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Cerebrosides of Human Aorta: Isolation and Fatty Acid Distribution

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CEREBROSIDES OF HUMAN AORTA: ISOLATION AND FATTY ACID DISTRIBUTION

by

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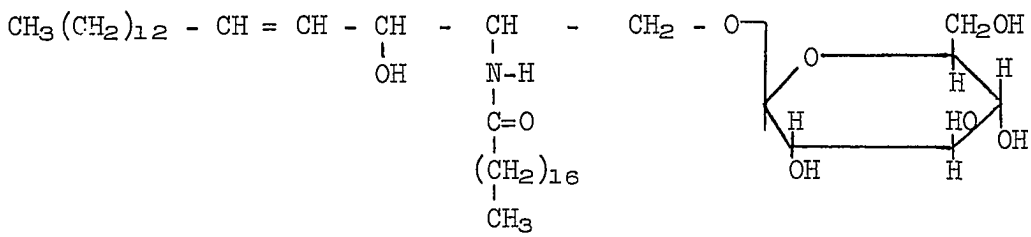
INTRODUCTION

Cerebrosides

Cerebrosides are a family of glycolipids, in which a sphingosine is linked by an amide bond to a long chain fatty acid and to a hexose through a glycosidic bond. The following structure can be named (1) . .



. . N-Stearyl-1-O-D-glucosyl-4-sphingenine.

The combination of fatty acid and sphingosine, is called ceramide. The cerebroside molecule can then be referred to as a glycosylceramide, which describes the hexose of the cerebroside.

There are many slight variations in naturally occurring cerebrosides. The name sphingosine is specific for 2-amino-4-octadecene-1,3-diol. If the double bond is not present, the compound is named dihydrosphingosine. Sphingosine and a small amount of dihydrosphingosine are found in animal cerebrosides. Plant cerebrosides contain only 4-hydroxysphingosine or 4-hydroxy-dihydrosphingosine. The sugar of animal cerebrosides is either galactose or, as represented above, glucose. By far the most common variation in cerebrosides, is found in the fatty acid. The fatty acid chain length varies from C_{12} to C_{28}, with even numbered chain lengths

between C_{16} and C_{24} predominating. The fatty acid can be saturated or unsaturated and occurs with or without a 2-hydroxy group.

Cerebrosides are found in highest concentration in brain (2). They have also been reported in kidney (3), spleen (4), plasma (5-6), serum lipoprotein (7), erythrocytes (6), leukocytes (8), and aorta (9-16). Their presence in aortic tissue led us to more fully investigate these cerebrosides and their possible relationship to atherosclerosis.

The objectives of this investigation, were to isolate cerebrosides from various types of atherosclerotic tissue and to determine their quantity and fatty acid distribution. From these data, it was hoped a correlation could be found between fatty acid distribution and or the quantity of cerebrosides and the advancement of atherosclerosis.

Cerebrosides in Aorta

The presence of cerebrosides has been reported in human aortic tissue (9-16). Böttcher (14) found cerebrosides decreasing with increasing atherosclerosis, in normal tissue, after removal of lesions, from 6.5 to 4.8% of total lipid. Streaks and spots contained 4.8% cerebrosides and fibrous plaques 2.4% cerebrosides.

Hausheer and Bernhard (13) found cerebrosides increasing with severity of atherosclerosis, from 2.2 to 3.4% of total lipid. They reported a galactose/glucose of 8:3 in cerebrosides, but their infrared spectrum corresponded to glucosylceramide (17). Their weight of cerebrosides was based on a fraction collected from a chromatography column which chromatographed with a cerebroside standard on TLC in

propanol ammonia. Experiments reported here indicate that this fraction contained substances besides cerebrosides. Our data suggests that these investigators had not fully purified the cerebrosides and therefore had cerebroside values that were too high.

