Cerebrospinal Fluid Lipids in Diabetes Mellitus

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SUMMARY

Abnormalities in cerebrospinal fluid (CSF) lipids have been found in certain diseases and have correlated with direct biochemical measurements of lipids in nervous system tissue. In the present study, total and free cholesterol, total lipid, and total phospholipid were determined by microanalytic technics in the serum and CSF of thirty subjects: eight normal controls, seven diabetic subjects with polyneuropathy, eight diabetic subjects without neuropathy, and seven patients with other neurological diseases. An elevation of free cholesterol ($p < 0.02$) was found in the CSF of all diabetics regardless of neurological involvement and independent of any simultaneous abnormality of serum. Phospholipid was probably elevated. The abnormality of CSF free cholesterol could be related to neurological dysfunction in the diabetic patient and to ganglion cell abnormality. DIABETES *15*:471-74, July, 1966.

The neuropathy associated with diabetes mellitus remains the least understood and the most inadequately studied of the degenerative complications of this disease. Despite the early clinical description of this disease, there remains a lack of information regarding the specific etiology for the degenerative changes in the nervous system associated with diabetes mellitus. In general, three main theories have prevailed as to etiology. 1. Vascular insufficiency, which may be due either to large vessel atherosclerosis,[1,2] or to specific diabetic angiopathy.[3-5] Recently, Pirart in an extensive clinical review of the subject has found little to support this theory.[6] 2. Nutritional vitamin deficiency has been considered but Rundles,[7] Jordan[1] and Goodman,[8] in their reviews of the subject have examined the evidence for this and found it lacking. 3. A defect in metabolism associated with diabetes mellitus itself[7,8] has been proposed by numerous investigators, yet little in the way of specific evidence has been presented to support this.

The anatomical studies of Dolman[9] and the recent review by Locke,[10] have served to emphasize the different and varying disorders of the neuromuscular system in diabetes mellitus.

The works of Tourtellotte et al.[11,12] have defined the normal spinal fluid lipid profile, the abnormalities which are noted in various neurological diseases, and their possible relation to disordered lipid metabolism in the nervous system. The purpose of this study is to examine the cerebrospinal fluid lipid profile in diabetic neuropathy with particular emphasis upon that form of neurological involvement which is most common, i.e., the distal symmetrical polyneuropathy.

MATERIALS AND METHODS

Clinical material. All patients were selected from the inpatient medicine and neurological services of the University of Texas Medical Branch Hospitals. Thirty patients were examined. Eight of these with no evidence of neurological disease, diabetes mellitus or family history of diabetes, served as normal controls. Seven diabetics with neuropathy and eight diabetics without neuropathy were studied. Seven patients with various other neurological diseases were also examined (herniated nucleus pulposus, Levy Russey syndrome, polyneuropathy due to arsenic, cerebral thrombosis due to polycythemia vera, early presenile dementia, asymptomatic CNS lues, cerebral arteriosclerosis). All patients received a complete history and neurological examination. A patient was judged to have neurological involvement only if he had symptoms, plus objective neurological findings in addition to the absence of deep tendon reflexes. All patients in the neuropathy group had distal symmetrical polyneuropathy. Patients with amyotrophy, mononeuropathy, radiculopathy or autonomic neuropathy were not included in this study. One patient did have symptoms of autonomic bowel and bladder involvement in addition to a polyneuropathy, however. No patient in the control or diabetic groups had positive serum or spinal fluid serological tests for syphilis.

Studies performed on spinal fluid

All patients received a lumbar puncture, performed in

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the fasting state, at which time 15 ml. of cerebrospinal fluid (CSF) were removed. Cell counts, determinations of protein, chloride and glucose and serological tests for syphilis were carried out in the hospital clinical laboratories. Spinal fluid protein was determined by the turbidimetric method of Looney and Walsh.[13] The remainder of the spinal fluid was used to determine total lipid, total phospholipid, free cholesterol and total cholesterol, according to the microtechnics of Tourtellotte et al.[14-16] All samples were run in either duplicate or triplicate and an average value obtained.

Studies performed on serum

All serum samples were drawn in the fasting state at the same time that spinal fluid samples were obtained. Total serum protein, fasting blood sugar, blood urea nitrogen and serological tests for syphilis were determined by the hospital clinical laboratories. Total lipid, total phospholipid, free cholesterol and total cholesterol were determined by the microtechnics which were used for spinal fluid after appropriate dilution of the serum. All samples were run in either duplicate or triplicate and an average value obtained.

