Lipid Class and Fatty Acid Composition of Rat Brain and Sciatic Nerve in Alloxan Diabetes

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SUMMARY

Quantitative analysis of major lipid classes and the fatty acids carried out on lipid extracts of brain from young and adult alloxan diabetic rats revealed decreases in total lipid, cholesterol, the more polar cerebroside, and phosphatidylethanolamine in brain from young diabetic rats. No significant changes in lipid class composition were found in brains from adult rats with alloxan diabetes. No significant changes in fatty acids were detected in the total brain lipids of young or adult rats as a result of alloxan diabetes. In contrast, similar studies on the lipids of sciatic nerve revealed an increase in cholesterol balanced by a decrease of triglycerides in young rats and a decrease of total lipid and the more polar cerebroside only in adult rats with alloxan diabetes. Compared to normal rats, total lipids from sciatic nerves of young alloxan diabetic rats showed relative decreases in palmitate (16:0), oleate (18:1), and linoleate (18:2) with increases in stearate (18:0), eicosanoate (20:0), eicosenoate (20:1), arachidonate (20:4), docosanoate (22:0), docosapentaenoate (22:5), docosahexaenoate (22:6), lignocerate (24:0). In lipid extracts from sciatic nerves of adult diabetic rats, only oleate (18:1) was increased and linoleate (18:2) decreased compared to normal. DIABETES *18*:556-61, August, 1969.

Diabetic neuropathy, which frequently accompanies diabetes mellitus, is associated with decreases in nerve conduction velocity in human diabetes and in alloxan treated rats.[1] Since the system composed of neuron, myelin sheath, and glial elements represents an active site of metabolism,[2] it should not be surprising to find changes in the lipid class or fatty acid composition of nervous tissue as is the case with other tissues associated with the diabetic state. Early studies by Jordan, Randall, and Bloor[3] on human nerves from autopsied cases of diabetes showed decreases in a number of lipids although the extraction solvents and methods used were not such as to guarantee quantitative extraction or determination of all lipids from nervous tissue. In vitro studies on the distribution of labeled precursors in nerves from alloxan diabetic rats indicated decreases in the incorporation of U-C-14-glucose or 1-C-14-acetate into total lipids,[4] decreased 1-C-14-acetate into all lipid classes except phospholipids[5] and specifically into cerebrosides.[1] No quantitative data on the recovery of lipids or concentration of component lipid classes were given and the lack of specific radioactivity data on a lipid class weight basis renders interpretation difficult. In only one of these studies[1] were young animals used, while adult animals were used for the other studies.[4,5] No corresponding investigations of brain lipids have been carried out other than on the effects of insulin on acetate or glucose incorporation in normal animals.[6]

The present study was developed to examine quantitatively the in vivo effect of alloxan diabetes on the lipid class and fatty acid composition of lipids extracted from brain and sciatic nerve of young and adult rats.

METHODS

Three-week and seven-month-old Sprague-Dawley rats from Holtzman Company were fasted for twenty-four hours and then injected intravenously through a tail vein with alloxan. Alloxan, 55 mg./kg., was administered to the adult animals, and 70 mg./kg. to the young animals. Nonfasting blood sugars of 180 mg./100 ml. or more on two different occasions were used to indicate attainment of diabetes. After one month, rats were sacrificed by decapitation and brains and sciatic nerves were quickly excised and frozen. At the time of sacrifice, alloxan treated young rats had a range of blood sugars of 380-816 mg./100 ml. compared to 68-144 mg./100 ml. for controls. Alloxan treated adult rats had

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a range of blood sugars of 336-948 mg./100 ml. compared to 58-104 mg./100 ml. for controls. Tissues were homogenized and extracted as previously described.[7]

Phospholipids were determined by two-dimensional thin-layer chromatography (TLC) followed by phosphorus analysis according to Rouser et al.[9] Cerebrosides were analyzed by densitometry of one-dimensional TLC plates by the method of Rouser et al.[10] Cholesterol was quantitated by the Abell-Kendall method,[11] and triglycerides were determined by the method of Carlson and Wadstrom,[12] as modified by Katsuki et al.[13]

Methyl esters of fatty acids were prepared by methanolysis for two hours with boron trifluoride in anhydrous methanol as described by Morrison and Smith.[8] Completeness of methanolysis and the lack of side reactions were confirmed by TLC. Losses of polyunsaturated fatty acids from standard mixtures were within the limits of error of column, detector, and integrator performance. Gas-liquid chromatographic analyses of methyl esters of fatty acids were carried out on polar and nonpolar columns and with the standardization procedures previously described.[14] Peak areas were determined by electronic integration.

