THE FATTY ACIDS OF BACTERIA

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I. Introduction

For many years the accumulation of knowledge regarding the chemistry and metabolism of lipids has lagged far behind the progress made in the studies of carbohydrates and proteins. One has only to examine any of the current textbooks or monographs dealing with bacterial physiology to perceive the paucity of readily available information on bacterial lipids and on their component fatty acids. There appear to have been three main reasons for this lack of activity and progress in the study of lipids. First of all, low water solubility and other inconvenient physical character-

istics of lipoid materials often make work with such compounds discouragingly difficult. Secondly, until relatively recently few really satisfactory techniques were available for the isolation, purification, and identification of lipids and their components. Lastly, and possibly to some extent as a consequence of the first two reasons, there has been a tendency to regard the lipids as less interesting than other cellular constituents, both from the chemical and physiological points of view.

Today a variety of techniques are available that make work with the lipids less arduous and more accurate than ever before. Also, it is now recognized that the lipids and fatty acids, and particularly those of microorganisms, are much more chemically complex and metabolically active than was suspected in the past. Consequently, in the years since World War II there has been a progressively increasing tempo of research in lipid chemistry and metabolism, and several reviews attest to the fact that this activity has extended to the microorganisms (9, 11, 12, 46, 153). As these reviews indicate, recent years have seen research done on many aspects of microbial lipids: synthesis and degradation, intracellular distribution and function, immunological properties, the natures of the various lipid complexes, and so on.

However, essential to real understanding of each of these aspects is an exact and detailed knowledge of those fatty acids which are the fundamental components of the bacterial lipids. Such knowledge is the *sine qua non* for further accurate work on microbial fats, and as such is deserving of compilation and study. Much research has been done on the fatty acids of bacteria, and a considerable body of information has been accumulated. Unfortunately, this information is scattered among many journals and monographs in the world's chemical and biological literature. It is, therefore, the purpose of this review to collect and summarize the presently available knowledge regarding bacterial fatty acids, including their chemical nature, certain biochemical considerations, and the methods now in use for the study of these compounds.

Those familiar with the subject of microbial fats will note that little is here reported on the lipids of the mycobacteria. As is well known, these organisms contain an astonishing variety of lipid constituents, a far more complex assortment than one finds in other bacteria. Indeed, the study of mycobacterial lipids could well constitute a subspecialty in itself. Fortunately the vast body of literature on this complicated subject has been excellently and exhaustively reviewed elsewhere (5, 9, 11, 12, 135). Therefore I have intentionally omitted all but a few references to the mycobacteria and have limited this review to consideration of the fatty acids of less well surveyed bacteria, particularly species of the orders *Eubacteriales* and *Pseudomonadales*.

II. Isolation and Analysis of Bacterial Fatty Acids

A. Extraction of Fatty Acids from Bacterial Cells

1. General considerations. To evaluate better the data available in this field and to avoid some of the pitfalls involved in this type of research, it is important to be aware of several characteristics peculiar to bacterial fats.

Of primary importance is the fact that the fatty acid composition of bacteria is markedly affected both quantitatively and qualitatively by the nature of the medium and by the conditions under which the culture is grown. Many authors have commented in detail on this phenomenon (12, 57, 117, 134, 153). Among the various nutritional and environmental factors which they have reported to influence the fatty acid content of bacteria are the amounts and relative proportions of acetate, glycerol, carbohydrate, lipid, and nitrogenous substances present in the medium; oxygen supply; pH; and the age of the culture when harvested. Unawareness of the profound effects that these factors can have on fatty acid composition has led to considerable confusion over the many inconsistencies and apparent contradictions that have appeared in the literature on bacterial fats. For many years these variations in fat content seemed largely random and unpredictable, and certainly there is still a great deal to be learned. However, as the body of knowledge regarding bacterial metabolism increases, many of the vagaries in the fat content of bacteria grown under different conditions are becoming more understandable. Conversely, in certain cases careful study of the variations in the fatty acid contents of cells grown under different but carefully defined conditions has led to increased understanding of metabolic processes. An example of this is the work of Hofmann and

his colleagues (61, 68) in which the fatty acid contents of different media were correlated with the fatty acids found in the bacteria grown in each medium to obtain an insight into the biosynthesis of lactobacillic acid. The accuracy of the predictions based on this work was later verified by other procedures (95, 112, 113).

From the foregoing it is obvious that a detailed knowledge of the medium and culture conditions employed is essential to any valid interpretation of data regarding bacterial fats, and that, to be comparable, successive experiments must duplicate conditions exactly. This means that particularly for initial studies of an organism a chemically defined and exactly reproducible medium is necessary for meaningful results. It should be borne in mind, however, that synthetic media may differ markedly from the natural environments of the organisms being studied, and that this difference may affect the composition of the bacterial cells. Asselineau and Lederer (12) and Lovern (97) have pointed out that the lipid contents of such "artificial bacteria" can be quite dissimilar to those of the same species grown in natural surroundings. For example, it has been observed that cells of *Mycobacterium tuberculosis* grown in lung tissue differ from cells of the same strain grown in laboratory medium with respect both to relative lipid content and to the utilization of various fatty acids (127). Therefore, after carefully studying the fatty acids of any given microorganism grown on synthetic media, it may be desirable to proceed to further studies of the organism grown under more nearly natural conditions or, in the case of the pathogens, to organisms grown in vivo.

Another important problem involved in studying bacterial fatty acids is the difficulty one encounters in extracting all of the fatty acids present in the cell. In every bacterial species some percentage of the fatty acids occurs free and can be readily removed from the cells simply by extracting with an appropriate organic solvent. The percentage of free fatty acids varies with the species and may be quite high (83). However, a significant proportion of the fatty acids is bound very firmly in various protein and carbohydrate complexes which are not soluble in organic solvents. Consequently, merely extracting bacterial cells with some lipid solvent will fail to recover considerable portions of fatty acids. To obtain all the fatty acids present, it is necessary first to subject the cells to some hydrolytic procedure which will free the so-called "bound" fatty acids so that they can be removed by the extracting solvent. Studies of bacterial fats in which this fact was not taken into consideration are of limited value, since they of necessity consider only those acids which occur free in the cell. There is no evidence to show that the free acids are quantitatively or even qualitatively representative of the total cellular content of fatty acids.

One further circumstance worthy of mention in any general discussion of bacterial fats is the fact that, with the notable exception of *Azotobacter chroococcum* (128), none of the true bacteria have been shown conclusively to contain steroids. In fact, most bacteria contain little unsaponifiable material of any kind. The absence of steroids in bacteria is particularly interesting in that various species have been shown to synthesize or to require for growth mevalonic acid (130, 137, 138), which is known to be a sterol precursor in other forms of life.

2. Extraction methods. In attempting a careful study of bacterial fats, the investigator is faced with somewhat of a dilemma. If extraction conditions are too mild, he will not recover all the constituents present; if, on the other hand, conditions are too rigorous, he may damage or destroy some of the more labile constituents. This is especially a problem when the object is to study specific lipid complexes. Fortunately, the fatty acids are relatively resistant to the hydrolytic procedures necessary to insure their complete removal from the cell. Nevertheless, exposure to any extreme conditions should be minimized to prevent alterations of the more labile acids such as the unsaturated and ring-containing compounds. Exposure to oxygen is particularly to be avoided and is prevented whenever possible by conducting procedures in an atmosphere of nitrogen. This precaution should include even the purging of solvents with nitrogen to remove dissolved oxygen.

Several references are available (26, 38, 58, 81, 119, 132) which discuss in much greater detail than is possible in the present paper the general principles and methods of lipid and fatty acid chemistry including extraction procedures, and these should be consulted for background information.

The extraction techniques employed for the

fatty acids of bacteria are typified by the method employed by Hofmann et al. (62) and by many others with generally satisfactory results. In this procedure, dried bacterial cells are suspended in 2 N sulfuric acid and hydrolyzed by autoclaving for 90 min. After the hydrolyzate has cooled and the insoluble cell debris has been removed by filtration, the crude lipoid material is extracted from the hydrolyzate with ether. The lipoid material is then saponified with potassium hydroxide in ethanol to form water-soluble potassium salts of the fatty acids. Water is added, and the unsaponifiable material present, if any, is removed by a second ether extraction. Finally, the aqueous phase, now containing only salts of fatty acids, is reacidified to convert the fatty acid salts back to free fatty acids. The acids are then recovered by ether extraction. Evaporation of the ether yields the mixed fatty acids which had been present in the bacterial cells.

In another method used successfully by several workers, the hydrolysis is accomplished using ethanolic potassium hydroxide instead of sulfuric acid (61). This both hydrolyzes and saponifies in one step, producing potassium salts of the cellular fatty acids which are then handled as described above. One advantage of this method is the saving in time as a consequence of fewer manipulations. However, an even more important advantage has recently been pointed out by Law (87), who, by applying various extraction procedures to *Escherichia coli*, found that acid hydrolysis sometimes produced artifacts in the recovered fatty acids. He found that the procedure that caused the least alteration of fatty acids was the use of methanolic potassium hydroxide for hydrolysis followed by extraction of unsaponifiable material, acidification, and recovery of the mixed acids.

