BACTERIOLOGICAL REVIEWS, Sept. 1967, p. 194–213
Vol. 31, No. 3

Extracellular Lipids of Yeasts

FRANK H. STODOLA, MARIA H. DEINEMA, AND J. F. T. SPENCER

Pioneering Laboratory for Microbiological Chemistry, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois 61604; Agricultural University, Wageningen, The Netherlands; and Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada

INTRODUCTION

Although lipids were among the first natural products to be examined in detail (30), their study lagged far behind that of the proteins and carbohydrates for over a century. This neglect was due in large part to the unattractive physical properties of the lipids, which sometimes rendered impossible, and always very tedious, the isolation of these products in a state of purity meeting the strict requirements of the chemist. This unfavorable situation began to change after World War II with the development of elegant new methods for the separation, analysis, characterization, and structural determination of organic compounds. At about the same time, there was an abandonment of the old view that the only function of importance for lipids was their role as long-term storage materials. This restricted outlook was replaced by recognition of the great significance of minor lipid constituents (41), such as phospholipids, sphingolipids, and lipoproteins, some of which were finally becoming available in the pure state as a result of the advances in technique. This combination of a fresh viewpoint and improved laboratory practice resulted in a resurgence of interest in lipid

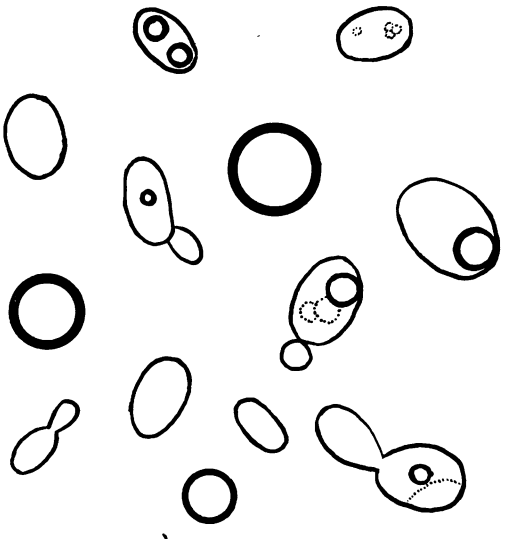


FIG. 1. *Rhodotorula graminis cells and extracellular fat drops; fat drops are heavily outlined circles.* × *2,500.*

chemistry and physiology, which has covered the whole range from the simplest to the most complex forms of life. One facet of the work with microorganisms is the subject of this review.

Intracellular lipids of yeasts have received considerable attention since the initial work of Nägeli and Loew (84) in 1878 on fat production by a beer yeast. The literature on this subject that has since accumulated was ably reviewed by Woodbine (145) in 1959. The study of extracellular lipids of yeasts, on the other hand, is of rather recent origin. No doubt many observers in the past encountered oily droplets in yeast cultures, but dismissed them as autolysis products. It was not until 1954 that Spencer obtained from a *Torulopsis* species lipids that were clearly extracellular. However, chemical work (38) on this product could not be initiated at the Canadian laboratory until 1960, so that the first report of extracellular lipid production by yeasts came from di Menna (78), who showed in 1958 that young cells of a new yeast from New Zealand pasture grass secrete lipids into the culture medium (Fig. 1).

Reports on the chemistry of extracellular lipids of yeasts soon followed from three widely separated laboratories: in 1960 from the Agricultural University at Wageningen, in the same year from the Northern Regional Research Laboratory at Peoria, and soon after from the Prairie Regional Laboratory at Saskatoon. These investigations disclosed a new aspect of yeast biochemistry, and paved the way for a series of papers which show that many yeasts form appreciable amounts of extracellular lipids that are markedly different chemically from intracellular ones.

ORGANISMS

During the past 9 years the formation of extracellular lipids has been observed in over 200 strains of yeasts (102, 103). Most of these have been isolated from a neglected habitat, the phyllosphere, the importance of which was first demonstrated by Last (61) during a study of fungus incidence on cereal leaves in England, and by Ruinen (99–101) in the course of an investigation of the relation between the different components of epiphytic vegetation in Indonesia and Surinam. The technique used by Ruinen for the isolation of yeasts from the phyllosphere has been described by Deinema (31) as follows: leaves were placed in 10 ml of sterile water containing some potassium dihydrogen phosphate; after 2 to 3 days, a drop of this solution was streaked on a nitrogen-free medium containing glucose, KH_2PO_4, $MgSO_4$, and agar.

One of the yeasts isolated by Ruinen (99) in Indonesia and Surinam was designated as *Rhodotorula graminis* di Menna CBS 3043 by Deinema (31) because of its similarity to the red yeast identified as *R. graminis* CBS 2826 by di Menna (78). The nature of the extracellular lipids produced by the Ruinen strain was described by Deinema and Landheer (32) in 1960 in a preliminary report. The following year, Deinema (31) gave the details of this work in her doctoral thesis, together with a description of the extracellular lipids formed by two strains of *R. glutinis* (Fres.) Harrison (CBS 3044 from the leaf surface of *Desmodium repens* and CBS 4648 from cacao plants) and by *Candida bogoriensis* CBS 4101, first isolated by Ruinen from the leaf surface of *Randia malleifera* grown in Indonesia.

In 1963, Ruinen (101) summarized her work on yeasts isolated from the phyllosphere of tropical foliage, noting that the 65 strains isolated belonged to 22 species of the genera *Hansenula*, *Cryptococcus*, *Candida*, *Sporobolomyces*, and *Rhodotorula*. Except for two strains of *Candida guillermondii*, all isolates produced extracellular lipids. Almost all strains which formed such lipids were lipolytic in action. *Cryptococcus* was the predominant genus recovered from the leaf surfaces examined, being represented by 30 strains of the 65 isolated.

In 1964, Ruinen and Deinema (104) described the extracellular lipids produced by strains of *R. glutinis* (CBS 3044 and 4648), *Cryptococcus*

laurentii (no. 45 = CBS 4932), *Candida javanica* (no. 33 = CBS 5236), and *C. bogoriensis* (no. 61 = CBS 4912).

Compared with the extensive work of the Dutch on the phyllosphere yeasts, the efforts of the American and Canadian investigators have been rather limited in scope insofar as the number and variety of organisms are concerned. Only two species of yeasts have been studied at the Peoria laboratory. The first included mating types of *Hansenula ciferri* NRRL Y-1031, particularly isolate F-60-10, which produces acetylated sphingosines (74, 123, 124, 144). The second species of yeast, NRRL YB-2501, which is closely related to *Torulopsis fujisanensis* NRRL YB-4824 but is a separate species (114), was isolated in 1950 from frass of white spruce (*Picea glauca*). It excretes 8,9,13-triacetoxydocosanoic acid (122), as well as 8,9-dihydroxy-13-oxodocosanoic acid, presumably in the acylated form (R. F. Vesonder and F. H. Stodola, *in preparation*).

The Canadian group (38, 116, 141) has worked mostly with an osmophilic yeast which forms hydroxy fatty acid glycosides of the disaccharide sophorose. This organism, first isolated from sow thistle petals and originally classified as *Torulopsis magnoliae*, is now believed to be *Torulopsis apicola* Hajsig (J. F. T. Spencer, *unpublished data*) and is designated as PRL 123-64. More recently, A. P. Tulloch and J. F. T. Spencer (*unpublished data*) studied the extracellular products of *R. glutinis* (CBS 4648 and a new isolate, 16 A8) and of *R. graminis* 6 CB. It has also been shown by these workers that an unidentified species of *Rhodotorula*, isolated in California from trout stomach contents, produces an 8,9,13-trihydroxydocosanoic acid identical with the one isolated by Stodola et al. (122).

Except for the trout organism, all the yeasts so far reported as producers of extracellular lipids have been isolated from plant sources. Some connection with insects is indicated in two cases: the yeast YB-2501, which resembles *T. fujisanensis*, having been isolated from frass produced by beetles, and *T. apicola*, from honey collected from bumblebees' nests. It is of interest that none of these organisms has been isolated from soil.

Yeasts capable of excreting lipids are distributed throughout the three main groups into which Lodder et al. (73) divided the yeasts. The genus *Hansenula* is placed with the ascosporogenous yeasts, the genus *Sporobolomyces* with the ballistosporic yeasts, and *Cryptococcus*, *Torulopsis*, *Candida*, and *Rhodotorula* appear with the asporogenous yeasts.

In addition to the true yeasts just considered, yeastlike smuts of the genus *Ustilago* and the yeastlike fungus *Pullularia pullulans* have been studied from the standpoint of extracellular lipid production.

PRODUCTION, ISOLATION, AND CHEMICAL NATURE OF EXTRACELLULAR LIPIDS OF YEASTS

Detailed chemical studies disclose that four different types of compounds are excreted by yeasts: polyol fatty acid esters, in which saturated, unsaturated, and hydroxy fatty acids are joined by ester linkages to C_5 and C_6 polyols; sophorosides of hydroxy fatty acids, in which saturated and unsaturated hydroxy fatty acids are linked glycosidically to the disaccharide sophorose; acetylated sphingosines, in which all of the hydroxy and amino groups of C_{18}-phytosphingosine and C_{18}-dihydrosphingosine are acetylated; C_{22} acids, in which trihydroxy and dihydroxyoxo fatty acids are acylated.

Polyol Fatty Acid Esters

Natural fatty acid esters of alcohols can be classified on the basis of the number of hydroxyl groups in the alcohol moiety: monohydric (e.g., hexadecyl hexadecanoate of spermaceti wax); dihydric, esters of ethylene glycol, propane-1,2-diol, propane-1,3-diol, butane-1,3-diol, and butane-1,4-diol, for which the general term diol lipids was suggested by Bergelson et al. (5); trihydric, esters of glycerol (e.g., common fats and oils); tetrahydric, none reported; pentahydric, the only member of this class, except for the *R. graminis* and *R. glutinis* lipids described below, being arabityl margarate, isolated by Yabuta et al. (146) in 1941 from inekoji; hexahydric, the yeast extracellular lipids from *R. graminis* and *R. glutinis* being the only examples of this group.

From Rhodotorula graminis. Strains of a red yeast isolated by Ruinen (99) from the leaf surface of citrus plants in Indonesia and Surinam were classified by Deinema (31) as *R. graminis* di Menna CBS 3043. For production of lipids by this organism, cultures were grown on a medium containing 4% glucose, 0.25% yeast extract, 0.1% KH_2PO_4, 0.02% $MgSO_4$, and tap water. After 7 days of incubation at 28 C in shaken 5-liter Erlenmeyer flasks containing 1 liter of medium, the yeast cells were separated by centrifugation and the cell-free supernatant fluid was extracted with ethyl ether. After removal of the solvent, the lipids were hydrolyzed and the free fatty acids were converted to methyl esters with diazomethane. The ester mixture was analyzed by gas-liquid chromatography (GLC) with the following results (percentage of weight of free acid): acetic (45.0), myristic (1.0), palmitic (3.0), stearic (1.0), C_{20}? (1.5), C_{22}? (31.0),

TABLE 1. *Component acids of extracellular lipids of Rhodotorula glutinis (Fres.) Harrison, CBS 3044 and 4648*[a]

Fatty acid	CBS 3044		CBS 4648	
	18 C	29 C	18 C	29 C
Acetic acid	55.0	40.0	50.0	40.0
Myristic acid	0.0	1.0	0.5	1.0
Palmitic acid	5.0	14.0	13.0	15.5
Stearic acid	3.0	1.5	2.5	4.0
Oleic acid	16.0	29.0	14.0	19.0
Linoleic acid	3.0	3.0	3.0	4.0
Linolenic acid	3.0	0.5	2.0	1.0
3-Hydroxy-hexadecanoic acid	11.5	8.5	7.5	12.5

[a] The alcoholic component in all cases was a mixture of C_5 and C_6 alcohols. The fermentations were conducted at the two incubation temperatures shown. The results are expressed as percentages by weight.

and C_{24}? (5.5). In later work, Van Ammers et al. (142 and *unpublished data*) established that the C_{22}? product was 3-hydroxyhexadecanoic acid and the C_{24}? material was 3-hydroxyoctadecanoic acid.