RESULTS

Table I indicates that the total brain weights were reduced in alloxan diabetic animals of either age group. The crude lipid weight of brain was decreased only in young animals with alloxan diabetes. Since these crude lipid extracts were not purified on Sephadex, they contain gangliosides, sulfatides, free fatty acids, minor components, and water-soluble nonlipids which would not contribute to the totals determined in tables 2 and 3. In contrast, there was no change from normal in nerve weight and weight or percentage of crude lipid in young alloxan treated rats. There was a significant increase in nerve weight and a decrease in percentage of crude lipid in alloxan treated adult rats.

TABLE 1

Mean weight, total crude lipid, and percentage crude lipid of rat brain and nerve (± standard deviation)

	Young			Adult		
	Normal	Diabetic	P	Normal	Diabetic	P
Brain wet weight, mg.	1,396±161	1,028±175	<0.005	1,608±212	1,368±216	<0.015
Total crude lipid, mg.	159± 16	137± 11	<0.007	193± 20	189± 15	N.S.
Per cent crude lipid	11.5±2.0	13.7±3.0	N.S.	12.1±1.5	14.1± 2.2	<0.025
Number of animals	5	8		7	13	
Nerve wet weight, mg.	34.4±4.2	34.5±5.5	N.S.	53.8±6.3	102.4±17.4	<0.01
Total crude lipid, mg.	9.8±0.8	10.2±1.0	N.S.	19.8±2.0	27.2± 2.2	<0.05
Per cent crude lipid	28.6±4.9	29.8±6.5	N.S.	36.7±4.5	20.9± 3.3	<0.02
Number of pooled groups	2	3		2	4	

TABLE 2

Lipid class composition of young rat brain (± standard deviation)

	Normal (five)			Diabetic (eight)				
	mg.	mg./100 mg. total lipid	Per cent P of total lipid P	mg.	P	mg./100 mg. total lipid	P	Per cent P of total lipid P
Cholesterol	33.6±4.20	30.5±2.60	—	25.0±3.19	<0.01	28.0±2.27	N.S.	—
Cerebroside								
(less polar)	3.1±0.65	2.8±0.50	—	2.4±0.51	N.S.	2.7±0.72	N.S.	—
(more polar)	8.3±0.85	7.5±0.51	—	6.5±0.90	<0.001	7.3±0.91	N.S.	—
PE	25.8±1.91	23.6±0.91	39.8±0.80	21.5±2.49	<0.01	23.5±1.01	N.S.	39.2±2.74
PC	25.6±2.11	23.3±1.36	39.5±2.89	22.8±2.02	N.S.	25.3±0.88	<0.001	41.5±1.95
Sphingomyelin	3.2±0.93	2.9±0.84	4.9±1.3	3.4±1.1	N.S.	3.6±1.2	<0.01	5.6±2.1
PS + PI	10.4±1.40	9.4±1.0	16.0±1.15	7.6±2.6	<0.05	9.5±0.99	N.S.	13.8±4.22
Total	109.9±8.30			89.1±8.32	<0.01			

Numbers in parentheses indicate the number of animals in each group.
Abbreviations used: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol. Less polar cerebroside migrates closer to the solvent front in both solvent systems and contains non-hydroxy- and more saturated fatty acids. The more polar cerebroside contains hydroxy- and more unsaturated fatty acids.

TABLE 3

Lipid class composition of adult rat brain
(± standard deviation)

	Normal (seven)			Diabetic (thirteen)				
	mg.	mg./100 mg. total lipid	Per cent P of total lipid P	mg.	P	mg./100 mg. total lipid	P	Per cent P of total lipid P
Cholesterol	41.1± 8.50	33.1±3.35	—	38.5± 7.43	N.S.	33.5±4.03	N.S.	—
Cerebroside								
(less polar)	2.7± 0.70	2.6±0.82	—	4.0± 1.3	N.S.	3.4±1.06	N.S.	—
(more polar)	9.3± 2.8	7.6±2.2	—	9.5± 1.6	N.S.	8.2±0.78	N.S.	—
PE	28.4± 4.79	22.8±0.93	41.4±2.15	25.8± 2.81	N.S.	22.1±1.40	N.S.	39.5±2.88
PC	26.7± 4.30	21.7±1.67	39.0±2.35	24.6± 3.77	N.S.	21.2±2.59	N.S.	39.1±2.29
Sphingomyelin	3.4± 0.93	2.9±0.88	4.7±1.7	3.5± 1.9	N.S.	2.9±1.4	N.S.	5.4±3.4
PS + PI	11.8± 3.90	9.6±3.2	14.8±2.36	10.4± 2.26	N.S.	8.8±1.3	N.S.	16.3±2.77
Total	123.6±17.57			119.5±14.15	N.S.			

