AN IMMUNOCHEMICAL STUDY OF THE PROTEINS IN CEREBROSPINAL FLUID

F. D. ROSENTHAL AND J. F. SOOTHILL

*From the Queen Elizabeth Hospital, and Department of Experimental
Pathology, University of Birmingham*

Quantitative gel diffusion precipitin techniques make possible rapid estimation of a wide range of serum proteins. We report a study of cerebrospinal fluid proteins using such a technique. The cerebrospinal fluid: serum concentration ratios for seven serum proteins were determined in patients investigated for a wide range of neurological disease.

METHODS

Cerebrospinal fluid was obtained by lumbar puncture. Only fluids containing fewer than 100 red blood cells/c.mm. were studied.

The cerebrospinal fluid protein was concentrated by the technique of Grant (1957), in most cases to between 500 and 1,000 mg./100 ml. Cerebrospinal fluids with protein concentrations already in this range were not further concentrated.

The details of the immunochemical gel diffusion precipitin technique used are described by Soothill (1962). It is an adaptation of the method of Gell (1957). Proteins studied were albumin, siderophilin, γ globulin, high molecular weight α_2 glycoprotein, β lipoprotein (arranged in order of molecular weight in Table I), and, in a few instances, caeruloplasmin and a component of complement.

The method entails visual matching of patterns of precipitate produced by suitable dilutions of cerebrospinal fluid and of serum, when they react with a standard series of dilutions of specific antiserum. Direct readings of the concentration ratios are obtained (after multiplication by the concentration factor). The error of the method, which entails a constant coefficient of variation for each protein over a wide range of concentrations, has been described in detail by Soothill (1962). It varies with different proteins, but the coefficient of variation of the estimation is about 18% (see Table I).

Reactions of identity were demonstrated by the technique of Ouchterlony (1948). Immuno-electro-

phoresis was performed by a modification of the technique of Grabar and Williams (1953).

A rabbit was immunized to cerebrospinal fluid protein, concentrated to 1 g./100 ml., using a Freund's adjuvant preparation subcutaneously and a saline solution intravenously, by the immunization course described by Soothill (1962). The antiserum (six volumes) was absorbed with one volume of human serum, with incubation at 37°C. for two hours and at 4°C. for 16 hours, after which the precipitate was removed by centrifugation.

RESULTS

The results are shown in Table II. The cerebrospinal fluid/serum concentration ratios for albumin are given directly, and each ratio for the globulins is expressed as a percentage of the albumin ratio. The patients are divided into six diagnostic groups: (1) No demonstrable organic neurological disease; (2) idiopathic and post-traumatic epilepsy; (3) disseminated sclerosis; (4) cerebrospinal fluid block; (5) peripheral neuropathy of Guillain-Barré type; and (6) miscellaneous. The average results for groups 1 to 5 are plotted in Fig. 1.

These show that the proteins studied were present in the cerebrospinal fluid in concentrations such that there was a selectivity in favour of proteins of smaller molecular weight. However, in all groups, except those with a very high cerebrospinal fluid protein concentration, the siderophilin was present in concentrations as high as or higher than the albumin, and, in cases of disseminated sclerosis, the γ globulin often exceeded the albumin. β lipoprotein was detected in the cerebrospinal fluid of some patients in all groups except the first. The results suggest that the failure to detect it in the majority of specimens was due to the limitation of volume of

TABLE I

ACCEPTED VALUES FOR MOLECULAR WEIGHTS OF PROTEINS AND COEFFICIENTS OF VARIATION
OF THE ESTIMATION FOR EACH

Protein	Albumin	Siderophilin	γ Globulin	Caeruloplasmin	α_2 Glycoprotein	β Lipoprotein
Molecular weight	68,000	90,000	150,000	150,000	840,000	1-3,000,000
Coefficient of variation (%)	19	19	13	15	25	18