The mixture of polyhydric alcohols obtained in the hydrolysis of the original oil was analyzed qualitatively by paper chromatography. Only pentitols and hexitols were found; they formed 20 to 30% of the crude lipid.

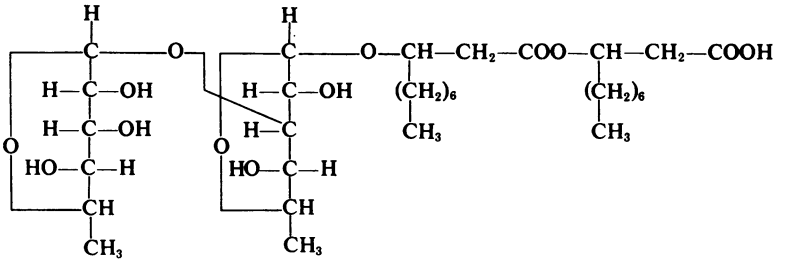
A strain of yeast (6 CB) similar to *R. graminis* was investigated by Tulloch and Spencer (140). On a glucose-yeast extract medium, this organism formed 1 to 2 g of extracellular lipid per liter when grown in aerated fermentors. Acid methanolysis of the lipid yielded ethyl acetate, hydroxy fatty acid esters, and polyols. Saponification of the hydroxy acid ester gave 3-D-hydroxyhexadecanoic acid (85%) and 3-D-hydroxyoctadecanoic acid (15%). The polyols were analyzed as acetates by GLC and consisted of D-mannitol (76.9%), D-arabinitol (15.6%), and xylitol (7.5%).

From Rhodotorula glutinis. Using conditions already described for *R. graminis*, Deinema (31) examined extracellular lipid production by *R. glutinis* (Fres.) Harrison CBS 3044, a red yeast isolated from the leaf surface of *Desmodium repens* grown in tropical greenhouses in Wageningen. The results are given in Table 1, together with data on another strain of *R. glutinis* (CBS 4648), from cacao plants. It will be noted that the acetic acid content of the extracellular lipids is considerably higher at 18 than at 29 C. The 3-hydroxyhexadecanoic acid was identified by Van Ammers et al. (142).

Tulloch and Spencer (140) compared the extracellular lipids produced by *R. glutinis* CBS 4648 and a strain of this organism (16 A8) isolated from Canadian flowers. In both, the lipids consisted of a mixture of mannitol and pentitol esters of two new acids, 3-D-hydroxyhexadecanoic and 3-D-hydroxyoctadecanoic. One molecule of the long-chain acid is attached to each polyol molecule, and most of the remaining hydroxyl groups, including the one on the fatty acid, are acetylated.

Sophorosides of Hydroxy Fatty Acids

The first natural product in which a hydroxyl group of a fatty acid is joined glycosidically to a carbohydrate moiety was pyolipic acid, reported by Bergström et al. (7) in 1946. This oily product of *Pseudomonas pyocyanea* (*P. aeruginosa*) is a rhamnoside of L-β-hydroxydecanoic acid. Three years later, Jarvis and Johnson (47) assigned the following structure to a crystalline acid from the same organism:



In 1950, Haskins (44) described the extracellular formation of a carbohydrate-containing lipid when corn smut (*Ustilago zeae*) was grown on glucose; later chemical studies showed the lipid to consist in part of hydroxy acids in the form of cellobiosides. Recently, Legler (64) isolated from the leaves of *Ipomoea fistulosa* a resin in which 7-hydroxydecanoic acid, 11-hydroxytetradecanoic acid, 11-hydroxyhexadecanoic acid, and 3,11-dihydroxytetradecanoic are bound glycosidically to several residues of D-glucose, D-fucose, D-quinovose, and L-rhamnose to form glycosidic

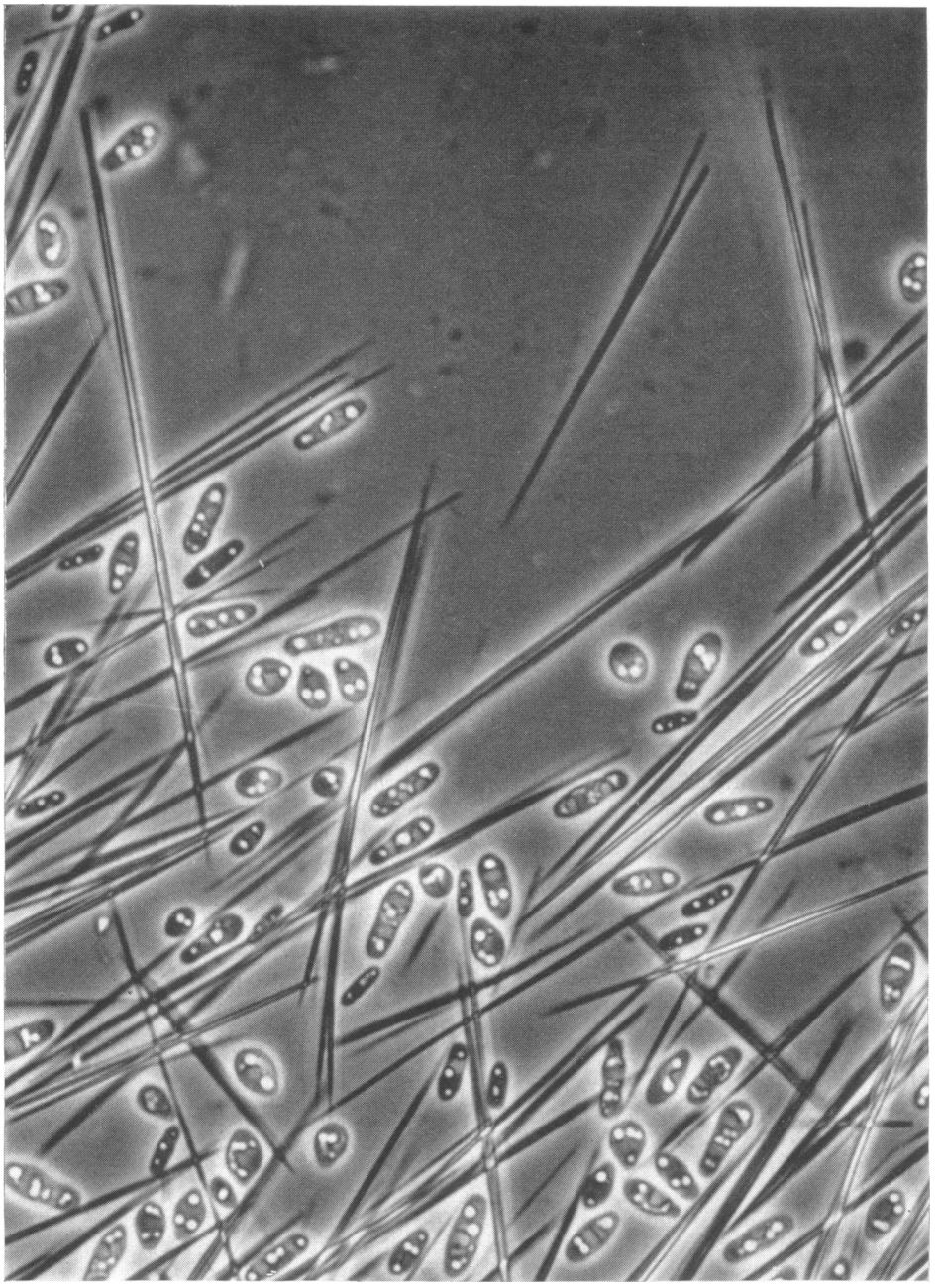


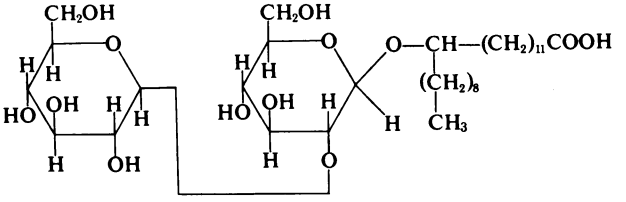
FIG. 2. *Cells of Candida bogoriensis and needles of compound which melts at 74 to 76 C. × 2,000. Phase contrast.*

acids. The lipids from *C. bogoriensis* and *T. apicola*, now to be described, provide further examples of this rather rare type of structure.

From Candida bogoriensis. A cream-colored yeast isolated from the leaf surface of the shrub *Randia malleifera* grown in Indonesia was shown by Deinema (31) to be a new species to which the name *Candida bogoriensis* was given. When grown on a glucose-yeast extract medium, the organism produced two types of extracellular

lipids. One was obtained as a liquid by solvent extraction of the cell-free culture left after centrifugation. The other product remained as crystals with the yeast cells (Fig. 2).

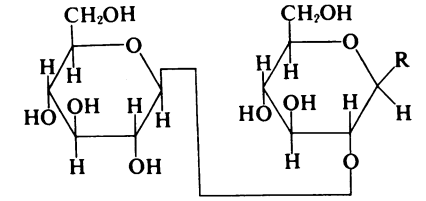
The crystalline lipid, separated from the yeast cells by acetone or alcohol extraction, melted at 74 to 76 C after recrystallization from aqueous acetone. Acid hydrolysis yielded 15 to 20% acetic acid and 25% glucose, but attempts in 1961 to determine the nature of the remainder of the molecule were unsuccessful. In 1965, however, it was shown by Tulloch, Deinema, and Spencer (*unpublished data*) that the crystalline lipid is composed of sophorose (2-*O*-β-glucopyranosyl-D-glucopyranose) linked glycosidically to 13-hydroxydocosanoic acid. Nuclear magnetic resonance spectral studies also indicated that two acetate groups are probably attached to the sophorose moiety in the 6 and 6′ positions. The structure of the deacetylated glycoside can be represented as follows:

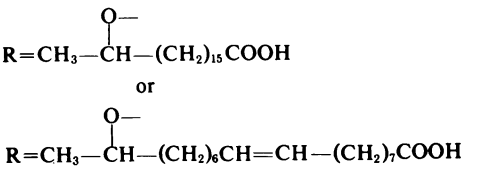


In the latter work it was noted that the lipid melts at 74 to 76 C when isolated from some fermentations, and at 59 to 61 C when obtained from others, a difference which is probably accounted for by a variation in the positions of the acetate groups.

From Torulopsis apicola. For some time, the osmophilic yeasts have been under investigation by Spencer and his associates at the Prairie Regional Laboratory (115). In the course of this work, a *Torulopsis* yeast (now known to be *T. apicola*), which converted glucose to a heavy oil (38), was isolated. The material was produced in highest yields in a medium consisting of glucose, yeast extract, and urea, the fermentation being conducted in 5-liter fermentors aerated for 8 to 10 days at 30 C.

Chemical studies (38) of the oil were carried out as follows: acetyl groups, about two per mole, were removed with methanolic sodium methoxide; the deacetylated product, containing approximately equal quantities of lactonic and acidic products, was reacted with methanolic HCl to yield 2 moles of methyl α-D-glucopyranoside and 1 mole of a mixture of hydroxy fatty acid esters. The structure of the disaccharide was established as that of sophorose, already mentioned as the sugar portion of the crystalline compound produced by *C. bogoriensis*. The hydroxy acids were found to be 17-L-hydroxyoctadecanoic and 17-L-hydroxyoctadecenoic acids. Hence, the structure of the deacetylated *T. apicola* glucoside would be the following:





It was later found by chromatography on silicic acid that the original oil consists of a mixture of acidic and neutral glycosides (A. P. Tulloch and J. F. T. Spencer, *unpublished data*). The acidic glycoside has two acetate groups, probably at the 6 and 6′ positions of the sugar. Thus, this glycoside is remarkably similar to that produced by *C. bogoriensis*, except that the hydroxy acids are different. The neutral glycoside is a very unusual, perhaps unique, type of macrocyclic lactone in which the carboxyl group of the hydroxy acid is esterified with a hydroxyl group of the sophorose. Two acetate groups are also present in the neutral glycoside.