As was mentioned above, cerebrosides are found in highest concentration in brain. Foote, Allen and Agranoff (18) reported cerebrosides of human brain to be 3% of gray matter and 11% of white matter, based on dry weight of tissue. O'Brien, Fillerup and Mead (19) found cerebrosides increasing with age in human brain, from .085% to .74% in gray matter and .085% to 3.5% in white matter. These percentages were based on weight of wet tissue. Similar results have been reported by other authors (20), when studying mature and immature human brains.

In spleen, Suomi and Agranoff (21) found cerebrosides to be between .1 and .28% of wet tissue. Only normal fatty acids and glucose were found in these cerebrosides.

Vance and Sweeley (6) analyzed glycolipids from human blood plasma and erythrocytes. In plasma they found .45 u mole/50 ml and from erythrocytes .25 u mole/50 ml. The hexose of these cerebrosides was predominantly glucose with galactosylceramide less than 0.1u mole/50 ml, in each case.

Classification of Atherosclerotic Tissue

For this investigation it was necessary to classify tissue in the human aorta as to severity of atherosclerosis in order to compare our quantitative data. Several methods were available.

Böttcher (14), in a study of phospholipids in atherosclerotic lesions, classified the aortas as follows; Stage 0: no lesions discernible at a

magnification of 10; Stage I: fatty streaks and/or spots present but no other lesions; Stage II: discrete or confluent plaques, either of the firm fibrous type or of the soft, fatty, yellow type, but no other evidence of ulceration or other complications; Stage III: additional complications, e.g., ulceration, necrosis and hemorrhage. Tissue was sectioned out of each aorta by this classification. Fatty streaks and spots were obtained from aortas in Stage I, II or III, and plaques from Stage II and III aortas. Normal intima was obtained from unaffected aortas, Stage I, or obtained after removal of lesions from diseased aortas. Böttcher checked the classification histochemically.

A second method of classifying atherosclerotic tissue, utilizes lipophilic dyes which are absorbed by the fat deposits in the aorta and make it easier to distinguish the types of tissue. This method is widely used by pathologists. A dying method was used by the World Health Organization (22) in an impressive study to measure the imprecision in assessing the degree of atherosclerosis and type of plaques found in human aortas. Aortas were first dyed in an alcohol solution of Sudan IV then graded by a team of pathologists as to types of lesions present and degree of coverage of aortic lesions. They defined four types of lesions. 1. calcified lesion: areas in which there is calcium deposition detectable either visually or by palpation without overlying hemorrhage, ulceration or thrombosis, 2. complicated lesion: areas in which there is ulceration, hemorrhage or thrombosis with or without calcium deposits, 3. fatty streak or spots: any intimal lesion that is stained distinctly by Sudan IV or other fat soluble dyes and does not show any other type of change underlying it

and 4. fibrous plaque: any firm, elevated intimal lesion which in the fresh state is pale gray, glistening and translucent. After staining fibrous plaques may be partially or totally covered by sudanophilic deposits. Using this system these observers had about 10% relative inter-observer variation in the assessment of type of plaque present.

For our work staining seemed unsuitable, since the dye reactions with cerebrosides are unknown. We therefore used a method similar to that described previously by Böttcher (14). Tissue from aortas was classified in four catagories; normal, streaks and spots, plaques and lesions. Classification was made by gross observation.

The Isolation of Cerebrosides

The isolation of cerebrosides from crude lipid has been done previously by two basic methods. One method involves the precipitation of cerebrosides from solution. Rosenberg and Chargaff (17) described two precipitation methods in isolating cerebrosides from patients with Gaucher's disease. One method involved a threefold extraction of tissue with hot acetone and then with anhydrous methanol. The methanol extract deposited crude crystals of cerebrosides for an initial yield. Additional yields were obtained by cooling both the acetone and methanol fractions at 0°C overnight. Another precipitation method, reported by these workers, involved extraction of tissue with chloroform: methanol (C:M), 2:1, followed by a partition dialysis. The upper phase and interphase were removed and the cerebrosides, precipitated in the lower phase, were collected by filtration. This crude material was recrystallized from a minimum volume of methanol:acetone, 2:1.

