STUDIES ON ACUTE DISSEMINATED ENCEPHALOMYELITIS PRODUCED EXPERIMENTALLY IN RHESUS MONKEYS*

VI. Changes in the Cerebrospinal Fluid Proteins

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Electrophoretic (1, 2) and immunochemical (3, 4) studies on human cerebrospinal fluid have established the relationship of the spinal fluid proteins to the serum proteins and have shown that, in a large proportion of cases of multiple sclerosis, the proportion of gamma globulin in the cerebrospinal fluid is increased; a similar increase occurs in neurosyphilis. In multiple sclerosis the total protein content of the cerebrospinal fluid is generally normal or but slightly elevated, while in neurosyphilis the protein content is usually slightly elevated. By contrast, however, a variety of other neurological conditions are characterized by an increase in the total protein content with little or no increase in the proportion of gamma globulin. In these latter conditions, the increase in total protein is thought to be derived from the serum as a consequence of increased permeability accompanying inflammation.

Since the experimentally produced acute disseminated encephalomyelitis (5–8) under investigation in this laboratory in *rhesus* monkeys presents similarities to the human demyelinating diseases (9, 10), a study of the total protein and gamma globulin was undertaken to ascertain the nature of the changes in the cerebrospinal fluid proteins in this disease. In general, it was found that the acute phase of the disease is usually characterized by a marked increase in white cell count, total protein, and in gamma globulin with a definite but less pronounced increase in the percentage of gamma globulin to total protein. In animals which show a more prolonged course, however, the total protein appears to return to normal more rapidly than does the gamma globulin and some animals show a striking increase in the ratio of gamma globulin to total protein.

EXPERIMENTAL

Cerebrospinal Fluid.—Cerebrospinal fluid was obtained by lumbar puncture from normal monkeys, from monkeys which had been injected with various emulsions with or without

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brain tissue plus the Freund adjuvants—aquaphor, paraffin oil, and killed tubercle bacilli (11)—and had shown no evidence of disseminated encephalomyelitis, and from monkeys similarly injected which showed signs and pathological changes characteristic of acute disseminated encephalomyelitis. Cerebrospinal fluid was collected with sterile precautions, the red and white cells counted, and the fluid kept frozen in solid CO_2 until analyses were performed. Fluids showing gross blood were discarded.

Monkey Gamma Globulin.—The gamma$_2$ globulin was prepared by ethanol fractionation in the cold as described by Nicol and Deutsch (12) except that after removal of beta and gamma$_1$ globulins (precipitate B (12)) at pH 5.1 in 15 per cent ethanol at 0° to −2° C., the bulk of the gamma$_2$ globulin precipitated at this ethanol concentration on adjusting the pH to 7.25; only a small additional quantity of gamma$_2$ globulin was obtained when the ethanol concentration was brought to 25 per cent. The monkey gamma$_2$ globulin was examined electrophoretically by Dr. Dan H. Moore in 0.15 M NaCl + 0.02 M PO_4 buffer at pH 7.4 and found to show a single broad peak of mobility -0.45×10^{-5} cm.^{2}/volt sec. with at most traces of other materials. In barbiturate buffer at pH 8.6, a single peak was seen of mobility -1.4×10^{-5} cm.^{2}/volt sec. with a sharp spike similar to the so called beta anomaly except that it appeared behind the gamma globulin boundary.

Antisera to Monkey Gamma$_2$ Globulin.—Antisera were prepared by injecting rabbits four times weekly for 4 weeks with alum-precipitated monkey gamma globulin, using a total of about 20 mg. for the course of sixteen injections. The animals were bled 5 to 7 days after the last injection. The rabbits received a second series of injections and additional serum was obtained. The agar diffusion technic of Oudin (13) was employed to find out whether or not the anti-monkey gamma globulin sera contained antibody only to a single antigen. Portions of the antisera were mixed with an equal volume of 1 per cent agar and allowed to solidify in 75 × 10 mm. tubes. Whole monkey serum was then layered on the agar and the tubes observed daily. As diffusion of the monkey serum proteins into the agar took place, only a single broad slowly moving band characteristic of gamma globulin was observed in all but one of the twelve samples of antiserum. The exceptional serum showed a very faint band moving more rapidly than the main gamma globulin band and was not used for determinations of cerebrospinal fluid gamma globulin; this band was no longer visible when the antiserum was diluted with an equal volume of saline before mixing with agar.

Immunochemical Estimation of Gamma Globulin in Monkey Cerebrospinal Fluid.—The microquantitative precipitin method of Heidelberger and MacPherson (14) was used except that the washed specific precipitates were analyzed by the Markham micro-Kjeldahl technic (*cf.* reference 15). The procedure employed was essentially that previously described for estimation of albumin and gamma globulin in human cerebrospinal fluid (3). Several antisera to monkey gamma globulin were pooled and diluted to contain about 100 to 150 μg. antibody N per ml. The antiserum pool is calibrated by addition of increasing known amounts of purified monkey gamma globulin to 1 ml. portions of antiserum. After 1 hour at 37° C. and 48 hours in the refrigerator, the precipitates are centrifuged off, washed twice in the cold with saline, and analyzed for nitrogen. Total N precipitated is plotted against quantity of antigen added. The gamma globulin in cerebrospinal fluid is determined by adding an appropriate volume of cerebrospinal fluid to 1 ml. of the antiserum such that the total N precipitated will fall on the calibration curve, measuring the total N in the washed precipitates and reading off the corresponding gamma globulin content from the curve. Analyses are valid only in the region of antibody excess (*cf.* references 16, 15, 3). Each serum supernatant is tested by dividing in half and adding antigen to one portion and antibody to the other to establish whether antibody or antigen is present in excess.

