SODIUM CHLORIDE CONTENT OF CEREBRO-SPINAL FLUID IN TUBERCULOUS MENINGITIS

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The normal sodium chloride content of the cerebro-spinal fluid is usually taken as 700 to 750 mg. per 100 c.cm., and diminution is practically a constant feature of all inflammatory conditions of the cerebro-spinal nervous system. Although the extent of this diminution is variable, all observers seem to be agreed that it is greatest in tuberculous meningitis. Nobécourt and Voisin (1903) directed attention to this low chloride content in tuberculous meningitis, and Mestrezat (1912) emphasized its diagnostic significance. More recently Fowweather (1930), studying cases of tuberculous meningitis, showed that there was a rapid fall in the cerebro-spinal fluid chlorides concomitant with the progress of the illness. Very divergent figures have been recorded by various investigators, as may be seen from the following table:

	Mg. per 100 c.cm.
Mestrezat (1912)	500-600
Fowweather (1930)	590 and over
Eskuchen (1924)	500-600
Fremont-Smith (1925)	Under 600
Wilcox and Lyttle (1925)	513-690
Neale and Esslemont (1928)	489-763
Benoliel (1925)	765-936
Riser (1929)	500-675
Lattes (1929)	570-640
Fontecilla and Sepulveda (1921)	580
Greenfield and Carmichael (1926)	500-600
Nowicka and Mestrezat (1924)	590
A. Prunell (1935)	504-678

Such a wide variation in figures seems to call for some explanation or reinvestigation.

In the course of routine work in this department 700 mg. per 100 c.cm. was obtained in one meningitic fluid containing tubercle bacilli, but on investigation this high figure was found to be due to the nurse having sterilized the lumbar puncture needle by boiling in saline. The examination was repeated on the following day with fluid withdrawn by a needle which had been sterilized in boiling distilled water, and the sodium chloride content was found to be only 510 mg. per 100 c.cm. On another occasion a high chloride figure resulted from the lumbar puncture needle having been sterilized in the autoclave, the water of which had not been changed for a long time. In other cases the cotton-wool test tube plugs and filter papers used in the test have been found responsible for a fallacious high chloride figure.

In estimating the sodium chloride content of the cerebro-spinal fluid the greatest care is essential to ensure that there is absolutely no possibility of the slightest trace of extraneous saline reaching the fluid from any source whatsoever—needle, test tube, etc., or apparatus used in the test.

The Investigation

With these facts in mind, and paying special attention to technique, the chloride content of the cerebro-spinal fluid of 126 patients was investigated. Of these, eighty-four suffered from tuberculous meningitis, all excepting two showing tubercle bacilli in the fluid, and forty-two were non-tuberculous cases. These latter served as controls and were made up of: (a) twenty-six cases of non-tuberculous meningitis—namely, meningococcal

meningitis (eleven cases), pneumococcal meningitis (six cases), streptococcal meningitis (three cases), aseptic meningitis (one case), syphilitic meningitis (one case), influenzal meningitis (four cases); (b) ten cases of non-tuberculous lesions of the nervous system other than meningitis—namely, poliomyelitis (two cases), polio-encephalitis (two cases), juvenile dementia paralytica (one case), mental defective (post-meningitic) (one case), infantile convulsions (one case), epilepsy (one case), cerebral softening (one case), paraplegia (one case); (c) six normal healthy individuals.

Results

NON-TUBERCULOUS CONTROL FLUIDS—CHLORIDE CONTENT

The fluids from the six normal healthy individuals gave the following figures (mg. per 100 c.cm.): 700, 720, 714, 740, 700—that is, an average sodium chloride content of 715.66 mg. (Four showed no cells, one showed 3 lymphocytes per c.mm., and another 2 lymphocytes.)

The fluids from the ten cases of non-tuberculous lesions of the nervous system—other than meningitis—gave the following figures (mg. per 100 c.cm.): 620, 670, 600, 650, 620, 650, 600, 640, 680, 600—that is, an average sodium chloride content of 633 mg. (The cell content varied from 5 to 920 per c.mm., and 100 per cent. lymphocytes were present in all cases except two—namely, the two cases of polio-encephalitis, which showed 80 per cent. and 85 per cent. lymphocytes.)

