Quantitative Estimation of the Total Protein in the Cerebrospinal Fluid

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BOSTON

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Increase in protein is probably the earliest and most constant abnormality noted in the cerebrospinal fluid under pathologic conditions. Not only does protein accompany even the most sluggish meningeal inflammatory process, but venous congestions are also accompanied by the appearance of protein in the cerebrospinal fluid in excess of normal. Protein excess may then be an index not only of an exudative meningeal process, but also of the degree of permeability of meningeal vessels under pathologic or abnormal physiologic states. In the one case the protein is of exudative, in the other of transudative origin.

With its diverse sources of origin, then, protein determination becomes of fundamental importance as a nondifferential test. No other test wholly supersedes it, and certainly no differential protein test wholly displaces it. And yet for years we have been satisfied with performing rough qualitative tests, the results of which were frequently construed as "positive" by one observer, "negative" by another, varying with the degree of experience, shades of light, size of test tubes, and other factors, more or less uncontrolled. It was the desire to place protein determinations in figures which led us to seek the aid of a chemist, and Dr.

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Denis\textsuperscript{1} elaborated a method which has proved satisfactory and not too difficult of application. Briefly, the method consists of precipitating the fluid protein in a colloidal state by means of sulphosalicylic acid, and reading by means of a colorimeter against a standard prepared at the same time from a blood serum solution of known protein content. The percentage of error by this method was found to be less than five.

This technic has been carried out exclusively by one of us (H. E. F.) during the last year, and approximately 2,100 readings have been made. The method has, we feel, abundantly justified the extra amount of time consumed; in many ways the figures obtained have been of value.

First, and obviously, the quantitative method has served to control the quicker and less accurate methods. Time and again preliminary estimations of protein said to be “normal” have been changed to a definite “increase,” and occasionally the reverse has been the case. While the borderline between normal and pathologic will always exist, we feel that by means of quantitation the line is more closely drawn.

Second, we have found a value in following the protein content of the fluids from patients under treatment for neurosyphilis. While much has been written concerning the cell count, colloidal gold test and Wassermann reaction under treatment, little has been said with reference to protein. By this method it is indicated that protein begins to fall early under treatment in all forms except paresis; in the latter it may even increase, although the cell count is dropping.

It was our interest in studying fluids from different loci of the subarachnoid space which made a more exact method of protein determination imperative, especially in the early diagnosis of spinal subarachnoid block by tumors and other pathologic states. Not infrequently a fluid is obtained by lumbar puncture which is slightly abnormal in protein content, but which might, and frequently does, pass as of no significance. When, by means of cistern puncture, we begin to compare the fluid above with that below, a difference of more than the slightest degree is found to be of importance. We have found such comparative readings of great value in the diagnosis of cord tumor, as significant as, and of more practical value than, the dramatic xanthochromic fluid with massive coagulation.

While protein values in different pathologic states are of only relative worth, certain disease entities or conditions do appear to give fairly constant readings. A preliminary table has already been published from this laboratory,\textsuperscript{2} the averages in which we now believe to have been too high. The accompanying table, based on a much larger number of tests, supersedes the previous one.

\textbf{Comments on Table}

From more than 2,100 quantitations from approximately 800 patients, we have used 429 of these from as many patients in making up the table. They were not chosen to conform to any preconceived idea, but only if the history, symptoms and laboratory findings permitted of a fairly definite and accurate diagnosis. Only one reading from each patient has been used, although many have had from six to twenty readings.

\textbf{I. Normal.}—The average is within the limits set by other observers, but somewhat wider range has been found in our series than most workers record. There are so many physiologic and non-neurologic pathologic conditions which exert a greater or lesser influence on the normal content of the spinal fluid that it is hard to draw a sharp line. However, we feel very strongly that any total protein quantitation above 40 mg. per hundred c.c. is distinctly pathologic.

\textbf{II. Syphilis of the Central Nervous System.}—

\textit{Tabes:} Although both the upper and lower limits in the treated active cases exceeds those of the untreated active cases, it will be noted that the average in the former is markedly lower. The average of the active cases presenting parietal colloidal gold curves—untreated and treated—seems to confirm the contention of many writers that Lange’s test is more or less of a quantitative protein test. To this we cannot accede, as the same curve is given in conditions other than general paresis, especially multiple sclerosis, in which disease the protein readings are more nearly normal. The inactive cases show some variation, the average being within normal limits.

