demics. This serves further to set influenza C apart from influenza A or B as a distinct entity. Studies on the biological and immunological characteristics of the GL 899C strain of influenza C indicate its close resemblance to the JJ strain of Francis(2). Its relationship to the 1233 strain of Taylor(1) is under study.

Neither influenza B nor C viruses isolated at Great Lakes during 1952 seemed to possess marked virulence as only a small fraction of men showing diagnostic antibody rises had clinical illness of any degree of severity. However, in view of the past experience with influenza A and B, it is conceivable that the pathogenicity of the influenza C virus may change in subsequent years or in different localities. Thus, it would appear justifiable to look for this influenza virus strain with other etiological agents when investigating epidemic respiratory disease.

Summary. Data have been presented on the occurrence of influenza in Navy recruits at Great Lakes Naval Training Center during the winter of 1951-52. Serological evidence of occurrence of epidemic influenza B was confirmed by the isolation of 11 viruses which appear to resemble the 1210 strain of influenza B. The isolation of 14 strains characterized as influenza C and the serological evidence that influenza C was also epidemic are presented. One of these viruses, the GL 899C strain, was studied in more detail. Its biological and immunological properties and its relation to previously isolated strains were studied. Data were also presented indicating that influenza C infections occurred in other localities throughout the United States. The importance of performing routinely the CRC agglutination and CRC agglutination-inhibition tests at 4°C for the detection of influenza C viruses and antibody titer is stressed.

1. Taylor, R. M., *Am. J. Pub. Health,* 1949, v39, 171.

2. Francis, T., Jr., Quilligan, J. J., and Minuse, E., *Science,* 1950, v112, 495.

3. Salk, J. E., *J. Immunol.,* 1944, v49, 87.

4. Hirst, G. K., and Pickels, E. G., *J. Immunol.,* 1942, v45, 273.

5. Report of the World Health Organization Influenza Study Program in the United States, July 1, 1951-June 30, 1952. Influenza Information Center, National Institutes of Health.

Received October 20, 1952. P.S.E.B.M., 1952, v81.

Electrophoretic Studies on Cerebrospinal Fluid.* (19966)

HANS HOCH AND ALFRED CHANUTIN.
(With the technical assistance of Titus Pankey, Jr.)
From the Biochemical Laboratory, School of Medicine, University of Virginia, Charlottesville, Va.

In a previous publication(1), a component (X-protein), migrating faster than albumin, was found to be a normal constituent of cerebrospinal fluid. This component has also been observed in a few cases by earlier workers(2,3). Labhart, Schweizer and Staub(4) analyzed 178 cerebrospinal fluids of healthy and diseased individuals with the aid of the Jamin interferometric optical system and failed to describe the presence of the rapidly migrating component. Recently Bücher, Matzelt and Pette(5), using paper strip electrophoresis, noted a rapidly migrating component in each of 10 cerebrospinal fluids which were classified as "normal". In the present paper, cerebrospinal fluids of patients with neurological disturbances were analyzed in the Tiselius apparatus in order to study the distribution of the components, particularly those migrating faster than albumin.

Method. Cerebrospinal fluids (50-220 ml) were obtained from patients undergoing pneumoencephalograms for diagnosis. Each fluid was centrifuged to remove cells and concentrated by pressure dialysis to final volumes varying from 3 to 5 ml. The sample was dialyzed against phosphate buffer of pH 7.85

* This investigation was supported by Research Contract, V100M-1074 Veterans Administration.

TABLE I. Determination of γ-Globulin in Cerebrospinal Fluid by Elimination of δ-Boundary.

