INCORPORATION OF ISOBUTYRATE AND VALERATE INTO CELLULAR PLASMALOGEN BY *BACTEROIDES SUCCINOGENES*

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ABSTRACT

WEGNER, G. H. (University of Wisconsin, Madison) AND E. M. FOSTER. Incorporation of isobutyrate and valerate into cellular plasmalogen by *Bacteroides succinogenes*. J. Bacteriol. **85**:53–61. 1963.—*Bacteroides succinogenes* was found to require both a branched-chain volatile fatty acid (e.g., isobutyric) and a straight-chain acid (e.g., valeric) for growth. The organism used the acids as precursors for the synthesis of long-chain fatty acids and fatty aldehydes, which in turn were employed in the synthesis of phospholipid, mainly ethanolamine plasmalogen. Isobutyric acid was incorporated primarily into branched-chain C_{14} and C_{16} acids (tentatively identified as 12-methyl tridecanoic and 14-methyl pentadecanoic acids, respectively), and into fatty aldehydes. Valeric acid was used mainly for the synthesis of n-C_{13} and n-C_{15} fatty acids and fatty aldehydes. Apparently the two short-chain fatty acids were built up by the addition of two-carbon units to form the long-chain acids and aldehydes of the plasmalogen.

Ruminal bacteria such as *Bacteroides succinogenes*, *Ruminococcus flavefaciens* (Sijpesteijn, 1951), *R. albus*, and certain *Borrelia* species require volatile fatty acids for growth (Bryant and Doetsch, 1955; Allison, Bryant, and Doetsch, 1958; Wegner and Foster, 1960). All cultures thus far studied require a branched-chain acid (isobutyric, isovaleric, or α-methylbutyric), and, in addition, certain species need one of the C_5 to C_8 n-acids. A combination of isobutyric and valeric acids satisfies the requirements of *B. succinogenes* S85, which was used in this work.

The purpose of the study was to determine how the acids are used by the organism. The small amounts required (less than 1.0 μmole/ml of medium) suggest that they are employed in the synthesis of a vital cell component. This idea is supported by the results of Allison, Bryant, and Doetsch (1959), who reported that *R. flavefaciens* incorporated isovalerate-1-C^{14} into leucine and an unidentified lipid material. To establish the metabolic fate of the acids, *B. succinogenes* was grown with C^{14}-labeled isobutyrate or valerate. Then the cells were fractionated and the radioactive components identified.

MATERIALS AND METHODS

B. succinogenes S85, and the anaerobic culture methods employed, have been described previously (Bryant and Doetsch, 1954, 1955; Bryant, Robinson, and Chu, 1959). The organism was grown in a basal medium that contained, in g/liter of distilled water: Trypticase (BBL), 10; yeast extract, 2.5; glucose, 5.0; KH_2PO_4, 0.9; $(NH_4)_2SO_4$, 0.9; NaCl, 0.9; $CoCl_2 \cdot 6H_2O$, 0.002; $CaCl_2$, 0.01; $MgCl_2 \cdot 6H_2O$, 0.024; $MnSO_4 \cdot H_2O$, 0.01; Na_2CO_3, 4.0; cysteine $HCl \cdot H_2O$, 1.0; and resazurin, 0.001. Isobutyrate was added at a concentration of 0.05 to 0.3 μmole/ml of medium and valerate from 0.1 to 1.0 μmole/ml of medium. The medium was adjusted to pH 6.6 to 6.7 before autoclaving.

Tracer studies. The organism was grown with either isobutyrate-1-C^{14} and unlabeled valerate, or with valerate-1-C^{14} and unlabeled isobutyrate. When the culture reached the late exponential phase of growth, the cells were harvested by centrifugation and fractionated into lipid, nucleic acid, and protein fractions as described by Roberts et al. (1957), except that 95% ethanol-ethyl ether (3:1; v/v) was used to extract the lipids. The amount of alcohol-ether mixture employed for each of three extractions was about ten times the volume of the packed wet cells. Extraction was accomplished on a magnetic stirrer at room temperature for 20 to 30 min, and the cells were removed by centrifugation.

The alcohol-ether extracts were combined and

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evaporated to about one-tenth of their original volume in a rotary evaporator at room temperature. The resulting watery residue was extracted three or four times with 1 to 2 volumes of petroleum ether (bp 60 to 68 C). The pooled petroleum ether extracts were washed four or five times with small portions of water and then evaporated to dryness. The residue, which contained the extracted lipids, was dissolved in 10 to 25 ml of chloroform and stored at −20 C.

