A Thesis

ON

Observations

ON THE

Cerebro-Spinal Fluid

in Mental Disease

BY

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Observations on the Cerebro-Spinal Fluid in Mental Disease.

Introduction.

In Asylum practice to-day examination of the cerebro-spinal fluid is recognised as a valuable aid in diagnosis, and by means of simple tests, an opinion can readily be expressed regarding the cellular and protein content of a given sample of the cerebro-spinal fluid.

These simple tests served the purpose of the clinician, whose main object was to make a rapid diagnosis, yet it was felt that improvements in methods and technique were desirable so that advance could be made in the field of cell differentiation.

Advance was rendered possible with the introduction by Alzheimer [1] of a method whereby the cerebro-spinal fluid could be treated along

the lines employed in the histopathology of the central nervous system.

Alzheimer's technique has been followed in this research, and this thesis incorporates the results obtained from the examination of the cerebro-spinal fluid of 100 cases of mental disease.

As a rule, at least 10 C.C. of cerebro-spinal fluid were withdrawn at each lumbar puncture, 5 C.C. were necessary for Alzheimer's method, and the surplus was utilised in confirming, as far as possible, the more important biochemical reactions which have recently been introduced. This has considerably extended the scope of the research.

Lastly, numerous observations have been made on the behaviour of the living cells of the cerebro-spinal fluid by means of the "Vital Jelly method" recently introduced by Ross.[20.]

I have been fortunate enough to possess the facilities afforded in the well-equipped laboratory of the Lancashire County Asylum, Winwick. This Asylum contains over 2,000 beds, and the acute admission rate is about 400 per annum.

Historical.

The general anatomy of the central nervous system, the course and branching of the peripheral nerves and the functions of the special sense organs were known to the ancients. From ancient times down to the middle of the last century, the prevailing idea as to the nature of nervous energy was that it was some sort of fluid, gas or spirit. This fluid of whatever degree of density or rarity, was carried to and fro through the nerves which must offer tubular or other passage ways for it. It may be that this idea had evolved as a result of the presence of a fluid in the brain.

However, first mention of such a fluid appears in the memoirs of Pacchioni[2] in 1721. In ascribing a secretory and excretory function to the bodies which bear his name, he mentions: "A lymphatic stream which flows between the membranes of the cerebrum, in order to bathe them incessantly, in order to maintain their constant movement in the same way that the lymph of the membrane which covers the heart serves a similar purpose."

It is noteworthy that these bodies were already known to Willis about the middle part of the seventeenth century and were considered by him to be glands. Therefore it is highly improbable that the fluid would escape the notice of such an acute observer as Willis.

To Cotugno[3] is given the credit of definitely pointing out the presence in the human body of a clear watery fluid that bathes the brain and spinal cord.

Majendi[4] in the year 1825 was the first to carry out important observations. He named the fluid "Cerebro-Spinal", affirmed its existence in man and mammals, noted variations in its pressure during respiration, and that withdrawal of a large quantity from an animal produced weakness and debility.

In 1838 Conquest[5] published the analysis of fluids obtained from cases of Hydrocelphalus. He was followed by Malgaigne,[6] Percy,[7] Landerer,[8] Battersby,[9] and Schlossberger[10] who analysed fluids obtained from such sources as Spina Bifida, Meningocele and Hydrocephalus. Schmidt[11] analysed the fluid from a healthy dog.

In 1852 Bussy[12] first demonstrated the presence of a reducing substance in the fluid of a horse.

Claude Bernard,[13] using the fluid of a rabbit, definitely identified this reducing substance as sugar, and stated that a reducing body is regularly present in the fluid of animals

The difficulty met with in obtaining human fluid, and the fact that the fluid in animals being only available in negligble quantities to permit of research determining the accurate characteristics of the fluid, necessarily left many gaps in the knowledge of its normal composition.

