A Novel Mechanism for the Biosynthesis of Unsaturated Fatty Acids*

GÜNTER SCHEUERBRANDT, HOWARD GOLDFINE,† PAUL E. BARONOWSKY,‡ AND KONRAD BLOCH

From the James Bryant Conant Laboratory, Harvard University, Cambridge, Massachusetts

(Received for publication, July 5, 1961)

In animal tissues (1) and yeast (2), oleic and palmitoleic acids are formed by desaturation of the corresponding saturated fatty acids. In yeast, these oxidative transformations require both molecular oxygen and reduced triphosphopyridine nucleotide (2). Realizing that an essential process requiring molecular oxygen cannot operate in anaerobic bacteria, we have considered alternative, anaerobic pathways for the synthesis of unsaturated fatty acids. One of the possibilities, an anaerobic dehydrogenation of the corresponding saturated fatty acids, has been ruled out; growing cells of *Lactobacillus plantarum* or *Clostridium butyricum* do not convert palmitate or stearate to unsaturated acids (3).

A clue to the nature of the anaerobic mechanism was obtained in a study of the metabolism of homologous saturated fatty acids in *C. butyricum*. In this organism, octanoate and decanoate serve as precursors of both saturated and unsaturated long chain acids, whereas laurate and the acids of greater chain length are converted almost exclusively to saturated fatty acids, all these processes involving primarily chain elongation (3). This suggested separate routes from the acids of medium length to the long chain saturated and unsaturated acids, the branch point occurring at the C_{10} and possibly also at the C_8 stage.

The monounsaturated acids of *C. butyricum* consist of two pairs of isomers, Δ^7- and Δ^9-hexadecenoic acids, and Δ^9- and Δ^{11}-octadecenoic acids.[1] To account for these structures, we propose the mechanism shown in the figure. It involves C_2 addition to octanoate or decanoate, followed by a β,γ elimination of water from the presumed β-hydroxy acid intermediates and addition of further C_2 units without reduction of the double bond. As the C_2 units are added, the distance between the double bond and the carboxyl end of the molecule increases. The data in Table I provide experimental support for this mechanism.

C. butyricum was grown in the presence of octanoate-1-C^{14} and decanoate-1-C^{14} and harvested as described previously (3). The whole cells were saponified at reflux temperature in 5% potassium hydroxide (weight per volume)-50% methanol (volume for volume) for 2 hours under nitrogen. The fatty acids were isolated by standard extraction procedures. The average composition of the long chain fatty acids (% of total weight) was: C_{12}, 0.6; C_{14}, 5.0; an unidentified fatty acid, possibly a C_{15}-cyclopropane acid, 9.5; C_{16}, 42.6; C_{16}-monounsaturated, 17.0; C_{17}-cyclopropane acid, 9.1; C_{18}, 2.8; C_{18}-monounsaturated, 3.2; C_{19}-cyclopropane acid, 4.5; and two other unidentified acids, 5.5.

TABLE I

C. butyricum was grown as described previously (3) for 36 hours in the medium of Broquist and Snell (4) with the addition of 0.2 mc of octanoate-1-C^{14} or decanoate-1-C^{14}. In the experiment with octanoate, the yield of total fatty acids was 31 mg, and they contained 0.17% of the added radioactivity. In the experiment with decanoate, the total fatty acids (35 mg) contained 2.63% of the added radioactivity. The methods used for determining specific activities are described in greater detail in other papers from this laboratory (3).[2] The results of individual determinations are given, but additional determinations and an analysis of the sources of error indicate that the over-all standard deviation of the specific activities is ±20%.

