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Title STUDIES ON THE LIPID AND FATTY ACID COMPOSITION OF MICROCOCCUS RADIODURANS

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Comparatively little work has been done on bacterial lipids. There are profound differences between bacterial lipids in general and lipids of the higher forms of life in such respects as the absence of sterols, phospholipids low in nitrogen but high in inositol and carbohydrate, the presence of large proportions of free fatty acids and the presence of certain fatty acids unique to microorganisms.

The purpose of this investigation was to characterize the fatty acids of Micrococcus radiodurans, a gram-positive, red-pigmented aerobe, which was isolated from meat, in this laboratory. Lipids were extracted from lyophilized cell extracts of this organism. Free fatty acids were separated using column chromatography methods. The pattern of the various lipid classes was observed on thin-layer chromatograms. Methyl esters of fatty acids were characterized using reversed-phase partition thin-layer chromatography and gas-liquid chromatography techniques.

The results of these investigations showed that the organism possesses a spectrum of fatty acids ranging from 12-C to 22-C chain length. The free fatty acid fraction contains a greater percentage of unsaturated fatty acids than saturated fatty acids. Some of these fatty acids have been reported for the first time in a microorganism. The investigation revealed the presence of several long chain poly-unsaturated fatty acids; this could be a major factor responsible for the high resistance of this organism to gamma radiation.

STUDIES ON THE LIPID AND FATTY ACID COMPOSITION OF *MICROCOCCUS RADIODURANS*

by

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Typed by Ruth Baines

DEDICATION

I dedicate this thesis to my father, Titus Francis, whose memory has been a constant source of inspiration to me.

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INTRODUCTION

Until recently, bacterial lipids and their component fatty acids did not receive as much attention as the carbohydrates and proteins of bacteria. The main reasons for this lack of research on lipids were the low water solubility and other inconvenient physical characteristics of lipid materials coupled with the fact that there were few satisfactory techniques available for the isolation, purification, and identification of micro quantities of lipids and their components.

With the advent of highly specialized techniques like thin-layer chromatography, gas-liquid chromatography, infra-red spectroscopy, etc., work with lipids has been greatly simplified and results obtained much more accurate. In recent years great strides have been made in several aspects of microbial lipids including synthesis and degradation, intracellular distribution and function, immunological properties and the nature of various lipid complexes.

The purpose of this investigation was to determine the fatty acid spectra of *Micrococcus radiodurans* grown in one particular medium and to examine the differences in fatty acid composition of this organism which occur by altering growth conditions. This organism was selected for study because of its comparatively high resistance to gamma radiation, in an attempt to correlate fatty acid composition with radiation resistance.

LITERATURE REVIEW

It was pointed out by Bloor in 1943 (19) that data on the lipid content of microorganisms are not reliable because a variable and sometimes considerable portion of the cellular lipid is bound with the protein and carbohydrate and is not extractable with fat solvents unless it has been liberated by hydrolysis. Several times more lipids were obtained from yeasts after hydrolysis with hot HCl than by direct extraction with solvents (93). Twelve to fifteen percent more lipid may be obtained from Mycobacterium tuberculosis when the cells, already 'defatted' with neutral solvents are treated with an acidified alcohol-ether mixture (62). Kates (53) has reviewed the content and overall composition of lipids in various bacteria. Comparatively little work has been done on the free fatty acid composition of bacteria.

Chemical Nature of Bacterial Lipids

Bacterial lipids may be divided into eight classes: (1) free fatty acids, (2) fatty acid polymers, (3) glycerides, (4) waxes, (5) phospholipids, (6) glycolipids, (7) lipopeptides and lipoproteins and (8) bound lipids.

Free fatty acids: The proportion of free fatty acids 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 Corynebacterium diphtheriae, Lactobacillus acidophilus,

Bacillus megatherium, Agrobacterium tumefaciens, Hemophilus (Bordetella) pertussis and Salmonella typhimurium (12, 62). The lipid fraction of S. typhimurium consists almost entirely of free fatty acids. The major constituents of the free fatty acid fraction are usually found to be palmitic, stearic, hexadecenoic, and octadecenoic acids although small amounts of lauric, myristic, lignoceric (tetracosanoic), cerotic, linoleic, butyric, caproic acids and modifications of these acids such as hydroxystearic and palmitostearic acids have been reported in bacteria (62).

Fatty acid polymers: Lipoid material consisting of polymerised β-hydroxybutyric acid has been isolated from many bacteria including species of Azotobacter (30, 69), Rhizobium, Chromobacterium, Pseudomonas (30), Bacillus (68, 102) and Micrococcus (57, 92).

Glycerides: Mono-, di-, and triglycerides have been reported in many bacteria (12, 86). The amount of glycerides in bacteria varies from trace quantities up to 80% of the neutral lipid content while some bacteria do not contain any triglycerides (11, 54, 62). The lipids of Mycobacteria contain a great deal of mono-, di-, and triglycerides, a few of which have been isolated.

Monoglycerides: A 1-glyceryl ester of C_{16} or C_{18} fatty acid has been characterized in the fat of a human strain of mycobacteria (81). Several workers (18, 80, 98) have shown that a wax fraction of human and BCG strains contain a large amount of 1-glyceryl mycolate, the synthesis of which has been described by Defaye and Lederer (27).

Diglycerides: A diglyceride (possibly 1-stearo-3-palmitin) has

been isolated from the fat of a human strain of mycobacteria (81), and diglycerides, some of which contain mycolic acid, from a wax preparation of BCG (18). From the neutral fat of a human strain of M. tuberculosis, a diglyceride was isolated, containing phthienoic acid and another fatty acid of about 18 C atoms (13).

Triglycerides: Many triglycerides have been observed in neutral fats (13, 81) and in wax fractions (18, 78) of various Mycobacteria. Oleotetracosanopalmitin was identified in a wax fraction of Mycobacterium marianum (78). Asselineau and Moron (13) isolated a triglyceride containing mycocerosic acid from the fat of a human strain of tubercle bacilli. These workers observed that in the case of neutral fats of human strains of M. tuberculosis, mycocerosic acid is contained in the triglyceride fraction whereas phthienoic acid is found in the di- or possibly monoglyceride fraction.

Waxes: The occurrence of true waxes i.e. esters of long chain fatty acids and long chain fatty alcohols is quite uncommon in bacteria (12). These substances appear to be limited primarily to the corynebacteria (24, 25) and mycobacteria (22, 35). In mycobacteria, two kinds of waxes are found; human and bovine strains of M. tuberculosis contain diesters of mycocerosic acid and the alcohols of the phthiocerol group (8, 80, 85, 91), Mycobacterium avium and Mycobacterium phlei contain, instead of phthiocerol, a mixture of 2-eicosanol and 2-octadecanol and it was assumed that these alcohols occurred as esters (83, 88). Corynebacterium diphtheriae contains esters of 'Corinnic alcohol' (35). On the other hand Asano and Takahashi

(5) have found plamitates and stearates of octadecanol and docosanol in a wax fraction.

Phospholipids: Representatives of this lipid class are widespread in bacteria and frequently comprise major portions of the total lipid contents of the cells. Carbohydrates, inositol and glycerol are common constituents of those bacterial phospholipids which have been studied (82). As regards nitrogenous components, choline has been found in a few cases only; ethanolamine is rather frequent, except in the phospholipids of mycobacteria. Amino acids have been found very often in phospholipid fractions, but with the possible exception of serine, it is not yet certain whether they are always bound to the phospholipids. In some cases at least, they may be part of the peptidolipids (12). Kates has reviewed the phospholipid composition in various bacteria (53).

Glycolipids: These compounds are characterized by the combination of one or more molecules of fatty acids or fatty alcohols and one or more molecules of sugar. Several carbohydrates have been detected in bacterial glycolipids, including glucose, galactose, arabinose, mannose, rhamnose and trehalose. Amino sugars have also been frequently observed (64,67).

Lipopeptides and lipoproteins: These compounds are complexes consisting of fatty acids and amino acids. It has been shown that individual complexes of this general type have fixed characteristic proportions of lipid and protein, so that they appear to have some specific structure (36). At present very little is known of the nature

of these substances in bacteria (82).

Bound lipids: 'Bound lipids' refer to the material extracted after acid or alklaine hydrolysis of the cell residue, which residue is obtained after extracting the free lipids from the cells by organic solvents. The bound lipids are generally understood to be lipoid constituents of the cell that are primarily combined with carbohydrates and proteins. The bound lipids constitute large proportions of the total cellular lipids of various bacteria (53).

Fatty Acids of Bacteria

Fatty acids found in bacteria may be divided into five classes (1) normal, saturated acids, (2) normal, unsaturated acids, (3) hydroxy acids, (4) branched-chain acids, and (5) cyclopropane acids.

1. Normal saturated acids: 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_{20-28} acids are encountered only in a few species. Arachidic acid has been reported in Pseudomonas aeruginosa (86), behenic in C. diphtheriae (5), lignoceric in C. diphtheriae (1, 4) and L. acidophilus (26) and octacosanoic in C. diphtheriae (4).

