THE CHOLESTEROL AND CHOLESTEROL ESTER CONTENT OF CEREBROSPINAL FLUID IN PATIENTS WITH MULTIPLE SCLEROSIS AND OTHER NEUROLOGICAL DISEASES*

BY

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Cumings (1953, 1955) showed that demyelination of the human brain caused by multiple sclerosis resulted in a loss of free cholesterol and the appearance of cholesterol esters in the involved areas. Cholesterol is a principal constituent of myelin and, according to Cumings, exists in the normal brain entirely as the free non-esterified sterol. It seemed of interest to determine the cholesterol content of the cerebrospinal fluid, and the proportion of free to esterified cholesterol normally present. The possibility existed that the total concentration and state of the cholesterol was different in the cerebrospinal fluid of patients with multiple sclerosis, reflecting the changes in the brain. The cerebrospinal fluid was chosen for this study rather than the blood serum because it is in direct contact with areas of demyelination. Also, small changes in the large serum "pool" of cholesterol might be difficult to evaluate.

A procedure for the accurate determination of small amounts of cholesterol and cholesterol ester in the cerebrospinal fluid was devised. This was based upon a new method for the extraction of cholesterol developed in this laboratory (Roboz, Hess, Di Nella, and Cevallos, 1958) and a new technique for separating free and esterified cholesterol by silicic acid column chromatography (Fillerup and Mead, 1953).

The cholesterol and cholesterol ester concentrations in the cerebrospinal fluid were determined in a group of patients with a firmly established diagnosis of multiple sclerosis. These results are compared with values obtained in normal individuals and in a series of patients with various neurological disorders.

Since the cerebrospinal fluid γ globulin concentration is thought to be an aid in the diagnosis of multiple sclerosis, this was determined and the results compared with the cholesterol concentration.

Materials and Methods

Cerebrospinal fluid was obtained by lumbar puncture from most of the 53 patients included in this study. In a minority of cases fluid removed during air studies was utilized. The diagnosis of each patient was established by a thorough history and neurological examination, combined with air studies and arteriography where indicated. Especially strict criteria were applied in the selection of 13 multiple sclerosis patients. A history of remission and exacerbation was present in most, and all had evidence of dissemination of lesions. The onset of the disease in all cases was before the age of 40 years.

Included in the sample of neurological diseases were eight patients with seizures. A few represented idiopathic epilepsy while most were probably symptomatic. Each had a pneumoencephalogram taken but this failed to demonstrate a neoplasm.

In addition to routine studies the γ -globulin concentration in the cerebrospinal fluid of the multiple sclerosis patients was determined by the zinc sulphate precipitation technique reported by Roboz, Hess, and Forster (1953). Values over 5 mg. per 100 ml. are considered elevated in this laboratory.

Cholesterol and cholesterol ester were extracted from 10 ml. of cerebrospinal fluid by the method of Roboz and others (1958) and determined by the chromatographic technique of Papadopoulos, Cevallos, and Hess (1958). This method utilizes a silicic acid column and the selective elution of free and ester cholesterol followed by their colorimetric estimation.

Results

Since it was desirable to perform the cholesterol analysis on 10 ml. of cerebrospinal fluid, it was difficult to survey a large number of normal individuals. The total cholesterol content in the cerebrospinal fluid of six patients diagnosed as

*This investigation was supported by a grant from the Public Health Service.

†Portions of this paper are taken from a dissertation to be presented to the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

neurotics with no neurological disease averaged 0.33 mg. per 100 ml., ranging from 0.26 to 0.43. Of this total, 0.17 mg. (range: 0.14 to 0.20) was in the free state, and 0.16 mg. (range: 0.12 to 0.23) represented cholesterol ester. It is recognized that the number of "normal" controls is quite small; emphasis will therefore be placed on a comparison of the levels obtained in the different diagnostic groupings.

Table I presents the cholesterol content—free, esterified, and total—of the cerebrospinal fluid of 13 patients with multiple sclerosis. When the averages of these values are compared with those of the normals, it is seen that the principal difference was in the cholesterol ester fraction. An increase in cholesterol ester was chiefly responsible for the higher total cholesterol levels in the patients with multiple sclerosis.

