CCLXXI. INORGANIC CONSTITUENTS OF THE CEREBROSPINAL FLUID.

VI. SULPHUR.

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A NUMBER of papers have appeared in recent years on the relationship between the constituents of the cerebrospinal fluid and the blood serum. Calcium, magnesium, potassium and inorganic phosphorus have all been studied more or less extensively, but inorganic sulphur, as Flexner [1934] has pointed out, has been largely neglected. Mestrezat [1912] was probably the first to estimate the sulphur of cerebrospinal fluid, but his values were probably much too high (49–90 mg. SO_3 per 100 ml.), and he did no parallel investigation of the serum. Meyer-Bisch [1922] stated that the cerebrospinal fluid sulphur was higher than that of the serum, both normally and in two pathological non-meningeal cases. The serum values ranged from 12 to 22 mg. H_2SO_4 per 100 ml. and the corresponding cerebrospinal fluid values were 20–98 mg. H_2SO_4 per 100 ml. These figures for serum were later shown to be too high [Heubner and Meyer-Bisch, 1926] but no correction was made for the cerebrospinal fluid. Haurowitz [1923] found an average value of 1.1 mg. S per 100 ml. for the total sulphur of the cerebrospinal fluid (*i.e.* inorganic sulphur + protein sulphur) of which he calculated that approximately four-fifths was inorganic. He compared this with the normal serum values given by Heubner and Meyer-Bisch [1921] and concluded that sulphur did not enter the cerebrospinal fluid solely by ultrafiltration. As stated above Heubner and Meyer-Bisch [1926] later pointed out that the figures taken for serum at that time were too high, and actually the value found by Haurowitz for the cerebrospinal fluid was about equal to that of normal serum which various workers have now established to be 0.5–1.5 mg. of S per 100 ml. The results of Haurowitz might therefore be taken to support the origin of cerebrospinal fluid by ultrafiltration.

Very little work also has been carried out on the partition of sulphur between serum and ocular fluids. Heubner and Meyer-Bisch [1921] concluded that ocular fluids contained less sulphur than serum ultrafiltrates, and Tron [1928] concluded similarly that ocular fluid contains less inorganic sulphur than the serum. Duke-Elder [1927, 1, 2], however, found rather more sulphur in the aqueous humour than in serum and considered that the aqueous humour represented a serum dialysate or ultrafiltrate. There is therefore experimental disagreement as to whether the aqueous humour should be regarded as obtaining its sulphur by secretion or by ultrafiltration, and the cerebrospinal fluid equilibrium clearly requires further investigation.

METHODS AND TECHNIQUE.

Human material only has been studied and all of it has been obtained from in- and out-patients of King's College Hospital. The blood was taken from an antecubital vein with the minimum amount of constriction, and the fluid was

withdrawn immediately afterwards. Cerebrospinal fluid was obtained by lumbar puncture. Ventricular fluid was taken by ventricular puncture under gas and oxygen anaesthesia, but otherwise no anaesthetics have been employed. The samples were not taken at any particular time of the day or in any definite relation to a meal. The blood was allowed to clot at room temperature and the serum was separated as soon as the clot had retracted. Serum and fluid were then transferred to small bottles and dispatched to Cambridge for analysis [McCance and Watchorn, 1931]. A description of the membranes, ultrafiltration technique and other points of detail may be found in previous papers by the authors [Watchorn and McCance, 1932; 1933; McCance and Watchorn, 1932].

The method used for estimating inorganic sulphur was essentially that of Cuthbertson and Tompsett [1931], but some modifications were introduced. The proteins were precipitated by adding an equal volume of 20% trichloroacetic acid to the undiluted serum [Hubbard, 1930; Cope, 1931]. The precipitate was filtered through acid-washed filter-papers. The commercial acid-washed papers were found to contain appreciable amounts of sulphur and were rewashed with dilute acetic acid. Considerable difficulty was encountered in the washing of the benzidine sulphate precipitate. No means of removing the supernatant wash-fluid could be devised which did not tend to disturb the precipitate and to lead to a loss which, though unimportant in relation to large precipitates, formed a considerable percentage error in the case of the small precipitates. The difficulty was finally fairly satisfactorily overcome by washing with a mixture of acetone and water (80 ml. of acetone made up to 100 ml. with sulphur-free distilled water). This modification has been followed also by Pirie [1934, 2]. The suggestion of Cope [1931] that the centrifuge-tube should be drained over filter-paper moistened with acetone was found to be useful, as it prevented evaporation of acetone before drainage was complete and consequent deposition of benzidine on the sides of the tube. The mouth of the tube should be wiped dry with filter-paper before turning the tube into the upright position. Alterations were also introduced into the colorimetric part of the determination, chiefly in order to avoid the awkward measurements of Cuthbertson and Tompsett. 1 ml. of 0.1% sodium nitrite was added to the benzidine sulphate dissolved in *N* HCl, followed by 5 ml. of alkaline thymol solution (0.5% thymol in 10% NaOH). As stated by Pirie [1934, 1, 2] the additional 15% NaOH is unnecessary and it also retards the colour production. When very small amounts of sulphur were being estimated 3 ml. of the alkaline thymol were used to obtain a smaller volume and consequently a deeper colour. With all possible precautions the method did not always give good duplicates, and this was particularly so when the amounts of sulphur were small.