The fluids from the twenty-six non-tuberculous meningitis cases gave the following figures (mg. per 100 c.cm.): 640, 670, 640, 644, 620, 600, 600, 680, 640, 650, 640, 690, 640, 600, 640, 600, 640, 620, 660, 620, 620, 640, 620, 580, 660, 660—that is, an average sodium chloride content of 632.84 mg.

The case of syphilitic meningitis showed 33 cells per c.mm. (100 per cent. lymphocytes), and the case of aseptic meningitis 2,450 cells per c.mm., 80 per cent. being lymphocytes. In all the others polymorphs predominated, the average percentage being 87.4. The lowest percentage of polymorphs in any case was 60; the highest was 98. The total cell count varied between 54 and 6,000 per c.mm.

TUBERCULOUS FLUIDS—CHLORIDE CONTENT

Of the eighty-four cases of tuberculous meningitis only two had a chloride content exceeding 550 mg. per 100 c.cm. The latter were the two cases which failed to show tubercle bacilli in the fluid as obtained by lumbar puncture, and they call for special comment. One was that of a man aged 45, the other that of a boy aged 11. Both patients suffered from miliary tuberculosis, particularly in the lungs, and eventually developed tuberculous meningitis and died. The findings were confirmed post mortem, and it is of special interest to note that in both cases thickening of the meninges at the base of the brain had given rise to internal hydrocephalus and there was no evidence of extension to the spinal meninges. The complete examination of their fluids were as follows:

Case 1 (man aged 45).—The fluid, bright and clear and of light amber colour, was examined two days before death. The cell count showed 3 per c.mm., all lymphocytes, and the globulin was + +. The sugar content was 85 mg., the protein 200 mg., and the chlorides 640 mg. per 100 c.cm. No organisms seen in stained films.

Case 2 (boy aged 11).—There were two examinations. In the first, on October 10, 1935, the fluid was bright and clear, and light amber in colour. The cell count gave 96 per c.mm., 65 per cent. being lymphocytes. There was no globulin. The sugar content was 45 mg. per 100 c.cm., the proteins 56 mg., and the chlorides 620 mg. A stained film showed no

organisms. The second examination, on October 19, revealed no change in the appearance of the cerebro-spinal fluid, but the cells had fallen to 22 per c.mm., 90 per cent. being lymphocytes. There was no globulin. The sugar content was 40 mg., the proteins 62 mg., and the chlorides 600 mg. per 100 c.cm.. No organisms were observed in a stained film. The patient died on October 24.

Chemical examination of the cerebro-spinal fluids containing tubercle bacilli in the eighty-two cases of tuberculous meningitis revealed a chloride content averaging 510.24 mg. per 100 c.cm. The highest chloride content in any field was 550 mg. and the lowest 420 mg. per 100 c.cm. The cell content varied within wide limits—from 20 per c.mm. (100 per cent. lymphocytes) in a bright clear fluid free from clot to 1,952 per c.mm. (96 per cent. lymphocytes) in a cloudy fluid showing a clot of fibrin.

Comments

A differential count of the cerebro-spinal fluid occupies about fifteen minutes and has a certain diagnostic value. In tuberculous meningitis lymphocytes predominate; the lowest percentage in any fluid of the present series (eighty-four cases) was 58, and the average 92. But a high percentage of lymphocytes may be found also in syphilitic meningitis, aseptic meningitis, and a number of lesions of the nervous system other than meningitis, and so is not pathognomonic. In all other forms of meningitis, however, polymorphs greatly predominate; in the twenty-six cases referred to above the lowest percentage of polymorphs was 60 and the highest 98, and the average percentage was 87.4.

Microscopical examination of the centrifuged deposit from a fluid with a chloride content not exceeding 550 mg. per 100 c.cm., if sufficiently prolonged, will always reveal the presence of tubercle bacilli. Only about 2 per cent. of cases of tuberculous meningitis may have a high chloride content (over 600 mg. per 100 c.cm.), and these will fail to show organisms even after two and a quarter hours' search. The finding of tubercle bacilli in the cerebro-spinal fluid of any case of meningitis is of course pathognomonic, but the time necessary for success is often very long, and so a negative result is frequently reported.

