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The cerebrospinal fluid protein

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THE CEREBROSPINAL FLUID PROTEIN

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B.U.S.M. iii
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MEMO TO: Dr. Franz J. Ingelfinger

FROM: Dr. Joseph Foley

SUBJECT: Thesis: "The Cerebrospinal Fluid Protein" by William J. Shapiro.

GRADE 93

An excellent paper providing a very critical review of modern knowledge of the subject. The student has not been afraid to criticize techniques and conclusions. I will discuss this paper personally with the author.

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INTRODUCTION

Interest in the alterations of the cerebrospinal fluid proteins dates back to 1891, when Quincke demonstrated the possibility of using the lumbar puncture as a diagnostic aid in the diseases of the central nervous system. Since that early date, the clinical importance of the protein content of the cerebrospinal fluid has attained such heights as to cause investigators like H. H. Merritt and F. Fremont-Smith (78) to state: "Perhaps the most frequent abnormality of the cerebrospinal fluid is an alteration of its protein content." Spiegel (95) records that "Increase in the protein content of cerebrospinal fluid is probably the earliest and most constant of the abnormalities noted in the fluid under pathological conditions," and A. V. Neel (64) calls attention to the fact that even small changes in the protein concentrations are of diagnostic significance. The cerebrospinal fluid protein pattern is a criterion of the progression of the disease process and of the efficacy of therapy in some neurologic disorders. In the diagnosis of neurosyphilis, three of the five procedures, considered to be essential by many clinicians, are concerned with the proteins. These substances are not only a focus of interest to the neurologist and syphilologist, but also to the orthopod, internist, pediatrician and epidemiologist. To be sure, a diagnosis seldom rests on protein determination alone, but taken together with other tests, a knowledge of the total protein content, globulin and albumin relationship and content of the cerebrospinal fluid is of great value in differential diagnosis.

In spite of the apparent paramount importance of the cerebro-

spinal fluid proteins, one finds very different and even opposed beliefs in regard to what may be considered as fundamental knowledge. There is considerable diversity of opinion in regard to the normal proteins of the cerebrospinal fluid, origin of normal and pathologically increased proteins, normal cerebrospinal protein concentration values, best methods of estimating protein concentration values, protein pattern alterations in diseased states, and lastly, the significance of altered protein patterns. This prevents the clinician from gaining full advantage from an examination of the fluid. It is the purpose of this paper to present and evaluate some of the views expressed on these aspects in order to derive a better understanding of the basic knowledge of another tool in the clinician's armamentarium.

THE PROTEINS OF THE CEREBROSPINAL FLUID

The proteins that have been reported to be found in the cerebrospinal fluid include the albumins, the globulins, fibrinogen, and the albumoses. Before entering upon a discussion of the normal and abnormal proteins, it would be advantageous to consider, briefly, each of these protein groups independently.

The albumins constitute the major portion of the total protein content of cerebrospinal fluid, and they are characterized by their solubility in salt-free water and half-saturated ammonium sulfate solution. Like the globulins, they are coagulated by heat, but unlike the former the albumins are of small molecular size (molecular weight 69,000) (43). It has been stated by several authors (26, 22, 60, 78) that the relatively large concentrations of albumin is due to the easy diffusability of the small albumin molecule through the hemangiothecal barrier.

The globulins are simple proteins which are insoluble in salt-free water, but soluble in neutral saline solutions. Euglobulins, is the term used to designate globulin reacting in such a manner, in contra-distinction to the pseudoglobulin. The latter constitute the globulins which have identical physical properties as the euglobulins but differ in that they are soluble in salt-free water (43). The electrophoretic studies of Tiselius (99) showed that the serum globulins could be broken down into three distinguishable components, designated as alpha, beta and gamma globulins. Later workers have demonstrated that these three globulin fractions may be resolved into further components which have been designated alpha $_1$, and alpha $_2$ globulin, etc.. The alpha globulins are those which in alkaline or neutral solutions have the greatest electrophoretic mobility of this group, and in this respect most nearly resemble the albumins. The beta globulins have an electrophoretic mobility intermediate between that of the alpha and the gamma globulins. The latter, has been shown conclusively to make up most of the antibodies. A typical electrophoretic pattern of human plasma and of normal cerebro-spinal fluid is diagramatically illustrated (fig. 1.). It is usually considered that all the globulins of plasma are precipitated upon half-saturation with ammonium sulfate, or by a 22% concentration of sodium sulfate at 57^0 C., and that the protein remaining in solution is albumin (36).

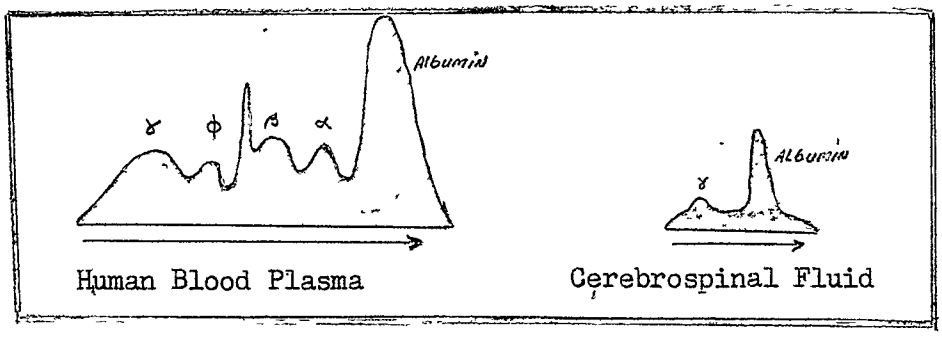

Fig. 1. The distance along the x-axis is a measure

of the relative velocity of movement of the various ion species present, and the height of the peaks corresponds to the differences in the refractive index between the moving boundary and the adjacent fluid. The area under each curve is proportional to the amount of material present moving with an average velocity represented by the position of the peak along the x-axis. This method enables one to distinguish ion species of different mobilities and to estimate the relative amounts of each type of ion present (99).

Fibrinogen is the most unstable protein of the plasma. When acted upon by thrombin, it is converted to a fibrin which separates out in the form of crystal-like needles or threads and forms a reticulum. Electrophoretically, the fibrinogen peak is usually indicated by the symbol ϕ, possessing a mobility which is intermediate between beta and gamma globulins. The only dependable standard method for demonstrating fibrinogen in spinal fluid, at present, is based on its spontaneous coagulation (67). The albumoses are modified proteins, which are similar to the albumins except that they are not heat coaguable.

In regard to the composition of the protein content of the normal fluid, it is generally agreed that albumin forms the major portion, and that there is an absence of fibrinogen. However, there are a few investigators (82, 70, 13) who contend that the globulins are the preponderant cerebrospinal fluid protein, but state that only small quantities are present. Solomon (94), Exton and Rose (25), Neel (84), and Spiegel (95) believe that normal fluid does not contain any globulin. Nickolson (86) states that trace amounts may be found, but most investigators report the presence of more than trace quantities. The electrophoretic studies of Kabat, Landow and Moore (52, 53) demonstrated that the protein components of the cerebrospinal fluid corresponds closely to those of

the blood except that alpha globulin and fibrinogen are absent. Euglobulin is not normally found in the spinal fluid (56, 85). Dattner (15) and Pappenheim (according to Izikowitz (49)) report the occurrence of albumoses but other investigators have not concurred or include them in the group of albumins. In summary, we can say that the normal componints of cerebrospinal fluid proteins are albumin, beta and gamma globulins.

The make-up of the fluid protein in pathological conditions is dependent upon the specific abnormal state. In the acute meningitides, one finds, in addition to the increased albumin and globulin, fibrinogen which exhibits its presence by clot formation in the fluid after removal from the subarachnoid space. Chronic and degenerative diseases of the central nervous system are characterized by increased gamma globulin. Alpha globulin may appear in disorders which present markedly elevated total protein (52, 53), and euglobulins occur in neurosyphilis (66, 15). However, the presence of "abnormal" proteins, i.e., fibrinogen, euglobulin and alpha globulin, is not clinically important as are the alterations of the total protein, globulin and albumin concentrations. Barring the demonstration of fibrinogen by clot formation, the determination of the "abnormal" proteins is infrequently, if ever, performed in a busy cerebrospinal fluid laboratory.

THE ORIGIN OF THE CEREBROSPINAL FLUID PROTEINS

This writer does not wish to enter into the controversy on the origin of the cerebrospinal fluid. Suffice it to say, one school holds to the thought that it is definitely secretory in its formation, based on the histological arrangement of the plexuses and the pharmaco-dynamic effect of certain reputed glandular stimulants on their structure and on

the rate of production of cerebrospinal fluid. Opposing this thought is a group who contend that cerebrospinal fluid is formed by dialysis through a semipermeable membrane, based on the similarity between the composition of an artificial blood ultrafiltrate or dialysate and that of the cerebrospinal fluid. Katzenelbogen (60) compromises both viewpoints and states: "There are, moreover, evidences of spontaneous changes in the function of the barrier between blood and cerebrospinal fluid which suggests a biological functioning, manifesting itself by physiochemical reactions. If neither 'secretion' nor 'dialysis' can be applied to the mechanisms of cerebrospinal fluid-formation, then the term 'physiological or biological permeability' appears to present an adequate expression of a viewpoint that would be a compromise between the concept of secretion and that of dialysis."

The question of the source of the protein, per se, in the cerebrospinal fluid, also, has not been fully answered. The beliefs in this regard differ only in degree, in that, some believe that cerebrospinal fluid protein is derived solely from blood plasma, whereas others feel there is a dual origin; a minor portion coming from the central nervous system proper. There is a great deal of evidence to support blood plasma origin whether it be the partial or the complete source of the protein. It is common knowledge that the liquor is practically a protein-free filtrate of the blood, containing about 0.4% as much protein as plasma, but possessing a higher albumin-globulin ratio. This latter fact is explained perhaps by the smaller molecular size and greater diffusability of albumins. Electrophoretic studies (52, 53) have been most revealing in demonstrating the relationship between blood plasma and cerebrospinal fluid proteins. It is apparent from table 1. (modified from Kabat, Moore and Landow (52)) that the mobilities of the

components in the cerebrospinal fluid correspond to those of the blood except that alpha globulin and fibringgen are absent. The effect of changes in concentration of plasma protein components on the cerebrospinal fluid protein is evident in the cases of cirrhosis and lymphopathia venereum: a reduction of the former resulted in a concomitant reduction of the latter. In the patient with multiple myeloma, an increase of beta globulin in the serum proved to have a similar rise of that protein in the liquor.

TABLE 1. Relationship of Electrophoretic Patterns of Serum and Cerebrospinal Fluid Proteins.

