AN ELECTROPHORETIC STUDY OF THE PROTEIN COMPONENTS IN CEREBROSPINAL FLUID AND THEIR RELATIONSHIP TO THE SERUM PROTEINS[1]

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(Received for publication May 2, 1942)

Increases in the total protein content of cerebrospinal fluid frequently occur in diseases of the nervous system, and the determination of total protein content is one of the most useful diagnostic laboratory procedures to the neurologist (1). The origin of the cerebrospinal fluid protein is usually considered to be the blood plasma and the increases in total protein content in meningitis are believed due to transudation of serum through the walls of inflamed vessels (1). In other conditions, such as polyneuritis, the reason for the elevated protein content is not clear (1). There are few data available regarding the composition of the cerebrospinal fluid proteins, chiefly because the small amounts of protein present make chemical studies difficult. Freund (2) has shown that the titre of antibody in the spinal fluid of passively immunized rabbits is about 1/300 that of the serum, which is of the same order as the ratio of the total protein of spinal fluid to that of serum. By the use of the Tiselius electrophoresis technic (3), the authors were able to demonstrate that the electrophoretic patterns of concentrated cerebrospinal fluids were similar to those of serum (4). The present report covers a more extensive study of the effect of variations in the serum proteins on the composition of the cerebrospinal fluid proteins, and of the changes in the proportions of the cerebrospinal fluid proteins in various neurological conditions. The fraction of cerebrospinal fluid associated with abnormal colloidal gold activity is identified, and the inhibiting effects of the albumin fraction on the colloidal gold reaction are considered.

EXPERIMENTAL

Cerebrospinal fluids were obtained from patients on whom encephalography or ventriculography was performed. Volumes of cerebrospinal fluid as large as 80 ml. were obtained in this manner. Samples were taken for the colloidal gold test and for determination of the total protein content. The rest of the fluid was then placed in a cellophane dialyzing membrane with a one hole rubber stopper at one end and a solid rubber stopper at the other. The membrane was connected by rubber and glass tubing to a small tank of nitrogen, and a gas pressure of 5 pounds per sq. inch (about 250 mm. Hg) was constantly maintained on the inside of the membrane. The membrane was immersed in saline so that it was completely covered and the entire apparatus was kept in the icebox at about 5° C. Under these conditions it was found possible to concentrate the cerebrospinal fluids from 70 ml. to about 2 ml. in about 2 to 3 days. Several fluids may be concentrated simultaneously by the use of a "Y" tube connecting to the nitrogen tank. It is necessary to test the membranes by applying 5 pounds pressure for several hours before adding the spinal fluid to avoid loss of the specimen in weak membranes or through the connections. After the fluid is concentrated to the desired volume, it is redialyzed against a solution containing 0.15 M NaCl + 0.02 M phosphate buffer at pH 7.4 and is studied in the Tiselius electrophoresis apparatus, using a microcell of 2 ml. capacity.

Serum samples were diluted about 1:4 and dialyzed against the same saline phosphate buffer used for the spinal fluids.

The Tiselius electrophoresis cell (3) consists of a U-tube having a rectangular cross-section, the end walls of which have high optical quality so that light refraction caused by concentration gradients in the solution can be detected accurately. The concentrated cerebrospinal fluid is placed in the bottom half of the U-tube underneath a buffer against which it has been previously dialyzed until its conductivity and pH have assumed approximately the same value as that of the buffer. To facilitate filling and recovery of material after separation, the electrophoresis cell (U-tube) is divided into sections with sliding flange plates, so that each section may be sealed off from the rest of the system. The tops of the U-tube connect to large buffer vessels containing electrodes. The whole is placed in a water bath thermostatically maintained at a low temperature (1.5° C. in our work). A boundary is formed by aligning the section containing the protein solution with the rest of the cell. A regulated voltage applied to the electrodes causes a constant current to flow. Convectional disturbances due to heat are mini-

[1] Aided in part by a grant from the William J. Matheson Commission.