The formation of sophorose derivatives by *T. apicola* is unique in that the yield of the product and the nature of the fatty acid portion of the molecule can be varied to a considerable extent by changing the substrate. When fatty acid esters, hydrocarbons, or glycerides were added over a period of 3 to 4 days to a 1-day-old culture containing glucose, yeast extract, and urea, the yields of crude lipid increased 5 to 28% by volume, and the composition of the product reflected directly the nature of the added lipid (116, 141). Addition of such compounds as heptadecane, octadecane, stearic or oleic acids, which are converted to a single hydroxy acid,

TABLE 2. *Composition of hydroxy fatty acid portion of fermentation products formed from fatty acid methyl esters and from hydrocarbons by Torulopsis apicola*

Added compound	Hydroxy fatty acid (% of total)									
	15-OH C16	16-OH C16	16-OH C17	17-OH C17	17-OH C18	17-OH[a] C18	18-OH[a] C18	18-OH C19	19-OH C20	20-OH C21
None	9	10			21	50	6			
$CH_3(CH_2)_{14}COOCH_3$	40	40			5	14	1			
$CH_3(CH_2)_{15}COOCH_3$			87	13						
$CH_3(CH_2)_{16}COOCH_3$	2	2			80	9				
$CH_3(CH_2)_{17}COOCH_3$	3	5	24	9	6			53		
$CH_3(CH_2)_{19}COOCH_3$	4	9	54	17	5			8		3
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_3$	2	2			6	77	13			
$CH_3(CH_2)_4CH{=}CH{-}CH_2CH{=}CH(CH_2)_7COOCH_3$	1	2			4	37	56			
$CH_3(CH_2)_7CH{=}CH(CH_2)_9COOCH_3$	2	3			9	56	11			
$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOCH_3$	2	4			5	65	12			
$CH_3(CH_2)_{14}CH_3$	36	43			5	13	2			
$CH_3(CH_2)_{16}CH_3$	2	4			85	4				
$CH_3(CH_2)_{18}CH_3$	8	7			57	8	2		14	
$CH_3(CH_2)_{20}CH_3$	8	9			67	9	3			
$CH_3(CH_2)_{22}CH_3$	8	8			63	16	5			

[a] Unsaturated.

gave good yields of a pure crystalline lactonic glycoside.

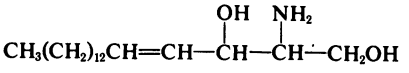
Table 2 gives the results obtained with fatty acid esters and hydrocarbons. The work with added glycerides is summarized in Table 5 of the paper by Spencer et al. (116).

From Table 2 it can be seen that: (i) when esters of acids with more than 18 carbons are used, there is a shortening of the chain as well as hydroxylation; (ii) unsaturated acids can be hydroxylated without interference with the double bonds; (iii) with the C_{20}, C_{22}, and C_{24} hydrocarbons, there is simultaneous hydroxylation, chain shortening, and acid formation.

It is interesting to note the correlation between chain length and the position of the hydroxyl group. ω-Hydroxylation did not occur with stearic acid (25.05 A in length) or with elaidic acid (24.91 A); however, with the shorter acids such as oleic (24.22 A), heptadecanoic (23.80 A), linoleic (23.31 A), palmitic (22.55 A), and linolenic (22.51 A), this reaction takes place to a considerable extent. The maximal ω-hydroxylation occurred with linoleic acid, which may indicate that the other acids are slightly too long or too short. It was first assumed that pentadecanoic and myristic acids, which are readily oxidized, are too short for proper attachment to the enzyme surface and subsequent biosynthesis. However, later work has shown that palmitoleic acid is rapidly hydroxylated. Since its length is very close to that of pentadecanoic acid, this indicates that other factors must be involved. Final elucidation of the problem must await studies with purified enzymes, isolation of which has proven difficult.

Acetylated Sphingosines from Hansenula ciferri

Although sphingosine (D-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-octadecane)

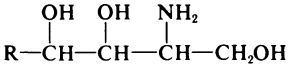


was discovered by Thudichum before 1880 (138), it was not until 1953 that all aspects of its structure were elucidated by the Carter group (18) and others, particularly Mislow, Kiss, Fodor, Jenny, and Grob. This amino alcohol is a component part of the so-called sphingolipids (15, 28, 88), found principally in the brain and spinal cord (23) and in smaller amounts in various animal tissues and fluids (20, 28). Sphingosine also occurs in the following: the ceramides, which are amides of sphingosine and higher fatty acids (134); the ceramide monohexosides (cerebrosides; 19, 26, 28, 135, 137); cerebroside sulfuric esters or sulfatides (ceramide-galactose-sulfate; 8, 125); sphingomyelins (ceramide-phosphate-choline; 19, 42, 139); ceramide dihexosides (11, 59, 93, 129); ceramide trihexosides (76, 128, 129); ceramide

polyhexosides (40, 57, 75, 92, 128, 147); and the many gangliosides, which are phosphorus-free glycosphingolipids containing sialic acid (neuraminic acid; 55, 58, 63, 127, 148). Among the lesser known natural derivatives of sphingosine are sphingosine phosphoryl chloride (54), ceramide aminoethylphosphonate (98), the cyclic acetal of Teets et al. (133), and the plasmalogens (60).

In addition to the well-known C_{18}-sphingosine, a number of related compounds have been reported: C_{20}-sphingosine, isolated from brain lipids by Proštenik and Majhofer-Oreščanin in 1960 (89), Klenk and Gielen in 1961 (56), and Stanacev and Chargaff in 1962 (118); C_{16}- and C_{17}-sphingosines, obtained by Karlsson in 1964 (49) from human plasma; and C_{19}-sphingosine, from corn in 1965 (51).

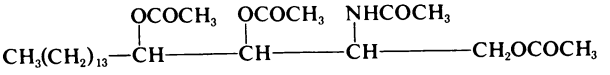
The sphingosines, so common in higher animals, have as their counterparts in the plant world the phytosphingosines, which have the basic structure



Surprisingly, a simple phytosphingosine derivative made its appearance during a taxonomic study of the genus *Hansenula*; the sequence of events was as follows.

Tetraacetyl C_{18}-phytosphingosine. In December 1955, Wickerham observed that mating types of *Hansenula ciferri* NRRL Y-1031 produce fine needles on agar within 24 hr after transfer. This finding was of interest because the parent heterothallic culture did not exhibit this behavior. In February 1957, chemical studies (144) were begun on material obtained from cultures grown on an agar medium containing yeast extract, malt extract, glucose, and peptone. The yeast cells were removed from the agar with water after growth for 10 days at 25 C, and the resulting yeast suspension was extracted with petroleum ether. Removal of solvent gave a crude crystalline product, which was separated by countercurrent distribution into a main component (90%), melting at 49 to 50 C, and another product, melting at 97 to 98 C.

Saponification of the main product yielded acetic acid and phytosphingosine; from the molecular formula $C_{26}H_{47}NO_3$, it was concluded that the material is tetraacetyl C_{18}-phytosphingosine (123):

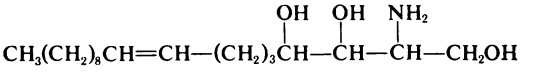


Large-scale production of the crude lipid presented no problems; as much as 175 g of the mixture was obtained in a single run in a 750-liter aerated tank on a 4% glucose solution (74).

The first phytosphingosine was isolated by Reindel (94) in 1930 from yeast, and by 1940 its structure had been only partially elucidated (95). In 1952, Oda (86) obtained from *Penicillium notatum* a phytosphingosine in which R = $CH_3(CH_2)_{13}$—; 2 years later, Carter and his students (17) reported the occurrence of this base in plant seeds, in which it is attached through phosphate ester linkages to an oligosaccharide containing inositol, glucuronic acid, glucosamine, arabinose, and mannose (16, 22, 25). In 1958, Proštenik and Stanacev (91), and Oda and Kamiya (87), demonstrated that the cerebrin of beer yeast yields upon hydrolysis a C_{20}-phytosphingosine.

More recently, Stanacev and Kates (120) reported C_{18}- and C_{20}-phytosphingosines as constituents of the cerebrins of *Candida utilis* (*Torulopsis utilis*), and Sastry and Kates (106, 107) found C_{18}-phytosphingosine in the lipids of runner bean leaves. In 1964, Karlsson (50) demonstrated for the first time that C_{18}- and C_{20}-phytosphingosines occur in animal tissues; this was confirmed by Michalec and Kolman (81). Evidence for a C_{19}-phytosphingosine was provided by Karlsson and Holm in 1965 (51). In 1966, Wagner and Zofcsik (143), in an examination of the sphingolipids of *Saccharomyces cerevisiae* and *Candida utilis*, showed them to have C_{18}- and C_{20}-phytosphingosines as components. In a recent study of the sphingolipids of protozoa, Carter, Gaver, and Yu (21) found, in addition to C20-phytosphingosine, a 19-methyl C_{20}-phytosphingosine.

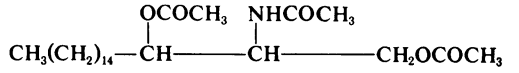
An unsaturated phytosphingosine (dehydrophytosphingosine) is also known. It was first isolated by Mueller (83) in 1953, and its structure was established in 1961 as



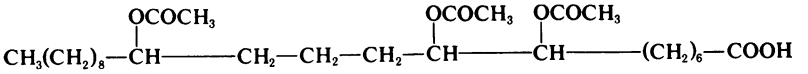
by Carter et al. (25) and by Proštenik and Majhofer-Oreščanin (90). It has been found in wheat flour (25), peanuts (24, 90), soybeans (24), corn (24), and runner bean leaves (106, 107). Of the naturally occurring phytosphingosines, only the one from *H. ciferri* is produced extracellularly.

Triacetyl C_{18}-dihydrosphingosine. The minor component accompanying the tetraacetyl phyto-

sphingosine from *H. ciferri* proved to be triacetyl C_{18}-dihydrosphingosine (124):



The parent base C_{18}-dihydrosphingosine was first obtained from a natural source by Lesuk and Anderson (72) in 1941, who found it in *Cysticercus fasciolaris* larvae. It was not isolated from the sphingolipids of brain and spinal cord until 1947 (27). With the advent of gas chromatography, the detection of dihydrosphingosine was greatly facilitated, and in 1959 Sweeley and Moscatelli (131) were able to demonstrate its presence in human plasma, as well as in bovine intestinal mucosa, spinal cord, and brain. These workers (131), in an examination of a yeast phosphatide, were also the first to show that this reduction product of sphingosine is produced by a microorganism. In 1961, Carter et al. (25) concluded from a careful study of wheat flour cerebrosides that dihydrosphingosine is likewise elaborated by higher plants. C_{16}- and C_{20}-dihydrosphingosines

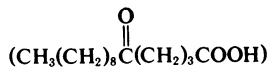


have also been reported recently (48, 80, 105, 119). All naturally occurring dihydrosphingosines except that from *H. ciferri* were intracellular in nature.

