have shown that occasionally the meningococcus may live on either starch or germ agar for as long as two months.

The extractive in the peafLOUR, therefore, would appear to be concerned rather with favouring the growth of meningococcus than with preserving its vitality.

That the extractive contained in the peafLOUR is probably of a vitaminic nature is shown also by the fact that the tryptic medium, after it has received the peafLOUR extract, deteriorates if autoclaved, and after submission to such treatment may absolutely fail to grow the meningococcus. The presence of vitaminic is difficult to prove chemically, but the above experiments clearly indicate that the essential constituent of the peafLOUR is a body of an extractive nature, not precipitated from solution by alcohol, and that it may provisionally be classified in the group of bodies known as "vitamines." The experiments in question suggest, further, that possibly a deficiency, to which attention has been drawn in the preceding paper, may be remedied by adding to the medium an extract of the germ of wheat.

EPIDEMIC CEREBRO-SPINAL MENINGITIS:

ITS BACTERIOLOGY AND PATHOLOGY.

BY

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THE extreme variability, the comparative uncertainty of our bacteriological findings in this disease, and the impossibility of making an absolute diagnosis on a clinical basis alone, especially in the milder types, have combined to influence me in publishing my conclusions from the examination of an enormous number of specimens in the laboratory from the 368 cases that have been admitted to the Alfred Hospital, Melbourne, during the past eleven months (to July 1st, 1916).

An endeavour is made in this article to give the laboratory findings in the Victorian epidemic, the cases dealt with being those occurring in the adult male population of the metropolitan district, together with a considerable number from country districts.

Of the 368 cases under review 207 occurred among soldiers in camps and the remainder among the civil population—the great majority within the first few months being from the military population, the civil population providing the greater number during the summer months. For the last three months the numbers are as follows: 31 soldiers, 50 civilians. These figures show how, with the increased number of foci as the epidemic proceeds, the civil population begins to suffer more severely.

First, in dealing with suspected cases, the examination of cerebro-spinal fluid is all-important. If the fluid is turbid the patient is admitted without delay, the fallacy of this proceeding being evidenced by the occasional presence of a pneumococcal meningitis or a septic meningitis, and, in a few cases, the turbidity has been due entirely to red blood corpuscles from accidental sources or from cases of cerebral haemorrhage.

Procedure.

In my experience the greatest cause of mistaken positive diagnosis has been in regarding a turbid or opalescent fluid, due to red blood corpuscles, as a cerebro-spinal fluid, turbid owing to the presence of polynuclear leucocytes or organisms, this fault lying with both hospital residents and private practitioners.

The microscopic examination of a wet specimen is the next procedure—(1) not centrifuged, (2) sedimented or centrifuged. The presence of polymorphonuclear leucocytes in appreciable numbers is taken as a positive diagnosis of an acute meningitis, not necessarily cerebro-spinal. Coccal forms can be seen even in the wet state. They are usually intracellular, contrary to the experience of Douglas¹ in early cases. Frequently also in the early cases mononuclears are quite as numerous as polynuclears. In only 3 per cent. of cases were mononuclears found to be the outstanding feature of the cerebro-spinal fluid, and in half of these polynuclears were very numerous also. The period when mononuclears were most in evidence certainly appeared to be in the early stage (that is, the early

part of septicaemic stage under Lundie's classification²) of the disease. This period immediately succeeds the stage of clear fluid with a positive potassium permanganate test, as described later.

The presence of fibrin is noted; the fibrin clot in a positive case usually settles in a few hours together with the majority of cells present. Excess of globulin is estimated by the simple ring test with saturated solution of ammonium sulphate. An enormous increase in globulin is a marked feature in the clear fluid after centrifuging. In all cases the potassium permanganate test is performed. Frequently one finds a clear fluid with no cell content, excepting, perhaps, a few mononuclears, no fibrin, and negative globulin test, and with no organisms, but giving an immediate potassium permanganate test (Lundie²).

In 51 different cases of this series the above result was obtained—that is, no abnormality was found except positive potassium permanganate test. The fallacy of this test is in a cerebro-spinal fluid containing red blood corpuscles; this invariably gives a positive reaction. Even if cerebro-spinal fluid is contaminated with red blood corpuscles, we still have an accurate means of judging the reaction to potassium permanganate.

If we centrifuge the cerebro-spinal fluid till we get a clear fluid free of haemoglobin and cells, we find in an early case of cerebro-spinal meningitis a positive potassium permanganate test, a negative test being obtained in non-meningitic cases.

I wish to add my recommendation of this test to that of Lundie and his co-workers.² Especially will it be of value to men in general practice, or in practice away from the advantages of laboratory diagnosis; for it appears to me that not only in cases where the fluid is turbid (with exceptions referred to) a diagnosis of some type of purulent meningitis can be made, but also a positive diagnosis of acute meningitis can be made with little risk of mistake in cases showing clear fluid if the potassium permanganate test is positive (avoiding the fallacy as above, as I suggest).

I do not wish to say that this test is specific in early cerebro-spinal meningitis, but after a large number of examinations of cerebro-spinal fluids from non-meningitic cases (128 examinations) I have come to this conclusion as to the value of the test, my only doubt being that a positive reaction may be obtained in septic non-meningitic cases.