The range of very low values for normal serum inorganic sulphur given by Cuthbertson and Tompsett [1931] have not been confirmed. The few normals examined fell within the range given by other workers [Cope, 1931; Denis, 1921; Loeb and Benedict, 1927; Udaonda *et al.*, 1933], *i.e.* 0.5–1.5 mg. of S per 100 ml.

Table I. *Serum compared with plasma.*

Serum mg. S per 100 ml.	Plasma mg. S per 100 ml.
3.2	4.3
19.2	19.5
2.2	2.1
0.7	0.6
9.3	10.0
1.4	1.4

Before starting the series of experiments described below, a number of comparisons were made between serum, obtained as above mentioned, and plasma. The latter was obtained by centrifuging the blood immediately on withdrawal, minimum amounts of pure sodium oxalate being used to prevent coagulation. Table I shows that it is immaterial whether serum or plasma is used for the estimation of inorganic sulphur and that no breakdown of organic sulphur takes place during the separation of the serum.

RESULTS.

In Table II are given comparisons of the inorganic sulphur in serum, serum ultrafiltrates and cerebrospinal (lumbar puncture) fluid. The cases have been arranged into three groups: Group (a) *General neurological diseases*. In these

Table II. *Comparison of the inorganic sulphur in serum, serum ultrafiltrates and cerebrospinal fluid.*

Diagnosis	Inorganic S (mg. per 100 ml.)		
	Serum	Serum ultra-filtrate	Cerebrospinal fluid
(a) General neurological diseases			
Cerebral tumour	2.4	2.1	0.7
"	2.2	2.9	0.6
"	1.4	1.4	0.5
Acute alcoholic poisoning	0.7	0.7	0.2
G.P.I.	2.0	2.7	0.9
Juvenile G.P.I.	2.6	—	0.4
Tabes	2.3	1.9	0.7
Congenital syphilis	3.5	2.9	0.9
Progressive muscular atrophy	1.6	—	0.9
Muscular atrophy	2.9	—	0.5
Disseminated sclerosis	1.5	1.2	0.7
"	1.8	2.6	0.7
Hepato-lenticular degeneration	2.6	2.4	1.0
Facio-scapulo-humeral myopathy	1.4	1.2	—
3rd nerve palsy	1.7	2.1	0.8
Cervical neuritis	1.2	1.2	0.3
Diabetic peripheral neuritis	2.2	2.5	0.5
Amyotrophic lateral sclerosis	1.8	2.2	0.3
Parathyroid tetany	1.6	1.8	0.3
Uncertain	1.5	—	0.7
"	2.9	—	0.7
"	1.8	1.9	0.3
Average of sera with corresponding C.S.F.	2.0	—	0.6
Average of ultrafiltered sera	1.9	2.0	—
(b) Chronic interstitial nephritis			
Uraemia	24.2	28.0	4.5
"	19.2	19.2	3.6
"	35.1	—	10.3
"	3.3	—	0.5
"	9.3	—	2.9
"	3.0	3.3	0.9
"	8.9	—	2.1
Average	14.7	—	3.5
(c) Meningeal inflammation			
Tuberculous meningitis	0.7	0.9	0.5
"	1.0	1.3	0.7
"	3.4	—	2.9
"	1.6	—	1.5
"	0.8	—	0.3
"	2.1	—	0.3
Average	1.6	—	1.1

the inorganic sulphur of the serum averaged 2 mg. of S. per 100 ml. and all of it was ultrafiltrable. The cerebrospinal fluid however averaged only 0.6 mg. S per 100 ml., which is between one-third and one-fourth of the serum or ultrafiltrable value. The range of cerebrospinal fluid values has been found to be wider than that shown by other inorganic constituents [McCance and Watchorn, 1931; Watchorn and McCance, 1932; 1933]. This may be due partly to a larger experimental error in the method of analysis, and this may also explain the greater fluctuations in individual ultrafiltrate figures compared with those for the corresponding sera. The range of serum values in these miscellaneous cases corresponds with those found by others [Loeb and Benedict, 1927; Udaonda *et al.*, 1933; Wakefield, 1929].

Group (b) *Chronic interstitial nephritis*. It has already been shown many times [Haurowitz, 1923; Loeb and Benedict, 1927; Meyer-Bisch, 1924; Kahn and Postmontier, 1925; Denis *et al.*, 1928; Wakefield and Power, 1931] that the inorganic sulphur of the serum is increased in renal insufficiency. The whole of it, however, appears to be ultrafiltrable. The cerebrospinal fluid sulphur, though higher than in Group (a), was still only a quarter that of the serum.