Attention was directed anew to the cerebro-spinal fluid by the introduction of "Lumbar Puncture" by Quinche[14] in 1891. By this means he withdrew fluid from the spinal canal in the hope of relieving intracranial pressure, also in such conditions as meningitis and general paralysis but with no beneficial therapeutic results.

The simple and safe nature of the operation, however, opened up a vast field for

investigation.

Widal, Sicard, Ravaut,[15] and Abadie[16] directed their attention to the cytology of the fluid in pathological conditions. The importance of their findings in various forms of meningitis led to the adoption of lumbar puncture as a valuable aid in clinical diagnosis.

About this time it was found that anaesthesia of the lower extremities could be produced by the introduction of such drugs as Cocaine, Stovaine, and Novocaine into the spinal canal. Further by the addition of strychnine it has been possible to extend this method to the upper part of the body, the strychnine counteracting the tendency to cardiac and respiratory failure which the action of the stovaine on the medullary centres may produce; this focussed surgical attention upon the cerebro-spinal fluid.

In epidemic cerebro-spinal meningitis, after the withdrawal of cerebro-spinal fluid, antiseptics have been injected with good results. Again, in cases of tetanus, remarkable recoveries have followed the injections of antitoxin and magnesium sulphate.

Uraemic coma and convulsions have been relieved by the withdrawal of cerebro-spinal fluid, thus proving the therapeutic value of lumbar puncture.

In 1903 Castellani [17] discovered the Trypanosoma gambiense in the cerebro-spinal fluid of cases of sleeping sickness. This has led to lumbar puncture being recognised as an essential method in the diagnosis of sleeping sickness.

Noguchi in a paper published in the "Journal of Experimental Medicine" (Feb. 1913) announces that he has succeeded in demonstrating the Treponema (spirochaeta) pallidum in the brain of 12 out of 70 cases of general paralysis.

Wassermann [18] has shown that in syphilis of the nervous system, and also in the parasyphilitic conditions general paralysis and tabes dorsalis, the cerebro-spinal fluid contains a specific antibody, which, by reason of its power of fixing complement, can be readily recognised.

Recently Robertson [19] has treated cases of general paralysis by intra-spinal injection of

anti-syphilitic serum. Before making the injection an amount of cerebro-spinal fluid was withdrawn by lumbar puncture equal to that of the serum which was to be injected into the spinal canal.

Physical and Chemical Properties.

The composition of the normal cerebro-spinal fluid has been very fully investigated by Mott,[21] and the following description is based on his writings.

The normal cerebro-spinal fluid is clear and colourless, with a specific gravity of 1·006 to 1·008. The fluid issues drop by drop, about 60 drops per minute, being the ordinary rate of flow. No correspondence has been found between the alkalinity and the rate of flow of the cerebro-spinal fluid.

The cryoscopic point of the fluid is from -0·51° to -0·56° C. — that is to say, the temperature of congelation is very near that of blood (-0·56°C.). Ravaut[23] points out that there is a lowering of the freezing point in meningitis, both

septic and tuberculous.

The fluid is alkaline in reaction, the alkalinity being only one-half that of blood (Cavazzani).[24] The alkalinity varies very slightly in different pathological conditions. Mott[25] states that the alkalinity corresponds to 0·1% sodium hydrate and gives the following table.

Alkalinity of Cerebro-spinal Fluid

Male	Dementia (general paralysis?)	0·1076	calculated as Na 6 H.
"	" (general paralysis?)	0·1056	" "
"	General paralysis	0·1104	" "
"	" "	0·1168	" "
"	" "	0·1249	" "
"	" "	0·1132	" "
"	Delusional insanity	0·1120	" "

(Fluids obtained by lumbar puncture during life, all about noon.)

The chief constituent is sodium chloride, but traces of carbonates, bicarbonates, phosphates, urea and dextrose are present. These phosphates, bicarbonates and carbonates probably contain a higher proportion of potassium than the salts of the blood, since Geoghegan[26] has shown

that the ash of the brain contained from 20 to 30 per cent of potassium as compared with 15 per cent. sodium salts. These proportions are reversed in all other tissues of the body.