These cases (with clear fluid giving positive potassium permanganate test) have at all times been admitted to hospital, and my experience is that, within twenty-four to forty-eight hours of admission, the cerebro-spinal fluid is found to be of the usual turbid type of cerebro-spinal meningitis.

Case 207.

Admitted October 10th, 1915. Died October 13th, 1915.
October 10th.—Cerebro-spinal fluid: No cells; no organisms. Fibrin —; globulin —; potassium permanganate + (immediately).
October 11th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 14th.—Autopsy: Brain shows pus along sulci over vertex and at base. Enormous distension of ventricles. General congestion of organs. Internal hydrocephalus.

Case 354.

Admitted June 14th, 1916. Relieved July 3rd, 1916.
June 15th.—Cerebro-spinal fluid: No cells; no organisms. Fibrin —; globulin —; potassium permanganate + (slow).
June 17th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear + +. Occasional red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Many intracellular and extracellular diplococci.
June 21st.—Swab: Staphylococci.

In the well-marked meningeal stage the potassium permanganate test, of course, is unnecessary from a diagnostic point of view; but, following Sophian,³ I attempted a quantitative test, as follows: Potassium permanganate (1 in 1,000) is poured from a measure into 1 c.cm. of cerebro-spinal fluid. The purple permanganate colour is usually immediately lost, the brown colour, due to reduction, replacing it. More potassium permanganate solution is added in small quantities until, on standing for three minutes, some purple colour remains. It was found that to the cerebro-spinal fluid of those most seriously ill seven to eleven times its volume could be added before the fluid ceased to reduce the potassium permanganate. Further work may determine a very definite prognostic value for this quantitative method.

The work done in connexion with well-marked cases shows the continued presence of polynuclear cells and meningococci for a considerable time. Examinations conducted in the following cases will give an idea of the findings:

Case 361.

Admitted June 24th, 1916. Died June 27th, 1916.
June 24th.—Cerebro-spinal fluid (yellow): Polymorphonuclear + + +. Mononuclear + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Many intracellular and few extracellular diplococci.
June 24th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Many intracellular and few extracellular diplococci.
June 25th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears and red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Many intracellular and few extracellular diplococci.
June 26th.—Autopsy: Brain: Intense general congestion; pus general; ependyma spoiled; ventricles moderately dilated. Heart: Pericardium; petechiae over pericardial surfaces; toxæmia.

Case 351.

Admitted March 11th, 1916. Died March 19th, 1916.
March 13th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
March 14th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
March 15th.—Culture from cerebro-spinal fluid: Meningococcus.
March 18th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Red blood corpuscles + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
March 19th.—Cerebro-spinal fluid: Polymorphonuclear +. Red blood corpuscles + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
Cerebro-spinal fluid, right ventricle: Polymorphonuclear +. Few red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
March 20th.—Autopsy: Brain shows marked congestion of surface; thick pus at base; ependyma spoiled; ventricles markedly dilated; no damage due to puncture. Spleen large, mushy. Internal hydrocephalus.

Case 371.

Admitted January 21st, 1916. Died March 10th, 1916.
January 22nd.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
February 6th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Fibrin +; globulin +; potassium permanganate + (in one minute). Intracellular and extracellular diplococci.
February 9th.—Cerebro-spinal fluid: Polymorphonuclear + +. Red blood corpuscles + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci. Some shadow cells.
Culture from cerebro-spinal fluid (24 hours): Gram + diplococci.
February 10th.—Culture from cerebro-spinal fluid (48 hours): Gram - diplococci; pure meningococci.
February 21st.—Cerebro-spinal fluid: Occasional polymorphonuclears and mononuclears. No organisms. Fibrin -; globulin +; potassium permanganate + (immediately).
February 26th.—Cerebro-spinal fluid: Polymorphonuclear +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
March 2nd.—Cerebro-spinal fluid: Polymorphonuclear +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
March 6th.—Cerebro-spinal fluid: Occasional mononuclears. No organisms. Fibrin, trace; globulin -; potassium permanganate -.
March 7th.—Cerebro-spinal fluid: Clear. Fibrin, trace; globulin +; potassium permanganate + (immediately).
March 9th.—Cerebro-spinal fluid: Occasional polymorphonuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
March 11th.—Autopsy: Brain shows general congestion over surface; gelatinous pus at base. Ventricles very dilated; ependyma spoiled. Spleen large, mushy. Large hydatid cyst in right kidney. Internal hydrocephalus. Toxæmia.

Case 215.

Admitted October 21st, 1915. Relieved April 27th, 1916. Readmitted May 18th, 1916. Died May 22nd.
October 22nd, 1915.—Cerebro-spinal fluid: Polymorphonuclear + + + (shadow cells). Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate +. Intracellular and extracellular diplococci.
October 23rd.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears and red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 26th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 27th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 28th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
November 4th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Occasional mononuclears and red blood cells. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
November 12th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Occasional red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.

November 13th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci.

November 24th.—Serum: Widal.

January 14th, 1916.—Swab: Staphylococci; Gram - and + diplococci.

March 23rd.—Swab: Staphylococci; Gram + diplococci.

April 26th.—Swab: Staphylococci; Gram + diplococci.

May 19th.—Cerebro-spinal fluid: Red blood corpuscles +. No organisms. Fibrin -; globulin +; potassium permanganate + (immediately).