	Mobility u x 10^5					Percent Composition					Diagnosis
	A	α	β	φ	γ	A	α	β	γ	A/G	
CSF	5.5		2.8		0.9	71.1		22.6	6.3	2.5	
Serum	5.2	3.7	2.6		0.8	60.7	6.5	12.6	20.2	1.5	normal
CSF	5.5	4.1	2.9		0.9	67.3	5.1	20.4	7.2	2.1	
Serum	4.9	3.5	2.7		0.7	61.0	8.5	13.5	14.1	1.6	normal
CSF	6.2		3.4	1.5	0.9	62.4		26.2	11.3	1.7	
Serum	4.8	3.2	3.2		0.7	63.3	9.9	8.8	14.0	2.1	normal
CSF	6.0		3.2		1.1	58.4		25.8	8.2	1.7	
Serum	5.1	3.8	2.7		0.9	61.0	7.9	7.8	17.3	1.6	normal
CSF	5.5	4.1	2.7		0.4	42.4	9.4	21.9	26.5	0.7	lymphopath.
Serum	4.8	3.3	2.9		0.5	34.0	3.6	9.0	38.4	0.5	venereum
CSF	5.1		2.8		1.0	50.6		19.5	29.9	1.0	
Serum	5.5	3.7	2.6		0.8	36.0	6.9	11.4	10.8	0.6	cirrhosis
CSF	5.4		2.5			66.4		33.7			multiple
Serum	5.4	3.7	2.8		0.9	67.9	9.9	17.2	4.9		myeloma

After Kabat, Moore, Landow (52).

Freund (36) carried out active and passive immunization in rabbits with intravenous injections of typhoid bacilli and concluded that antibodies penetrate from the general circulation into the cerebrospinal fluid, even in the absence of inflammatory processes in the

meninges. He does not believe that there is production of antibodies, which are for the most part gamma globulins, in the central nervous system. This is significant, as consequently the positive Wassermann reaction of the cerebrospinal fluid would be due to antibodies that come from the blood. Katzenelbogen (60) believes that the cerebrospinal tissues are capable of responding by autonomous elaboration of immune bodies when stimulated by the antigen. It is his contention that antibodies present in the cerebrospinal fluid are likely to be of two-fold origin, from the blood and from the cerebrospinal tissues. Kabat and his associates (52,55) found in a number of patients with neurosyphilis and multiple sclerosis that the albumin-globulin ratio of the fluid was much lower than that of the blood and the per cent of gamma globulin was much higher than that in the serum which suggests that not all spinal fluid protein is derived from blood. They agree with Katzenelbogen and state that it would be: "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." This writer is in favor of the dual origin of the cerebrospinal fluid protein and most evidence supports this view.

The origin of the altered spinal fluid protein pattern has been the subject of speculation in some diseased states and of known certainty in other abnormal conditions. One of the earliest responses to inflammation is an increased permeability of the regional blood vessels such that they become more permeable to proteins. Such an increased permeability of the meningeal blood vessels occurs in the earliest stages of meningitis resulting in an increase in the albumin and globulin content of the fluid and the appearance of fibrinogen.

The albumin molecule is small and a minor increase in the permeability of the hemangiothecal barrier might allow the passage of albumin and bar the plasma globulins so that wide variations in their relationship is conceivable. Gradwohl (39) is of the opinion that an accumulation of the polymorphonuclear leukocyte proteins accounts for the major portion of cerebrospinal fluid protein increase in suppurative diseases of the meninges. In all probability, the breakdown of cellular exudate may contribute to the protein increase but it definitely plays a minor role. When a part of the subarachnoid space is cut off from the general subarachnoid space, the enclosed fluid undergoes certain changes. Venous congestion causes the transudation of blood plasma into the loculated fluid, perineural lymph stasis also results in the addition of lymph so that the fluid exhibits a marked increase of protein in the neighborhood of 500-1000 mgms.%, or more. In addition, the fluid may coagulate spontaneously and exhibit xanthochromia, but there is no increase in cells. These are the changes that occur in Froin's syndrome and are found in the fluid of the isolated cul-de-sac below a subarachnoid block and also in the fluid immediately above. The cause of the increased gamma globulin that is noted in the more chronic and degenerative diseases of the nervous system, especially syphilis and multiple sclerosis, is more controversial than the causes of the protein alterations just described. As previously stated there are investigators who believe that the gamma globulin is produced by the cerebrospinal tissue (52, 60). Bing and Neel (9) compared the protein content of cerebrospinal fluid and the cellular reaction accompanying the various affections of the central nervous system, and that an increased globulin content unaccompanied by corresponding albumin increase occurs only in diseases which pathologically are characterized by accumulation of plasma cells. They

believe that the plasma cells are responsible for the increase of gamma globulin in diseases such as multiple sclerosis and neurosyphilis. Barr (6) points out that recent evidence seems to favor the production of antibodies by the plasma cells wherever present in the body. This would support Bing and Neel's views, but Barr also makes it clear that the lymphocyte may be the source of antibodies. Both types of cells are usually present in these chronic diseases of the nervous system and if either were to be responsible for increased gamma globulin of the spinal fluid, it would more likely be the lymphocytes as they are usually more numerous. It must be emphasized that there is only a relative increase of gamma globulin in diseases characterized principally by diffuse degeneration of the parenchyma, in contradistinction to the absolute increases of gamma globulin as would be expected in diseases characterized by increased permeability of the hemato-encephalic barrier. Dattner (15) has summed this up by saying: "...in all pathologic processes involving the nervous parenchyma (ganglion cells and glia, i.e., ectodermal structures) globulins are more than proportionately increased in the spinal fluid, whereas the involvement of interstitial tissues (meninges and vessels, i.e., mesodermal structures) causes an increase in albumin." In multiple sclerosis, Kabat, et al (55) have shown that there is an increase of the percentage of gamma globulin to the total protein in 80% of the patients studied. In the disorders that affect the meninges and blood vessels, there is a marked elevation of total protein and gamma globulin; but there is an even greater elevation of albumin, and the percentage of gamma globulin to the total protein does not change appreciably. At present, there is insufficient evidence to enable one to state whether the plasma cells, lymphocytes, break down of nervous tissue, or autonomous elaboration by cerebrospinal tissue is the cause of relative

increased gamma globulins in chronic and degenerative diseases.

THE PHYSIOLOGICAL CEREBROSPINAL FLUID PROTEIN VALUES

The protein pattern of the cerebrospinal fluid involves a consideration of (1) the total protein concentration, (2) the albumin content, (3) the globulin content, (4) the ratio of albumin to globulin, and (5) the quality of the globulins. It must be admitted that a reliable knowledge of physiologic values for the above is of fundamental importance for all clinical investigations on the spinal fluid. Upon reviewing the literature, two facts become immediately apparent. First, all aluthors are in accord regarding the establishment of maximum normal values, and second, very little unanimity exists as to what constitutes maximum normal values. The reader is referred to tables 2. and 3. in which the author has compiled some of the recommended normal values that are to be found in the literature.

TABLE 2. Total Protein Content of Cerebrospinal Fluid from "Normal" Cases as Presented in the Literature

Authors	Year	Min. mgms. %	Max mgms.%	Av. mgms.%	Cases	Method
Mestrezat	1912	6.0	32.0	18.6	49	Mestrezat
Denis & Ayer	1920	35.0	100.0		*	Denis & Ayer
Ayer & Foster	1921	16.0	38.0	28.0	48	Denis & Ayer
Lange	1922	18.0	19.0	18.0	*	Lange's modification of Denige
Fremont-Smith & Ayer	1926	20.0	40.0	28.0	13	Denis & Ayer
Ling	1926	18.8	37.9	25.5	6	Ling
Spurling & Maddock	1926	21.0	47.0	37.0	*	modification of Denis & Ayer
Kafka & Samson	1928	19.2	28.2	24.0	10	Kafka
Grey	1930	20.0	40.0	28.0	69	Grey
Matz & Novick	1930	27.0	62.0	48.0	10	Matz & Novick
Kral, Stary & Winternitz	1931	23.1	34.4	28.7	77	Winternitz, Stary, Kral &
Spiegel	1932	7.0	21.0	11.0	15	Exton & Rose
Merritt & Fremont-Smith	1937	15.0	45.0	28.0	*	Denis-Ayer

TABLE 2. (continued)

Authors	Year	Min. mgms.%	Max. mgms.%	Av. mgms.%	Cases	Method
Neel	1939	12.0	28.0		7500	Roberts-Stolnikow -Brandberg-Bisgaard
Marron	1941	15.4	46.0	31.6	100	Johnson & Gibson tyrosine
Izikowitz	1941	14.0	65.0	40.0	45 (m)	Izikowitz
Kabat, Moore & Landow	1942	23.0	38.0	31.6	9	Electrophoresis
Kabat, Glusman & Knaub	1948	25.0	38.0	33.4	10	Precipitin
Lange & Miller	1950	16.0	30.0	26.0	500	

* number of cases studied not mentioned.

Although table 2. contains some of the major contributions in the study of total protein of cerebrospinal fluid, one can not extrapolate to any great extent at this time. The considerable variation of values is quite evident. These discrepancies were the very impetus for this paper and some of the reasons for such include: differences in technics, the number of supposed "normal" cases, the amount of fluid removed at puncture, and the site of puncture. The table is included merely to acquaint the reader with the many values presented by investigators and it is hoped that the subsequent discussion will enable us to discard many of their findings. At present, we may say that the investigators of Scandinavia and the European mainland place the maximum limit for the total protein concentration in lumbar fluid at about 30 mgms.%. Higher values are considered as definitely pathological (84). In England and the United States, the physiologic limit of total protein is on the average greater (Merritt and Fremont-Smith (78), 45 mgms.%; Spurling and Maddock (96), 47 mgms.%; Matz and Novick (75), 62 mgms.%; and Brain (11), 40 mgms.%). Considerably lower values than that of the Scandinavian investigators have been proposed by Spiegel (95), Young

et al (104), 21 mgms.% and 20 mgms.%, respectively.