Centrifugalization of the fluid and making and staining a film occupy fifteen to twenty-five minutes or more, and the subsequent microscopical examination may entail a prolonged search. In the present series of eighty-four cases the organism was demonstrated in eighty-two (approximately 98 per cent.)—a high proportion of positives—but the time expended on the microscopical examination varied from two minutes to two and a quarter hours. As a rule twenty-five to thirty minutes' searching was necessary, but a longer time was often required, and in at least five cases the search had to be prolonged to two and a quarter hours. Few workers can afford sufficient time for success.

The estimation of the sodium chloride content of the cerebro-spinal fluid takes only fifteen minutes. In eighty-two of the eighty-four cases investigated—that is, approximately 98 per cent.—the sodium chloride content was found greatly reduced, averaging 510.24 mg. per 100 c.cm. The lowest and highest limits were 420 mg. and 550 mg. respectively. In none of the forty-two controls did the sodium chloride content fall below 580 mg. per 100 c.cm., the vast majority having over 600 mg. Technique must be accurate, however, and all precautions must be taken to avoid contamination with extraneous chlorides. With this proviso it may be said that a sodium chloride content

of 550 mg. per 100 c.cm. and under is found only in tuberculous meningitis, and is practically pathognomonic of the disease.

Method of Estimating the Sodium Chloride Content

The method adopted in estimating the sodium chloride content of the cerebro-spinal fluid is a modification of that described by Whitehorn (1921). The following is the apparatus required, all of which must be chemically clean:

Two 5 c.cm. microburettes calibrated in 0.02 c.cm.; Grade B, N.P.L.
One 10 c.cm. bulb pipette; Grade B, N.P.L.
Two 2 c.cm. bulb pipettes; Grade B, N.P.L.
Two 1 c.cm. bulb pipettes; Grade B, N.P.L.
One 10 c.cm. graduated pipette; Grade B, N.P.L.
One 50 c.cm. conical flask (pyrex).
One 5 c.cm. measuring cylinder.
One glass funnel, ribbed, 2 inches diameter.
Two test tubes.
One box Whatman's filter papers No. 1, 11 cm. diameter.

The reagents necessary are:

Powdered iron alum, chlorine-free.
Concentrated nitric acid, chlorine-free.
2/3 normal sulphuric acid.
10 per cent. aqueous solution of sodium tungstate.
Distilled water prepared in a pyrex all-glass still.
Standard solution of sodium chloride: 1 c.cm. = 2 mg. NaCl.
Standard solution of silver nitrate: 1 c.cm. = 2 mg. NaCl.
Standard solution of potassium thiocyanate: 1 c.cm. = 2 mg. NaCl.

These three standard solutions are prepared as follows.

Preparation and Standardization of Solutions

Standard Solution of Sodium Chloride.—A stock solution is first prepared by dissolving 10 grammes pure dry sodium chloride in 500 c.cm. of distilled water. Its strength, therefore, is 1 c.cm. = 20 mg. sodium chloride. The standard solution is prepared, as required, by diluting the stock solution with distilled water ten times, so that 1 c.cm. = 2 mg. sodium chloride.

Standard Solution of Silver Nitrate.—Dissolve 5.81 grammes of pure silver nitrate in distilled water and make up the volume to 1,000 c.cm. (1 c.cm. = 2 mg. sodium chloride). Store in amber bottle.

Standard Solution of Potassium Thiocyanate.—Dissolve about 6 grammes of pure potassium thiocyanate in 1,000 c.cm. of distilled water, mix thoroughly, and standardize as follows:

Measure 10 c.cm. of distilled water into a 50 c.cm. flask and add 5 c.cm. of standard silver nitrate solution from a 5 c.cm. microburette and 3 c.cm. of concentrated nitric acid (chlorine-free). Shake, cool under water-tap, and allow to stand for two minutes. Then add about 0.3 gramme of powdered iron alum. Titrate with the potassium thiocyanate solution from a 5 c.cm. microburette until the last trace of silver has been precipitated. This is indicated by the development of a pink colour due to the formation of ferric thiocyanate. Adjust the concentration of the potassium thiocyanate solution until 5 c.cm. corresponds with 5 c.cm. of silver nitrate solution.

The standard solutions having thus been prepared the strength of the standard solution of silver nitrate, in the interests of accuracy, should now be checked as follows:

Measure 5 c.cm. of distilled water into a 50 c.cm. flask and add 5 c.cm. standard sodium chloride solution, 10 c.cm. standard silver nitrate solution, and 3 c.cm. concentrated nitric acid. Shake, cool under water-tap, allow to stand for two minutes, and then add about 0.3 gramme iron alum. (The 5 c.cm. sodium chloride will combine with the 5 c.cm. of silver nitrate and so leave 5 c.cm. of silver nitrate to be titrated.)