May 23rd.—Autopsy: Brain shows recent marked congestion; ependyma clear; no pus; at base dry and matted; enormous dilatation of ventricles. Four punctures show no damage. Spleen enlarged. Aortic atheroma. Internal hydrocephalus.

Here the clinical characteristics are usually sufficient to give a diagnosis, but help is given in those cases with few, if any, other signs except coma or headache.

In the cerebro-spinal fluid of a typical case we usually find an enormous deposit of fibrin and cells, which settles and may occupy as much as one-third of the total volume.

In the markedly purulent fluids intracellular diplococci occur almost constantly, rare exceptions showing no organisms. In the examination of 700 specimens of cerebro-spinal fluid obtained at various stages of the disease I was able to demonstrate organisms in 82 per cent. In 80 per cent. intracellular organisms were seen, the numbers varying vastly in different specimens; in 8 per cent. enormous numbers were present.

In 24 per cent. intracellular diplococci occurred alone, and, in the remaining 56 per cent., both intracellular and extracellular diplococci were present, the extracellular variety usually being in scanty numbers. Extracellular diplococci occurred alone in only 2 per cent. of the specimens, so that in 58 per cent. one finds some extracellular organisms, but rarely in sufficient numbers to warrant the assertion that the name *Diplococcus intracellularis* is a false one. In this series of examinations it was rare to find a large number of extracellular diplococci, only 5 per cent. of specimens showing them in numbers sufficient to make them a feature.

In the remaining 18 per cent. of specimens I found it impossible to demonstrate any organisms.

The wonderful increase noted in the numbers of organisms after incubating the cerebro-spinal fluid alone at 37° C. for twelve or twenty-four hours is worthy of mention. A fluid which appeared on a first examination to be free from organisms, in twelve hours showed an enormous number of intracellular and extracellular diplococci, the increase apparently going on within the leucocytes as well as in the fluid media. Some observers report an extracellular increase only, but repeatedly I have found a marked intracellular increase also. This, however, agrees with the result of Lundie's work, but it seems to me that the addition of citrate and glucose is unnecessary, for, if cultured overnight, the number of organisms, both intracellular and extracellular, is usually very large.

The incubation of cerebro-spinal fluid alone, as indicated above, suggested an easy means of making primary cultures, and the results have been as successful as recently reported elsewhere. I found that if the number of organisms in a non-incubated cerebro-spinal fluid was very large the patient's condition was very serious, and in nearly all cases death ensued very soon after the fluid was obtained. Especially does the presence of many extracellular organisms seem to indicate a very severe case, and usually a hopeless prognosis.

Some writers say that when the disease is becoming less severe and the patient approaching convalescence, the organisms become extracellular. This has not been my experience, and, if it does occur, the number of extracellular organisms must be so small as to make it of no prognostic value.

Contrary to the experience of Webster¹¹ and others, I have never been able to satisfy myself that the number of extracellular organisms was increased by allowing cerebro-spinal fluid to stand at room temperature. Even in twenty-four hours no alteration of site of organisms could be found.

A few cases have shown a peculiar feature in that no cells (or very few cells) were present, even in the lower layers of a centrifuged specimen, but the fluid showed a general opalescence similar to that seen in a saline emulsion of organisms. These fluids, on microscopic examination, show the presence of an enormous number of diplococci from which cultures could be made. The disease period of such specimens was not constant, but was usually towards

the end of the disease. These fluids gave a positive reaction to globulin test, and also a positive potassium permanganate test, but this reaction was not as marked as with a cell-containing fluid.

Cultural Characteristics.

It was during this stage of the fully developed disease that the cultural characteristics were studied. The meningococcus grew readily on blood serum, the infection of the culture media being carried out by (1) pouring sediment of cerebro-spinal fluid over it and immediately withdrawing it, (2) pouring sediment into a culture tube and incubating for twelve to twenty-four hours before withdrawing the fluid, and then reincubating for twenty-four hours, or (3) with a platinum loop making streak cultures.

With the first plan a uniform sheet of growth was frequently found in twenty-four to forty-eight hours, usually the longer period elapsing before a well-marked growth was found. With the second and third plans the usual type of circular discrete colony was found, although occasionally a sheet culture was seen in (2).

For the purposes of vaccine, subcultures or other media were necessary.

Just at this stage, owing to the failure of cultures in three consecutive litres of ascitic agar and ascitic glucose agar, the culture, primary and secondary, on 3 per cent. glucose agar was attempted. This proved to be quite a reliable medium, and the growth, though often delayed till forty-eight hours, was sufficiently profuse for vaccine purposes. An alteration in technique was instrumental in saving time and media. On incubating the cerebro spinal fluid for twelve to twenty-four hours (usually overnight) a streak or sheet culture on glucose agar was almost invariably positive. A second subculture was then made from the latter, and a forty-eight hour culture was used for vaccine purposes.

I have kept cerebro-spinal fluids in the incubator at 37° C. for three to six days before subculturing, and yet a profuse growth was even then obtained on glucose agar.

I have impressed on the ward staff the necessity for incubation of specimens within a very short time of withdrawal from the body. The fact that during the earlier part of the epidemic no cultures were obtainable in many cases was due to the long period that elapsed between the withdrawal of fluid from the body and incubation. The ideal would, of course, be to incubate immediately, so keeping up the body temperature without a break.