It must be borne in mind that the methods just described are for the recovery of total cellular fatty acids only, without regard to the complexes in which they occur. Naturally, for the recovery of specific lipid complexes such as the glycerides, phospholipids, glycolipids, waxes, and so on much more gentle and selective procedures must be employed. The classic example of lipid class fractionation in bacteria is the elegant scheme originally devised for the study of mycobacterial lipids (5, 12).

B. Separation and Identification of Bacterial Fatty Acids

As mentioned above, the inability readily and reliably to separate and identify fatty acids was for many years a major reason for the limited activity and progress in lipid research. Fortunately, today a number of techniques are available which, in various combinations with each other and with the standard methods of analytical organic chemistry, make possible quite accurate analyses of fatty acid mixtures. Methods for separation and identification will be discussed together in this review since in many cases the characteristics which make it possible to separate one fatty acid from others are also some of the characteristics by which it can be identified.

1. Distillation. Highly efficient fractional distillation can be used to separate fatty acids on the basis of the lengths of their carbon chains. It is usual to convert fatty acids to lower boiling methyl esters and to perform the distillations in vacuo to use as low temperatures as possible. The various types of apparatus suitable for this use and their operation have been described by Bowman and Tipson (18) and Murray (105).

The temperature at which each component is obtained indicates the number of carbon atoms present in the molecules being distilled. However, one cannot distinguish between saturated and unsaturated compounds having the same chain length. Thus each fraction must be checked by other techniques to determine whether more than one compound is present. Because the general shape of the distillation curve can be predicted, any unusual departure from the expected curve can aid in the detection of new or unusual compounds. It was in this way that cyclopropane ring-containing fatty acids were first discovered in bacterial lipids (65, 66).

In addition to being useful for the separation and analysis of fatty acids, distillation is also a valuable means of obtaining amounts of individual fractions large enough to be used for further chemical and biological purposes. Most of the other methods to be described below yield amounts that are impractically small for any further manipulation.

Paradoxically, however, its relatively large scale is the major disadvantage of distillation in the study of bacterial fatty acids. That is, the size of the fatty acid sample required is quite appreciable in terms of the amount of cells that

must be amassed. Because its sample requirements are relatively modest in comparison with those of other distillation assemblies, the semimicro spinning band still, particularly the Piros-Glover model, has been preferred for this type of work. The use of this device for the analysis of bacterial fats has been described in detail by Lucas (98). However, even for the spinning band apparatus, one still must have from 1 to 10 g of mixed fatty acid esters. Since the total lipid content of most bacteria is less, usually much less, than 10% of the dry cell weight, such sample requirements entail the collection of large amounts of bacteria. Though this may be reasonable for preparative purposes, it is obviously not convenient for routine analyses.

2. Crystallization. For many years a standard procedure in fatty acid chemistry has been the separation of saturated acids from unsaturated acids by differential crystallization of their lead salts (81, 119). Today, increasing use is being made of low temperature fractional crystallization of free fatty acids (20). With this procedure it is possible to achieve quite satisfactory separations of saturated from unsaturated acids, and to resolve further each of these two major groups. Low temperature crystallization procedures have the considerable advantage of simplicity, requiring only facilities for working at −20 to −70 C and appropriate solvents. Also, there is little danger of any chemical alteration of fatty acids occurring during the crystallization operations. There is, however, the problem of possible cocrystallization of similar compounds which can make precise separations difficult in some cases. Earlier methods required considerable amounts of starting material, but techniques have now been devised which require as little as 20 mg of sample (41, 139).

Another useful crystallization technique involves the formation of urea inclusion complexes of mixed fatty acids (125). These inclusion complexes, or adducts, can be separated by crystallization at higher temperatures than the free fatty acids (i.e., around 0 C). After fractionation, the adducts of the individual fatty acids can be easily decomposed by warming, thus liberating the unaltered fatty acids. In addition to resolving fatty acids with respect to degree of unsaturation, urea adducts are useful in separating straight chain compounds from those with branched chains.

3. Countercurrent distribution. Extensive descriptions of countercurrent distribution and of its application to fatty acids can be found in papers by Craig and Craig (29), Dutton (35), and Ahrens (1). In essence this method consists of subjecting a sample to a large number of successive liquid-liquid extractions in a multiple-tube apparatus which permits one of the solvents to advance to the next tube of the series independently of the other solvent. The components of the sample are distributed according to their relative solubilities in each solvent.

The separation of fatty acids by countercurrent distribution, as well as other liquid-liquid partition procedures, is affected by both the chain length and the degree of unsaturation of the compounds in the sample. The presence of one double bond has the same solubility effect as does shortening the chain by two carbon atoms. Thus a monounsaturated 18-carbon acid will migrate in the same way as the saturated 16-carbon acid. Aside from this problem for which there are adequate remedies including crystallization procedures, countercurrent distribution can separate complex mixtures into their various constituents in high purity. Further, there are the advantages of accomplishing this separation under the mildest of conditions and of being able to obtain relatively large amounts of sample components should the need arise. Countercurrent distribution does have the disadvantages of requiring complicated and expensive equipment and of yielding a large number of individual liquid fractions which must be further manipulated.

4. Chromatography. A surprisingly large number of chromatographic methods have been devised for fatty acids, and these have been reviewed periodically by various authors (8, 15, 17, 74, 90). These techniques, which vary widely in application and effectiveness, for present purposes may be divided into three groups: adsorption chromatography, partition chromatography, and gas chromatography.

Adsorption techniques using charcoal, silicic acid, or alumina are widely used for the separation of lipid complexes (38). However, such methods have not proved as successful in the analysis of the fatty acids themselves, and consequently they are not as commonly employed for this purpose as are partition and gas chromatographic techniques. One variation of adsorption chromatography that has been successfully used

for fatty acid analysis is the displacement technique employed by Holman (74) and others. Two new methods that appear quite promising are the chromatography of fatty acid esters on glass fiber paper impregnated with silica gel (52) and the so-called "thin-layer chromatography" which has been used in Europe for some time and is now finding enthusiastic supporters in this country (152, 154).

Partition chromatography both in columns and on paper has been very useful for fatty acid research, and this is particularly true of reversed-phase procedures. Boldingh's basic technique (16), using powdered rubber as a support for the nonpolar stationary phase and acetone-water mixtures as eluting solvents, has found many applications. A modification of this method devised by Hofmann and his associates (62) has given excellent results in the study of the fats of many bacteria (62, 68, 112, 113). Unsaturated acids in the sample are hydroxylated so that they can be separated from the shorter saturated acids with which they would otherwise be eluted. The eluate is monitored by titration of each fraction. Another useful variation of Boldingh's technique is that of Hirsch (59) in which methyl esters rather than the free acids are passed through rather lengthy columns. In this procedure the eluate is continuously monitored by a recording differential refractometer.

Reversed-phase paper chromatography is useful when dealing with very small samples. Unfortunately, it is necessary to impregnate the paper with various materials such as latex (16), hydrocarbons (7, 81), or silicones (126), which introduce other variables into an already complex operation. Spot development is also more complicated than in other types of paper chromatography (7, 124).

All the chromatographic methods mentioned so far, although exhibiting the same order of efficiency customarily associated with other chromatographic procedures, generally fail to separate an unsaturated acid from a saturated acid having two less carbon atoms (e.g., oleic acid cannot be separated from palmitic acid). For this reason, it is necessary to remove one group of acids before chromatographing the other, or to alter chemically the unsaturated acids so that they can be resolved. One such chemical alteration is the hydroxylation employed in Hofmann's reversed-phase column chromatography described above. Several other methods have also been devised (38). If the aim of the investigator is solely analysis of a fatty acid mixture, such chemical alterations pose no problem. But, if further use is to be made of the individual fatty acids, chemical changes may be undesirable.

Gas-liquid chromatography is actually another form of partition chromatography. This technique, first introduced by James and Martin (78), has been widely and enthusiastically adopted by lipid chemists as the most nearly ideal analytical method now available for fatty acids. A number of reviews have appeared discussing the principles of this technique and in particular its application to fatty acid analysis (10, 54, 77, 94, 115).

In this method, methyl esters of fatty acids are introduced in minute amounts into a heated column containing the nonpolar stationary phase coated on an inert support such as Celite or even simply the column wall. The esters are vaporized and moved through the column by a stream of gas such as nitrogen or helium. As the individual esters emerge from the column at times reflecting their affinities for the stationary phase, they pass through a detection device which is connected to a recorder. The time after injection of sample at which each peak appears on the recorder identifies the ester, and quantitative data can be obtained from the heights or areas of each peak. The individual esters can be recovered after leaving the detector. Several types of detectors and many columns are available, each with varying applications and efficiencies (54, 116).

Very high efficiencies can be obtained with gas chromatographic apparatus which not only facilitate the detection of trace amounts of fatty acids, but also make it possible to separate unsaturated acids from saturated acids without preliminary chemical alterations.

The advantages of this method are: very small sample requirements, speed and ease in performing an analysis that is simultaneously quantitative and qualitative, and recovery of unaltered sample constituents. The major disadvantage, which is consequent to a small sample size, is that only minute amounts of each constituent can be recovered. Thus, gas chromatography is primarily an analytical method, although preparative scale gas chromatography can be per-

formed in which capacity is increased at the expense of efficiency.