TABLE 3. Globulin and Albumin Content in Lumbar Fluid from "Normal" Cases as Presented in the Literature

Author	Year	Glob. Conc. mgms.%			Alb. Conc. mgms.%			A/G Ratio			Cases	Method
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.		
Kafka & Samson	1928	2.4	4.8		16.8	24.0		5.0	7.0	6.0	10	Kafka's centrifuge
Matz & Novick	1930	6.0	20.0	13.0	21.0	52.0	34.0	2.6	3.5	2.6	10	Matz & Novick
Kral, Stary & Winternitz	1931	3.8	10.0	6.7	16.9	28.7	22.0	2.9	4.4	3.3	7	Stary, Winternitz & Kral
Izikowitz	1941	1.7	11.0	7.7	11.0	52.0	31.7	4.7	6.5	4.1	45	Izikowitz
Kabat, Moore & Landow	1942	7.0	13.0	10.6	15.0	26.0	20.0	1.5	2.5	1.8	9	Electro-phoresis

Table 3. contains some of the different values offered as the physiologic limit for albumin and unqualified globulin. The sparcity of values are in the main explained by the fact that most clinicians have been contented with the results of qualitative tests for globulin, expressed as 0, plus 1, plus 2, etc., and were not interested in the concentration of globulin in terms of milligrams per 100 cc. of spinal fluid. On the basis of the qualitative test results, many believe that there is no globulin in normal fluid. One should not assume that Merritt (79) believes that there is an absence of globulin in normal fluid when he states: "The various globulin tests are negative in normal fluid." He is cognizant, as are many clinicians, of the fact that a certain concentration above that found in normal spinal fluid is required to yield clinically positive globulin-test results. The findings of Simmons and Gentzkow (93) have been excluded from this table because this writer feels that either a gross error in their technic or identification of the precipitated protein may account for their consideration of 30

mgms.% as the normal limit of globulin in the spinal fluid.

Since the introduction of the colloidal gold reaction by Lange, in 1912, there have been numerous additional colloidal reactions. Lange's test is most widely employed and the only colloidal reaction discussed in this paper. The normal reaction to gold sol is negative, i.e., there is no reduction in any dilution (0000000000). Occasionally, a normal fluid may give a slight reaction in one or more dilutions so that many clinicians consider any change in the color of the solution less than lilac ("2") of no significance. The interpretation of a lilac color change ("2") is somewhat difficult, since it occurs in many otherwise normal fluid specimenssand in those from patients with no evidence of any abnormality in the central nervous system so that only a color change corresponding to "3" is considered significant by other investigators. Lange has modified the original gold sol reaction for both qualitative and quantitative purposes, and considers the normal gold curve, by this technic, to be characterized by four factors: (1) a sum total from 20-40, (2) maximum reaction at dilution 1:51 or 1:76, (3) degree of coagulation in first dilution does not exceed a color value of 3.0, and (4) the absence of plateau (61). This normal curve is not to be confused with the "negative" gold reactions obtained by the standard qualitative test. These factors have been derived from the studies of 500 presumed normal persons by Lange and Miller (61).

A great deal of significance has been attributed to the albumin-globulin ratio, particularly in regard to the colloidal reactions. According to Dattner (15), Kafka and Lange were among the first investigators to demonstrate that changes produced in the gold sol reaction are related to the albumin-globulin ratio. The values quoted in the liter-

ature range from 2.5 to 7.0 as the upper limit of normal, and most American authors believe that the ratio of albumin to globulin is on the average of 5 to 1. However, more recent emphasis has been placed on gamma globulin since its role of precipitating gold sol was established (38, 52). By use of precipitin technic, Kabat, Glusman and Knaub (54) found that the gamma globulin concentrations ranged from 1.7 to 3.8 mgms.%, the albumin content between 11-19 mgms.%, and the albumin-gamma globulin ratio was between 5.0 and 8.8 in ten healthy medical students. Earlier electrophoretic studies revealed gamma globulin values to range from 2-6 mgms.%, albumin concentration was between 15-26 mgms.%, and the albumin-gamma globulin ratio varied from 7.5 to 12.0 in nine presumed normal cases (52).

Before discussing some of the reasons for the discrepancies in the normal values, this writer wishes to call attention to the fact that there is no sharp demarcation between pathological and physiological protein concentration. Assuming that 45 mgms.% is an accurate maximum value for total protein content, determinations that reveal slightly higher results can not always be used as an indication of a pathalogical process in the central nervous system. The greater the difference is between the protein concentration value of the fluid under examination and the presumed maximum normal value, the more likely is the chance that pathology exists. On the other hand, there may be physiological protein concentrations of globulin, albumin and total protein but they do not necessarily imply physiological protein relations. The protein components may all be within their respective normal ranges, but the globulin may be markedly increased in relation to the total protein or to the albumins. Such a condition may exist in neurosyphilis or in multiple sclerosis.

Of the many factors that influence the physiological protein values, one of the earliest recognized by investigators was the contamination of the fluid with blood. As early as 1909, Ross and Jones (91) stated: "Whichever test be applied, it is essential first of all to be sure that no blood or pus has contaminated the fluid to be examined; results are of little value... ." Denis and Ayer (17) list three factors which render any test for protein worthless; (1) fluid contaminated by blood, (2) fluid with bacterial contamination, and (3) fluid standing for a long period uncorked. These conditions have been found to influence results by every technician studying cerebrospinal fluid. Blood admixture, in particular, is of such importance that Lange (62) considers it to be the main error in practice. He points out that even the smallest quantity of blood in spinal fluid renders total protein determination valueless, because blood plasma contains about three hundred times more protein than spinal fluid; consequently an admixture of one part plasma to three hundred parts of normal spinal fluid would double the protein content.

Most of the early writers and many of the more recent ones fail to specify the amount of fluid removed at puncture and the site of the puncture. Ayer and Solomon (4) were the early investigators who called attention to the differences of protein content in the lumbar, cisternal and ventricular fluid. Using the Denis-Ayer method, they found that the lumbar fluid contained about 30 mgms.% of total protein, the cisternal fluid had 25 mgms.% and 10 mgms.% was found in the ventricular fluid. Broader ranges of normal total protein have been advocated by Merritt and Fremont-Smith (78), who contend that lumbar fluid has 15-45 mgms.%, cisternal fluid has 15-25 mgms.%, and 5-10 mgms.% is contained in ventricular fluid. It has been suggested that the increased protein

found in the lumbar focus may be due to increased number of perivascular spaces emptying into the subarachnoid space (32). One could readily see that if large quantities of fluid are removed at lumbar puncture, that the fluid would contain a considerable admixture of cisternal and possibly ventricular fluid with their respective low protein content. The end-result would be a lower protein concentration value than would be determined on only lumbar fluid, and pathologically increased protein would be obscured. Izikowitz (49) performed forty-five fractional lumbar punctures on healthy persons, using the same quantity of fluid in the initial and subsequent punctures, He found that the total protein, globulin and albumin concentrations in the initial portions in all cases showed higher values than in the second portions; corresponding procedures were carried out on 180 abnormal patients with similar results. He also found that the protein does not reach the original concentration until a fortnight after the initial puncture. This is a significant finding as many clinicians are prone to make frequent taps in order to study protein alterations without considering the time interval between the subsequent punctures.

Another relevant factor affecting the physiologic protein values is the people upon whom the determinations were performed. Indeed, very few investigators used healthy persons, and this writer recalls but six studies wherein healthy individuals were utilized; all other investigations were on presumed "normal" patients. One is aware of the difficulty of getting normal people to submit to lumbar puncture as such a procedure is not unattended by occasional serious complications. However, the use of presumed "normal" patients implies that the investigators have preconceived ideas as to what constitutes normal cerebrospinal fluid. There are numerous examples of studies where psychiatric and

neurological patients with "normal" spinal fluid are used as prototypes of presumed "normal" patients and it appears that these patients had cerebrospinal fluid protein concentrations compatible with the investigators' ideas of normalcy. Conversely, patients who exhibit no neurologic signs or symptoms and are serologically negative have also been used as normal subjects. The fallacy in this regard is quite apparent. It is well known that in asymptomatic neurosyphilis, absence of neurologic signs and symptoms may be associated with marked abnormality of the cerebrospinal fluid, and many non-neurological systemic diseases may cause gross alterations of the protein of the spinal fluid. This author feels that the lack of healthy subjects in the studies has definitely influenced the findings for normal protein patterns.

Less important factors bearing upon the over-all physiologic values are age and sex. Izikowitz investigated forty-five men and twenty-seven females, all of whom were healthy. His data is summarized in table 4. Marron (74) examined the spinal fluid of 100 persons with the Johnston and Gibson tyrosine equivalent method and found that the 70 males in this series had an average total protein content of 32.6 mgms.% whereas the 30 females had a corresponding value of 27.5 mgms.%. In regard to age, Marron (74) found that in children under one year old, the total protein concentration in the majority was less than 20 mgms. % and the concentration in fluids from persons over 60 years of age had a tendency to be high. He does not state how many cases were younger than one year or older than 60 years of age and the tendency to be high implies about 40 mgms.%. Levinson (71) contentds that normal cerebrospinal fluid protein of infants and children range from 5-45 mgms.%, with an average of 25 mgms.%.

TABLE 4. Normal Protein Values in Lumbar Fluid

	Men (45 cases)			Women (27 cases)		
Total protein mgms.%	39.46	1.29	σ = 8.58	30.99	1.16	σ = 5.90
Globulin mgms.%	7.71	0.30	σ = 1.99	6.27	0.29	σ = 1.47
Albumin mgms.%	31.75	1.03	σ = 6.83	24.80	0.91	σ = 4.53
G/A quotient	0.24	0.005	σ = 0.03	0.25	0.007	σ = 0.034

After Izikowitz (49).

In the final analysis, this author believes that the most significant factor influencing the values of cerebrospinal fluid protein is the method employed in its estimation. The other determinants such as fluid contamination, site of puncture and amount of fluid can easily be controlled by the diligent investigator. If the clinician is to derive any benefit from his protein determinations, a knowledge of the liabilities as well as the assets of the tests that are utilized is imperative. Therefore, a more lengthy discussion of the methods of examination of cerebrospinal fluid is undertaken in this paper.

THE METHODS OF STUDY

Historical: The presence of fluid enveloping the central nervous system has been known since Galen, but the credit for the recognition of the cerebrospinal fluid as a circulating medium goes to the eighteenth century investigators, Valsalva, Haller and Cotugno (98). The latter, who is often given the sole honor for this description was the first to mention the presence of small amounts of protein in the fluid. At first, increased protein contents was simply demonstrated by boiling the fluid, but soon the early French investigators employed precipitating reagents to estimate the protein present. Guillain demonstrated the occurrence of globulin by its precipitation following saturation of

cerebrospinal fluid with magnesium sulfate, and in the filtrate, albumin was determined by boiling after careful acidification (15). Ammonium sulfate was used by Nissel in Germany to precipitate the globulins, and this reagent was also utilized by Nonne and Apelt (87), and later by Ross and Jones (91) in their respective tests. Pandy (88) used phenol to differentiate and estimate the components of fluid protein. These tests have been called qualitative methods by some authors, whereas others refer to them as "semi-quantitative" technics.

The first method for quantitative protein determination in the cerebrospinal fluid was developed by Roberts in 1876, based upon Heller's nitric acid test. This method was further modified by Stolnikow, Brandberg, Bertelsen and Bisgaard so that it now possesses the long title of Roberts-Stolnikow-Brandberg-Bisgaard method for determination of total protein (84). Nissel used Esbach's picric acid reagent to precipitate the proteins and measured the height of the sediment after centrifuging in a special type of graduated centrifuge tube. The principle was modified by Kafka and his collaborators (56) in order that globulin and albumin content may be determined as well as the total protein.