In furtherance of this scheme of uninterrupted heat up to 37° C. the transference of sediment from tube to primary and secondary culture tubes was carried out without delay, only a few minutes elapsing before the culture was returned to the incubator.

Again, I discovered by accident a means of obtaining a more rapid and profuse growth. On several occasions a trayful of glucose agar slants was incubated immediately after pouring into tubes and allowing them to settle in order to test their sterility. The tubes at blood heat (that is, direct from the incubator) have been used for primary and secondary culture of cerebro-spinal fluid. An earlier appearance of growth, as well as an increased number of colonies, was constantly noticed. In a few cases the growth appeared in fourteen to sixteen hours. This agrees with the generally accepted fact that the meningococcus does not grow at a temperature below 23° C. (room temperature being as a rule below this).

Apparently this subjection of the meningococci to room temperature for a period, perhaps only brief, is a frequent cause of non-growth or of scanty growth. I would suggest that this is a possible cause of the findings of several observers⁵ that the meningococci give only a scanty growth on agar. My conclusion as to the cultural power of glucose agar for meningococci is based on the uniformity of the results of cultures for vaccine purposes. Successful cultures approximate to 60 per cent. and 70 per cent.; cultures from cerebro spinal fluid of 32 cases have been used in the preparation of vaccine for prophylactic purposes and for use in chronic cases.

In the identification of the organism, acid production has always been obtained in solid and fluid media containing glucose and maltose, the reaction certainly being a little slower with maltose.

On two occasions I have seen a mixed infection of what appeared to be definite pneumococci with meningococci.

This calls to mind an experience of five cases in which cultures on blood serum or glucose agar in twenty-four hours showed Gram-positive diplococci, while twenty-four hours later, on re-examining before emulsifying for vaccine purposes, the organisms appeared Gram-negative. A control of *Staphylococcus albus*, *B. coli communis*, and *B. typhosus* was done to ensure accuracy of staining in one case, the four smears being placed on the one slide.

Some observers report irregular staining, which is of course marked in smears of ordinary cultures; but my experience has been that, though the depth of staining varies, the organisms (except as above stated) have always been Gram-negative. I cannot agree with Shaw⁶ that old cultures are Gram-positive. I have repeatedly had cultures in the incubator at 37° C. for many days and still the Gram-negative staining was obtained.

The staining methods employed for films of sediment from cerebro-spinal fluid were Gram's, and Leishman's modification of Romanowsky's method, the latter showing up the deeply-stained organisms on a pale ground of leucocytic protoplasm and making the detection of the organisms much easier than by the Gram method. Similar methods were employed in the examination of blood films. Wet specimens of blood were also examined.

Cultures have been made not only from lumbar puncture fluids but from cerebro-spinal fluids obtained by ventricular puncture. On three occasions blood cultures have been obtained. In two cases of cerebral abscess meningococci were cultured, and in a case of ulcerative endocarditis complicating cerebro-spinal meningitis a pure culture was obtained.

The presence of vacuolated polynuclear leucocytes in the cerebro-spinal fluid (so-called shadow cells) is not frequent, but their presence seems to indicate the approach of death. I have notes of thirteen cases in which shadow cells were present in the cerebro-spinal fluid. Death occurred in all. The following two cases (128 and 279) demonstrate this peculiarity.

Case 128.

Admitted September 2nd, 1915. Died October 23rd, 1915.
September 3rd.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci + +, and extracellular diplococci + + +.
September 21st.—Cerebro-spinal fluid: Polymorphonuclear + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
September 27th.—Cerebro spinal fluid, right ventricle: Polymorphonuclear + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci + + +.
September 30th.—Cerebro-spinal fluid: Polymorphonuclear + +. Fibrin +; globulin +; potassium permanganate + (immediately).
October 8th.—Cerebro-spinal fluid: Polymorphonuclear + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
October 15th.—Cerebro-spinal fluid, left ventricle: Polymorphonuclear +; globulin +; potassium permanganate + (immediately). Few intracellular and extracellular diplococci.
October 17th.—Cerebro-spinal fluid, right ventricle: Polymorphonuclear + + + (many shadow cells). Mononuclear + + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 19th.—Cerebro spinal fluid, left ventricle: Polymorphonuclear + + +. Occasional mononuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 21st.—Cerebro-spinal fluid: Polymorphonuclear + + + (many shadow cells). Occasional mononuclears and red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 24th.—Autopsy: Brain shows some congestion. Ventricles show marked dilatation with thin turbid fluid. Internal hydrocephalus.

Case 279.

Admitted March 2nd, 1916. Died March 21st, 1916.
March 3rd.—Cerebro-spinal fluid: Polymorphonuclear + + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci.
March 4th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci.
March 7th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
March 9th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Few mononuclears. Occasional red blood corpuscles. Fibrin +; globulin +; potassium permanganate (immediately). Intracellular and extracellular diplococci.
March 13th.—Cerebro spinal fluid: Polymorphonuclear + + +. Few red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.
March 14th.—Cerebro spinal fluid: Polymorphonuclear + + +. Few mononuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and many extracellular diplococci.

March 15th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Few mononuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci. Culture from cerebro-spinal fluid: Meningococcus; some Gram + in twenty-four hours.