5. Infrared spectroscopy. In recent years this technique has become one of the major tools of fatty acid analysis (104, 148). The examination of infrared absorption spectra has three main applications: in determining the details of molecular structure of a compound, in detecting the presence of unidentified substances even in complex mixtures, and in identifying individual compounds by comparing their spectra with those of known compounds. This method has a number of advantages including the fact that a considerable amount of information can be obtained without affecting the sample (which consequently can be recovered and used for other purposes), the ability to use a very small sample (as little as 0.5 mg) if necessary, and the ability to differentiate readily between *cis* and *trans* isomers, which is of prime importance in the study of fatty acids containing double bonds or cyclopropane rings.

It has become common practice to apply infrared spectroscopy to gas chromatographic fractions as a means of obtaining a maximum of information from amounts of material too minute for any appreciable treatment by conventional "wet" analytical procedures.

6. Other procedures. In addition to those methods already described, there are several other instrumental procedures that are in use or that are now coming into use for special purposes in the analysis of fatty acids. These procedures include X-ray diffraction, ultraviolet spectroscopy, microwave analysis, nuclear magnetic resonance, and mass spectroscopy. Space limitations preclude any detailed description of these rather complex methods here, but they have been recently and thoroughly discussed by O'Connor (109) and by Ryhage and Stenhagen (120).

In any study in which one is attempting to identify fatty acids, the techniques described so far should, of course, be used in conjunction with the classical methods of lipid chemistry and organic analysis to arrive at an unequivocal identification (50, 53, 58, 144). These methods include, among many, determinations of the various physical properties for both the fatty acid as isolated and for appropriate derivatives; elemental analysis; determinations of neutral equivalent; iodine number; hydrogenation uptake; and identification of the hydrogenation product. In studying compounds containing double bonds, cyclic configurations, and other labile structural features, the molecule should be degraded and the resultant fragments identified to perceive the structure of the original molecule. In many cases microbiological assay procedures can be used to advantage for both quantitative and qualitative studies of fatty acids (61, 70).

Whenever possible, it is best to compare the various properties of the fatty acids under study with those determined simultaneously for high purity preparations of known compounds (i.e., "authentic samples") rather than to rely exclusively on values appearing in the literature.

The ultimate proof of structure is accomplished by synthesizing with certainty the suspected molecular configuration and demonstrating that the synthetic compound is identical with the compound originally isolated (42, 49). This procedure is particularly important in the study of new or unusual fatty acids when authentic samples are not available.

7. Evaluation. The particular methods of separation and identification that should be employed in any given study depend on many different considerations. Prominent among these are: the amount of sample available, whether or not significant amounts of each sample component must be recovered, whether some chemical alteration of some components is acceptable, the information that each method used is capable of supplying, and, most important, the amount of information that is necessary to establish unequivocally the identity to be reported for any given compound.

The importance of this last consideration cannot be stressed too much since the lack of sufficiently rigorous criteria in the identification of fatty acids has impaired the value of much of the literature in the field. For example, there are many reports in the older literature of the occurrence of oleic acid in various species of bacteria. In many if not most cases, the "identification" of this compound was based on insufficient data rarely exceeding a neutral equivalent, determination of hydrogen uptake, and perhaps the identification of the hydrogenation product as stearic acid. Actually, even with the a priori assumption that the substance under study was a straight chain, monocarboxylic fatty acid, such information only indicated that the compound could be an 18-carbon acid with one double bond. This description is equally applicable to several posi-

tional isomers of octadecenoic acid, only one of which is oleic acid (i.e., *cis*-9-octadecenoic acid). More elaborate investigations in which the location of the double bond was established with certainty have shown that in many bacteria the sole or major unsaturated fatty acid is *cis*-vaccenic acid in which the double bond is in the 11-12 position rather than in the 9-10 position as is the case in oleic acid. This is not to say that oleic acid does not occur in bacteria, but it does point out the necessity of excluding other possible compounds before making a specific identification. The literature records a number of instances in which fatty acids have been incorrectly identified or in which the opportunity to discover an unusual and important compound has been missed because of insufficiently critical evaluation of analytical data.

It is, therefore, essential that the investigator give constant consideration both to the criteria that must be satisfied for the unequivocal identification of any given compound, and to the limitations as well as the capabilities of each procedure he is employing.

III. Fatty Acids of Bacteria

Much of the earlier work on bacterial lipids was limited to determinations of "per cent total lipid" for various organisms. Because figures for any species can vary considerably depending on the strains studied and the culture conditions employed, such data are of limited value and significance. However, from these studies one may make the general observation that, with a few exceptions, the total lipid content in most species of bacteria ranges between 1 and 10% of the dry cell weight (83, 117).

Of considerably more interest and utility are reports of the chemical composition of bacterial lipids, particularly with respect to the component fatty acids. Porter (117) has reviewed the work in this field up to 1946, and further information on bacterial fatty acids has been included in several more recent lipid reviews (9, 11, 12, 60, 153). Of parallel interest are the reviews on the lipids of the yeasts (36) and of the molds (40).

One purpose of the present review is to catalogue and to describe briefly those fatty acids which, on the basis of reasonably satisfactory evidence, are believed to occur in bacteria. The reader should bear in mind that the same amount of analytical information has not been accumulated for each of the fatty acids to be listed. The identities of most of these compounds has been conclusively established, but some still require further study and may be subject to revision in the light of later findings. Also, with the advent of the newer analytical methods, the number of fatty acids known to occur in bacteria has been increasing rapidly. For this reason, it is probable that by the time this review appears in print the catalogue of bacterial fatty acids which it contains will already be incomplete.

The fatty acids to be listed are grouped according to their chemical natures. Their structural formulas are given in Table 1.

A. Saturated Straight Chain Acids

These compounds have long been known to occur in bacteria and are identical to the saturated fatty acids found in other forms of life.

Acids having chains of less than 12 carbon atoms have been detected in small amounts in virtually all species in which they have been sought. Included in this group are the following acids: formic (C_1), acetic (C_2), propionic (C_3), butyric (C_4), caproic (C_6), caprylic (C_8), and capric (C_{10}).

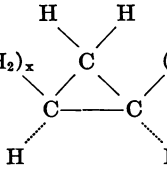
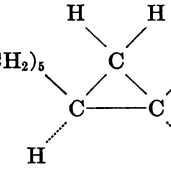
The higher fatty acids constitute a much larger proportion of the total fatty acid content of the cell. The acids included in this group are lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), arachidic (C_{20}), behenic (C_{22}), lignoceric (C_{24}), and octacosanoic (C_{28}). No straight chain saturated acids longer than C_{28} have been reported other than in the mycobacteria. Palmitic acid occurs more frequently and usually in larger amounts than any other saturated acid found in bacteria. Stearic, myristic, and lauric acids are also common though lesser constituents of microbial lipids, whereas the $C_{20\text{-}28}$ acids are encountered only in a few species. Arachidic acid has been reported in *Pseudomonas aeruginosa* (117), behenic in *Corynebacterium diphtheriae* (6), lignoceric in *C. diphtheriae* (3, 6) and *Lactobacillus acidophilus* (30), and octacosanoic in *C. diphtheriae* (6).

It will be noted that the higher acids listed above all contain even numbers of carbon atoms, as is usually expected in fatty acids extracted from biological materials. However, there have been a few reports of fatty acids with odd numbers of carbon atoms occurring in bacteria. In their study of fatty acids recovered from cultures of *P. aeruginosa*, James and Martin (79) found

TABLE 1. *Fatty acids of bacteria*

Common name	Systematic name	Carbon atoms	Formula
Higher straight chain saturated acids			
Lauric	Dodecanoic	12	$CH_3-(CH_2)_{10}-COOH$
Myristic	Tetradecanoic	14	$CH_3-(CH_2)_{12}-COOH$
Palmitic	Hexadecanoic	16	$CH_3-(CH_2)_{14}-COOH$
Stearic	Octadecanoic	18	$CH_3-(CH_2)_{16}-COOH$
Arachidic	Eicosanoic	20	$CH_3-(CH_2)_{18}-COOH$
Behenic	Docosanoic	22	$CH_3-(CH_2)_{20}-COOH$
Lignoceric	Tetracosanoic	24	$CH_3-(CH_2)_{22}-COOH$
Montanic	Octacosanoic	28	$CH_3-(CH_2)_{26}-COOH$
Hydroxy acids			
β - Hydroxy-butyric	3-Hydroxybutanoic	4	$CH_3-CH(OH)-CH_2-COOH$
Mevalonic (β - δ - Dihy-hydroxy - β - methyl-valeric)	3,5 - Dihydroxy - 3 - methyl pentanoic	6	$HO-CH_2-CH_2-C(OH)(CH_3)-CH_2-COOH$
	β - Hydroxyoctanoic (3 - hydroxyocta-noic)	8	$CH_3-(CH_2)_4-CH(OH)-CH_2-COOH$
	β - Hydroxydecanoic (3 - hydroxydeca-noic)	10	$CH_3-(CH_2)_6-CH(OH)-CH_2-COOH$
	β - Hydroxydodeca-noic (3 - hydroxy-dodecanoic)	12	$CH_3-(CH_2)_8-CH(OH)-CH_2-COOH$
β - Hydroxy-myristic	3 - Hydroxytetra-decanoic	14	$CH_3-(CH_2)_{10}-CH(OH)-CH_2-COOH$
Di - hydroxy-stearic	Di - hydroxyocta-decanoic	18	$C_{17}H_{33}(OH)_2COOH$
Corynomycolic		32	$CH_3-(CH_2)_{14}-CH(OH)-CH(C_{14}H_{29})-COOH$
Corynomyco-lenic		32	$CH_3-(CH_2)_5-CH{=}CH-(CH_2)_7-CH(OH)-CH(C_{14}H_{29})-COOH$
Corynolic		52	$CH_3-CH(OH)-(CH_2)_7-CH(CH_3)-CH(OH)-CH(CH_3)-CH(CH_3)-$ linked to C of $CH_3-(CH_2)_{14}-CH(CH_3)-(CH_2)_{17}-C(H)-COOH$

