Distribution of Protein, Lipid and Administered Bromide Between Serum and CSF in Myxedema

By Richard A. Schacht, Wallace W. Tourtellotte,

Boy Frame and Stewart N. Nickel

An analysis of the blood and CSF protein concentration and lipid profiles in 14 patients with clinical myxedema has been presented. In addition, the serum and CSF bromide concentrations were measured following the oral administration of sodium bromide during the pre- and post-treatment period. While the serum lipids and protein concentrations were noted to be elevated prior to treatment, the CSF constituents were elevated to a greater degree when compared to normal values than serum constituents, and treatment caused a return in concentration towards normal. Both serum and CSF bromide concentrations were elevated in the myxedematous state and returned toward normal following treatment, though a disproportionately greater rise in CSF than serum concentration was noted irrespective of treatment. That the evidence may reflect a breakdown in the "blood-CSF barrier" in myxedema, which is partially corrected by treatment, is discussed as a likely explanation for these observations. (Metabolism 17: No. 9, September, 786-793, 1968)

NEUROPSYCHIATRIC MANIFESTATIONS occur frequently in patients with myxedema and occasionally are accompanied by a marked elevation of the cerebrospinal fluid (CSF) protein.[1] The cause of the elevated CSF protein in myxedema is not known but one possibility is an altered permeability of the blood-CSF barrier which allows protein to enter the CSF from the serum. In an attempt to learn more about the integrity of this barrier in myxedema, simultaneous assessments of the protein and lipid fractions in both the serum and CSF were carried out in association with a study of the distribution of orally administered bromide between the serum and CSF compartments. Evidence compatible with an altered blood-CSF barrier in myxedematous patients was obtained. Studies were performed before treatment and again three to twenty months after thyroid replacement therapy to see the effect of treatment on the observed abnormalities.

MATERIALS AND METHODS

Fourteen patients in whom primary myxedema was clinically apparent comprise the basis for this report. The diagnosis of myxedema was made from the characteristic physical findings and laboratory studies of an abnormally depressed protein bound iodine (below 3.5 µg. per cent and/or radioactive iodine uptake (less than 15 per cent in 24 hours). All patients were examined before treatment was begun and at regular intervals for periods up to 20 months after treatment when the clinical signs of myxedema had subsided.

Patients were carefully questioned to verify that none had taken bromides in any form for a reasonable period of time preceding the study. Prior to treatment and in the fasting state, 10 ml. of venous blood was drawn before the administration of sodium bromide, this specimen being utilized to assure that substances which might interfere with the subsequent bromide determinations were not present in the serum. During the next 48 hours, patients were given a total oral dose of sodium bromide equal to 0.1 Gm. per kilogram of body weight in six divided doses immediately following meals, the sodium bromide having been dissolved in a glass of water.

Thirty-six to 48 hours following the last dose of sodium bromide, in the fasting state, 10 ml. of venous blood was drawn, allowed to clot and retract, was centrifuged at 4° C and the supernatant fluid was carefully removed and stored under sterile conditions for the subsequent determinations of the total protein, lipid fractions and bromide levels.

CSF was obtained by lumbar puncture at the time venous blood was sampled. In three separate chemically cleaned tubes, 2, 15 and 2 ml., respectively, of CSF were collected. Cell counts were done on tubes 1 and 3 to assure that contamination of the specimens due to a "traumatic tap" was not present and the contents of tube 2 were centrifuged for ten to twenty minutes at 900 to 1100 relative centrifugal force (RCF) at 4° C, and the cell-free supernatant fluid was removed and stored under sterile conditions. This fluid was used for the total protein, lipid profile and bromide determinations.

After a period of up to twenty months following these initial studies and following or during treatment of their myxedema, patients were again prepared as described above and the same procedures carried out.

Chemical Procedures

The determination of total protein was accomplished by a modification of the biuret procedure.[2] Tourtellotte[3] presented in a previous report an analytical scheme for the determination of cephalins, cephalins plus lecithins, sphingomyelins, total cholesterol, free cholesterol and total lipids. From these determinations the lecithin, non-phosphorus sphingolipid, cholesterol ester, total phospholipid and neutral fat concentrations can be calculated.