		Planimeter units			
		γ + δ or ε	Other components	Total	γ + δ or ε fraction of total area
Control*	Asc.	67, 77	205, 257	298, 334	.225, .230
	Desc.	26, 27	264, 308	290, 335	.099, .081
12% H_2O	Asc.	42	264	306	.137
	Desc.	17	255	272	.063
14% H_2O beginning convections	Asc.	22	255	277	.079
	Desc.	16	250	266	.060
16% H_2O	Asc.	21	242	263	.080
	Desc.	12	235	247	.049

	Calculated (from ascending limb) γ-globulin	
	(I)†	(II)†
Control	.04, .04	.12, .11

* Total protein conc., 1.15 g %.

† Calculated from equations: (I) $x = c.f.$; (II) $x^2 - x + \frac{A_A \cdot c.f.}{A_t} = 0$, where x is the size of the δ-boundary expressed as the fraction of the total area, A_A and A_t are the areas for the albumin and total pattern respectively, c is the protein concentration in g % and f is the size of the δ-boundary for a 1% albumin solution, expressed as the fraction of the total area.

and ionic strength 0.05. The protein concentrations of these samples, estimated with an Abbe refractometer, varied between 0.4 and 1.3 g %. The concentrated fluids were subjected to electrophoresis in a micro cell of 1.5 x 15 mm cross section and 50 mm height for 45 minutes at 10 volts/cm in a Klett model of the Tiselius apparatus, which was equipped with the Longsworth schlieren scanning mechanism. The ascending patterns only were evaluated and the components were classified in the same manner as are those of plasma.

Results. Interpretation of patterns. It has been shown previously that boundary anomalies may be utilized for demonstrating and separating rapidly migrating trace components (6). These anomalies are pronounced in respect to the albumin and the faster components in a phosphate buffer at pH 7.85 and ionic strength of 0.05 and they cause the apparent concentrations of the faster components to increase at the expense of the slower components(7,8). This phenomenon accounts for the present success in demonstrating at least one fast migrating component in every cerebrospinal fluid, in contrast to the results obtained with other buffers by earlier workers (2,3).

The cerebrospinal fluid patterns could not be analyzed by the standard procedure because the γ-globulin did not separate from the salt boundaries. The values for γ-globulin were obtained by subtracting the values for the δ-boundary which could be bracketed between two possible limits. These limits were obtained by assuming that, I) all proteins produce as large a δ-boundary as would be given by the same concentration of albumin, or II) the albumin alone contributes to the δ-boundary. The relative size of the area of the δ-boundary was determined for 4 bovine albumin (Armour) solutions, varying

TABLE II. Apparent Concentrations of Components at Different Protein Concentrations (% of Total Area).

	Total protein conc. (g %)								
	.50		.67		1.1		2		
	I	II	I	II	I	II	I	II	III
X	5	5	8	7	8	8	9	9	9
A	58	56	58	54	66	60	73	73	70
α_2	9	9	12	11	9	8	7	6	6
β	9	9	11	11	14	13	11	11	10
γ	19	21	11	17	3	11	0	1†	5*

* γ-globulin separated from δ-boundary; δ-boundary measured directly.

† If the error in the total protein were 10%, the value for the γ-globulin would be raised to 5%.

TABLE III. Comparison of Analyses of Cerebrospinal Fluids in Phosphate pH 7.8, Γ/2, .05 and in Veronal pH 8.5, Γ/2, 0.1.

Total protein, mg %	Buffer	Protein conc. in cell, g %	Limb	X I	X II	A I	A II	α_1	α_2 I	α_2 II	β I	β II	τ I	τ II	γ I	γ II
400	P*	1	Asc.	3	3	53	48		(a)		6	5	4	4	34	40
	V	1.40	Asc.	0		56.3		2.3	2.6		4		(b)		34.8	
			Desc.	0		56		2.8	2.5		5.6		(b)		33.1	
36	P	1.36	Asc.	16	14	60	50		9	7	11	9	4	4	0	16
		.55	Asc.	11	10	56	54		10	9	10	9	4	4	9	14
	V	1.	Asc.	2.8		55.4		8	9.2		20.4		(b)		4	
			Desc.	Not evaluated												
35	P	.55	Asc.	9	9	68	67		9	9	11	10	3	3	0	2
	V	.8	Asc.	4.1		55.8		7.7	9.8		14.4		(c)		8.2	
			Desc.	2.8		63.7		8.4	9.5		10		(c)		5.6	