TABLE 1.—(*Continued*)

Common name	Systematic name	Carbon atoms	Formula
Branched chain acids			
Isooctanoic	Methylheptanoic	8	$CH_3-(CH_2)_x-CH(CH_3)-(CH_2)_y-COOH$ $(x + y = 4)$
	6-Methyloctanoic	9	$CH_3-CH_2-CH(CH_3)-(CH_2)_4-COOH$
	13 - Methyltetradecanoic	15	$CH_3-CH(CH_3)-(CH_2)_{11}-COOH$
	15 - Methylhexadecanoic	17	$CH_3-CH(CH_3)-(CH_2)_{13}-COOH$
Corinnic		35	$C_{35}H_{68}O_2$
Diphtheric		35	$C_{35}H_{68}O_2$
Cyclopropane acids			
	Methylene - hexadecanoic	17	$CH_3-(CH_2)_x$  $(CH_2)_y-COOH$ $(x + y = 12)$
Lactobacillic	*cis* - 11,12 - Methylene - octadecanoic	19	$CH_3-(CH_2)_5$  $(CH_2)_9-COOH$
Unsaturated acids			
	Tetradecenoic	14	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 10)$
	Hexadecenoic	16	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 12)$
Palmitoleic	*cis*-9-Hexadecenoic	16	$CH_3-(CH_2)_5-CH=CH-(CH_2)_7-COOH$
Palmitvaccenic	*cis*-11-Hexadecenoic	16	$CH_3-(CH_2)_3-CH=CH-(CH_2)_9-COOH$
	Octadecenoic	18	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 14)$
Oleic	*cis*-9-Octadecenoic	18	$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH$
cis-Vaccenic	*cis*-11-Octadecenoic	18	$CH_3-(CH_2)_5-CH=CH-(CH_2)_9-COOH$
	Eicosenoic	20	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 16)$
	Heneicosenoic	21	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 17)$
	Docosenoic	22	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 18)$
	Tetracosenoic	24	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 20)$
	Octacosenoic	28	$CH_3-(CH_2)_x-CH=CH-(CH_2)_y-COOH$ $(x + y = 24)$

evidence of C_{15} and C_{17} straight chain saturated acids. Goldfine and Bloch (45) noted what may be a C_{15} acid of this type in *Clostridium butyricum*. None of these compounds has yet been identified with certainty.

B. Hydroxy Acids

β-Hydroxybutyric acid has been found in several bacteria including species of *Azotobacter* (39, 92), *Chromobacterium* (39), *Bacillus* (91, 146, 151), *Pseudomonas* (39), and *Micrococcus* (131).

Mevalonic acid (β-δ-dihydroxy-β methylvaleric acid) has been isolated from cultures of lactobacilli and other microorganisms (137, 138). Since it is known that some bacteria require only very small amounts of this compound for maximal growth, it may well be that it has escaped detection in many organisms.

β-Hydroxydecanoic acid occurs in *E. coli* (87), *Serratia* sp. (23), and in various species of *Pseudomonas* (14, 80). Small amounts of β-hydroxyoctanoic and β-hydroxydodecanoic acids have been detected in *Pseudomonas pyocyanea* (*P. aeruginosa*) (14). β-Hydroxymyristic acid has been found in *E. coli* by Ikawa et al. (76) and by Law (87).

Dihydroxystearic acid occurs, apparently as the free acid, in *L. acidophilus*. Although 30 years have elapsed since this compound was discovered by Crowder and Anderson (31), the location of the hydroxyl groups is still unknown.

Several complex hydroxy acids are peculiar to *C. diphtheriae*. These compounds, which have been discussed in detail by Asselineau (9) and Asselineau and Lederer (12), are corynomycolic acid ($C_{32}H_{64}O_3$), its monounsaturated form called corynomycolenic acid ($C_{32}H_{62}O_3$), and corynolic acid, a dihydroxy compound also known as corynine ($C_{52}H_{104}O_4$). These substances, as their names would suggest, have many similarities to the mycolic acid found in mycobacteria. In fact, the complicated lipid composition of the corynebacteria is in many ways more like that of the mycobacteria than that of the species in the orders *Eubacteriales* and *Pseudomonadales*. An interesting summary of the many lipoid substances isolated from the corynebacteria is that of Pustovalov (118).

C. Branched Chain Acids

Mevalonic acid, discussed in the previous section with the hydroxy fatty acids, should be mentioned here as it is also a branched compound.

Hausmann and Craig (56) have shown that isooctanoic, or methylheptanoic, acid ($C_8H_{12}O_2$) is produced by *Bacillus polymyxa* as a component of polymyxin B. The same paper reported that the major fatty acid constituent of the various polymyxins is a methyloctanoic acid. The accumulated results of numerous workers have shown that this compound is (+)-6-(L)methyloctanoic acid.

Akashi and Saito (2) detected a branched chain C_{15} acid in the lipids of a strain of *Sarcina*. This compound was tentatively named sarcinic acid. A similar C_{15} acid and a C_{17} compound were found to "abound" in *Bacillus subtilis* and *Bacillus natto*. In subsequent studies on the acids found in the *Bacillus* species, Saito (121, 122) showed that the C_{15} acid was 13-methyltetradecanoic acid and that the C_{17} acid was 15-methylhexadecanoic acid. Whether the C_{15} acid found in *Bacillus* species is the same as that detected in *Sarcina* species is not yet known.

In a paper dealing with gas chromatographic analyses of the fatty acids from several microbial species, Asselineau (10) has reported finding two branched chain acids in *B. subtilis*, one a C_{15} compound and the other a C_{16} substance. These were not further characterized. In the same paper there is mention of a C_{19} branched acid which occurs in *Pasteurella pestis*. This also has not been studied in greater detail.

A number of complex branched chain acids have been isolated from the corynebacteria (9, 12, 48). These include corinnic and diphtheric acids, both of which have the same empirical formula, $C_{35}H_{68}O_2$, although they appear to differ in structural detail. Several derivatives of these acids have been found in the same organisms. Further, the reader will recall that corynomycolic, corynomycolenic, and corynolic acids, which were discussed with the hydroxy acids, are also branched chain compounds.

D. Cyclopropane Acids

The first acid of this type to be discovered in bacteria, and the only one for which any detailed structural information is available, has been named lactobacillic acid after the organisms from which it was originally isolated. This compound was first reported in 1950 by Hofmann and Lucas (65) and is now known to be *cis*-11,12-methyleneoctadecanoic acid (63, 67, 69, 70). It has not yet been possible to determine whether this acid occurs naturally as the D or the L isomer. Organic

synthesis of this molecule (69) produces a DL mixture, and resolution studies are impeded by the unfortunate fact that the optical rotation of the natural material is so small as to be undetectable.

Lactobacillic acid has been unequivocally identified in *Lactobacillus arabinosus* (62, 66), *Lactobacillus casei* (62, 71), *Lactobacillus delbrueckii* (62), and *Agrobacterium tumefaciens* (62, 73). The report on *A. tumefaciens* is of particular interest. In 1944 Velick (141, 142) reported the discovery in *Phytomonas* (now *Agrobacterium*) *tumefaciens* of a 10- or 11-methylnonadecanoic acid which he named "phytomonic acid." Eleven years later, Hofmann and Tausig (73) demonstrated that this substance was a 19-carbon cyclopropane compound rather than a branched methyl acid, and that, in fact, phytomonic and lactobacillic acids were identical.

Other organisms have been shown to contain C_{19} acids similar to lactobacillic acid although they have not been completely characterized. The bacteria in which such compounds have been reported are *E. coli* (87, 113), *C. butyricum* (44, 45), and possibly in *P. pestis* (10).

For some time it was believed that only C_{19} cyclopropane acids occurred in bacteria. This belief was based on the fact that no other such acids had been encountered in all the organisms that had been studied. However, more sensitive techniques have shown in recent years that other cyclopropane acids do occur in microorganisms. In 1959 the author detected what appeared to be a C_{17} cyclopropane acid in the lipids of *E. coli* (113). Shortly thereafter, Dauchy and Asselineau (32) showed that such an acid was indeed present in *E. coli*. The location of the ring in this compound, as well as other structural details are unknown. C_{17} cyclopropane acids have since been found in *B. subtilis* and *P. pestis* (10), and in *C. butyricum* (44, 45). Recently evidence has appeared suggesting the possible occurrence of C_{13} and C_{15} cyclopropane acids in the lipids of *C. butyricum* (44, 45).

These shorter cyclopropane acids occur in only small amounts and are most difficult to separate from other acids of similar chemical and physical characteristics. These considerations probably account for the fact that they are being encountered only now with the advent of highly efficient separation techniques. One cannot help but speculate on the acids that may not yet have come to light. Of interest in this respect is the paper of James and Martin (79) in which they report the detection in extracts of a culture of *P. aeruginosa* of a number of branched chain acids containing an odd number of carbon atoms ranging in length from C_8 to C_{19}. None of these acids was specifically identified, nor was it shown whether any of these compounds did, in fact, contain a cyclopropane ring. This would be a most interesting matter to investigate further.