A second general method uses chromatography as the basic tool. Radin, Lavin and Brown (23) described a method for the isolation of cerebrosides using a combination of Dualite A-7, Dowex 50-Xa resin and Florisil, an activated magnesium silicate. The mixture of resins was first packed into a chromatography column and the Florisil layered on top of the resins. Crude lipid extracts in C:M, 2:1 were then added to the column. Additional C:M, 2:1 was eluted through and the eluant dried to obtain crude cerebrosides. Cerebrosides of 80% purity were obtained.

Ställberg-Stenhagen and Svennerholm (24) isolated sulfitides and cerebrosides from brain by first subjecting crude lipid to alkaline methanolysis and then a solvent partition. The chloroform layer was dried and chromatographed on a silicic acid column. Cerebrosides were eluted in the first fraction by C:M, 4:1.

Foote, Allen and Agranoff (18) used a series of columns to purify cerebrosides from brain. The crude lipid was first applied to a Florisil column. The chloroform-methanol fraction from the first column was rechromatographed on a silicic acid column. Cholesterol was eluted with C:M, 98:2, after which cerebrosides were eluted with C:M, 94:6.

Vance and Sweeley (6) used a TLC method developed by Svennerholm and Svennerholm (5), for the final purification of a mixture of glycolipids obtained from blood plasma and erythrocytes. The glycolipids were separated from crude lipid by elution of a silicic acid column. Neutral lipids were eluted with chloroform after which polyglycosylceramides were eluted with methanol:acetone; 9:1. The

various polyglycosylceramides were separated by preparative silica gel thin layer chromatography (T.L.C.) in C:M:water (C:M:W), 100:42:6. Cerebrosides had an R_f of 0.8.

The method chosen for the isolation of cerebrosides from a crude lipid sample, will vary with 1. the ratio of neutral to polar lipids, and 2. the percentage of cerebrosides in total lipid. The method chosen for isolation of cerebrosides in this investigation consisted of 1. a Florisil column, 2. mild alkaline methanolysis, 3. a second Florisil column and 4. preparative TLC, in that order. The choice of method will be discussed in results and discussion.

MATERIALS AND METHODS

Materials

Human aortas were obtained from autopsy and stored immersed in 0.9% sodium chloride at -20° C. All specimens were from adults with no record of metabolic defects.

Solvents were redistilled and other reagents were analytical grade unless otherwise noted. Silica Gel G for TLC was obtained from Brinkmann Instruments Inc., Westbury, New York. Florisil, for column chromatography was purchased from Fisher Scientific, Fair Lawn, New Jersey.

Solutions are expressed on a volume:volume basis unless otherwise noted.

Tissue Classification

The inner portions of the aortas were divided into four classes based on severity of atherosclerosis: normal tissue (N); spots and streaks (S); plaques (P) and lesions (L). The classes of tissue were sectioned out for crude lipid extraction. Samples were designated by two letters; the first identifying the aorta and the second identifying the class of tissue.

Extraction of Lipid from Tissue

The method of Folch (25) was used to obtain crude lipid from tissue samples. The tissue was homogenized in C:M, 2:1, 19 ml of

solution per gram of wet tissue, in a glass hand homogenizer. The mixture was then filtered through sintered glass and the residue washed with C:M, 2:1, 3.5 ml per gram wet tissue and then with C:M, 1:2, 3.5 ml per gram (26). 3.5 ml per gram of chloroform was then added to the filtrate. The filtrate was washed with one fifth the volume of 0.1 M KCl. The upper layer was removed and the lower layer washed again with C:M:W; 3:48:47. The upper layers were combined and saved. The washed lower layer was dried on a rotary evaporator, in a water bath at 37°C. To remove traces of water remaining in the crude lipid, a few ml of benzene: absolute ethanol, 1:1 was added and evaporation continued. This drying procedure was repeated until no foaming was evident and the lipid clear. The dried lipid was weighed and then stored under nitrogen at -20°C.