TABLE II

RESULTS OF C.S.F./SERUM CONCENTRATION RATIOS FOR SEVEN SERUM PROTEINS IN 50 PATIENTS

No.	Age (yr.)	Sex	Diagnosis	Concentration Factor	C.S.F./Serum Concentration Ratios for						
					Albumin	Globulins as Percentage of Albumin					
						Sidero- philin	γ Globulin	α_2 Glyco- protein	β Lipo- protein	Caerulo- plasma	Comple- ment
Group 1 No demonstrable neurological disease											
1	21	M	Schizophrenia	100	1/300	150	50	19	— ¹	—	—
2	24	F	Hysteria	28	1/675	240	50	75	<11	150	—
3	16	F	Vertigo	70	1/550	266	67	33	—	50	—
4	34	M	Hysteria	60	1/80	100	33	17	—	37	—
5	30	M	Hysteria	23	1/550	100	37	25	—	—	87
6	28	M	Migraine	46	1/640	100	50	22	—	—	35
Group 2 Idiopathic and post-traumatic epilepsy											
7	29	F	Idiopathic epilepsy	45	1/270	160	17	33	<6	—	—
8	38	M	Post-traumatic epilepsy	30	1/240	160	57	33	2	114	—
9	18	F	Post-traumatic epilepsy	27	1/650	200	60	50	—	87	—
10	47	M	Post-traumatic epilepsy	46	1/360	200	33	50	—	114	—
11	56	M	Post-traumatic epilepsy	68	1/280	200	33	25	<3	40	25
12	29	F	Birth injury epilepsy	48	1/400	160	66	33	<6	66	—
Group 3 Disseminated sclerosis											
13	55	F	Disseminated sclerosis	50	1/150	150	75	15	<2	37	—
14	36	M	Disseminated sclerosis	40	1/200	125	100	10	—	—	—
15	40	F	Disseminated sclerosis	40	1/320	100	133	33	—	50	—
16	49	F	Disseminated sclerosis	22	1/175	125	200	17	2	100	100
17	37	M	Disseminated sclerosis	49	1/300	100	100	18	—	—	—
18	22	F	Disseminated sclerosis	26	1/310	100	170	37	—	—	—
19	51	F	Disseminated sclerosis	10	1/32	100	64	13	—	—	—
20	54	M	Disseminated sclerosis	57	1/350	100	87	29	—	—	—
Group 4 Peripheral neuropathy											
21	51	M	Peripheral neuropathy	4	1/16	66	40	17	50	50	—
22	37	M	Peripheral neuropathy	27	1/150	100	50	18	<0.5	—	—
23	54	F	Peripheral neuropathy	1	1/8	50	57	8	6	66	—
24	30	F	Peripheral neuropathy	6	1/30	100	62	25	21	—	71
25	58	M	Peripheral neuropathy	5 $\frac{1}{2}$	1/100	160	66	50	14	—	100
26	48	M	Peripheral neuropathy	4	1/32	100	66	33	25	—	—
27	38	F	Peripheral neuropathy	3	1/40	117	58	29	22	—	—
28	52	M	Peripheral neuropathy	3	1/36	60	50	19	6	—	—
29	49	M	Peripheral neuropathy (atypical)	1	1/7	117	100	25	12	140	—
Group 5 Block to flow of C.S.F.											
30	20	M	Aqueduct stenosis	12	1/32	66	53	22	4	22	—
31	63	M	Cervical spondylosis	18	1/125	117	50	25	3	—	58
32	50	F	Spinal meningioma	10	1/140	100	29	15	<5	—	—
Group 6 Miscellaneous											
33	52	M	Cerebral atrophy ? arteriosclerosis	120	1/480	200	50	50	4	—	—
34	47	M	Cerebral atrophy ? alcoholic	150	1/750	167	62	31	>5	167	—
35	65	F	Cerebral atrophy ? familial	150	1/450	300	37	60	>10	75	—
36	32	M	Leber's optic atrophy	24	1/240	167	21	31	>4	83	62
37	56	M	Chiasmal lesion ? cause	55	1/165	150	75	37	1	100	—
38	60	F	Arteriosclerosis	40	1/480	200	41	31	<2	41	—
39	57	M	Cerebral atrophy	186	1/560	200	50	37	10	75	—
40	21	M	Cerebral atrophy following cerebral abscess	25	1/200	200	40	13	<1	67	—
41	49	F	Syringomyelia	36	1/210	160	57	33	—	75	—
42	42	F	Left hemiparesis ? cause	39	1/240	100	50	25	>0.5	50	—
43	61	F	Hemiplegia ? cause	47	1/150	150	50	37	2	60	—
44	48	F	Cerebral atrophy ? cause	31	1/180	117	30	25	1	50	—
45	50	M	Motor neurone disease	35	1/175	200	50	31	2	—	—
46	56	M	Midbrain vascular syndrome	55	1/330	100	30	25	—	—	75
47	44	F	Hemiplegia vascular	45	1/450	167	41	21	—	—	—
48	54	F	Congenital tremor	42	1/300	200	58	29	—	—	35
49	62	M	Depression C.S.F. protein high	38	1/190	143	36	16	1.5	—	—
50	30	M	Migraine C.S.F. protein high	26	1/200	133	50	14	—	67	—

¹(—) indicates that analysis was not performed owing to insufficient C.S.F.