The presence of Choline in the blood and cerebro-spinal fluid in conditions where a large amount of nervous tissue was undergoing degeneration has been demonstrated by Mott and Halliburton.[27] Later observations caused Mott to doubt the accuracy of the micro-chemical tests employed. Further he was unable to obtain Choline in fluid withdrawn during life, even in conditions where gross brain destruction must have been in progress. Later, however, Hebb,[28] using the method of Halliburton and Rosenheim,[29] obtained cholin-platinum crystals from the cerebro-spinal fluid in cases of cerebral haemorrhage, syringomyelia and disseminated sclerosis.

After prolonged investigation and discussion it is now generally admitted that glucose is the Fehling-reducing substance present in the cerebro-spinal fluid. The amount of glucose varies from 1·2 to 2·5 parts per 1000.

The normal fluid contains no albumen, but traces of serum-globulin and albumose are

present. The active principle in the Wassermann reaction is probably present in the form of eu-globulin. Pighini[30] asserts that cholesterol is essential for the Wassermann reaction, but Mott[31] has found cholesterol present in fluids of disease which do not give the Wassermann reaction.

Cholesterol is one of the forms in which lipoids are found in degenerative conditions of the nervous system. Lipoids are not present in normal fluid. Besides cholesterol there are phosphatides, the results of cleavage products of the lecithins and sphingomyelin.

Cellular elements are scanty in the normal fluid, only an occasional small mono-nucleated cell with a few endothelial plates being found. But in certain organic diseases of the central nervous system or its membranes, however, the number of cells may show a considerable increase. Thus, in acute suppurative meningitis the fluid contains numerous polymorpho-nuclear leucocytes.

Purves Stewart[32] states that it is the acuteness of the inflammatory process, not its microbic origin, which appears to be the chief-

factor in producing polynuclear leucocytosis. As recovery sets in, the polymorpho-nuclear leucocytes are replaced by lymphocytes, and the lymphocytes in turn gradually disappear as convalescence advances. A lymphocytosis is usually found in subacute and chronic affections of the meninges, whether tubercular, syphilitic or otherwise, also in certain chronic degenerative lesions of the central nervous system. The type of cell found in these conditions is the small mononucleated lymphocyte with a small proportion of large mononucleated lymphocytes.

Glycyl-tryptophane Test.

In 1909 Neubauer and Fischer[53] proposed a new laboratory test for the further assistance of the clinician in the diagnosis of carcinoma of the stomach.

It consists of the action of gastric contents upon a polypeptide. Hall and Williamson,[54] while not agreeing entirely with the basis upon which the reaction rests, used the test in

connection with an investigation upon the peptide-splitting action of pathological fluids, and stated that in their hands the test had yielded results suggesting an advocacy for that extended use which alone can determine its permanent utility.

These authors found that when 1 C.C. of the di-peptide, glycyl-tryptophane, was used, so small a quantity as 0·2 C.C. of blood effects a cleavage of the peptide. When equal quantities of glycyl-tryptophane and blood are employed, 0·01 C.C. of blood suffices to yield free tryptophane. The blood serum contains, therefore, a ferment capable of splitting a synthetic di-peptide, glycyl-tryptophane, into its two components.

Williamson,[55] in a preliminary communication found that the ferment was not present in normal cerebro-spinal fluid, of which he was able to obtain 16 specimens.

However, he found that the ferment did appear in cerebro-spinal fluid and synovial fluid under the stimulus of a local irritant, and obtained positive results in such diseases as tubercular meningitis,

tabes dorsalis, uraemia, pneumonia, and septicaemias.

Williamson states that it seems probable that the presence of this ferment in the cerebro-spinal fluid indicates an irritative transudation, though not necessarily inflammatory. The continued presence of this ferment would mean a progressive irritation.