A great number of methods have been developed based upon colorimetric or nephelometric principles, and they vary in the method of protein precipitation. Sulfosalicylic acid may be the precipitating agent (Denis and Ayer (17); Kingsbury, et al. (61); Spurling and Maddock (96); Fennel (27); Grey (38)), or phosphotungstic acid (Young, et al. (103, 104); Ling (72)), or trichloroacetic acid (Izikowitz (49)), or alcohol as used by Hempel and Giese (46). Aside from the use of different precipitating reagent, these turbidometric methods also differ in the

types of standards with which the treated spinal fluid is compared.

In 1912, stimulated by the work of Schultz and Zsigmondy, C. Lange opened the era of colloidal reactions by introducing the diagnostic gold reaction of the cerebrospinal fluid (65). Since Lange's first ingenious application of gold sol, numerous modifications and numerous tests based upon the same principle ensued. In 1915, Emanuel introduced the mastic reaction which was the first test to replace colloidal gold; followed by Berhold and Kerchberg's Berlin blue reaction in 1917; then in 1920, Guillain, Laroche and Lechelle brought out their benzoin resin method (15). Other colloidal reagents that have been employed include: paraffin (Kafka), Guaiac (Thurzo) and sublimate fuchsin (Takata-Ara) (15). Most of these tests undergo continual modification of the original technic to increase their sensitivity, specificity and reproducibility.

Recently, electrophoresis and immunochemistry have been introduced by Kabat and his coworkers (52-55) as new technics for the study of the cerebrospinal fluid protein.

Classification: There is universal agreement that the only exact and perfect quantitative test for total protein is the micro-Kjeldahl method. However, this technic has proved to be impractical in clinical work because spinal fluid is protein-poor, only small amounts of fluid are usually available for examination, there is a great deal more non-protein nitrogen than protein nitrogen in spinal fluid, there is considerable expenditure of time and the procedure is quite intricate. One could readily imagine the obstacles involved for the separate determination of the albumin and globulin concentration, if the above difficulties are connected with the determination of total protein. Thus, a very

large number of methods for determination of protein concentrations have appeared in the literature in search of an ideal procedure, and for the most part, these methods may be classified under one of the following main headings.

Group I. Methods in which the total protein or globulin concentration is determined by establishing the highest degree of dilution at which protein precipitation may be observed as an opalescence or as a "ring" at the contact surface between spinal fluid and the precipitating medium, or by precipitating colloidal suspensions.

Group II. Methods in which the volumes of the protein precipitated are measured after sedimentation in certain "albuminmeters" for a period of time (volumetric), or after centrifugation in special calibrated centrifuge tubes (gravimetric).

Group III. Methods which are based upon an estimation of the degree of turbidity of the solution after addition of a protein precipitating agent (nephelometric).

Group IV. Methods which are based on immunochemical reactions, physical reactions (electrophoresis) or determination of one of the more regular components of the protein molecule (e.g. tyrosine determination).

Group I.

The methods that fall into Group I. have been called qualitative or semi-quantitative tests for cerebrospinal fluid protein. They include the phenol test (Pandy), the ammonium sulfate tests (Nonne-Apelt and

Ross-Jones), the nitric acid test (Roberts-Stolnikow-Brandberg-Bisgaard), the colloidal reactions (Gold and Mastix), the sublimate test (Weichbrodt) and the zinc sulfate and thymol turbidity tests for gamma globulin. This discription will be limited to the more commonly used and more fully evaluated tests.

In 1910, Pandy (88) described a phenol method for the estimation of protein that has become one of the most popular tests performed on cerebrospinal fluid. It is accomplished by adding one to five drops of spinal fluid (varies with the investigator) to one or two cubic centimeters of Pandy's solution which is 100 grams of carbolic acid in 1000 c.c. of distilled water. The immediate formation of a clearly observable bluish-white cloud or turbidity is regarded as a positive reaction. In addition to indicating the presence of globulins, Pandy claimed that the test also showed presence of albumins and albumoses when 1% or stronger solutions are employed. Exton and Rose (25), Merritt and Fremont-Smith (78) and Neel (85) agree with this, but consider that the positive reactions are mainly due to the globulins. Although most authors consider the phenol method to be a very sensitive globulin test, it should be kept in mind that moderate and larger amounts of albumin give very strong reactions even in the complete absence of globulins. It is the opinion of many clinicians that positive Pandy reactions do not occur with physiological protein values, and that such reactions thus constitute proof of pathological protein increase in the examined fluid (40, 42, 78). These investigators believe the converse to be true, that is, negative Pandy reactions imply normal protein values and that consequently negative reactions do not occur with pathologically increased protein concentration. Madonick and Savitsky (73) were interested in this question and

attempted to correlate the total protein values of spinal fluid with the Pandy reaction in 1000 neurologic patients. They investigated the total protein by the Ling method (a modification of the Johnston and Gibson tyrosine equivalent method), but do not mention the technic of Pandy or a modification that they may have employed. The maximum normal value by the Ling method is 37.9 mgms.% (72), although Madonick and Savitsky consider values below 50 mgms.% as still within the normal range of total protein. They found that 57% of the cases had a negative Pandy reaction with a total protein of 50 mgms.% or greater, and 17% of the cases had a negative Pandy test with 100 mgms.% or greater. The protein level at which the Pandy reaction became positive in more than 50% of the cases was 70 to 75 mgms.%. Table 5. summarizes their data.

TABLE 5. The Relationship Between the Pandy and Total Protein Content of Spinal Fluid.

	Cases	%		Cases	%
Total Number With Positive Pandy	195	19.5	Total Number With Negative Pandy	805	80.5
Number With Positive Pandy & Protein Below 50 mgms.%	38	6.0	Number With Negative Pandy & Protein Below 50 mgms.%	600	94.0
Number With Positive Pandy & Protein Above 50 mgms.%	157	43.0	Number With Negative Pandy & Protein Above 50 mgms.%	205	57.0
Number With Positive Pandy & Protein Above 100 mgms.%	62	83.0	Number With Negative Pandy & Protein Above 100 mgms.%	13	17.0

After Madonick and Savitsky (73).

Izikowitz (49) examined 1000 persons with the Pandy test, and differentiated negative, trace, one plus, two plus and three plus reactions. The two former reaction values are considered clinically negative and constituted 618 cases out of the 1000 cases examined. He attempted to

correlate the total protein concentration with the various reaction values, and found that there was a wide range of total protein concentration that gave the same reaction value and that there was considerable overlapping. The total protein was determined by Izikowitz' technic (the principle of which is described in Group II. methods), and the maximum value for total protein by this method is 65.2 mgms.% (for men). He found that the range of total protein concentration simultaneously determined for each Pandy reaction value was as follows: negative, 10.44-62.21 mgms.%; trace, 20.52-71.28 mgms.%; one plus, 25.23-125.31 mgms.%; two plus, 30.84-155.90 mgms.%; three plus, 48.84-187.26 mgms.%. The extent of overlapping is evident, as for example, a total protein concentration between 30-60 mgms.% would yield a negative, trace, one plus, two plus and possibly three plus reactions. Therefore, the Pandy reaction may often give misleading and erroneous information to the clinician as to the total protein of a single lumbar fluid. If one were to consider the mean values of total protein concentration corresponding to the various reaction results of the Pandy test, it is true that the reaction becomes more positive as the mean total protein concentration is increased. This may be applied generally to a group of patients and not to the single fluid under examination in which the clinician is primarily interested. Izikowitz found that negative reactions do occur, although infrequently, in lumbar fluids that have higher than physiological total protein. He also found that positive Pandy reactions occurred in 12 out of 62 healthy persons. In addition, Izikowitz correlated the concentration of globulin with the various reactions of the phenol test as it is believed by many to be a globulin test. Again, a wide range of values was found for each reaction result, there was overlapping, and in general as the concentration of globulin increased so did the posi-

tivity of the Pandy reaction. The globulin concentration was estimated by Izikowitz' method which places the maximum value for globulin at 13.61 mgms.% in lumbar fluid. There were a small number of cases that showed negative phenol reaction results at globulin concentration considered pathological by this quantitative method. Izikowitz (49) states that "negative Pandy test does not with absolute certainty exclude pathological increase in globulin concentration, and they do not prove the presence of physiological globulin concentration values."

Epstein (24), using Lehmann's modification of biuret method for total protein, also compared the total protein values with Pandy reaction results. He found that a negative Pandy in about 70% of the cases meant a total protein content of less than 40 mgms.%, whereas in trace and positive reaction of the Pandy corresponded to total protein concentration of over 40 mgms.% in 85% of the cases. Epstein does not make it clear as to the number of cases considered in this study. The normal limit for total protein by this modification of the biuret method is 40 mgms.%. Fennel (27) found the phenol test highly inaccurate and there was little correlation with other globulin tests so his laboratory has discarded its use completely.

We can conclude from these studies that positive Pandy reactions are no proof of pathological protein increase, nor do negative reactions constitute a proof of physiological protein values. It is best to follow the advice of Neel (84) and Merritt and Fremont-Smith (78) who advocate quantitative protein determination regardless of the Pandy reaction, especially if it is negative so as not to be misled.