(a) Low concentration of α_2-globulin, included in β-area.
(b) " " " τ-component, " " γ- " .
(c) Not resolved.
* P = Phosphate; V = Veronal.

in concentration between 0.40 and 1.25 g %. A plot of the value for the fraction of the total area occupied by the δ-boundary versus the albumin concentration yielded 0.171 per 1 g % change in albumin concentration. The γ-globulin concentration was also determined directly after eliminating the δ-boundary by adding water to the dialyzed concentrated cerebrospinal fluid(9). With increasing amounts of water, the (γ + δ)-peak decreased until at 14% water, the appearance of small spikes at the rear ends of the patterns indicated convection currents due to the negative salt boundaries (Table I). At this point, the values for γ-globulin fell between 4 and 12% of the total area, the range bracketed by (I) and (II).

The apparent percentages of the components at different concentrations of total protein of the same sample of fluid are shown in Table II. The percentage areas for α_2 and β-globulins are approximately the same for all protein concentrations. At the highest protein concentration (2%), the X-protein is about twice and the γ-globulin about one fourth as large as observed at the lowest concentration (0.5%). The data in Tables II and III are uncorrected for boundary anomalies. It would be difficult to apply more than crude corrections to the apparent concentrations unless the corrections were obtained experimentally from sets of measurements on similarly constituted fluids. From the data shown in Table II, the true values for X-protein and γ-globulins in Tables III, V and VI may be estimated to be ½ to ⅔ and 1 to 2 times their respective apparent values.

Analyses in phosphate and veronal buffers are compared in Table III. The corrected values for X-protein in phosphate are slightly greater than those in veronal. The uncertainty in determining the size of the δ-boundary in the phosphate buffer is reflected in the lack of agreement of the γ-globulin values in the two buffers.

The relative migration velocity of X-protein with reference to the velocity of albumin V_X/V_{Alb}, depended on the concentration of albumin (Table IV). This relationship agreed qualitatively with theoretical considerations(7,8). The relative velocities of the other components were as follows: α_2, 0.79; β, 0.57; τ, 0.42; the γ-globulin boundary was poorly resolved from the σ-boundary. The corresponding values for plasma were: α_2; 0.84; β, 0.55; ø, 0.30 and γ, 0.24. In one cerebrospinal fluid, the γ-globulin migrated much more slowly than the γ-globulin of

TABLE IV. Relative Migration Velocities of X-Protein and Albumin.

Conc. in cell, g %				
Protein	Albumin	V_A*	V_X*	V_X/V_A
.5	.3	1	1.24	1.24
1.3	.7	1.08	1.25	1.15
2.6	1.4	1.14	1.28	1.12

* Arbitrary units.

TABLE V. "General Type" Cerebrospinal Fluid Patterns.

Total protein, Original (In cell) Range	Mean		% I Range	% I Mean	% II Range	% II Mean	mg protein/100 ml I Range	mg protein/100 ml I Mean	mg protein/100 ml II Range	mg protein/100 ml II Mean
0-29 (400-1300)	20 (800)	X	7-17	11.7	6-16	11 (36)	1.3- 3.8	2.4	1.2- 3.5	2.2
		A	47-72	58.9	43-69	55.4(36)	4.4-19.5	12.1	4.2-18.7	11.4
		α_2	4-16	8.5	4-15	7.7(33)	.8- 2.8	1.7	.8- 2.6	1.6
		β	6-22	12.1	5-20	11.2(33)	.8- 5.1	2.5	.8- 4.6	2.3
		γ	0-18	8.9	8-27	14.7(36)	0 - 4.4	1.7	1 - 5.4	2.9
30-39 (600-1300)	35 (1100)	X	6-20	11.2	5-13	10.1(13)	2.1- 5.9	3.9	1.9- 5.9	3.5
		A	50-70	61.8	43-67	56.6(13)	16.5-25	21.3	14.2-22.8	19.5
		α_2	5-12	8.5	5-11	7.8(13)	1.8- 4.3	2.9	1.8- 4	2.7
		β	8-21	13.8	8-18	12.8(13)	2.4- 7	4.8	2.4- 6.2	4.4
		γ	0-14	4.7	5-23	12.7(13)	0 - 5.2	1.7	1.6- 9	4.3

