THE ESTIMATION OF THE FATTY ACID COMPOSITION OF BACTERIAL LIPIDES*

BY KLAUS HOFMANN, CHAO-YING YUAN HSIAO, DOV B. HENIS, AND CHARLES PANOS

(From the Biochemistry Department, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania)

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As the initial phase in our attempts to elucidate the mechanism of the stimulation of bacterial growth by lipides, we have investigated the chemical nature of the fatty acids of Lactobacillus arabinosus (1), Lactobacillus casei (2), a Streptococcus species (3), and Agrobacterium tumefaciens (4). Large quantities of these various organisms were grown under controlled conditions, and the fatty acids were characterized by both chemical and physical methods. Although providing information on the chemical nature of the various fatty acids, these preliminary studies gave only a crude estimate of the quantitative distribution of these substances in the bacterial lipides.

A method for the estimation of individual fatty acids in small samples of bacterial lipides was required in order to permit insight into the metabolic interplay among the various fatty acids in bacteria, and to allow investigation of the effect of alterations of the culture medium upon their fatty acid spectrum.

In 1950, Boldingh (5) devised a chromatographic microtechnique for the determination of the composition of mixtures of straight chain, saturated fatty acids. We have now developed a modification of this procedure which allows determination of monounsaturated, saturated, and branched chain fatty acids in small samples of bacterial lipides. It is the purpose of this communication to describe our technique and its application to the analysis of synthetic fatty acid mixtures and to the determination of the fatty acid spectrum of five microorganisms.

EXPERIMENTAL

Preparation of Solvents and General Comments

Acetone was purified by distillation from a mixture of solid potassium permanganate and anhydrous potassium carbonate. Petroleum ether

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(low and high boiling) was washed with concentrated sulfuric acid and water, dried over potassium hydroxide pellets, and distilled. Freshly distilled, sodium-dried ether was employed.

The acetone-water mixtures were prepared by placing in a 1 liter volumetric flask the desired volume of acetone (i.e. 350 ml. for Solvent A35, 500 ml. for Solvent A50, 580 ml. for Solvent A58, and 640 ml. for Solvent A64) and filling to the mark with doubly distilled water. One analysis requires approximately the following quantities of these solutions: A35, 1000 ml.; A50, 500 ml.; A58, 500 ml.; and A64, 2000 ml. Solvents A58 and A64 were equilibrated with petroleum ether (b.p. 100–140°) on a mechanical shaker for 1 hour at room temperature, and the hydrocarbon was left in contact with these solvents during storage. The titrations were carried out (under a stream of nitrogen) with 0.01 N sodium hydroxide, 1 drop of a 1 per cent solution of phenolphthalein in absolute ethanol serving as the indicator. The extraction and washing procedures employed in the isolation of the fatty acids from the bacteria were performed in countercurrent fashion by the use of three separatory funnels (under nitrogen whenever possible).

Cultivation of Microorganisms

The organisms (L. arabinosus and L. casei) were cultured under the conditions previously described (1, 2). The medium described later was employed for the mass culturing of Lactobacillus delbrueckii; the Tween was omitted. After an incubation time of 48 hours at 35°, the cells were collected in a Sharples supercentrifuge, washed with distilled water, and lyophilized. From 10 liters of medium, 10 to 12 gm. of dried cells were obtained.