Radioactivity was determined with a Mylar window gas-flow counter. All samples except those from chromatography columns were mixed with equal volumes of 2% gelatin in 0.02 N NaOH, and 1 ml of the mixture was applied to a 1-in. copper planchet. Other samples were examined at infinite thinness to eliminate self-absorption. All counts were corrected for background.

Chromatography. Reverse phase paper chromatography was used to separate the C_{12} to C_{18} saturated fatty acids (Buchanan, 1959). Acetic acid-formic acid (88%)-H_2O_2 (30%)-water (9:1:1:1) was the solvent.

The nitrogen-containing component of the phospholipid was freed by hydrolysis with 6 N HCl for 3 hr at 108 C and identified by paper chromatography essentially as described by Block, LeStrange, and Zweig (1952). The solvent system was butanol-acetic acid-water (75:10:25).

Phospholipids were separated on a silicic acid-Hyflo Super-Cel (2:1; w/w) column adapted from the procedure described by Hanahan, Dittmer, and Warachina (1957).

Aldehyde *p*-nitrophenylhydrazones (aldehyde *p*-NPH), prepared essentially as described by Wittenberg, Korey, and Sevenson (1956), were separated from other C^{14}-labeled material on a silicic acid (Mallinckrodt, A. R.) column. The column was washed successively with 3 to 4 volumes each of methanol, ethyl ether, and petroleum ether before the sample was applied. The aldehyde *p*-NPH were eluted by 50 to 70 ml of 15% ethyl ether in petroleum ether.

Gas chromatography of the methyl esters of fatty acids was performed with a 5-ft column containing 30% Apiezon L on 60 to 80 mesh, acid- and alkali-washed Celite. The operating temperature was 221 C. The methyl esters in the effluent gas were detected with a thermal conductivity detector cell. To confirm further the identity of certain peaks, the methyl esters also were analyzed on a polyester column (20% LAc-3r-728 on 60 to 80 mesh, acid-washed Chromosorb W) at 210 C. Helium was the carrier gas for both columns. The peaks in unknown samples were identified both by comparing their *r* values (retention times relative to methyl stearate) with the *r* values of known fatty acids analyzed on the same column, and by comparing the observed values with published data (Farquhar et al., 1959). The identification of branched-chain fatty acids was facilitated by comparing the chromatogram of the unknown acids with a chromatogram of acids from milk fat, which contains small amounts of branched-chain acids. To determine which peaks contained C^{14}, fractions of the vapor from the Apiezon L column were collected in 12-in. lengths of Teflon tubing containing a small amount of glass wool. The tubes were dipped in *n*-hexane just before they were attached to the outlet of the thermal conductivity detector cell. The trapped methyl esters were rinsed from the tubes, and the rinsings were assayed for radioactivity. The amounts of fatty acids were estimated from the areas of the individual peaks as calculated by triangulation (Farquhar et al., 1959). Methyl esters of fatty acids were prepared from saponified phospholipid (0.5 N KOH at 108 C for 12 hr) as described by Stoffel, Chu, and Ahrens (1959), except that benzene was not used in the reaction mixture.

Analytical methods. Phosphorus was determined by the method of King (1932). Ester bonds were measured by the hydroxamate color test of Stern and Shapiro, as described by Entenman (1957). Soybean lecithin was used as the standard. The ester content of lecithin was assumed to be twice the molar phosphate content.

Fatty aldehydes were determined by the *p*-nitrophenylhydrazine method of Wittenberg et al. (1956). Lauraldehyde *p*-nitrophenylhydrazone was the standard. The latter material, which was prepared as described by Zilversmit, Marcus, and Ullman (1961), had a molecular extinction coefficient in 95% ethanol of 22,400 at 390 mμ with a slit width of 0.1 mm (Beckman DU spectrophotometer).

Glycerol in phospholipid samples was determined, as described by Neish (1952), after hydrolysis with 2 N HCl (Hanahan et al., 1957). Total nitrogen was measured by the Kjeldahl procedure.

RESULTS

Incorporation of isobutyrate and valerate into phospholipid. When *B. succinogenes* S85 was grown with either isobutyrate-1-C^{14} or valerate-1-C^{14}, at least 94% of the incorporated label was recovered in the lipid fraction of the cells (Table 1). Unlike *R. flavefaciens* (Allison et al., 1959), *B. succinogenes* did not incorporate label from isovalerate-1-C^{14}, nor did this acid satisfy the branched-chain requirement for growth of *B. succinogenes* (Wegner, 1962).