Fatty acids with odd number of C atoms have also been reported to be present in some microorganisms. James and Martin (47) reported the presence of C_{15} and C_{17} acids in P. aeruginosa. Cason and Tavs (23) reported the presence of C_{15}, C_{17}, C_{19} in M. tuberculosis.

2. Normal unsaturated acids: Cis-vaccenic acid has been reported to be widely distributed in bacteria (39). It is the sole octadecenoic acid present in Lactobacillus arabinosus (41), in Lactobacillus casei (42), and A. tumefaciens (43) and it has also been found to be the major octadecenoid acid of a Group C Streptococcus species (44). The presence of cis-vaccenic acid as well as palmitoleic acid has been reported in Escherichia coli (65). The presence of palmitoleic (9, 10-hexadecenoic acid) acid as well as its isomer palmitvaccenic acid (11, 12-hexadecenoic acid) were reported in a Group C Streptococcus species (43). Palmitoleic acid is also reported to be present in Sarcina lutea (45). The presence of C_{20} and C_{21} unsaturated acids was also reported in S. lutea (45).

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 (5). There is evidence for the presence of a C_{10} or C_{12} unsaturated acid in Brucella suis (34). Unsaturated fatty acids with chain lengths greater than C_{18} have also

been noted in Corynebacteria. C. diphtheriae was found to contain C_{20} and C_{22-24} unsaturated acids (1, 87), as well as C_{21} and C_{28} unsaturated acids (5).

3. Hydroxy acids: β-hydroxy acid has been found in several bacteria including species of Azotobacter (30, 69), Rhizobium, Chromobacterium, Pseudomonas (30), Bacillus (68, 102), Micrococcus (57, 92). Mevalonic acid (β-δdihydroxy-βmethylvaleric acid) has been isolated from cultures of lactobacilli and other microorganisms (94, 95).

β-Hydroxydecanoic acid occurs in E. coli (65), Serratia sp. (22) and in various species of Pseudomonas (15, 49). Small amounts of β-hydroxyoctanoic and β-hydroxydodecanoic acids have been detected in P. aeruginosa (15). β-hydroxymyristic acid has been found in E. coli (46, 64). Several complex hydroxy acids have been isolated from C. diphtheriae (12, 87). These are corynomycolic acid ($C_{32}H_{64}O_3$), its monounsaturated form called corynomycolenic acid ($C_{32}H_{62}O_3$) and corynolic acid ($C_{52}H_{104}O_4$) a dihydroxy acid.

4. Branched-chain fatty acids: The presence of branched-chain acids in microorganisms has been reported by several workers (12, 52, 82, 87). Asselineau and Lederer (12) identified hydroxy branched-chain, high molecular weight acids namely corynolic acid ($C_{52}H_{104}O_4$), corynomycolic acid ($C_{32}H_{64}O_3$), and corynomycolenic acid ($C_{32}H_{62}O_3$) in C. diphtheriae. Kaneda (52) demonstrated the presence of iso-C_{15} and -C_{17} branched acids in B. subtilis.

5. Cyclopropane acids: The presence of lactobacillic acid, a C_{19} fatty acid containing a cyclopropane ring, was demonstrated in

L. arabinosus (40, 41), L. casei (42), L. delbrueckii (40) and A. tumefaciens (44). C_{17}- and C_{19}- cyclopropane acids have been found in the lipids of E. coli and S. marcescens (12) and were also reported present in A. aerogenes and P. vulgaris (53).

Effect of Culture Conditions on Composition of the Lipids

Several workers (6, 12, 104) have demonstrated that the content and composition of bacterial lipids vary with the composition of the culture medium, the age of the culture and incubation temperature.

1. Effect of age of culture: Several workers (66, 76) found that young cultures of E. coli had high proportions of C_{16}- and C_{18}-monoenoic acids and low values for C_{17}- and C_{19}-cyclopropane acids. The former were converted rapidly to the corresponding cyclopropane acids during the late logarithmic growth phase, conversion being complete before or just after maximal growth was reached. Lennarz et al. (70) investigated the fatty acid composition of M. phlei during the growth cycle. In the early stages of growth, the fatty acids had high proportions of oleic and low proportions of 10-methyl-stearic acids; while analysis of the fatty acids during the stationary phase revealed a marked increase in the proportion of 10-methylstearic acid and a comparable decrease in oleic acid.

2. Effect of temperature: Early studies (32, 41, 51, 84, 96, 97) on the effect of temperature on the lipids of microorganisms showed that at low temperatures the lipids contained higher proportions of unsaturated fatty acids or more highly unsaturated acids than at

optimum temperatures. Marr and Ingraham (76) investigated the effect of temperature on fatty acid composition of E. coli grown over the range 43°C to 10°C; they found that the proportion of hexadeconoic and octadecenoic acids increased continuously with decreasing temperature resulting in a high degree of unsaturation of the total fatty acids at the lowest temperature and a low degree of unsaturation at the highest temperature; the proportions of cyclopropane acids also decreased to very low values at the low temperature. Similar changes in the proportion of monoenoic and cyclopropane acids were observed when Serratia marcescens was grown at 30°C and 20°C (17) and at 30°C and 10°C (56). This observation, namely the low content of cyclopropane acids and high content of monoenoic acids in E. coli and S. marcescens grown at low temperatures has not been explained but it is hypothesized (53) that enzymatic transfer of methylene group to double bond of monoenoic acids is either very slow or inhibited at low temperatures.

3. Effect of changes in composition of growth medium: Marr and Ingraham (75) investigated the influence of nutrition on the fatty acid composition of E. coli. They observed that cells grown at 30°C in a nitrogen-limited medium had much higher proportion of palmitic and methylene hexadecanoic acids and lower values for unsaturated fatty acids than cells grown at the same rate in a glucose-limited medium. These workers also demonstrated that supplementation of Casamino acids or yeast extract to a glucose-minimal media resulted in higher proportions of unsaturated acids and lower values for palmitic acid.

EXPERIMENTAL PROCEDURE

Source of Organism

The microorganism used in this study was M. radiodurans, a red-pigmented gram-positive aerobe, isolated in this laboratory from irradiated meat by Anderson et al. (2).

Reagents

1. Bacto-Tryptone --Difco
2. Dextrose, anhydrous -- 'Baker Analyzed' reagent grade
3. Bacto yeast extract -- 'Difco'
4. DL-Methionine -- Matheson, Coleman and Bell

The above reagents were used in the preparation of tryptone-glucose-yeast extract-methionine medium (TGYM medium) which had the following composition: 5 g. bacto-tryptone: 1 g, dextrose; 1 g, yeast extract; 20 mg, DL-methionine; made to 1000 ml using tap water. The pH of this solution was adjusted to 7.0 before sterilization.

5. Silica gel 'G' -- Stahl. Used in the preparation of thin-layer chromatographic plates.
6. Absolute methanol -- reagent grade
7. Chloroform -- reagent grade
8. n-Hexane -- High purity. This solvent was glass-distilled twice before use.
9. Ether, anhydrous -- Baker and Adamson

10. 2 N Potassium hydroxide
11. Sulphuric acid -- Baker and Adamson, Reagent grade
12. Glacial acetic acid --DuPont, Reagent grade
13. 2 N Hydrochloric acid
14. Formic acid -- 'Baker analyzed' Reagent grade
15. Whatman No. 1 filter paper used for all filtrations.

Culture Conditions

Cells of M. radiodurans were grown under two different conditions. In both instances, the inoculum consisted of a 44-hr culture of the organism grown in TGYM medium under aerobic conditions at 30°C in a shaker. In the first instance, 1% inoculum was added to TGYM broth contained in 500 ml Erlenmeyer flasks. The flasks were kept on a shaker maintained at 30°C and incubation was allowed to proceed for 44 hours. In the second instance, 1% inoculum was added to TGYM broth contained in a 10 liter glass carboy. Sterile air, obtained by circulating air through a sterilized glass tube (7" x 1") packed with cotton, was bubbled continuously through the medium which was maintained at 20°C and incubation was continued for 44 hours. A 44-hour culture corresponds to the stationary phase of the growth curve (63).

Cells were harvested by centrifugation at 7000 x g for 20 minutes in a Servall centrifuge and then washed three times with distilled water. The cells were then suspended in a minimum quantity of distilled water to give a thick homogeneous slurry which was then

freeze-dried for 48 hours.

Extraction of Lipids

Lipids were extracted according to the following procedures based on those of Huston and Albro (45).

(a) Extraction of free lipids: One gram lyophilized cells were shaken in 100 ml acetone for one hour. The cell residue obtained after filtering off the acetone was shaken with 100 ml of a mixture of chloroform and methanol (2:1 v/v) for 2 hours. The cell residue obtained after filtering off the solvent mixture was again shaken with 100 ml of a mixture of chloroform and methanol (2:1 v/v) for 2 hours. The cell debris obtained after filtration was then extracted with 100 ml of a mixture of chloroform and methanol (1:1 v/v) for one hour. The acetone extract and the three chloroform-methanol extracts were pooled and evaporated to dryness in an atmosphere of nitrogen in a water bath at 50°C. The 'free' lipid residue was taken up in 9 ml of chloroform-methanol (2:1 v/v). This solution was washed free of non-lipid contaminants according to the method of Folch, Lees and Sloan-Stanley (29). The resulting solution was evaporated to dryness by a stream of nitrogen after removing traces of water using anhydrous Na_2SO_4. The dried residue was taken up in ether.