March 16th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Few mononuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci.

Cerebro-spinal fluid: Polymorphonuclear + + +. Few mononuclears. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

Culture from cerebro-spinal fluid: Meningococcus (48 hours) vaccine (Gram -).

March 17th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and few extracellular diplococci.

March 18th.—Cerebro-spinal fluid, right ventricle: Few polymorphonuclears. Red blood corpuscles + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

Cerebro-spinal fluid, left ventricle: Few polymorphonuclears (some shadow cells). Fibrin -; globulin +; potassium permanganate + (immediately). Intracellular diplococci.

Cerebro-spinal fluid, right ventricle, culture: Meningococcus vaccine.

March 20th.—Cerebro-spinal fluid, left ventricle: Few polymorphonuclears. Red blood corpuscles + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

Cerebro-spinal fluid, right ventricle: Polymorphonuclear + + +. Red blood corpuscles + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

March 21st.—Cerebro-spinal fluid, right ventricle: Polymorphonuclear +. Red blood corpuscles +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

March 21st.—Autopsy: Brain shows marked congestion; haemorrhage near right trephine hole; pus marked at base. Ependyma spoiled with petechiae showing. Enormous dilatation of ventricles with turbid fluid. Serous membranes petechial. Internal hydrocephalus.

The general tendency to haemorrhage is evidenced (1) by the presence of red blood corpuscles in the cerebro-spinal fluid, often in great numbers; and (2) by the frequent appearance *post mortem* of a haemorrhagic condition of the surface of the brain, especially the base. This will be dealt with more fully later.

Dr. Mary Williams¹⁰ states that "if there were any symptoms showing involvement of the nervous system we found the germ in the blood."

I have examined films of incubated and non-incubated blood and have been unable to demonstrate organisms. These examinations were all from cases with marked meningeal and septicaemic signs and symptoms, some even being moribund when blood was taken. I must admit that when one does discover the organisms in the blood film (a frequent occurrence) the picture is very typical, as the polymorphonuclear neutrophile leucocytosis is so marked that the need for an actual leucocyte count is unnecessary.

I have not found time to enter into the question of the disease period when the leucocytosis appears, but intend to study this phenomenon with the view of determining if it be an early diagnostic feature.

I have records of three cases in which I have been able to demonstrate extracellular organisms as well as intracellular in blood films. The positive fermentation tests with glucose and maltose media were obtained in the case of one blood culture, these being performed in order to confirm the morphological characters. Gram-negative diplococci have been obtained from pericardial fluids both *ante mortem* and *post mortem*.

Fluids obtained during life from elbow and knee joints have been found to contain many polynuclears with intracellular Gram-negative diplococci. These joint conditions have cleared up without having to be treated as other pyogenic joints.

In a swab from an aural discharge I found Gram-negative diplococci; but again, in this case, the organism present may have been the *Micrococcus catarrhalis*, no time having been available to carry out differential tests.

The sputum examinations done were mainly in cases of complicating pneumonia or in suspected cases of tuberculosis. In nineteen examinations made from different patients five showed Gram negative diplococci indistinguishable morphologically from the meningococcus.

Out of a large number of microscopical examinations of urine from patients with definite cerebro-spinal meningitis, many pus cells were found in seven, and in three cases chemical tests showed the presence of sugar in moderate amount (1.5 per cent. average). As convalescence approached the glycosuria disappeared. Since July 1st several cases of

glycosuria with definite cerebro-spinal meningitis have come under notice.

No systematic examination of throat swabs has been undertaken in the early stages; but in the stage of convalescence, when the patient is fit to leave hospital, swabs free from Gram-negative diplococci have been required before discharge is allowed. Time has not permitted a closer study of swabs than to culture for twenty-four to forty-eight hours and determine the presence or absence of Gram-negative diplococci. It was astonishing how many tubes showed no growth after twenty-four hours' culture. Many of them, however, gave positive results after forty-eight hours. With regard to those giving no growth, I think the explanation lies in the fact that not only were argyrol and hydrogen peroxide used locally with a short free period before swabbing, but the patients themselves obtained throat tablets, many containing free formalin, which successfully inhibited growth for some time—a patient eager to leave the ward taking care to administer to himself a liberal dose of such tablets immediately before the swabbing was performed.

In these convalescents a series of 190 swabs was examined. Of 93 first swabs, Gram-negative diplococci were obtained in 28 (that is, 30 per cent.). Of the remaining 97 swabs, taken usually at intervals of three to four days after first swabs, 42 (that is, 43.3 per cent.) contained Gram-negative diplococci. In some, great difficulty was experienced in obtaining cultures free from the organisms.

Cases 294, 269, and 254 show this difficulty.

Case 294.

May 11th, 1916.—Swab: No growth.
May 13th.—Swab: Staphylococcus, and Gram - and + diplococcus.
May 19th.—Swab: Gram - diplococcus.
May 21st.—Swab: Gram - diplococcus.
May 25th.—Swab: Gram - diplococcus.
May 31st.—Swab: No growth.
June 2nd.—Swab: Staphylococcus.

Case 269.