Cyclopropene acids, such as sterculic acid (*cis*-9,10-methylene-9-octadecenoic acid) which has been isolated from the oil of *Sterculia foetida* (108), have not been demonstrated in the true bacteria although such compounds have been suggested as intermediates in the formation of saturated cyclopropane acids. However, recent studies indicate that cyclopropene acids may occur in the lipids of pleuropneumonia-like organisms (114*a*).

E. Unsaturated Acids

These compounds are among the most important fatty acids from a biochemical standpoint and constitute a major portion of the fatty acids of all bacteria. Yet it is with respect to these acids that the literature is least reliable. There is a curious readiness on the part of many investigators to "identify" unsaturated fatty acids on the basis of the most superficial analytical data. This is by no means always the case, but it happens often enough to warrant the most critical examination of papers purporting to list the unsaturated fatty acids of bacteria.

Let us first consider the octadecenoic acids. The reader has already been cautioned at some length about the tendency in the past, and to no small extent in the present, to call any monounsaturated C_{18} fatty acid "oleic acid." In 1952 it was found (66) that the octadecenoic acid of *L. arabinosus* was in actuality *cis*-vaccenic acid (i.e., *cis*-11-octadecenoic) rather than oleic acid (*cis*-9-octadecenoic acid). In rapid succession it was shown that *cis*-vaccenic acid was the sole octadecenoic acid present in *L. casei* (71) and in *A. tumefaciens* (73), two organisms that differ greatly biologically. In a study of the lipids of a group C streptococcus, Hofmann and Tausig (72) found that the major octadecenoic acid was, again, *cis*-vaccenic accompanied by a small amount of oleic. Law reported that the

octadecenoic acid in *E. coli* was *cis*-vaccenic acid (87).

Oleic acid has been definitely identified in bacteria in certain instances. For example, this compound was unequivocally detected in streptococci (72). Also there is the most interesting and conclusive identification of this compound in the tubercle bacillus by Cason and Tavs (24). Other cases in which oleic acid was specifically identified have been listed by Asselineau (9).

Hexadecenoic acids are almost as frequently encountered in bacteria as are the octadecenoic acids. On occasion palmitoleic acid (*cis*-9-hexadecenoic acid) has been identified (6, 72, 87, 118), but in most studies the evidence justifies only the generic term "hexadecenoic acid." There has been one report of *cis*-11-hexadecenoic acid in streptococci (72). This compound was named palmitvaccenic acid because of the similarity between the carboxyl end of its molecule and that of *cis*-vaccenic acid.

There are few reports of unsaturated acids of fewer than 16 carbon atoms in bacteria. The occurrence of an unidentified tetradecenoic acid in *C. diphtheriae* has been reported by Asano and Takahashi (6). Gubarev et al. (47) have evidence of a C_{10} or C_{12} unsaturated acid in *Brucella suis*.

Unsaturated fatty acids with chain lengths greater than C_{18} have been noted only in the corynebacteria; C_{20} acids have been reported by Alimova (3) and by Pustovalov (118), C_{21} by Asano and Takahashi (6), C_{22-24} by Alimova (3), and C_{28} again by Asano and Takahashi (6).

IV. Fatty Acid Spectra of Some Representative Bacteria

Although the identification of specific fatty acids in various bacterial species is interesting and important if not indispensable, the most useful information in this field is the exhaustive analysis of all the fatty acids present in a given organism. Such catalogues of the fatty acid composition of individual species are often referred to as fatty acid spectra. At the present time, only a few such spectra are available. The organisms which have been most thoroughly characterized in this respect are *A. tumefaciens* (73), *C. butyricum* (45), *E. coli* (32, 87, 113), *L. arabinosus* (66), *L. casei* (71), *L. delbrueckii* (62), and a group C streptococcus (72). The fatty acid composition of these organisms is shown in Table 2. In addition, there are several other bacteria whose lipids have been somewhat less intensively studied but regarding which there is nevertheless considerable information available. Among these are *B. subtilis* (2, 10, 121), *B. suis* (47), *C. diphtheriae* (3, 4, 6, 10, 48, 118), *Corynebacterium ovis* (10), *P. pestis* (10), and a species of *Sarcina* (2).

TABLE 2. *Fatty acid spectra of some representative bacteria*

Fatty acids	*Lactobacillus arabinosus* (62, 66)[a]	*Lactobacillus casei* (62, 71)	*Lactobacillus delbrueckii* (61)	*Streptococcus* sp. (62, 72)	*Clostridium butyricum* (45)	*Escherichia coli* (113)	*Agrobacterium tumefaciens* (62, 73)
Saturated							
C_{10}	1.1[b]	2.1	0.5	0.5	0.5	0.3	0.9
C_{12}	2.3	2.8	1.1	5.2	2.4	0.3	4.0
C_{14}	1.2	2.1	2.5	4.4	0.4	0.7	1.1
C_{16}	18.7	24.3	27.5	26.6	49.0		8.2
$C_{16} + C_{17}$[c]						85.4	
C_{18}		7.0	10.8	18.0	6.2		
$C_{18} + C_{19}$[c]						0.5	
Unsaturated							
C_{16}					17.0		
C_{18}					7.9		
$C_{16} + C_{18}$	35.6	37.6	45.5	38.0		11.6	63.6
Cyclopropane							
C_{13}					0.4		
C_{15}					1.5		
C_{17}					9.0	+[d]	
C_{19}	30.1	12.6	6.4		5.2	+[d]	9.4

[a] References.
[b] All figures refer to per cent of total fatty acids.
[c] Cyclopropane acid.
[d] + = Present but amount not determined.

In discussing determinations of the total complement of fatty acids in various bacterial species, it is germane to mention again briefly the fact that marked quantitative and sometimes even qualitative changes can be brought about in fatty acid spectra by alterations in media or growth procedures (see, for example, (61) and (68)).

V. Chemical Nature of Bacterial Lipids

It is not strictly within the purview of this paper to discuss in any detail the chemical combinations in which bacterial fatty acids occur.

Several reviews have appeared in recent years which have dealt with the chemical nature of bacterial lipid complexes (9, 11, 12), and the interested reader is referred to these articles for extensive information on this subject. However, for the purposes of the present discussion, it seems apropos at least to enumerate and describe briefly the various forms in which bacterial fatty acids are encountered.

Various authors including Asselineau and Lederer (12) and Lovern (97) have emphasized the fact that bacterial lipids differ radically from those of higher forms of life in several respects. These include: the presence of uncommonly large proportions of free fatty acids, the frequent presence of unusual fatty acids not seen in other organisms, the absence of sterols, and the absence of classical lecithins and cephalins. In many strains the phospholipids are markedly low in nitrogen and rich in carbohydrate. These general characteristics are reflected in the discussions of the different classes of bacterial lipids that follow.

A few reports have appeared from time to time regarding the specific fatty acid(s) detected in individual lipid complexes, but in general there is little information on the distribution of the fatty acids among the various types of lipids found in bacteria. This is understandable when one considers that investigators are still striving to establish merely the identity and relative abundance of each fatty acid in the bacterial cell as a whole. Now that a clearer picture is emerging of the fatty acids that one can expect to encounter, in all likelihood one of the next steps in this field will be to determine which acids are present in which lipids.

A. Free Fatty Acids

As just noted, the proportion of free, or unesterified, fatty acids found in bacterial cells is unusually high in comparison with the amounts found in the cells of other forms of life. A number of bacteria are known in which more than 20% of the total fatty acids occur free. Among these are *C. diphtheriae*, *L. acidophilus*, *Bacillus megatherium*, *A. tumefaciens*, *Hemophilus* (*Bordetella*) *pertussis*, and *Salmonella typhimurium* (12, 83). The lipid fraction of the last named organism has been reported to consist almost entirely of free fatty acids.

The major constituents of free fatty acid fractions are usually found to be palmitic, stearic, and octadecenoic acids, although small amounts of many more fatty acids have been detected in one organism or another in the unesterified form (83). The purposes which unesterified fatty acids serve in bacteria are not known. Equally uncertain are the cellular sites at which they may be located.

B. Fatty Acid Polymers

Lipoid material consisting of polymerized β-hydroxybutyric acid has been isolated from many bacteria including species of *Azotobacter* (39, 92), *Chromobacterium* (39), *Pseudomonas* (39), *Bacillus* (91, 146, 151), and *Micrococcus* (131). Law and Slepecky (88) have summarized a number of recent papers dealing with poly-β-hydroxybutyric lipid. With respect to the possible function of this polymer, Luria (99) has commented, "This is an interesting storage device, which makes available a metabolite less rich in energy, but more readily utilizable than the fully reduced chains of saturated fatty acids." There is at present, however, no detailed information on the metabolism of this type of lipid.

C. Glycerides

Mono-, di-, and triglycerides have been reported in many bacteria (12, 117). However, it is noteworthy that the concentrations of these neutral lipids are much lower in bacteria than in plant and animal cells, and that, in fact, many bacteria apparently contain no glycerides at all (11, 83).