The above extractions were all carried out at room temperature to prevent alterations to complex lipid structures (75) and as rapidly as possible to avoid prolonged exposure to methanol (71).

(b) Extraction of bound lipids: The cell residue remaining after final extraction of the free lipids with chloroform-methanol was subjected to a 2-hour reflux with 2 N aqueous KOH. The resulting material was acidified and extracted four times with 200 ml of chloroform (4 x 50 ml). The chloroform extract was evaporated to dryness in a stream of nitrogen. The bound lipid was treated as in the case of the free lipid fraction and finally taken up in ether.

Separation of Free Fatty Acids

The free lipid fraction was fractionated into a free fatty acid fraction, a glyceride fraction and a phospholipid fraction on an isopropanol-KOH treated silicic acid column as described by McCarthy and Duthie (77) with a slight modification. McCarthy and Duthie state that the above method was not desirable for the isolation of phospholipids since methanol which was used for their elution would also strip potassium salts from the column. However, this difficulty was overcome in the following manner: The methanol fraction containing phospholipid as well as the precipitate of potassium formate was evaporated to dryness and then taken up in chloroform. To this chloroform fraction was added dilute HCl which dissolves the precipitate of potassium formate. The $CHCl_3$ fraction was separated from the aqueous phase and evaporated to dryness in a stream of nitrogen. The phospholipid residue was taken up in ether.

Preparation of Methyl Ester Derivatives of Fatty Acids

The triglyceride, phospholipid and bound lipid fractions were combined into a 'total lipid' fraction. Fatty acid methyl esters were prepared by refluxing the total lipid material for one hour in 100 ml of a solution containing methanol: benzene: conc.H_2SO_4 17 : 2 : 1 (v/v/v), benzene serving the purpose of a water scavenger. After stopping the reaction with water, the solution was made slightly alkaline by addition of dilute NaOH solution. The fatty acid methyl esters were then extracted with 200 ml of double-distilled n-hexane (4 x 50 ml). Making the solution alkaline helped to remove most of the pigment material which moved into the aqueous phase. The free fatty acids were also converted into their respective methyl ester derivatives according to the procedure outlined above.

Thin-layer Chromatography

The TLC technique as described by Mangold (73) was used to characterize the different classes of lipids. A 'total lipid' aliquot was monitored side by side with known lipid standards on thin layers (250μ) of silica gel G (Stahl) on standard glass plates (20 x 20 cm). Monostearin, glyceryl distearate and glyceryl tristearate were each found to contain small proportions of the other two, hence these compounds were run on thin-layer plates and individual components were scraped off the plates after development, and used as standards. Other standards used were oleic acid, methyl linolenate, and 2-heptanone, all of which were given by Dr. E. A. Day, Department of Food Technology.

All plates were spread with a Desaga-Brinkman adjustable applicator; activated for one hour at 110° and developed by the ascending method in unlined tanks. The plates were developed in n-hexane-ethyl ether-glacial acetic acid (90:10:1 v/v/v) (72). Lipid material was also characterized on prefabricated thin layers of silica gel G (Stahl) on mylar sheets (20 x 20 cm)[3]. However, in this case, the plates were developed on n-hexane-ethyl ether-glacial acetic acid (80:20:1 v/v/v) in jars lined with filter paper (74). Various indicators were used to examine lipid classes, including bromothymol blue (40 mg in 100 ml of 0.01N NaOH solution) (50), and Rhodamine B (0.05% in 96% ethanol) (58, 100) for detection of most lipids; iodine vapors (72, 74) for identification of all unsaturated lipids and some saturated nitrogenous lipids; ninhydrin (0.2% in n-butanol) (100) for observation of aminophosphatides; 2',7'-dichlorofluorescein (0.2% in 96% ethanol) for visualization of both the saturated and unsaturated nonpolar lipids (72,74) and chromic-sulfuric acid solution, followed by charring for detection of all organic matter (61, 79).

Reversed-Phase Partition Thin-Layer Chromatography

To characterize free fatty acids and their methyl ester derivatives use was made of the reversed-phase partition TLC technique using siliconized chromatoplates obtained by slowly immersing silicic acid plates at room temperature into a 5% solution of silicone (Dow-Corning '200', fluid viscosity 10 cs) in diethyl ether (72). Plates

[3] Supplied by Eastman Organic Chemicals

were developed at room temperature in acetic acid-acetonitrile-water (2:14:5 v/v/v) (72) and at 5°C in acetic acid-formic acid-water (2:2:1 v/v/v) (72). Fatty acids were observed using iodine vapors and spraying with chromic-sulfuric acid followed by heating. Tentative identification of the saturated fatty acids was made by matching the spots on the plate with those of known fatty acids run side by side on the plate.

In the preparation of siliconized chromatoplates, it is important that the plates be immersed very slowly into a solution of silicone in ether. Also, the chromatoplates to be impregnated should be of the same temperature of the silicone solution. Disregarding either of these precautions leads to crumbling of the thin layers (73). Before reusing siliconized chromatoplates, the glass plates have to be thoroughly cleaned. After scrubbing the plates several times with cleansing powder, the plates are kept for 48 hours in a solution of Haemo-sol,[1] then rinsed and dried. During this investigation several thin-layers disintegrated because of 'apparently-clean' glass plates.

Gas Chromatographic Analysis of Methyl Ester Derivatives of Fatty Acids

Preparation of inert support: Celite 545[2] of mesh size 120-140 was obtained by using a standard 'ASTM' sieve. This fraction was treated with 1N NaOH for 15-30 minutes with frequent stirring. After

[1] Haemo-sol is the trade name for a detergent used in cleaning laboratory glassware.

[2] Celite 545 consists of amorphous diatomaceous silica plus varying percentages of inverted microcrystalline silica, Johns-Manville, U. S. A.

washing with distilled water to neutrality, the fraction was treated with 1N HCl and stirred at intervals for 30 minutes. Again it was washed until neutral. It was then filtered by suction and washed with methanol to remove water. Finally, the celite fraction was dried in an oven at about 105°C for two to three days. After this acid-base treatment the celite was size-graded again through a standard 8" diameter sieve in a mechanical shaker.

Coating and packing of columns: The choice of the most desirable particle size of celite is dependent on a number of factors. There is a theoretical basis for use of very small particles for maximum column efficiency (60). The column efficiency is dependent on the particle size of the packing, the smaller size yielding a greater efficiency, however the time to carry out a run is longer with the smaller particle sizes. The stationary phase selected was diethylene-glycolsuccinate (LAC-3R-728)[1] 18/100 (w/w) of DEGS[2] Celite was chosen as the ratio of stationary phase to inert support. Diethylene-glycol-succinate (1.8 g) was weighed into a beaker. To this was added a minimum amount of chloroform. With stirring and heating over a hot plate the stationary phase was dissolved. To this solution 10 g of acid-base treated celite 545, 120-140 mesh was gradually added with gentle stirring so as to prevent breaking-up of the particles. The chloroform was evaporated in the presence of N_2 by warming the mass over a hot plate. After the solvent had been removed, the packing

[1] Cambridge Industries Co., Inc., 101 Potter Street, Cambridge, Mass.

[2] diethylene-glycol-succinate

material was dried in an oven at a temperature of 105°C for two days. The dry packing material was then cooled and weighed and packed into a seven feet 1/8 inch O.D. stainless steel column using a vibration tool. The packed column was conditioned for 24 hours by heating at 200°C and flushing with nitrogen at a flow rate of 20 ml/min. It was found that prolonged heating resulted in gradually decreased efficiency of the polyester column.

Chromatographic instrument: An Aerograph "Hy-Fi" model A-600-B gas chromatograph, equipped with a hydrogen flame ionization detector was used to establish the retention times of the methyl ester mixtures. The detector was calibrated by fatty acid methyl esters (Standard Mixture D)[1]. As shown in Table I, hydrogen flame ionization detector gives quantitative detection in terms of actual wieght percent to within ($\pm$2) for some esters and ($\pm$3) for others for the range of molecular weight tested.

Instrumental operation conditions for this investigation were set as follows for all samples:

Column: 7 ft. x 1/8 inch O.D. (18/100) on Celite 545
Column Temperature: 185°C
Injector Temperature: 240°C
Carrier Gas: Nitrogen
Flow rate of Carrier Gas: 20 ml/min
Size: 3.0 μl

Column characteristics: The number of theoretical plates of this column was measured according to the following definition of a theoretical plate (28). Theoretical plate=n=16(tr/W)^2 where W=the base width of the component (in same units as tr), in this case 18:0 was

[1] Standard Mixture D was supplied by Metabolism Study Section, National Institutes of Health, Bethesda, Maryland

arbitrarily chosen as reference peak for the characterization of the column. The uncorrected retention time=Tr = time from sample injection to the midpoint of the symmetric elution curve of a component. Conditions for the measurement of retention times were the same as the operation conditions for sample runs. The calculated theoretical plates in reference to 18:0 was 1972.