Numbers in parentheses indicate the number of animals in each group.
Abbreviations used: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol. Less polar cerebroside migrates closer to the solvent front in both solvent systems and contains non-hydroxy- and more saturated fatty acids. The more polar cerebroside contains hydroxy- and more unsaturated fatty acids.

Table 2 shows that there was a significant decrease in the total brain lipid of young alloxan diabetic rats. This is due to decreases in cholesterol, the more polar cerebroside, and phosphatidylethanolamine (PE). The relative concentrations of these lipid classes are unchanged when results are expressed on the basis of the same amount of total lipid and slight, but significant, increases of phosphatidylcholine (PC) and sphingomyelin are noted.

In contrast, the results of table 3 indicate that the lipid class composition of adult rat brain is more refractile to the effects of alloxan diabetes than is the case with young rat brain since no significant changes were noted in any of the lipid classes studied or in the total lipid as a result of alloxan treatment.

TABLE 4

Fatty acid composition of lipid extracts of rat brain
(± standard deviation)
(Values expressed as per cent of total fatty acid methyl esters)

Fatty acids	Young		Adult	
	Normal	Diabetic	Normal	Diabetic
14:0	2.4±1.1	1.7±0.8	2.2±0.7	1.9±0.6
16:0	32.6±4.5	32.7±2.9	29.6±5.4	30.6±5.4
18:0	15.2±2.5	16.1±4.4	13.0±1.1	14.1±1.9
18:1	25.2±3.9	26.2±3.5	27.4±1.6	27.2±2.3
20:4	8.1±2.2	7.8±2.9	7.8±1.5	7.9±1.8
22:5	1.3±0.3	0.8±0.4	1.9±0.7	0.9±0.3
22:6	11.8±2.6	11.5±4.4	13.3±4.2	12.9±3.0

Only fatty acids (as methyl esters) are included which exceed 1 per cent of the total or which undergo significant changes.
Number before colon indicates the number of carbon atoms. Number after colon indicates the number of double bonds.
The numbers of animals were as indicated in table 1.

As indicated in table 4, the fatty acid composition of rat brain lipid extracts was unchanged by alloxan diabetes in either the young or adult animal.

Since nerves had to be pooled in each group in order to obtain adequate amounts of lipid for analyses, results in table 5 were not expressed as mg. of lipid or mg. of lipid per nerve since this would assume nerves to be of identical size or that size differences would be reflected in weight differences. In the young animal, the amount of lipid extracted from the normal nerve (1.72 mg./nerve) was the same as that extracted from the diabetic nerve (1.71 mg./nerve) so that differences in the relative distribution of lipid classes are representative. The decrease of 62 per cent in triglycerides in diabetic nerve is balanced by the 76 per cent increase in cholesterol in sciatic nerve lipid extracts from the alloxan treated rats. No other significant changes were found. In all cases, the distribution of phospholipid classes as percentage of total lipid phosphorus is in good agreement with that obtained by Evans and Finean[15] for peripheral nerve myelin.

Table 6 shows that the only significant change from normal in relative lipid class composition of nerve lipid extracts from alloxan treated adult rats was a 31 per cent decrease in the more polar cerebroside. The amount of lipid extracted in the alloxan diabetic adult animals (2.42 mg. per nerve) was 35 per cent less ($p < 0.01$) than that extracted from normal adult nerves (3.71 mg. per nerve).

Table 7 shows that in lipid extracts of sciatic nerves from young alloxan diabetic rats, palmitate (16:0), oleate (18:1), and linoleate (18:2) are decreased com-

TABLE 5

Lipid class composition of young rat sciatic nerve
(± standard deviation)

	Normal (two)		Diabetic (three)		
	mg./100 mg. total lipid	Per cent P of total lipid P	mg./100 mg. total lipid	P	Per cent P of total lipid P
Triglyceride	18.0±2.2	—	6.9±0.9	<0.01	—
Cholesterol	18.6±1.5	—	32.8±2.7	<0.01	—
Cerebroside					
(less polar)	7.0±1.5	—	5.7±1.2	N.S.	—
(more polar)	11.6±1.1	—	10.1±1.0	N.S.	—
PE	15.7±0.7	35.0	15.0±0.6	N.S.	34.2
PC	12.2±0.5	27.2	12.6±0.6	N.S.	28.7
Sphingomyelin	8.7±2.7	19.4	8.1±2.6	N.S.	18.4
PS + PI	8.1±0.8	18.1	8.1±0.8	N.S.	18.4