Substituted C_{22} Acids from an Unidentified Yeast

The naturally occurring aliphatic hydroxy acids have been reviewed by Downing (33), who pointed out that until recently they have been regarded as curiosities, their isolation being incidental to other work. However, as a result of the growing realization that these acids may be of importance as structural materials in plants and animals, they are now commanding more attention; this is especially true of the monohydroxy acid components of the lipids obtained from the central nervous system. Of the dihydroxy acids, only a few have been isolated and studied chemically: corynolic acid ($C_{52}H_{104}O_4$; 29, 132); mycolic acid ($C_{88}H_{176}O_4$; 4, 121); 9,10-dihydroxyoctadecanoic acid (53); 2,3-dihydroxytetracosanoic acid (86); 15,16-dihydroxyhexadecanoic acid (66); 10,16-dihydroxyhexadecanoic acid (77); 10,18-dihydroxyoctadecanoic acid (77); and 3,11-dihydroxytetradecanoic acid (110). The four trihydroxy acids of known structure have been tabulated by Stodola et al. (122); a new member of this series was encountered in the work on the lipids of the yeast NRRL YB-2501.

Erythro-8,9,13-triacetoxydocosanoic acid. The yeast NRRL YB-2501, isolated from frass, formed extracellular lipid in a yield of 1.42 g per liter when grown for 7 days at 25 C in shaken flasks on a medium containing malt extract and glucose (122). The crude lipid, obtained by extraction of the culture liquor with hexane, gave, on silicic acid chromatography, a pure liquid having the composition $C_{28}H_{50}O_8$. Saponification yielded acetic acid and a $C_{22}H_{44}O_5$ acid melting at 157 to 158 C. The C_{22} acid was shown to be 8,9,13-trihydroxydocosanoic acid by conversion to suberic acid [$HOOC(CH_2)_6COOH$] and 5-oxotetradecanoic acid



The structure of the C_{28} compound is represented by the following:

The *erythro* configuration of the 8,9-hydroxyls was suggested by the slow reaction of the trihydroxy acid with acetone containing dry HCl (R. F. Vesonder and F. H. Stodola, *unpublished data*).

Tulloch and Spencer (*unpublished data*) established that a lightly pigmented species of *Rhodotorula*, strain 62-506, isolated from trout stomach contents, produces an 8,9,13-trihydroxydocosanoic acid that is partially acetylated and partially esterified with long-chain fatty acids. The free trihydroxy acid was shown to be identical with the one of Stodola et al. (122).

It is of interest that the only naturally occurring tetrahydroxy fatty acids have been isolated from lichens (112, 113).

Erythro-8,9-dihydroxy-13-oxodocosanoic acid. During the isolation of the 8,9,13-trihydroxy acid, the existence of a closely related acid was indicated. To obtain enough of the material for study, about 100 g of the crude lipid was prepared. A portion of this (48.3 g) was saponified, the trihydroxy acid (34.0 g) was removed by crystallization, and a new acid (5.0 g) with a melting point of 129 to 130 C was obtained from the mother liquors. Its structure

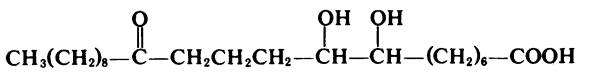


TABLE 3. *Fatty acid composition[a] of palm oil and some intracellular microbial lipids*

Acid	Palm oil (145)	*Rhodotorula graminis* (43)	*R. gracilis* (46)	*Aspergillus nidulans* (111)
Myristic	2.4	3.9	1.1	0.7
Palmitic	41.6	31.9	29.8	20.9
Stearic	6.3	3.2	8.8	15.9
Oleic	38.9	37.2	40.1	40.3
Linoleic	9.5	10.2	11.2	17.0
Linolenic	0.4	4.6	4.8	0.2

[a] Expressed as percentages of total weight.

was proven by its preparation from the trihydroxy acid by oxidation of the 13-hydroxyl group after protection of the other hydroxyls as an acetonide (R. F. Vesonder and F. H. Stodola, *in preparation*). The *erythro* configuration was established by application of the method of Ames and Bowman (2) to 8,9-dihydroxydocosanoic acid obtained from the oxo dihydroxy acid by reductive desulfurization.

This appears to be the only naturally occurring dihydroxyoxo aliphatic fatty acid so far reported. The only related compounds are the prostaglandins, which are C_{20} dihydroxyoxo acids containing a cyclopentane ring (6).

COMPARISON OF INTRA- AND EXTRACELLULAR LIPIDS OF YEASTS

The types of compounds excreted by yeasts are structurally quite different from those found inside the cell.

Esters

The esters within the yeast cell are almost entirely in the form of typical glycerides, in which the glycerol is esterified with unsubstituted fatty acids, mainly palmitic and oleic acids. Table 3 gives analytical data on intracellular fatty acids from microorganisms, and shows their similarity to those of palm oil. In the extracellular lipids of *R. graminis* and *R. glutinis*, the main acids are acetic, 3-hydroxyhexadecanoic, and 3-hydroxyoctadecanoic acids, and the alcohol components are mannitol, arabinitol, and xylitol.

Glycosides

No glycosides corresponding to those from *C. bogoriensis* and *T. apicola* have been isolated from the yeast cell.

Sphingosines

Sphingosines, which are excreted by *H. ciferri* in the form of acetyl derivatives, exist within the yeast cell in quite different form. Preliminary work by Kaufman and Roseman (52) indicated that a cerebroside consisting of phytosphingosine, fatty acids, and a neutral carbohydrate is present within the *H. ciferri* cell.

Recently, Stanacev and Kates (120) surveyed the literature on the nature of the sphingosines occurring in yeasts. They also carefully studied a cerebrin from *T. utilis* and showed the presence of C_{18}- and C_{20}-phytosphingosine and C_{18}-dihydrosphingosine. The acids with which these bases were combined had hydroxyl groups in the 2 position and contained 18, 24, or 26 carbon atoms.

Hydroxy Acids

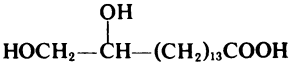
There have been no reports of the isolation of trihydroxy or dihydroxyoxo fatty acids from the intracellular lipids of yeasts.

EXTRACELLULAR LIPIDS FROM YEASTLIKE ORGANISMS

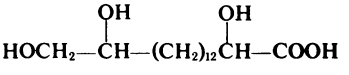
The only organisms resembling yeasts that have been examined for lipid excretion are the yeastlike smut, *U. zeae*, and the yeastlike fungus, *P. pullulans*.

Ustilago zeae

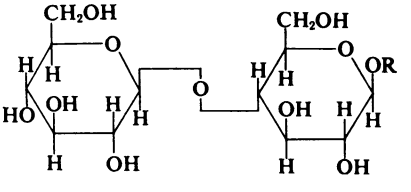
In 1950, Haskins (44) at the Prairie Regional Laboratory reported the formation of a crystalline product by the corn smut, *Ustilago zeae* PRL 119. Chemical studies by Lemieux and his associates (65, 66, 68–71) disclosed that the material was a mixture of acylated acids A and B, in which ustilic acid A



and ustilic acid B



are joined by glycosidic linkages to β-D cellobiopyranoside units as follows:



For glucoustilic acid A, $R = C_{16}H_{31}O_3$

For glucoustilic acid B, $R = C_{16}H_{31}O_4$

These acids are closely related structurally to

TABLE 4. *Acids of the intra- and extracellular lipids of Pullularia pullulans grown at 20 C*[a]

Fatty acid	Intracellular	Extracellular
Myristic	0	4.5
Palmitic	30.5	17.0
Stearic	17.0	6.5
Oleic	37.0	20.0
Linoleic	15.0	26.0
Linolenic	0	19.5

[a] Results expressed as percentages of the total weight of acids.

the *C. bogoriensis* and *T. apicola* acids in which monohydroxy acids are linked glycosidically to the disaccharide sophorose.

The high yields and ease of preparation of the *U. zeae* products suggest their possible industrial use, perhaps in the synthesis of rare and expensive perfume bases as suggested by Lemieux (67).

Ustilago sp. PRL 627

In later studies on the *Ustilaginales*, Haskins noted that a *Ustilago* sp., PRL 627, produced an extracellular oil when grown in submerged culture. It was characterized as a mixture of compounds in which D-mannopyranosyl-1-*meso*-erythritol is esterified with acetic, lauric, myristic, palmitic, and stearic acids (45). Other natural carbohydrate esters are the cord factor of Noll and Bloch (3, 85) from the tubercle bacillus, and the toxic trehalose fatty acid esters of Alimova (1) from *Corynebacterium diphtheriae*.

Pullularia pullulans

The intra- and extracellular lipids of *P. pullulans* 272 were isolated by Ruinen and Deinema (104) and analyzed for acid and alcohol components. Glycerol was found in the intracellular lipid as expected; the alcohol in the extracellular lipid was a hexitol. The distribution of the fatty acids is shown in Table 4.

In a recent study of the fatty acids from intracellular lipids of *P. pullulans*, Merdinger (79) also found that the predominant acids are palmitic and oleic.

BIOSYNTHESIS OF EXTRACELLULAR LIPIDS OF YEASTS

Polyol Fatty Acid Esters

Very little is known about the biosynthesis of extracellular esters produced by yeasts. A few experiments have been carried out by Deinema (*unpublished data*) with several strains of *Rhodotorula glutinis*. Since the products obtained from this yeast contain mannitol esterified with fatty acids, it was of interest to determine the concentration of mannitol dehydrogenase in cell-free extracts. The cells were found to contain two mannitol dehydrogenases, one specific for nicotinamide adenine dinucleotide (NAD) and one for nicotinamide adenine dinucleotide phosphate (NADP). Strains of yeast which produce extracellular lipids all showed an NADP activity of the mannitol dehydrogenase that was two to three times higher than the activity with the coenzyme NAD. With strains of *Rhodotorula* which do not produce extracellular lipids, the results were the reverse of these, and the total activity was always lower. Cells cultivated on mannitol yielded higher values for dehydrogenase activity than those grown on glucose, demonstrating an adaptation of the organism to the substrate.

In another series of experiments by Deinema and Tulloch (*in preparation*), glucose, mannitol, arabinitol, and glycerol were found to be suitable carbon sources, whereas erythritol was not (Table 5).

Table 5 shows yields of extracellular lipids obtained from glucose that are lower than those reported by Deinema (31) in 1961, indicating a change in the organisms as a result of repeated transfer on malt-agar. The higher yields of extracellular lipids from mannitol and arabinitol are not surprising, since these polyalcohols form part of the glycolipid synthesized by the organisms. On the other hand, before glucose and glycerol can

TABLE 5. *Effect of different carbon sources on yield of cells and extracellular lipids produced by Rhodotorula glutinis CBS 4648 at 30 C*[a]

C source (28 to 30 g/liter)	Dry yeast	Extracellular lipids	Acetic acid	Polyalcohol of lipid
	g/liter	*g/liter*	%	
Glucose	6.0–6.5	0.35–0.40	35–40	C_6, trace C_5
Mannitol	6.0–6.5	1.0 –1.20	45–50	C_6, trace C_5
Arabinitol	5.5–6.0	1.0 –1.20	45–50	C_5, trace C_6
Glycerol	5.5–6.0	0.2 –0.25	45–50	C_6, trace C_5

[a] The same experiments were carried out with *R. glutinis* CBS 3044, and gave similar results.