TOTAL PROTEIN IN CEREBROSPINAL FLUID

<table>
<thead>
<tr>
<th>Cases</th>
<th>High</th>
<th>Low</th>
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<td>Mg.</td>
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I. Normal (no affection of central nervous system demonstrable)................11 | 38 | 15 | 25 |
II. Syphilitis of the Central Nervous System:
1. Active, untreated..................| 31 | 20 | 25 |
2. Active, treated (tests still positive)..................| 34 | 17 | 26 |
3. Active, with general parasympathetic curve (untreated and treated)..................| 19 | 15 | 25 |
4. Inactive (symptoms stationary, tests essentially negative)..................| 10 | 4 | 17 |
5. Paralytic (untreated and treated)..................| 7 | 4 | 10 |
6. Optic atrophy (untreated and treated)..................| 18 | 17 | 18 |
7. Cerebrospinal Syphilis:
   1. Meningitic type (average, 253 cells)..................| 4 | 118 | 89 | 99 |
   2. Active (average, 36 cells)..................| 12 | 95 | 59 | 73 |
   3. Inactive (average, 2.5 cells)..................| 6 | 36 | 26 | 42 |
   4. Latent syphilis..................| 3 | 111 | 71 | 85 |
III. Non-neuritic Syphilis:
1. Primary, active (blood positive)..................| 3 | 47 | 31 | 38 |
2. Secondary, active (blood positive)..................| 11 | 60 | 39 | 81 |
3. Tertiary, active (blood positive)..................| 10 | 31 | 18 | 23 |
4. Inactive (blood negative)..................| 13 | 36 | 20 | 36 |
IV. Vascular Hypertension:
1. With symptoms of old cerebral lesions (hemiplegia, etc.)..................| 18 | 150 | 90 | 105 |
2. With symptoms denoting recent cerebrovascular lesions..................| 12 | 95 | 59 | 73 |
3. Without evidence of cerebral lesions (cerebrospinal, etc.)..................| 14 | 138 | 82 | 96 |
V. Vascular Disease with Hypertension:
1. Chronic myocardial insufficiency..................| 1 | 10 | 10 | 10 |
2. Stokes-Adams syndrome (old cerebral infarction—hypotension)..................| 1 | 10 | 10 | 10 |
V. Epidemic Encephalitis:
1. Active stage..................| 6 | 50 | 30 | 36 |
2. Convalescent..................| 11 | 36 | 25 | 31 |
VI. Poliomyelitis:
1. Acute..................| 3 | 81 | 67 | 75 |
2. Inactive..................| 1 | 23 | 23 | 23 |
VII. Meningitis:
1. Tuberculous..................| 10 | 239 | 68 | 70 |
2. Acute forms (scleronecrosis and pneumonitic meningitis)..................| 5 | 923 | 64 | 76 |
3. "Serous"..................| 2 | 87 | 67 | 77 |
VIII. Degenerations of Central Nervous System:
1. Polymyelitis agitans..................| 5 | 74 | 59 | 81 |
2. Multiple Sclerosis:
   1. Progressive..................| 15 | 106 | 15 | 48 |
   2. Inactive..................| 4 | 95 | 31 | 48 |
   3. Poliomyelitis (general paralysis of spinal cord)..................| 3 | 247 | 42 | 119 |
   4. Friedreich's ataxia..................| 1 | 10 | 10 | 10 |
IX. Brain tumor, with increased intracranial pressure..................| 1 | 100 | 55 | 64 |
X. Brain abscesses..................| 1 | 100 | 55 | 64 |
XI. Miscellaneous:
1. Epilepsy (no pathologic lesion known)..................| 11 | 50 | 17 | 29 |
2. Meningitis gravis..................| 1 | 20 | 10 | 19 |
3. Toxic subacute encephalitis..................| 1 | 87 | 24 | 51 |
4. Subacute meningitis..................| 3 | 24 | 12 | 20 |
5. Hydrophobia (commencing acute systemic infections)..................| 3 | 21 | 19 | 20 |
XII. Combined Clusters and Lumbar:*
1. Normal..................| 7 | 29 | 15 | 25 |
2. Cluster..................| 7 | 29 | 15 | 25 |
3. Block, with hydrodynamic evidence..................| 11 | 33 | 20 | 25 |
4. Progressive myelitis..................| 11 | 33 | 20 | 25 |
5. Block, without hydrodynamic evidence..................| 6 | 150 | 45 | 70 |
6. Progressive myelitis..................| 11 | 33 | 20 | 25 |