As the first step toward identifying the labeled material, lipid extract from cells grown with isobutyrate-1-C^{14} was evaporated to dryness, the residue was dissolved in a minimal amount of ethyl ether, 10 volumes of acetone were added, and the mixture was held at −20 C overnight. The precipitated phospholipids contained 85 to 90% of the total radioactivity of the lipid fraction.

Phospholipid also was isolated from the extract by chromatography (Hanahan et al., 1957). Figure 1 shows that most of the radioactivity and phosphorus were eluted in a single peak by chloroform-methanol (4:1; v/v). Similar results were obtained with extracts of cells grown with valerate-1-C^{14}.

TABLE 1. *Incorporation of C^{14} from isobutyrate and valerate into cellular lipids of Bacteroides succinogenes*

Fraction	Counts per min per fraction from	
	Isobutyrate*	Valerate†
Whole culture	700,200	5,150,000
Cells	136,300	1,236,000
Cell fractions		
Lipid	134,500	1,160,000
Nucleic acid	140	—‡
Protein wash	500	—
Protein	530	—
Percent of cell radioactivity in lipid fraction	98.7	94.0

* The medium contained 1.0 μmole of valerate and 0.1 μmole of isobutyrate-1-C^{14}/ml (specific activity: 2.2 mc/mmole).

† The medium contained 0.2 μmole of isobutyrate, 0.3 μmole of valerate, and 0.0027 μmole of valerate-1-C^{14}/ml (specific activity: 2.65 mc/mmole).

‡ The cells were not fractioned further.

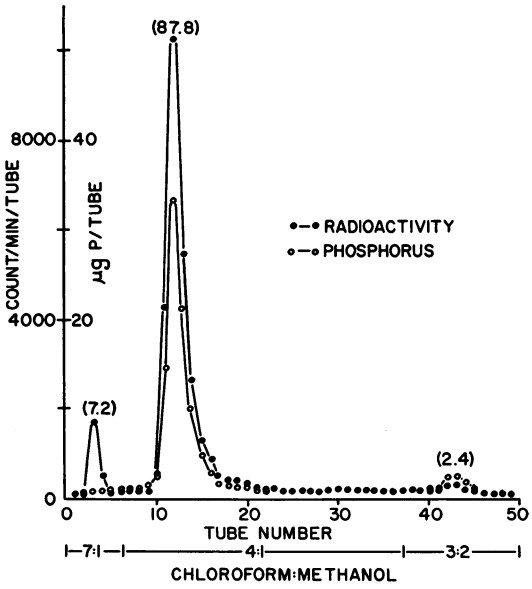
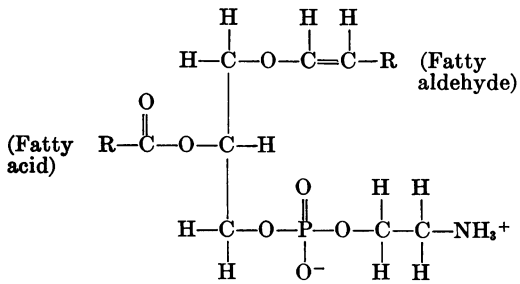


FIG. 1. *Chromatogram of lipids from cells grown with isobutyrate-1-C^{14}. The numbers in parentheses represent the percentage of the radioactivity applied to the column that was recovered in the peak. The packed column (11 × 140 mm) contained 4 g of silicic acid and 2 g of Hyflo-Super-Cel; 4-ml fractions were collected.*

Identification of phospholipids. According to Hanahan et al. (1957), chloroform-methanol (4:1) should elute the cephalins, that is, phosphatides containing ethanolamine or serine. Paper chromatography of acid-hydrolyzed phospholipid (Block et al., 1952) revealed ethanolamine as the only nitrogenous component of the extract, thus suggesting that the material was phosphatidyl ethanolamine. However, a test for aldehydes was positive, indicating the presence of a plasmalogen. These results are not necessarily inconsistent, for Gray and MacFarlane (1958) found that plasmalogens were not separated from the corresponding ester phosphatides on a silicic acid column similar to that used for Fig. 1.