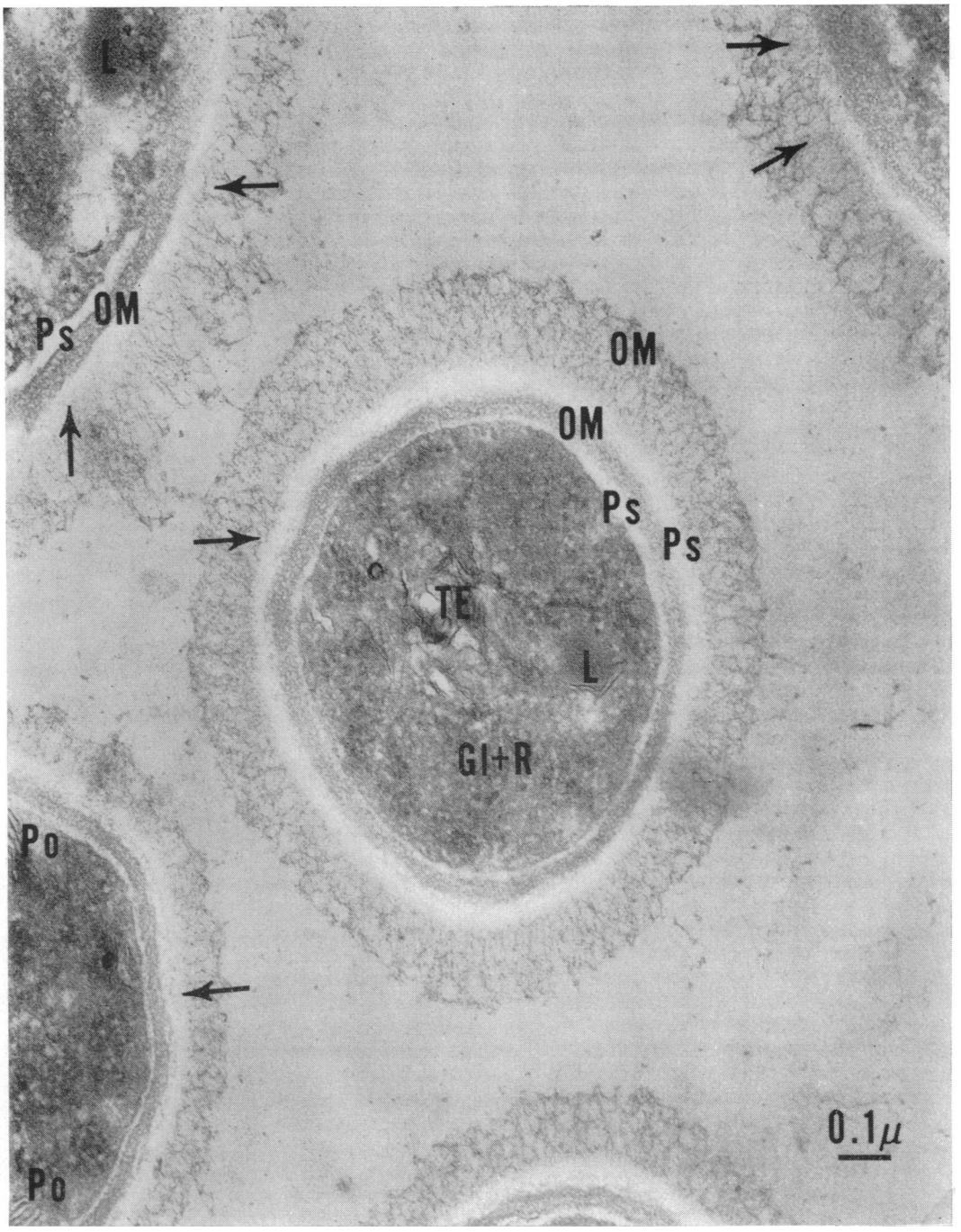


FIG. 3. *Rhodotorula glutinis CBS 4648. The cells are surrounded by a fringe of osmiophilic material; at some places, it is connected with the same material in the cell wall (arrows). Air bubble. Osmium, uranyl acetate, and lead citrate staining. For abbreviations see legend to Fig. 4.*

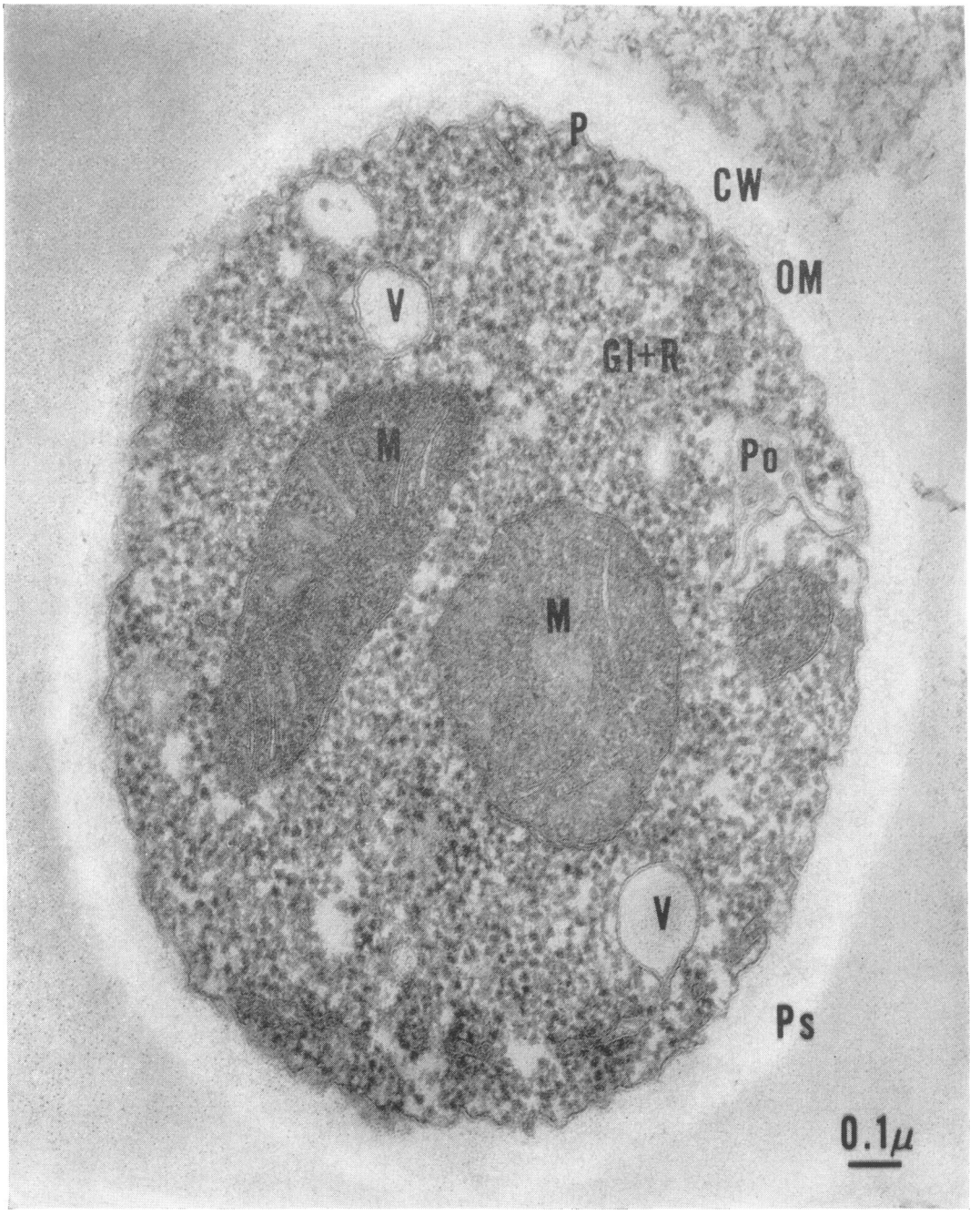


FIG. 4. *Rhodotorula gracilis (Fres.) Harrison. The osmiophilic material in the cell wall is only faintly visible; no fringe. Agar strip. Osmium and lead citrate staining. CW, cell wall; Gl, glycogen, which can be seen as black spots; L, lipid; M, mitochondrion, OM, osmiophilic material; P, plasmalemma; Po, plasmalemmosome; R, ribosomes, which can be seen as very small gray spots; Ps, polysaccharide; TE, tubular elements; and V, vacuole.*

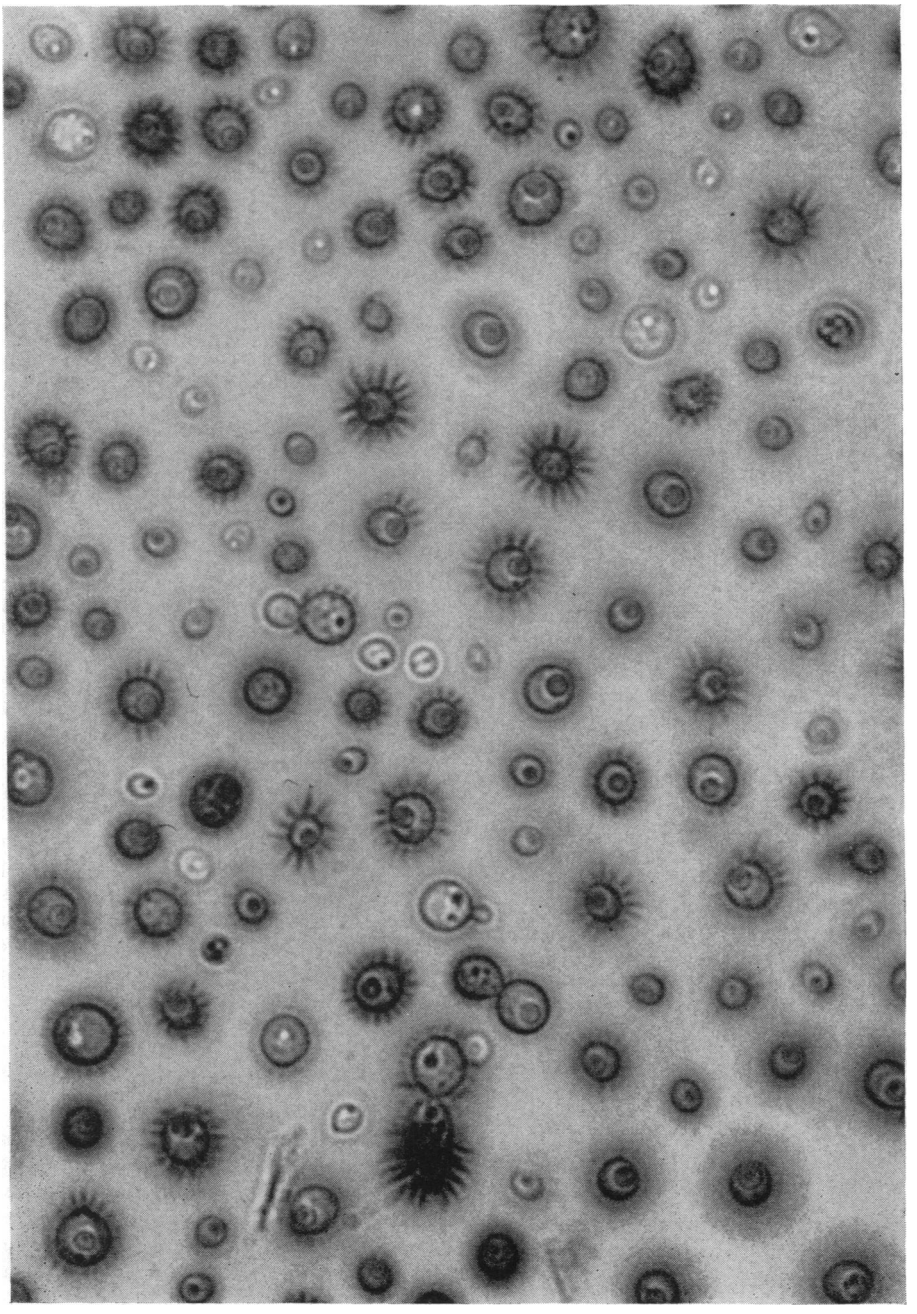


FIG. 5. *Cryptococcus laurentii (Kufferath) Skinner strain 45 grown on malt-agar at 25 C for 1 week. Yeast cells surrounded by a slime capsule in which the lipid material is imbedded and made visible by staining with Nile Blue sulfate.* × *2,000.*

be utilized they must be converted to mannitol, which necessarily involves losses.

Sophorosides of Hydroxy Fatty Acids

The biosynthesis of the sophorosides from *C. bogoriensis* and *T. apicola* has not been investigated. Such studies have, however, been made on the rhamnose-containing compound from *Pseudomonas aeruginosa* by Burger et al. (14) and on ustilagic acid from *U. zeae* PRRL 119, by Boothroyd et al. (9).