The Nonne-Apelt test is another old and commonly employed method

of protein study which was described in 1907 (87). It is performed by adding to a given quantity of spinal fluid in a test tube an equal amount of saturated ammonium sulfate. The opalescence or turbidity produced is read in exactly three minutes. As regards the nature of the protein precipitate, most authors are in accord with Nonne (87), Dattner (15) and Izikowitz (49) that this is a globulin reaction. However, Bertelsen and Bisgaard (8), the noted Scandinavian investigators, do not believe that the major portion of the precipitated protein is globulin. Many believe that the Nonne-Apelt test may be used also in estimating total protein. In respect to the reliability of this test, Greenfield (40) and Eagle (22) consider the Nonne-Apelt test to be the most satisfactory test for globulin estimation in spinal fluid. Neel (84) has compared the results of the Nonne-Apelt test with the Ammonium Sulfate ring test and the Nitric Acid test. He found good parallelism with one reservation, in that, the Nonne-Apelt test was more sensitive to small quantities of globulin. Bisgaard, according to Izikowitz, is of the opposite opinion; he feels that the Nonne-Apelt test serves only to confuse the clinician and should be discarded. In parallel studies with the Ammonium Sulfate ring test, Bisgaard found the Nonne-Apelt test to be negative when the former showed ten-fold increase in globulin. Many authors (25, 40, 79, 87, 94, 95) state that the Nonne-Apelt test is negative in healthy persons. If this were the case, the technic would be of extreme clinical importance, for it would mean that a positive reaction proved the existence of a pathological process in the central nervous system. According to Nonne (87) a positive reaction proved the existence of an organic pathological central nervous system process in those cases where artificial admixture of blood with spinal fluid had not occurred. He states that there are no positive reactions in functional

disease of the central nervous system. Contrary to this belief, Izikowitz found that 19 cases out of 72 healthy men and women showed a positive Nonne-Apelt test; the reaction in each case being one plus. Re-punctures of several of these subjects yielded similar results. Izikowitz states: "Positive results of Nonne-Apelt test on lumbar fluid are consequently no proof of pathological increase in globulin concentration... ." There is widespread opinion, as was the case with the Pandy reaction, that increase or decrease in the strength of the positive reaction is a proof of increase or decrease, respectively, of the globulin or total protein of the spinal fluid, or as a proof of intensification or diminution in the strength of the pathologic process that has given rise to the changes. Izikowitz studied 1000 cases with the purpose of determining any correlation between the reaction of the Nonne-Apelt test and the globulin concentration as determined by his method. He found that there was essentially the same basic fault as was present with the Pandy reaction, namely, when the Nonne-Apelt test is applied to the single lumbar fluid, which is of prime interest to the clinician, it falters in giving a good approximation of the globulin concentration dependent upon the particular reaction value. A wide range of globulin concentration was found when the same Nonne-Apelt reaction was present and overlapping was evident. He found that on the average an increase in the reaction indicated an increase in the globulin concentration, but with single fluids a more intense reaction in one than in another by no means necessarily indicates higher globulin concentration in the former; the concentration may actually be similar or even considerably lower. All that one can say, is that the different reactions constitute a relatively weak indication of globulin concentration of the spinal fluid, but no more. Similar findings were found by Izikowitz in correlating the total protein concentration and the Nonne-Apelt

reaction. It is obvious from these studies that the Nonne-Apelt test is highly inadequate as a clinical guide.

Another technic employing ammonium sulfate is the Ross-Jones test. The authors of this method sublayered one c.c. of spinal fluid in a test tube with two c.c. of neutral saturated ammonium sulfate, and read the results in three minutes. A bluish-white or white ring at the junction of the two fluids is a positive reaction (91). According to Izikowitz (49), Bisgaard modified this test by using equal volumes of spinal fluid and reagent, and claimed that albumin does not give a positive result even in a concentration of 200 mgms.%. Neel (84) believes that albumins are precipitated in some cases but Irvine (48) agrees with Bisgaard, in that, this is a globulin specific test. Ross and Jones (91) indicate that the method is quite reliable in ascertaining increase in globulins and many authors agree that the test is negative when normal fluid is examined. Fennel (27) found the Ross-Jones test to be negative in spinal fluids containing 7-8 mgms.% globulin; one plus with 8-12 mgms.%; four plus indicated 50 mgms.% or more. He measured the globulin turbidometrically after precipitation with equal parts of spinal fluid and saturated solution of ammonium sulfate. The relationship between the dilution values obtained in performing the Ross-Jones test and the globulin concentration was studied by Izikowitz, who found in 300 cases that the increased dilution figures, in general, corresponded to increased globulin concentration values. As with the Pandy and Nonne-Apelt tests, there was a wide range of concentration values with each dilution figure and a great deal of overlapping so that evaluation in regard to a specific spinal fluid is rather difficult. Izikowitz concludes, as does Fennel, that the method of Ross and Jones is not quantitatively trustworthy.

The inaccuracies of any globulin test involving 50% saturation with ammonium sulfate as illuminated by the work of Lange (67) must also be considered. Experimentally, if a known mixture of albumin and globulin, prepared from normal blood sera, were serially diluted, and each dilution precipitated with 50% ammonium sulfate, the greatest deviation from this known albumin-globulin ratio would be found in dilutions where the total protein contents ranged from 15 mgms.% to about 150 mgms.% (67). This source of error may lead to incorrect results in routine examinations, since protein concentration of specimens submitted for study frequently falls within the above range. Marked increases in total protein content may result in positive Ross-Jones reactions and this should be considered in the interpretation of any positive test. Only after one is aware of the above facts, does this writer feel that the Ross-Jones test is a good procedure for the indication of abnormal quantities of globulin in the spinal fluid.

When comparing the Pandy, Nonne-Apelt and the Ross-Jones tests as to reliability and sensitivity, we are confronted again by numerous beliefs. Epstein (24) and Izikowitz (49) compared the Pandy and the Nonne-Apelt tests, and both authors state that the Pandy test gave clinically positive reactions at lower globulin and total protein concentrations than the Nonne-Apelt test. The former method also yielded fewer trace r reactions and more clinically positive results than the latter which indicates that the Pandy test is the more sensitive test. Neel (84) found that there was good parallelism between the results of the Nonne-Apelt and the Ross-Jones test, and he performs them in conjunction with one another. Demme, according to Izikowitz (49), found the Ross-Jones test to be less exact than the Nonne-Apelt, whereas Epstein (24) con-

cluded that the former test will exhibit positive reactions, on the average, with lower globulin concentration, and also was better able to differentiate globulin concentrations than the Nonne-Apelt test. The writer desires to postpone, momentarily, further discussion on the comparison of qualitative tests in order to first consider some other technics.

Since Heller's original publication, in 1852, of a method for the determination of total protein in urine, numerous modifications have been instituted, especially by the Scandinavians, in order to make the nitric acid test applicable for total protein estimation of spinal fluid. In principle, the test is based upon the assumption that the highest degree of dilution of a spinal fluid with normal saline that will give a positive reaction (white disc or ring at the junction between nitric acid and cerebrospinal fluid) after three minutes, affords a measure of the total protein content of the fluid examined. Neel (84) is the greatest exponent of this method and employed it in all determinations referred to in his book, The Content of Cells and Proteins in the Normal Cerebrospinal Fluid. He states that the technic "allows of an exact quantitative determination, both with a normal content of protein and with small changes when an exact determination is of importance" and that "this method ought to be used more extensively than has hitherto been done." Lange (62) advocates his own modification of this technic as a simple, fairly exact and economical standard method for total protein determination. However, there is great controversy regarding the subjectivity of this test and many authors claim and disclaim good parallel and simultaneous results. Of far greater importance than the problem of subjectivity is the difficulty in converting the dilution

values into total protein concentration, i.e., milligrams of total protein per 100 c.c. of spinal fluid. Many attempts have been made to establish the magnitude of a factor which will equal the protein content divided by the dilution figures. This factor has been referred to by Neel and others as the Q factor. Neel (84) published many reports on its determination, and at first, had considerable faith in such factors, but since, has lost confidence. On page 113 of his book, he states: "As regards the question of giving a figure (constant factor) by which it is possible to convert the dilution figures to protein content, quoted in mgms.%, this must, as emphasized already by Bisgaard, be said to be impossible." In spite of such a statement, he continues to describe the method, present a Q factor and laud the exactness of the technic. Lange (62), on the other hand, condemns the use of Q factors and advises that the final dilution at which the positive ring or disc sign just disappears be recorded. He considers a Heller dilution of 1:5 as denoting normal protein content, and goes on to point out that in Froin's syndrome one may get a Heller 1:200 and in paresis it may be 1:25. The latter two dilutions indicate an elevated protein content but can not be interpreted as demonstrating a protein content forty or five times, respectively, greater than physiologic spinal fluid.

The Nitric Acid test is not recommended for the determination of total protein because it is not very accurate, it possesses a large subjective component, it must be interpreted in terms of awkward dilution values, and it offers no advantages that can not be derived from some of the better quantitative methods for total protein estimation.

The development of the colloidal gold reaction by Lange 39 years ago opened an entirely new approach to the study of the cerebro-

spinal fluid protein. Basically, the various colloidal reactions are qualitative or "semi-quantitative" methods to determine alterations in the components of the protein, and therefore they are discussed under this group of methods. The gold sol reaction, which is most commonly employed, is the only colloidal reaction described here as the principles applicable to one type of reaction generally apply to the others. A discussion of the differences in sensitivity, reproducibility and specificity of the various colloidal reactions, is not germane to the general subject of this paper and is therefore not undertaken.

The gold sol test involves the precipitation of colloidal gold by serially diluted spinal fluid set up in ten test tubes, all treated in the same manner, and the results read after a standard period of time. Dependent upon the degree of precipitation in each tube there will be concomitant changes in the color of the original ruby red gold sol added to each tube. Full precipitation of the colloidal gold sol results in complete discoloration of the sol, leaving a supernatant water-clear fluid above the precipitated gold at the bottom of the test tube. To this degree of change, the digit 5 is assigned, while unchanged ruby red gold sol is designated by the digit 0. The intermediate colors are labeled 4, 3, 2, 1.

In regard to the mechanism of the production of color change and precipitation of the colloidal gold, it was first shown by both Kafka (58) and Lange (65) that colloidal reactions depend on the presence of an elevation of the euglobulin fraction of the cerebrospinal fluid protein. They further observed that albumin protects colloidal solutions from precipitation by euglobulin. Thus, the precipitation of the colloidal solution was demonstrated to be dependent upon the ratio between albumin

and euglobulin in the precipitating solution. Further clarification of this reaction was obtained from the electrophoretic studies of Gray (38) and Kabat, et al. (52, 53), who demonstrated conclusively that the precipitating activity of globulin is present in the gamma globulin fraction, and the ability to protect colloidal solution from precipitation was within the albumin fraction. Table 6. summarizes the data obtained by Kabat and his associates (52) on the colloidal reaction of pure albumin, pure gamma globulin and a mixed fraction obtained from the electrophoretic cell. There is no colloidal activity found in the albumin fraction, whereas the separated gamma globulin had a higher activity than either the original spinal fluid or the middle mixed fraction. This indicates the inhibiting effect of albumin on the colloidal gold reaction as well as the activity of gamma globulin.

TABLE 6. Colloidal Gold Curves on Electrophoretic Fractions of Concentrated Spinal Fluids

Diagnosis	Original Unconc. C.S.F.	Colloidal Gold Curves		
		Albumin	Middle	Globulin
Idiopathic grand mal	1100000000	0000000000	0000000000	1112210000
Post-traumatic headache	1100000000	0000000000	0000000000	0000000000
Cirrhosis	1100000000	0000000000	1111222110	1243210000
Multiple sclerosis	2222100000	0000000000	3322221100	5553321000
Neurosyphilis	5555544250	0000000000	5554332110	5555432100
Neurosyphilis	1111122211	0000000000	1112221000	5554321000

After Kabat, Moore and Landow (52).