Extraction of Fatty Acids

The dry bacteria (10 gm.) are suspended in 100 ml. of 2 N sulfuric acid and the mixture is autoclaved at a pressure of 15 pounds per sq. in. for 90 minutes. Hyflo Super-Cel (10 gm.) is added to the cooled hydrolysate and suspended throughout the solution by swirling. The mixture is filtered through two layers of Whatman No. 41 filter paper on an 8 cm. Büchner funnel, and the filter cake washed with two 10 ml. portions of water and sucked as dry as possible. The filter cake plus filter paper is then refluxed for 2 hours with 100 ml. of boiling acetone, the mixture filtered, and the solids reextracted with two 100 ml. portions of acetone and one 100 ml. portion of ether. The acetone extracts are combined, water (100 ml.) is added, and the bulk of the acetone is removed under diminished pressure. The resulting suspension is extracted with ether, the ether layer is combined with the ether extract of the filter cake, and the ethereal solution washed
successively with 5 per cent sodium bicarbonate and water, and dried with anhydrous sodium sulfate. Evaporation of the ether affords the crude lipides, which are then refluxed for 4 hours with a solution of potassium hydroxide (2.8 gm.) in water (2.8 ml.) plus ethanol (12 ml.). The bulk of the ethanol is removed by blowing a stream of nitrogen through the solution at 80°. The residue is cooled to room temperature, transferred to a separatory funnel, and extracted with ether. The ether extract is discarded and the aqueous layer acidified to Congo red with 50 per cent sulfuric acid, and the fatty acids are isolated in the usual manner and dried to constant weight over phosphorus pentoxide in vacuo. The yield of free fatty acids from 10 gm. of the various organisms was L. arabinosus 190 mg., L. casei 199 mg., and L. delbrueckii 180 mg. Streptococcal and Phytoomonas fatty acids were available from our large scale experiments (3, 4).

**Hydroxylation of Fatty Acids**

A sample of the fatty acids (100 mg.) is mixed with 0.5 ml. of 88 per cent formic acid and the solution is cooled to 0°. Hydrogen peroxide (0.1 ml. of 30 per cent) is added and the mixture is shaken for 15 minutes at 0° and then kept at 40° for 2 hours. Mixing is effected by bubbling a stream of nitrogen through the solution. The formic acid is removed in vacuo, 3 N potassium hydroxide (8 ml.) is added, and the mixture is refluxed for 2½ hours. The solution is acidified with 20 ml. of 2 N hydrochloric acid, and the hydroxylated fatty acids are isolated in the usual manner and dried to constant weight over phosphorus pentoxide in vacuo. Samples of oleic acid were hydroxylated by this procedure and the ensuing dihydroxy acids were chromatographed. The recoveries of “dihydroxy” acid (expressed as oleic acid) from the column ranged from 90 to 95 per cent.

**Chromatographic Procedure**

**Purification and Swelling of Mealorub**—The Mealorub (100 gm.) is suspended in 400 ml. of acetone (containing 1 per cent, by volume, of concentrated hydrochloric acid), and the suspension is agitated on a mechanical shaker for 1 hour and then kept at room temperature for 12 hours. The acetone is decanted and the rubber powder reextracted with two 300 ml. portions of the acetone-hydrochloric acid mixture, followed by four 300 ml. portions of pure acetone. The washed Mealorub is collected on a bed of glass wool in a large glass funnel and percolated with acetone until the filtrate becomes clear. The acetone-saturated rubber is then stored in a glass-stoppered bottle. An 8 month-old sample gave excellent

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1 The powdered, vulcanized Hevea rubber, Mealorub, was obtained through the courtesy of Mr. J. F. Frank, 120 Wall Street, New York 5, New York.
ESTIMATION OF FATTY ACIDS

performance. For chromatographic use, the acetone-saturated Mealorub (60 gm.) is placed in a mortar, layered with petroleum ether (b.p. 40–60°), and ground with a pestle until an even suspension results. This suspension is then worked through a 20 mesh sieve, the powder being covered with petroleum ether during the entire operation. The strained particles are collected and stored in a glass-stoppered bottle. For the preparation of a chromatographic column, a suitable sample of the “swelled rubber” is removed, and the excess petroleum ether is evaporated in vacuo.

Preparation of Mealorub Column—The processed Mealorub (9.0 gm.) is evenly suspended in a mixture of acetone (84 ml.) and petroleum ether (b.p. 100–140°) (12 ml.); water (45 ml.) is added and the suspension is poured into a chromatogram tube of 13 mm. inside diameter and 64 cm. length. A plug of glass wool is employed to keep the Mealorub column in place. The stop-cock at the lower end of the chromatogram tube is kept open during the pouring of the column, the effluent serving to transfer the Mealorub quantitatively into the tube. Gentle tapping of the walls of the tube during the procedure insures even settling of the particles. A second plug of glass wool is then inserted, and the column is gently pressed with a glass rod to insure firm packing. The column of material must be kept covered with solvent throughout the entire procedure. The finished column has a height of 42–48 cm. and should not contain any entrapped air. It is washed with 200 ml. of Solvent A64 and is left in contact with this solvent at room temperature for 12 hours. An additional 200 ml. portion of Solvent A64 is then passed through the column, followed by 100 ml. of Solvent A35. A 400 drop sample (11 ml.) of these last washings should give a blank titration not exceeding 0.05 ml. of 0.01 N sodium hydroxide. The last washing is drained until the meniscus of the solvent has reached a point approximately 1 cm. above the top of the Mealorub.