Isolation of Cerebrosides

Cerebrosides were purified from crude lipid by a four step procedure. Crude lipid was first chromatographed on a Florisil column, using 10 g of Florisil (8% water by weight) per gram of crude lipid. Elution was carried out with hexane: ether (H:E), 8:2, 25 ml per gram of Florisil followed by C:M, 3:1, 30 ml per gram of Florisil (27). Solvent was removed from both fractions and the lipid residue weighed. The H:E fraction was labelled F-1 and contained relatively nonpolar lipids. The C:M fraction was labelled F-2 and contained most of the more polar lipids. Sphingomyelin was not recovered from the column. The F-2 fraction was subjected to mild alkaline methanolysis (28) in a mixture of chloroform; .21 M NaOH in methanol, 2:1, using

10 ml of reagent per 30 mg of F_2. The mixture was stirred at room temperature for one hr after which 1/5 volume of .36 M acetic acid was added. After mixing, the upper aqueous layer, which had a pH 6-7, was removed and discarded. The solvent was removed from the lower layer on the rotary evaporator.

This dried lipid was chromatographed on a second Florisil column, exactly like the first except for the ratio of Florisil to lipid which was 40:1. At least 1 g of Florisil was used for this column. The eluants of this column were dried and weighed. They were labelled F_{2-1} and F_{2-2} respectively.

The F_{2-2} sample was streaked on a preparative TLC plate, 0.6 mm thick. Not more than 10 mg of F_{2-2} was used per plate. A standard cerebroside, obtained from Applied Science Lab., State College, Pa., was also spotted on the plate and used as a marker. The plate was developed with C:M:W, 24:7:1. The bands were visualized by spraying with bromthymol blue. The cerebroside band, that band corresponding to the standard, was scraped from the plate and the cerebrosides eluted from the gel with C:M:W, 7:7:1, 40 ml per gram of scraped silica gel. To each 20 ml eluant, 9.3 ml of chloroform and 6 ml of 0.1 M KCl were added. After mixing, the upper layer was discarded. The lower layer was washed again with C:M:0.1 M KCl, 3:48:47. The upper layer was again discarded and the lower layer dried and weighed. This fraction was labelled T_2.

Acid Methanolysis of Cerebrosides

The isolated cerebrosides were treated with 5% dry HCl in methanol

for 15 hrs at 75°C in a sealed tube. One ml solution was used for 5 mg cerebrosides. The methyl esters were then extracted with four 1 ml portions of hexane. The hexane was removed on a rotary evaporator and the dried esters were streaked on a preparative Silica Gel G TLC plate, and developed in hexane:ether (85:15). The normal and hydroxy fatty acid bands were then visualized with bromthymol blue and scraped from the dried plate within one hour. The esters were eluted from the gel with ether, 10 ml per gram gel.

The 2-hydroxy esters were acetylated with .05 ml of a solution containing 4 mg para-toluene sulfonic acid per ml isopropenyl acetate (Eastman Organic Chemicals, Rochester, N.Y.) for 30 min at 60° in a tightly closed screw cap tube (18).

Gas-Liquid Chromatography of Fatty Acid Esters

Unsubstituted esters were dissolved in an appropriate volume of carbon disulfide and chromatographed. The 2-acetoxy methyl esters were chromatographed directly from their acetylating mixture.

GLC was performed using an F and M model 402R-00, dual column, flame ionization detector, gas chromatograph. The samples were chromatographed on a pair of U-shaped, 6 ft. x 3 mm columns, packed with 6% polydiethylene glycol succinate on 80-100 mesh Diatoport S (F and M Scientific Division of Hewlett Packard, Avondale, Pa.). Some ester samples were also chromatographed on a pair of 4 ft x 3 mm columns, packed with 17% Apiezon L on 60-80 mesh Gas Chrom Z (Applied Science Laboratories, State College, Pa.). The unsubstituted esters were chromatographed between 160° and 220°C with a temperature gradient of

2.0°/min. The 2-acetoxy methyl esters were chromatographed between 200° and 220°C with a 1.0°/min temperature gradient. Standards KD and KF (Applied Science Laboratories) were chromatographed periodically to check the accuracy of the instrument and aid in the identification of the unsubstituted esters. Acetoxy methyl esters were identified using 18h:0, 19h:0 and 22h:0 acetylated methyl esters. Plots of retention time versus carbon number were made for identification purposes.