The supernatant tests provided further evidence that the antiserum contained antibody only to gamma globulin in that single supernatants did not show tests for the presence of

both antigen and antibody (*cf.* references 17, 15, 18). In addition, the same values for gamma globulin, within experimental error, were obtained when the same spinal fluid was analyzed with different calibrated antiserum pools.

The total protein content of the cerebrospinal fluids was determined turbidimetrically after addition of sulfosalicylic acid. Results of total protein and gamma globulin are reported in mg. per 100 ml. of cerebrospinal fluid; the percentage of gamma globulin to total protein is also given.

In a considerable number of instances the quantity of cerebrospinal fluid obtained was not sufficient for analyses for gamma globulin to be performed in duplicate.

To evaluate the effect of possible abnormalities in proportions of the serum proteins on the composition of the cerebrospinal fluid, 8 serum samples, 2 from animals whose signs had disappeared and 6 from animals with signs of encephalomyelitis but with varying cerebrospinal fluid total proteins, were examined electrophoretically in 0.15 M NaCl plus 0.02 M phosphate buffer at pH 7.4 by the electrophoresis laboratory at the College of Physicians and Surgeons. The gamma globulin on a suitable dilution of these sera was also determined immunochemically as described above (*cf.* references 4, 15, 17) and the total protein was determined by micro-Kjeldahl technique. The percentage of gamma globulin in the sera was estimated both from the electrophoretic patterns and from the values of gamma globulin and total protein. Serum samples were obtained immediately after lumbar puncture.

RESULTS

Table I summarizes data on white blood cells, cerebrospinal fluid protein, gamma globulin, and percentage of gamma globulin in 22 presumably normal monkeys. Results are listed in order of increasing total protein. Total protein ranged from 8 to 50 mg. per 100 ml., gamma globulin from 0.4 to 6.3 mg. per 100 ml., and the percentage of gamma globulin from 3 to 14 per cent with one grossly aberrant value of 26 per cent.

In Table II are presented cerebrospinal fluid findings on 27 monkeys which had been injected with various emulsions with adjuvants and which remained symptom-free and at necropsy showed no evidence of pathological changes of acute disseminated encephalomyelitis in the central nervous system. Only 3 of these animals had been injected with emulsions which had no encephalitogenic potency. Many of the others, however, received injections of emulsions of diluted brain or of fractions of brain tissue given for the purpose of assaying these materials for encephalitogenic potency. Total protein ranged from 9 to 39 mg. per 100 ml., gamma globulin from 0.4 to 4.1 mg. per 100 ml., and the percentage of gamma globulin to total protein from 3 to 18 per cent. These values correspond quite closely to the values obtained in Table I with cerebrospinal fluid from animals presumed to be normal. The two highest values for percentage gamma globulin were obtained in 2 animals (2–78 and 3–48) in which the total protein was very low (9 mg./100 ml.) so that the experimental error was considerably greater than usual. The number of white blood cells in the normal and negative groups were comparable and varied from 1 to 24 per mm^3.

Data on 15 monkeys which showed evidence of acute disseminated enceph-

alomyelitis at necropsy but which had either remained free of symptoms owing to the mildness of the disease or which had become symptom-free before the sample of cerebrospinal fluid was taken are given in Table III. All these animals showed total protein levels from 11 to 33 mg. per 100 ml., gamma globulin levels from 0.9 to 6.0 mg. per 100 ml., and percentages of gamma globulin to total protein ranged from 6 to 23. By comparison with the data in Tables I and II, excluding the aberrant value in Table I, 3 of the animals (5–58, 2–38,

TABLE I

Total Protein and Gamma Globulin in Normal Monkey Cerebrospinal Fluid

Monkey No.	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
	RBC/mm.³	WBC/mm.³			
			mg./100 ml.	*mg./100 ml.*	*per cent*
3-67	28	1	8	0.9	11
3-68	0	1	11	0.6	6
3-29	0	24	13	0.4	3
3-49			13	0.6	5
3-51	1	2	13	0.9	7
3-53	370	4	14	0.7	5
3-52	1	1	14	1.8	13
3-66	0	1	14	0.4	3
3-39	0	2	16	4.1	26
Ch 47	0	17	16	0.5	3
3-28		3	17	0.6	4
3-32	0	2	17	0.9	5
3-65	0	2	21	3.0	14
3-36	370	8	21	1.0	5
3-33	20	3	21	0.8	4
3-34	270	14	24	2.4	10
3-54	700	16	24	2.1	9
3-30		2	24	1.3	5
3-38	220	0	32	2.3	7
3-48	Slight amount blood		34	4.5	13
Ch 27	5	4	47	4.6	10
3-50	Clear		50	6.3	13

2–41) showed increases in the proportion of gamma globulin greater than any of those in normal and negative animals. The number of white blood cells in the fluids was similar to that of the normal and negative animals and ranged from 1 to 25 per mm.³.

Table IV gives data obtained in the cerebrospinal fluid of 44 monkeys showing signs of disseminated encephalomyelitis. It is evident that a large majority of these fluids showed increases in total protein and in gamma globulin and 21 of the 44 fluids showed percentages of gamma globulin to total protein of 19 or greater.