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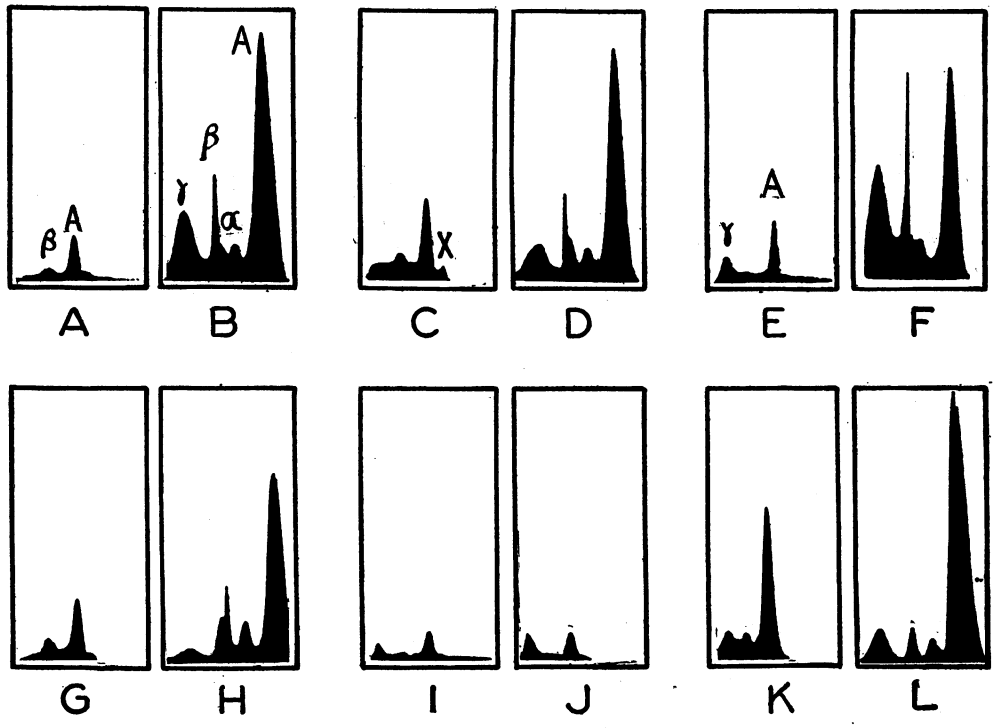

FIG. 1. ELECTROPHORETIC PATTERNS

Case number 661981—Idiopathic grand mal (normal)	A Spinal fluid	B Serum
647635—Anxiety state (normal)	C Spinal fluid	D Serum
652282—Lymphopathia venereum	E Spinal fluid	F Serum
661095—Multiple myeloma	G Spinal fluid	H Serum
WH 138—Multiple sclerosis	I Spinal fluid	
655751—Neurosyphilis	J Spinal fluid	
635202—Diabetic neuritis	K Spinal fluid	
652371—Left frontal cystic astrocytoma	L Cyst fluid	

mized by maintaining the mean temperature (temperature is highest at center and lowest at walls of cells) of the solution at the point where the change of density with temperature is least (temperature of maximum density). Convection limits the wattage which may be dissipated in the cell.

The current produces an electrical field throughout the cell. If the cell has a uniform cross-sectional area, Acm^2, the field E equals I/KA where I is the current in amperes and K is the specific conductivity of the solution in reciprocal ohms. This electrical field causes the different protein components of the solution to migrate at a rate proportional to the statistical surface charge per unit area of the particles or molecules, and in a direction determined by the sign of this charge. This rate of migration, in cm. per second per unit field in volts per cm., is designated as the mobility of the component.

Bending of light rays caused by concentration gradients at the boundaries of each component may be detected by various optical methods; in this study, the Toepler *"Schlieren"* method, as modified by Longsworth (5), was used. With this method each electrophoretically distinct protein component gives a symmetrical curve, the area of which is the total refractive index change and is proportional to the concentration of the protein component. By integrating the area for each component in the patterns (Figure 1), the relative concentrations may be obtained, assuming that the specific index of refraction of all components is the same.

After the migration is complete, samples of pure individual components may be removed from the U-tube and tested for colloidal gold activity, etc.

The total protein content of the cerebrospinal fluid was determined turbidimetrically by precipitation with sulfosalicylic acid. The concentration of any electrophoretic component in the original spinal fluid was obtained by multiplying the total protein content of the fluid by the percentage composition of that component as calculated from the electrophoretic pattern.