FIG. 1. Average values for each of the first five groups of patients of concentration ratios of five of the proteins (expressed as a percentage of albumin) plotted against the molecular weight of the protein (log-log scale). Molecular weight of each protein is given on abscissa axis: A = albumin; S = siderophilin; γ = γ globulin; α = high molecular weight α_2 glycoprotein; and β = β lipoprotein. Numbers of the groups, as given in Table II, are used as symbols for marking the points on the graph.

cerebrospinal fluid available so that the degree of concentration was insufficient for it to be detectable. In no instance was it demonstrated to be less than 0.5% of the albumin. It was relatively raised in the cerebrospinal fluid of patients with peripheral

neuropathy, who had a very high total cerebrospinal fluid protein concentration, and in two of the three patients with cerebrospinal fluid block, in whom the cerebrospinal fluid protein was only slightly raised. Ratios for caeruloplasmin were rather variable. In one patient with Wilson's disease, in whom the serum concentration of caeruloplasmin was 16% of that of a standard serum from a healthy adult male, only trace quantities were detected in the cerebrospinal fluid. The ratios for the component of complement were also rather variable, but there was no evidence of deficiency in the one case of disseminated sclerosis studied.

To exclude the possibility that the excess of siderophilin in the cerebrospinal fluid might be an artifact of the concentration technique, the cerebrospinal fluid/serum concentration ratios for albumin, siderophilin, and γ globulin were estimated for three patients, using unconcentrated cerebrospinal fluid. The results, which are given in Table III, confirm that siderophilin was present in cerebrospinal fluid at a higher relative concentration than albumin.

TABLE III

CEREBROSPINAL FLUID/SERUM CONCENTRATION RATIOS OF THREE SERUM PROTEINS FOR THREE PATIENTS OBTAINED USING UNCONCENTRATED CEREBROSPINAL FLUID

Diagnosis	C.S.F./Serum Concentration Ratios		
	Albumin	Siderophilin	γ globulin
Motor neurone disease	1/160	1/64	1/256
Paralysis agitans	1/160	1/56	1/320
Cerebral vascular disease	1/112	1/96	1/160

Reactions of identity were obtained for albumin, siderophilin, γ globulin, and α_2 glycoprotein between

FIG. 2. Reactions of identity between serum (left) and concentrated cerebrospinal fluid protein (right) for five serum proteins.

cerebrospinal fluid and serum from two of the patients with no demonstrable neurological disease, for γ globulin from the case of disseminated sclerosis, and for γ globulin and β lipoprotein from the case of peripheral neuropathy. These are shown in Fig. 2.

The rabbit antiserum against concentrated cerebrospinal fluid protein, when reacted against normal human serum and concentrated cerebrospinal fluid by immunoelectrophoresis, gave a number of precipitin lines including albumin, γ globulin, and several α and β globulins. After absorption with human serum, this antiserum gave no precipitation either with human serum or with concentrated cerebrospinal fluid. Thus no antibodies were obtained to proteins which were in cerebrospinal fluid but were not present in the serum.

DISCUSSION

Immunoelectrophoretic study of cerebrospinal fluid proteins has demonstrated many plasma proteins in the cerebrospinal fluid in health and disease (Frick, 1959; Burtin, 1960). Quantitative estimations of albumin and γ globulin have been reported by various techniques, including the very precise immunochemical study of Kabat, Glusman, and Knaub (1948) who demonstrated that, in the normal cerebrospinal fluid, albumin is present in a higher concentration than γ globulin relative to serum, suggesting selective ultrafiltration from serum, favouring the smaller molecule. Reports that β lipoprotein, the largest serum protein, is absent from cerebrospinal fluid (Baudouin, Lewin, and Hillion, 1953; Burtin, 1960) are in keeping with this concept, but quantitative data of other proteins are required to confirm this hypothesis, and to confirm the observation made by immunoelectrophoresis (Gavrilenco, Courcon, Hillion, Uriel, Lewin, and Grabar, 1955; Burtin, 1960) that siderophilin is in relatively high concentration in cerebrospinal fluid.