In 20 monkeys with disseminated encephalomyelitis, it was possible to obtain 2 or more specimens of cerebrospinal fluid at various stages in the course of the disease. These data are presented in Table V and show several types of

TABLE II

Total Protein and Gamma Globulin in Cerebrospinal Fluid from Injected Monkeys Which Remained Negative for Disseminated Encephalomyelitis

Monkey No.	Cel lcount		Day of CSF sample*	Total protein	Gamma globulin	Gamma globulin / Total protein
	RBC/mm.³	WBC/mm.³				
				mg./100 ml.	*mg./100 ml.*	*per cent*
2-78	0	6	164	9	1.6	18
3-48	0	10	191	9	1.6	18
2-74	0	1	128	10	1.2	10
3-13	0	2	62	10	0.4	4
2-80	25	4	166	11	1.6	14
4-09	0	3	200	12	1.3	11
3-21	20	12	139	12	1.0	8
2-92		5	155	13	0.4	3
3-31	0	3	86	13	1.3	10
5-65	0	2	66	14	0.9	6
5-68	15	4	70	14	0.7	5
5-64	0	5	70	15	1.4	10
3-27	360	5	172	17	2.2	13
3-34	0	1	87	18	2.3	13
2-63	70	22	297	18	2.4	13
2-93	140	4	155	19	0.7	4
2-95	120	2	156	20	1.1	6
2-75	Clear		159	20	1.4	7
3-43	0	9	68	20	1.8	9
212‡	Clear		188	22	2.9	13
4-89‡	—	—		22	1.3	6
3-18	5	9		23	3.1	13
2-29	0	3	185	25	2.6	10
2-85	95	4	165	26	2.4	9
2-34	130	5	186	27	2.7	10
3-47	1840	16	57	27	2.8	10
2-11‡	Clear		189	39	4.1	11

* Days after first injection of emulsions.

‡ Non-encephalitogenic emulsion.

change in cerebrospinal fluid in the acute phase of the disease. In 7 instances (monkeys 3–85, 3–53, 4–99, 5–73, 4–76, 4–37, 4–54), the acute phase of the disease was characterized by a striking rise in total protein and in gamma globulin but without any great change in the percentage of gamma globulin to total protein, while in 9 other instances (4-53, 3–84, 4–05, 5–60, 4–67, 4–98, 4–78, 5–72, 5–28) a significant increase in the percentage of gamma globulin to

total protein was found as well. Nine of these 16 fluid specimens were obtained on the day of onset, 3 one day, 2 three days, and 2 four days after onset of the disease.

As the disease process continued, those animals in which improvement was noted showed a drop in total cerebrospinal fluid protein frequently to normal levels (3–85, 3–84, 3–53, 5–60, 4–67, 4–98, 4–54, 5–28), while in 5 animals in which the illness became more severe, the total protein remained elevated or even increased (5–69, 4–99, 4–76, 4–37, 4–55). In 6 of the 8 animals which improved, the gamma globulin also decreased. In only 3 of these, however, did

TABLE III

Total Protein and Gamma Globulin in Cerebrospinal Fluid from Monkeys with Disseminated Encephalomyelitis Which Remained or Had Become Symptom-Free

Monkey No.	Cell count		Day fluid obtained after first injection	Day fluid obtained after first symptoms	Day fluid obtained after symptoms disappeared	Total protein	Gamma globulin	Gamma globulin / Total protein
	RBC/mm.³	WBC/mm.³						
						mg./100 ml.	*mg./100 ml.*	*per cent*
2-59		12	153	No symptoms observed		11	1.0	9
3-71	0	12	85	55	38	12	2.1	17
2-84	0	3	169	No symptoms observed		13	0.9	7
5-58	0	7	54	No symptoms observed		14	2.9	21
2-76	13	5	154	No symptoms observed		14	2.3	16
2-62	0	7	157	No symptoms observed		14	0.9	6
2-38	0	1	316	263	249	15	3.2	21
2-83			355	213	98	17	1.8	11
2-60	28	25	156	No symptoms observed		18	1.3	7
3-69	600	21	104	74	64	21	1.8	9
2-39	82	17	317	262	250	24	3.5	15
2-41	0	25	312	273	252	26	6.0	23
5-70	0	25	64	No symptoms observed		31	3.6	12
2-94	4700	12	156	No symptoms observed		32	4.4	14
2-03	0	1	346	287	217*	33	4.4	13

All animals in this group showed pathological changes characteristic of acute disseminated encephalomyelitis.

*All symptoms had disappeared except blindness.

the percentage of gamma globulin decrease (3–84, 5–60, 4–98) as well, so that the cerebrospinal fluid appeared completely normal. These 3 animals ultimately became negative for signs of encephalomyelitis, although necropsy showed typical pathological changes. In the other 3, the decrease in gamma globulin was less marked than was the decline in total protein so that the percentage of gamma globulin was ultimately increased (3–85, 3–53, 4–67). The remaining 2 animals showed a considerable increase both in gamma globulin and percentage of gamma globulin (4–54, 5–28).

Of the 5 animals showing more severe symptoms, the gamma globulin rose in each instance, but the percentage of gamma globulin was increased in only 3 of the 5 cases.