Introduction of Sample and Elution—A sample of hydroxylated fatty acids (25 to 60 mg.) is dissolved in 0.8 ml. of a 1:1 mixture of acetone and petroleum ether (b.p. 100–140°) with slight warming. The liquid meniscus in the column is then lowered until it reaches a point approximately 1 cm. below the top of the Mealorub and the solution of the hydroxylated fatty acids is introduced with a pipette. To insure quantitative transfer of the sample, the container and pipette are rinsed with several 1 ml. portions of Solvent A35, which are then added to the column. The column is mounted on a Technicon automatic fraction collector and set to provide a drop rate of approximately 90 drops per minute. The impulse counter is adjusted to collect fractions of 400 drops each (approximately 11 ml.). The column is then eluted with the acetone-water mixtures in the sequence A35, A50, A58, and A64. The individual fractions are collected in rimmed test-tubes (6 inches X ½ inch) and titrated with 0.01 N sodium hydroxide.
The meniscus of each solvent is allowed to fall to the top of the Mealorub prior to the introduction of the next solvent. In these experiments, we have usually collected forty to 60 fractions of A3S, twenty fractions of A50, twenty fractions of A58, and 70 fractions of A64. However, the volume necessary in order to insure quantitative elution of a given fatty acid in a mixture of unknown composition must be predetermined by special experiment. The columns were operated at a temperature of 27° ± 2°.

**Microbiological Evaluation of the \( (C_{18} + C_{19}) \) Fraction**

*Preparation of Samples for Assay*—The contents of the titrated tubes containing the \( (C_{18} + C_{19}) \) eluates are combined, acidified to Congo red with 50 per cent sulfuric acid, and concentrated under nitrogen to a volume of approximately 150 ml. in vacuo (bath temperature, 80°). The residue is extracted with one 60 ml. and two 30 ml. portions of ether, and the extracts washed successively with 5 per cent sodium bicarbonate and water, and dried over anhydrous sodium sulfate. The ether is evaporated and the fatty acids are dried to constant weight in vacuo. The fatty acids are dissolved in 10 ml. of 70 per cent ethanol, and suitable aliquots of the solution are added to the assay tubes. One aliquot of the solution is titrated with 0.01 N sodium hydroxide for determination of its fatty acid content.

**Assay Procedure**—The lactobacillic acid content was determined with *L. delbrueckii* (ATCC 9649). The medium of Craig and Snell (6) was em-

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2 Care must be exercised with Solvents A58 and 64, which are layered with petroleum ether. The petroleum ether layer must not come in contact with the top of the Mealorub since this causes swelling of the rubber with consequent clogging of the column.
ployed, with the addition of calcium pantothenate (80 μg per 100 ml. of double strength medium) and the omission of oleic acid and biotin. A vitamin-free casein hydrolysate3 (10 ml. per 100 ml. of double strength medium) was added, and the enzymatic casein digest was omitted. After an incubation time of 25 ± 4 hours at 35°, growth was determined turbidimetrically with a Klett-Summerson colorimeter equipped with a No. 66 filter. The uninoculated controls were set at zero. The amount of lactobacillic acid was calculated from a standard lactobacillic acid curve (Fig. 1) determined simultaneously. The stearic acid content was found by subtracting from the total milliequivalents of fatty acid, estimated by titration, the milliequivalents of lactobacillic acid as measured by microbiological assay.