* One case presumably belonging to this group but undoubtedly complicated, gave 223 mg.
† Only one case so low; next lowest, 125.

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Paresis, Untreated and Treated: The striking feature of these figures is that while the high limit is not as high as in some other central nervous system syphilitic conditions, the low is the highest of the entire syphilitic groups, excepting the meningitic type, and the average is also the highest of the infectious disease encountered except the meningitides.

Optic Atrophy, Untreated and Treated: The wide variation here is due to the inclusion of both early untreated and treated cases, some of which have been quiescent for years. In patients favorably responding to treatment, the protein decrease is the first sign usually noted, and the quantitation here is especially valuable in prognosis.

Cerebrospinal Syphilis (clinically and from laboratory findings, progressive and nonprogressive types): The meningitic type with an average cell count of 253 gives the highest average, although the range is much less than Group 2, with an average of 26 cells. Both of these types usually have positive spinal fluid Wassermann reactions. The inactive type with low normal cell count and usually negative fluid Wassermann reaction, nevertheless, gives a protein average over 50 per cent. higher than normal.

Latent Syphilis of the Nervous System: Patients never having had any neurologic symptoms, but with positive laboratory findings, are included in this group. The average number of cells of the three patients was 18; while a wide protein range is seen, the average is over two and one-half times the normal average.

III. Non-Neurologic Syphilis (no sign of symptoms of neurologic syphilis)—the patients in the three classical stages all had positive blood Wassermann reactions. The inactive cases had received enough treatment to give negative blood Wassermann reactions. The spinal fluids gave a normal cell count and negative Wassermann reactions, and no globulin ring was recorded. It is of interest to note that while the average total protein of each of the four subdivisions was within our normal, the cases presenting a primary lesion had the highest average. The greatest variation was found to be in the secondary stage of the disease.

IV. Vascular Hypertension.—With symptoms of old cerebral lesions, such as hemiplegia and internal
ophthalmoplegia, cases range from low normal to six times normal, averaging over two and one-half average normal.

With symptoms denoting recent cerebrospinal lesions, a shorter range is given with a somewhat lower average.

The fourteen patients without evidence of cerebral lesions (uremia, cardiorenal vascular, etc.) had an average systolic blood pressure of 179, average diastolic of 105. In these cases the protein range is wide and the average over twice normal.

Of the thirty-nine cases in these hypertension groups, only nine gave normal readings.

Vascular Disease with Hypotension.—One of these patients had chronic myocardial insufficiency and one Stokes-Adams syndrome with cerebral ischemia. The former had increased proteins, which may have been caused by former arterial hypertension or damage. The latter at necropsy showed a cerebral infarction undoubtedly following a slowly forming thrombus. His symptoms had been noted for a year and a half with remissions.

V. Epidemic Encephalitis.—The wide extremes in the active stage are worthy of passing note. The average here is two and one-half times normal. In the convalescent patients the average is high normal.

VI. Poliomyelitis.—The three acute cases give a fairly uniform high total protein content, with average three times normal.

VII. Meningitis.—Tuberculous: These fluids were taken from early and late cases, but even in the early cases showing slight pleocytosis the lowest recorded protein was double a high normal, the average being six times average normal.

Acute Forms: Protein regularly increased, giving as a group the second highest average of the entire series. The one patient with the low protein content had a proved streptococcic meningitis. Three protein determinations made on this patient within eight days were all low. But for this case the average of the other four would have been 524 mg. per hundred c.c.