This column also had HETP of 0.11 cm using the definition of HETP (Height Equivalent to a Theoretical Plate) = column length (in cm) ÷ Number of theoretical plates.

The number of theoretical plates does not indicate how efficient a column may be in separating certain compounds. In polyester columns such as DEGS the separation of 18:0 and 18:1 is less easily achieved than in a non-polar column, therefore the efficiency of separating these two compounds may be used as another index to measure the efficiency of a polar column. Component resolution is measured by the formula $2\Delta Y/Ya+Yb$ (28) where ΔY=distance in mm between two peak maxima, Ya and Yb=base widths in mm of triangles fitted to the two peaks at their points of inflection. A number greater than one indicates complete resolution. Using fatty acid methyl esters Standard Mixture D the above mentioned quantities were measured and component resolution between 18:0 and 18:1 was calculated to be 1.6. This indicates the efficiency of the column in separating 18:0 and 18:1 when the column was new. However, it was observed that the efficiency decreased gradually and constantly as the column was repeatedly used at this high temperature.

Table 1

Calibration of detector by a known fatty acid mixture (Standard 'D')[1]

Fatty Acids[2]	Actual Weight %	Determined % Composition	% Of Determined Composition to Actual Weight
14:0	11.82	11.90	101
16:0	23.61	24.20	102
16:1	6.84	6.64	97
18:0	13.08	13.40	102
18:1	44.62	43.80	98

[1] This mixture was provided by the National Institutes of Health, Maryland.

[2] Number of carbon atoms in acid:number of double bonds.

Identification and Quantitation of Methyl Esters of Fatty Acids

Approximately 3.0 μl samples were chromatographed in triplicate. The identification of components were based either on comparison of relative retention times of known esters when known esters were available or tentatively identified using carbon numbers as suggested by Woodford and VanGent (105). The following pure esters were obtained[5]: 8:0, 10:0 12:0, 14:0, 16:0 18:0, 20:0 of the saturated series, 16:1, 18:1, 18:2, 18:3, 20:4, of the unsaturated series. Higher unsaturated esters of 20:1, 20:5, 22:6 were provided by National Institutes of Health, Metabolism Study Section. It was observed that as the column was repeatedly used, the relative retention times of polyunsaturated esters changed slightly; therefore known esters were run frequently between sample runs to obtain more accurate relative retention times and carbon numbers of the components of the known mixture, which were tabulated in Table II.

Quantitation was achieved by triangulation technique which is a procedure used routinely by some investigators (48). Straight lines were drawn by eye along the two slopes of a curve so as to contact these slopes at their points of inflection. The area under a curve was calculated as 1/2 base x height. The areas were totalled and the percentage of each component was calculated. Quantitative percentages of each component were tabulated in Tables III, IV, V and VI.

[5]Applied Science Laboratory, State College, Pa.

Table II

Relative retention time and carbon number of standard fatty acid mixture[1]

Rel. Ret. Time Tr/Tr	Compound[2]	Carbon Number
0.303	14:0	14.00
0.550	16:0	16.00
0.645	16:1	16.55
1.000	18:0	18.00
1.170	18:1	18.55
1.475	18:2	19.30
2.040	18:3	20.43
3.540	20:4	22.20
4.800	20:5	23.20
9.850	22:6	25.60

[1] This mixture was provided by the National Institutes of Health, Maryland

[2] Number of carbon atoms in acid:number of double bonds

Table III

Free fatty acid composition of M. radiodurans grown at 30°C in TGYM medium

Peak No.	Rel. Ret. Time Tr/Tr	Compound[1]	Carbon Number	% Composition
1	0.169	12:0	12.0	1.15
2	0.191	un[2]	12.4	0.30
3	0.224	13:0	13.0	trace[3]
4	0.266	14:0br[4]	13.5	0.81
5	0.300	14:0	14.0	2.56
6	0.364	un	14.6	0.65
7	0.404	15:0	15.0	1.60
8	0.477	16:0br	15.5	2.01
9	0.550	16:0	16.0	21.40
10	0.655	16:1	16.6	17.35
11	0.735	17:0	17.0	3.22
12	0.870	16:2	17.5	1.77
13	1.000	18:0	18.0	6.80
14	1.150	18:1	18.5	8.55
15	1.220	un	18.7	1.82
16	1.470	18:2	19.3	1.86
17	1.810	20:0	20.0	2.29
18	3.340	20:4	21.9	5.45
19	4.380	un	22.9	1.56
20	4.970	20:5	23.3	6.30
21	6.150	22:4	24.1	3.66
22	8.850	22:5	25.3	2.30
23	10.500	22:6	25.7	7.15

[1] Number of carbon atoms in acid:number of double bonds

[2] un= Unidentified

[3] Trace= less than 0.1%

[4] br = branched

Table IV

Total fatty acid composition of M. radiodurans grown at 30°C in TGYM medium

Peak No.	Rel. Ret. Time Tr/Tr	Compound[1]	Carbon number	% Composition
1	0.189	12:0	12.0	8.36
2	0.250	14:0br[2]	13.4	0.45
3	0.297	14:0	14.0	2.23
4	0.363	un[3]	14.6	0.40
5	0.400	15:0	15.0	0.76
6	0.484	16:0br	15.5	0.65
7	0.550	16:0	16.0	24.70
8	0.632	16:1	16.5	1.19
9	0.737	17:0	17.0	0.55
10	0.870	16:2	17.5	0.52
11	1.000	18:0	18.0	15.00
12	1.140	18:1	18.5	9.55
13	1.220	un	18.7	0.83
14	1.490	18:2	19.4	1.66
15	1.840	20:0	20.0	2.19
16	2.050	18:3	20.5	2.02
17	2.600	20:2	21.2	3.58
18	3.480	20:4	22.1	6.05
19	4.660	20:5	23.1	11.80
20	5.600	22:4	23.7	1.09
21	8.400	22:5	25.1	2.39
22	9.800	22:6	25.6	4.04

[1] Number of carbon atoms in acid:number of double bonds

[2] br = Branched

[3] un = Unidentified

Table V

Free fatty acid composition of M. radiodurans grown at 20°C in TGYM medium

Peak No.	Rel. Ret. Time Tr/Tr	Compound[1]	Carbon Number	% Composition
1	0.149	un[2]	11.6	0.48
2	0.171	12:0	12.0	0.63
3	0.201	un	12.6	0.53
4	0.228	13:0	13.0	0.34
5	0.265	14:0 br[3]	13.5	1.18
6	0.305	14:0	14.0	1.99
7	0.350	un	14.5	1.55
8	0.402	15:0	15.0	4.06
9	0.471	16:0 br	15.5	7.45
10	0.550	16:0	16.0	25.10
11	0.653	16:1	16.6	36.70
12	0.733	17:0	17.0	6.45
13	0.864	16:2	17.5	4.43
14	1.000	18:0	18.0	3.28
15	1.130	18:1	18.5	4.04
16	1.425	18:2	19.3	0.63
17	3.220	20:4	21.9	0.52
18	4.220	20:5	22.8	0.31
19	5.900	22:4	23.9	0.44

[1] Number of carbon atoms in acid:number of double bonds

[2] un =unidentified

[3] br= branched

Table VI

Total fatty acid composition of M. radiodurans grown at 20°C in TGYM medium

Peak No.	Rel. Ret. Time Tr/Tr	Compound[1]	Carbon number	% Composition
1	0.148	un[2]	11.6	0.06
2	0.163	12:0	12.0	0.22
3	0.195	un	12.5	0.11
4	0.226	13:0	13.0	0.13
5	0.259	14:0 br[3]	13.5	0.48
6	0.298	14:0	14.0	1.07
7	0.350	un	14.5	0.88
8	0.399	15:0	15.0	2.34
9	0.475	16:0 br	15.5	4.66
10	0.550	16:0	16.0	22.20
11	0.660	16:1	16.6	47.30
12	0.740	17:0	17.0	4.96
13	0.875	16:2	17.5	5.10
14	1.000	18:0	18.0	2.21
15	1.140	18:1	18.5	5.17
16	1.220	un	18.7	0.32
17	1.430	18:2	19.3	0.17
18	5.000	20:5	23.3	2.16
19	6.500	un	24.2	0.52

[1] Number of carbon atoms in acid:number of double bonds

[2] un = Unidentified

[3] br = Branched

RESULTS AND DISCUSSION

The total lipid fraction (free lipid and bound lipid) of M. radiodurans was resolved into its component lipid classes on thin-layer plates. Each band, representing an individual lipid class was matched against reference lipid standards run side by side on the chromatoplate. The lipid fraction was resolved into various classes all of which could not be identified (Fig. 5). There was tentative evidence for the presence of free fatty acids, phospholipids which did not move from the origin, triglycerides, and hydrocarbons which travelled with the solvent front. The presence of aminophosphatides was not indicated; ninhydrin indicator did not give a positive test. Huston and Albro (45) recently reported analyses of the lipids of S. lutea, a gram-positive, aerobic, pigment-producing micrococcus. They demonstrated the presence of hydrocarbons, free fatty acids, mono-, di-, and triglycerides and a complex mixture of highly polar phosphorous-containing compounds in the lipid material.