According to Rossita and Strickland (1960), there still is uncertainty about the exact structure of plasmalogens, but the following construction usually is accepted:



In this phosphatide a fatty aldehyde is believed to be linked to the α-carbon of the glycerol by an α, β-unsaturated ether linkage, and a fatty acid is linked to the β-carbon by a conventional ester linkage. From the above structure it is apparent, therefore, that the molar ratios should be 1.0 for aldehyde-phosphorus, ester bond-phosphorus, nitrogen-phosphorus, and glycerol-phosphorus. By contrast, phosphatidyl ethanolamine contains two fatty acid molecules per glycerol; hence, the ester bond-phosphorus molar ratio is 2.0.

Phospholipid extracted from cells grown with isobutyrate-1-C^{14} and separated by chromatography was precipitated three times with cold acetone and assayed for ester bond, total nitrogen, glycerol, phosphorus, and aldehyde. The resulting molar ratios (Table 2) do not eliminate the possibility of phosphatidyl ethanolamine, but their proximity to 1.0 clearly shows that the material was rich in ethanolamine plasmalogen.

By treating the "cephalin" fraction of ox heart with 90% acetic acid (v/v) at 38 C for 18 hr, Gray (1958) cleaved the α, β-unsaturated ether linkage of the plasmalogen in the preparation to yield free aldehydes and "lysocephalin." This treatment did not hydrolyze ester bonds, thus leaving the fatty acid linked to the β-carbon of the glycerol. The resulting mixture of free aldehydes, cephalins (ester bond-phosphorus ratio of 2.0), and lysocephalins (ester bond-phosphorus ratio of 1.0) then was separated by chromatography. Thus, Gray's procedure should make it possible to (i) confirm the presence of plasmalogen in the extracted lipids of *B. succinogenes*, (ii) determine whether the label from isobutyrate-1-C^{14} is incorporated into both fatty aldehydes and fatty acids, and (iii) determine the original amount of plasmalogen from the amount of lysocephalin, in this instance lysophosphatidyl ethanolamine, that resulted from the acetic acid treatment.

TABLE 2. *Characterization of the phospholipid from Bacteroides succinogenes grown with isobutyrate-1-C^{14}*

Components	Molar ratio
Nitrogen-phosphorus	1.00
Glycerol-phosphorus	0.93
Ester bond-phosphorus	0.98
Aldehyde-phosphorus	0.70–0.72
Nitrogen compound	Ethanolamine

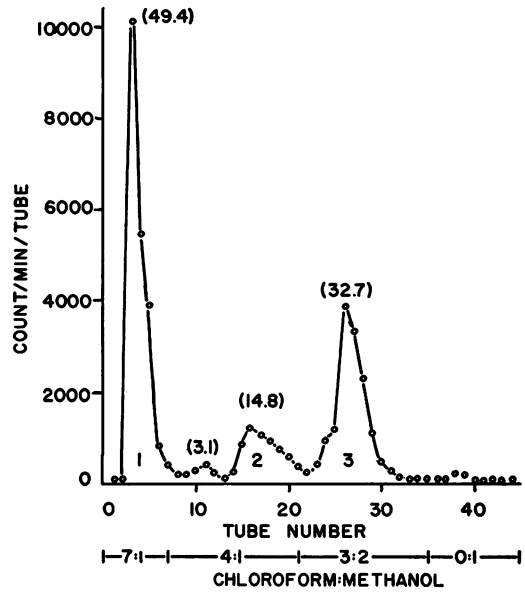


FIG. 2. *Chromatogram of phospholipid from Bacteroides succinogenes grown with isobutyrate-1-C^{14}. Phospholipid was treated with 90% acetic acid at 38 C for 18 hr and analyzed on the column used in Fig. 1. The number in parentheses above each peak represents the percentage of the radioactivity applied to the column that was recovered in the peak.*

If the phospholipid consisted entirely of plasmalogen, and if the acetic acid cleavage and chromatographic separation were complete, one would expect to find only two components, free aldehydes and lysophosphatidyl ethanolamine. However, if the phospholipid were a mixture of plasmalogen and other phosphatides, more than two components should be revealed after acetic acid treatment and chromatography.