RESULTS AND DISCUSSION

The analytical method which is the subject of this communication was devised as a tool to study fatty acid interconversions in microorganisms; in particular, to determine palmitic, stearic, cis-vaccenic, and lactobacillic acids in small samples of bacterial lipides. We wish to emphasize that, in its present stage of development, the method may not prove suitable for the separation of other complex fatty acid mixtures. Future experience in this and other laboratories will undoubtedly serve to indicate its general potentialities and limitations.

Boldingh (5) achieved the quantitative separation of small quantities of even-numbered, saturated, straight chain fatty acids containing from 8 to 18 carbon atoms, by reversed phase chromatography, using benzene-swelled Mealorub as the stationary and acetone-methanol-water mixtures as the mobile phase. We were unable to duplicate Boldingh's results, and selected for extensive study the system of Mealorub swollen with petroleum ether as the stationary and aqueous acetone as the mobile phase.4 A variety of synthetic mixtures composed of different proportions of even-numbered, straight chain, saturated fatty acids ranging from 8 to 18 carbon atoms were separated by the use of this system, with excellent recoveries of the individual components. Initially, we employed the solvent sequence A35, A50, A58, A64, A69, and A74 for the elution of C₈, C₁₀, C₁₂, C₁₄, C₁₆, and C₁₈ straight chain fatty acids, respectively. The results of a typical experiment demonstrating the high resolving power of this system are given in Table I (Experiment 1). More experience with the method led to the observation that Solvent A64 brings about the quan-

3 “Vitamin-free” casein hydrolysate, General Biochemicals, Inc., Chagrin Falls, Ohio.
4 We wish to express our gratitude to Dr. J. Boldingh for suggesting this system to us.
titative elution of myristic, palmitic, and stearic acids in three well defined bands, rendering unnecessary the use of solvents of higher acetone content for the separation of these components. We have consequently omitted Solvents A69 and A74 in all further experiments.

Since lactobacillic acid is a major constituent of certain bacterial lipides, we attempted the separation of this branched chain component from certain straight chain fatty acids. As shown in Table I (Experiment 2), it was found that Solvent A64 separates lactobacillic acid from palmitic and

<table>
<thead>
<tr>
<th>Acid</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Experiment 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic</td>
<td>22.0 (95)‡</td>
<td>30.2 (96)‡</td>
<td>35.7 (92)</td>
<td>12.7 (95)‡</td>
<td>6.5 (86)</td>
</tr>
<tr>
<td>Capric</td>
<td>27.8 (98)‡</td>
<td>29.5 (97)‡</td>
<td>33.4 (90)‡</td>
<td>1.1 (100)‡</td>
<td>1.2 (78)</td>
</tr>
<tr>
<td>Lauric</td>
<td>33.0 (91)‡</td>
<td>26.9 (87)‡</td>
<td>35.8 (90)‡</td>
<td>2.1 (107)‡</td>
<td>4.1 (95)‡</td>
</tr>
<tr>
<td>Myristic</td>
<td>35.8 (90)‡</td>
<td>30.3 (106)‡</td>
<td>35.7 (92)‡</td>
<td>12.8 (105)‡</td>
<td>1.6 (92)‡</td>
</tr>
<tr>
<td>Palmitic</td>
<td>41.2 (94)‡</td>
<td>31.2 (95)‡</td>
<td>33.4 (90)‡</td>
<td>4.8 (90)‡</td>
<td>1.2 (78)</td>
</tr>
<tr>
<td>Stearic</td>
<td>47.0 (92)‡</td>
<td>51.2 (86)‡</td>
<td>35.7 (110)‡</td>
<td>4.1 (95)‡</td>
<td>1.2 (78)</td>
</tr>
<tr>
<td>Lactobacillic</td>
<td>11,12-Dihydroxy-octadecanoic</td>
<td>35.7 (110)‡</td>
<td>10.0 (92)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In our earlier experiments (Experiments 1 to 3), we applied 200 to 300 mg. samples to the rubber column. Poor resolution of the C16 and (C18 + C19) bands was observed with large samples containing lactobacillic acid. Optimal resolution was achieved in these instances with samples weighing 40 to 60 mg. or less.
† The (C18 + C19) fraction (1.2 mg. of stearic acid and 6.5 mg. of lactobacillic acid) corresponds to 0.0262 m.eq. of fatty acid. The recovery from the column was 0.0222 m.eq. (85 per cent). Microbiological evaluation of this material (see the text) gave the stearic and lactobacillic acid recoveries shown in parentheses.
‡ The figures in parentheses represent per cent recoveries.

lower even-numbered, straight chain saturated fatty acids. Numerous attempts to achieve the separation of stearic acid from lactobacillic acid with our system were fruitless since both acids invariably accumulated in the same fraction, designated as the (C18 + C19) fraction.