The method used for the determination of bromide concentrations in the serum and CSF is a modification previously described,[4] of the procedures of Hunter and associates[5] and Friedman.[6] Gamma globulins were quantitated utilizing the immunochemical technique of Kabat and Mayer[7] and expressed as a percentage of the total protein.

RESULTS

CSF

In Table 1, the mean values obtained for the concentrations of the protein and lipid constituents in the CSF of the 14 myxedematous patients are summarized. Their CSF profiles are compared with similar constituents observed in normal individuals, the later data having been presented and discussed in detail elsewhere.[8] In addition, similar observations made on the CSF after a period of treatment (mean of 12 months) in nine of these patients is presented. Both the pre- and post-treatment values are compared with the normal values and represented as the ratio patient/normal or P/N. With the exception of the per cent free cholesterol, each consti-

Table 1.—*Analysis of Cerebrospinal Fluid Constituents*
Before and After Treatment of Myxedema

Cerebrospinal Fluid Constituents	Normal Mean	± S.D.	Pre-treatment Mean	± S.D.	P/N*	Post-treatment Mean	± S.D.	P/N
Total Phospholipids								
(μM/100 ml.)	521	± 89	889	±393†	1.71	531	±255††	1.02
Cephalins	142	± 39	228	± 80†	1.61	173	± 49††	1.22
Lecithins	228	± 54	407	±150†	1.78	327	± 93	1.43
Sphingomyelin	119	± 29	235	± 98†	1.97	156	± 46	1.31
Nonphosphorus Sphingo-								
lipids (μM/100 ml.)	94	± 55	154	± 67†	1.64	111	± 23††	1.18
Total Cholesterol								
(mg./100 ml.)	395	± 87	593	±268†	1.50	369	± 89††	0.93
% Free	33	± 9	33	± 6	0.99	33	± 2	0.99
Neutral Fats								
(mg./100 ml.)	434	±238	480	±554	1.11	267	±240	0.62
Total Lipids								
(mg./100 ml.)	1252	±239	1881	±853†	1.50	1239	±363††	0.99
Total Protein								
(mg./100 ml.)	36	± 10	72	± 40†	1.95	46	± 15††	1.26
% Gamma Globulin	10	± 3	13	± 4†	1.36	13	± 3††	1.30

*Patient/Normal
†Significantly increased from normal, at 99% confidence level
††Not significantly increased from normal, at 99% confidence level

tuent measured was elevated in the pre-treatment period and returned towards normal following treatment.

A statistical analysis of this data was performed by means of the student t-test. Nine of 11 parameters measured in the pretreatment period were significantly altered, this alteration being significant at the 99 per cent confidence limits. Only the mean values obtained for the per cent free cholesterol and the concentration of neutral fat were not significantly elevated. Following treatment, seven of the nine values unquestionably elevated were no longer significantly different from normal values. The values obtained for lecithin and sphingomyelin concentrations remained elevated to a significant degree.

In Table 2 the CSF constituents of normal individuals, myxedematous patients prior to treatment and myxedematous patients after a period of treatment are presented in terms of the lipid fractions present per mg. of protein. When expressed in these terms, the values are significantly reduced in the myxedematous patient and treatment does not substantially alter this reduction.

Serum Constituents

The values obtained for serum constituents of normal individuals are compared with those of myxedematous patients during the pre- and post-treatment period in Table 3, and the ratio of patient/normal (P/N) is presented. All parameters but the per cent free cholesterol and concentration of neutral fat showed some degree of elevation prior to treatment and in most

Table 2.—*Cerebrospinal Fluid Lipids per mg. Protein
Before and After Treatment of Myxedema*