TABLE IV

*Total Protein and Gamma Globulin in Cerebrospinal Fluid from Monkeys Showing Signs of Acute Disseminated Encephalomeylitis**

Monkey No.	Cell count		Day fluid obtained after first injection	Day fluid obtained after first signs	Symptoms when cerebrospinal fluid taken	Total protein	Gamma globulin	Gamma globulin / Total protein
	RBC/mm.³	WBC/mm.³						
						mg./100 ml.	*mg./100 ml.*	*per cent*
4-19	0	2	50	0‡	Slight	27	2.9	11
5-51	0	13	62	1	Moderate	30	5.8	19
4-43	0	12	165	1	Severe	31	8.3	27
3-70	86	14	35	0	Moderate	33	3.0	9
3-82	0	2	27	0	Severe	34	3.6	11
5-35	0	15	22	1	Prostrate	38	9.2	25
4-96	480	7	66	1	Moderate	39	5.0	13
5-90	6	24	37	2	Moderate	42	5.7	14
5-57	Clear		16	0	Moderate	42	7.1	17
4-72	0	10	23	0	Moderate	45	5.5	12
6-06	0	65	23	2	Moderate	46	13	28
5-00	0	130	23	0	Prostrate	46	7.6	17
3-81	Slightly bloody		16	0	Severe	50	9.6	19
2-24	Clear		53	1	Prostrate	52	7.2	14
3-80	0	36	29	2	Prostrate	54	10	19
4-75	250	31	21	0	Severe	55	16	29
2-77	1480	1032	26	1	Prostrate	58	14	24
5-18	14	9	29	0	Moderate	62	8.2	13
4-95	130	650	32	1	Blindness only	66	>16	>25
2-51			20	0	Prostrate	66	7.8	12
4-16	0	33	30	0	Moderate	70	10	14
3-57	24	112	23	0	Severe	72	11	15
5-24	0	3	42	2	Prostrate	77	6.5	8
6-07	3	66	25	4	Slight	78	16	20
5-62	Clear		22	2	Severe	79	12	15
5-74	Clear		44	19	Prostrate	82	29	35
2-67	Clear		59	23	Moderate	89	7.0	8
2-89			23	3	Prostrate	99	19	19
2-98			19	1	Prostrate	99	21	21
3-54	280	55	22	2	Severe	99	17	17
3-02	Clear		22	1	Severe	104	20	19
2-66	282	200	26	3	Prostrate	112	18	16
5-48	Clear		26	0	Prostrate	117	23	20
5-85	0	10	39	1	Prostrate	128	25	19
3-94	24	30	24	0	Moderate	131	27	21
2-68	950	1500	60	9	Prostrate	135	50	37
4-94	0	68	32	1	Moderate	135	21	16
2-71	Clear		58	27	Prostrate	137	19	14
5-36	0	400	22	1	Prostrate	151	35	23
2-72			51	32	Prostrate	152	22	14
4-03	350	284	24	1	Moderate	155	21	13
3-00	640	27	24	1	Prostrate	180	47	26
2-88			27	1	Prostrate	180	37	21
2-73			20	19	Prostrate	212	39	18

* Each animal was shown to have acute disseminated encephalomyelitis at necropsy.

‡ Cerebrospinal fluid sample taken the same day as symptoms first noted.

TABLE V

Total Protein and Gamma Globulin in Cerebrospinal Fluid from Monkeys with Disseminated Encephalomyelitis in Relation to the Course of the Disease

Monkey No.	Days after first injection	Signs of disease noted	Day fluid obtained after first signs	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
				RBC/mm.³	WBC/mm.³			
						mg./100 ml.	*mg./100 ml.*	*per cent*
2-90	96	Onset with slight progressive ataxia						
	149	reaching peak of severe ataxia						
	184	subsiding to slight ataxia	88		5	19	3.8	20
	204	second attack of severe ataxia	108	570	26	24*	5.8*	24*
	213	slight improvement	117	0	1	17	2.7	16
	269	moderate ataxia continuing with	173	0	11	10	1.4	14
	313	fluctuations and finally	217	Slightly bloody		22	4.3	20
	323	becoming slight	227	35	8	14	2.6	19
4-53	31	Slow in movements, visual disturbance						
	36	continuing with slight ataxia,						
		dilated pupils, and	5	0	73	32*	10*	31*
	71	blindness	40	0	21	33	12	36
	73	vision returned continued	42					
	75‡	ataxic	44	0	1	32	9.7	30
	79‡	until	48	12	32	49	16	33
	127	reported symptom-free						
	239	continued negative	208	0	6	17§	1.9§	11§

* Plotted in Fig. 1 C.

‡ It is uncertain as to which of these fluids was taken on 75 and which on 79 days.

§ Plotted in Fig. 1 B.

TABLE V—*Continued*

Monkey No.	Days after first injection	Signs of disease noted	Day fluid obtained after first signs	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
				RBC/mm.³	WBC/mm.³			
						mg./100 ml.	*mg./100 ml.*	*per cent*
3-85	36	Moderate ataxia becoming						
	37	severe	1	0	8	116*	10*	9*
	63	subsiding to moderate	27	0	27	15	5.1	33
	72	becoming negative						
	88	remained negative	52	12	8	16§	2.2§	14§
4-62	60	Blindness widely dilated pupils						
	79	no other symptoms continued	19	0	1	16§	2.1§	13§
	258	blind until sacrificed	198	12	1	12	1.5	12
3-84	37	Moderate ataxia becoming						
	38	very severe with generalized tremors	1	100	85	67	14	21
	39	severe ataxia rotation of head and chin						
	51	subsiding to slight ataxia						
	67	very slight ataxia	30	0	6	12	2.0	17
4-05	30	Severe ataxia continuing	0	17	178	29*	4.5*	16*
	44	through entire course	14	0	6	14	1.3	9
3-53‖	26	Moderate ataxia subsiding to slight, negative, slight	0	Clear		79*	3.9*	5*
	92	negative continued						
	165	negative	139	462	7	15	1.9	13

‖ Cerebrospinal fluid findings before injection in Table I.