In attempts to separate oleic acid from palmitic and stearic acids, Boldingh (5) observed that the unsaturated acid leaves the column between the palmitic and stearic acid bands, and he was unable to achieve the separation of these three compounds. In agreement with Boldingh, we have observed that the dihydroxy derivatives of oleic or cis-vaccenic acid are readily separable by chromatography from even-numbered, straight chain, saturated fatty acids possessing a chain of 10 to 18 carbon
The dihydroxy derivatives are eluted by Solvent A35, but Solvent A50 is required in order to recover capric acid from the column. A separation of myristic, palmitic, and 11,12-dihydroxyoctadecanoic acids is shown in Table I (Experiment 3). For the separation of the mono-ethenoid octadecanoic acids from the saturated acids, we subject the mixture of fatty acids to oxidation with performic acid prior to chromatography. The application of this procedure to the separation of a mixture of palmitic, stearic, and oleic acids is shown in Table I (Experiment 4). A representative chromatographic pattern illustrating the separation of oleic acid, four even-numbered, straight chain fatty acids, and lactobacillic acid is shown in Fig. 2, which demonstrates the high resolving power of the Mealorub column.

![Chromatographic pattern of a synthetic fatty acid mixture](http://www.jbc.org/)

**Fig. 2.** Chromatographic pattern of a synthetic fatty acid mixture (47.8 mg.) composed of oleic acid 17.6 mg. (81 per cent), capric acid 1.6 mg. (103 per cent), lauric acid 2.2 mg. (110 per cent), myristic acid 2.5 mg. (96 per cent), palmitic acid 13.0 mg. (98 per cent), lactobacillic acid 10.9 mg. (97 per cent); the figures in parentheses represent recoveries from the column. The solvents were changed at the positions indicated by the dotted lines.

The procedure fails to distinguish between oleic and cis-vaccenic acids, and knowledge regarding the exact composition of the mono-ethenoid, octadecanoic acid fraction in an unknown mixture of fatty acids must be obtained by other means (2).

As has been mentioned above, the chromatographic method is incapable of separating stearic acid from lactobacillic acid. Despite this limitation, determination of the lactobacillic acid content of an unknown mixture by microbiological assay is possible. We have recently demonstrated (7) that lactobacillic acid and a number of closely related fatty acids containing the cyclopropane ring have the ability to stimulate growth of a number of organisms. One of these organisms, *L. delbrueckii*, has now been employed for the microbiological determination of lactobacillic acid. A typical growth curve is illustrated in Fig. 1. Mixtures of lactobacillic and stearic acids were assayed with *L. delbrueckii*, and the results given in Table II demonstrate that the growth-promoting activity of such mixtures is a true
reflection of their content of lactobacillic acid. Within the range of concentration studied, the added stearic acid did not exhibit any synergistic or inhibitory effects. The lactobacillic acid content of an unknown mixture of fatty acids is thus readily determined by microbiological assay of the \((C_{18} + C_{19})\) eluate fraction.

The stearic acid content of the \((C_{18} + C_{19})\) fraction was determined by difference. The losses involved in the isolation of the fatty acids from the combined eluates and the error (±10 per cent) of the microbiological assay limit the accuracy of these determinations. A stearic acid content in the \((C_{18} + C_{19})\) fraction of less than 15 per cent cannot be detected by this method. The combination of oxidation with performic acid, rubber chromatography, and microbiological assay has resulted in a method which enables us to determine the composition of complex mixtures of fatty acids in small samples. The determination of caprylic, capric, lauric, myristic, palmitic, stearic, and lactobacillic acids in a 29.3 mg. sample is illustrated in Table I (Experiment 5).