Fatty Acid Resolution on Siliconized Chromatoplates

A solution of 5% paraffin in diethyl ether as an impregnating agent (59, 103) did not give as good a separation as that obtained using silicone; further, paraffin-coated chromatoplates have the disadvantage that they cannot be sprayed with sulfuric acid, since the whole plate chars following heating. Using siliconized chromatoplates, better separation of both fatty acids and their corresponding methyl ester derivatives was obtained by developing the plates at room

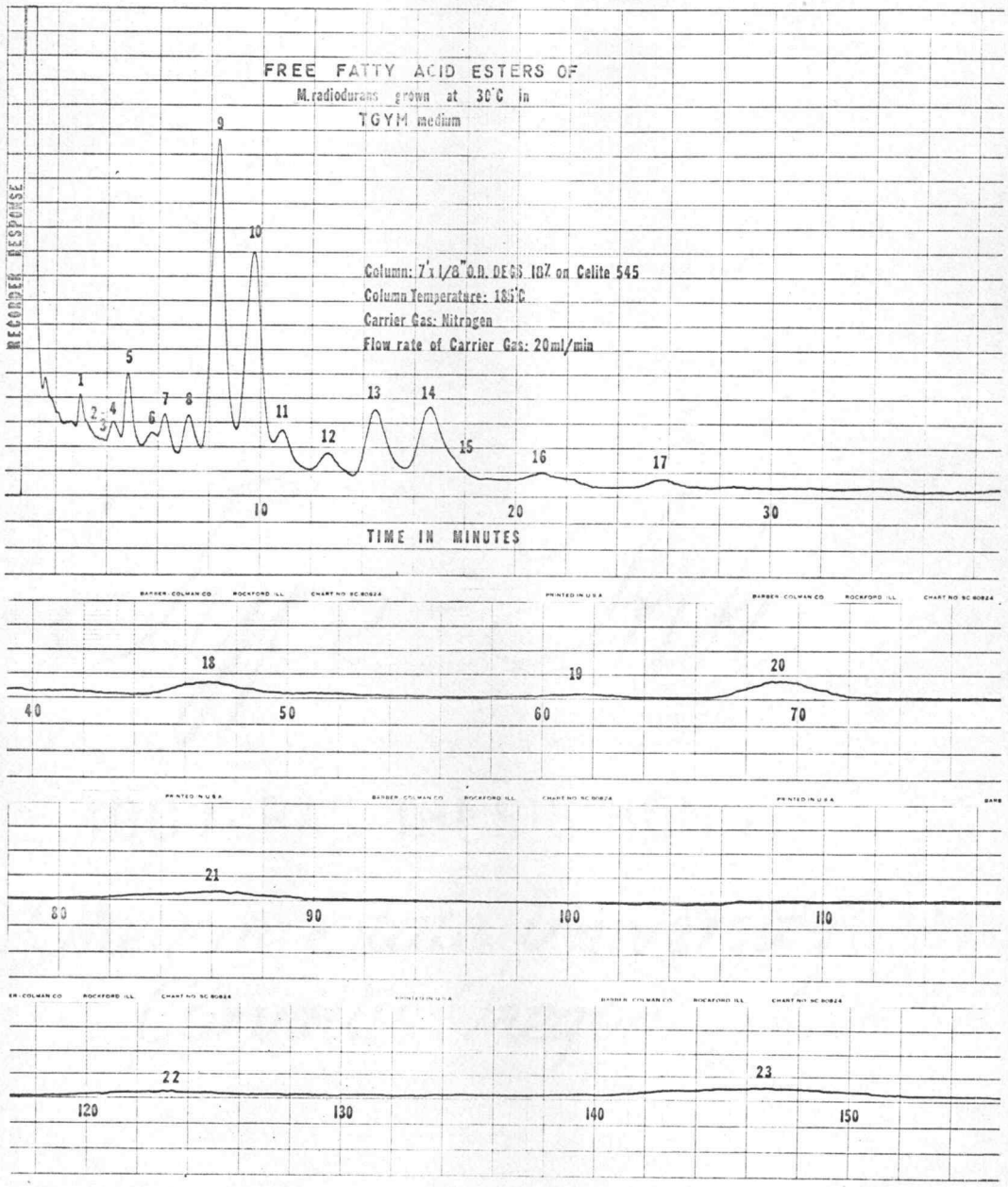

Figure 1

Gas chromatogram of methyl esters of free fatty acids of M. radiodurans grown at 30°C

temperature in a solvent system containing acetic acid=acetonitrile-water (2:14:5 v/v/v) than in a system containing acetic acid-water (3:1, v/v) (72). Tentative identification of 18:0, 16:0, 14:0 fatty acids was possible using known saturated fatty acids (Fig. 6). However, a single spot on the chromatogram does not necessarily mean the presence of a single acid but may sometimes represent a mixture of two fatty acids (72).

The fatty acid fractions of M. radiodurans were also resolved into their individual components in a system of acetic acid-formic acid-water (2:2:1, v/v/v). The chromatogram took 5 hours to develop at 5°C as compared to a development time of 50 minutes at room temperature; with no appreciable difference in fatty acid resolution (Fig.7). Using reversed-phase column chromatography, Saito (89,90) separated the fatty acids of acetone soluble lipids derived from five kinds of bacterium. Boldingh (20) 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 column chromatography. Hofmann et al. (40) modified the above technique and were able to determine monounsaturated, saturated and branched-chain fatty acids in small samples of bacterial lipids. However, there is no report of bacterial fatty acid characterization using reversed-phase partition TLC.

GLC Analysis of Fatty Acids

The free fatty acids of M. radiodurans comprised a large percentage of the 'Free' lipids. Separation of the free fatty acids from

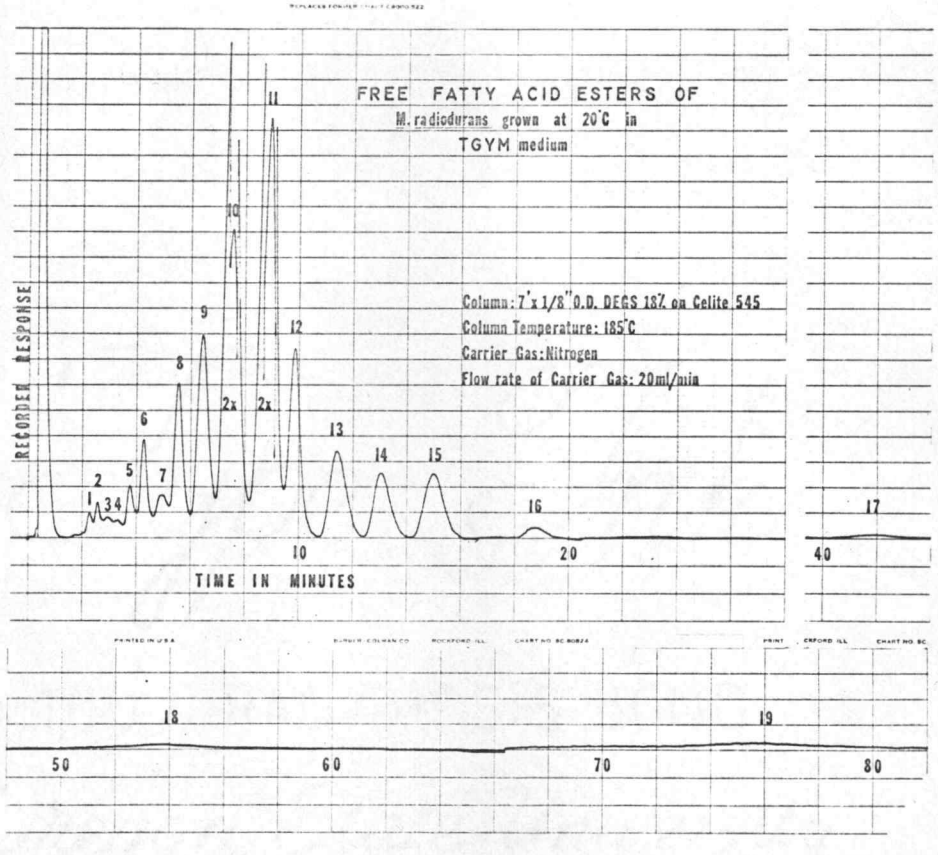

Figure 2

Gas chromatogram of methyl esters of free fatty acids of M. radiodurans grown at 20°C