D. Waxes

As has been pointed out recently by Asselineau and Lederer (12), the occurrence of true waxes (i.e., esters of long chain fatty acids and long chain fatty alcohols) is quite uncommon in bacteria. Such substances appear to be limited primarily to the mycobacteria and to the corynebacteria.

E. Phospholipids

For a discussion of the chemistry of the phospholipids, the reader is referred to a monograph by Lovern (96). Representatives of this lipid class are widespread in bacteria and frequently comprise major portions of the total lipid contents of the cells. Unfortunately, few

bacterial phospholipids, or phosphatides as they are also called, have been studied thoroughly by modern analytical methods. Carbohydrates, inositol, and glycerol are common constituents of those bacterial phospholipids which have been studied. Of the nitrogen-containing components, ethanolamine is most frequently detected, choline being found in some instances (12). Bases such as sphingosine have not been found in bacteria. Lovern (97) has commented on the relative simplicity of many bacterial phospholipids which he describes as being essentially fatty acid esters of phosphorylated carbohydrates. Phosphatidic acids (diacyl glycerophosphoric acids) are also commonly found in microorganisms.

F. Glycolipids

These important substances have been reviewed recently by Lederer (89) and Law (86). Such compounds are characterized by the combination of one or more molecules of fatty acids or fatty alcohols and one or more molecules of sugar. Many carbohydrates have been detected in bacterial glycolipids including glucose, galactose, arabinose, mannose, rhamnose, and trehalose. Amino sugars are also frequently observed.

Strictly speaking, the glycolipids are compounds which are soluble in such solvents as ether or benzene, but not soluble in water. However, the water-soluble lipopolysaccharides are usually considered in discussions of glycolipids because the lipoid moieties of the lipopolysaccharides are themselves glycolipids. Many of the glycolipids have pronounced biological properties as, for example, the antibiotic substances that have been isolated from various species of *Pseudomonas* and the lipopolysaccharide endotoxins of gram-negative bacteria (89).

G. Lipopeptides and Lipoproteins

As their names suggest, these are complexes consisting of fatty acids and amino acids. Purists require that these terms be used only for complexes having the solubility characteristics of peptides or proteins, whereas complexes with lipid solubilities should be called peptidolipids and proteolipids. It has been shown that individual complexes of this general type have fixed, characteristic proportions of lipid and protein, so that they seem to have some specific structure (51). At present, however, very little is known of the nature of these substances in bacteria.

H. Bound Lipids

This is a frequently encountered but ill defined term that refers more to solubility characteristics than to chemical nature. "Bound lipids" are generally understood to be lipoid constituents of the cell that are firmly combined with carbohydrates and proteins and that, consequently, cannot be extracted with organic solvents until first liberated by some hydrolytic procedure. As has been noted in a previous section, these bound lipids often constitute large proportions of the total cellular lipids.

VI. Intracellular Distribution of Fatty Acids

Information regarding the chemical distribution of bacterial fatty acids is scanty, but even less is known about the intracellular distribution of these compounds. Certain cellular structures are known to be relatively rich in lipid, but even in these instances little is known of the specific fatty acids present.

A. Cytoplasmic Inclusions

Lipid inclusions are commonly detected by differential staining using a dye such as Sudan black B. However, as Lamanna and Mallette (85) have pointed out, a globule staining with such a dye is not necessarily composed exclusively of lipid. As lipids have a tendency to collect at exposed surfaces, it is possible that many "lipid inclusions" may be nonlipid granules coated by lipid.

In any event, lipid inclusions have been reported in many bacteria. According to Burdon (21), the accumulation of lipid globules within the cell is a consistent characteristic of various species. Such globules are especially conspicuous in species of *Azotobacter*, *Rhizobium*, *Spirillum*, and *Bacillus*. Stainable intracellular lipid has also been found in *C. diphtheriae*, certain species of both gram-positive and gram-negative cocci, and in some anaerobic bacilli. Few gram-negative rods have been found to contain visible depositions of lipoid material. Murray (106) has pointed out that the number of lipid globules per cell appears to increase with increasing physiological

age and is influenced to some extent by the nature of the medium.

With respect to the chemical nature of lipid inclusions, the literature appears to be limited to the demonstrations by Lemoigne and his associates (91) and by Williamson and Wilkinson (151) that the lipoid granules characteristic of *Bacillus* species are composed almost exclusively of polymerized β-hydroxybutyric acid.

B. Cell Walls

In his recent and authoritative reviews on the cell wall (123, 124), Salton has pointed out, as have many others, that there is a marked difference in lipid content between the cell walls of gram-positive and gram-negative bacteria. Only small amounts of lipid have been found associated with the isolated cell walls of gram-positive organisms, usually on the order of 1 to 3%; it has been suggested that such a small quantity of lipid might be merely contamination from the rest of the cell and that in actuality the gram-positive cell wall may not contain any lipid at all. In contrast, gram-negative cell walls contain between 10 to 20% lipid. The physiological significance of this difference is not known.

In 1956, Salton (123) stated bluntly, "There is no information about the chemical nature of cell wall lipids." Unfortunately, little has happened in the intervening years to modify this bleak evaluation. Asselineau and Lederer (12) have commented that gram-negative cell walls seem to be composed largely of lipoprotein and lipopolysaccharide complexes. According to Weidel and Primosigh (147), the cell wall of *E. coli* contains 80% lipoprotein. In what may be the first attempts at actual analyses of cell wall lipids, Alimova (3, 4) has found that the walls of *C. diphtheriae* contain a variety of fatty acids ranging in chain length from C_{14} to C_{24}. The acids occur free, as esters of trehalose, or in both forms (3, 4).

C. Protoplasmic Membranes

These structures, which are believed to be the selective, semipermeable osmotic barriers of bacterial cells, are notably rich in lipid materials. Studies of a variety of bacteria show that the lipid content of their protoplasmic membranes varies between 10 and 30% (12, 106). Gilby and his associates (43) found, for example, that the protoplasmic membranes of *Micrococcus lysodeikticus* contained 28% lipid which consisted largely of phosphatidic acids. Weibull (146) has shown that the membranes from *B. megaterium* strain M contained 55 to 75% of the total cellular lipid despite the fact that the membranes represented only about 15% of the total cell weight. These membrane lipids consisted of free fatty acids, neutral fats, and phosphatidic acids.

D. Protoplasts and L-Forms

It is of interest that these protoplasmic elements, whose chief characteristic is the lack of a cell wall, frequently contain large amounts of lipids (102). Vendreley and Tulasne (143) have shown, for example, that L-forms derived from *Proteus* species contain 24% lipid although the bacterial forms contain only 6 to 8%. As yet no data are available regarding the chemical nature of the lipids found in bacterial protoplasts, L-forms, or mycoplasmas (pleuropneumonia-like organisms; PPLO). This is doubtless due to the great difficulty of growing sizable amounts of these entities.

VII. Aspects of Fatty Acid Biochemistry and Metabolism

The burgeoning activities in fatty acid chemistry have been paralleled by equally intense research on the metabolism of these compounds. As has been the case in the chemical studies, metabolic research has been greatly facilitated and accelerated by the availability of new instruments and techniques. Of particular importance have been the various isotopic tracer techniques, using both stable and radioactive nuclides, which have permitted the following of atoms and molecules through many of the intricate pathways of fatty acid metabolism. Mead and Howton (103) have discussed in a recent and excellent monograph the many uses of radioisotopes in this field.

The studies of fatty acid biochemistry and metabolism in bacteria not only have contributed to our knowledge of bacteria per se, but also have been most useful in providing clues to the details of fat metabolism in higher animals. In spite of the progress that has been made, however, there still exists a great deal of uncertainty regarding this aspect of bacterial physiology. Prior to the 1950's, very little was known about the synthesis or utilization of fatty acids in

bacteria, or in other organisms for that matter. Then, with the discovery of the coenzyme A (CoA) cycle and the elucidation of many details regarding it, there was a widespread feeling that the mystery of fatty acid metabolism had been suddenly and largely solved. In recent years, however, as data accumulated and promising hypotheses were tested and found inadequate, it has become increasingly apparent that the picture is much more complex than was originally believed, and that many pieces of the puzzle have yet to be found and fitted into place.

We are fortunate that a number of excellent reviews have appeared lately discussing the general field of fatty acid metabolism in some detail (28, 82, 100, 101, 136). It is my intent, therefore, to discuss below only certain aspects of the subject that are today receiving particular attention from workers in the field.

A. Degradation and Synthesis

The β-oxidative reactions associated with CoA have been demonstrated in various bacteria (133, 136), and it is generally assumed that this mechanism is operative in the bacterial degradation of fatty acids. Indeed, much of our present knowledge of β-oxidation and of the functioning of the CoA cycle has been obtained from studies using microorganisms (133).

Although a large amount of information has been gathered regarding the oxidation of fatty acids, considerably less is known about the synthesis of these compounds. Since the various reactions involved in β-oxidation are known to be individually reversible, it was a popular concept for some years that synthesis was merely the reverse of degradation. It is now evident that the synthesis of fatty acids is a more complicated process than was previously suspected, and that it involves considerably more than merely "backing up" the β-oxidation pathway. Wakil (145) has discussed this problem exhaustively and, on the basis of studies so far limited to preparations of animal tissues or of yeast, has proposed the existence of at least two synthetic mechanisms. One of these appears to involve enzymes of the β-oxidation system and is believed to be primarily responsible for the elongation of existing fatty acids. The other system converts acetyl-CoA to malonyl-CoA which is then condensed with itself, acetyl-CoA, or propionyl-CoA to form long chain fatty acids. The malonyl-CoA is formed by condensing HCO_3^- with acetyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase (a biotin-containing enzyme). Condensations involving propionyl-CoA are of special interest in this context in that they could account for branched chain acids which, as has been pointed out, are common in bacteria.