RESULTS

Table 1 reviews the CSF and serum lipid data in the various groups studied. The control values are in agreement with those of Tourtellotte et al.[12] The findings reveal a significant elevation of CSF free cholesterol in all diabetics. Possibly there is also a significant elevation of total phospholipid in spinal fluid of diabetics with neuropathy. There was more variability in cerebrospinal fluid lipids in the various patient groups than there was in our control group. It seems likely that multiple factors, possibly unrelated to the diabetes, may be responsible for some of the variations. Consequently, in the lipid analysis where large differences in average values were noted in a comparison of controls with other groups, our study simply indicates that differences were not significant for the number of individuals which we studied and for the particular means of classification which we utilized. It is certainly possible that some of these differences might prove significant as more precise means, either clinically or biochemically, become available for the evaluation and classification of patients with diabetic neuropathies.

There is no statistical difference encountered when comparing the spinal fluid lipids of diabetics with and without neuropathy. Similarly a comparison of control values with the groups of other neurological diseases reveals no significant differences.

A comparison of spinal fluid and total serum protein values between the control group and diabetic neuropathy group (table 2) reveals that the neuropathy group had a significant elevation of spinal fluid protein and a significant depression of total serum protein. These same differences were also noted when diabetics without neuropathy were compared with those with neurological involvement.

The clinical characteristics of the two groups of diabetic patients are listed in table 3. The average age of the patients in each group was fifty years. In the neuropathy group, diagnosis of diabetes was made at an earlier

TABLE 1

Results of lipid analyses in the various patient groups

Subjects	Total lipid mg. per 100 ml. Serum	Total lipid mg. per 100 ml. CSF	Total phospholipid† Serum	Total phospholipid† CSF	Total cholesterol mg. per 100 ml. Serum	Total cholesterol mg. per 100 ml. CSF	Free cholesterol mg. per 100 ml. Serum	Free cholesterol mg. per 100 ml. CSF	Per cent free cholesterol Serum	Per cent free cholesterol CSF
Controls	565 (±52)	1.105 (±0.109)	227 (±36)	0.433 (±0.052)	159 (±20)	0.295 (±0.036)	49 (±5)	0.110 (±0.007)	30	37
Neurological diseases	558 (±87)	2.929 (±1.160)	163 (±25)	0.502 (±0.074)	138 (±24)	0.359 (±0.083)	50 (±6)	0.121 (±0.013)	36	33
Diabetic patients with neuropathy	772 (±103)	3.148 (±1.304)	210 (±47)	0.732 (±0.129) p=0.05	173 (±18)	0.608 (±0.165)	60 (±9)	0.189 (±0.028) p<0.02	34	31
Diabetic patients without neuropathy	644 (±61)	1.370 (±0.061)	181 (±19)	0.558 (±0.076)	177 (±16)	0.358 (±0.031)	57 (±4)	0.156 (±0.012) p<0.01	32	43
All diabetic patients	712 (±62)	2.318 (±0.714)	197 (±30)	0.651 (±0.078)	175 (±12)	0.512 (±0.105)	59 (±5)	0.173 (±0.016) p<0.02	33	33

*Listed values are average ± the standard deviation. Probability values (Student *t* test) in comparing controls with other patient groups are listed in the appropriate column, where significant.

†Total phospholipid expressed as μM./100 ml.

TABLE 2

Comparison of serum and cerebrospinal fluid protein concentrations in control and diabetic patients

	Control (Average)	Diabetic with neuropathy (Average)	Diabetic without neuropathy (Average)
Spinal fluid protein (mg. per cent)	32	75($p<0.01$)	41 (N.S.)
Total serum protein (gm. per cent)	7.5	5.8($p<0.01$)	6.8 (N.S.)

N.S.: Not significant.

TABLE 3

Clinical characteristics of diabetic patients

Age (years)	Treatment (U. insulin)	Known duration of diabetes (years)
	Patients with neuropathy	
58	60-80	20
67	50	17
41	120-140	7
16	125	6
54	30	14
67	Diet only	Newly discovered
71	40	13
26	60	23
50 avg.		12.5 avg.
	Patients without neuropathy	
51	Tolbutamide	7
51	15	6
32	Diet only	1
40	60	14
55	25	3
63	Tolbutamide	Newly discovered
60	None	Newly discovered
50 avg.		4 avg.

average age, and the duration of known diabetes was longer than that of the group without neuropathy. Similarly, the neuropathy group tended to be more "severe" in terms of daily insulin dosage and ease of control of carbohydrate metabolism. In general there were no marked differences between the groups in the degree of gross control of carbohydrate metabolism at the time the spinal fluid samples were obtained, although there was considerable variation within each group.