Quantitation of unsubstituted methyl esters was done using methyl arachidate as an internal standard. Corrections were made for the amount of methyl arachidate present before addition of the internal standard, by a preliminary chromatogram, which used from 1 to 5% of the total sample as measured by volume.

Stated and found composition in methyl ester standards KD and KF, agreed with a relative error of 3% or less. Duplicate chromatograms of samples had a relative deviation of 5% or less for all components comprising 10% or more of the total sample. Fatty acid ester distribution of duplicate preparations of cerebrosides had a relative deviation of 8% or less for components comprising more than 10% of the sample. Reported percentages for fatty acids are mean values.

The quantity of cerebrosides in total lipid was based on quantitation of fatty acid esters. The percentage of fatty acid in the cerebroside molecule was based on an average carbon chain length of 18.52 for the fatty acid, sphingosine and a hexose.

RESULTS AND DISCUSSION

Analysis of Isolation Procedure

As described in the introduction, there were various methods available for the isolation of cerebrosides. The method chosen was based on the composition of the crude lipid from aorta. Aorta is known to contain large amounts of relatively nonpolar lipids, e.g. triglycerides and cholesterol, and generally lesser amounts of the relatively polar lipids, e.g. phospholipids and cerebrosides (14). A method which would easily separate large quantities of nonpolar lipids from small amounts of cerebrosides was therefore desirable.

Triglycerides and cholesterol were readily eluted from Florisil with H:E, free from any detectable cerebrosides. Cerebrosides are known to be eluted from Florisil with C:M (27). TLC showed this fraction to contain some cholesterol and phospholipid. Alkaline methanolysis was employed to hydrolyze the glyceryl phosphatides (28). The products of the methanolysis are methyl esters and water soluble substances, (e.g. glycerol, phosphate and choline). Methyl esters as well as the remaining cholesterol were separated from cerebrosides with the second Florisil column. At this stage of purification the crude cerebroside fraction was about 10 mg.

The final step in the isolation, preparative TLC, separates cerebrosides from similar components carried through the isolation. This step also provides a means of identification for cerebrosides when a standard cerebroside is used on the same plate.

Recovery of Cerebrosides

To determine the efficiency of the isolation, one mg of standard glucosylceramide containing only stearic acid, was added to one gram of IP-F_1. This mixture was treated as crude lipid and the cerebroside was isolated as described before. After isolation, the cerebroside was subjected to acid methanolysis. After methanolysis the fatty acid ester was extracted and methyl arachidate added as an internal standard. The esters were chromatographed on GLC and the quantity of methyl stearate calculated. The recovery was 289 ug or 77.7%. To test recovery from the acid methanolysis alone, a second methanolysis was done on a one mg sample of the glucosylceramide. The recovery by GLC was 371 ug of fatty acid or 99.7%.

These data suggest that there is little loss due to the methanolysis. The 22% loss probably occurs during the Folch partitions after alkaline methanolysis and when removing bromthymol blue after TLC. Some loss could be from numerous transfers and from TLC. There was no detectable loss during column chromatography. The F_1 and F_{2-1} fractions were checked for cerebrosides and additional solvent was eluted through these columns after the usual C:M elution. No additional cerebrosides were obtained.

