Occurrence of Glycolipids in the Lens of the Human Eye

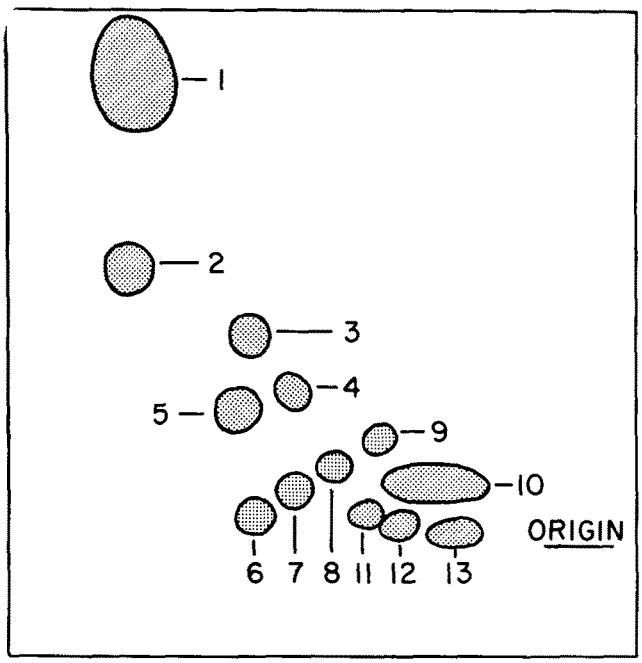
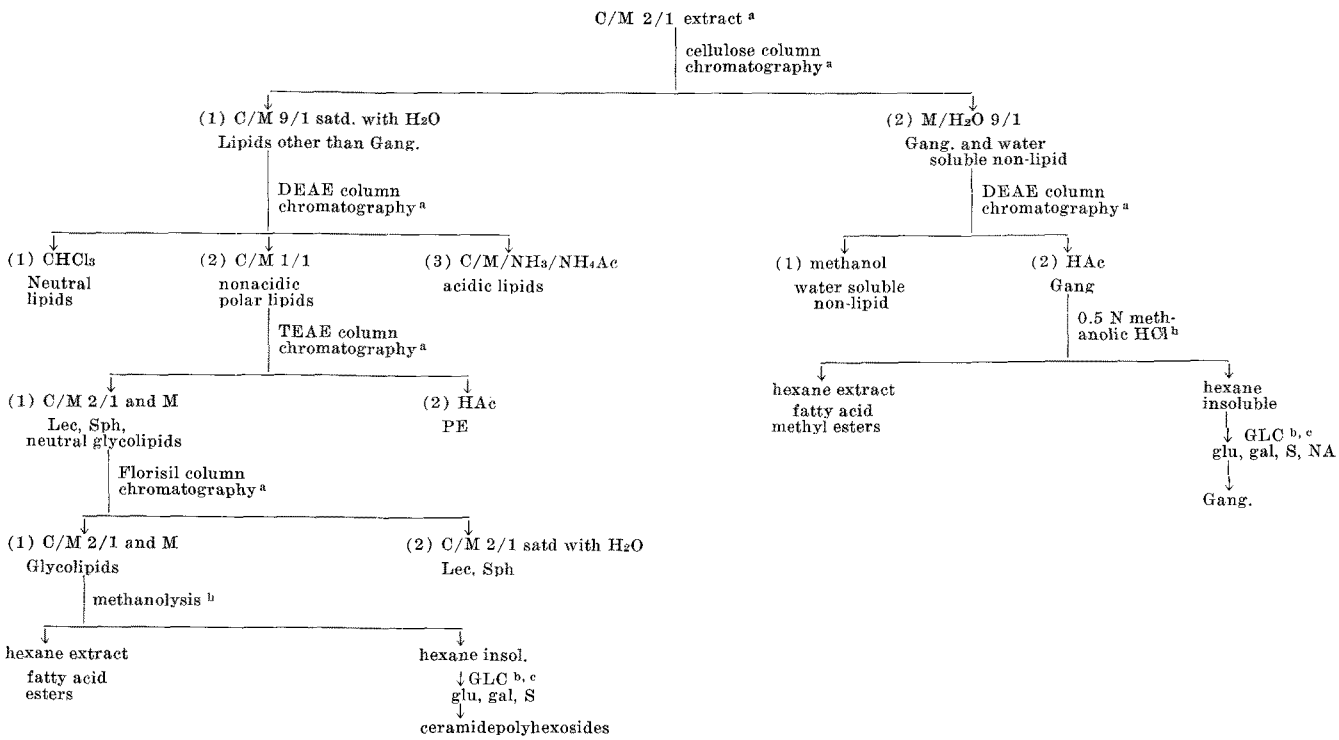