DISCUSSION

In diabetic polyneuropathy, the sensory loss and associated motor weakness do not relate to a single nerve root or peripheral nerve, but rather to the distribution of the overlapping peripheral terminals of many segmental nerves. Demyelination is found primarily in the peripheral nerve and less often in the nerve root and posterior columns.[9] The axon is usually intact, and supravital staining with methylene blue has revealed degenerative changes of the end-plate.[17]

It has been postulated that neuronal cells elaborate axoplasm which is propagated along the axon to the distal terminals.[18] It is conceivable that a primary metabolic abnormality of the dorsal root ganglion cell could result in impairment of function of the peripheral nerve and terminals, thus producing the typical polyneuropathy with predominately sensory and reflex abnormalities. It is also conceivable that this abnormality of metabolism of the dorsal root ganglion cell may be reflected in abnormalities of the spinal fluid lipids.

The spinal ganglia lie either partly or completely within the spinal canal, except C_1 and the coccygeal nerves which usually do not have spinal ganglia. The pia, arachnoid and dural membranes continue to cover the nerve roots until the spinal ganglia are passed, at which location they fuse to form the connective tissue sheaths of the peripheral nerves. The nerves perforate the dura mater as they pass through the intervertebral foramen. Therefore, the dorsal root ganglia have the same anatomical relations to the sub-arachnoid space as the spinal cord and brain.[19]

The elevation of free cholesterol, noted in spinal fluid of all diabetics regardless of neurological involvement, occurred independently of any lipid abnormality in serum. Further study will be required to establish the significance of the elevation of total phospholipids in the polyneuropathy group of diabetics. The significance of this elevation of free cholesterol must await further information regarding the origin and formation of spinal fluid lipids and the importance of lipid metabolism to the functional integrity of the spinal cord and peripheral nerves. The fact that diabetic patients with and without polyneuropathy showed a similar abnormality does not exclude the possibility that the abnormality is in some way related to the neuropathy. The gross clinical methods for evaluating neuropathy certainly do not exclude minimal degrees of involvement. Striking pathological changes have been demonstrated in peripheral nerve in the presence of minimal or absent neurological signs and symptoms.[9] An abnormality in nervous system lipid metabolism may produce functional and degenerative changes only as a function of time. The fact that the neuropathy patients may have contracted their disease earlier and had their disease longer would favor this view. Pirart[6] on the basis of a large clinical experience has reached similar conclusions.

Eliasson and Hughes[20] observed the incorporation of acetate-1-C-14 into fatty acids and cholesterol of spinal cord and nerves of normal rats and rats made diabetic by alloxan. They found a decrease in cholesterol synthesis in diabetic cord and nerve, while fatty acid synthesis

was depressed in spinal cord, but elevated in peripheral nerve of the diabetic rats. The addition of pyridine nucleotides had no effect on the depressed cholesterol and fatty acid synthesis in diabetic animals. These authors have also studied homogenates of cat sciatic nerve.[21] A fraction containing axons and myelin showed the most active fatty acid synthesis. A sheath fraction containing primarily fibrous tissues and Schwann cell nuclei was found to utilize acetate for cholesterol synthesis almost exclusively. Thomas and Lascelles have presented pathological evidence for an abnormality in Schwann cell function and metabolism in diabetic neuropathy.[22] Insulin has been found to stimulate lipogenesis in peripheral nerve in vitro.[23]

The fact that no significant abnormalities in CSF lipids were demonstrated in the group of miscellaneous neurological diseases suggests that the changes observed in the diabetic subjects are not merely secondary to degenerative changes within the nervous system. This conclusion could be supported by the fact that several distinct abnormalities in CSF lipids have been found in different neurological diseases.[12]

The elevation of cerebrospinal fluid protein content in patients with neuropathy has been well recognized. Its significance and its possible relation to the neurological lesions remain to be clarified, however.[24,25] The depression of total serum protein in the neuropathy group of diabetic subjects was an unexpected finding. Its significance will require more extensive and detailed study. This finding cannot be explained on the basis of renal disease in the present patients.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Dr. John Overall of the Research Computation Center of the University of Texas Medical Branch for help in preparing the statistical analysis of the data. We are grateful to Mrs. Violet Vassberg Geiz for technical assistance.

REFERENCES

[1] Jordan, W. R.: Neuritic manifestations in diabetes mellitus. Arch. Int. Med. *57*:307, 1936.