TABLE I
CHOLESTEROL CONTENT IN CEREBROSPINAL FLUID IN MULTIPLE SCLEROSIS

Patient	γ -Globulin (mg. %)	Cholesterol			
		Free (mg. %)	Ester (mg. %)	Total (mg. %)	Percentage as Ester
1	5.6	0.35	0.27	0.62	43
2	10.5	0.16	0.30	0.46	65
3	0.8	0.21	0.24	0.45	53
4	13.0	0.26	0.50	0.76	65
5	1.1	0.23	0.45	0.68	66
6	13.1	0.20	0.21	0.41	50
7	0.8	0.18	0.49	0.67	73
8	10.6	0.18	0.21	0.40	52
9	1.7	0.23	0.22	0.45	53
10*	1.7	0.13	0.15	0.28	54
11	3.4	0.23	0.27	0.50	54
12	5.4	0.16	0.19	0.35	54
13	10.2	0.23	0.37	0.60	61
Mean	6.7	0.21	0.30	0.51	57

* Acute disseminated encephalomyelitis.

Table II lists the cerebrospinal fluid cholesterol levels in patients with a variety of neurological disorders. The free cholesterol content was found to vary more than the ester. For example, free cholesterol ranged from 0.18 to 0.35 mg. per 100 ml. in patients with a convulsive disorder, while the ester varied between 0.14 and 0.26 mg. per 100 ml. in this group. High levels of total cholesterol were found in cases of cerebral infarction (1.04, 0.96, 0.71), severe head trauma (0.68, 0.65), intracerebral haematoma (1.08), and glioblastoma multiforme (1.35). Augmented amounts of both free and esterified cholesterol appear to be responsible for these high totals.

The calculation of the percentage of the total cholesterol as ester revealed a significant difference between the multiple sclerosis patients and those with other diseases (Tables I and II). Five patients with multiple sclerosis had cholesterol ester levels

TABLE II
CHOLESTEROL CONTENT IN CEREBROSPINAL FLUID IN VARIOUS NEUROLOGICAL DISORDERS

Diagnosis	Cholesterol			Percentage as Ester	Comment
	Free (mg. %)	Ester (mg. %)	Total (mg. %)		
Convulsive disorder	0.35	0.20	0.55	36	See text
	0.21	0.25	0.47	53	
	0.19	0.35	0.44	56	
	0.22	0.20	0.42	47	
	0.25	0.14	0.39	36	
	0.22	0.23	0.45	51	
	0.21	0.26	0.47	55	
	0.18	0.23	0.41	53	
Head trauma	0.17	0.12	0.29	41	Cerebral concussion Prolonged coma Cerebral concussion Subdural haematoma
	0.26	0.39	0.65	60	
	0.30	0.21	0.52	40	
	0.37	0.31	0.68	44	
Cerebral infarction	0.45	0.59	1.04	57	Died
	0.29	0.42	0.71	59	
	0.17	0.21	0.38	55	
					Two months after onset
	0.24	0.31	0.55	56	
	0.21	0.23	0.48	48	
	0.21	0.21	0.42	50	Severe deficit Rapid recovery
	0.44	0.52	0.96	54	
	0.20	0.16	0.36	44	
Aneurysm	0.28	0.23	0.51	45	Clear fluid Intracerebral haematoma
	0.61	0.47	1.08	43	
Brain tumour	0.20	0.21	0.41	51	Glioblastoma
	0.60	0.75	1.35	56	
Pre-senile dementia	0.24	0.27	0.51	53	Cerebral atrophy (P.E.G.)
	0.32	0.29	0.61	47	
C.N.S. syphilis	0.31	0.20	0.52	38	Tabes dorsalis Paresis
	0.27	0.22	0.49	45	
Peripheral neuropathy	0.18	0.18	0.36	50	Aetiology undetermined " "
	0.26	0.14	0.40	35	
	0.29	0.31	0.60	52	
Osteo-arthritis of cervical spine	0.17	0.16	0.33	48	Root compression Long tract signs
	0.37	0.18	0.55	33	
Subacute combined degeneration (pernicious anaemia)	0.25	0.22	0.47	47	Severe cord changes

which were in excess of 60% of the total cholesterol. Of the 40 patients bearing other diagnoses (including the six normals), none had more than 60% of the total cholesterol as ester, and only one had a level of 60%.