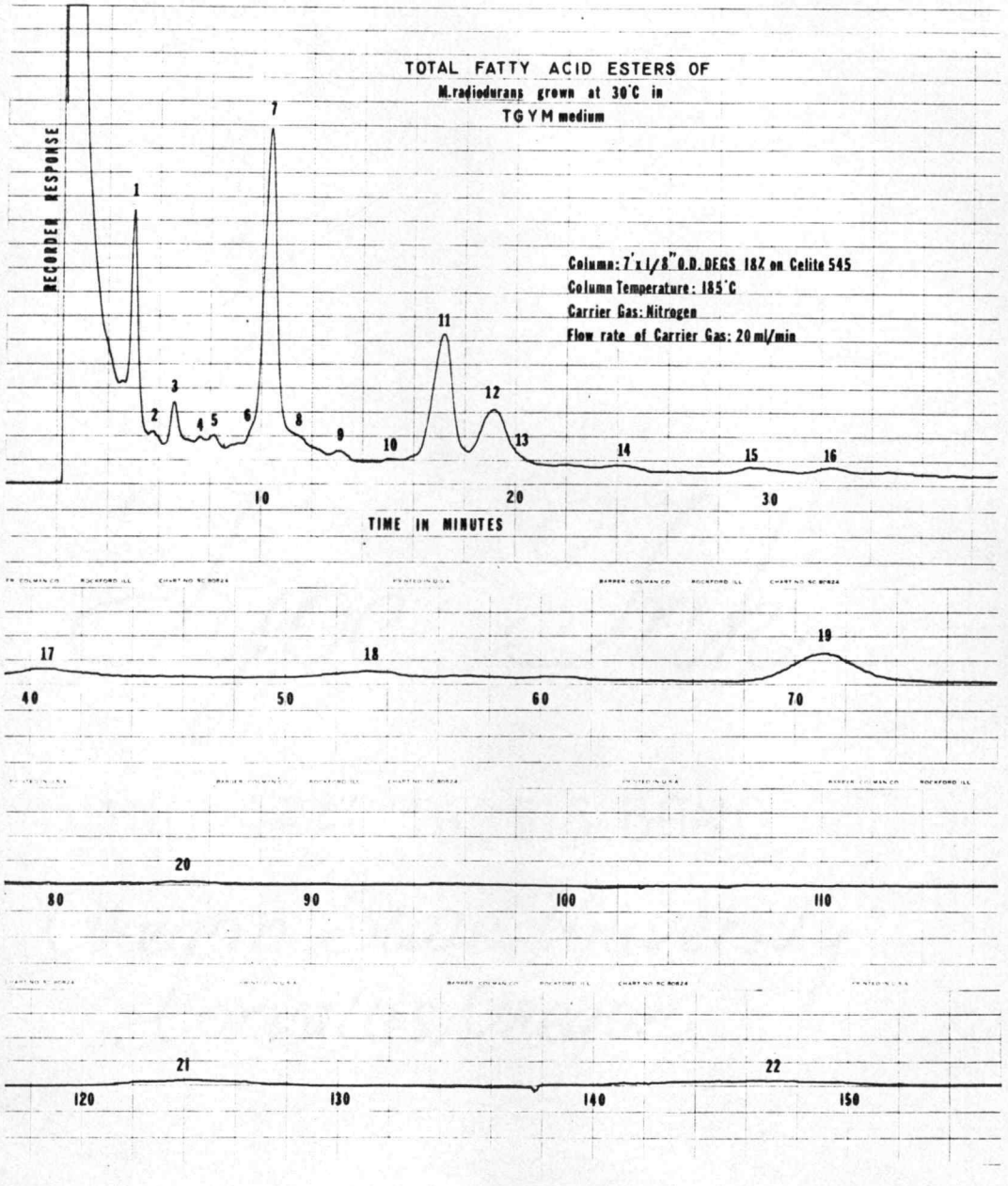

Figure 3

Gas chromatogram of methyl esters of total fatty acids of M. radiodurans grown at 30°C

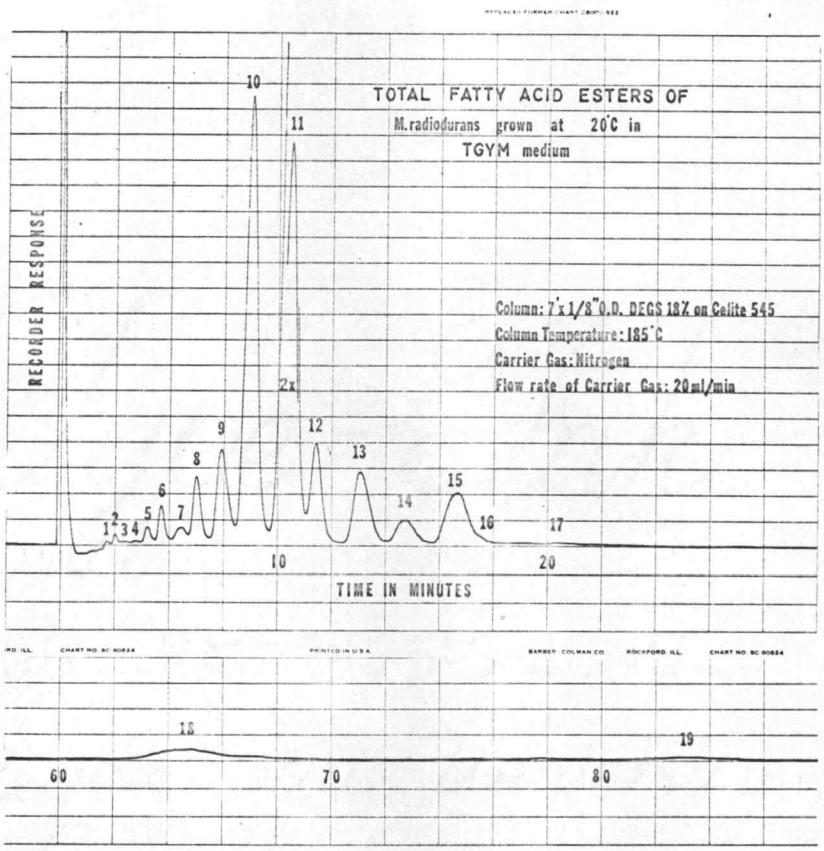

Figure 4

Gas chromatogram of methyl esters of total fatty acids of M. radiodurans grown at 20°C

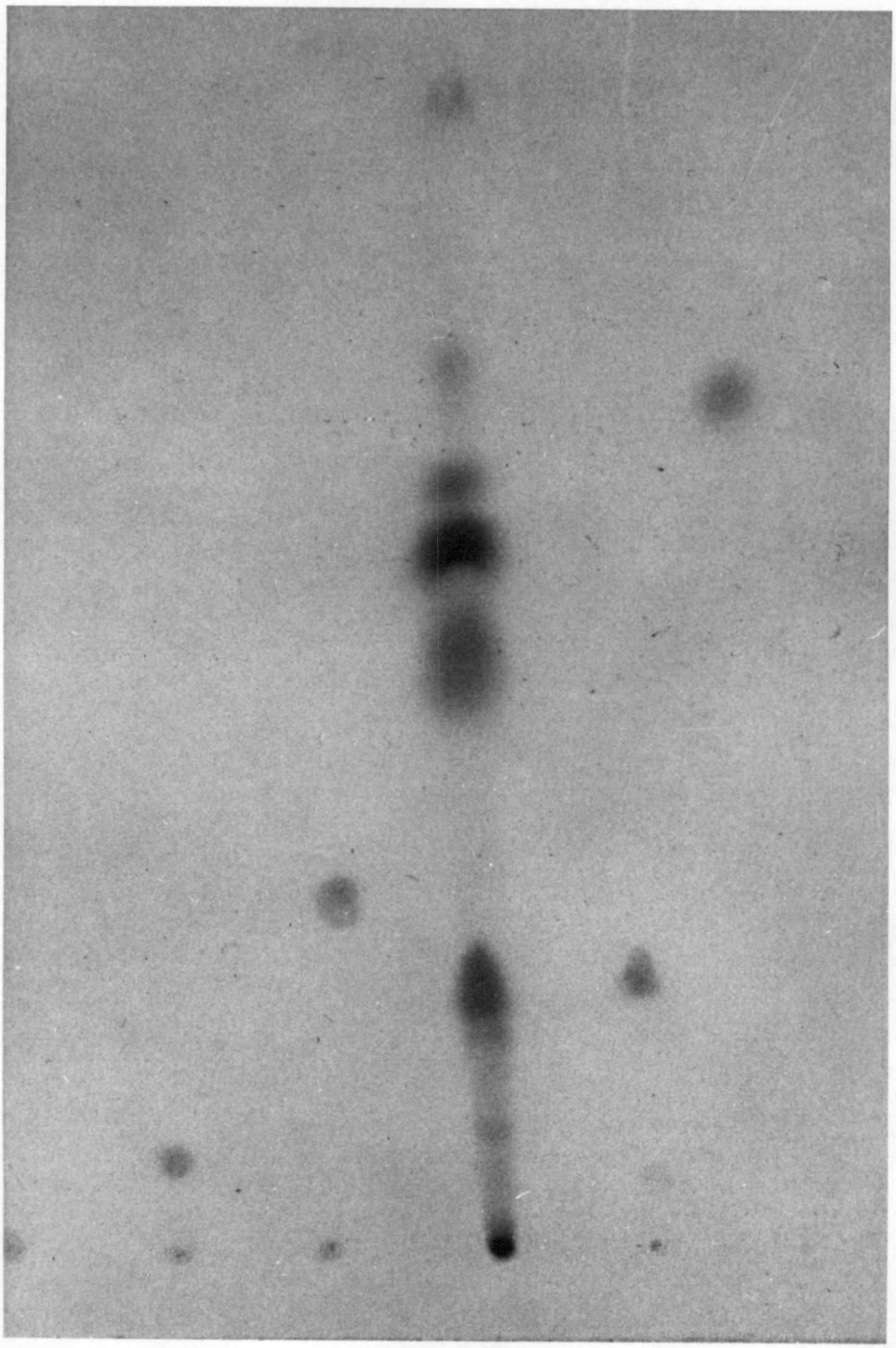

Figure 5. Thin-layer adsorption chromatography of lipid classes on Silica Gel. Solvent: n-hexane-diethyl ether-acetic acid, 90:10:1, v/v/v. Development time: 50 min. at 25°C. Indicator: Iodine vapors. Amounts: 10 γ each. 1) Monostearin 2) Glyceryl distearate 3) Glyceryl tristearate 4) Lipid sample of M. radiodurans 5) Oleic acid 6) Methyl linolenate