So-called “serous meningitis” includes those cases with meningitic symptoms but without a corresponding pleocytosis or positive cultures, in which recovery took place. It is assumed that in these cases the protein increase is of transudative rather than exudative origin.

VIII. Degenerations of the Central Nervous System.—Paralysis Agitans: While the average in these patients is not high, it is sufficiently high not to be ignored. Some of the patients were advanced in years, and a cardiorenal vascular condition may partially account for the high readings.

Multiple Sclerosis: The variations in both the progressive and the inactive cases are similarly marked, and the two averages coincide. In seven of the nineteen cases the content was within normal limits.

Infection (?) of the Spinal Cord: These were cases showing a rapidly progressive, diffuse and extensive—myelitic process of unknown etiology.

Friedreich’s Ataxia: The one case shows a low normal protein reading.

IX and X. Brain Tumor and Brain Abscess.—To these groups we desire to add more determinations before making any definite statements. However, from the brief series we are led to surmise that tumors and abscesses located in or very near the cortex will give an increased total protein.

XI. Miscellaneous.—Epilepsy: All cases of known or demonstrable pathologic lesion were omitted, the so-called “idiopathic” epilepsies alone being chosen. An average only slightly higher than our normal average is given. It would be interesting to know what variation exists in the individual cases immediately following an attack and at some later period. Unfortunately, the time relation of rachicentesis is unknown to us in this series.

The remaining cases of this group need no comment.

XII. Combined Cistern and Lumbar 2.—These patients presented clinical signs and symptoms which might have been interpreted in the light of cord compression, partial or complete. Included in the “normal” are cases which later were diagnosed syringomyelia, multiple sclerosis, etc. A normal difference in protein content between ventricular and lumbar fluids has been known to exist. We found very constantly a normal difference between cisterna magna and cisterna magna, cases range from low normal to six times normal, averaging over two and one-half average normal.
ternal terminalis fluids to be from 4 to 8 mg. per hundred c.c. In the second subdivision of this group the extreme differences are found. These patients frequently had a previous rachicentesis with a marked increase of total protein present. The combined punctures were made to determine the relative difference hydrodynamically and chemically. Several patients with cord compression operated on during the past year did not have the combined puncture for the reason that the lumbar fluid alone—showing in some cases the complete syndrome of Froin, with a total protein of from 2,100 to 2,400 mg. per hundred c.c.—made, in view of the clinical findings, an evident diagnosis.

In the cases without hydrodynamic evidence of block, protein, quantitation plays a most important part. The clinical evidence, coupled with the marked differences in protein from the two loci, completed the picture on which operation was advised.

**COMMENT**

What is the value of careful quantitative protein estimation? We believe that in many neurologic diseases the appearance of abnormal amounts of protein in the spinal fluid is one of the earliest detectable signs of pathology or altered physiology, and that it constitutes a nondifferential reaction of fundamental importance. If this is so it becomes necessary to know the normal amounts, and if possible, the amounts to be expected in different pathologic states. It is therefore obvious that an estimation of protein by an accurate quantitative method becomes essential.

For some years the French, pioneers in cerebrospinal fluid pathology, have expressed their protein determinations in figures, using for the most part the Mestrezat trichloracetic acid method, and more recently that advocated by Ravaut and Boyer, in which silver chloride is used as standard. While we have not used these methods, if it is felt that our method, elaborated by Dr. Denis, should be even more accurate than these, in that the standard employed is a protein, and that an unknown protein precipitate is compared with a known protein precipitate prepared at the same time.

While all who examine spinal fluid form a rough conception of the amount of protein to be expected in normal fluids, and some authors give figures, it is well known that the normal variation is considerable. To one only slightly familiar with such examinations, the estimation of normal and pathologic amounts of protein is bewildering. It was with the hope of establishing normal and pathologic groups that this method has been followed by us for the last year and a half. Can we establish normal, and separate pathologic states according to protein content? To a certain extent we believe that we can, as is apparent in the accompanying table, of which a few of the most significant figures will be discussed.