A sample of extracted lipids labeled with 42,800 counts/min of C^{14} from isobutyrate and containing about 4 mg of phospholipid was treated with acetic acid as described by Gray

TABLE 3. *Analysis of peaks from Fig. 2*

Component	Amount (μmoles/sample or peak)			
	Before hydrolysis	After hydrolysis		
		Peak 1	Peak 2	Peak 3
Ester bonds	30.1	4.7	4.3	18.2
Phosphorus	24.0	1.3	2.0	19.8
Aldehyde	18.1	9.6	0.9	0.4
Main component		Free aldehydes	Cephalin	Lysocephalin

(1958). The acetic acid was removed with a rotary evaporator, and the residue was dissolved in chloroform and subjected to chromatography. Figure 2 shows separation into three main peaks, and Table 3 gives the ester bond, phosphorus, and aldehyde content of the material in each peak. Recoveries of ester bonds and phosphorus were satisfactory (90.5 and 96.2%, respectively), but only 60% of the aldehyde in the original sample was recovered by chromatography. Aldehydes are known to undergo polymerization reactions and it is possible that the polymers, if formed, do not react with *p*-nitrophenylhydrazine to the same extent as do aldehydes that are bound in the phospholipid molecule until they are coupled to the hydrazine.

Peak 1 accounted for practically all of the free aldehydes. In addition, it contained a small amount of unidentified material having phosphorus and intact ester bonds. Peak 2, with an ester bond-phosphorus molar ratio of 2.15, represents a cephalin, phosphatidyl ethanolamine. Peak 3 represents lysophosphatidyl ethanolamine. It accounted for the bulk of the ester bonds and phosphorus in a molar ratio of 0.92, and contained only a trace of aldehyde. Thus, on the basis of phosphorus recovery it is apparent from the amount of lysophosphatidyl ethanolamine in peak 3 that ethanolamine plasmalogen comprised at least 80% of the phospholipid in the original cell extract.

The material in peak 1, consisting mainly of free aldehydes, contained almost one-half of all the radioactivity in the original sample (Fig. 2). Peaks 2 and 3, which contained fatty acids but very little aldehyde, accounted for the other half. This observation suggests that *B. succinogenes* uses isobutyrate in the synthesis of both fatty acids and fatty aldehydes.

Identification of fatty acids. Phospholipid from cells grown with isobutyrate-1-C^{14} or valerate-1-C^{14} was saponified with 0.5 N KOH at 108 C for 12 hr. After acidification, samples of the free fatty acids were extracted with ethyl ether and subjected to reverse phase chromatography. Radioautograms prepared from the paper chromatograms showed label from valerate-grown cells in C_{13} and C_{15} fatty acids, whereas radioactivity

TABLE 4. *Gas chromatography of methyl esters of fatty acids prepared from lipid extracts of Bacteroides succinogenes grown with valerate-1-C^{14}*

Fraction	Tentative identification of major components	Counts per min per fraction	Per cent of total radioactivity
1	$<C_{12}$	410	
2	C_{12} and C_{13}	53,350*	36.0
3	iso-C_{14}	246	
4	C_{14}	112	
5	C_{15}	93,550	63.1
6	iso-C_{16}	312	
7	C_{16}	28	
8	$C_{18:1}$ and $C_{18:2}$	156	
9	C_{18}	12	

* Radioactivity in a C_{13} acid shown by paper chromatography.

TABLE 5. *Gas chromatography of methyl esters of fatty acids prepared from lipid extracts of Bacteroides succinogenes grown with isobutyrate-1-C^{14}*

Fraction	Tentative identification of major components	Per cent of total acids	Counts per min per fraction	Per cent of total radioactivity
1	$<C_{12}$	—	95	
	C_{12}	3.2		
2	Unknown	2.1	7,630	15
	Unknown	2.4		
	C_{13}	11.4		
3	iso-C_{14}	7.3	26,350	52
4	C_{14}	5.4	1,080	
5	brC_{15}*	1.2	400	
	C_{15}	45.5		
6	iso-C_{16}	4.8	14,600	29
7	C_{16}	5.6	440	
8	C_{17}	1.6	240	
9	$C_{18:2}$	1.4	50	
	$C_{18:1}$	3.6		
	C_{18}	3.4		
	Unknown	1.1		

* Branched chain C_{15} acid.

from isobutyrate-grown cells was found in C_{14} and C_{16} fatty acids.