Numbers in parentheses indicate numbers of groups into which nerves were pooled.
Abbreviations used: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol. Less polar cerebroside migrates closer to the solvent front in both solvent systems and contains non-hydroxy- and more saturated fatty acids. The more polar cerebroside contains hydroxy- and more unsaturated fatty acids.

pared to normal. Stearate (18:0), eicosanoate (20:0), eicosenoate (20:1), arachidonate (20:4), docosanoate (22:0), docosapentaenoate (22:5), docosaphexaenoate (22:6), lignocerate (24:0) were all increased. In lipid extracts of adult nerve from alloxan diabetic rats, only oleate (18:1) was increased and linoleate (18:2) decreased.

DISCUSSION

Nervous tissue lipids play an important role in maintaining structural integrity which facilitates proper neuronal function. Since neuronal function is impaired in diabetes, quantitative determination of the lipid class and fatty acid composition of nervous tissue in experimental diabetes may provide insight into the pathogenesis of diabetic neuropathy.

In the present study, although brain weights and the crude lipid weights were reduced from normal in alloxan diabetic rats, the proportion of lipid remained unaltered. Since this occurred in both young and adult animals, the effect is probably not nutritionally related to myelination as in the studies of Dobbing.[16]

The loss of total lipid was accompanied by losses of cholesterol, the more polar cerebroside, and PE from brain lipids of the young diabetic rat and is similar to the results obtained in diabetic human sciatic nerve by Jordan et al.[3] These changes were not found in the relative distribution of lipid classes. The relative increases of PC and sphingomyelin may reflect the opposite of the effects of insulin which depressed P-32-orthophosphate incorporation in PC[17] and into its precursor, phosphorylcholine, in young rat brain.[18] These

TABLE 6

Lipid class composition of adult rat sciatic nerve
(± standard deviation)

	Normal (two)		Diabetic (four)		
	mg./100 mg. total lipid	Per cent P of total lipid P	mg./100 mg. total lipid	P	Per cent P of total lipid P
Triglyceride	17.3±1.8	—	15.4±1.6	N.S.	—
Cholesterol	23.6±2.6	—	25.7±2.8	N.S.	—
Cerebroside					
(less polar)	6.9±2.1	—	4.8±1.5	N.S.	—
(more polar)	12.5±1.4	—	8.6±1.0	<0.05	—
PE	14.9±0.8	37.5	15.7±0.8	N.S.	34.4
PC	10.2±1.0	25.7	12.7±1.3	N.S.	27.8
Sphingomyelin	6.7±2.6	16.9	8.6±3.3	N.S.	18.9
PS + PI	7.9±1.9	19.9	8.6±2.0	N.S.	18.9

Numbers in parentheses indicate numbers of groups into which nerves were pooled.
Abbreviations used: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol. Less polar cerebroside migrates closer to the solvent front in both solvent systems and contains non-hydroxy- and more saturated fatty acids. The more polar cerebroside contains hydroxy- and more unsaturated fatty acids.

TABLE 7

Fatty acid composition of lipid extracts of rat sciatic nerves (± standard deviation)
(Values expressed as per cent of total fatty acid methyl esters)

	Young		Adult	
Fatty acids	Normal (5)	Diabetic (8)	Normal (7)	Diabetic (13)
14:0	2.3±0.3	2.2±0.7	2.5±1.0	1.3±0.9
16:0	37.0±0.1	29.1±0.5	31.3±0.8	32.7±0.2
18:0	6.5±0.4	8.9±0.1	8.3±0.3	8.2±0.3
18:1	41.5±0.6	35.9±0.3	42.7±1.8	48.8±1.9
18:2	10.1±0.4	0.9±0.1	9.8±1.0	4.5±0.3
20:0	0.1	1.9±0.1	0.1	0.1
20:1	0.1	1.0	0.2	0.2±0.1
20:4	1.3±0.2	5.9±1.4	2.0±0.4	1.9±0.4
22:0	0.2±0.1	2.8	0.6±0.1	0.4±0.2
22:5	0.3±0.1	3.9±0.5	0.7±0.2	0.5±0.2
22:6	0.6±0.2	2.7±0.1	0.4	0.3±0.2
24:0	0.2±0.1	1.7±0.1	0.4±0.2	0.4±0.2

Only fatty acids (as methyl esters) are included which exceed 1 per cent of the total or which undergo significant changes.