Bernsohn and Borman (7) have found gamma globulin to be significant in the causation of abnormal colloidal gold reactions, but they contend that albumin is inert and not protective in the reaction. Beta globulins are the proteins in blood serum which possess the protective ability in the colloidal reaction according to these observers. The

fact that the albumin may vary in its capacity to protect the solutions has also been noted by other investigators (31). However, there has been no confirmation of the role of beta globulin in protecting the solution, in fact, according to Freedman and Merritt (31), Scheid found that beta globulin in some dilutions has the ability to precipitate colloidal solutions but to a lesser degree than does gamma globulin. We can state that the ability of a given spinal fluid to precipitate a colloidal suspension is dependent upon the amount of gamma globulin present and the amount of albumin present to protect the solution.

Precipitation and the greatest color changes in the tubes with the highest concentration of spinal fluid occur when there is predominantly a globulin excess and this has been termed a first-zone curve. The greatest color change occurring in the third to the sixth tube is designated as a mid-zone curve and has no value in a differential diagnosis. In the presence of very high protein content, especially when the albumin-globulin ratio is high and fibrinogen may be demonstrated, maximum precipitation is found in tubes with the least concentration of spinal fluid, i.e., an end-zone curve. There has been sufficient condemnation of the use of "paretic", "tabetic" and "meningitic" curves that investigators make a point to include such terms in quotation marks even though they may continue to think along such lines. Only the first-zone curve may be considered significant, and it must be remembered that it is a manifestation of several nervous disorders such as neurosyphilis, multiple sclerosis, post-infectious encephalomyetides, virus encephalitides, etc..

A review of the literature reveals that there are many investigators who consider the standard colloidal reactions to be the best methods for differentiating qualitative and quantitative changes in the

spinal fluid protein (16, 27, 62). However, this writer believes that the diagnostic value of these reactions have been greatly exaggerated. In spite of Lange's excellent work (63, 64, 65) to control the factors that influence gold sol, such as pH, electrolytes and despersity of the colloidal particles, there are often disheartening results in regard to sensitivity and reproducibility. Two very reputable and busy cerebrospinal fluid laboratories in Boston which use the same procedures frequently do not even come close to the same results on simultaneous spinal fluid examinations (28). The performance of the examination by Lange's technic is very intricate and there are numerous avenues for error even after dependable reagents are prepared. The clinician persists in the use of this technic. Perhaps it is simply due to routine or there is something exhilarating in a macabre sort of way in discovering that a patient has "numbers in his spinal fluid." It is more stimulating to see a report that states "colloidal gold 5555443211" than one that says "total protein 75 mgms.%." Instead of depending upon the end-result of a test that is due to the interaction of two factors, namely, the absolute and relative concentrations of albumin and globulin, and which in no way informs the investigator as to which is more prominent in the fluid under examination, this author favors a quantitative examination, however rough, of these two proteins. A determination of the gamma globulin, by such technics as the thymol turbidity or the zinc sulfate test (19), and the albumin by numerous methods would give one a better understanding of changes occurring in pathological processes of the nervous system than would a finding of a first zone curve which occurs in many diseases. I do not believe that the clinician need develop a complex formula, as advocated by Eagle (22), based upon globulin and albumin determination so that he may calculate and plot a colloidal curve.

Realizing the inadequacies of the standard gold sol reaction, Lange published a quantitatively standardized gold test in 1939 (62). He utilizes a color scheme with corresponding values of 0-20; complete discoloration is 18-20, and unchanged gold sol is designated as 0. In addition to noting the dilutions at which there is maximal color change, a quantitative value is obtained by adding the figures of all the tubes. The gold curves are classified as types A, AB, B, BC, C, CD and D, and are not comparable with the first, second and third zone curves of the standard gold sol reaction. Under the ideal conditions that prevail in Dr. Lange's laboratory, this test has been shown to possess considerable sensitivity and reproducibility. Freedman and Merritt (31) compared the colloidal gold changes, both the standard and Lange's quantitative technic which was performed by its author, with Kabat's gamma globulin determination (54) in 22 cases of multiple sclerosis. Of this small group, 91 per cent showed abnormally high gamma globulins in their cerebrospinal fluid, 71 per cent had abnormal colloidal gold tests (a type D curve) according to Lange, and in only 33 per cent was the conventional gold test abnormal. Unfortunately, all the work lauding the attributes of Lange's quantitative colloidal reaction have come from his own laboratory (101, 102) and one can not evaluate this procedure until other clinicians have also employed the procedure. However, it must be remembered that the ability of a protein mixture to precipitate a colloidal suspension will depend upon absolute and relative amounts of gamma globulin and albumin. No colloidal test, no matter how carefully set up and performed can get beyond these limitations.

A consideration of the defectiveness of the qualitative tests as a group has caused this writer to have little credence in their results.

Subjectivity plays an important role in each of these qualitative tests. Considerable experience with a particular procedure is required of the investigator in order that he may differentiate various degrees of positivity. The subjective element interposed is roughly proportional to the degree of differentiation used in recording results. A test that utilizes values of zero to plus six has a greater subjective error than a test that distinguishes between zero to plus three reaction-values. When a clinician attempts to compare one qualitative test with another, in simultaneous determinations, invariably, the method with which he is most familiar yields the best results. Another inadequacy of qualitative methods is that most of them have exhibited the occurrence of negative reactions in the presence of abnormal protein values and positive reactions in normal cerebrospinal fluid. The degree of positivity can often be misleading and should not be used as a criterion of progression or remission of a disease process. It has been frequently demonstrated that a particular reaction-value may correspond to a wide range of the specific protein or total protein when the test is applied to a single spinal fluid. Many authors are cognizant of the faults of qualitative tests and advocate their use only for screening purposes. If this be the procedure used, this writer would like to remind the reader that the screen is made of a rather coarse mesh.

Group II.

The Kafka-Samson centrifuge method or the Eiweissrelation method is a standard technic used on the European mainland and will be the only volumetric procedure discussed in this paper. Eiweissrelation has never been defined by Kafka, Reibling and Samson; at most one can gather that they mean it is their method of determining the total proteins,

globulin and albumin in cerebrospinal fluid (56). This technic is a modification of the old Nissel centrifuge method and is, accordingly, based upon the assumption that the height of the protein precipitate pillar in the capillary part of Kafka's special centrifuge tube obtained under certain conditions after the addition of Esbach's reagent, represents the protein concentration of the spinal fluid investigated. The capillary part of each tube is marked off in divisions and the distance between each graduation mark is 1 mm. (56). Precipitation heights are read in tenths of the divisions and this means that it is possible to differentiate 0.1 mm. of precipitated protein. Kafka and his associates refer to the so-called 1st Figure which represents total protein concentration, the 2nd Figure, which according to these authors represents the hydration of the globulins and the 3rd Figure represents the globulin concentration. The difference between the 1st and the 3rd Figures is the Division Figure of the albumins, and information about the globulin/albumin quotient can be obtained by dividing the 3rd Figure by that difference. Most European and Scandinavian method utilize G/A quotients rather than the A/G ratio employed in English speaking countries.

Much of the European work on globulin, albumin and total protein concentrations of spinal fluid in the different diseases of the central nervous system is based on the investigations carried out with the volumetric centrifuge method of these authors. There has been little or no negative criticism of this method in the literature, and Kafka (58) claims that the procedure yields "a faithful picture of protein relationships in cerebrospinal fluid that is not possible to obtain with any other method." The error is no greater than 10% in determining protein concentration between 10-70 mgms.%, i.e., 3 divisions (56). It is apparent from the procedure that the Eiweissrelation method is an

indirect method of protein determination as a "value" or "factor" must be found in order to convert "Division Values" into protein concentration values. The authors of the method determined this factor to be 2.41, so that the protein concentration may be obtained in mgms.% by multiplying the number of 0.1 Division Values by this factor. Kafka (57) later implied that this factor may be slightly different with various centrifuges. The main disadvantages involved with this method are that special apparatus is required; it is an indirect technic requiring certain constants in order to get actual concentrations, temperature control and centrifuge speeds must be regulated and that high concentrations of total protein increases the per cent of error in the determinations.

Group III.

In 1920, Denis and Ayer (17) described their nephelometric technic for the determination of total protein, and it has since become the most commonly utilized method in the United States. Ayer, Dailey and Fremont-Smith (5), in 1931, modified the original method, the principal of which involves the addition of sulfosalicylic acid solution to the spinal fluid under certain conditions and the degree of turbidity that develops is compared to a standard in a colorimeter. In reference to the accuracy of this procedure, Denis and Ayer (17) state that it is "accurate within approximately 5%," in which connection 5% applies to the actual amount of the protein present and not to comparative readings between different fluids. Ten complete determinations on the same fluid, gave five readings of 34 mgms.% and five readings of 35 mgms.% for the total proteins (32). A comparison between determinations with the micro-Kjeldahl method and values obtained with the Denis-Ayer method yielded results that "agree as well as can be expected" (5). In this investigation,

the comparison was made on six lumbar fluids; table 7. summarizes the findings which evidently do not agree as well as one would like to expect. The Denis-Ayer technic resulted in higher values in all cases of this study, whereas Brown, Gilden and Man (12) found that the nephelometric procedure tended to yield lower values than the micro-Kjeldahl technic. They found that there was agreement usually within 12 mgms.% which still leaves a high percentage of error when it is considered that often a total protein value is not twice that figure. Irvine (48) found that the Denis-Ayer method checked accurately with the micro-Kjeldahl and Izikowitz (49) obtained excellent agreement when parallel examinations with his technic were performed on 200 lumbar fluids.

TABLE 7. Total Protein Values of Six Spinal Fluids

Denis-Ayer Method	Micro-Kjeldahl Method	Difference
17 mgms.%	14 mgms.%	3 mgms.%
63	61	2
64	57	7
151	147	4
216	197	19
260	214	16

After Ayer, Dailey and Fremont-Smith (5).

It is the opinion of most American investigators that the Denis-Ayer technic is the method of choice in the determination of total protein. It possesses almost all the characteristics of an ideal method, namely, accuracy, reliability, rapidity, facility, conservation of spinal fluid and expense. This writer feels that the Denis-Ayer technic is the best method for total protein determination that has been described, and advocates its universal use.

Group IV.

The methods that belong to this group are in this writer's opinion of more value in the biochemistry laboratory than in the clinical laboratory, but they are described because of their possible importance in scientific investigation and in hope that modifications may adapt these methods for practical clinical use. Izikowitz describes an exact quantitative method for the determination of total protein, globulin and albumin concentrations in his thesis (49). In principle, his method involves a precipitation of the cerebrospinal fluid protein with trichloroacetic acid, followed by several centrifugations and sucking up of the centrifugate and the washings. Combustion in an electrically heated sand-bath with tungstic acid and hydrogen peroxide is the next step in the procedure and this is finally succeeded by the determination of the nitrogen content of the sample by an iodimetrical titration. A special constructed centrifuge is required for the numerous centrifugations and the entire procedure takes at least 15 hours. From this brief resume of the principle, it is evident that the procedure is time consuming, complicated, tedious and very expensive. There has been no evaluation of this technic by other investigators and it is doubtful if such would be forthcoming.