These results were confirmed and extended by gas chromatography of the methyl esters of the fatty acids. Table 4 shows that 99% of the radioactivity recovered from valerate-grown cells was in the fractions containing n-C_{13} (tridecanoic) and n-C_{15} (pentadecanoic) fatty acids. For cells grown with isobutyrate-1-C^{14} (Table 5), 81% of the radioactivity was recovered in fractions containing iso-C_{14} (12-methyl tridecanoic) and iso-C_{16} (14-methyl pentadecanoic) acids. In this sample, 15% of the radioactivity was recovered in a fraction that should not contain branched-chain acids with an even number of carbon atoms (fraction 2). The labeled component in this fraction was not identified.

Separation of fatty aldehydes. Phospholipid was treated with *p*-nitrophenylhydrazine (Wittenberg et al., 1956), and the resulting aldehyde *p*NPH were subjected to chromatography. Figure 3 shows that the aldehyde *p*NPH peak from a sample labeled by valerate-1-C^{14} was radioactive. A similar chromatogram was obtained with phospholipid labeled by isobutyrate-1-C^{14}. This procedure did not resolve the individual aldehyde *p*NPH, but it did separate the hydrazones from other labeled lipid material (fatty acids and phospholipids).

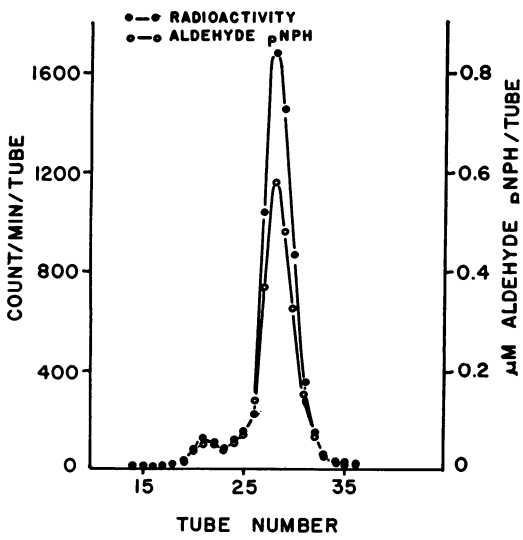


FIG. 3. *Part of the chromatogram of aldehyde p-nitrophenylhydrazones (pNPH) prepared from the phospholipid of Bacteroides succinogenes grown with valerate-1-C^{14}. The packed column (11 × 140 mm) contained 7 g of silicic acid; 2-ml fractions were collected.*

DISCUSSION

This report amplifies and expands an emerging pattern of requirement for and utilization of short-chain volatile fatty acids by bacteria from the bovine rumen. Originally, Bryant and Doetsch (1955) reported that *B. succinogenes* required two saturated fatty acid components for good growth: one a branched-chain acid (isobutyric, isovaleric, or α-methyl-butyric), and the other a straight-chain acid (valeric, caproic, heptanoic, or caprylic). We have confirmed these results with respect to isobutyric, α-methyl-butyric, valeric, and caproic acids, but not with isovaleric acid. Initial trials with a commercial isovaleric acid (Wegner and Foster, 1960) allowed good growth of the organism, but the commercial product proved to be contaminated with α-methyl-butyric acid (Wegner, 1962). Pure isovaleric acid did not support growth. Thus, this organism apparently can utilize the five-carbon acid when the methyl group is attached to the α-carbon atom but not to the β-carbon.

B. succinogenes apparently uses both the branched-chain and the straight-chain acids for the synthesis of long-chain fatty acids and fatty aldehydes, which in turn are used in the formation of phospholipids. Our results thus far indicate that a *Borrelia* species from the rumen behaves like *B. succinogenes* (Wegner and Foster, 1960; Wegner, 1962).

A second pattern of utilization is shown by *Ruminococcus* species, which require a branched-chain but not a straight-chain volatile fatty acid (Allison et al., 1958, 1962*a*). *R. flavefaciens*, strain C-94, uses either isobutyric acid or isovaleric acid for the synthesis of both lipid material and amino acids, the latter having one carbon atom more than the starting unit. For example, isobutyric acid gives rise to valine, and isovaleric acid to leucine (Allison et al., 1962*a*; Wegner, 1962). *R. albus*, strain 7, can use isobutyric acid, but not isovaleric. Like *B. succinogenes*, it employs the short-chain acid only to synthesize lipids (Allison et al., 1962*b*).