TABLE VI. Atypical Cerebrospinal Fluid Patterns. The italicized figures represent deviations from "*general type.*"

Protein conc., mg %	Apparent concentrations (%) X I	X II	A I	A II	α_2 I	α_2 II	β I	β II	τ I	τ II	γ I	γ II	Diagnosis
54	10	10	*69*	*64*	5	4	11	10	5	5	0	7	Encephalitis
400	*3*	*3*	53	48	*		6	5	4	4	*34*	*40*	Lymphocytic meningitis
19	15	14	*40*	*37*	14	13	10	9	6	6	15	21	Epilepsy
26	*21*	*19*	56	48	9	7	9	7	3	3	2	16	Convulsions, undiagnosed
29	10	9	53	48	8	8	16	14	**		*13*	*21*†	" "
29	15	12	56	47	8	7	*3*	*3*	1	1	*17*	*30*	Cortical atrophy
29	11	10	53	47	11	10	10	9	3	3	*12*	*21*	Jacksonian epilepsy
34	*4*	*3*	35	30	17	14	12	10	**		*32*	*43*	Headache, undiagnosed
28	*7*	*6*	48	43	10	9	8	8	**		*27*	*34*	Pia arachnoiditis
30	11	10	52	47	9	8	9	8	**		*19*	*27*	Epilepsy
18	*6*	*6*	45	39	12	11	10	9	5	4	*22*	*31*‡	"
22	*7*	*7*	58	56	9	8	9	8	**		*17*	*21*§	Non-thrombocytopenic purpura
28	10	9	59	54	*10*	*9*‖	9	8	4	3	8	17	Meningitis
28	16	15	66	59	5	5	10	9¶	**		3	12	Encephalopathy
22	10	9	62	58	9	8	19	18¶	**		0	7	Subdural hemorrhage
26	12	11	60	54	8	7	18	17¶	**		2	11	Epilepsy

* Low conc. of α_2, included in β-globulin area. † High γ-globulin, separated from δ-boundary. ‡ 3 γ-globulins observed. § 3 γ-globulins observed, separated from δ-boundary. ‖ α_1- and α_2-globulins with increased mobility. ¶ Additional slow β-globulin. ** τ-component included in β-globulin area.

plasma in both phosphate or veronal.

A component, designated as X_1-protein, which migrated 8 to 14% faster than X-protein, was observed in 9 cerebrospinal fluids. A small elevation or a spike, representing either a low concentration of X_1-protein or a convectional disturbance, was present in 4 samples.

Electrophoretic analyses. The majority of cerebrospinal fluids gave patterns of the type shown in Fig. 1a. The ranges in relative areas and protein concentrations of the components are summarized in Table V; these data were arbitrarily divided into 2 groups according to the protein concentrations of the original samples. The relative concentrations of the components of these 2 groups differed very little. Since the ranges did not differ either, this indicated that no one particular component was responsible for the higher protein concentrations.