Cerebrospinal Fluid Constituent	Normal Mean	Patients with Myxedema			
		Pre-treatment		Post-treatment	
		Mean	P/N*	Mean	P/N
Total Phospholipids					
(mμM/100 ml./mg. Prot)	14.3	12.4	0.87	11.5	0.80
Cephalins	3.9	3.2	0.82	3.8	0.97
Lecithins	6.3	5.7	0.90	7.1	1.13
Sphingomyelin	3.3	3.3	1.00	3.4	1.03
Non-Phosphorus Sphingolipids					
(mμM/100 ml./mg. Prot)	2.6	2.2	0.85	2.4	0.92
Total Cholesterol					
(μg./100 ml./mg. Prot)	10.9	8.3	0.76	8.0	0.73
Neutral Fats					
(μg./100 ml./mg. Prot)	11.9	6.7	0.56	5.8	0.49
Total Lipids					
(μg./100 ml./mg. Prot)	34.4	26.3	0.77	26.9	0.78

*Patient/Normal

instances more closely approximated normal values following treatment.

A statistical analysis of the data was performed by means of the student t-test. Eight of 11 parameters measured in myxedematous patients showed a statistically significant elevation when compared to normal individuals at the 99 per cent limits of confidence. Seven of these eight constituents were no longer significantly elevated after a period of treatment. The mean serum cephalin concentration remained significantly elevated following treatment. Conversely, the non-phosphorus sphingolipid concentration which was not previously statistically elevated attained statistical significance following treatment. The per cent free cholesterol and total protein concentrations were not significantly elevated prior to or following treatment.

Bromides

The mean bromide concentrations in the serum and CSF of myxedematous patients during the pre- and post-treatment periods are presented and compared to similar data obtained in normal individuals[4] in Table 4. In addition, the serum/CSF ratios of the bromide concentrations are presented, the normal for our laboratory being 3.30. Both the serum and CSF bromide ion concentrations were elevated in the myxedematous state and returned toward normal following treatment. Conversely, the serum/CSF ratios of 2.5 and 2.7 during the pre- and post-treatment periods respectively indicate that no significant change in ratios resulted from treatment and reflect a disproportionately greater elevation in the CSF than serum bromide concentration in both instances.

DISCUSSION

The increasing frequency with which the neurologic features of myxedema are being documented warrants investigation into the close envi-

Table 3.—*Analysis of Various Serum Constituents
Before and After Treatment of Myxedema*

| | Normal | | Patients with Myxedema | | | | | |
| | | | Pre-treatment | | | Post-treatment | | |
Serum Constituents	Mean	± S.D.	Mean	± S.D.	P/N*	Mean	± S.D.	P/N
Total Phospholipids								
(mμM/100 ml.)	262	± 35	361	± 62†	1.38	267	± 51††	1.02
Cephalins	26	± 7	35	± 6†	1.36	42	± 13	1.61
Lecithins	182	±.25	259	± 40†	1.42	178	± 35††	0.98
Sphingomyelin	53	± 9	81	± 14†	1.53	57	± 8††	1.07
Nonphosphorus Sphingo-								
lipids (mμM/100 ml.)	26	± 18	31	± 10	1.19	53	± 22	2.04
Total Cholesterol								
(mg./100 ml.)	180	± 32	280	± 48†	1.56	189	± 28††	1.05
% Free	30	± 5	26	± 2	0.88	26	± 32	0.87
Neutral Fats								
(mg./100 ml.)	489	±111	438	±120†	0.90	330	±125††	0.67
Total Lipids								
(mg./100 ml.)	828	±201	1016	±190†	1.23	775	±165††	0.93
Total Protein								
(mg./100 ml.)	7.1 ±	0.1	7.6 ±	.50	1.07	6.6 ±	0.8	0.93
% Gamma Globulin	18.8 ±	3.3	31.8 ±	6.1†	1.69	18 ±	3.3††	0.96

*Patient/Normal
†Significantly increased from normal, at 99% confidence level
††Not significantly increased from normal, at 99% confidence level

ronment of the brain tissues, namely, the CSF. Elevation of the spinal fluid protein is frequently observed in myxedematous patients and may present diagnostic difficulties in the presence of neuromuscular signs and symptoms when the usual clinical features of myxedema are minimal or unrecognized.

