive diunsaturated fatty acid formed from uniformly labelled stearate. This hypothesis may be summarized schematically as follows:

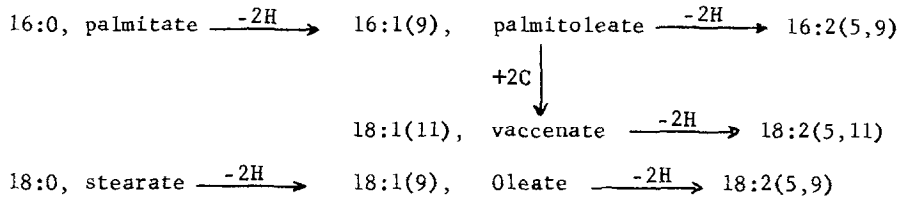

TABLE II

Incorporation of Fatty Acid Precursors into the Major Fatty Acids of *D. discoideum*

Precursor*	Fatty Acid Fraction					
	16:0**	16:1***	16:2	18:0	18:1****	18:2
	Relative specific activities					
Acetate	13	17	19	6	29	35
Hexanoate	3	11	18	4	29	30
Octanoate	3	7	11	14	26	21
Decanoate	2	8	11	6	20	17
Laurate	5	21	35	6	33	33
Myristate	13	48	64	7	52	49
Palmitate	8	11	30	6	21	26

* All 1-C^{14}-labelled.

** The low specific activities of the intracellular palmitate (especially striking where 1-C^{14}-palmitate was the radioactive precursor) may be due to changes in the relative rates of synthesis of the acids during the experiment or to relatively slow uptake of non-radioactive palmitate from the medium (bacterial residue or Dow Antifoam).

*** The specific activities of the 16:1 fractions are only approximate due to incomplete resolution by GLC and the low mass and radioactivity of the fractions.

**** Over 90% of the counts were in vaccenate, 18:1(11), the remainder in oleic acid, 18:1(9).

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