The colloidal gold tests were carried out in the usual manner, by addition of 2.5 ml. colloidal gold solution to 0.5 ml. of spinal fluid dilutions of 1:10, 1:20, 1:40 . . . 1:5120. The tubes were allowed to stand at room temperature and read after 24 hours. The color changes

TABLE I

Mobilities and concentrations of protein components in cerebrospinal fluid and in serum

Case number	Diagnosis	Volume of fluid used	pH	Mobility $u \times 10^5$						Per cent composition							Concentration in original spinal fluid						Colloidal gold reaction on original spinal fluid
				χ	A	α	β	ϕ	γ	χ	A	α	β	ϕ	γ	A/G	Total protein (found)	A	α	β	ϕ	γ	
		ml.															*mgm. per 100 ml.*						
661981	Idiopathic grand mal	65	7.4		5.5		2.8		0.9(5)		71.1		22.6		6.3	2.5	27	19		6		2	1100000000
661981 (serum)			7.4		5.2	3.7	2.6		0.8		60.7	6.5	12.6		20.2	1.5							
371706	Anxiety state	63	7.4	7.0	5.5	4.1	2.9		0.9	5.2	67.3	5.1	20.4		7.2	2.1	23	15		5		2	1100000000
371706 (serum)			7.4		4.9	3.4	2.7 2.4		0.7		61.0	8.5	2.9 13.5		14.1	1.6							
661917	Idiopathic cortical seizures	67	7.4		6.2		3.4	1.5	0.9		62.4		26.2	5.4	11.3	1.7	30	19		7	2	3	1100000000
661917 (serum)			7.4		4.8	3.2	2.2		0.7		67.3	9.9	8.8		14.0	2.1							
647635	Anxiety state	60	7.4	8.1	6.0		3.2		1.1	7.6	58.4		25.8		8.2	1.7	38	22		10		3	1100000000
647635 (serum)			7.4		5.1	3.8	2.7 2.4		0.9		61.0	7.9	6.1 7.8		17.3	1.6							
652282	Lymphopathia venereum	16	7.4		5.9		3.1		1.0		56.3		12.0		31.8	1.3	90	51		11		29	1111222100
652282 (serum)			7.5		5.0	3.2	2.3		0.6		39.6	8.4	17.7		34.3	.7							
643966	Lymphopathia venereum	25	7.3		5.5	4.1	2.7		0.4		42.4	9.4	21.9		26.5	0.7	27	12	3	6		7	1100000000
643966 (serum)			7.3		4.8	3.3	2.9 2.4	1.6*	0.5		34.0	3.6	3.3 9.0	11.7	38.4	0.5							
655646	Neurosyphilis	34	7.3		5.4	4.7 3.9	2.5		0.2		49.6	5.0	7.2		38.2	1.0	55	27	3	4		21	4334421100
655646 (serum)			7.3		5.4	3.6	2.9 2.5		0.8		60.7	12.3	5.2 16.9		11.0	1.6							
660660	Cirrhosis	25	7.4		5.7		2.8		1.0		50.6		19.5		29.9	1.0	37	19		7		11	1110000000
660660 (serum)			7.4		5.5	3.7	2.6 2.0	1.2*	0.8		36.0	6.9	25.6 11.4	9.3	10.8	.6							
657549	Lues; arsenical encephalitis	11.5	7.4		5.3		2.5		0.5		61.4		13.7		25.0	1.6	92	56		13		23	1112211000
657549 (serum)			7.3		4.9	3.1	2.6		0.5		45.2	9.1	13.8		31.9	.8							
661095	Multiple myeloma	33	7.4		5.4		2.5				66.4		33.7				38	25		13			0000000000
661095 (serum)			7.4		5.4	3.7	2.8 2.5		0.9		67.9	9.9	9.8 7.5		4.9								

* Not fibrinogen.

Where two values are given, the values refer to mobility or composition of α_1 and α_2 or β_1 and β_2 components.

were expressed by numbers as follows: 0—no change, 1—reddish blue, 2—lilac, 3—blue, 4—pale blue, 5—colorless. The results are given by the values recorded for each tube, beginning with the highest concentration of spinal fluid, as in the usual procedure.