The problem of evaluating the nature of altered central nervous system function and changes in the spinal fluid in systemic disease is complex. Study of the CSF offers an avenue of approach since abnormalities observed here may reflect either a breakdown in the protective "barrier" to assault from without or an intrinsic alteration within the central nervous system. In Tay-Sachs disease, for example, evidence indicates a primary abnormality in lipid metabolism within the central nervous system which is reflected in the CSF compartment alone.[2] In myxedema, diffuse organ system involvement may encompass changes in both the serum and CSF constituents.

The present report presents data on the abnormalities observed in the

Table 4.—*Mean Bromide Concentrations in Serum and CSF
Before and After Treatment of Myxedema*

| | Serum Bromide (mg./100 ml.) | | | CSF Bromide (mg./100 ml.) | | | Ratio Serum/CSF | | |
	N	Mean	σ	N	Mean	σ	N	Mean	σ
Normal	20	23.0	3.24	20	7.1	0.44	20	3.3	0.42
Pre-	14	34.5	12.03	14	13.3	4.61	14	2.5	0.37
Post-	7	25.8	10.9	7	9.6	4.62	7	2.7	0.21

serum and CSF protein and lipid fractions in myxedema. We have been able to document not only a rise in the mean CSF lipid fractions in myxedema, but a disproportionately greater rise of the CSF lipids than of serum lipids. This is evident when the P/N values for CSF and serum constituents are compared.

Thompson et al.[9] have documented a markedly increased protein content of the cerebrospinal fluid in patients with myxedema and further were able to show a return to normal of the protein content after adequate thyroid replacement therapy.

There is strong evidence that the spinal fluid lipids in myxedema are closely associated with the CSF protein for when the data are expressed in terms of CSF lipid concentration per mg. of protein, there is no increase in the observed ratios irrespective of the lipid fraction being considered. More striking is the fact that the lipid/protein ratios are slightly reduced reflecting a deficit in lipid transport or diffusion as compared to protein distribution. This deficit is perhaps more apparent than real since we do not know precisely the specific protein fraction with which the lipids might be associated. These observations suggest a movement along a concentration gradient of proteins and lipids from serum to CSF in myxedema. Final proof of this thesis would entail the introduction of tagged lipids and proteins into the serum compartment with subsequent recovery from the CSF.

Evidence to favor a breakdown in the blood-CSF barrier is obtained from an examination of the serum and CSF bromide ion concentrations. Cognizant of the limitations upon the application of bromide distribution studies emphasized by Hunter et al.[5] the bromide studies are of particular interest for two reasons. First, bromides are not found normally in the serum or CSF as are lipids and proteins and their presence in the CSF indicates a migration from the serum into which they are introduced. Second, bromide ions are considerably smaller particles than lipid or protein-bound lipid molecules and, because of their smaller size, might be expected to reflect more accurately minor alterations in the permeability of a "barrier." Finally, as Wallace and Brodie[10] demonstrated, bromide ions do not enter the cerebrospinal fluid compartment as freely as other extracellular fluid spaces. With this in mind, the observation that both serum and CSF bromide ion concentrations are elevated in myxedema and that the serum/CSF ratio is depressed, indicating a disproportionately greater rise in CSF bromide concentrations takes on greater significance. Whereas the mean lipid serum to CSF ratio returns toward normal following treatment, the bromide ion serum to CSF ratio is unaltered. It is possible that treatment which renders the patient clinically normal is unable to effect complete repair of a damaged serum-CSF barrier, the smaller bromide ions still being able to cross a damaged barrier. Perhaps more prolonged treatment in the patients under study would have restored the "barrier" to normal.

The exact mechanism whereby alterations of the CSF lipid profile in myxedema are affected remains obscure. Several theories might be forwarded to explain these observations. Primary among these is whether the

observations are the direct result of active or passive transfer across the blood-CSF barrier. Lange[11] has demonstrated an increase in capillary permeability in the skin in myxedematous patients which was reversed by thyroid replacement therapy. The possibility that a damaged "barrier" allows passive transfer of the constituents measured from the serum to the CSF seems most likely since virtually every constituent measured, whether of endogenous or exogenous origin, was found in greater concentrations in the CSF in the untreated myxedematous state. There seems to be a general breakdown of the "barrier" rather than a selective defect in active transfer for selected substances.