With regard to synthesis of lipid materials, the reactions shown in Table 6 have been reported for ruminal bacteria. In every instance, it will

TABLE 6. *Synthesis of lipid materials by ruminal bacteria*

Precursor	Products	Organism	Reference
Iso-C_4 (isobutyric)	Iso-C_{14} + iso-C_{16} acids + fatty aldehydes	*B. succinogenes*	This report
		R. albus	Allison et al., 1962*b*
		Borrelia A-10*	Wegner, 1962
Iso-C_5 (isovaleric)	Iso-C_{15} + iso-C_{17} acids + fatty aldehydes	*R. flavefaciens*	Allison et al., 1962*b*
n-C_5 (valeric)	*n*-C_{13} + *n*-C_{15} acids + fatty aldehydes	*B. succinogenes*	This report
	n-C_{15} + *n*-C_{17} acids	*Borrelia* A-10*	Wegner, 1962
n-C_6 (caproic)	C_{14} + C_{16} acids†	*B. succinogenes*	Wegner, 1962

* No test for aldehydes in *Borrelia* A-10.

† Acid configuration not determined.

be observed, a branched-chain precursor gave rise to a branched-chain product. Furthermore, precursors with odd numbers of carbon atoms yielded products with odd numbers, and those with even numbers of carbon atoms gave rise to even-numbered products. These observations suggest that the precursors serve as starting blocks, which are built up by the addition of two-carbon units to the carboxyl end of the molecules. Horning et al. (1961) described an enzyme system, from rat adipose tissue, that synthesizes a variety of branched-chain and odd-numbered-chain fatty acids by this mechanism.

If short-chain acids serve as precursors of essential long-chain acids, one might expect direct utilization of the long-chain compounds, provided they can enter the cell. Allison et al. (1962*b*) tried unsuccessfully with *R. flavefaciens* to replace the branched-chain fatty acid requirement with various lipid fractions. With *B. succinogenes*, however, this replacement of the volatile fatty acid requirement has, in fact, been demonstrated. Bryant and Doetsch (1955) observed that palmitic acid could partly substitute for volatile fatty acids in the medium for *B. succinogenes*. More specifically, Wegner (1962) demonstrated that pentadecanoate-1-C^{14} could replace valeric acid in the medium for *B. succinogenes* and was incorporated intact into the phospholipid of the cell. The failure of the lipid fractions to replace the branched-chain fatty acid requirement of *R. flavefaciens* may be involved with the amino acid metabolism of this culture, for, unlike *B. succinogenes*, *R. flavefaciens* uses the volatile fatty acids for synthesis of both lipids and amino acids.

It is clear that *B. succinogenes* synthesizes fatty aldehydes as well as fatty acids from the short-chain precursor acids. These aldehydes have not been studied extensively, but presumably they follow the same pattern as the acid products. Allison et al. (1962*b*) found that isovaleric acid was incorporated into both a branched-chain C_{15} aldehyde and a branched-chain C_{15} fatty acid.

Plasmalogens have only recently been identified in bacteria (Allison et al., 1962*b*), although they are common in animal lipids. The function of plasmalogens, or for that matter, phospholipids in general, is uncertain. Phosphatidyl ethanolamine has been identified as the major lipid component in electron-transport particles from *Azotobacter agilis* and in chromatophores from *Rhodospirillum rubrum* (Marr, 1960). In these particles the phospholipid may serve as a "cement" or structural component. Phosphatidyl ethanolamine has also been found as a major component in the lipids of *Bacillus cereus* (Kates, Kushner, and James, 1962).

It is not entirely clear why some ruminal bacteria require short-chain volatile fatty acids as growth factors and others do not. It may be, as Bryant and Doetsch (1955) suggested, that these organisms have lost the ability to synthesize the short-chain starting units from which they make long-chain fatty acids (and aldehydes). Some support for this idea can be obtained from the results of Akashi and Saito (1960) and Saito (1960), who found long-chain iso- and anteiso-

acids as the main ones in the lipids of several bacterial species. Although odd-numbered and branched-chain fatty acids are not uncommon in bacteria, only certain organisms from the rumen have been shown to require short-chain, volatile fatty acid precursors for synthesis of the long-chain acids.

Even-numbered iso-acids, odd-numbered iso- and anteiso-acids, and odd-numbered straight-chain acids, as well as many branched-chain and odd-numbered fatty aldehydes, have been isolated from a variety of ruminant fats (Markley, 1960; Gray, 1960, 1961). Although no direct evidence is available, these long-chain acids and aldehydes might be synthesized from isobutyrate, isovalerate, α-methyl-butyrate, and valerate, all of which occur in the rumen, by bacteria living in the gut of the animal.

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