Although the \((C_{18} + C_{19})\) fraction from a natural source is free from microbiologically active, unsaturated fatty acids (these are converted into the inactive dihydroxy derivatives by the performic acid treatment and are removed by chromatography), it may contain "saturated" fatty acids, with growth-promoting properties for *L. delbrueckii*, which differ structurally from lactobacillic acid.

For example, in *L. delbrueckii* assays of *dl*-9-methyl-, *dl*-10-methyl-, *dl*-11-methyl-, and *dl*-12-methyloctadecanoic acids, we have found that the 9- and 10-methyl derivatives exhibit 41 and 25 per cent, respectively, of

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**TABLE II**

<table>
<thead>
<tr>
<th>Material assayed</th>
<th>Lactobacillic acid</th>
<th>Found</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>Inactive</td>
<td>3.6, 3.9</td>
<td>100, 110</td>
</tr>
<tr>
<td>(S) (0.0) + L (3.6)</td>
<td></td>
<td>5.3, 5.3</td>
<td>93, 93</td>
</tr>
<tr>
<td>&quot; (1.0) + &quot; (5.7)</td>
<td></td>
<td>2.9, 2.9</td>
<td>105, 105</td>
</tr>
<tr>
<td>&quot; (1.8) + &quot; (2.8)</td>
<td></td>
<td>2.6, 2.7</td>
<td>106, 109</td>
</tr>
<tr>
<td>&quot; (2.5) + &quot; (2.5)</td>
<td></td>
<td>1.0, 0.9</td>
<td>100, 90</td>
</tr>
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</table>

\(S\), stearic acid; \(L\), lactobacillic acid.

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These compounds were obtained through the courtesy of Dr. J. Cason, Department of Chemistry, University of California, Berkeley 4, California.
the growth-promoting activity of lactobacillic acid; the 11- and 12-methyl derivatives were inactive. Thus, it becomes apparent that the growth-promoting activity, for \( L. \) delbrueckii, of the \((C_{18} + C_{19})\) fraction derived from a natural source does not prove the presence of lactobacillic acid. The identity of the growth promoter must be verified by isolation and chemical characterization.

The encouraging results with synthetic mixtures prompted application of our technique to the analysis of bacterial lipides. Three lactobacilli, \( A. \) tumefaciens, and a Group C Streptococcus species were selected for study. With the exception of \( L. \) delbrueckii, the chemical nature of the fatty acids of these organisms had been previously explored in our laboratory (1-4). The fatty acids were isolated from the acid-hydrolyzed

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fatty acid composition, per cent</th>
<th>Recovery, per cent</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>“Dihydroxy”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( C_{10} )</td>
<td>( C_{12} )</td>
</tr>
<tr>
<td>( L. ) arabinosus</td>
<td>35.6</td>
<td>1.1</td>
</tr>
<tr>
<td>( L. ) casei</td>
<td>37.5</td>
<td>2.1</td>
</tr>
<tr>
<td>( L. ) delbrueckii</td>
<td>45.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( A. ) tumefaciens</td>
<td>38.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Below detectable limits.

TABLE III
Fatty Acid Composition of Lipides Derived from Number of Microorganisms

cells and were then hydroxylated with performic acid. The resulting hydroxylated, fatty acid mixture was then subjected to rubber chromatography. The \((C_{18} + C_{19})\) fraction was isolated from the respective eluates and assayed with \( L. \) delbrueckii. The results are summarized in Table III. Individual, fatty acid samples were reanalyzed repeatedly by the same procedure, and the figures for the “dihydroxy,” palmitic acid and \((C_{18} + C_{19})\) fraction were reproducible with an error of ±10 per cent. Greater variation was observed with those individual fatty acids which were present in small proportions. The “dihydroxy” values of the \( L. \) arabinosus, \( L. \) casei, and \( A. \) tumefaciens lipides reflect the cis-vaccenic acid content of these organisms. Previous work (1, 2, 4) has demonstrated that cis-vaccenic acid is the only major monoenoic component of their lipides.