Table III. *Inorganic sulphur in sera and non-purulent effusions.*

Nature of fluid	Diagnosis	Inorganic S (mg. per 100 ml.)		
		Serum	Serum ultrafiltrate	Effusion fluid
Pleural	Neoplasm	2.9	—	2.9
"	"	4.2	—	6.9
"	Tuberculosis	2.4	—	5.7
"	"	2.2	2.8	2.4
"	"	1.2	—	1.6
"	"	2.0	—	2.3
"	"	3.6	—	3.3
Hydrocele	—	1.1	1.1	1.5
"	—	2.6	—	2.3
"	—	2.8	—	2.6
"	—	1.2	—	1.1
Ascitic	Nephrosis	3.2	—	2.9
"	"	1.3	—	1.1
	Average	2.4	—	2.8

Group (c) *Meningitic cases*. The inorganic sulphur of the serum tended on the whole to be lower and that of the cerebrospinal fluid higher than in group (a) but exceptions have been found in both directions. The cerebrospinal fluid sulphur was never actually higher than the serum level but the average was only slightly lower. Meyer-Bisch [1924] stated that in tubercular meningitis the cerebrospinal fluid sulphur was generally higher than normal.

A number of simple effusions of various types were compared with serum (Tables III). On an average the fluids contained slightly more inorganic sulphur than the sera and in this respect resemble ultrafiltrates. Heubner and Meyer-Bisch [1926] stated that only two-thirds of the serum inorganic sulphur was found in pleural and ascitic fluids, but these authors did not examine serum and fluid from the same patient.

A short series of sulphur determinations was made in ventricular fluids (Table II). In one case the corresponding fluid obtained by lumbar puncture was withdrawn within a few minutes of the ventricular puncture. The sulphur contents of these two were identical, and the average of the ventricular fluids was exactly the same as that of the lumbar fluids in group (a) of Table II.

Table IV. *Ventricular fluids.*

Diagnosis	mg. S per 100 ml.	
Cerebellar tumour	0.7	
Cerebral tumour	0.6	
"	0.8	
"	0.6	
"	0.5	S in C.S.F. = 0.5 mg. per 100 ml.
"	0.6	
"	0.4	

DISCUSSION.

The present results support those of Hayman and Johnston [1932] in showing that the whole of the serum inorganic sulphur is ultrafiltrable. In this connection it is interesting to note that both Meyer-Bisch [1924] and de Boer [1917] determined the sulphur in serum ultrafiltrates as their method of measuring the inorganic sulphur content of the serum. The amount of sulphur found in effusions from various sites and with various pathological causation also resembles the amount found in serum ultrafiltrates. It is reasonable therefore to suggest that inorganic sulphur reaches these fluids by ultrafiltration. Gilligan *et al.* [1934] consider that this is true also of all other ions. A study of Table II makes it evident that the sulphur in cerebrospinal fluid cannot be explained on the same basis. Since moreover ventricular fluids contain the same amounts as the lumbar puncture fluid there can be no suggestion that the amounts of sulphur in the latter are due to changes in this fluid during its passage from the ventricles. One must assume therefore that as regards sulphur the cerebrospinal fluid cannot be regarded as an ultrafiltrate. This is in keeping with what has been found for other constituents, both organic and inorganic [Flexner, 1934; McCance and Watchorn, 1931; 1932; 1934; Watchorn and McCance, 1932; 1933; Gilligan *et al.*, 1934; Walker, 1933], but owing to the extensive variations in serum inorganic sulphur which are encountered pathologically the sulphur ion provides new and valuable evidence. In this small series it is to be noted that when the serum inorganic sulphur rises very greatly, as in uraemia, that of the cerebrospinal fluid also rises and the normal ratio between the two tends to be maintained. This is being further investigated. In many cases of meningitis the "normal" fluid/serum ratio seems to be lost and the relationship approaches that of the ultrafiltrate/serum. It is impossible to say from this small series whether a fall in the serum value plays a significant part in this, but the problem will be further studied. The rise in the cerebrospinal fluid sulphur is probably more significant. The change is to be compared with the rise in cerebrospinal fluid phosphorus [Cohen, 1923-24] and the fall in magnesium [McCance and Watchorn, 1932] which accompanies meningitis. The fall in chlorides may or may not be comparable, but the evidence is accumulating that in meningitis (especially tuberculous meningitis) changes take place in the secretory activity of the choroid plexus which are reflected by changes in a number of the chemical constituents of the cerebrospinal fluid.

SUMMARY.

1. The serum inorganic sulphur in non-nephritic patients lay between 1 and 4 mg. per 100 ml. In chronic interstitial nephritis values up to 35 mg. per 100 ml. were found.
2. The whole of the serum inorganic sulphur was ultrafiltrable.

3. The inorganic sulphur in ventricular and cerebrospinal (lumbar puncture) fluids was approximately one-quarter to one-third of the serum value. These fluids, therefore, do not represent serum ultrafiltrates.

4. In tuberculous meningitis the cerebrospinal fluid sulphur tended to rise and to approach the serum value.

5. The inorganic sulphur in pathological effusions could be accounted for satisfactorily by supposing that such fluids had been formed by ultrafiltration.

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