Identification of Linoleic Acid

Methyl linoleate was identified by its retention time relative to methyl stearate (29). Since this acid has not generally been found in cerebrosides, it was desirable to have further identification. For this purpose, methyl oleate and methyl linoleate were collected from

the exit port, of the GLC column, in a glass-wool filled tube. The flame of the detector was extinguished in the middle of the methyl oleate peak and the tube placed over the exit port for the time needed to elute the methyl linoleate. The esters were washed from the tube with hexane and rechromatographed. The two expected peaks (18:1 and 18:2) were obtained. The remainder of the esters were hydrogenated by bubbling hydrogen gas through a solution of the esters in ethanol, with a platinum oxide catalyst. After hydrogenation only one peak, corresponding to methyl stearate, was obtained on GLC.

Percent Lipid in Tissue

The quantity of lipid in each sample is represented in Table I as percent of wet tissue and percent of total dry weight. Dry weight as used here was total lipid plus the weight of dried tissue residue after lipid extraction. This weight does not include those substances which first dissolved in C:M but were subsequently extracted into the aqueous layer.

The total lipid trends can best be seen by comparing tissue of one aorta. In aorta J, the N tissue was quite low in lipid as compared to S tissue (3.6 vs 19.9%). Aorta J had relatively mild atherosclerosis. In aortas D, G and I, which were all more severely atherosclerotic, the normal tissues were all higher in lipid than JN, but lower in lipid than their corresponding P or L tissue. Aortas H and E were also severely atherosclerotic and only L tissue was obtained from each. Aorta L is very low in percent lipid of wet weight. This was due to the high degree of calcification in this aorta, which gave an unusually high wet weight.

Since the percent water in each tissue could be a variable affecting the percent lipid in each sample, a second calculation was made of percent lipid based on total dry weight. These figures are represented in Table I. The same trends are observed here between N and S, N and P, and N and L tissue as was observed above.

Table I

Concentration of Lipid and Cerebrosides in Human Aorta

Sample	Crude Lipid		Cerebrosides
	Percent of Wet Tissue	Percent of Dry Tissue	Percent of Crude Lipid
DN	40.0%	72.4%	0.14%
GN	7.71	45.5	0.38
IN	26.6	66.1	0.01
JN	3.58	15.6	0.26
JS	19.9	55.8	0.22
LS	4.70	9.7	0.51
FP	52.8	94.0	0.01
IP	57.3	89.4	0.02
CL	36.5	——	0.08
DL	51.0	91.0	0.02
EL	24.0	——	0.03
GL	23.9	75.4	0.48
HL	70.1	——	0.33

Percent Cerebrosides of Crude Lipid

The percentages of cerebrosides shown in Table I, show no definite trend between N tissue and more severely atherosclerotic tissue. Groups S and P show little variation but group N and L vary greatly. It appears that there are other factors that control the quantity of cerebrosides in aorta, besides the severity of atherosclerosis.

Normal Fatty Acid Ester Distribution in Cerebrosides

Table II lists the fatty acid distribution of the cerebrosides and the ratio of stearic to oleic acid in each sample. There are several patterns correlated with the severity of atherosclerosis. The ratio (18:0/18:1) decreases first, from an aorta with mild atherosclerosis to one more severely diseased and second, from N tissue to a diseased area of the aorta. Aorta J was the only aorta with mild atherosclerosis. The (18:0/18:1) ratio in JN was 2.14 while in JS the ratio was 0.29. In more severely atherosclerotic aortas, G and D, (18:0/18:1) from "normal tissue" were 0.20 and 0.39 respectively. This is smaller than JN but larger than the corresponding L tissue from these aortas which were 0.09 and 0.28 respectively. Aorta I showed the same trend, only much less pronounced than aortas J, G and D.

A similar trend can be seen with the percent of linoleic acid, which increases as (18:0/18:1) decreases. Aorta D is an exception. It contains large quantities of 18:2 in its N tissue with about the same quantity in the L tissue. These two trends lead to an increase of total unsaturated fatty acids in more severely atherosclerotic tissue.