The streptococcal lipides contain both \( C_{16} \) and \( C_{18} \) monounsaturated fatty acids (3). Since the dihydroxy derivatives of hexadecanoic and octadecanoic acids are not separable by our present method, the “dihy-
droxy" fraction derived from this organism is representative of its content of both hexadecenoic and octadecenoic acids. The lipides of *L. delbrueckii* have not been investigated previously, and the composition of its "dihydroxy" fraction is unknown.

The ratio of C\textsubscript{16} and C\textsubscript{18} dihydroxy fatty acids in the "dihydroxy" fraction may be ascertained by chromatography of a duplicate sample which is subjected to hydrogenation prior to analysis. Hydrogenation converts the C\textsubscript{16} unsaturated fatty acids into palmitic acid, whereas the unsaturated C\textsubscript{18} acids are transformed into stearic acid. Thus, the difference in the C\textsubscript{16} and the (C\textsubscript{18} + C\textsubscript{19}) bands between a hydroxylated and a hydrogenated sample of a mixture of fatty acids of unknown composition provides a measure of its content of both C\textsubscript{16} and C\textsubscript{18} unsaturated fatty acids.

The (C\textsubscript{18} + C\textsubscript{19}) eluates from the three lactobacilli and *A. tumefaciens* exhibited microbiological activity when assayed with *L. delbrueckii*. The (C\textsubscript{18} + C\textsubscript{19}) eluates from the streptococcal lipides were inactive. These findings are in agreement with our previous demonstration (3) that the lipides of the streptococcus species are devoid of lactobacillic acid. The microbiological activity of the (C\textsubscript{18} + C\textsubscript{19}) eluates from *L. arabinosus*, *L. casei*, and *A. tumefaciens* is a true reflection of their lactobacillic acid content. The growth-promoting activity of the (C\textsubscript{18} + C\textsubscript{19}) eluates from *L. delbrueckii* may be caused by lactobacillic acid or another microbiologically active "saturated" fatty acid. From our large scale isolation experiments, we estimated a lactobacillic acid content for *L. arabinosus*, *L. casei*, and *A. tumefaciens* of 31, 16, and 13 per cent, respectively. We now find values of 30.1, 12.6, and 9.4 per cent, respectively.

As a check on the efficiency of the rubber column, we have isolated the C\textsubscript{16} acid fraction in a number of experiments and have subjected the regenerated fatty acids to *L. delbrueckii* assay. In all these instances, this material was devoid of growth-promoting activity. In addition to these major components, we have now demonstrated the presence of small quantities of C\textsubscript{10}, C\textsubscript{12}, and C\textsubscript{14} fatty acids in the various bacterial lipides. The presence of small proportions of lauric and myristic acids in the lipides of the *Streptococcus* species has been reported previously (3). Fatty acids lower than C\textsubscript{16} had not been identified in the lipides of the other organisms.

The present method offers a convenient tool for investigation of the fatty acid composition of microorganisms, and, in addition, provides a screening technique for microbiologically active "saturated" fatty acids in natural materials.

**SUMMARY**

A method is described for the determination of certain fatty acids in small samples of lipides. The method involves (1) the hydroxylation of
the fatty acid mixture with performic acid and (2) a separation of the resulting mixture of hydroxylated fatty acids by reversed phase chromatography on rubber columns. Stearic acid and lactobacillic acid are not separable from one another by this procedure and accumulate in the same eluate fraction from the chromatogram. The lactobacillic acid content of this fraction is determined by microbiological assay with *Lactobacillus delbrueckii*.

The application of this method to the separation of synthetic, fatty acid mixtures and to the determination of the fatty acid spectrum of five microorganisms is given. The scope of the method is critically evaluated. The lipides of *L. delbrueckii* are shown to contain a microbiologically active "saturated" fatty acid.

**BIBLIOGRAPHY**

THE ESTIMATION OF THE FATTY ACID COMPOSITION OF BACTERIAL LIPIDES
Klaus Hofmann, Chao-Ying Yuan Hsiao, Dov B. Henis and Charles Panos


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