TABLE V—*Continued*

Monkey No.	Days after first injection	Signs of disease noted	Day fluid obtained after first signs	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
				RBC/mm.³	WBC/mm.³			
						mg./100 ml.	*mg./100 ml.*	*per cent*
	220	received second course of injections						
	247 (27) ¶	ataxia, unequal pupils	220(0)	0	15	129	29	22
5-60	19	Moderate ataxia became	0			88*	17*	20*
	20	severe diminished became						
	42	negative						
	44	continued negative	25	0	7	17§	1.9§	11§
4-67	48	Marked ataxia became	0	117	64	46*	13*	28*
	62	more severe, moribund	14	Clear		22	7.8	35
4-98	31	Slight ataxia becoming						
	34	severe	3			85*	17*	20*
	44	improvement continuing						
	51	slight ataxia	20	2500	22	38	3.1	8
	140	negative continuing						
	179	negative	148	0	3	24§	2.2§	9§
4-78	21	Dilated pupils right internal strabismus moderate ataxia	0	0	85	48*	8.4*	18*
	22	became blind						
	48	ataxia decreased in severity						
	78	blind otherwise negative						
	192	continuing blindness	171	315	16	53	9.0	17
5-69	32	Blindness lasting 6 days						

¶ Days after second course.

TABLE V—*Continued*

Monkey No.	Days after first injection	Signs of disease noted	Day fluid obtained after first signs	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
				RBC/mm.³	WBC/mm.³			
						mg./100 ml.	*mg./100 ml.*	*per cent*
	50	vision regained, ataxia	18	0	94	50*	9.0*	18*
	62	visual disturbances and ataxia continuing	30	0	29	49	11	21
5-72	25	Ataxia and visual disturbance						
	28	continuing	3		308	76*	21*	28*
	31	continuing	6	27	34	52	11	20
4-99	20	Severe ataxia	0	0	13	68*	11*	16*
	21	more severe ataxia						
	24	continued ataxia, constant clonic movements	4	0	34	117	18	15
5 73	31	Pupils dilated						
	33	blind slight ataxia						
	35	continued	4	0	117	100*	16*	16*
	48	more severe ataxia	17	0	187	70	11	16
4-76	19	Moderate ataxia tremors						
	20		1	37	166	88*	13*	15*
	23	blind, ataxia increasing in intensity, ptosis						
	29	both eyelids						
	32	prostrate, left pupil pin point, right pupil dilated	13	180	13	163	17	10
4-37	33	Visual disturbance						
	37	ataxia horizontal nystagmus, delivered dead infant blind	4	4300	50	109	19	17

TABLE V—*Concluded*

Monkey No.	Days after first injection	Signs of disease noted	Day fluid obtained after first signs	Cell count		Total protein	Gamma globulin	Gamma globulin / Total protein
				RBC/mm.³	WBC/mm.³			
						mg./100 ml.	*mg./100 ml.*	*per cent*
		marked ataxia						
	41	prostrate	8	660	160	114*	21*	19*
4-54	23	Widely dilated pupils	0	0	7	216	9.6	5
	24	blind, moderate ataxia, ptosis both eyelids, pupils dilated						
	25	continuing with severe ataxia	2	1300	150	114*	>42*	>37*
5-28	29	Moderate ataxia	0	300	190	216*	41*	19*
	75	decreasing to slight ataxia	46	67	13	186	59	32
4-55	30	Slight ataxia, weakness left side of mouth						
	45	continuing unchanged	15	48	58	25	4.8	19
	64	severe ataxia increasing						
	67	ataxia with intention and head tremors	37	350	124	180*	39*	22*

One animal (4–62) in which blindness was the only evidence of disease noted throughout the entire course showed normal cerebrospinal fluid 19 and 198 days after the onset of the disease.

Another animal, 2–90, showed normal total protein levels 88 to 227 days after signs of encephalomyelitis were noted; the percentages of gamma globulin fluctuated frequently being abnormal and declining to the upper levels of normal.

Monkey 4–53 showed a very marked increase in gamma globulin and in percentage of gamma globulin from 5 to 38 days after signs developed, but several months after the signs disappeared and the animal appeared well, the cerebrospinal fluid findings were completely normal.

Monkey 4–78 showed values for total protein, gamma globulin, and percentage of gamma globulin just at the upper limit of normal over a period of 171

days. Two animals (5–72, 5–73) in which the disease persisted in severity showed, during 3 and 13 day intervals respectively, drops in total protein and in gamma globulin; in one case the percentage of gamma globulin declined from 28 to 20 per cent and in the other remained constant at 16 per cent.

The cerebrospinal fluid findings are shown graphically in Fig. 1, in which the values for total protein, gamma globulin, and percentage of gamma globulin are given in A for 22 normal plus 27 negative animals, in B for 20 animals which recovered and appeared well, and in C for 63 animals showing signs of disease.

In 44 animals with signs of encephalomyelitis (Tables IV and V), the number of white cells in the cerebrospinal fluid was determined. Values varied from

TABLE VI

Comparison of the Proportions of Gamma Globulin to Total Protein in Cerebrospinal Fluid and Serum

Monkey No.	Total protein in cerebrospinal fluid	Gamma globulin / Total protein		
		Cerebrospinal fluid	Serum	
			Immunochemical	Electrophoretic*
	mg. /100 ml.	*per cent*	*per cent*	*per cent*
5-68	14	5	30	30
2-11	39	11	17	16
3-70	33	9	22	24
2-77	58	24	24	23
5-74	82	35	41	37
2-67	89	8	27	26
2-68	135	37	39	34
5-36	151	23	23	26

* Electrophoretic patterns were obtained in 0.15 M NaCl + 0.02 M PO_4 by the Electrophoresis Laboratory of Columbia University.