Numbers in parentheses indicate numbers of animals in each group.

Number before colon represents the number of carbon atoms in the fatty acid chain. Number after colon represents the number of double bonds.

observations may be related to turnover and may not result in net changes of lipid composition.

The lipid class composition of adult rat brain was completely resistant to alloxan treatment. Similarly, the fatty acid composition of the lipids of both young and adult rat brain was unaffected by alloxan treatment.

The losses of triglyceride from the sciatic nerve lipids of young diabetic rats is in keeping with the corresponding triglyceride loss from other tissues in alloxan diabetes.[19] The increase of cholesterol is in contrast to the findings with brain and in contrast to the decreased incorporation of 1-C-14-acetate into cholesterol.[1,5] In view of the reported decrease of acetic thiokinase activity in alloxan diabetic nerve,[20] a decrease in labeled acetate incorporation into lipids might be expected. Since the previous studies with labeled acetate do not present specific radioactivity data, it is difficult to interpret whether the net concentration of cholesterol is affected. The same results would have been obtained in alloxan diabetic nerve if cholesterol formation from preformed precursors were stimulated and the pool size became large without going through total synthesis from acetate.

In the sciatic nerve lipid extracts from alloxan diabetic adult rats, only the more polar cerebroside was significantly decreased. This is in agreement with the observations of Jordan et al.[3] On the basis of relative rates of 1-C-14-acetate incorporation,[1] it would be expected in sciatic nerve lipids that in alloxan diabetes the more polar cerebroside containing unsaturated and hydroxy-fatty acids would increase and the less polar cerebroside containing saturated fatty acids would decrease.

Benjamin and Gellhorn[21] reported a depression in the conversion of labeled stearic acid to oleic acid in the synthesis of triglycerides by adipose tissue of alloxan diabetic rats. Similarly, Mercuri, Peluffo, and Brenner[22] noted in alloxan diabetic rats a defect in the ability of rat liver microsomes to desaturate stearic acid to oleic acid and linoleic acid to δ-linolenic acid. Observations made from the present study in sciatic nerve lipids of young diabetic rats are consistent with these reports with increases of stearate and decreases of oleate and linoleate. Since decreases of palmitate and increases of eicosenoate, docosanoate, lignocerate, and all of the polyunsaturated fatty acids were also found, it appears more likely that the fatty acids which decrease relatively are associated with the triglycerides which decrease, while those which relatively appear to increase are probably associated with phospholipids which do not change in concentration. A similar explanation may apply to the relative increase of oleate in the sciatic nerve lipids of adult diabetic rats and the relative decrease of linoleate. If linoleate were present in cerebrosides of adult sciatic nerve, it would be associated with the more polar cerebroside, which decreases in alloxan diabetes.

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Manganese and Glucose Tolerance

...In South Africa, extracts of lucerne (alfalfa or *Medicago sativa*) are sometimes used as folk remedies to treat diabetes. A. H. Rubinstein, N. W. Levin, and G. A. Elliott (Nature 194:188, 1962; Lancet 2:1348, 1962) studied a young man with juvenile onset diabetes who claimed that his diabetes was better controlled by extracts of lucerne than by large doses of insulin. Observations were made on twelve occasions following oral administration of an extract prepared by boiling the green leaves of alfalfa in water, and a dramatic hypoglycemic response consistently occurred.

Since lucerne contains a high concentration of manganese (45.5 mg. per kilogram), the effects of oral administration of 5 to 10 mg. manganese chloride were studied in this patient. On each of fourteen occasions, a hypoglycemic effect, maximal at two to four hours, was noted, with hypoglycemic coma on three occasions when blood glucose levels fell to between 10 and 20 mg. per 100 ml. Satisfactory control of the diabetes could not be maintained by the oral administration of manganese salts. Since hypoglycemia occurred spontaneously and an islet cell tumor of the pancreas was suspected, partial pancreatectomy was performed, but no such tumor was found. After pancreatectomy, manganese no longer had a hypoglycemic effect.

Because of the profound hypoglycemic effect of manganese in this single subject, Rubinstein et al. performed preliminary studies in normal control subjects and in diabetics of both juvenile and obese adult onset type, and in one patient with diabetes secondary to chronic calcific pancreatitis. Even with large doses of manganese, a hypoglycemic effect was never observed.

Trace elements other than manganese may be involved in maintaining normal glucose metabolism (Nutrition Reviews 26:223, 1968). Reversible impairment of glucose tolerance, similar to that found by Everson and Shrader in the manganese deficient guinea pigs, occurs in chromium deficient rats (Ibid. 25:49, 1967;

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