[2] Woltman, H. W., and Wilder, R. M.: Diabetes mellitus, pathologic changes in the spinal cord and peripheral nerves. Arch. Int. Med. *44*:576-603, 1929.

[3] Fagerberg, S. E.: Studies on the pathogenesis of diabetic neuropathy. Part I. Review of the literature and own working hypothesis. Acta. Med. Scandinav. *154*:145, 1956.

[4] Fagerberg, S. E.: Studies on the pathogenesis of diabetic neuropathy. Part II. Relation between clinically demonstrable neuropathy and pathoanatomic investigation of nerve. Acta. Med. Scandinav. *156*:295-302, 1956.

[5] Fagerberg, S. E.: Studies on the pathogenesis of diabetic neuropathy. Part IV. Angiopathia diabetica vasae nervorum. Acta. Med. Scandinav. *159*:59-62, 1957.

[6] Pirart, J.: Diabetic neuropathy. A metabolic or a vascular disease? Diabetes *14*:1-9, 1965.

[7] Rundles, R. W.: Diabetic neuropathy: General review with report of 125 cases. Medicine, Baltimore, *24*:111, 1945.

[8] Goodman, J. I.: The Diabetic Neuropathies. Springfield, Ill., Charles C Thomas, 1953.

[9] Dolman, C. L.: The morbid anatomy of diabetic neuropathy. Neurology (Minneap.) *13*:135-42, 1963.

[10] Locke, S.: The peripheral nervous system in diabetes mellitus. Diabetes *13*:307-11, 1964.

[11] Tourtellotte, W. W.: Study of lipids in cerebrospinal fluid. VI. The normal lipid profile. Neurology (Minneap.), *9*:375-83, 1959.

[12] Tourtellotte, W. W., Haerer, A. F., DeJong, R. N.: Cerebrospinal Fluid Lipid Profiles in Various Diseases. Proceedings IV, International Congress of Neuropathology, Vol. 4-8, September, 1961.

[13] Looney, J. M., and Walsh, A. I.: The determination of spinal fluid protein with the photoelectric colorimeter. J. Biol. Chem. *127*:117-21, 1939.

[14] Tourtellotte, W. W., Vander, A. J., Skrentny, B. A., and DeJong, R. N.: A study of lipids in the cerebrospinal fluid, II. The determination of total lipids. J. Lab. Clin. Med. *52*: 481-90, 1958.

[15] Tourtellotte, W. W., Parker, F. M., and DeJong, R. N.: A study of lipids in the cerebrospinal fluid. III. The determinations of total phospholipids. J. Lab. Clin. Med. *52*:491-95, 1958.

[16] Tourtellotte, W. W., Skrentny, B. A., and DeJong, R. N.: A study of lipids in the cerebrospinal fluid. IV. The determination of free and total cholesterol. J. Lab. Clin. Med. *54*: 197-206, 1959.

[17] Woolf, A. L., and Malins, J. M.: Changes in the intramuscular nerve endings in diabetic neuropathy. A biopsy study. J. Path. Bact. *73*:316-18, 1957.

[18] Weiss, P.: Genetic Neurology. P. Weiss, Ed., Chicago, University of Chicago Press, 1950, Chapter 1.

[19] Spalteholz, Werner: Hand Atlas of Human Anatomy, Vol. III. Viscera, Brain, Nerves, Sense Organs, L. F. Barker, Ed., Philadelphia and London, J. P. Lippincott & Co., 1903, pp. 623, 712.

[20] Eliasson, S. G., and Hughes, A. H.: Cholesterol and fatty acid synthesis in diabetic nerve and spinal cord. Neurology (Minneap.) *10*:143-47, 1960.

[21] Hughes, A. H., and Eliasson, S. G.: Synthesis of cholesterol and fatty acids in fractions of peripheral nerve. J. Clin. Invest. *39*:111-15, 1960.

[22] Thomas, P. K., and Lascelles, R. G.: Schwann-cell abnormalities in diabetic neuropathy. Lancet *1*:1355, 1965.

[23] Field, R. A., and Levy, C. D.: The effect of insulin on lipid formation in isolated sciatic nerves from normal and alloxan diabetic rats. 4th Congress Fed. Int. Diabete, Geneva, 1961, p. 603.

[24] Kutt, H., Hurwitz, L., Ginsburg, S., and McDowell, F.: Cerebrospinal fluid protein in diabetes mellitus. Arch. Neurol. 4:31-36, 1961.

[25] Ives, Elinor R.: Protein content in the cerebrospinal fluid of diabetic patients. Bull. Los Angeles Neurol. Soc. *22*:95-111, 1957.