Titrate with the potassium thiocyanate standard solution until the definite pink colour first appears. This should require 5 c.cm. potassium thiocyanate if the strength of the silver nitrate solution is correct.

TECHNIQUE OF TEST

(All apparatus must be chemically clean.) The lumbar puncture needle must be sterilized in boiling distilled water, alcohol, or by dry heat. No saline or tap-water should be used. The test tube for collecting the cerebro-spinal fluid must be chemically clean, sterilized by dry heat, and closed with a sterile cork covered with tin-foil (not cotton-wool plug). At least 10 c.cm. of cerebro-spinal fluid is required. The following is the procedure:

(i) Observe appearance of fluid, then shake and pour about 1 c.cm. into a small test tube for estimation of white-cell content.

(ii) Centrifuge remainder for twenty minutes at high speed in a conically bottomed centrifuge tube.

(iii) Remove supernatant fluid—without interfering with any clot present—using a fine-pointed capillary pipette and rubber teat. The top end of the pipette should be guarded with a small plug of cotton-wool which has been previously washed in distilled water, dried, and sterilized. As much supernatant fluid as possible is transferred to a chemically cleaned sterile test tube.

(iv) All the deposit is then transferred by means of the capillary pipette to the centre of a microscope slide and, without having been spread, the drop is dried by gently heating. The conical part of the centrifuge tube is then washed with two or three drops of the supernatant fluid and the washing transferred to the dried drop on the slide. Without spreading, the slide is then dried for ten minutes in the 55° C. incubator, and in this way a highly concentrated film is obtained. Afterwards it is stained in the usual manner by Ziehl-Neelsen's method.

(v) Precipitate the proteins from the supernatant cerebro-spinal fluid obtained in (iii) above by Folin and Wu's method, as follows: Measure 16 c.cm. distilled water into a test tube and add 2 c.cm. of the cerebro-spinal fluid, 1 c.cm. sodium tungstate, and 1 c.cm. 2/3 normal sulphuric acid; mix gently, allow to stand for five minutes, then filter into another test tube, using a funnel and Whatman's No. 1 filter paper. Estimate the sodium chloride content of the filtrate as follows:

(vi) With 10 c.cm. bulb pipette transfer 10 c.cm. of the filtrate to a 50 c.cm. conical flask and add 5 c.cm. standard silver nitrate solution (using burette) and 3 c.cm. concentrated nitric acid.

Shake, cool under water-tap, allow to stand for two minutes. Add about 0.3 gramme iron alum, then titrate with standard potassium thiocyanate solution from 5 c.cm. burette until definite pink colour is obtained.

The formula upon which the calculation is made is: (5 c.cm. minus volume of potassium thiocyanate used) $\times 200$ = No. of mg. sodium chloride in 100 c.cm. cerebro-spinal fluid.

Summary

1. Diminution of the sodium chloride content of the cerebro-spinal fluid—practically a constant feature of all inflammatory conditions of the cerebro-spinal nervous system—is greatest in tuberculous meningitis.

2. As different observers have given very divergent figures for this diminution a reinvestigation of the question has been undertaken and the results are here set out.

3. The chloride content of the cerebro-spinal fluid of 126 patients was investigated. Eighty-four suffered from tuberculous meningitis, and forty-two were non-tuberculous and served as controls. None of the controls showed a sodium chloride content below 580 mg. per 100 c.cm., the vast majority having over 600 mg.

4. A sodium chloride content of 550 mg. per 100 c.cm. and under was found only in tuberculous meningitis, and may be considered practically pathognomonic of this disease. Microscopical examination of such a fluid, if sufficiently prolonged, will always reveal tubercle bacilli.

5. Only about 2 per cent. of cases of tuberculous meningitis may show a high chloride content (over 600 mg. per 100 c.cm.), and these will fail to reveal organisms even after two and a quarters hours' search.

6. The technique of the test—a modification of that described by Whitehorn—is given in detail, and its performance occupies fifteen minutes. It is of the utmost importance to exclude any extraneous source of chloride contamination, and accurate technique is essential.

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