I considered it desirable to carry out a series of observations in the hope that further information could be gained upon this interesting reaction.

Method.

1. Glycyl-tryptophane, sold as "Ferment Diagnostikum" by Kalle of Biebrich.
2. A solution of Bromine water, 3 parts; glacial acetic acid (10%), 5 parts.
3. Toluol.

1 C.C. of glycyl-tryptophane is added to 5 C.C. of cerebro-spinal fluid in a test-tube, a layer of toluol is then poured over the mixture, and the whole placed in an incubator for 24 hours at 37°C.

A small quantity of the mixture is pipetted from below the layer of toluol, and the bromine-glacial acetic acid solution added drop by drop until a rose pink or lilac colour appears, disappearing on further addition of bromine water.

The appearance of a lilac or rose pink colour indicates free tryptophane, and a positive reaction.

Blood, bile, trypsin and tryptophane decompose the polypeptide, and must be excluded, if possible, when applying the test.

In dealing with the cerebro-spinal fluid, blood contamination is to be guarded against. In collecting fluids at lumbar puncture, a drop of blood issuing in the first flow rendered that particular fluid useless for the purpose of the test, and was discarded. 10 C.C. of fluid were centrifuged, and 5 C.C. were taken from the upper part of tube. If the Widal film made from the deposit showed any red blood corpuscles, the fluid was discarded.

I was able to collect 45 blood-free fluids and Table IV shows the results in tabular form.

Results.

The series of cases is numerically a small one but it was very difficult to obtain fluid in sufficient quantity and of sufficient purity for the performance of the test.

The test was positive in 16 out of 45 cases. This is very different from the result obtained by Williamson,[56] who records positive results in 44 out of 52 cases of insanity. Our results agree in so far that patients suffering from imbecility or idiocy gave negative reactions

Negative results were, however, obtained in diseases in which one could be fairly certain that an irritave transudation was probably present in the fluid.

One patient suffering from acute delirious mania from whose blood and cerebro-spinal fluid a pure culture of a gram-positive diplococcus had been obtained, gave a negative glycyl-tryptophane reading.

Again in the case of a general paralytic who had had a succession of seizures over night, the cerebro-spinal fluid withdrawn the following morning gave a negative result.

Positive results were only obtained in

50 per cent of the cases of general paralysis.

A case of meningeal haemorrhage, whose clinical history is given on page 67 is worthy of special comment. The reaction was the most distinctly positive one in the whole series. Here, one can trace the source of the ferment which was present in the cerebro-spinal fluid. It had most probably arisen as a product of disintegration of the white corpuscles of the blood that had escaped from the vessel walls.

The test is exceedingly delicate, the slightest excess of bromine causing the rose or lilac colour to pass so quickly that even with practice the reading of positive and negative in many cases is often of a doubtful nature.

Future research alone will reveal the errors in technique to be avoided, the necessary controls to be carried out, and the general utility of the reaction as an aid to diagnosis and treatment.

Protein Content.

The normal cerebro-spinal fluid contains only a trace of protein in the form of serum-globulin and albumose, the total amounting to about 0·03 per cent. Albumin is absent.

There is an increase in the protein content in acute and chronic inflammatory affections of the central nervous system, but the excess is especially marked in progressive degeneration of nervous tissue, viz:- General Paralysis and Tabes Dorsalis and also in Brain Syphilis.

This excess consists of globulin, nucleoprotein, and a small amount of albumin, the greater part being coagulable by heat between 73°-80°C. The protein increase usually bears some relation to the cellular increase, but this is not invariable.

Several methods have been employed for the precise observation of the globulin increase.

Guillain[33] recommends that the fluid be saturated with magnesium sulphate and then boiled; a precipitate indicates the presence of globulin. Nissl[34], Nonne, and Apelt[35] add to the fluid an equal quantity of a saturated ammonium sulphate solution.

Cimbal[36] adds a saturated zinc sulphate solution.