The percentage of ester also correlated best with the degree of functional deficit possessed by individual patients with multiple sclerosis. The patients numbered 2, 4, 5, and 13 in Table I had the most severe involvement clinically; these patients all had raised percentages of cholesterol ester. However,

patient number 7 had the largest amount of cholesterol in the ester form, yet had a mild illness, with retrobulbar neuritis as the chief manifestation. Conversely, the eighth patient, although severely disabled by a disseminated disease, had relatively low cholesterol and cholesterol ester values in the cerebrospinal fluid. Examinations of the cerebrospinal fluid in this case during the one week after an exacerbation revealed almost identical levels.

The γ -globulin concentration was elevated in seven of the patients with multiple sclerosis. The γ -globulin and percentage of cholesterol as ester were both increased in three patients. Abnormalities of one or the other of these two determinations were present in nine of the 13 cases.

Although this report is not primarily concerned with serum cholesterol levels, the relationship between serum and cerebrospinal fluid cholesterol content is of interest. A patient with myxoedema had a serum cholesterol level of 406 mg. per 100 ml. (normal: 50 to 250), and a cerebrospinal fluid level of 0.55 mg. per 100 ml. Two other patients with serum levels of 288 and 256 mg. per 100 ml. had cerebrospinal fluid values of 0.57 and 0.50 respectively.

Discussion

Plaut and Rudy (1933) investigated the cholesterol content of the cerebrospinal fluid in neurological diseases but did not determine the cholesterol ester fraction. They reported a normal level varying from 0.06 to 0.22 mg. per 100 ml., although they indicated that cholesterol may be absent from normal cerebrospinal fluid. They found an increase of cholesterol in patients with cerebral arteriosclerosis, dementia paralytica, brain tumour, brain abscess, and other diseases. Patients with multiple sclerosis were evidently not studied. It is significant that Plaut and Rudy found a lower level of total cholesterol in their controls than we did. The method of determination employed by these investigators was less sensitive than the modern methods utilized in this study, a fact which was emphasized by their occasional failure to find cholesterol in the cerebrospinal fluid samples. The separation and determination of very small quantities of cholesterol and cholesterol ester has only recently been made practicable by the application of column chromatography.

It is not surprising that destruction of brain or spinal cord tissue by various disease processes was accompanied by an increased cholesterol content of the cerebrospinal fluid. The human brain, and especially the white matter, is rich in cholesterol which may be released into the cerebrospinal fluid. Both this study, and the work of Plaut and Rudy,

indicate that increased serum cholesterol levels do not influence the cerebrospinal fluid concentration in patients without neurological disease.

Increased levels of both free and esterified cholesterol were found in the cerebrospinal fluid of patients with cerebral infarction, brain tumour, and head trauma, as well as in multiple sclerosis. The multiple sclerosis group was separated from the other diagnostic categories by the presence in several cases of a cholesterol ester fraction which constituted over 60% of the total cholesterol. This finding correlates well with the observations of Cumings (1955). The decrease in free cholesterol and the appearance of cholesterol ester in demyelinated areas of the brains of patients with multiple sclerosis, reported by this worker, is thus reflected by an augmented proportion of cholesterol as ester in the cerebrospinal fluid. The data are insufficient to establish a correlation between the cerebrospinal fluid results and the activity of demyelination; more patients must be studied. An extension of this series is also desirable since the low γ -globulin levels of several of the multiple sclerosis patients are contrary to previous experience in this laboratory and may indicate that this group was not fully representative of the disease. If further studies now in progress confirm this report, the determination of the percentage of esterified cholesterol in the cerebrospinal fluid may well be a valuable aid to the diagnosis of multiple sclerosis.

Summary

The cholesterol and cholesterol ester content of the cerebrospinal fluid was determined in six normal individuals, 13 patients with multiple sclerosis, and 34 patients with other neurological diseases. While levels of free and esterified cholesterol were raised in the cerebrospinal fluid of patients with a variety of neurological diseases, only multiple sclerosis patients were characterized by a proportion of ester which was over 60% of the total cholesterol. This finding correlates well with previous investigations of demyelinated tissue by Cumings. It is concluded that the determination of the percentage of esterified cholesterol in the cerebrospinal fluid is a promising adjunct in the diagnosis of multiple sclerosis.

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