FIG. 1. TLC of human lens lipids, according to Rouser et al., JAOCS *41*, 836–840 (1964). Sample application in the lower right hand corner with chloroform/methanol/water 65/25/4 in the vertical direction followed by n-butanol/acetic acid/water 60/20/20 in the horizontal direction.

THE LENS OF THE HUMAN eye contains a complex mixture of lipids comprising about 2% of the tissue wet weight. Two-dimensional thin layer chromatography (TLC) disclosed the presence of phospholipids and previously unrecognized glycolipids (Fig. 1). The reactions of the spots of polar lipids separated by TLC to various spray reagents are shown in Table I. These characteristics differentiate neutral (less polar, nonionic) lipids, phospholipids and polar glycolipids. After isolation by column chromatography according to the scheme below, TLC and gas-liquid chromatography (GLC) demonstrated the occurrence of glucose, galactose, fatty acids, sphingosine, and dihydrosphingosine in a nonionic glycolipid fraction thus demonstrating the presence of ceramide polyhexosides (Fig. 2) GLC of the acidic glycolipid fraction demonstrated the presence of fatty acids, sphingosine, dihydrosphingosine, glucose, galactose, and neuraminic acid thus demonstrating the presence of ceramide polyhexosides containing neuraminic acid (gangliosides) but no hexosamine. Lipids of this type have been referred to as hematosides.

The presence of both ionic and nonionic glycolipids is clearly established by the procedures used since the chromatographic characteristics by TLC and column chromatography on DEAE and TEAE celluloses and magnesium silicate (Florisil) are in keeping with these identifications and GLC has disclosed the presence of the proper hydrolytic products.

FIG. 2



[a] According to Rouser et al., JAOCS *40*, 425–454 (1963).
[b] According to Sweeley and Walker Anal. Chem. *36*, 1461–1466 (1964).
[c] Sphingosine analysis by GLC according to Gaver and Sweeley JAOCS *42*, 294–298 (1965).
Abbreviations: C, $CHCl_3$; M, CH_3OH; DEAE, diethylaminoethyl cellulose; NH_4Ac, ammonium acetate; HAc, glacial acetic acid; TEAE, triethyl aminoethyl cellulose; PE, phosphatidy ethanolamine; Lec, lecithin; Sph, sphingomyelin; S, sphingosine; NA, neuraminic acid; Gang, gangliosides.

TABLE I

Spray Reagent Characteristics of Human Lenticular Lipid Classes

Spot No. [a]	Reagent				Lipid classes
	Char [b]	PLS [c]	Nin [d]	α-Nap [e]	
1	+	−	−	+	Neutral lipids (cholesterol and glycerides)
2	+	−	−	−	Free fatty acid
3	+	+	+	+	Phosphatidyl ethanolamine and glycolipid
4	+	−	−	+	Glycolipid
5	+	−	−	+	Glycolipid
6	+	+	−	−	Phosphatidic acid
7	+	+	+	−	Phosphatidyl serine
8	+	+	−	−	Phosphatidyl inositol
9	+	+	−	−	Phosphatidyl choline
10	+	+	−	−	Sphingomyelin
11	+	−	+	+	Glycolipid
12	+	−	+	+	Glycolipid
13	+	−	+	+	Glycolipid

[a] Numbers refer to spots in Figure 1.
[b] 55% sulfuric acid plus 0.6% potassium dichromate reagent (Rouser et al. JAOCS *41*, 836–840 (1964).
[c] Specific for phospholipids (Dittmer and Lester, J. Lipid Res. *5*, 126–127 (1964).
[d] Ninhydrin reagent (0.1% in n-*butanol*). Color developed by heating for 3–5 min at 120C.
[e] Specific glycolipid spray of 0.2% α-naphthol in ethanol followed by a light spray with 95% H_2SO_4 and heating at 120C. (Siakotos and Rouser, unpublished). Cholesterol gives a color.