January 29th, 1916.—Swab: Staphylococcus, and Gram - and + diplococcus.
February 2nd.—Swab: Gram - and + diplococcus.
February 7th.—Swab: Staphylococcus, and Gram - and + diplococcus.
February 12th.—Swab: Staphylococcus, and Gram - and + diplococcus.
February 14th.—Swab: Staphylococcus, and Gram + diplococcus.

Case 254.

January 14th, 1916.—Swab: Staphylococcus, and Gram - and + diplococcus.
Relapse.
February 5th.—Swab: Gram - and + diplococcus.
February 12th.—Swab: Staphylococcus, and Gram - and + diplococcus.
February 14th.—Swab: Gram - and + diplococcus.
February 19th.—Swab: Gram - and + diplococcus.
February 23rd.—Swab: Gram - and + diplococcus.
February 27th.—Swab: Staphylococcus, and Gram + diplococcus.

Contacts.

As to examination of contacts, during November and December, 1915, under the direction of Major R. J. Bull, Director of the Bacteriological Department, University of Melbourne, I, with three others, examined the direct nasal and pharyngeal smears from the 800 employees of the Commonwealth Clothing Factory, where there had been two cases of cerebro-spinal meningitis. In 179 of these Gram-negative diplococci were obtained, some showing the organisms in both smears, but many only in the pharyngeal smear. These employees were not allowed to resume work until negative swabs on two successive days were obtained.

The work of treating these patients and subsequently examining the swabs was handed to me by the Principal Medical Officer of Victoria. Treatment consisted in allowing various fluids to run gently into each nostril and syringing the posterior pharyngeal wall also. It was found that the results were too slow with saline alone, so that potassium permanganate (1 in 1,000) was tried, the result being that some of the patients felt a marked soreness of nose and throat, showing red glazed mucous membrane. Also my negative results were still slower in appearing. Therefore I found it necessary, under these circumstances, to seek a fresh means of treatment. I chose a solution of sodium bicarbonate (1 drachm in a pint of normal saline) as a possible improvement. The results justified my choice, for I obtained negative results sooner than previously (working on batches from 20 to 40 previously untreated), and without causing any inconvenience

to the patients. From the nose 500 to 600 direct smears were examined, and a similar number from the pharynx, negative returns from both nose and throat simultaneously being required on two successive days.

Post-mortem Investigations.

In the study of the results of 70 autopsies done in cases of cerebro-spinal meningitis during the last nine months, I am forced to agree with Lundie that not only is there a meningitis present, but that a panencephalitis is a feature in the acute cases, the macroscopic evidence in favour of this view being the general spoiling of the brain substance (the organ is flabby and falls flat, losing its dome shape when placed on the examining bench). It tears if held in the hand, no matter how gently one handles it, and the intracerebral vessels are widely dilated and stand out very markedly on the cut surface. Also the ependyma lining the ventricles is spoiled in nearly every case, being petechial in many. This spoiling of the ependyma seems to me a very useful diagnostic point in favour of cerebro-spinal meningitis as against other cerebral lesions, especially tuberculosis.

Mode of Death.

As regards the mode of death, I decided that toxæmia was the main factor in 48 cases (69 per cent.), while in 11 cases (16 per cent.) internal hydrocephalus was the immediate cause. This cause is divisible into two varieties: (1) Those occurring in the acute stage, with meningitis still present, and caused partly by adhesions and partly by plugging of the foramina of Magendie and Luschka by thick tenacious pus and fibrin. I have not included under this head those cases of general hydrocephalus in which, on opening the skull, there is an enormous excess of fluid, both within and without the brain, with apparently a free communication between. This phenomenon occurred in several cases, the deaths being included under the head of toxæmia. (2) Internal hydrocephalus was most marked in those cases which had recovered from the meningitis and showed clear meninges with no pus and usually very little congestion. These were due directly to adhesions closing the foramina.

Considering internal hydrocephalus, both as an early and a late complication, I must agree with Horder⁷ that "lack of mechanical resistance on the part of the ventricular walls" is a factor, but my experience forces me to the conclusion that this is decidedly more important in the early cases (that is, those showing what is really a panencephalitis) rather than in the chronic cases as suggested by Horder.

Heiman's idea, to which Horder makes reference, "that the weight of fluid in the posterior horns of the lateral ventricles, pressing down the cerebellum over the bulb, temporarily obliterates the outflow from the fourth ventricle," seems quite feasible, especially as we find on *post-mortem* examination that (1) the brain substance covering the posterior horns of the lateral ventricles always suffers most severely in internal hydrocephalus, the dilatation and consequent thinning being most marked here; (2) the posterior horns always contain the greatest sediment; and (3) the posterior horn, being the lowest part of the lateral ventricular system, is the site selected for ventricular puncture.

Ventricular puncture was done as a therapeutic measure in all cases of internal hydrocephalus as soon as symptoms arose and was repeated as occasion demanded. Many of the fluids obtained by ventricular puncture were perfectly clear and gave negative globulin and potassium permanganate tests. I consider that this indicates a freedom from meningitis. Dr. Stewart, medical superintendent, who was performing the clinical work, reports success from this treatment in a few cases.