So far only the entry of materials into a system or compartment has been considered. Equally as important in any dynamic system is the facility with which materials can be cleared or eliminated from that system or compartment. The possibility that substances are removed from the CSF at a slower rate and their elimination from the body as a whole is prolonged cannot be excluded.

In the foregoing, emphasis has been placed upon a migration of the constituents measured from the blood to the CSF. Shanker[12] has summarized the evidence to support the concept that entry into the CSF and brain is dependent upon the passage of constituents through intact cells, i.e., those lining the choroid plexus, ependyma and glial sheaths of brain capillaries. The possibility that entry into the CSF occurs via the brain tissues and not directly from blood to CSF cannot be excluded. Thus, a brain-CSF breakdown is possible and only studies at the cellular level can provide the necessary evidence to substantiate the exact mechanisms whereby the observed alterations occur.

A breakdown in the blood-CSF barrier in myxedema has potential clinical significance. It is a known clinical fact that patients with myxedema may be unduly sensitive to the administration of various drugs such as sedatives and narcotics. In addition to a reduced metabolic breakdown, it is possible that these drugs are able to reach the brain in greater concentrations through a damaged blood-CSF barrier allowing for a greater and more prolonged effect of these drugs upon the central nervous system. This could be tested by measuring the concentration of such orally administered agents in the serum and CSF of patients with myxedema and make comparisons with the concentrations in normal subjects.

Further investigation of the blood-CSF barrier in myxedema as well as in other systemic diseases is warranted in view of the possible therapeutic implications.

REFERENCES

1. Nickel, S. N., Frame, B., Bebin, J., Tourtellotte, W. W., Parker, J. A., and Hughes, B. R.: Myxedema neuropathy and myopathy: A clinical and pathologic study. Neurology 11:125, 1961.

2. Tourtellotte, W. W., Allen, R. J.,

Haerer, A. F., and Bryan, E. R.: Study of lipids in cerebrospinal fluid and serum, X, in Tay-Sachs' disease. Arch. Neurol. 12: 300, 1965.

3. Tourtellotte, W. W.: Study of lipids in cerebrospinal fluid. VI. The normal lipid profile. Neurology 9:375, 1959.

4. Haerer, A. F., Tourtellotte, W. W., Richard, K. A., Gustafson, G. M., and Bryan, E. R.: A study of the blood-cerebrospinal fluid-brain barrier in multiple sclerosis. I. Blood-cerebrospinal fluid barrier to sodium bromide. Neurology 14:345, 1964.

5. Hunter, G., Smith, H. V., and Taylor, L. M.: On the bromide test of permeability of the barrier between blood and cerebrospinal fluid—an assessment. Biochem. J. 56: 588, 1954.

6. Friedman, M. M.: Simplified bromide determination in blood and urine. J. Biol. Chem. 144:519, 1942.

7. Kabat, E. A., and Mayer, M. M.: Experimental Immunochemistry (ed. 2), Springfield, Ill., Charles C Thomas, Publisher, 1961.

8. Tourtellotte, W. W., DeJong, R. N., Janich, S., and Gustafson, K.: A study of lipids in the cerebrospinal fluid (and serum). VIII. Further comments on the normal lipid profile. Univ. Mich. Med. Bull. 28:114, 1962.

9. Thompson, W. O., Thompson, P. K., Silveus, E., and Dailey, M. E.: The protein content of the cerebrospinal fluid in myxedema. J. Clin. Invest. 6:251, 1928.

10. Wallace, G. B., and Brodie, B. B.: The distribution of iodide, thiocyanate, bromide and chloride in the central nervous system and spinal fluid. J. Pharmacol. Exp. Therap. 65:220, 1939.

11. Lange, K.: Capillary permeability in myxedema. Amer. J. Med. Sci. 208:5, 1944.

12. Schanker, L. S.: Mechanisms of drug absorption and distribution. Ann. Rev. Pharmacol. 1:29, 1961.