In 13 of the 36 fluids in the lower protein concentration group (0-29 mg %) and in 8 of the 13 fluids in the higher group (30-39 mg %), the component between β- and γ-globulin, designated as τ, formed a distinct peak and its area ranged between 3 and 8% of the total area. This component was either missing or present in small amounts in the remaining fluids. In a few cases, a component migrated between albumin and X-protein (Fig. 1b). In one instance, a distinct α_1-component was

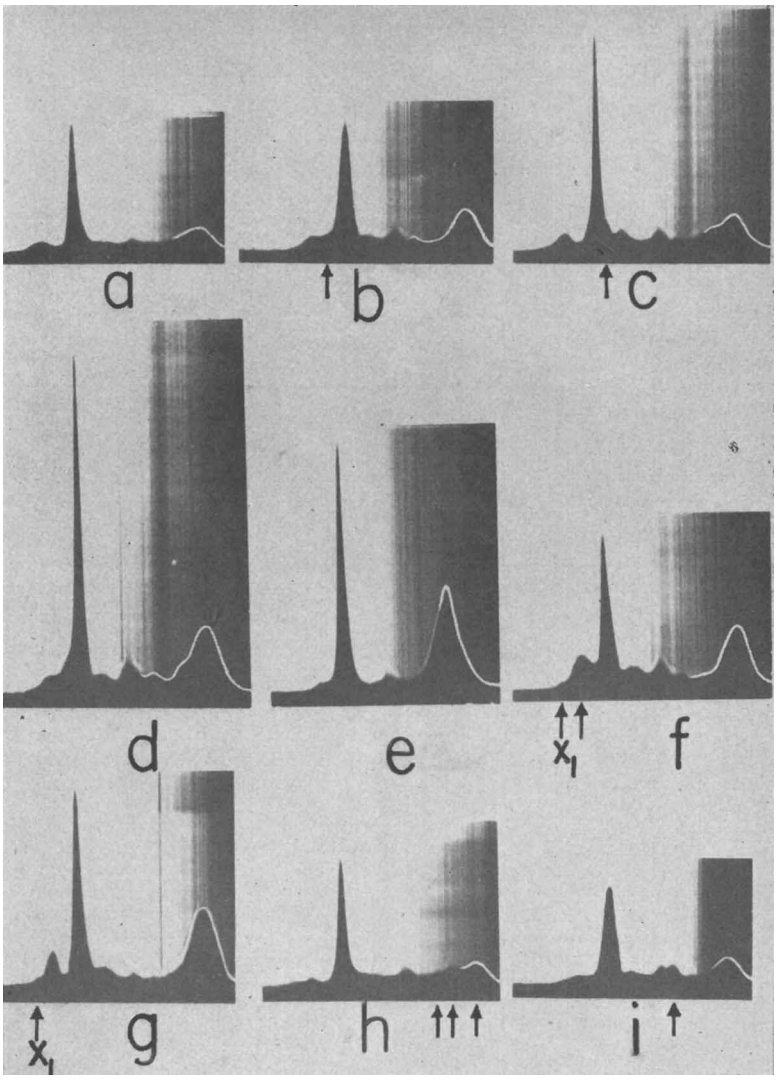

FIG. 1. Atypical electrophoretic patterns of cerebrospinal fluid: (a) Typical pattern; (b) component between albumin and X-protein; (c) α_1 globulin; (d) high total protein; (e) low X-protein, high γ-globulin; (f) high X-protein; (g) low β-globulin, high γ; (h) low X-protein, 3 γ-globulins; (i) additional slow β-globulin.

observed (Fig. 1c).

The incidence of the X_1-component and of the X_1-convection disturbance in the patterns of the cerebrospinal fluids of patients with and without a history of convulsions follows:

	Absent	X_1-protein	Trace of X_1-protein or "X_1-convection"
Convulsive	7	6	3
Non-convulsive	16	3	1

If either X_1-protein or X_1-convections are observed, the patient is more likely to have had convulsions than if X_1-protein is absent ($\chi^2 = 5.0$, $P = 0.02$).

Analytical data for 16 atypical patterns are presented in Table VI. The most frequent changes were: γ-globulin was elevated in 9 fluids, X-protein was decreased in 5 and an additional β-globulin component was present in 3 cases. No relationship could be established between any of these changes and the disease.