2 to 1500 per $mm.^3$. The median value for white cells was 66 per $mm.^3$ as compared with a median value of 4 cells per $mm.^3$. for the normal and negative animals in Tables I and II and of 7 cells per $mm.^3$. for the recovered animals in Table III. Thirty-one of the 44 animals with encephalomyelitis had white cell counts higher than the highest value recorded for the normal, negative, or recovered groups. No correlation was apparent between the white cell count and the total protein, gamma globulin, or percentage of gamma globulin.

A comparison of the proportion of gamma globulin in the cerebrospinal fluid and serum of 8 monkeys is given in Table VI. The first 2 of these were free of signs of encephalomyelitis at the time the serum and cerebrospinal fluids were drawn (*cf.* Table II). In these instances the proportion of gamma globulin to total protein was considerably lower in the cerebrospinal fluid than in the serum

despite a high proportion of gamma globulin in the serum of monkey 5–68. Similar results were also obtained with 2 monkeys showing signs of encephalomyelitis; one of these (3–70) had a normal total protein in the cerebrospinal

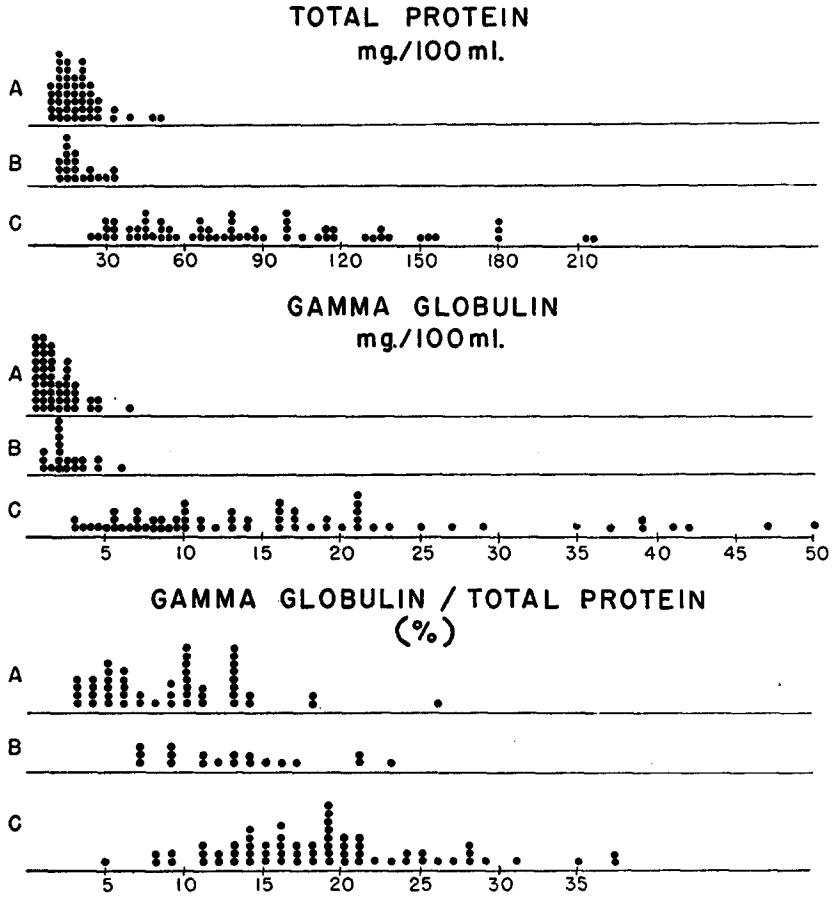

FIG. 1. Total protein, gamma globulin, and percentage of gamma globulin to total protein in the cerebrospinal fluids of: A, 22 normal and 27 negative monkeys; B, 20 animals with encephalomyelitis which had recovered and appeared well; C, 63 monkeys showing signs of acute disseminated encephalomyelitis.

fluid while in the other (2–67), the total protein was considerably elevated. In 2 of the remaining 4 animals with active disease in Table VI, the proportion of gamma globulin to total protein in the cerebrospinal fluid, regardless of the total protein level, was strikingly elevated and was equal to that in the serum and in the other 2 was but slightly lower. These results bear a striking similarity

to those already reported (1, 3, 4), comparing serum and cerebrospinal fluid gamma globulin/total protein ratios in normal human subjects and in cases of inflammatory disease of the central nervous system with those of neurosyphilis and multiple sclerosis. In the former group the cerebrospinal fluid gamma globulin/total protein ratio was consistently less than the corresponding serum ratio,