**Normal**.—We find to lie between 38 and 16 mg. per hundred c.c. Perhaps a larger number of cases would make the limits a little greater, but it appears safe to say that more than 40 mg. is always suspicious of an underlying pathologic process. True, many figures below this occur in the different pathologic groups, but for the most part these low readings are found in convalescence or in inactive stages of chronic diseases.

**Pathologic**.—In considering pathologic states, we must admit at least two sources of spinal fluid protein: that which accompanies exudative processes, and that which comes from the blood by transudation or increased permeability of the membranes. The purest example of the former is an acute pyogenic meningitis, and of the latter the fluid below a cord tumor. In the milder inflammations, such as syphilitic meningitis, an amount of protein will be expected less than in the acute meningitides, and this is usually the case; but within the syphilitic group itself there are subgroups which show slight cell reaction and relatively great protein content. It is reasonable to suppose that this considerable protein output is not all the product of meningeal irritation, but is also a product of transudation, and perhaps a manifestation of deep-seated parenchymatous disease. Thus, we find the higher readings in the syphilitic group are either associated with acute meningitic types (exudative in origin) or with the more profound degenerative types, such as paresis (transudative in origin). We have, then, within the syphilitic
Of special note are several groups of cases in which the spinal fluid is generally said to be “negative,” but which are shown to be abnormal on careful protein determination. Of the group of cases presenting vascular hypertension, thirty out of thirty-nine presented hyperalbuminosis. Some of these patients had evidence of cerebral vascular accidents, and some did not. It is not too much to say that in these cases there is usually to be found protein excess which, because of lack of cells, is of transudative origin. This is a point but seldom emphasized, and one of considerable diagnostic significance. Presumably in a similar manner a limited number of cases of purely degenerative character, such as multiple sclerosis and paralysis agitans, have presented increased protein. The fluids from brain tumors and abscesses also are not necessarily “negative,” but may present considerable protein excess. French writers have for several years paid considerable attention to this isolated protein reaction, but in America its significance appears to have been generally overlooked. With the quantitative method, a moderate but distinct protein increase is brought to the attention and cannot be discarded as of no significance.

As is well known, the acute forms of meningitis yield a very great amount of protein, but it was not realized by us until the present method was employed that a typical acute meningitis might be associated with a comparatively slight protein output, even in the advanced stages of the disease. The amount of protein obtained in tuberculous meningitis agrees with preconceived ideas that the protein is moderately increased, but less than in the pyogenic forms. Comment on the serous meningitic types must be withheld until more cases have been investigated.

Since first called to attention by Froin⁴ in 1903, the syndrome which bears his name (the chief characteristic of which is excessive protein) has been considered almost pathognomonic of spinal cord compression. But it is only recently that the importance of lesser amounts of protein in the spinal fluid below cord tumors has been emphasized. For more than a year, cases suspected of having cord tumor have been systematically studied by combined cistern and lumbar puncture in this clinic, an opportunity being thereby given to compare the spinal fluid above and below the obstruction. It has been found normally that the cistern fluid shows slightly less protein than that obtained from the lumbar sac. In almost every case in which tumor has been demonstrated there has been a distinct difference in the protein content of the two fluids. Further, when it was possible by means of manometric readings to demonstrate a block in the spinal meninges, the protein content from a lumbar puncture was usually found increased to a marked degree, in some cases presenting the complete syndrome of Froin. It is our belief that even slight increase in protein in suspected cord tumor is an almost constant finding and a sign of great value. Another paper will deal with this subject.

A point of interest, but one not brought out in the table, is the effect of repeated lumbar puncture on subsequent protein determination. In a number of cases it was clearly demonstrated that in cases in which spinal fluid was withdrawn at an interval of a few days, the second fluid regularly presented lower readings. If the interval was increased to a week or more, this was not the case. This fact suggests a hypothetic hydromere and an interesting correlation with so-called lumbar puncture headache.

CONCLUSIONS

While we do not pretend that quantitative protein determination is a necessity in the hands of a competent and experienced observer, there is no question but that accurate estimation gives results in more certain and intelligent form, that slighter degrees of abnormality tend to assume unsuspected significance, and that in consequence diagnosis is rendered more acute.

⁴ Froin: Gaz. d. hôp. 76: 1005, 1903.