In nine cases which came to *post-mortem* examination I have noted the presence of trephine hole with ventricular punctures; in one case seven punctures were found. It is remarkable that these punctures show no damage to the brain substance. They pierce the most dependent part of the lateral ventricular system—that is, the posterior horn—and one finds merely a needle track, usually blood-stained, without any damage to the brain substance. Even in the case of multiple puncture, which is usual, a collection of puncture wounds can be seen on the ependymal surface with no evidence of damage. In one case the lateral ventricles were distended with markedly

blood-stained fluid, but both the lumbar and ventricular puncture fluids were, even before ventricular puncture, markedly blood-stained. In this case a further evidence of the hæmorrhagic tendency was shown by the petechial ependyma. In another case a large dark clot lay immediately within the skull (extradural), due to accidental rupture of a vessel in this situation.

These were the only two examples in which any abnormality was traced to ventricular puncture.

Complications.

Pneumonia, usually of the lobular type, seemed to be the actual cause of death in seven cases; probably the condition was accentuated by the continued presence in the majority of the original meningitis. In one or two other cases a less marked bronchopneumonia was present, but death seemed to have occurred from other causes.

Apparently the pneumococcus was the causative organism, although in five cases of pneumonia complicating cerebro-spinal meningitis, in which the sputum was examined, Gram-negative diplococci were present in appreciable numbers. In three cases recent cerebral hæmorrhage (capsular) was present. In two cases rupture had occurred into a ventricle. Apparently the congestion due to early meningitis was the determining factor in causing vascular rupture, in each case chronic nephritis and diseased blood vessels being present. In two cases old encapsuled cerebral hæmorrhages were seen. These had no causal relation to death.

In this connexion it is necessary to mention the presence of chronic nephritis in 14 cases (20 per cent.). These all occurred in patients past middle age, and it seems that cerebro-spinal meningitis is much more serious in a person past middle age with chronic nephritis than at any other period of life, not even excepting the very young.

In case No. 214, male, aged 84, the diagnosis of cerebro-spinal meningitis is open to doubt, as at autopsy chronic nephritis was found and an empyema of the gall bladder with suppurative cholangitis, the latter without any apparent gross local cause. I did not personally conduct this examination, and as no cultures were made from either gall bladder or meninges I must label this case as of doubtful origin.

In reviewing the cases that came to autopsy the brain conditions not yet considered include two cases in which cerebral abscess was present. In both cases the meningococcus was isolated from the pus.

In case No. 348, male, aged 50, death occurred within three days of onset. At autopsy the main points were: (1) Marked general meningeal congestion. (2) Profuse pus at base. (3) Dilated ventricles. (4) Abscess, which had ruptured into the right lateral ventricle, present in right cerebral hemisphere. (5) Heart clear. (6) Culture from pus of cerebral abscess—meningococcus. Culture from pus under meninges—meningococcus.

The laboratory examinations in this case were as follows: The cerebro-spinal fluid on May 14th showed an enormous number of polynuclear and mononuclear leucocytes with only a few intracellular and occasional extracellular diplococci. On May 15th the result was as on the 14th, with the exception that many intracellular and extracellular diplococci were present.

The second case showed a temporo-sphenoidal abscess, but this was secondary to mitral ulcerative endocarditis. In this case pericarditis was also present, also multiple emboli in other organs. In two cases ulcerative endocarditis was found, the first being as described above, the second affecting the aortic valve, the laboratory record being as follows:

Case 327.

Admitted May 27th, 1916. Died June 25th, 1916.

May 28th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear +. Occasional red blood cells. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.

June 1st.—Cerebro-spinal fluid: Occasional polymorphonuclears. Few mononuclears and red blood cells. Fibrin +; globulin +; potassium permanganate + (immediately). No organisms.

June 8th.—Cerebro-spinal fluid: Occasional polymorphonuclears (some shadow cells). Few mononuclears. Red blood corpuscles + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular diplococci.

June 26th.—Culture from aortic valve: Meningococci.

June 26th.—Autopsy: Brain shows marked general congestion. Ependyma clear; ventricles not dilated. Softening of right occipital lobe due to embolism. Endocardium and pericardium petechial; the two posterior flaps of the aortic valve show acute deep ulceration with vegetations. Petechial spots present over kidneys. Emboli present in brain, right brachial artery (1), spleen, and left lung. Ulcerative aortic valvulitis with multiple emboli. Toxæmia.

In two cases *ante mortem* cardiac thrombi were found in the right heart and haemorrhagic infarcts were present in the lungs. In one of these polyserositis was found, the pericarditis being especially well marked.

Pyopericardium was seen in 8 cases (11 per cent.), usually being the greatest factor in the causation of a toxic death. Pericardial and pleural adhesions were often seen but did not seem to have any importance.

The serous membranes frequently (13 per cent.) showed petechial haemorrhages. The ependyma lining the lateral ventricles was petechial in a few cases.

The choroid plexus on several occasions showed hypertrophy and vesiculation. This was more apparent in the more chronic cases.

Pyelitis was marked in two cases, but many showed turbid urine coming away from the pelvis on cutting the ureter.

The spleen showed appreciable enlargement in 29 cases (41 per cent.). Most writers seem to form too low an estimate of this feature. Milligan⁹ reports enlargement of the lymphatic glands in this condition at autopsy in a very great number. I found the mesenteric glands enlarged and reddened in the majority.

Aortic atheroma was present in 15 cases (22 per cent.). In 5 cases (aged 13, 15, 20, 21, 25) atheroma was present, and the organs were firm. In one case, aged 13 years, the aorta was clear, and the organs did not show any undue firmness.