Cellular fatty acids isolated	Specific activities	
	Octanoate-1-C^{14}	Decanoate-1-C^{14}
	c.p.m./μg	
Hexadecenoic acids		
Before oxidation	16	60
Oxidation products		
C_7-dicarboxylic acid	0	187
C_9-dicarboxylic acid	25	3
C_7-monocarboxylic acid	0	0
C_9-monocarboxylic acid	5	0
Octadecenoic acids		
Before oxidation	12	70
Oxidation products		
C_9-dicarboxylic acid	0	195
C_{11}-dicarboxylic acid	18	12

After gas chromatographic separation, the hexadecenoate and the octadecenoate fractions, each consisting of the two isomers described above, were individually oxidized by a micro adaptation of the KIO_4-$KMnO_4$ procedure (5), and the pairs of dicarboxylic acids resulting in each case were separated by gas chromatography of the dimethyl esters. The relative proportions of these esters indicated that the monounsaturated fatty acid isomers were present in the following ratios: Δ^7-hexadecenoic acid to Δ^9-hexadecenoic acid, 60:40; and Δ^9-octadecenoic acid to Δ^{11}-octadecenoic acid, 37:63. When octanoate-1-C^{14} was the precursor, only the C_9-dicarboxylic acid from the hexadecenoic acids and the C_{11}-dicarboxylic acid from the octadecenoic acids were radioactive, whereas in the experiment with decanoate-1-C^{14}, C^{14} was found predominantly in the C_7-dicarboxylic acid from the hexadecenoic acids and in the C_9-dicarboxylic acid from the octadecenoic fraction.

These data rule out the direct interconversion of double bond isomers, and show that octanoate is the precursor of the unsaturated acids which contain the double bond between carbon atoms 7 and 8, and that decanoate is the precursor of the two corresponding isomeric acids which have the double bond between carbon atoms 9 and 10, counting from the *methyl* end of the molecule in each case. It will be seen that the eventual location of the double bond is determined by the length of the medium sized acid at the point where the pathways to saturated and

* Supported by grants-in-aid from the National Science Foundation, the United States Public Health Service, the Life Insurance Medical Research Fund, and the Eugene Higgins Trust Fund of Harvard University.

† Scholar of the American Cancer Society.

‡ Predoctoral Research Fellow of the Division of General Medical Sciences, National Institutes of Health.

[1] Unpublished experiments.

[2] W. J. Lennarz, G. Scheuerbrandt, and K. Bloch, J. Biol. Chem., in press.

This is an Open Access article under the CC BY license.