Electrophoresis is an excellent research procedure, but the large quantities of fluid required for analysis precludes its routine diagnostic use even when the Tiselius apparatus is available. Therefore, further discussion of this technic is curtailed.

Because of the discrepancies in the protein determinations by the usual chemical means, Kabat, Glusman and Knaub (54) proposed a quantitative immunochemical method for the estimation of the small

amounts of cerebrospinal fluid protein. There have been earlier reports on the precipitin method for the quantitation of albumin and globulin in serum, lymph, ascitic and edema fluid in dogs (37), and Kabat et al. adapted this method for spinal fluid determinations. Their method involves preparation of rabbit antisera to crystalline human serum albumin and to purified human gamma globulin. The albumin and gamma globulin in cerebrospinal fluid is determined by adding an appropriate dilution of cerebrospinal fluid to the albumin and globulin antisera, such that, the total nitrogen precipitated by their interaction will fall on previously prepared calibration curves. The total nitrogen is measured in the washed precipitates and the corresponding albumin and gamma globulin values are read from the calibration curves. The comparison of this method with Lange's quantitative modification of the colloidal gold reaction in 22 cases of multiple sclerosis has already been described. This study demonstrates the efficacy of quantitative gamma globulin tests and their need in clinical evaluation of disease. Von Storch, Harris and Lawyer (101) believe that the quantitative gold reaction has greater speed and accuracy than the immunochemical method, but these workers are associates in Dr. Lange's laboratory and therefore, have the optimal environment for the quantitative colloidal gold reactions. This writer favors the immunochemical method to Lange's method because it provides protein values which are independent of the other proteins present in the cerebrospinal fluid. However, the disadvantages of the precipitin technic renders its impractical for clinical procedure. It requires a minimum of three days, from the receipt of the sample to the completion of the analysis. Standardization of the antisera is often difficult and the performance of the technic is intricate. The test does not reveal any additional information with spinal fluids containing

high concentrations of total protein. In spite of these faults, the authors claim results comparable to that obtained by electrophoresis (55). Until the much awaited modification for its practical employment is described, this technic remains an excellent method for investigative purposes.

PROTEIN PATTERNS OF SOME DISORDERS OF THE CENTRAL NERVOUS SYSTEM

The alteration of the cerebrospinal fluid protein pattern is simply a reflection of a process which may be intrinsic or extrinsic in regard to the central nervous system. General metabolic disorders, e.g. myxedema, influence the spinal protein concentrations as do the more specific neurological diseases. Such modifications that may occur are entirely non-specific; the same protein changes may be present in a number of diseases. Not unlike the other cerebrospinal fluid constituents, the protein changes are not stereotyped in character, in that, there may be a wide range of variation in the same disease, depending upon the severity and the stage of the organic disturbance. Yet, there are many changes that are generally constant for a given disease, and this pattern, in addition to other spinal fluid findings, gives the clinician a picture of the struggle between body defenses and the causative agent, enabling him, often, to prognosticate the outcome. I shall attempt to describe these protein alterations in only a few diseases of the nervous system. Some patterns characteristic of other disorders have been alluded to in other sections of this paper, and the reader is referred to the texts of Neurology as well as those concerned solely with the cerebrospinal fluid (78, 70, 32) for more exhaustive reviews of the spinal fluid syndromes.

Neurosyphilis

There are five tests which are advocated for the diagnosis of neurosyphilis; (1) total protein determination, (2) globulin determination, (3) cell count, (4) colloidal reaction, and (5) specific test for syphilis, e.g., Wassermann, Hinton, Kahn, etc.. In the natural course of syphilis, involvement of the central nervous system occurs very early after infection and first manifests itself by a lymphocytosis in the spinal fluid, followed by increased protein, then by changes of the specific luetic tests and colloidal reactions. The percentage of patients with alterations in the spinal fluid rises during the first year, reaches its peak about the eighteenth month, and falls off considerably in the next two or three years. Rarely does the first manifestation of spinal fluid changes occur after the fourth year.

In general paresis, the total protein is usually high; according to Merritt and Fremont-Smith (78) it ranged between 29-498 mgms.%, but over 50% of the cases had a protein content between 50-100 mgms.%. Merritt, Adams and Solomon (81) found the total protein to be between 29-498 mgms.% in a series of 100 cases of paretic neurosyphilis which had received very little or no treatment. Approximately 25% of the fluids had more than 100 mgms.%, and a similar per cent had less than 45 mgms.%. In Lange's experience (66) the total protein ranges from 100-150 mgms.%, and infrequently exceeds 200 mgms.%. Madonick and Savitsky (73) studied 18 cases by the tyrosine method; 2 patients had less than 50 mgms.%, 13 patients had a total protein content greater than 50 mgms.% and 3 patients had greater than 100 mgms.%. All investigators report almost 100% positive globulin reactions, and Kafka (58) believes that the globulin/albumin ratio is higher in general paresis than in any other disease of

the central nervous system. In three paretic patients, Kabat, et al. (54) determined the quantity of gamma globulin and found it to be 15, 16, 15 mgms.%, respectively (normal range 2.5-6.5 mgms.%). The colloidal reactions exhibit the maximal precipitation in the highest concentrations of spinal fluid in about 70-80% of paretics (78, 81, 77). Similar reactions may be present in other forms of neurosyphilis, multiple sclerosis, post-infectious encephalomyelitides, etc., so that, the presence of a first-zone curve is not diagnostic of paretic neurosyphilis.

The abnormalities in tabes dorsalis differ from that of general paresis, in that, they are less marked, tend to decrease with the duration of the disease, are much more amenable to treatment and exhibit considerable variation dependent upon activity. Early untreated cases may have a moderate or marked degree of meningeal reaction resulting in a protein pattern comparable to that of dementia paralytica, whereas inactive cases or cases that have received a moderate amount of treatment the spinal fluid may be normal. In one series the total protein varied between 17 and 320 mgms.%, with an average of 61 mgms.%; it was less than 45 mgms.% in 46% and greater than 100 mgms.% in 12% of the patients (78). Another study, utilizing the same method, exhibited a range from 14 to 250 mgms.%; it was less than 45 mgms.% in 47%, greater than 75 mgms.% in 9%, and greater than 100 mgms.% in 3% of the cases (81). These studies reveal that in slightly less than 50% of tabetic patients a normal total protein may be found (45 mgms.% is the upper limit of normal total protein by the method that was employed). Madonick and Savitsky (73), using another technic, also found a normal total protein content in about 50% of the patients in their series. Positive globulin reactions occur in about 90-95% of tabetic patients (15). Kafka (58) states that the average globulin/albumin quotient is 0.8 in these patients,

whereas it is 2.0 in general paresis. The colloidal reactions appear to be much weaker and all types of curves may be evident. Menninger and Bromberg (77) found a so-called "paretic curve" in 3% of the 500 neurosyphilitic cases that were studied. A first-zone curve was found in 7 fluids, a mid-zone curve in 26 fluids, and a negative or lilac color change in 39 fluids examined by Merritt and Fremont-Smith (78). A greater number of abnormal colloidal gold reactions were obtained in another study of 100 patients; 75% were abnormal, 20% possessed a first-zone curve and 55% had a mid-zone curve (81).

Protein patterns in meningovascular syphilis exhibits great variation because of the different pathological processes included in this type of lues. In 80 cases of acute or subacute syphilitic meningitis, Merritt, Adams and Solomon (81) report that the total protein ranged from 23 to 380 mgms.%; 13% contained normal concentrations, 50% possessed less than 100 mgms.%, and about 78% ranged between 46-200 mgms.%. The globulin content is rarely very high, so that the gold rarely shows complete precipitation. A first-zone curve was found in 40%, a mid-zone curve in 50%, and an end-zone curve in 5% of the 80 cases reported by Merritt, Adams and Solomon (81). The vascular form of neurosyphilis may have an entirely negative fluid in respect to protein alteration, however, changes may occur and they are expressions of participation of the meninges or parenchymatous tissue in the pathological process.

We note that in neurosyphilis there is an increase in total protein, increase in globulin, abnormal colloidal gold reaction, in addition to pleocytosis and a positive specific test for syphilis. Variation in the protein pattern is dependent upon the structures involved in the pathological process, the degree of activity and individual differences.

The clinician should not rely upon the spinal fluid syndrome to differentiate the different forms of neurosyphilis, but rather the clinical picture which is the most important guide.

Multiple Sclerosis

Multiple sclerosis is a degenerative disease of the nervous system which results in abnormalities of the spinal fluid that are limited to the cell count, protein and the colloidal reactions. The fluid may be normal, or it may show moderate increases in lymphocytes, a moderate increase in protein, and a slightly or markedly abnormal colloidal gold reaction. However, there are no alterations in the fluid that are pathognomonic of multiple sclerosis. Freedman and Merritt (31), in a review of the literature since 1934, found that 78% (2,247 cases) of a series of 2,629 patients had spinal fluid abnormalities. An elevated protein was present in 33%, positive qualitative globulin tests occurred in 35% and positive colloidal reactions in 58% of the patients. Some of the work that was reviewed differentiated types of curves, and first-zone curves were reported in only 24% of 2,035 fluids. Electrophoretic studies of the cerebrospinal fluid protein have demonstrated that the gamma globulin is elevated in multiple sclerosis. Kabat and his associates (55) studied 100 patients with multiple sclerosis by their immunochemical method and demonstrated that 85% of the cases exhibited a pathologic increase of cerebrospinal fluid gamma globulin by one or more of their three criteria. They found that the absolute value of the gamma globulin and the percentage of gamma globulin to total protein are the two most sensitive criteria, whereas, albumin/gamma globulin ratio is less indicative. It is important to note that high gamma globulin is not specifically characteristic for multiple sclerosis, but that it was also

observed in neurosyphilis, periarteritis nodosum, post-vaccinial encephalomyelitis and Raynaud's disease (55). It is also well to record that no relationship between the activity of the disease and the cerebrospinal fluid gamma globulin is at present available. The zinc sulfate reaction which was recently introduced as a qualitative test for gamma globulin also showed a lack of correlation between clinical activity and the presence or absence of significantly positive zinc reactions (19). The total protein content may be normal or slightly elevated. Most reports indicate only few cases of total protein greater than 100 mgms.%, about 1/3 of the cases have normal values, and $\frac{1}{2}$ of the cases exhibit between 45-75 mgms.% of total protein. Von Storch, Harris and Lawyer (101) using Lange's quantitative gold reaction, found a colloidal curve designated as "D" in 93% of 100 cases considered to be "clinically proven" multiple sclerosis. The electrophoretic studies of Kabat, et al. (52) showed that this gold curve type D indicates protein pattern characterized by increased gamma globulin without a corresponding increase of albumin. Conventional colloidal gold or mastic tests were carried out on 61 fluids from patients with clinically proven multiple sclerosis, and only 28% or 17 fluids showed changes with readings of "2" or more in any tube. All but two of these cases showed significant elevations in the gamma globulin by immunochemical technic (55).