Our results on cerebrospinal fluid from patients with no demonstrable neurological disease confirm such a selectivity for albumin, γ globulin, α_2 glycoprotein, and β lipoprotein, and the reported relative excess of siderophilin.

It seems unlikely that siderophilin would be made within the central nervous system, but it is possible that the return of proteins to the serum may also be selective. Available isotope data (Frick and Scheid-Seydel, 1958a) suggest that albumin is not broken down in the cerebrospinal fluid so that the excess of siderophilin is unlikely to be due to relatively slow destruction.

Selectivity, according to molecular size, suggests filtration through a semi-permeable membrane, but

it is difficult to see where such a structure would lie unless the proteins pass through the intact ependymal cells. If this is so, it is conceivable that there could be active concentration of siderophilin in a way similar to the active concentration of chloride but such a process for a protein would be unique.

A similar selectivity was demonstrated in all pathological cerebrospinal fluids studied, except for the excess of γ globulin in the cerebrospinal fluid of cases of disseminated sclerosis previously reported by Kabat *et al.* (1948). (We studied no patients with neurosyphilis in whom high cerebrospinal fluid γ globulin has also been reported.) Frick and Scheid-Seydel (1958b) have reported evidence that this is due to formation of γ globulin within the central nervous system. There was no evidence of deficiency of the component of complement in the cerebrospinal fluid in the one case of disseminated sclerosis which we studied for this protein; this might have arisen if the γ globulin were being involved in an antigen-antibody reaction in the cerebrospinal fluid.

Siderophilin was present in excess in all the groups of patients, except those with peripheral neuropathy with a very high cerebrospinal fluid protein concentration, and one case with block to cerebrospinal fluid flow.

β lipoprotein was present in considerable concentration in the cerebrospinal fluid of the patients with peripheral neuropathy, though it was still the protein present in the least relative concentration. Certain cerebrospinal fluids in all the other diagnostic groups except the first contained β lipoprotein, which is contrary to the reports of Baudouin *et al.* (1953) and Burtin (1960). Our data suggest that, if the volume of cerebrospinal fluid is sufficient to allow adequate concentration, β lipoprotein might well be found.

Our data are insufficient for an assessment of the diagnostic value of these tests, but the speed and ease of the determination make them practicable for routine use. It seems likely that the albumin/ γ globulin concentration ratio would be of value in the diagnosis of patients in whom disseminated sclerosis is suspected, and this can be determined on unconcentrated cerebrospinal fluid; this application will be studied further. The various empirical diagnostic tests on cerebrospinal fluid, such as the Lange curve, probably depend on the relative concentrations of various proteins, just as the empirical tests of liver function depend on the elevation of γ globulin concentration and depression of the β lipoprotein concentration. It is probable that direct measurement of the concentration of the relevant proteins in cerebrospinal fluid would be a more satisfactory diagnostic procedure.

The reactions of identity for albumin, sidero-

philin, and γ globulin in normal cerebrospinal fluids, and for γ globulin in that from the case of disseminated sclerosis confirm the results of Frick and Scheid-Seydel (1957) that, within the limits of this technique, these proteins, when in the cerebrospinal fluid, are identical with those in the serum. Reactions of identity were also demonstrated for α_2 glycoprotein in normal cerebrospinal fluid and for γ globulin and β lipoprotein in the cerebrospinal fluid from the cases of peripheral neuropathy. The failure to obtain antibodies to non-serum proteins when a rabbit was immunized with concentrated cerebrospinal fluid protein suggests, though it does not prove, that the protein of cerebrospinal fluid is entirely, or almost entirely, composed of serum proteins.

SUMMARY

Quantitative immunochemical analyses of seven serum proteins in cerebrospinal fluid from patients with a wide range of neurological abnormalities demonstrated an overall tendency to selection of the smaller molecules.

Exceptions were the excess of siderophilin in all fluids studied and the excess of γ globulin in patients with disseminated sclerosis, as has been previously reported.

β lipoprotein was present in the cerebrospinal fluid of patients with peripheral neuropathy with high cerebrospinal fluid protein concentration and in several other individuals.

No evidence was obtained of non-serum cerebrospinal fluid protein.

The possible clinical value of this procedure is suggested.

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