Table II Percentage Composition of Normal Fatty Acids in Aorta Cerebrosides

Fatty Acid	Sample												
	DN	GN	IN	JN	JS	LS	FP	IP	CL	DL	EL	GL	HL
12:0-13:1	tr.[A]	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
14:0	3.66	1.78	2.38	1.32	2.34	2.84	1.64	1.04	2.74	2.70	3.82	tr.	2.20
14:1	2.95	tr.	2.11	tr.	tr.	tr.	1.92	tr.	tr.	tr.	tr.	tr.	tr.
15:0	3.54	tr.	1.88	1.08	tr.	1.78	1.58	tr.	1.42	tr.	tr.	tr.	tr.
15:1	2.24	tr.	1.62	tr.	tr.	tr.	2.37	tr.	tr.	tr.	tr.	tr.	tr.
16:0	23.04	22.42	23.48	20.68	20.76	22.91	21.54	12.12	22.04	23.00	22.20	9.85	22.86
16:1	9.27	7.75	6.68	1.33	3.38	4.99	6.72	1.88	3.60	4.85	4.27	5.72	5.16
17:0	tr.	tr.	1.50	tr.	tr.	tr.	1.72	tr.	18.68	tr.	tr.	tr.	tr.
17:1	2.43	tr.	tr.	tr.	tr.	tr.	2.44	tr.	tr.	tr.	tr.	tr.	tr.
18:0	8.66	9.49	15.30	12.55	7.23	8.22	18.98	18.96	11.39	11.10	15.42	5.66	8.77
18:1	21.99	48.54	13.72	5.86	25.01	12.70	23.59	17.75	20.82	39.77	21.08	61.80	44.46
18:2	6.87	8.87			3.75	2.08	2.85	tr.	3.30	6.53	1.43	13.68	10.10
19:0	tr.	tr.	3.42	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.		
19:1	tr.		tr.	tr.	tr.	tr.		tr.					
20:0	1.62	1.10	4.86	1.54	tr.	1.09	2.86	1.08	tr.	tr.	tr.	tr.	tr.

Table II Continued

Fatty Acid	Sample												
	DN	GN	IN	JN	JS	LS	FP	IP	CL	DL	EL	GL	HL
20:1	tr.	tr.	tr.	tr.	tr.	tr.	tr.	3.00	tr.	tr.	2.12	1.86	1.92
21:0	tr.	tr.		tr.	tr.	tr.	1.66	tr.	tr.	tr.		tr.	1.28
21:1	tr.	tr.	tr.	tr.	tr.	tr.	2.35	tr.		tr.	tr.	tr.	tr.
22:0	1.72	tr.	1.04	10.40	6.56	6.70	tr.	1.07	tr.	tr.		tr.	tr.
22:1	tr.	tr.	2.04	tr.	tr.	1.06	2.40	2.55	tr.	tr.	6.18	1.42	2.15
23:0	tr.	tr.	3.82	9.80	4.17	3.87	tr.	13.47	tr.	1.37		tr.	1.10
23:1	tr.	tr.	tr.	1.33	tr.	tr.	5.37	1.55	tr.	tr.	4.00	tr.	tr.
24:0	8.94	tr.	8.78	17.33	10.62	12.13	tr.	5.42	15.98	10.62	12.60	tr.	tr.
24:1	tr.	tr.	2.70	11.60	12.65	17.58		8.42	tr.		2.51	tr.	tr.
25:0	tr.	tr.		tr.	tr.	tr.		3.52	tr.	tr.	tr.	tr.	tr.
25:1	tr.	tr.		1.96	2.13	tr.		2.10	tr.		tr.	tr.	tr.
26:0	3.16		4.56	3.22	1.40	tr.		3.60	tr.	tr.	4.36	tr.	tr.
26:1			tr.		tr.	2.04		2.46	tr.		tr.	tr.	tr.
27:0										tr.			tr.
18:0/18:1	0.39	0.20	1.12	2.14	0.29	0.65	0.84	1.07	0.55	0.28	0.73	0.09	0.20

A Trace (less than 1% of sample)

Although little is known about cerebroside synthesis or transport, several explanations can be postulated for these fatty acid differences. The permeability of the aortic wall might change with atherosclerosis to favor cerebrosides with unsaturated fatty acids, or to favor free fatty acids that are unsaturated. The postulate that the membrane changes in permeability with increasing atherosclerosis, is supported by the increased linoleic acid with severity of the disease. Since there is no known synthesis of linoleic acid in man, it must be absorbed through the aortic wall.