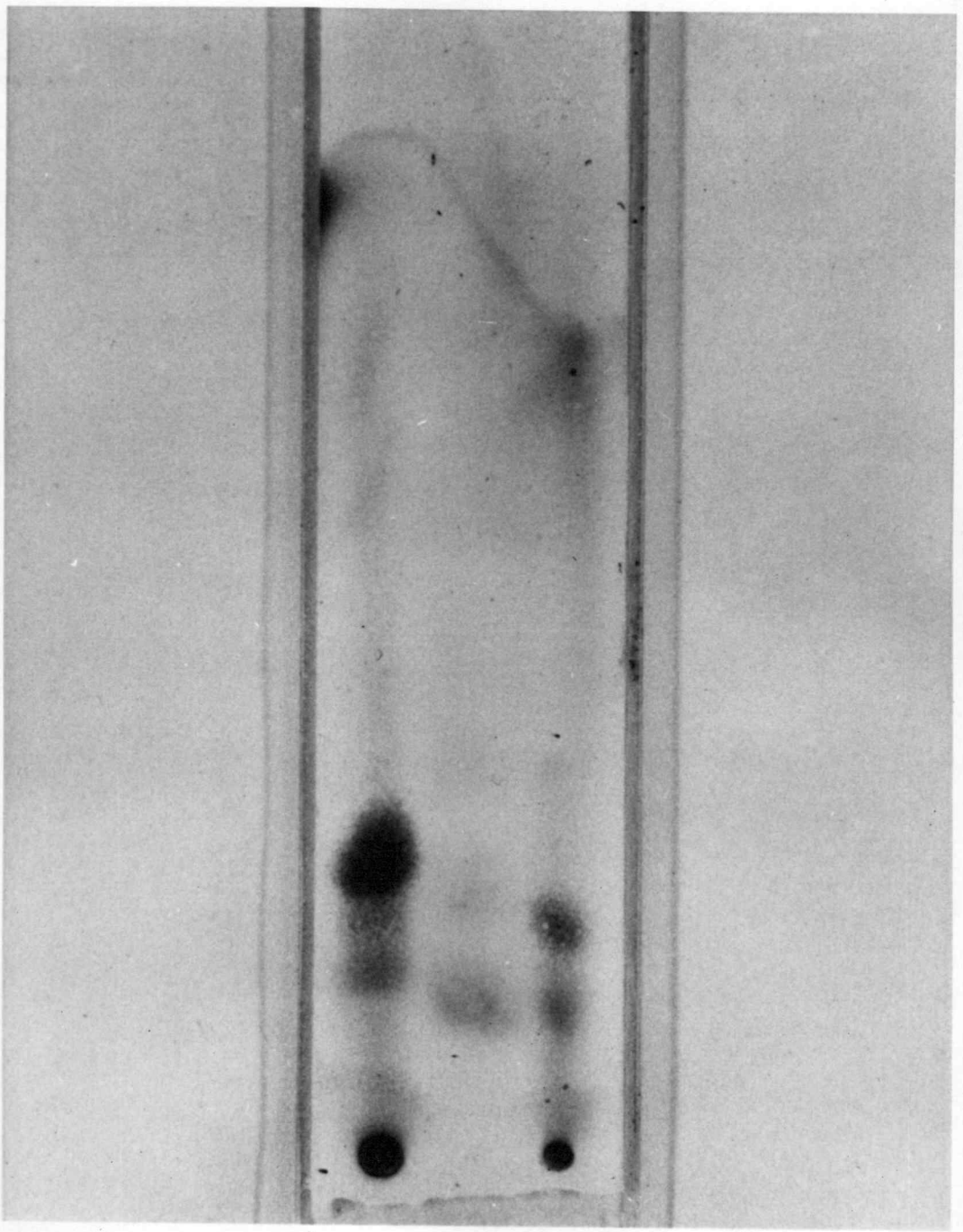

Figure 6. Reversed-phase partition TLC of methyl ester derivatives of fatty acids on a siliconized chromatoplate. Solvent: Acetonitrile-acetic acid-water, 70:10:25, v/v/v. Development time: 50 Min. at 25°C. Indicator: charring with chromic-sulfuric acid. Amounts: 10 γ each.
1) Total fatty acids of M. radiodurans. 2) Standard fatty acid mixture containing 18:0, 16:0, 14:0 12:0 and 10:0 acids. 3) Free fatty acids of M. radiodurans

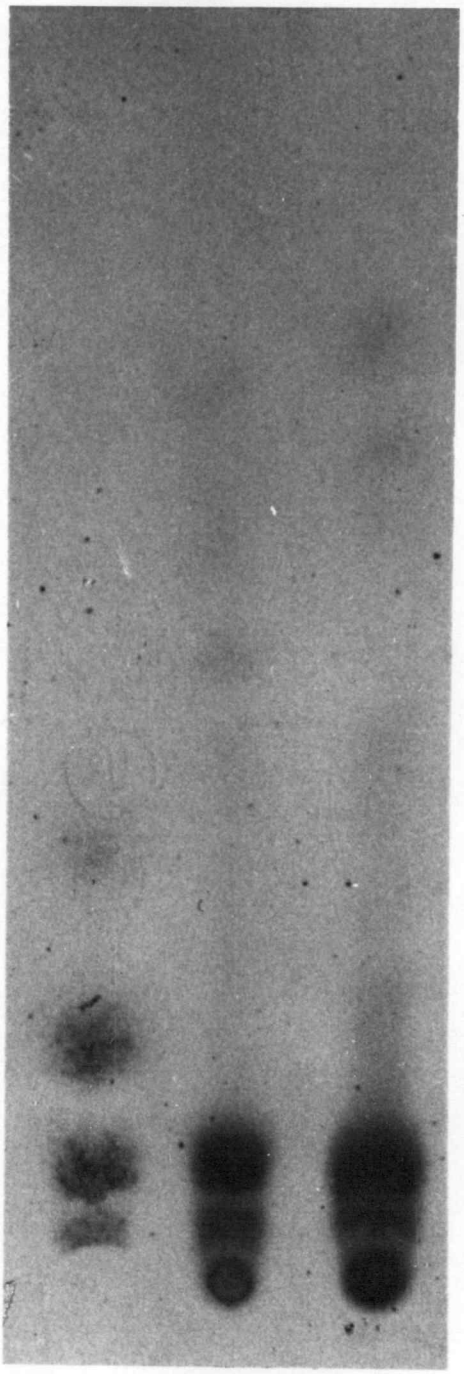

Figure 7. Reversed-phase partition TLC of methyl ester derivatives of fatty acids on a siliconized chromatoplate. Solvent: Acetic acid-formic acid-water, 40:40:20, v/v/v. Development time: 5 hr. at 5°C. Indicator: charring with chromic-sulfuric acid. Amounts: 10 γ each.
1) Standard fatty acid mixture containing 18:0, 16:0, 14:0, 12:0 and 10:0 acids. 2) Total fatty acids of M. radiodurans. 3) Free fatty acids of M. radiodurans

the 'free' lipid fraction on a KOH-treated silicic acid column as described by McCarthy and Duthie (77) gave almost complete recovery. A total of twenty-three fatty acids were detected in the free fatty acid fraction isolated from cells of M. radiodurans grown at 30°C (Fig. 1, Table III). Of these, nineteen were tentatively identified by comparing their relative retention times with those of known fatty acids (Table II). On the other hand, nineteen free fatty acids were observed in the free fatty acid fraction obtained from cells of M. radiodurans grown at 20°C (Fig. 2, Table V); sixteen of which were tentatively identified. Asselineau and Lederer (12) have reported that the free fatty acids constitute 25-40% of lipids isolated from various strains of mycobacteria. These include saturated straight-chain fatty acids up to 24:0 in saprophytic strains (14) and up to 26:0 in human and bovine strains (7), unsaturated straight-chain fatty acids, mainly oleic acid (3) and branched-chain fatty acids. Asselineau (9, 10) has recently investigated the lipids of C. diphtheriae. He has reported the presence of 44% free fatty acids, including 14:0, 16:0, 18:0, palmitoleic and oleic acids and 28% free hydroxy acids in the lipids. The unusually high quantities of free fatty acids may have arisen from enzymatic hydrolysis of glycerides (24, 25). Recent studies (38) suggest that reports of high free fatty acid contents in lipid material may be due to lipolysis during extraction and chromatography on silicic acid. As ether is known to activate certain lipolytic enzymes (37), the use of this solvent for extraction of lipids was avoided in this investigation. Moreover,

ketones are known to inhibit the action of certain lipases (101). Thus the use of acetone as the initial extracting solvent may have prevented the lipolysis of M. radiodurans lipids.