Another dichotomy in bacterial lipid metabolism seems to exist between the synthesis of saturated and unsaturated fatty acids. It has been found that in animal metabolism long chain unsaturated fatty acids are formed largely by enzymatic dehydrogenation of the corresponding long chain saturated acid, e.g., oleic acid is produced by dehydrogenation of stearic acid.

For some time it was assumed that bacteria formed their long chain unsaturated fatty acids by a similar procedure, and there have been a few reports of such dehydrogenations in a small number of species. However, the infrequent observation of this reaction in bacteria seemed curious in view of the fact that unsaturated fatty acids comprise large portions of the total fatty acid content of most microorganisms. Consequently, many investigators began to suspect that in bacteria dehydrogenation was not the major pathway to unsaturated acids, that instead some other more complicated mechanism was involved. The literature pertaining to this problem has been reviewed elsewhere (111).

Investigations of lactobacilli, in which the major unsaturated fatty acid is *cis*-vaccenic acid, have suggested the interesting possibility that this compound may be formed by the progressive lengthening, two carbons at a time, of some unknown, already unsaturated short chain precursor (68, 114). As will be discussed below, it has been shown that *cis*-vaccenic acid is the precursor of lactobacillic acid in these organisms. These observations suggest the possibility of a pathway such as is outlined in Fig. 1.

It will be noted that in each of the compounds indicated, the double bond is located in the same position with respect to the methyl end of the chain. Thus lengthening the chains on the carboxyl ends would eventually produce palmitoleic and *cis*-vaccenic acids, both of which are routinely found in lactobacilli.

Although this tentative pathway is an interesting and plausible explanation of unsaturated fatty acid biosynthesis in bacteria, it is em-

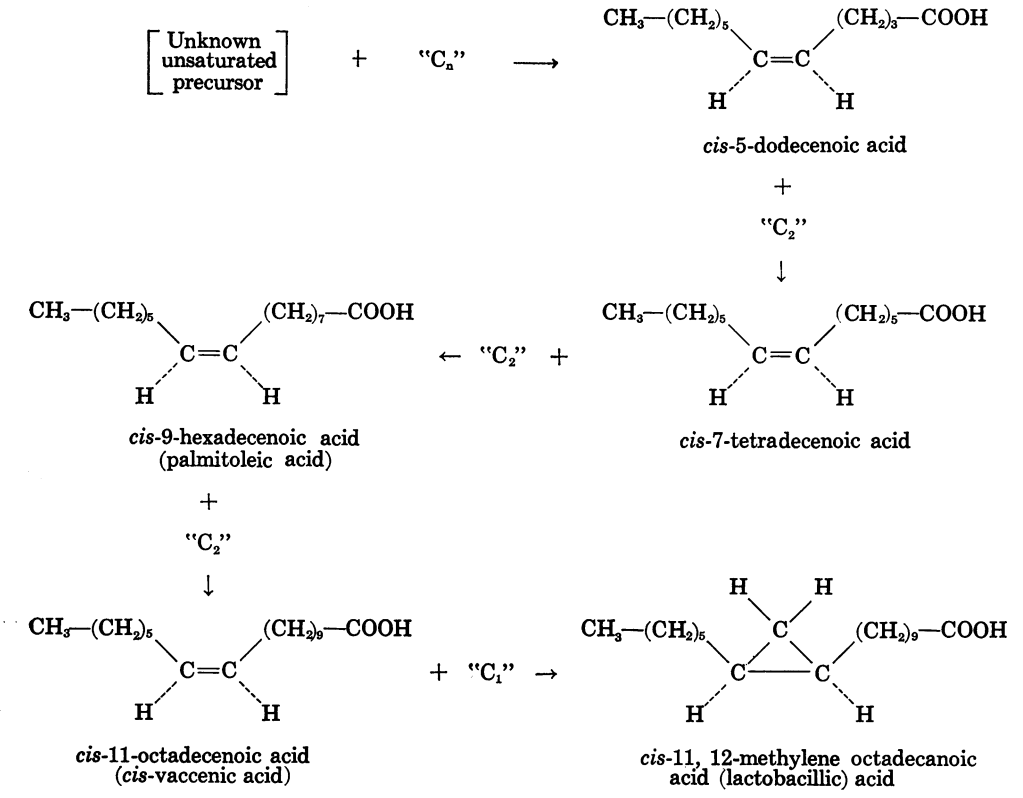


FIG. 1. *Possible pathway for the biosynthesis of unsaturated and cyclopropane fatty acids in bacteria.*

phasized that these studies are as yet preliminary, not conclusive, and that further investigation of this matter is essential. Some recent studies of *C. butyricum* and several other organisms have provided further evidence for separate and distinct biosynthetic pathways for saturated and unsaturated fatty acids (44, 45).

A third divergence in bacterial lipid metabolism is the fact that the metabolic pathways of the lower fatty acids (C_2 to C_{10}) differ in many ways from those of the higher acids (C_{12} to C_{18}). It is, for example, possible to inhibit the oxidation of higher acids without affecting oxidation of the shorter compounds. Further, unsaturated fatty acids ranging in chain length from C_{18} down to C_{12} have been shown to stimulate growth of certain bacteria, whereas shorter unsaturated acids do not possess this capability (68, 111). Oginsky and Umbreit (110) have suggested a spatial arrangement of the fatty acid molecule which, if correct, would make it improbable that the longer acids would be suitable substrates for enzymes that could affect the shorter compounds. Also, of course, there are marked differences in the solubility characteristics of the two groups that might influence their metabolism.

B. Stimulation and Inhibition of Growth

It has been known for some time that fatty acids in low concentrations can have marked effects on the growth of bacteria. Such phenomena have been reviewed by Kodicek (84), Nieman (107), and Deuel (33). The nature and magnitude of the effect in any given instance depend upon the specific fatty acid, on its concentration, and upon the bacterial species involved. In general, gram-positive bacteria are more sensitive to all effects of fatty acids than are the gram-negative organisms. Both saturated and unsaturated fatty acids can cause inhibition of growth. In the unsaturated acids, inhibitory effects increase with the number of double bonds in the molecule. Also, *cis* isomers are more inhibitory than the corresponding *trans* forms. Stimulation of growth is a property largely

limited to unsaturated long chain acids (i.e., C_{12} to C_{18}) and to certain cyclopropane acids. Stimulatory effects decrease with increasing unsaturation, and, once again, *cis* isomers are more effective than *trans*. Acids which are stimulatory in low concentrations are nevertheless inhibitory if present in only slightly higher concentrations.

There has been much disagreement regarding the nature of the mechanisms by which fatty acids affect bacterial growth. These arguments are discussed in detail in the references just cited. Here, for purposes of brevity, they can be reduced to two schools of thought, one favoring nonspecific physicochemical mechanisms and the other favoring more specific effects on metabolic reactions. For example, it has been generally accepted that inhibitory effects of fatty acids and their compounds are due to their surface-active properties, which cause nonspecific alterations in cell permeability and consequent loss of essential cellular constituents. However, two reports have appeared (22, 68) which indicate that, at least in lactobacilli, inhibition of growth by saturated acids and the reversal of this inhibition by unsaturated acids may be specific antimetabolite-metabolite interrelationships.

Similarly, some have chosen to regard the growth-stimulating effects of unsaturated fatty acids as merely the consequences of nonspecific enhancement of cell permeability, which facilitates the assimilation of essential nutrients. Others, including the author, feel that the preponderance of experimental evidence shows that fatty acids stimulate growth because they are useful and even essential metabolites for the organisms so affected.

The ability of unsaturated fatty acids to replace biotin in the nutrition of certain bacteria is pertinent in this respect. The literature regarding this interesting aspect of bacterial lipid metabolism has been reviewed extensively elsewhere (107, 111). Many fatty acids have been found to replace biotin including oleic, elaidic, *cis*- and *trans*-vaccenic, linoleic, linolenic, and other unsaturated C_{18} acids (13, 25, 70, 149), hexadecenoic acid (55, 68), dodecenoic and tetradecenoic acids (68), and even certain cyclopropane acids (70). The ability of each acid to serve in lieu of biotin depends upon the bacterial species, but is quite constant for any given organism.

Considerable evidence has been accumulated in support of the concept that biotin is somehow involved in the biosynthesis of long chain unsaturated fatty acids in many bacteria, possibly in the formation of the short chain unsaturated precursors (see (68)), and that if the unsaturated acids are supplied preformed, the biotin requirement is correspondingly reduced (19, 68, 70).

Though this concept has gained considerable credence and currency, it does not satisfy equally all students of the subject (84, 93, 140, 150). It may well be that the observed effect of exogenous fatty acid on bacterial growth in any given instance is actually the summation of changes brought about by many diverse mechanisms. Only further studies can say.