These methods have, however, proved unreliable, and sometimes fail even after twelve hours to give a precipitate with fluids that at once give one in the two tests next to be described.

In the present research the following methods have been used; the butyric acid test of Noguchi[37] and the ammonium sulphate ring test of Ross and Jones.[38]

Before applying these tests care must be taken that the cerebro-spinal fluid is free from blood; although the fluid may be perfectly clear to the naked eye, yet minute traces of blood may be present owing to the wounding of a small vessel in lumbar puncture. The fluid was therefore centrifuged and the supernatant layers used for the tests.

These tests depend upon precipitation of the globulin present, which constitutes the main bulk of the protein, at least in the parasyphilitic diseases.

The procedure of these tests is as follows:-

1. Noguchi. To 2 c.c. of cerebro-spinal fluid add 5 c. cm. of 10% butyric acid in normal saline, boil for a few seconds, then add 1 c. cm. of 4% solution of sodium hydrate and reboil.

A positive result is indicated by the appearance in a few minutes of distinct flocculi, which are very fine at first, gradually become coarser, and ultimately falling to the bottom of the tube in the form of a precipitate.

It has been claimed that this test is specific for general paralysis and tabes.

2. Greenfield (22) has used this test for quantitative purposes. He performed the test in a graduated centrifuge tube in exactly the same way as in the original method, but the fluid is allowed to stand for 12 hours and the height of the deposit in the graduated tube is then read off.

3. Ross Jones. One c. cm. of cerebro-spinal fluid is taken up in a fine pipette and is allowed to gently flow on to the surface of 1 inch of saturated solution of pure ammonium sulphate.

A positive reaction is indicated by the appearance of a ring at the junction of the

two fluids, the ring is clear cut and of the thickness of a sheet of paper. When viewed from above it has the appearance of a fine cobweb.

As regards the time it was held to be positive if the ring appeared within five minutes.

This is a delicate test and much depends upon the back ground and illumination used. A faint ring may be completely missed if proper precautions are not taken.

It is best to use indirect illumination from an electric lamp, the test tube being held against a black background.

A. Boyd.[39] The test as thus used merely shows whether or not an excess of protein is present, but gives no information as to the exact amount of the excess. Boyd has devised a method by means of which this test is made a quantitative as well as a qualitative one. In many cases a ring is still obtained when the fluid is diluted, and by determining the degree of dilution with which a ring still appeared, he was able to form an estimate of the amount of protein present. This method appears to be

a simple one for quantitative estimation, and it seemed to me desirable to test it on a further series of cases.

As far as I am aware this is the first time that this method has been verified.

Results.

Before discussing results, it must be remarked that it is generally admitted that an increase in the protein content of the cerebro-spinal fluid is one of the most constant features in general paralysis and tabes dorsalis. Thus, Cornell [40.] found an increase of albumen in all the cases of general paralysis which he examined. Marr [41.] states that serum-albumen was present in every case of general paralysis. Jones [42.] found that there was increase without exception in all cases of syphilis or parasyphilis that had not had recent treatment, and that there was no increase in all other cases examined.

Winifred Muirhead [43.] using the Noguchi and Ross-Jones tests obtained a positive result in 33 out of 35 cases of general paralysis and a partial reaction in the remaining two cases.

Williamson [44] obtained a positive Noguchi reaction in 20 out of 22 cases of general paralysis, in 2 out of 4 cases of tabo-paresis, and in 4 out of 5 cases of tabes.

With these results my own are in perfect accord.

Thus the Noguchi test was performed in 98 cases including 30 cases of general paralysis, 2 of tabes dorsalis and 1 of brain syphilis.

From Table 1 it will be seen that in every case of general paralysis a well marked positive result was obtained; the same is true of 2 cases of tabes, but the case of brain syphilis did not give a positive reaction.

One case of psychasthenia gave a positive result — this case is fully discussed in the section on bytology.