The total lipid material extracted from cells of M. radiodurans cultured at 30°C was fractionated into twenty-two fatty acids - twenty of which were tentatively identified on a DEGS column on a Barber-Coleman gas chromatograph (Fig. 3, Table IV). Fourteen fatty acids were tentative identified out of nineteen fatty acids detected in the total lipid fraction isolated from cells of M. radiodurans grown at 20°C (Fig. 4, Table VI).

The major group of fatty acids found in M. radiodurans was the normal unsaturated fatty acids, comprising over 50% of the total fatty acids. Based on their relative retention time in the gas chromatograph, two of the acids have been tentatively identified as 14:0 branched and 16:0 branched acids. These branched acids have been reported in S. lutea (45). Saito (89, 90) investigated the lipids of Bacillus subtilis and showed the presence of branched-C_{15} and -C_{17} acids which were identified as the iso acids. Kaneda (52) recently found the branched-C_{15} and -C_{17} acids to consist of both the iso and anteiso acids. Asselineau (10) has reported the presence of a branched-C_{16} acid in addition to the major branched-C_{15} acid, in B. subtilis. Branched-chain acids belonging to a homologous series were reported present in Bacillus cereus (55). The major component was a C_{15}-branched-chain acid, followed in decreasing order by a C_{17}-branched, a C_{13}-branched acid and a C_{16}-branched acid. Of the unsaturated

acids, a hexadecenoic acid, corresponding to palmitoleic acid is predominant. The presence of a hexadecenoic acid in bacteria, in most cases identified as palmitoleic acid, has been reported by several workers (16, 21, 31, 43, 54, 65).

The other major unsaturated fatty acid is an octadecenoid acid comprising about 15% of the total unsaturated fatty acids of M. radiodurans. This acid has been identified as oleic acid. Huston and Albro (45) reported the presence of oleic acid and cis-vaccenic acid in the gram-positive, pigment-producing micrococcus S. lutea. In another micrococcus, Micrococcus halodenitrificans, (57), the C_{18}-monoenic acid was twice as much as the C_{16}-monoenic acid, while the reverse is true in M. radiodurans. The presence of octadecenoic acid has been reported in several other bacteria (9, 10, 41, 57, 65). Besides palmitoleic and oleic acids, gas chromatographic analysis revealed the presence of several highly unsaturated fatty acids, most of which have been reported for the first time. The presence of 16:2, 18:2, 18:3, 20:2, 20:4, 20:5, 22:4, 22:5 and 22:6 fatty acids has been tentatively identified. Identification of 16:2, 18:2, 18:3, 20:4, 20:5, 22:6 fatty acids was made on the basis of their relative retention times and their 'Carbon Numbers' from plots of equivalent chain length versus retention time for n-saturated methyl ester standards. The 20:2, 22:4 and 22:5 acids, for which standards were not available, were identified on the basis of their relative retention time as compared with those obtained by Farquhar et al. (28) in their investigations in the fatty acid composition of Menhaden oil.

Huston and Albro (45) reported the presence of 16:2 and 18:2 fatty acids totalling 0.4% of the bound lipid fraction of S. lutea. In the same paper the presence of 20:4 fatty acid in the free fatty acid and glyceride fractions of S. lutea was also reported. The presence of these highly unsaturated fatty acids, many of them in appreciable quantities as free fatty acids could probably play an important role in the unusually high resistance of this organism to gamma radiation by acting as free radical acceptors.

The possession of this unusual complement of unsaturated fatty acids is yet another of the unique characteristics of this micro-organism. A new mucopeptide has been recently isolated from the cell wall of M. radiodurans (106). A recent report (33) indicates that the cell wall and sheath of M. radiodurans are more complex than any so far described for a bacterium.

With the exception of 19:0, M. radiodurans has been found to possess all the odd as well as even-numbered fatty acids ranging from C_{12} to C_{20}. Palmitic acid constitutes the major portion (50%) of the saturated acids, followed by stearic acid (30%). The presence of the even-numbered saturated fatty acids has been reported by several workers (23, 70, 89, 90) in various bacteria. However, the odd-numbered saturated fatty acids have been reported in comparatively few bacteria (23, 57). The presence of comparatively large amounts of 15:0 and 17:0 acids is another unique facet of the fatty acid complement of M. radiodurans. At present, the biochemical significance of these acids is not known.

Effect of changes in growth conditions: M. radiodurans was cultured under two different conditions with a view to observe any possible changes in the composition of lipids. In one experiment the organism was grown aerobically on a shaker maintained at 30°C, while in the other experiment, the organism was grown at 20°C with air bubbled continuously through the medium. There were several pronounced changes both in the content and composition of free fatty acids as well as the total fatty acids present in the lipid material extracted from cells grown under the above-mentioned conditions (Tables III, IV, V, and VI). Cells grown at 20°C did not contain the unsaturated free fatty acids 22:5 and 22:6 or the saturated free fatty acid 20:0, all of which were present in cells grown at 30°C. Further, there was a considerable decrease in the quantity of the long chain unsaturated fatty acids in cells grown at 20°C; octadecenoic acid was decreased by 50%, octadecadienoic acid by 66%, while 20:4, 20:5 and 22:4 were reduced ten, twenty and nine-fold respectively. On the other hand, the hexadecenoic acid was twice as much in cells grown at 20°C than at 30°C, while the hexadecadienoic acid was increased two and a half-fold. As regards the saturated free fatty acids, the odd-number acids 13:0, 15:0 and 17:0 showed approximately a two-fold increase in cells grown at the lower temperature, while the even-numbered fatty acids 14:0 and 18:0 were present in larger amounts in cells grown at the higher temperature. The palmitic acid content was not appreciably altered in cells grown at either temperature.

Profound changes were also observed in the content and composition of the total fatty acids (obtained by hydrolysis of the total lipid fraction) of M. radiodurans grown under the two different conditions specified above. Six of the unsaturated fatty acids tentatively identified as 18:3, 20:2, 20:4, 22:4, 22:5, and 22:6 in the cells grown at 30°C were lacking in cells grown at 20°C. However, GLC analysis of the fatty acid fraction of cells grown at 20°C indicated the presence of two long chain unsaturated fatty acids which have not been identified. The long chain saturated fatty acid 20:0 was present only in cells grown at 30°C. There was a notable change in the hexadecenoic acid content of the cells grown at the two different temperatures; this acid which comprised only 1% of the total fatty acid of cells grown at 30°C increased to 47% of the total fatty acid content of cells grown at 20°C. The hexadecadienoic acid also increased tenfold at the lower temperature. However, the 18:2 and 20:5 acids were present in much less concentration in cells grown at 20°C as compared to those grown at 30°C. Among the saturated fatty acids, the odd-numbered fatty acids 13:0, 15:0 and 17:0 were present in much larger concentrations in cells grown at 20°C, than the corresponding acids in cells grown at 30°C. However, the saturated fatty acids 12:0, 14:0, 16:0 and 18:0 were higher in cells grown at 30°C, than at 20°C. The biochemical significance of the presence of highly unsaturated fatty acids and large quantity of odd-numbered saturated fatty acids, as well as difference of the fatty acid pattern in cells grown under the two different conditions has yet to be assessed. Several workers (32, 51, 84, 96, 97) have demonstrated that the lipids of

microorganisms grown at low temperatures contained higher proportions of unsaturated fatty acids, or more highly unsaturated acids than those at optimum temperatures. In this investigation there was a notable increase in the hexadecenoic and octadecenoic acid content of cells grown at the lower temperature. However, the cells grown at the lower temperature were found to lack some of the higher unsaturated fatty acids; this may be due to the differences in oxygen tension in the two different growth conditions.

SUMMARY AND CONCLUSIONS

Studies were conducted on the lipid and fatty acid composition of M. radiodurans grown under two different conditions, the variable parameters being temperature and oxygen tension.

Lipids were classified into individual classes on thin-layer chromatoplates while fatty acids were characterized using reversed-phase partition TLC in conjunction with GLC techniques. The organism was found to possess the entire spectrum of saturated fatty acids ranging from C_{12} to C_{20} with the exception of C_{19}. Branched-C_{14} and branched-C_{16} fatty acids, and several highly unsaturated fatty acids some of which have never been reported so far in a microorganism, were also observed. The fatty acid pattern was different under the two culture conditions. The C_{16}-monoenoic and dienoic acids increased considerably in cells grown at the lower temperature. However, the C_{18} and C_{20} unsaturated fatty acids were in lesser concentrations, while the highly unsaturated long chain fatty acids were absent. As regards the saturated fatty acids, the odd-numbered fatty acids increased while the even-numbered fatty acids decreased in cells grown at the lower temperature.

The highly unsaturated fatty acids could presumably play an important role in the high resistance of the organism to gamma radiation by acting as free radical acceptors.

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