It is also possible that the fatty acid synthesis in the aorta is altered to produce more mono-unsaturated fatty acids. The most likely step for this to take place is the desaturation of stearic to oleic acid. An alteration of fatty acid synthesis has been shown in pigeon (30) and in rabbits (31). In both cases the authors showed fatty acid synthesis to increase in diseased aortas.

A change to more unsaturated fatty acids has been observed by others. Böttcher and Haute (32) found an increase in total linoleate in human arteries, with increasing atherosclerosis. Nelson, Werthessen and Holman (33) reported an increase in total unsaturation of fatty acids, comparing normal and fatty streaks from human aorta.

Of the thirteen tissue samples, eleven show an almost constant percentage of 16:0 while percentages of other major fatty acids vary markedly. The two exceptions are IP, which was the only sample to contain galactose (34), and hydroxy fatty acids, and GL which contained a relatively large quantity of 18:1, thus lowering the percentages of other fatty acids.

Hydroxy Fatty Acids

Hydroxy fatty acids were found only in sample IP. The three found were; 22:0, 79.1%; 23:0, 5.6% and 24:0, 15.3%. The quantity was so small that the whole sample was used in two runs on GLC. An estimate of sample size would be 4 ug, less than 1% of total fatty acids for IP. The apparent anomalous composition of IP cannot be explained.

Comparison with Fatty Acids of Other Cerebrosides

The distribution of normal and hydroxy fatty acids in cerebrosides reported from other tissues differs noticeably from data presented here. Human brain cerebrosides contain 42% hydroxy fatty acids (35) while the normal fatty acid esters are of considerably longer chain length; 24:0 and 24:1 comprise 55% of normal fatty acids while 25:1 and 26:1 comprise another 23% (36).

Cerebrosides of spleen (21) show similarities to aortic cerebrosides, in that no hydroxy fatty acids were present. The main normal fatty acids were 16:0, 30%; 22:0, 13%; 24:0, 15% and 24:1, 15%. It appears from these data that cerebrosides from spleen and brain have at least partially separate origin from aortic cerebrosides. It would be interesting to compare fatty acids from blood cerebrosides to aortic cerebrosides.

It should be noted that these aortas came from elderly patients who probably varied greatly with respect to diet. This may be a determining factor in atherosclerosis and could account for some of the variation observed in aortic cerebrosides.

SUMMARY

An efficient method was used to isolate cerebrosides from lipids of atherosclerotic and normal human aortic tissue. The quantity of these cerebrosides was found not to be a function of increasing severity of atherosclerosis. However, the distribution of fatty acids did show some correlation with the disease. It was found that the ratio 18:0/18:1 decreased from a mildly atherosclerotic aorta to a more diseased aorta. This ratio also decreased from N tissue to S, P or L tissue in an aorta. The quantity of 18:2 and total unsaturation were found to increase from N tissue to diseased tissue in an aorta. At the same time the amount of 16:0 remained relatively constant.

It is suggested that the variations mentioned could come from an alteration of synthetic pathways for fatty acids in the aorta, or from changes in the absorption characteristics of the aortic wall, or a combination of both.

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VITA

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ADDENDUM

Description of Patients from which Aortas were Obtained

Aorta	Age	Sex	Cause of Death
C	60	M	Undifferentiated bronchogenic carcinoma
D	50	M	Malignant hypertension
E	70	F	Transmural myocardial infarct
F	34	M	Acute lymphocytic leukemia
G	88	F	Mitral stenosis and insufficiency
I	76	M	Bronchogenic carcinoma and bronchopneumonia
J[A]	73	M	Coronary thrombosis
L	74	M	Bilateral branched pneumonia

[A] J was Negroid, all the others were Caucasian