C. Metabolism of Cyclopropane Acids

When lactobacillic acid was discovered in 1950 (65), it was the first fatty acid containing a cyclopropane ring to be encountered in biological materials. Even today, as has already been pointed out, only a few compounds of this type are known. Also, lactobacillic acid was the first compound other than long chain unsaturated fatty acids shown to have a biotin-like activity for certain bacteria (70). Understandably, in the relatively short time that they have been known to exist, the cyclopropane acids have posed a number of interesting metabolic questions.

One major problem associated with these compounds has been the manner in which they are biosynthesized. Some years ago Hofmann and his co-workers (61) proposed, on the basis of indirect evidence, that lactobacillic acid might be produced in bacteria by the addition of a "C_1" fragment across the double bond of *cis*-vaccenic acid. This addition accompanied by reduction of the double bond would then result in a cyclopropane ring in the correct chain location. It was later shown by radioisotope techniques that when *cis*-vaccenic acid is supplied under appropriate conditions to actively growing lactobacilli, it is taken into the cells unaltered and there used as the chain moiety of lactobacillic acid (112). This, then, accounted for the chain and 18 of the 19 carbon atoms of lactobacillic acid. Shortly thereafter, also using tracer procedures, it was shown that the 19th carbon atom, i.e., the one added to form the cyclopropane

ring, was derived from the methyl group of methionine (113). Subsequent studies have confirmed and expanded these observations (64, 95).

The concept of *cis*-vaccenic acid serving as the immediate precursor of lactobacillic acid is consonant with the fact that the double bond in *cis*-vaccenic acid and the cyclopropane ring in lactobacillic acid are located at the same place in the chain, i.e., between the 11th and 12th carbon atoms. Seemingly at variance with this is the observation that labeled oleic acid supplied in the medium also gives rise to labeled octadecenoic and cyclopropane acids in lactobacilli (112). It will be recalled that in oleic acid the double bond is located between the 9th and 10th carbon atoms. Preliminary data presented in this paper suggest the interesting possibility that even when oleic acid is supplied, the acids in the bacterial cells are *cis*-vaccenic and lactobacillic. This possible interconversion merits further investigation. In their studies of fatty acid metabolism in clostridia, Goldfine and Bloch (45) have also observed the conversion of oleic acid to a C_{19} cyclopropane fatty acid.

An even more intriguing problem is the use to which lactobacillic acid is put by bacteria which contain it. Various pieces of evidence argue, somewhat teleologically it is true, in favor of some specific and important function. The nature of the molecule requires the expenditure of considerable energy in its synthesis. The molecule is a highly specific structure in that alterations in chain length, *cis-trans* isomerism, or positional isomerism have quite marked effects on its biological activity (70). Studies of synthetic DL mixtures indicate that the active material is exclusively either the D or the L configuration, the contrary form being without biological activity (69). Lastly, it has been shown that, in bacteria containing this compound, it is possible by appropriate cultural procedures to increase, decrease, or even eliminate altogether the amounts of the other major acids both saturated and unsaturated. However, it is not possible by any means so far tried to eliminate or even to reduce below certain levels the amount of lactobacillic acid in these cells (61, 68). All of these observations alike suggest some important, indispensable function for lactobacillic acid. The nature of this function is totally unknown at present. It has been suggested, in view of the rather labile ring carbon which is the conspicuous and unusual feature of this molecule, that lactobacillic acid may function as a carrier of 1-carbon fragments or even as a storage form of such units, but there is at this time no experimental evidence to support such hypotheses.

The information summarized in the preceding paragraphs pertains largely to lactobacillic acid, and most of the data have been gathered in studies on the lactobacilli. It is still too early to tell how much of this will be applicable to the shorter cyclopropane compounds found in other genera. We now predicate that the synthesis of lactobacillic acid is accomplished by the formation of an 18-carbon unsaturated acid with a double bond between the 11th and 12th carbon atoms and then the bridging of this double bond with a methylene group by some process resulting in a saturated ring. Are each of the shorter acids terminal products of similar syntheses in which the immediate precursors are corresponding unsaturated acids, or is some other mechanism involved? Much will depend upon the as yet undetermined locations of the cyclopropane rings in the shorter compounds. Are the shorter acids capable of replacing biotin or of producing any other growth effects? Do the various cyclopropane compounds have similar or different functions? What are the details of the biosynthetic reactions involved in constructing a cyclopropane ring on a carbon-to-carbon double bond? Obviously many interesting findings lie ahead in the study of these substances.

D. Other Findings of Interest

In this section are included several phenomena associated with bacterial fats which may well prove to be of major importance but regarding which little is definitely known at the present time. Recently two reports have appeared regarding the possible involvement of bacterial fatty acids and their complexes in the transport of amino acids. Hunter and Goodsall (75) have presented evidence indicating that in *B. megaterium* lipid complexes transport amino acids from the site of activation to the site of protein synthesis. Silberman and Gaby (129) have published some more detailed observations on a similar action in *P. aeruginosa*. These reports

are quite recent, and as yet no further elucidation of these observations has appeared.

The continuing search for molecular explanations of the mode of action of antibiotics has provided evidence that reactions with lipoid materials may play a role in the actions of some of the antibiotics. Cooper (27) has shown that the so-called penicillin combining component in many gram-positive bacteria is a lipid substance which is still unidentified. A similar reaction appears to be involved in the initial combination of the polymyxins with sensitive bacteria (37). It might be mentioned here that at least one antibiotic substance, pyolipic acid isolated from *P. pyocyanea* (*aeruginosa*), is a glycolipid consisting of 1 molecule of L-rhamnose and 1 molecule of D-β-hydroxydecanoic acid (14).

The ability of bacterial fatty acids and their complexes to produce a variety of pharmacological and immunological effects in higher animals is another fertile field for further investigation. Asselineau and Lederer (12) have recently reviewed these effects which include enzyme inhibition, inhibitory and stimulatory effects on leukocytes, local tissue reactions, general toxicity (as exhibited by the lipopolysaccharide endotoxins of gram-negative bacteria), antigenicity, and adjuvant action. Observations on these properties, although numerous, are almost without exception empirical. Little is known of the chemical composition of the fractions producing these effects, of the mechanisms whereby the effects are elicited, or of the relationships between specific composition and specific effect.

A recent paper by Dubrovskaia et al. (34) suggests that one aspect of the effect of phage on microorganisms may be a disturbance of lipid metabolism. In studies employing *Brucella abortus*, these workers noted that cells infected with phage contained 26% more lipid than did normal cells. At the same time they observed a shift in the proportions of different type of lipids in that phage infection caused an increase in phosphatide and a concomitant decrease in neutral fat.

VIII. Conclusion

The very considerable increase in interest, research, and knowledge that has taken place in recent years regarding the fatty acids of bacteria is indeed encouraging, especially in comparison with the years of relative quiescence in this field. Nevertheless, as has been aptly said in another context, all we know is still infinitely less than all that yet remains to be known.

Many intriguing problems still await attention and solution. Among these, one may cite several as being of particular and immediate interest: the manner in which the various acids are distributed among the different lipid classes, the cytological distribution of fatty acids, the details of biosynthesis and metabolism particularly with respect to the unsaturated and cyclopropane compounds, and the specific functions of the fatty acids and their compounds in the bacterial cell.

The complexity and importance of these questions are ample indication that, in spite of the progress made thus far, the major accomplishments in the study of bacterial fatty acids and lipids still lie ahead. The writer, for one, awaits future developments with enthused anticipation.

IX. Addendum

Since the completion of this review, numerous papers have appeared dealing with various aspects of microbial lipids. Among these, the following are of especial interest.

Two papers have added significantly to knowledge of the cyclopropane acids. In one, Liu and Hofmann (95*a*) demonstrated unequivocally that lactobacillic acid biosynthesis involves the addition of a 1-carbon fragment across the double bond of *cis*-vaccenic acid. In the other, Kaneshiro and Marr (80*a*) have shown that the C_{17} cyclopropane acid found in the phospholipids of *E. coli* is *cis*-9,10-methylene hexadecanoic acid, a compound analogous to palmitoleic acid.

Problems of the biosynthesis of unsaturated fatty acids have been extensively discussed by Bloch and his associates (14*a*). Their findings indicate that in microorganisms there are two pathways for the synthesis of monoethenoic acids. One involves oxidative desaturation and occurs in yeast, blue-green algae, and some aerobic bacteria, whereas the other is an anaerobic mechanism involving elongation of already unsaturated acids. The latter pathway is found in anaerobes, facultative aerobes, and some obligate aerobes. In a later paper, Scheuerbrandt and Bloch (124*a*) postulate the involvement of octenoic and decenoic acids in the biosynthesis

of higher unsaturated and cyclopropane acids in a series of pathways similar to the scheme shown in Fig. 1 although much expanded in details.

Lastly, there have been attempts recently to analyze the fatty acids of microorganisms smaller than the true bacteria. Examination of the lipids of PPLO strain 07 (114*a*) has revealed a fatty acid composition differing both qualitatively and quantitatively from that of most bacteria. In PPLO, the concentrations of unsaturated and cyclopropane acids are much lower than in bacteria, and the concentration of palmitic acid is correspondingly increased. Kates and coworkers (80*b*) have catalogued the fatty acids found in influenza virus and have concluded that this virus incorporates lipid components present in the host cells before infection as well as those synthesized after infection. They further concluded that influenza virus is not able to direct cellular lipid synthesis as it does nucleic acid and protein synthesis.

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