Acetylated Sphingosines

In recent years, there has been a greatly increased interest in the chemistry of the nervous system which has given rise to the new discipline of neurochemistry (62). This branch of chemistry "is unique largely because it functions in a complex cytoarchitectural environment." So new is this subject that in 1963 Roberts and Baxter (96) could state that "Only recently pharmacology, electrophysiology, electron microscopy, and ultramicro analytical biochemistry have begun to achieve sufficient power to enable the biological and chemical disciplines to begin to meet at the level of the neurons and glia to attempt to delineate and integrate those properties which are germane to their unique function, the generation, conduction, and regulation of nerve impulses."

One concern of neurochemistry has been with the biosynthesis of sphingosine, which is a component part of all nerve tissue (10, 97). Knowledge of this pathway and identification of necessary cofactors was needed for a better understanding of problems of nerve regeneration and the lipidoses (126) such as Gaucher's (34), Niemann-Pick (35), Tay-Sachs (36), and Fabry's (130) diseases, the Pfaundler-Hurler syndrome (109), and the leukodystrophies (82). This group of clinical conditions is characterized by the accumulation of excessive quantities of sphingolipids in brain, spleen, liver, lung, and other organs.

The first work on the biosynthesis of sphingosine and dihydrosphingosine was carried out on whole animals by Zabin and Mead (150, 151) and Sprinson and Coulon (117). These studies, in conjunction with others on tissue homogenates (12, 13, 37, 149), have established that the sphingosines are produced through the condensation of serine or a serine derivative with some form of palmitaldehyde or palmitic acid. Because the study of the enzyme systems from animal brain presents some difficulties, Greene et al. (39) turned to the simpler system *H. ciferri* as a source of extracts that would carry out the enzymatic synthesis of sphingolipid bases at an appreciable rate. Unfortunately, this goal was not realized, but at least it was possible to show that the pathway to sphingosine in whole yeast cells is similar to that in animal systems. In a recent elaborate study of the lipids formed by *H. ciferri*, Thorpe and Sweeley (136) were able to eliminate those pathways to phytosphingosine which involve either water or molecular oxygen as a precursor of the hydroxyl group at carbon four. The exact source of this oxygen remains to be determined.

Substituted C_{22} Acids

There have been no reports on the biosynthesis of di- and trihydroxy fatty acids from any source.

Electron Microscopy and Extracellular Lipid Formation

A study has been made by Ruinen, Deinema, and van der Scheer (*in preparation*) of the fine structure of two yeasts which secrete lipids and two which do not, in order to ascertain whether there is a difference between these two types of yeasts. The electron micrographs, prepared by a special fixation and embedding technique (108), gave some information about structural details of the slime capsule and parts of the yeast cell, but no definite conclusions can be drawn about the structure of the cell wall.

In Fig. 3 and 4, the fine structure of examples of the two groups of yeasts can be seen. In the lipid-excreting yeast *R. glutinis*, the lipid material in the fringe is connected with the same material in the cell wall (Fig. 3). In the nonexcreting yeast *R. gracilis*, the osmiophilic material is present in layers parallel to the plasmalemma (Fig. 4). The cell wall in this case appears to consist mainly of polysaccharides (electron-transparent), only a small amount of lipid being present.

Extracellular Lipid Formation in Yeast Taxonomy

The occurrence of extracellular lipids can be a useful criterion in the classification of yeasts, since oily droplets or crystals in the culture medium are easily recognized. In some cases, as with certain *Cryptococcus* species (104), the appearance of the yeast on a solid medium is quite characteristic, minute droplets being visible in the enveloping slime capsules radiating in rows from the margin of the cell (Fig. 5).

Summary

The yeasts, which until recently were known to form mainly intracellular triglycerides of a very ordinary sort, have now been shown to excrete a number of unusual lipids. Polyol esters of 3-

hydroxy fatty acids from *Rhodotorula* species, sophorosides of hydroxy fatty acids from *Candida bogoriensis* and *Torulopsis apicola*, acetylated sphingosines from *Hansenula ciferri*, and hydroxylated C_{22} acids from a yeast resembling *Torulopsis fujisanensis* have all been observed and characterized. Yeastlike organisms also produce similar extracellular lipids. Little is known about the biogenesis of these compounds and nothing about where they are synthesized in the yeast cell.

LITERATURE CITED

1. ALIMOVA, E. K. 1960. Some biological properties of lipids and their complexes isolated from diphtheria bacteria. Zh. Mikrobiol. Epidemiol. i Immunobiol. **31**(no. 3):88–92.
2. AMES, D. E., AND R. E. BOWMAN. 1951. Synthetic long-chain aliphatic compounds. Part III. A critical examination of two methods of synthesis of olefinic acids. J. Chem. Soc., p. 1079–1086.
3. ASSELINEAU, J., AND E. LEDERER. 1955. Sur la constitution du "cord factor" isolé d'une souche humain de bacille tuberculeux. Biochim. Biophys. Acta **17**:161–168.
4. ASSELINEAU, J., AND E. LEDERER. 1960. Chemistry and metabolism of bacterial lipids, p. 337–406. *In* K. Bloch [ed.], Lipide metabolism. John Wiley & Sons, Inc., New York.
5. BERGELSON, L. D., V. A. VAVER, N. V. PROKAZOVA, A. N. USHAKOV, AND G. A. POPKOVA. 1966. Diol lipids. Biochim. Biophys. Acta **116**:511–520.
6. BERGSTRÖM, S., AND B. SAMUELSON. 1965. Prostaglandins. Ann. Rev. Biochem. **34**:101–108.
7. BERGSTRÖM, S., H. THEORELL, AND H. DAVIDE. 1946. Metabolic product of *Pseudomonas pyocyanea*, pyolipic acid, active against *Mycobacterium tuberculosis*. Arkiv Kemi Mineral. Geol. **A23**, no. 13.
8. BLIX, G. 1933. Zur Kenntnis der schwefelhaltigen Lipoidstoffe des Gehirns. Z. Physiol. Chem. **219**:82–98.
9. BOOTHROYD, B., J. A. THORN, AND R. H. HASKINS. 1955. Biochemistry of the Ustilaginales. X. The biosynthesis of ustilagic acid. Can. J. Biochem. Physiol. **33**:289–296.
10. BRADY, R. O. 1964. Biosynthesis of lipids, p. 95–109. *In* R. M. C. Dawson and D. N. Rhodes [ed.], Metabolism and physiological significance of lipids. John Wiley & Sons, Inc., New York.
11. BRADY, R. O. 1966. Immunochemical properties of glycolipids. J. Am. Oil Chemists' Soc. **43**: 67–69.
12. BRADY, R. O., J. V. FORMICA, AND G. J. KOVAL. 1958. The enzymatic synthesis of sphingosine. II. Further studies on the mechanism of the reaction. J. Biol. Chem. **233**:1072–1076.
13. BRADY, R. O., AND G. J. KOVAL. 1958. The enzymatic synthesis of sphingosine. J. Biol. Chem. **233**:26–31.
14. BURGER, M. M., L. GLASER, AND R. M. BURTON. 1963. The enzymatic synthesis of a rhamnose-containing glycolipid by extracts of *Pseudomonas aeruginosa*. J. Biol. Chem. **238**:2595–2602.
15. CARTER, H. E. 1958. Sphingolipides, p. 82–94. *In* I. H. Page [ed.], Chemistry of lipides as related to atherosclerosis. Charles C Thomas, Publisher, Springfield, Ill.
16. CARTER, H. E., W. D. CELMER, D. S. GALANOS, R. H. GIGG, W. E. M. LANDS, J. H. LAW, K. L. MUELLER, T. NAKAYAMA, H. H. TOMIZAWA, AND E. WEBER. 1958. Biochemistry of the sphingolipides. X. Phytoglycolipide, a complex phytosphingosine-containing lipide from plant seeds. J. Am. Oil Chemists' Soc. **35**:335–343.
17. CARTER, H. E., W. D. CELMER, W. E. M. LANDS, K. L. MUELLER, AND H. H. TOMIZAWA. 1954. Biochemistry of the sphingolipides. VIII. Occurrence of a long chain base in plant phosphatides. J. Biol. Chem. **206**:613–623.
18. CARTER, H. E., AND Y. FUJINO. 1956. Biochemistry of the sphingolipides. IX. Configuration of cerebrosides. J. Biol. Chem. **221**:879–884.
19. CARTER, H. E., D. S. GALANOS, AND Y. FUJINO. 1956. Chemistry of the sphingolipides. Can. J. Biochem. Physiol. **34**:320–330.
20. CARTER, H. E., D. S. GALANOS, R. H. GIGG, J. H. LAW, T. NAKAYAMA, D. B. SMITH, AND E. J. WEBER. 1957. Sphingolipides. Federation Proc. **16**:817–824.
21. CARTER, H. E., R. C. GAVER, AND R. K. YU. 1966. Novel branched-chain sphingolipid base from *Crithidia fasciculata*. Biochem. Biophys. Res. Commun. **22**:316–320.
22. CARTER, H. E., R. H. GIGG, J. H. LAW, T. NAKAYAMA, AND E. WEBER. 1958. Biochemistry of the sphingolipides. XI. Structure of phytoglycolipide. J. Biol. Chem. **233**:1309–1314.
23. CARTER, H. E., W. J. HAINES, W. E. LEDYARD, AND W. P. NORRIS. 1947. Biochemistry of the sphingolipides. I. Preparation of sphingolipides from beef brain and spinal cord. J. Biol. Chem. **169**:77–82.
24. CARTER, H. E., AND H. S. HENDRICKSON. 1963. Biochemistry of the sphingolipids. XV. Structure of phytosphingosine and dehydrophytosphingosine. Biochemistry **2**:389–393.
25. CARTER, H. E., R. A. HENDRY, S. NOJIMA, N. Z. STANACEV, AND K. OHNO. 1961. Biochemistry of the sphingolipids. XIII. Determination of the structure of cerebrosides from wheat flour. J. Biol. Chem. **236**:1912–1916.
26. CARTER, H. E., P. JOHNSON, AND E. J. WEBER. 1965. Glycolipids. Ann. Rev. Biochem. **34**: 109–142.
27. CARTER, H. E., W. P. NORRIS, F. J. GLICK, G. E. PHILLIPS, AND R. HARRIS. 1947. Biochemistry of the sphingolipides. II. Isolation of di-

hydrosphingosine from the cerebroside fractions of beef brain and spinal cord. J. Biol. Chem. **170**:269–283.