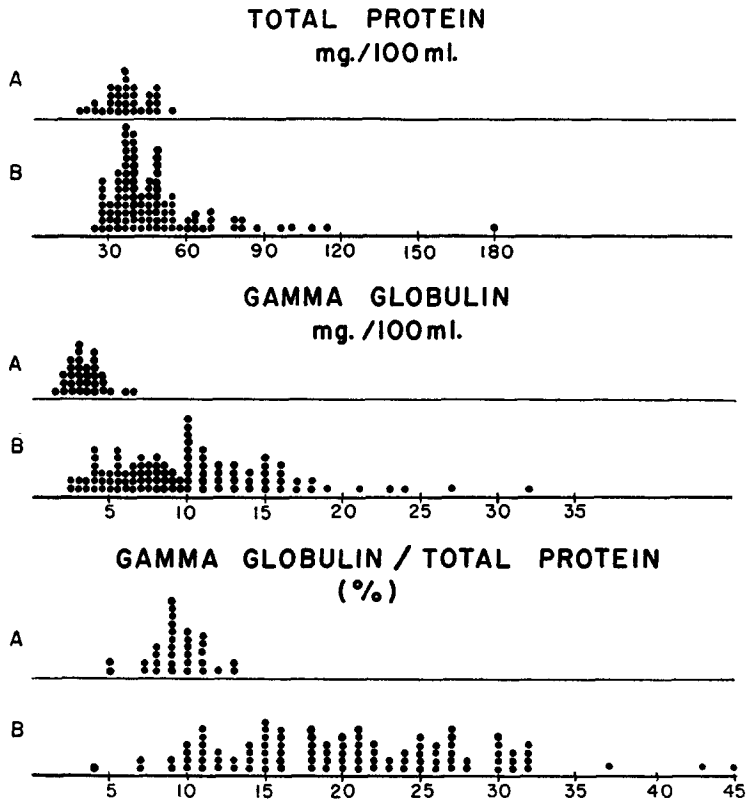

FIG. 2. Total protein, gamma globulin, and percentage of gamma globulin to total protein in the cerebrospinal fluids of: A, 32 individuals with normal cerebrospinal fluid proteins; B, 100 individuals with well established multiple sclerosis; data from reference 4.

while in many cases of neurosyphilis and multiple sclerosis, the ratio in the cerebrospinal fluid (regardless of the total protein content) was equal to or higher than that found simultaneously in the serum. These observations coupled with the findings in monkeys are consistent with the belief that in these diseases a large proportion of the gamma globulin of the cerebrospinal fluid is probably formed within the central nervous system.

The abnormally high proportion of serum gamma globulin in 3 of the monkeys

(2–68, 5–68, 5–74) was probably due to the trypanosomiasis which the monkeys were discovered to have (19). The proportion of gamma globulin to total protein in the sera of the 8 monkeys by immunochemical assay and by electrophoresis was in very close agreement. This contrasts sharply with the findings of Jager, Smith, Nickerson, and Brown (20) who reported considerably higher immunochemical values for gamma globulin in human serum as compared with the electrophoretic values. Moore (21) had previously reported the electrophoretic patterns of 3 sera from *rhesus* monkeys and had found 16, 26, and 29 per cent of gamma globulin.

A comparison of the changes in the cerebrospinal fluid proteins with the pathological findings in the central nervous system revealed the following: Normal or low values for cerebrospinal fluid total protein and gamma globulin were noted in those animals which had relatively few and small lesions. Such values occurred less frequently when moderate or large numbers of small lesions were present and were seen most often when the pathological process was chronic and many of the lesions were healed even though some might be large. With increasing numbers of lesions there is a progressive increase in cerebrospinal fluid protein and as the lesions increase in size and as more acute necrotizing lesions occur, the protein increase is more pronounced and a rise in the absolute gamma globulin is noted.

Focal inflammatory involvement of the choroid plexuses and leptomeninges by acute or subacute lesions and the presence of acute or subacute lesions in the walls of the ventricles often lead to considerable protein increases and high gamma globulin especially when lesions are large and numerous throughout. With involvement of the leptomeninges or choroid plexus alone, this effect is usually much less marked. The high ratios of gamma globulin to total protein are found evenly scattered among the lesions without regard to size, severity, age, and distribution or combinations of these and may therefore be related to the underlying mechanisms in this experimental disease.

DISCUSSION

The data presented clearly establish that changes in the white cell count and in the cerebrospinal fluid proteins occur in *rhesus* monkeys with experimentally induced acute disseminated encephalomyelitis and that these changes are most pronounced when signs of this condition are present. As remission of various signs occurs, the cerebrospinal fluid picture tends to return to normal. (Tables I to V, Fig. 1.)

The changes in the cerebrospinal fluid may be of two general types. The first type is illustrated by monkey 4–53 (Table V) which showed total protein levels in the cerebrospinal fluid ranging from 32 to 49 mg. per 100 ml. from the 5th to the 48th day after neurological signs were noted. These values would fall on the high side of normal. (Fig. 1.) The gamma globulin and the percent-

age of gamma globulin were strikingly elevated, however, and varied from 9.6 to 16 mg. per 100 ml. and from 30 to 36 per cent, respectively. This pattern conforms closely with that found in the cerebrospinal fluid in the majority of cases of human multiple sclerosis (3, 4) and neurosyphilis. Several months after the complete disappearance of signs in this monkey normal values for total protein, gamma globulin, and percentage of gamma globulin were found.