RESULTS

Table I shows the mobilities and concentrations of the protein components in cerebrospinal fluid and serum from a number of individuals. The first 4 cases listed showed relatively normal levels of total spinal fluid protein and may be taken to represent the composition of normal cerebrospinal fluids. It can be seen from the table that the mobilities of the components in the cerebrospinal fluid correspond closely to those of the blood, except that alpha globulin and fibrinogen are frequently absent. In addition, some spinal fluids contained a small amount of a component designated as "x," with a mobility faster than that of the albumin (Figure 1, A and C). The percentage composition and the amount of each fraction in mgm. per 100 ml. are also included. The effect of changes in the amounts of the serum protein components on the composition of the spinal fluid proteins is illustrated in the cases of lymphogranuloma venereum (Figure 1, E and F) and cirrhosis (Table I). It will be noted that increases in the gamma globulin of the serum are accompanied by an elevated gamma globulin in the spinal fluid and a lower A/G ratio, but that the spinal fluid changes are not as marked as are those of the blood. Similarly, in the case of multiple myeloma, an increased beta globulin in the serum was accompanied by an increase in beta globulin in the spinal fluid (Figure 1, G and H). In the case of organic disease of the nervous system, such as neurosyphilis (case 655646), which showed colloidal gold changes in the spinal fluid, the increase in gamma globulin was not a reflection of the blood picture.

The results obtained by electrophoretic analysis of a series of normal and pathological spinal fluids are shown in Table II. The first 5 fluids may be considered as normal. As in Table I, it may be noted that alpha globulin and fibrinogen are not usually seen in normal cerebrospinal fluids, but appear in those with elevated total protein content. The data obtained with fluids from 5 cases of multiple sclerosis showed no marked change from the normal except in one case which showed colloidal gold changes (WH138), and which was found to have a high gamma globulin (Figure 2) and an A/G ratio of 0.8. The serum of this case also showed a high globulin. Three of the 4 cases of neurosyphilis in Table II, as well as the case in Table I, showed a high gamma globulin (Figure 1, J) and colloidal gold changes were observed in those cases with the highest gamma globulins. In these cases, the A/G ratio of the spinal fluid was also very low.

Four cases of brain tumors showed elevated total protein and it was found that the bulk of this increase was due to albumin, with slight increases in the amount of the other components. A fifth case showed a relatively normal cerebrospinal fluid pattern and the diagnosis of brain tumor was not made from the cerebrospinal fluid. The last 3 values in the table were obtained by using the fluids from cystic tumors. These fluids had protein contents of 5, 4, and 6 per cent respectively and were diluted with buffer to about 2 per cent. These cyst fluids also showed very high albumin peaks and high A/G ratios (Figure 1, L).

The data obtained with 5 cases of peripheral neuritis, with a high total protein, also showed that the bulk of this increase was due to albumin, with some increase in the other protein components (Figure 1, K). In all but one of these cases, alpha globulin was present. In one case complicated by syphilis, an elevated gamma globulin was also found.

A case of meningitis of unknown etiology showed a normal A/G ratio with a very high total protein content and an increase in all components.

The relationship of the colloidal gold activity to the composition of cerebrospinal fluid is shown in Table III. Pure albumin and gamma globulin fractions were obtained from the electrophoresis cell and tested for colloidal gold activity. The middle fraction was a slightly changed spinal fluid due to the migration of the various components. It can be seen that in no case was activity found in the albumin fraction, and that the separated gamma globulin had a higher activity than either the original spinal fluid or the middle fraction. This indicates that the colloidal gold activity migrated with the gamma globulin fraction, and that

TABLE II

Mobilities and concentrations of the protein components of normal and pathological cerebrospinal fluids