28. CELMER, W. D., AND H. E. CARTER. 1952. Chemistry of phosphatides and cerebrosides. Physiol. Rev. **32**:167–196.
29. CHARGAFF, E. 1933. Chemistry of bacteria. Fat and phosphatide of diphtheria bacteria. Z. Physiol. Chem. **218**:223–240.
30. CHEVREUL, M. E. 1823. Recherches chimiques sur les corps gras d'origine animale. Levrault, Paris.
31. DEINEMA, M. H. 1961. Intra- and extracellular lipid production by yeasts. Thesis, Mededel. Landbouwhogeschool Wageningen **61**(2):1–54.
32. DEINEMA, M. H., AND C. A. LANDHEER. 1960. Extracellular lipid production by a strain of *Rhodotorula graminis*. Biochim. Biophys. Acta **37**:178–179.
33. DOWNING, D. T. 1961. Naturally occurring aliphatic hydroxyacids. Rev. Pure Appl. Chem. **11**:196–211.
34. FREDRICKSON, D. S. 1965. Cerebroside lipidosis: Gaucher's disease, p. 565–585. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson [ed.], The metabolic basis of inherited disease, 2nd ed. McGraw-Hill Book Co., New York.
35. FREDRICKSON, D. S. 1965. Sphingomyelin lipidosis: Niemann-Pick disease, p. 586–617. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson [ed.], The metabolic basis of inherited disease, 2nd ed. McGraw-Hill Book Co., New York.
36. FREDRICKSON, D. S., AND E. G. TRAMS. 1965. Ganglioside lipidosis: Tay-Sachs disease, p. 523–538. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson [ed.], The metabolic basis of inherited disease, 2nd ed. McGraw-Hill Book Co., New York.
37. FUJINO, Y., AND I. ZABIN. 1962. The configuration of sphingosine synthesized in rat brain homogenates. J. Biol. Chem. **237**:2069–2071.
38. GORIN, P. A. J., J. F. T. SPENCER, AND A. P. TULLOCH. 1961. Hydroxy fatty acid glycosides of sophorose from *Torulopsis magnoliae*. Can. J. Chem. **39**:846–855.
39. GREENE, M. L., T. KANESHIRO, AND J. H. LAW. 1965. Studies on the production of sphingolipid bases by the yeast, *Hansenula ciferrii*. Biochim. Biophys. Acta **98**:582–588.
40. HAKOMORI, S., AND R. W. JEANLOZ. 1961. Isolation and characterization of glycolipids from erythrocytes of human blood A (plus) and B (plus). J. Biol. Chem. **236**:2827–2834.
41. HANAHAN, D. J. 1960. Lipide chemistry. John Wiley & Sons, Inc., New York.
42. HANAHAN, D. J., AND G. A. THOMPSON, JR. 1963. Complex lipids. Ann. Rev. Biochem. **32**:215–240.
43. HARTMAN, L., J. C. HAWKE, F. B. SHORLAND, AND M. E. DI MENNA. 1959. The fatty acid composition of *Rhodotorula graminis* fat. Arch. Biochem. Biophys. **81**:346–352.
44. HASKINS, R. H. 1950. Biochemistry of the Ustilaginales. I. Preliminary cultural studies of *Ustilago zeae*. Can. J. Res. **28**(C):213–223.
45. HASKINS, R. H., J. A. THORN, AND B. BOOTHROYD. 1955. Biochemistry of the Ustilaginales. XI. Metabolic products of *Ustilago zeae* in submerged culture. Can. J. Microbiol. **1**:749–756.
46. HOLMBERG, J. 1948. Yeast lipids. I. The component acids of *Rhodotorula gracilis* fat. Svensk. Kem. Tidskr. **60**:14–20.
47. JARVIS, F. G., AND M. J. JOHNSON. 1949. A glyco-lipide produced by *Pseudomonas aeruginosa*. J. Am. Chem. Soc. **71**:4124–4126.
48. KARLSSON, K. A. 1964. Sphingosines. III. C_{20}-dihydrosphingosine, a hitherto unknown sphingosine. Acta Chem. Scand. **18**:565–566.
49. KARLSSON, K. A. 1964. Studies on sphingosines. 6. C_{16}- and C_{17}-sphingosines, hitherto unknown sphingosines. Acta Chem. Scand. **18**: 2395–2396.
50. KARLSSON, K. A. 1964. Studies on sphingosines. 7. The existence of C_{18}- and C_{20}-phytosphingosine in animal tissues. Acta Chem. Scand. **18**:2397–2398.
51. KARLSSON, K. A., AND G. A. L. HOLM. 1965. Sphingosines. IX. C_{19}-sphingosines, hitherto unknown sphingosines. Acta Chem. Scand. **19**:2423–2425.
52. KAUFMAN, B., AND S. ROSEMAN. 1962. Isolation of cerebroside analogues from yeast. Bacteriol. Proc., p. 35.
53. KING, G. 1942. The dihydroxystearic acid of castor oil: its constitution and structural relationship to the 9:10-dihydroxystearic acids, m.p.'s 132° and 95°. J. Chem. Soc., p. 387–391.
54. KING, E. J., AND C. W. SMALL. 1939. The phosphoric esters of the pancreas: sphingosine choline phosphate. Biochem. J. **33**:1135–1139.
55. KLENK, E. 1942. Über die Ganglioside, eine neue Gruppe von zuckerhaltigen Gehirnlipoiden. Z. Physiol. Chem. **237**:76–86.
56. KLENK, E., AND W. GIELEN. 1961. Über ein chromatographisch einheitliches hexosaminhaltiges Gangliosid aus Menschengehirn. Z. Physiol. Chem. **326**:158–161.
57. KLENK, E., AND K. LAUENSTEIN. 1952. Über die zuckerhaltigen Lipoide des Erythrocytenstroma von Mensch und Rind. Z. Physiol. Chem. **291**:249–258.
58. KLENK, E., AND G. PADBERG. 1962. Über die Ganglioside von Pferdeerythrocyten. Z Physiol. Chem. **327**:249–255.
59. KLENK, E., AND F. RENNKAMP. 1942. Über die Ganglioside und Cerebroside der Rindermilz. Z. Physiol. Chem. **273**:253–268.
60. KOCHETKOV, N. K., I. G. ZHUKOVA, AND I. S. GLUKHODED. 1963. Sphingoplasmalogens. A new type of sphingolipids. Biochim. Biophys. Acta **70**:716–719.

61. LAST, F. T. 1955. Seasonal incidence of *Sporobolomyces* on cereal leaves. Brit. Mycol. Soc. Trans. **38**:221–239.
62. LE BARON, F. N. 1959. Neurochemistry. Ann. Rev. Biochem. **28**:579–604.
63. LEDEEN, R. 1966. The chemistry of gangliosides: a review. J. Am. Oil Chemists' Soc. **43**:57–66.
64. LEGLER, G. 1965. Die Bestandteile des giftigen Glykosidharzes aus *Ipomoea fistulosa* Mart. et Chois. Phytochemistry **4**:29–41.
65. LEMIEUX, R. U. 1951. The biochemistry of the Ustilaginales. III. The degradation products and proof of the chemical heterogeneity of ustilagic acid. Can. J. Chem. **29**:415–425.
66. LEMIEUX, R. U. 1953. Biochemistry of the Ustilaginales. VIII. The structures and configurations of the ustilic acids. Can. J. Chem. **31**:396–417.
67. LEMIEUX, R. U. 1953. Synthetic musks from a metabolic product of corn smut in artificial culture. Perfumery Essent. Oil Record **44**:136–139.
68. LEMIEUX, R. U., AND R. CHARANDUK. 1951. Biochemistry of the Ustilaginales. VI. The acyl groups of ustilagic acid. Can. J. Chem. **29**:759–766.
69. LEMIEUX, R. U., AND J. GIGUERE. 1951. Biochemistry of the Ustilaginales. IV. The configurations of some β-hydroxyacids and the bioreduction of β-ketoacids. Can. J. Chem. **29**:678–690.
70. LEMIEUX, R. U., J. A. THORN, AND H. F. BAUER. 1953. Biochemistry of the Ustilaginales. IX. The β-D-cellobioside units of the ustilagic acids. Can. J. Chem. **31**:1054–1059.
71. LEMIEUX, R. U., J. A. THORN, C. BRICE, AND R. H. HASKINS. 1951. Biochemistry of the Ustilaginales. II. Isolation and partial characterization of the ustilagic acid. Can. J. Chem. **29**: 409–414.
72. LESUK, A., AND R. J. ANDERSON. 1941. Concerning the chemical composition of *Cysticerrcus fasciolaris*. II. The occurrence of a cerebroside containing dihydrosphingosine and of hydrolecithin in *Cysticercus larvae*. J. Biol. Chem. **139**:457–469.
73. LODDER, J., W. C. SLOOFF, AND N. J. W. KREGER-VAN RIJ. 1958. The classification of yeasts, p. 1–62. *In* A. H. Cook [ed.], The chemistry and biology of yeasts. Academic Press, Inc., New York.
74. MAISTER, H. G., S. P. ROGOVIN, F. H. STODOLA, AND L. J. WICKERHAM. 1962. Formation of extracellular sphingolipids by microorganisms. IV. Pilot-plant production of tetraacetylphytosphingosine by *Hansenula ciferrii*. Appl. Microbiol. **10**:401–406.
75. MAKITA, A., M. IWANAGO, AND T. YAMAKAWA. 1964. Biochemistry of organ glycolipids. IV. The chemical structure of human kidney globoside. J. Biochem. (Tokyo) **55**:202–204.
76. MÅRTENSSON, E. 1963. On the neutral glycolipids of human kidney. Acta Chem. Scand. **17**:2356–2358.
77. MATIC, M. 1956. The chemistry of plant cuticles: a study of cutin from *Agave americana* L. Biochem. J. **63**:168–176.
78. DI MENNA, M. E. 1958. Two new species of yeasts from New Zealand. J. Gen. Microbiol. **18**:269–272.
79. MERDINGER, E. 1966. Lipids of *Pullularia pullulans*. Can. J. Microbiol. **12**:206–208.
80. MICHALEC, Č. 1965. Biochemistry of sphingolipids. I. Thin-layer chromatography of sphingosines. Biochim. Biophys. Acta **106**: 197–199.
81. MICHALEC, Č., AND Z. KOLMAN. 1966. Biochemistry of sphingolipids. Chromatographic study of sphingolipids and sphingosine bases in normal human kidney. Clin. Chim. Acta **13**: 529–532.
82. MOSER, H. W., AND M. LEES. 1965. Sulfatide lipidosis: metachromatic leukodystrophy, p. 539–564. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson [ed.], The metabolic basis of inherited disease, 2nd ed. McGraw-Hill Book Co., New York.
83. MUELLER, K. L. 1953. Isolation of cerebrin base from plant phosphatides. M.S. Thesis, Univ. Illinois, Urbana.
84. NÄGELI, C., AND O. LOEW. 1878. Ueber die chemische zusammensetzung der Hefe. Ann. Chem. **193**:322–348.
85. NOLL, H., AND H. BLOCH. 1955. Studies on the chemistry of the cord factor of *Mycobacterium tuberculosis*. J. Biol. Chem. **214**:251–265.
86. ODA, T. 1952. Studies on the components of penicillin-producing molds. II. On fungus cerebrin (1). J. Pharm. Soc. Japan **72**:136–139.
87. ODA, T., AND H. KAMIYA. 1958. On the complex lipid, cerebrin phosphate, of yeast. Chem. Pharm. Bull. (Tokyo) **6**:682–687.
88. POLONOVSKI, J. 1964. Les glycolipides. Bull. Soc. Chim. Biol. **46**:833–857.
89. PROŠTENIK, M., AND B. MAJHOFER-OREŠČANIN. 1960. Occurrence of a new sphingolipide base in horse and beef brain. Naturwissenschaften **47**:399–400.
90. PROŠTENIK, M., AND B. MAJHOFER-OREŠČANIN. 1961. The structure of C_{18}-dehydrophytosphingosine of peanut phosphatides. Naturwissenschaften **48**:500–501.
91. PROŠTENIK, M., AND N. Z. STANACEV. 1958. Studien in der Reihe der Sphingolipoide. X. Über die Struktur der Cerebrin-Base aus Hefe. Chem. Ber. **91**:961–965.
92. RAPPORT, M. M., L. GRAF, AND H. SCHNEIDER. 1964. Immunochemical studies of organ and tumor lipids. XIII. Isolation of cytolipin K, a glycosphingolipid hapten present in human kidney. Arch. Biochem. Biophys. **105**:431–438.
93. RAPPORT, M. M., H. SCHNEIDER, AND L. GRAF. 1962. Immunochemical studies of organ and tumor lipids. XI. A simplified preparation of cytolipin H. J. Biol. Chem. **237**:1056–1059.
94. REINDEL, F. 1930. Über Pilzcerebrin. I. Ann. Chem. **480**:76–92.