Discussion. Bücher *et al.*(5) have demon-

strated the presence of a rapidly migrating component (V) and of a material with a mobility between β- and γ-globulin (τ) in each of 10 cerebrospinal fluids studied with the paper-electrophoresis technic. The rapidly migrating component, designated as X-protein in this laboratory, has been previously described(1) and has been found in each fluid studied in the present investigation. The τ component was noted in less than half of the fluids analyzed. This disagreement with the results of the German workers may possibly be due to the types of patients studied or to the difference in the procedures used for concentrating the cerebrospinal fluid.

Summary. 1. The results of a detailed investigation of the distribution of two rapidly migrating components and of other protein components of cerebrospinal fluids have been presented. A component (X-protein) migrating about 20% faster than the albumin could be demonstrated in every fluid by electrophoresis in phosphate at pH 7.85 and ionic strength 0.05. In addition a component (X_1) migrating 8-14% faster than the X-protein was observed in 20% of the cases. 2. Analyses of 49 patterns of the most frequently encountered type and 16 atypical patterns are presented. No individual protein component was responsible for the higher protein concentration observed in some of the fluids. A possible relationship between the X_1 component and the occurrence of convulsion in patients was indicated.

The authors wish to acknowledge the cooperation of Dr. W. O. Klingman and his associates of the Neurology Clinic; the staff of the Neurosurgery Clinic and the staff of the Roanoke Veterans Hospital for supplying the cerebrospinal fluids.

1. Fisk, A. A., Chanutin, A., and Klingman, W. O., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 1.
2. Kabat, E. A., Moore, D. H., and Landow, H., *J. Clin. Invest.*, 1942, v21, 571.
3. Scheid, K. F., and Scheid, L., *Arch. Psychiat. Nervenkrankh.*, 1944, v117, 219, 641.
4. Labhart, H., Schweizer, W., and Staub, H., *Confinia neurol.*, 1951, v11, 325.
5. Bücher, T., Matzelt, D., and Pette, D., *Klin. Wchnschr.*, 1952, v30, 325.
6. Hoch. H., *Biochem. J.*, 1950, v46, 199.
7. Svensson, H., *Ark. Kemi*, 1946, v22A, No. 10.
8. Dole, V. P., *J. Amer. Chem. Soc.*, 1945, v67, 1119.
9. Longsworth, L. G., *Chem. Rev.*, 1942, v30, 323.

Received October 24, 1952. P.S.E.B.M., 1952, v81.

Simultaneous Multiplication of Two Different Rickettsiae in the Same Cell. (19967)

N. TAKEMORI, M. HENMI, AND M. KITAOKA. (Introduced by H. R. Cox.)
From the National Institute of Health of Japan, Shiba Shirokane Daimachi, Minato-ku, Tokyo, Japan.

The observations of many investigators have amply shown that infection of a host (bacterial, plant, or animal) by one virus may, under certain conditions, afford temporary protection against infection by another, *i.e.*, interference phenomenon. However, interference is not the universal result of duplicate infections, since many instances of dual virus infections have been described(1,2). Several investigators have reported that two different animal viruses can multiply simultaneously in the same cell. By using viruses that form recognizable intracellular inclusions, studies(3,6) were made to demonstrate cytological evidence that individual cells may be invaded by, and become host to, two different viruses. Levaditi and Schoen(4) observed simultaneous appearance of Negri and Guarnieri bodies in the epithelial cells of rabbit cornea. Syverton and Berry(5) showed that cells of the Shope rabbit papilloma can be superinfected by extraneous viruses. McWhorter and Price(7) demonstrated the presence of inclusions of both tobacco mosaic and tobacco etch viruses in the same cells of doubly infected tobacco plants, and concluded that their presence together indicates simultaneous growth of two plant viruses in the