Case number	Diagnosis	Volume of fluid used	pH	Mobilities $u \times 10^5$					Per cent composition						Concentration in original spinal fluid						Colloidal gold curve on whole spinal fluid
				A	α	β	φ	γ	A	α	β	φ	γ	A/G	Total protein (found)	A	α (calculated)	β (calculated)	φ (calculated)	γ (calculated)	
		ml.													*mgm. per 100 ml.*						
658957	Post traumatic headache	29	7.3	5.9		3.1		0.8	68.6		19.8		11.6	2.2	38	26		7		4	1100000000
652366	Convulsive disorder	60	7.3	5.9		2.9		0.7	62.4	6.7	13.4		17.4	1.7	31	19	2	4		6	1100000000
644432	Pituitary chromophobe adenoma	46	7.4	6.0		3.0		0.5	58.4		23.4		18.2	1.4	31	18		7		6	1100000000
658958	Post traumatic headache	58	7.4	5.9		3.0		0.8	59.6		22.7		13.1	1.5	30	18		7		4	1100000000
647565	Right hemiparesis unexplained	65	7.3	6.0		3.0		1.0	64.0		20.3		15.6	1.8	37	24		7		6	
649400	Chronic encephalitis	70	7.4	6.6	3.8	2.9		0.8	62.5		22.9		14.6	1.7	16	10		4		2	1100000000
646398	Multiple sclerosis	37	7.3	5.8		3.0		0.4	56.3		21.1		22.7	1.3	29	16		6		7	1100000000
643497	Multiple sclerosis	64	7.3	6.2		3.3		1.2	63.8		22.9		13.3	1.8	39	25		9		5	1100000000
WH 138	Multiple sclerosis	18.5	7.4	5.5	3.8	2.8		0.3	45.3	6.0	15.5		33.4	0.8	40	18	2	6		13	2222100000
661808	Multiple sclerosis	16	7.4	5.7		3.1		1.1	66.7		18.2		15.1	2.0	72	48		13		11	1100000000
646550	Multiple sclerosis	60	7.3	5.4	3.9	3.1	1.9	0.7	73.9	5.5	9.3	1.7	9.6	3.0	110	81	6	10		11	1100000000
649471	Neurosyphilis	58	7.3	5.6		3.0		1.1	75.4		17.3		7.3	3.1	52	39		9		4	1100000000
WH 115	Meningovascular neurosyphilis	18.5	7.4	5.7	3.8	3.0		0.6	53.7	7.4	13.3		25.6	1.2	60	32	4	8		16	1100000000
659043	Neurosyphilis	33	7.4	5.8	3.9	2.9		0.9	43.5	4.1	9.6		42.8	0.8	70	30	3	7		30	1122100000
655751	Neurosyphilis	10	7.3	5.8				0.8	43.8				56.2	0.8	92	40				52	5555544210
649463	Tuberculum sellae meningioma	43	7.3	5.8		3.0		0.9	67.4		19.8		12.7	2.1	39	26		8		5	1100000000
654204	Glioblastoma multiforme	45	7.3	5.5	3.7	2.9	1.9	0.7	67.4	6.9	7.9	5.2	12.5	2.1	70	47	5	6	4	9	1100000000
653185	Right occipital meningioma	44	7.2	5.2		2.7		0.7	73.6		15.4		11.0	2.8	68	50		10		8	1100000000
652211	Right temporal parietal astrocytoma	50	7.3	5.2	4.0	2.9		0.7	80.6		9.8		9.6	4.2	116	94		11		11	1100000000
625056	4th ventricle astrocytoma	9	7.4	5.5	4.0	3.0	1.8	0.6	64.6	11.4	6.5	4.1	13.4	2.1	210	136	24	14	9	28	1100000000
655632	Myelo-radiculitis of lumbo-sacral region	17	7.3	5.4	4.0	2.8		0.8	68.8	5.9	8.3		7.1	2.2	94	65	6	8		7	1100000000
652235	Diabetic neuritis	15	7.4	6.5		3.6	1.6	0.5	69.2		17.1	7.4	6.4	3.0	154	106		26	11	10	1100000000
644499	Polyneuritis	16.5	7.4	5.8	3.9	2.8	1.9	0.7	65.9	8.5	11.4	3.8	10.4	2.2	198	130	17	23	7	23	1100000000
635202I	Diabetic neuritis	13	7.3	5.4	4.0	2.7	1.8	0.6	80.2	3.3	7.7	3.3	5.5	4.9	216	173	7	17	12	11	1100000000
635202II	Diabetic neuritis	7.9	7.3	5.3	4.1	2.7	2.1	0.7	76.1	4.2	5.6	4.0	10.1	3.2	226	172	9	12	9	23	
661381	Peripheral neuritis of syphilitic origin	4.5	7.4	5.9	4.6	3.0		0.4	64.5	8.4	14.0		13.1	1.8	400	258	34	56		52	1112221110*
659002	Meningitis of unknown etiology	2	7.4	5.1	4.0	2.7		0.2	67.3	5.1	10.2		17.4	2.1	768	518	39	79		134	1111122211
648238**	Cerebellar tumor	2	7.3	5.2	3.9	2.7		0.7	66.1	6.7	7.9		14.1	2.3							
652374‡	Left frontal cystic astrocytoma	2	7.3	5.0	3.6	2.5		0.8	71.8	6.1	8.0		14.1	2.6							
655651†	Left temporo-parietal cystic astrocytoma	2	7.7	6.8	5.3	3.7		0.3	71.1	4.8	10.5		12.0	2.6							

* On specimen of spinal fluid taken a week earlier. **Showed 5.3 per cent of a component of mobility 8.4. ‡Cyst fluid.
† Cyst fluid; barbital buffer used.