Though these cases are very few, they bear out the opinions of some authorities who state that in the majority of adolescents dying from acute infectious diseases there is a syphilitic basis. The positive evidence in these five cases is at least very suggestive.

Purulent bronchitis was present in the majority of cases coming to autopsy, thick creamy pus often being expressed from the bronchi with ease. This seems to invite further study. In view of its great frequency I would suggest the possibility of this being the primary site of the infection, with dissemination taking place therefrom.

Haemorrhages.

I have purposely left the consideration of the haemorrhagic tendency in this disease (as evidenced in the laboratory and *post-mortem* room) to the last.

I have already remarked on the great frequency of red blood corpuscles in the cerebro-spinal fluid—a condition which is unlikely to be always due to accidental causes or faulty technique, especially in view of the dexterity and perfection which those constantly performing lumbar puncture attain.

The occurrence, in 3 cases, of cerebral haemorrhage (capsular), in one of multiple small meningeal (sub-arachnoid) haemorrhages, and in many cases the presence of a general haemorrhagic condition of the base of the brain, give further evidence of this tendency.

I have referred above, also, to the frequency of petechial haemorrhages under the ependyma and the serous membranes. The purpuric skin condition is frequently seen *post mortem*, most often in fulminant and hyperacute cases. On two occasions multiple haemorrhages into lung tissue were seen, without apparent gross cause. In one case the intestinal canal was filled with blood clot. There was no lesion in the intestinal wall to account for this condition. Horder⁸ states that small haemorrhages into the intestinal canal may be conspicuous, especially in acute cases. This case, however, demonstrates the possibility of a large haemorrhage occurring. Recently, in two examinations conducted in fulminant cases, the presence of a haemorrhagic enlargement of the adrenals was noted, this being very conspicuous.

In two other cases (since July 1st) I have observed the same condition, apparently clearing up, the cases being of the acute type which usually ends in death after a few days. It would appear, therefore, that this is an early feature of fulminant, hyperacute, and acute cases. The occurrence in so many situations of haemorrhages is in accord with the existence of the purpuric rash which gave to the disease its name, spotted fever.

Conclusion.

In conclusion, I wish to add that I hope by the publication of these deductions to stimulate discussion, especially as to the points of importance in early diagnosis and their relation to treatment.

It will be seen that I have disagreed with some of the usual bacteriological findings. Though this may not meet with approval, I think such variations from what we at present regard to be the normal may prove to be much more common than formerly supposed, and may possibly help us to a better understanding and more efficient treatment of such a fatal disease.

My thanks are due to Lance-Corporal (now Staff Sergeant) Weir, Australian Imperial Force, who was detailed as my laboratory assistant, retaining that position and rendering me much valuable aid during six months out of the eleven months under review. I must also thank Drs. A. V. M. Anderson and G. T. Howard for their encouragement to continue a work the routine of which at times became very irksome.

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OBSERVATIONS ON THE TREATMENT OF CEREBRO-SPINAL FEVER.

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As a definite line of treatment in cerebro-spinal fever (meningococcal meningitis) is as yet not satisfactorily determined, methods of considerable diversity being still employed by different observers, any record of a number of consecutive cases treated by a particular method may be of some value in the ultimate elucidation of the best means to combat the disease. There is still much difference of opinion as to whether serum is or is not of any efficacy, whether cases are best treated by lumbar puncture alone, or if vaccines assist in contributing towards a successful issue.

The average mortality in epidemics of cerebro-spinal fever from 1904 to 1905 was 80 per cent.¹ In 1913 Flexner² published a summary of 1,294 cases treated with the serum bearing his name and yielding a mortality of only 30 per cent. Unfortunately, however, the cases occurring in this country from the end of 1914 onwards did not apparently show the good results with serum that were expected. In the cases occurring at Royal Naval dépôts (1914-15) the mortality of serum-treated cases was 64 per cent.,³ but in many instances the serum treatment was not commenced until late in the disease, often the fourth to the seventh day, sometimes even later. The doses, also, in certain cases were quite inadequate. In the report of the Medical Research Committee⁴ on cerebro-spinal fever (1916), the majority of reporters seemed unable to decide how much benefit accrued from serum and how much from the associated lumbar puncture. The average mortality appears to have been as high as 59 per cent. in the serum-treated cases mentioned in this report. More recently, however, F. W. Andrewes,⁵ using Lister Institute serum (1916) on 11 consecutive cases, had a mortality of 4—that is, 36.36 per cent.; two of the fatal cases were under a year old.

On the other hand, Gaskell and Foster¹ treated 30 cases of cerebro-spinal fever by frequent lumbar puncture only; their mortality was 30 per cent. Morgan⁶ in 1908 had a mortality of 44 per cent. in 18 cases treated in the same manner.

During 1915 vaccines were frequently employed concurrently with other methods of treatment. The few cases referred to by Rolleston⁷ in which vaccine was used, many of which I had the opportunity of observing, gave an apparent mortality of only 25 per cent. Most of these cases, however, were well on the way to recovery before the vaccine was given; they were also, in addition to lumbar puncture, treated with either serum or soamin. Of other observers, Warren Crowe⁸ considered that autogenous vaccines did good in influencing temperature.