The spinal fluid syndrome that is highly suggestive <u>but not specific for</u> multiple sclerosis is: a clear fluid with normal or slightly elevated mononuclear count, a normal or moderately elevated total protein concentration (usually less than 75 mgms.%), a negative complement-fixation reaction both in blood and spinal fluid, a type D gold curve or elevated gamma globulin by one of the three criteria advocated by <u>Kabat and his coworkers.</u>

Intracranial Tumors

The typical findings in the cerebrospinal fluid of patients with a brain tumor are an increased pressure and an increased total protein content (78). The other components of the cerebrospinal fluid are usually normal, but not infrequently xanthochromia, moderate pleocytosis and abnormal colloidal reactions may also be observed. The latter is practically always associated with a high protein content usually greater than 100 mgms.%, and it is often a mid-zone curve but end-zone and first-zone curves also are found. Ayer (2) reported an increase in total protein content in 73% of 67 patients with verified tumors. Merritt and Fremont-Smith (78) found that the total protein was greater than 45 mgms.% in 69% and greater than 100 mgms.% in 32% of 182 cases that were studied. Hare, according to Merritt and Fremont-Smith found a protein increase in 55% of his 186 cases. Shannon and Morgan (92) found in a series of 43 cases of metastatic brain tumor that the average protein content of lumbar fluid was 99 mgms.%, and that 91% of the patients had a protein content greater than 40 mgms.%. Almost every investigator reporting on this fluid component incident to brain tumor note that about 50% or more of the patients exhibit increase protein content. The reader is referred to table 8. which is taken from Shannon and Morgan (92) to represent total protein findings associated with different brain tumors without regard to location, and to table 9. which is taken from Merritt and Fremont-Smith (78) wherein location of the tumor is considered also.

TABLE 8. Spinal Fluid Protein in Cases of Brain Tumor

Type of Tumor	Protein content (mgms.%) 39 or less Cases	%	40-99 Cases	%	100-199 Cases	%	200 or more Cases	%	Cases	Av. prot. content
Metastatic tumors	4	9	23	54	12	28	4	9	43	99 mgms.%
Primary tumors										
Astrocytoma	12	50	8	34	4	16	0		24	55
Glioblastoma multiforme	7	19	19	53	6	17	4	11	36	126
Meningioma	8	26	15	48	7	23	1	3	31	78
Medullablastoma	3	60	1	20	1	20	0		5	70
Acoustic neuroma	0 0		1	8	5	38	7	54	13	272

After Shannon and Morgan (92).

Of the several factors which may be the possible cause of this increased protein, the location of the tumor, perhaps, is the most important to be considered since definitive treatment is dependent upon it. Shannon and Morgan (92) found that patients with supratentorial metastases had a higher protein content than did those with subtentorial masses, and the highest concentrations were found in cases with superficial cerebral tumors. Spurling and Maddock (96) would seem to agree with this latter finding as they believe that the more intimate contact the tumor has with the cerebrospinal fluid, the more marked are the variations in the fluid. Tumors which invade the ventricular walls characteristically are manifested by extremely high total protein concentrations. However, the protein content, solely, can not be used for locating the intracranial tumor since the size, vascularity, necrosis and hemorrhage from the mass, and histology of the tumor also influence the fluid protein. Acoustic neuroma and glioblastoma multiforme are generally associated with high total protein content, whereas metastatic tumors from the lung, bone, breast or kidney appear to have no correlation between histology and protein over-production.

TABLE 9. The Protein Content of the Lumbar Cerebrospinal Fluid in 182 Cases of Cerebral Tumor

Location and type of tumor	Lumbar cerebrospinal fluid protein content (mgms. per 100 cc.)					
	Under 45	45-100	100-200	200-500	500-1500	Total
Supratentorial						
Glioma of cerebral hemisphere	23	27	15	5	1	71
Glioma of 3rd ventricle	0	1	0	1	1	33
Glioma of corpus callosum	0	4	0	3	0	7
Meningioma	4	7	4	0	0	15
Metastatic & other tumors of cerebrum	10	13	5	2	0	30
Pituitary & suprasellar tumor	2	3	0	0	0	5
Subtentorial						
Acoustic neuroma	0	2	3	11	0	16
Other cerebellopontine angle tumors	2	1	2	1	0	6
Glioma of cerebellum or 4th ventricle	12	7	3	1	0	23
Glioma of brain stem	3	2	0	1	0	6

After Merritt and Fremont-Smith (78).

Ayer and Solomon (4) pointed out the importance of comparing the protein content from different loci as a diagnostic aid in tumors of the central nervous system. An exhaustive study of this procedure in differently located intracranial tumors may be found in Merritt and Fremont-Smith's monograph (78). Since the advent of radiographic procedures for the localization of intracranial lesions, this technic has assumed much less significance.

In patients with intracranial tumors, an abnormal colloidal gold curve is noted, invariably, in the presence of high protein content, usually greater than 100 mgms.%. A series of 159 patients is reported by Merritt and Fremont-Smith (78) in which 62% (110 cases) exhibited a normal colloidal reaction by their criteria, 4% (6 cases) a first-

zone curve, 24% (38 cases) a mid-zone curve, and 4% (5 cases) an end-zone curve. There was no evidence of neurosyphilis in the six patients whose cerebrospinal fluid yielded first-zone curves.

Thus, the most significant abnormality of the protein pattern of cerebrospinal fluid in patients with intracranial tumors is the elevation of the total protein content. Any alteration of the globulin content which manifests itself by colloidal gold reaction or globulin test abnormalities will be dependent upon the increased total protein.

Acute Anterior Poliomyelitis

The protein, both albumin and globulin, in the spinal fluid of poliomyelitic patients has been shown by Frazer (30), Zingher (105) and Andelman et al (1) to be increased for as long as five to eight weeks after the onset of the disease. The peak of this abnormal protein increase occurs about the second week, whereas very early in the disease the protein content may be normal or slightly elevated. Merritt and Fremont-Smith's study of 98 patients revealed an average protein content between 47-50 mgms.% for the first six days after onset of paralysis; after the sixth day there was a sharp rise which reached a peak of 164 mgms.% between the twentieth and the thirtieth day. A gradual return to normal was observed after the second month. Andelman et al, using the Denis-Ayer method for total protein estimation, found the spinal fluid protein to average about 70.4 mgms.% in 44 children with paralytic poliomyelitis, 63.3 mgms.% in 11 children with meningitic poliomyelitis, and 55.1 mgms.% in 19 children with subclinical poliomyelitis examined 11-45 days after the onset of fever and/or gastrointestinal symptoms. They found that it takes approximately 55 days after exposure for the

protein concentration to return to normal in aborted cases. Thus, the protein pattern is a useful aid in the retrospective diagnosis of subclinical poliomyelitis in suspected cases. Frazer (30) estimated the globulin content in 362 lumbar fluids of 126 patients by the Noguchi butyric acid method and his results are tabulated in table 10. The increased globulin appears to be associated with the increased total protein, having its peak about the second to the third week after onset of the disease. The colloidal gold reaction is usually normal in all stages of the disorder, although a slight mid-zone change may occasionally be observed. Only 18 patients exhibited a significant color change, all being mid-zone, in a group of 99 patients studied (78).

TABLE 10. Per Cent of Abnormal Spinal Fluid Globulin in Poliomyelitis

Duration	Per cent of cases with abnormal globulin	Total number of cases examined
1 week	49 %	84
2 weeks	68%	79
3 weeks	70%	56
4 weeks	56%	37

Thus, the cerebrospinal fluid protein pattern of acute anterior poliomyelitis consists of normal or slightly elevated total protein during the early stages, maximal increase about the second week, gradual return to normal over the course of two months. In contrast to the total protein, there is a marked pleocytosis in the early stages and rapid decline in the cell count as the disease continues so a so-called albumin-cytologic dissociation is evident in the late stages of poliomyelitis. The globulin appears to vary in proportion to the total protein and, usually, there is no abnormality of the colloidal gold reaction.

SUMMARY

1. The cerebrospinal fluid protein is composed of albumin, beta and gamma globulin which are derived, for the most part, from the blood plasma and to a minor extent from the cerebrospinal tissues. Under pathological conditions, there is an increase of the normal protein components in addition to the appearance of alpha globulin and fibrinogen, presumably, due to increased permeability at the hemato-encephalic barrier. There is insufficient evidence to support either the plasma cells, lymphocytes, nervous tissue breakdown or autonomous elaboration by nervous tissue as the cause of the relative increase of gamma globulin in the chronic and degenerative neurologic diseases.

2. The concentration of the fluid protein differs in the three loci, being greatest in the lumbar fluid and least in the ventricular fluid. Contamination with blood or bacteria, and removal of large quantities of fluid will alter the protein content of the specimen.

3. The maximal normal values for the total protein and each of the components differ with the method employed in their estimation. The upper total protein level is 45 mgms. % (Denis-Ayer method), and is the only value of consequence, at the present time, since a good practical method for total protein estimation is available. There are no adequate technics for the quantitative determination of albumin, globulin and gamma globulin, and hence, a maximal physiological value for these constituents is without significance.

4. It can be concluded from the discussion of the qualitative protein tests that very little heed should be paid to their results. Clinically

positive reactions occur with normal fluids, clinically negative reactions occur with pathologically increased proteins, and the extent of the protein alteration can not be estimated by the degree of the positivity of the reaction. The importance of the colloidal gold test has been over-exaggerated. Regardless of the refinement of the technic, all results will be dependent upon the ability of the absolute and relative amounts of gamma globulin and albumin in the cerebrospinal fluid to precipitate the colloidal suspension.

5. The ability to quantitate some variable and correlate it to the patho-physiological picture of a disease entity.is one of the ultimate goals of the investigator. Due to the impracticability of the micro-Kjeldahl technic, numerous quantitative protein methods have been published, employed and discarded in pursuance of the ideal procedure. It is believed that the Denis-Ayer technic is the best clinical method for measuring the total protein concentration of the cerebrospinal fluid. Some quantitative means for determining the protein constituents, although not clinically useful, have been described, and it is hoped that subsequent modifications may make them applicable in the ward and the clinic.

6. The altered protein pattern is simply a reflection of a pathological process and such changes that may be exhibited are not specific; there may be a wide range of variation even within a given disease, depending upon the stage and severity of that disorder. The protein changes which generally occur in neurosyphilis, multiple sclerosis, intracranial tumors, and acute anterior poliomyelitis have been discussed.

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