Bovine lens was found to contain similar components. It is interesting that sphingolipids are the predominant polar lipid components in lens of man and cattle and it appears that sphingolipids have special significance in the structure and function of the lens. It seems probable that these lipids are largely components of membrane structures.

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Reduction of Hydroperoxide Interference in the 2,4-DNP Determination of Carbonyls

The 2,4-dinitrophenyl hydrazine (2,4-DNPH) method of Henick et al. (1) for the determination of carbonyl in fats has been widely used because it is relatively simple and quite sensitive. These positive attributes are offset by the fact that the strong acid used (trichloroacetic) and the high temperature (60C) cause the decomposition of hydroperoxides and the formation of additional carbonyls.

This fact has been reported in the literature (2, 3) and has been verified in this laboratory in our work on autoxidized and thermally oxidized oils. Hence, reduction of the hydroperoxides in the oils with $NaHSO_3$ or HI has given substantially lower results. Reduction, however, does not seem to be a complete solution to the problem for, besides being time-consuming, it generates additional carbonyls (4).

TABLE I

Decomposition of *t*-Butyl Hyproperoxide [a] by Trichloroacetic Acid

Reaction conditions		Optical density of basic 2,4-DNP's formed	
Time	Temperature	430 mμ	460 mμ
30 min	60C	0.345	0.317
2 hr	23C	0.118	0.073
20 hr	5C	0.115	0.090
7 days	−18C	0.081	0.068

[a] Concentration of *t*-Butyl Hydroperoxide = 6.84 μM/5cc.

The decomposition of hydroperoxides to carbonyl is apparently pH-dependent since Schwartz and coworkers have shown that the use of phosphoric acid, on a Celite column to form the DNP's, does not cause the decomposition of methyl linoleate hydroperoxide (5). Table I shows that it is also very much dependent on the temperature at which the reaction is carried out. Similar results were also obtained using cumene hydroperoxide in the presence of hexanal and crotonaldehyde.

These results show that interference can be reduced drastically by using lower temperatures. An added advantage is the fact that ketones give higher derivatization (greater color formation) at the lower temperatures. Table II shows this. Table III indicates that at the lower temperatures crotonaldehyde, hexanal and 2-butanone, representing three types of carbonyl known to occur abundantly in heated fats, can be determined in the presence of one another with greater accuracy.

As modified, the method is identical to that of Henick et al. (1) except for the use of purified tertiary butyl alcohol to dissolve the DNPH reagent in order to obtain lower blanks (6). The reaction is carried out for 20 hours at 5 ± 1C, and the 10.0 ml alcoholic KOH is added with shaking as suggested by Chipault et al. (7). Optical density values

TABLE II

Influence of Temperature on the Molar Extinction of Alkaline Carbonyl 2,4-DNP's

Carbonyl	Molar extinction (E)							
	30 Min, 60C		2 Hr, 23C		20 Hr, 5C		Literature values	
	430 mμ	460 mμ	430 mμ	460 mμ	430 mμ	460 mμ	430 mμ	460 mμ
Hexanal	18,700	14,400	18,800	14,600	19,500	14,950	20,930 [a]	15,290
Crotonaldehyde	25,400	28,050	22,000	28,000	21,950	28,250	23,670 [a]	30,670
Acetone	11,600	8,700	12,300	9,400	20,400	15,700	19,000 [b]	.....
2-Butanone	5,770	4,500	6,760	5,250	18,950	13,600		
Levulinic acid	3,850	2,600	4,650	3,100	12,460	8,900	17,000 [b]	.....
2,3 Pentanedione [c]	9,600	13,750	10,300	13,800	10,620	17,600		

[a] See reference 8.
[b] Reference 9.
[c] Maximum wave-length = 505; E = 17,200; 18,400 and 23,600 at 60, 23 and 5C, respectively (mono-derivative E500 = 23,500 [b]).