The second type of change in the cerebrospinal fluid, illustrated by monkey 3–85, is characterized by an initial rise in total protein and in gamma globulin with the percentage of gamma globulin remaining normal or rising slightly. One day after onset monkey 3–85 had a total protein of 116 mg./100 ml., a gamma globulin of 10 mg./100 ml. with 9 per cent gamma globulin. 26 days later, however, as the acute process subsided, the total protein had dropped to 15 mg./100 ml. and the gamma globulin to 5.1 mg./100 ml. giving a value of 33 per cent gamma globulin. With the further subsidence of the disease process, all values returned to normal. The high values for total protein and gamma globulin with a normal percentage of gamma globulin in the acute stage of the disease resemble the findings in a variety of human neurological diseases characterized primarily by inflammation (3, 4) in which the increase in cerebrospinal fluid proteins results from transudation of serum protein. The necropsy findings in many of the monkeys with acute disseminated encephalomyelitis frequently show intense inflammatory changes, especially in those animals dying or sacrificed in the acute stages of the disease. With the subsidence of these acute inflammatory changes the increase in the proportion of gamma globulin to total protein becomes evident. This increase in the percentage of gamma globulin to total protein, characteristically occurring in human neurosyphilis and in multiple sclerosis, has been shown not to be due to increased permeability of the blood-brain barrier to serum protein, but has been attributed to synthesis of gamma globulin within the central nervous system (1, 3, 4). It may be supposed that in the acute disseminated encephalomyelitis in the monkey a similar mechanism operates to increase the percentage of cerebrospinal fluid gamma globulin in the animals showing an increased proportion of cerebrospinal fluid gamma globulin throughout the course of the disease (*e.g.* 4–53) as well as in those showing such a change only after the acute stage of the disease has passed (3–85). Needless to say, individual monkeys may show either of these types of cerebrospinal fluid change, or some intermediate pattern; occasional animals may show no cerebrospinal fluid changes. (*cf.* monkey 4–62, Table V.)

To provide a comparison on a statistical basis, the total protein, gamma globulin, and percentage of gamma globulin in the cerebrospinal fluid from 32 individuals with presumably normal cerebrospinal fluid proteins (3) and from 100 patients with well established multiple sclerosis (4) are given in Fig. 2. By comparison with Fig. 1, it is immediately evident that in multiple sclerosis the vast majority of patients have a normal total protein in the cerebrospinal fluid

(Fig. 2 B) while in the monkey with active disseminated encephalomyelitis the total protein is usually considerably increased (Fig. 1 C). A very large proportion of both human beings and monkeys show increased gamma globulin levels in the cerebrospinal fluid, the values in the monkeys being elevated over a broader range. The human cases of multiple sclerosis, however, showed a very sharp increase in the percentage of gamma globulin as compared with the monkeys. This would be expected since the total proteins in the cerebrospinal fluid of the human cases were generally normal. Advanced multiple sclerosis is not complicated by the acute inflammatory picture seen in monkeys in the early stages of acute disseminated encephalomyelitis. As already noted the changes in the cerebrospinal fluid in the human being would, therefore, result from production of gamma globulin within the central nervous system.

SUMMARY

The white cell count, total protein, gamma globulin, and percentage of gamma globulin in the cerebrospinal fluid of monkeys with acute disseminated encephalomyelitis produced by the injection of brain emulsions with adjuvants have been studied.

The acute phase of the disease is characterized by a rise in the white cell count, total protein, and gamma globulin in the cerebrospinal fluid. In some instances the percentage of gamma globulin to the total protein may be normal while in others it is elevated.

As the acute process subsides, the total protein declines and animals frequently show an increase in the percentage of gamma globulin to total protein.

The relation of the cerebrospinal fluid findings in acute disseminated encephalomyelitis in the *rhesus* monkey to those in human multiple sclerosis is discussed.

BIBLIOGRAPHY

1. Kabat, E. A., Moore, D. H., and Landow, H., *J. Clin. Inv.*, 1942, **21,** 571.
2. Scheid, K. F., and Scheid, L., *Arch. Psychiat. u. Nervenkrankh.*, 1944, **117,** 219, 312, 641; 1948, **118,** 179; *Med. Z.*, 1944, **1,** 48.
   Scheid, K. F., Scheid, L., and Schneidt, W., *Arch. Psychiat. u. Nervenkrankh.*, 1948, **118,** 337.
3. Kabat, E. A., Glusman, M., and Knaub, V., *Am. J. Med.*, 1948, **4,** 653.
4. Kabat, E. A., Freedman, D., Murray, J. P., and Knaub, V., *Am. J. Med. Sc.*, 1950, **219,** 55.
5. Rivers, T. M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, **58,** 39.
   Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1935, **61,** 689.
6. Ferraro, A., and Jervis, G. A., *Arch. Neurol. and Psychiat.*, 1940, **43,** 195.
7. Morgan, I. M., *J. Exp. Med.*, 1947, **85,** 131.
8. Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, **85,** 117.
9. Wolf, A., Kabat, E. A., and Bezer, A. E., *J. Neuropath. and Exp. Neurol.*, 1947, **6,** 333.

10. Kabat, E. A., Wolf, A., and Bezer, A. E., *Research Pub., Assn. Research Nerv. and Ment. Dis. Pub.*, 1950, **28,** 113.
11. Freund, J., and McDermott, K., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49,** 548.
12. Nicol, J. C., and Deutsch, H. F., *J. Am. Chem. Soc.*, 1948, **70,** 80.
13. Oudin, J., *Bull. Soc. chim. biol.*, 1947, **29,** 140; *Ann. Inst. Pasteur*, 1948, **75,** 30, 109.
14. Heidelberger, M., and MacPherson, C. F. C., *Science*, 1943, **97,** 405; 1943, **98,** 63.
15. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas Co., 1948.
16. Heidelberger, M. and Kendall, F. E., *J. Exp. Med.*, 1932, **55,** 555.
17. Kendall, F. E., *J. Clin. Inv.*, 1937, **16,** 921.
18. Munoz, J., and Becker, E. L., *J. Immunol.*, 1950, **65,** 47.
19. Wolf, A., Kabat, E. A., Bezer, A. E., and Fonseca, J. R. C., *Fed. Proc.*, 1951, **10,** 375.
20. Jager, B. V., Smith, E. L., Nickerson, M., and Brown, D. M., *J. Biol. Chem.*, 1948, **176,** 1187.
21. Moore, D. H., *J. Biol. Chem.* 1945, **161,** 21.