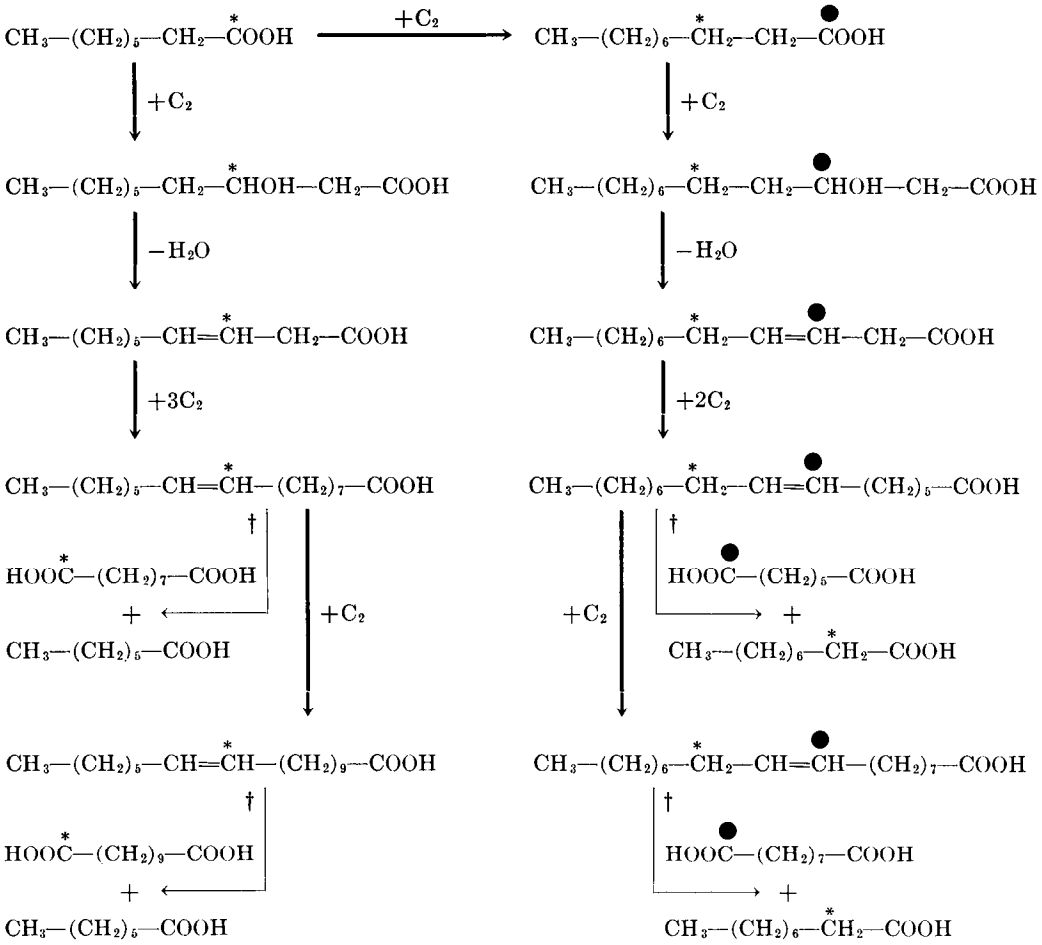

FIG. 1. Conversions of octanoate-1-C^{14} and decanoate-3-C^{14}. *, originates from carbon 1 of octanoic acid; ●, originates from carbon 1 of decanoic acid; †, chemical degradation with KIO_4-$KMnO_4$ (5).

unsaturated acids diverge. Since the chain length of this intermediate is variable, more than one double bond isomer can be produced. This is in contrast to the oxidative mechanism of yeast, which uses the corresponding long chain saturated fatty acid as precursor and affords only a single monounsaturated acid.

The monocarboxylic acids resulting from the degradation of the hexadecenoic acids were also chromatographed in the vapor phase, and their C^{14} contents were analyzed. The proposed mechanism for the synthesis of the unsaturated long chain acids leads to the prediction that no radioactivity would be found in these degradation products. The data in the table show this to be true in all but one case. The nonanoic acid but not the heptanoic acid isolated on degradation of the C_{16}-unsaturated fraction from cells grown with octanoate-1-C^{14} had a small, but measurable amount of C^{14}. This can be explained by the conversion of octanoate-1-C^{14} to decanoate-3-C^{14} in the cells followed by transformation to the Δ^7-C_{16}-unsaturated acid (see Fig. 1).

The present findings provide a mechanistic basis for the observations of Hofmann *et al.* (6) that acids of the general structure $CH_3(CH_2)_5CH{=}CH(CH_2)_nCOOH$ can replace vaccenic acid as growth factors for lactobacilli on biotin-free media. These authors have in fact made the suggestion that "the biosynthesis of unsaturated fatty acids in bacteria may . . . involve elongation of a carbon chain of an already unsaturated or potentially unsaturated precursor."

An anaerobic pathway not involving dehydrogenation of long chain saturated fatty acids has been demonstrated also in *L. plantarum*, *Escherichia coli* and two species of *Sarcina*.[1] It is therefore probable that the above mechanism is generally employed by members of the order Eubacteriales.

REFERENCES

1. SCHOENHEIMER, R., AND RITTENBERG, D., *J. Biol. Chem.*, **113**, 505 (1936).
2. BLOOMFIELD, D. K., AND BLOCH, K., *J. Biol. Chem.*, **235**, 337 (1960).
3. GOLDFINE, H., AND BLOCH, K., *J. Biol. Chem.*, **236**: 2596 (1961).
4. BROQUIST, H. P., AND SNELL, E. E., *J. Biol. Chem.*, **188**, 431 (1951).
5. VON RUDLOFF, E., *Can. J. Chem.*, **34**, 1413 (1956).
6. HOFMANN, K., O'LEARY, W. M., YOHO, C. W., AND LIU, T. -Y., *J. Biol. Chem.*, **234**, 1672 (1959).