95. REINDEL, F., A. WEICKMANN, S. PICARD, K. LUBER, AND P. TURULA. 1940. Über Pilzcerebrin. II. Ann. Chem. **544**:116–137.
96. ROBERTS, E., AND C. F. BAXTER. 1963. Neurochemistry. Ann. Rev. Biochem. **32**:513–552.
97. ROSSITER, R. J. 1964. Biosynthesis of lipids of the nervous system, p. 511–525. *In* R. M. C. Dawson and D. N. Rhodes [ed.], Metabolism and physiological significance of lipids. John Wiley & Sons, Inc., New York.
98. ROUSER, G., G. KRITCHEVSKY, D. HELLER, AND E. LIEBER. 1963. Lipid composition of beef brain, beef liver, and the sea anemone: two approaches to quantitative fractionation of complex lipid mixtures. J. Am. Oil Chemists' Soc. **40**:425–454.
99. RUINEN, J. 1956. Occurrence of *Beijerinckia* species in the phyllosphere. Nature **177**:220–221.
100. RUINEN, J. 1961. The phyllosphere. I. An ecologically neglected milieu. Plant Soil **15**: 81–109.
101. RUINEN, J. 1963. The phyllosphere. II. Yeasts from the phyllosphere of tropical foliage. Antonie van Leeuwenhoek J. Microbiol. Serol. **29**:425–438.
102. RUINEN, J. 1963. Cuticle decomposition by micro-organisms in the phyllosphere. J. Gen. Microbiol. **32**:IV.
103. RUINEN, J. 1966. The phyllosphere. IV. Cuticle decomposition by microorganisms in the phyllosphere. Ann. Inst. Pasteur Lille Suppl. **111**:342–346.
104. RUINEN, J., AND M. H. DEINEMA. 1964. Composition and properties of the extracellular lipids of yeast species from the phyllosphere. Antonie van Leeuwenhoek J. Microbiol. Serol. **30**:377–384.
105. SAMBASIVARAO, K., AND R. H. MCCLUER. 1964. Lipid components of gangliosides. J. Lipid Res. **5**:103–108.
106. SASTRY, P. S., AND M. KATES. 1964. Lipid components of leaves. IV. Occurrence of phytosphingosine and dehydrophytosphingosine-containing glucocerebrosides. Biochim. Biophys. Acta **84**:231–233.
107. SASTRY, P. S., AND M. KATES. 1964. Lipid components of leaves. V. Galactolipids, cerebrosides, and lecithin of runner-bean leaves. Biochemistry **3**:1271–1280.
108. VAN DER SCHEER, C. 1966. Fixation and embedding of yeast cell suspensions in air bubbles in agar. Proc. 6th Intern. Congr. Electron Microscopy, Kyoto, p. 37–39.
109. SCHNABEL, R. 1961. Histochemical and biochemical investigations in gargoylism (Pfaundler-Hurler syndrome). Arch. Pathol. Anat. Physiol. **334**:379–398.
110. SHELLARD, E. J. 1961. The chemistry of some convolulaceous resins. I. Vera Cruz jalap. Plant Med. **9**:102–116.
111. SINGH, J., T. K. WALKER, AND M. L. MEARA. 1955. The component acids of the fat of *Aspergillus nidulans*. Biochem. J. **61**:85–88.
112. SOLBERG, Y. J. 1957. Studies on the chemistry of lichens. II. Chemical components of *Haematomma ventosum* (L.) Mass. var. *lapponicum* (Räs.). Acta Chem. Scand. **11**: 1477–1484.
113. SOLBERG, Y. J. 1960. Studies on the chemistry of lichens. III. Long-chain tetrahydroxy fatty acids from some Norwegian lichens. Acta Chem. Scand. **14**:2152–2160.
114. SONEDA, M. 1959. Studies on animal-dung inhabiting yeast. Nagaoa, no. 6, p. 1–24.
115. SPENCER, J. F. T., AND H. R. SALLANS. 1956. Production of polyhydric alcohols by osmophilic yeasts. Can. J. Microbiol. **2**:72–79.
116. SPENCER, J. F. T., A. P. TULLOCH, AND P. A. J. GORIN. 1962. Fermentation of long-chain compounds by *Torulopsis magnoliae*. II. Factors influencing production of hydroxy fatty acid glycosides. Biotechnol. Bioeng. **4**: 271–279.
117. SPRINSON, D. B., AND A. COULON. 1954. The precursors of sphingosine in brain tissue. J. Biol. Chem. **207**:585–592.
118. STANACEV, N. Z., AND E. CHARGAFF. 1962. Icosisphingosine, a long-chain base constituent of mucolipids. Biochim. Biophys. Acta **59**: 733–734.
119. STANACEV, N. Z., AND E. CHARGAFF. 1965. Studies on the chemistry of mucolipids: occurrence of the long-chain base icosisphingosine, composition of fatty acids, fractionation attempts. Biochim. Biophys. Acta **98**:168–181.
120. STANACEV, N. Z., AND M. KATES. 1963. Constitution of cerebrin from the yeast *Torulopsis utilis*. Can. J. Biochem. Physiol. **41**:1330–1334
121. STODOLA, F. H., A. LESUK, AND R. J. ANDERSON. 1938. The chemistry of the lipids of the tubercle bacillus. LIV. The isolation and properties of mycolic acid. J. Biol. Chem. **126**:505–513.
122. STODOLA, F. H., R. F. VESONDER, AND L. J. WICKERHAM. 1965. 8,9,13-Triacetoxydocosanoic acid, an extracellular lipid produced by a yeast. Biochemistry **4**:1390–1394.
123. STODOLA, F. H., AND L. J. WICKERHAM. 1960. Formation of extracellular sphingolipids by microorganisms. II. Structural studies on tetraacetylphytosphingosine from the yeast *Hansenula ciferrii*. J. Biol. Chem. **235**:2584–2585.
124. STODOLA, F. H., L. J. WICKERHAM, C. R. SCHOLFIELD, AND H. J. DUTTON. 1962. Formation of extracellular sphingolipids by microorganisms. III. Triacetyl dihydrosphingosine, a metabolic product of the yeast *Hansenula ciferrii*. Arch. Biochem. Biophys. **98**:176.
125. STOFFYN, P. J. 1966. The structure and chemistry of sulfatides. J. Am. Oil Chemists' Soc. **43**: 69–74.
126. SVENNERHOLM, L. 1964. Lipidoses, p. 553–567. *In* R. M. C. Dawson and D. N. Rhodes [ed.], Metabolism and physiological significance of lipids. John Wiley & Sons, Inc., New York.
127. SVENNERHOLM, L. 1964. The gangliosides. J. Lipid Res. **5**:145–155.

128. SVENNERHOLM, E., AND L. SVENNERHOLM. 1963. The separation of neutral blood-serum glycolipids by thin-layer chromatography. Biochim. Biophys. Acta **70**:432–441.
129. SWEELEY, C. C., AND B. KLIONSKY. 1963. Fabry's disease: classification as a sphingolipidosis and partial characterization of a novel glycolipid. J. Biol. Chem. **238**:PC3148–3150.
130. SWEELEY, C. C., AND B. KLIONSKY. 1965. Glycolipid lipidosis: Fabry's disease, p. 618–632. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson [ed.], The metabolic basis of inherited disease, 2nd ed. McGraw-Hill Book Co., New York.
131. SWEELEY, C. C., AND E. A. MOSCATELLI. 1959. Qualitative microanalysis and estimation of sphingolipid bases. J. Lipid Res. **1**:40–47.
132. TAKAHASHI, H. 1948. Bacterial components of *Corynebacterium diphtheriae*. V. Phospholipides. 2. Structure of Chargaff's corynin. J. Pharm. Soc. Japan **68**:292–296.
133. TEETS, D. W., P. JOHNSON, C. L. TIPTON, AND H. E. CARTER. 1963. Isolation of a new type of sphingolipid. Federation Proc. **22**:414.
134. THANNHAUSER, S. J., AND E. FRÄNKEL. 1931. Über das Lignocerylsphingosine. Z. Physiol. Chem. **203**:183–188.
135. THIERFELDER, H., AND E. KLENK. 1930. Die Chemie der Cerebroside und Phosphatide. J. Springer, Berlin.
136. THORPE, S. R., AND C. C. SWEELEY. 1967. Chemistry and metabolism of sphingolipids. On the biosynthesis of phytosphingosine by yeast. Biochemistry (*in press*).
137. THUDICHUM, J. L. W. 1874. Researches on the chemical constitution of the brain. Report of the Medical Officer of Privy Council a. Local Government Board. New series no. III, 113, London.
138. THUDICHUM, J. L. W. 1880. Further researches on the chemical constitution of the brain, and of the organoplastic substances. 9th Annual Report of the Local Government Board, 1879–1880. Supplement B, no. 3, Report of the Medical Officer for 1879, London, p. 143–188.
139. THUDICHUM, J. L. W. 1884. A treatise on the chemical constitution of the brain. Baillière, Tindall and Cox, London, p. 105.
140. TULLOCH, A. P., AND J. F. T. SPENCER. 1964. Extracellular glycolipids of *Rhodotorula* species. The isolation and synthesis of 3-*D*-hydroxypalmitic and 3-D-hydroxystearic acids. Can. J. Chem. **42**:830–835.
141. TULLOCH, A. P., J. F. T. SPENCER, AND P. A. J. GORIN. 1962. The fermentation of long-chain compounds by *Torulopsis magnoliae*. I. Structures of the hydroxy fatty acids obtained by the fermentation of fatty acids and hydrocarbons. Can. J. Chem. **40**:1326–1338.
142. VAN AMMERS, M., M. H. DEINEMA, C. A. LANDHEER, AND M. H. M. VAN ROOYEN. 1964. Note on the isolation of β-hydroxypalmitic acid from the extracellular lipids of *Rhodotorula glutinis*. Rec. Trav. Chim. **83**:708–710.
143. WAGNER, H., AND W. SOFCSIK. 1966. Über neue Sphingolipide der Hefe. Biochem. Z. **344**: 314–316.
144. WICKERHAM, L. J., AND F. H. STODOLA. 1960. Formation of extracellular sphingolipids by microorganisms. I. Tetraacetylphytosphingosine from *Hansenula ciferri*. J. Bacteriol. **80**: 484–491.
145. WOODBINE, M. 1959. Microbial fat: microorganisms as potential fat producers. Prog. Ind. Microbiol. **1**:181–245.
146. YABUTA, T., Y. SUMIKI, AND K. TAMARI. 1941. Chemical constituents of inekoji. VIII. Arabityl margarate. J. Agr. Chem. Soc. Japan **17**:307–310.
147. YAMAKAWA, T., S. YOKOYAMA, AND N. KISO. 1962. Structure of main globoside of human erythrocytes. J. Biochem. (Tokyo) **52**:228–229.
148. YAMAKAWA, T., AND S. SUZUKI. 1951. The chemistry of the lipids of post-hemolytic residue or stroma of erythrocytes. I. Concerning the ether-soluble lipids of lyophilized horse blood stroma. J. Biochem. (Tokyo) **38**:199–212.
149. ZABIN, I. 1957. Biosynthesis of ceramide by rat brain homogenates. J. Am. Chem. Soc. **79**: 5834–5835.
150. ZABIN, I., AND J. F. MEAD. 1953. The biosynthesis of sphingosine. I. The utilization of carboxyl-labeled acetate. J. Biol. Chem. **205**: 271–277.
151. ZABIN, I., AND J. F. MEAD. 1954. The biosynthesis of sphingosine. II. The utilization of methyl-labeled acetate, formate, and ethanolamine. J. Biol. Chem. **211**:87–93.

ERRATUM

Epidemiology of Airborne Staphylococcal Infections

R. E. O. WILLIAMS

Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, England

Volume 30, no. 3, p. 664, column 2: on Fig. 4, the left-hand scale should read "cols. per 0.2 sq. ft. per 24 hour" and the figures on the right-hand scale should read, from top to bottom, "0.05, 0.025, and 0.005."