TABLE III

Colloidal gold curves on electrophoretic fractions of concentrated spinal fluids

Case number	Diagnosis	Colloidal gold curves			
		Original unconcentrated spinal fluid	Albumin	Middle	Gamma
661981	Idiopathic grand mal	1100000000	0000000000	0000000000	1112210000
661917	Idiopathic cortical seizures	1100000000	0000000000	0000000000	3321000000
647635	Anxiety state	1100000000	0000000000		0000000000*
658958	Post traumatic headache	1100000000	0000000000	0000000000	0000000000
635202	Polyneuritis	1100000000	0000000000	0000000000	1112332210
660660	Cirrhosis	1110000000	0000000000	1111222110	1243210000
661808	Multiple sclerosis	1100000000	0000000000	2112221100	5432100000
WH 138	Multiple sclerosis	2222100000	0000000000	3322221100	5553321000
655751	Neurosyphilis	5555544210	0000000000	5554332110	5555432100
659043	Neurosyphilis	1122100000	0000000000	3332233321	5554431100
657549	Lues; arsenical encephalitis	1112211000	0000000000	1111222210	5555432100
659002	Neurosyphilis	1111122211	0000000000	1112221000	5554321000

* Whole globulin.

the albumin had an inhibiting effect on the colloidal gold reaction. In gamma globulin samples obtained from sera showing colloidal gold changes, it was possible completely to abolish the colloidal gold reaction by adding albumin. Details will be published in a separate communication.

DISCUSSION

The observation that changes in the electrophoretic patterns of the spinal fluid are influenced by changes in the composition of the serum proteins strongly indicates that much of the spinal fluid protein is derived from the blood. The order of magnitudes of the ratios of spinal fluid gamma globulin to serum gamma globulin are in agreement with the average of 1:300, found by Freund, for the distribution of antityphoid agglutinins between the spinal fluid and serum of rabbits. This is not surprising as human antipneumococcus antibody and rabbit antibodies to several types of antigens were found to have the same molecular weights as their respective gamma globulins (6), and hence would be distributed on both sides of the hemato-encephalic barrier to the same extent.

The data obtained in neurosyphilis (case 655646), however, suggest that not all of the spinal fluid protein is derived from the blood. In this case, the A/G ratio of the spinal fluid was much lower than that of the blood and the percentage of gamma globulin was much higher than that in the serum. This spinal fluid also showed colloidal gold changes which are associated with increases in the gamma globulin. The data would suggest that some formation of gamma globulin could take place within the tissues of the central nervous system and be poured into cerebrospinal fluid, since it is difficult to imagine an altered permeability of the hemato-encephalic barrier which could produce an increase in gamma globulin without producing the same or an even greater increase in the smaller albumin molecule. The formation of some gamma globulin in the central nervous system is in accord with the views of Katzenelbogen (7) that cerebrospinal tissues are capable of producing antibodies, and that the origin of the antibodies in the cerebrospinal fluid is twofold, from the blood and from the cerebrospinal tissues. It also agrees with the views of Sabin (8) who has suggested that antibodies and normal gamma globulin are formed in the cells of the reticulo-endothelial system by a partial shedding of their surface cytoplasm. This hypothesis will explain the electrophoretic data as well as the fact that positive Wasserman reactions may be obtained frequently in spinal fluid, but not in the serum, in cases of neurosyphilis.

SUMMARY

1. The electrophoretic pattern of cerebrospinal fluid resembles that of serum.

2. Alterations in the composition of the protein components of serum are reflected in the cerebrospinal fluid, but the changes are not as marked.

In neurosyphilis, however, an increased gamma globulin occurs in cerebrospinal fluid, without similar changes in the blood stream.

3. Colloidal gold activity is associated with the gamma globulin fraction, and albumin has an inhibiting effect on the colloidal gold reaction.

The authors are indebted to Drs. Tracy J. Putnam and Robert F. Loeb for many suggestions and for assistance in obtaining material. The colloidal gold tests were carried out by Miss Ruth Shivitz of the Department of Bacteriology, and Misses Sheila Goldsmith and Helen Sikorski assisted in the electrophoretic experiments.

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