In all the other cases of insanity the Noguchi reaction was negative with the exception of a female case of alcoholic insanity which gave a positive reaction. Unfortunately she died before a Wassermann test could be carried out. From a review of the above cases one may say that the Noguchi test is strong

presumptive evidence of a para-syphilitic lesion, although apparently a positive reaction may be occasionally obtained in other forms of insanity.

Greenfields' test was performed on 37 fluids and the results are embodied in Table III.

It will be seen that in general paralysis the average precipitate reached 0.3 and in no case did the reading fall below 0.15.

In tabes it was 0.3 and in psychasthenia 0·15, whilst in non-syphilitic conditions the average was 0.05, the highest reading being obtained in an alcoholic dement-0.15.

This test may be of some value in enabling one to decide whether a doubtful Noguchi may be regarded as positive or negative.

The Ross-Jones test was performed in 96 cases including 30 cases of general paralysis, 2 of tabes dorsalis and 1 of brain syphilis. It will be seen from Table II that in none of these conditions was a negative result obtained and in only three cases, 2 of general paralysis and one of brain syphilis, did a ring appear when the undiluted fluid was used, and in one case of general paralysis a positive

result was obtained with as high a dilution as 1 in 18, the highest dilution that Boyd records being 1 in 13.

Very different are the results obtained in the other insanities. In one case of alcoholic insanity a ring was obtained with a dilution of 1 in 4, in another case of alcoholic insanity, in one case of psychasthenia and in one case of chronic mania with a dilution of 1 in 2, but with these exceptions no other case gave a ring when diluted with saline solution.

From the above it will be seen that when undiluted fluid is used a positive result is obtained in a considerable number of cases — whereas when Boyd's dilution method is used a greater degree of diagnostic accuracy is obtained.

As regards the relation between the two tests it may be noted :—

1. That in no case was the Noguchi test positive without the ammonium sulphate test giving a ring with 1 in 2.

2. That in a number of cases the ammonium sulphate test was positive with a dilution of 1 in 2 without a corresponding Noguchi, but

in dilutions above this the Noguchi reaction was always present.

To sum up, therefore, I consider that the Noguchi reaction is practically pathognomonic of the parasyphilitic conditions but that the Ross-Jones test in its original form is not of the same value, for in 14 cases of ordinary insanity I obtained positive results with this test when using the undiluted fluid. In this I am in agreement with Boyd,[45] who records positive results in the undiluted fluid in 26 cases out of 77 cases of ordinary insanity.

Jones on the other hand states — "The ammonium sulphate test is always negative in all other forms of mental disease," and Turner[46] - "The Ross-Jones test in my hands only gave a positive result in general paralysis and cerebral syphilis"; statements which can only be explained by assuming that these two observers did not use the black background and indirect illumination which makes the faint ring so much more evident.

Fehling Reduction.

The normal quantity of sugar present in fluid withdrawn during life is 0.15 to 0.18 per cent. It is generally stated that the usual conditions in which there is a marked change in this substance are diabetes, general paralysis and acute meningitis.

In diabetes it is greatly increased, in acute meningitis and general paralysis it is greatly diminished or altogether absent. Williamson[48] found that reduction was absent in 15 out of 23 cases of general paralysis, 3 out of 25 cases of other insanities, 3 out of 6 cases of syphilis, and one case of pernicious anaemia. On the other hand Winifred Muirhead[49] did not get a single negative result in 35 cases of general paralysis and 77 cases of other insanities.

In 1889 Halliburton[50] made the statement that he had succeeded in producing crystals from a hydrocephalic fluid, which crystals seemed to answer to the reducing element, and according to their appearance and characteristics corresponded to Pyrocatechin.

The crystals were soluble in water, alcohol

and ether, were completely precipitable by neutral lead-acetate, would reduce copper-oxide but not bismuth oxide, and showed the iron-chloride reaction characteristic of Pyrocatechin.

Nawratzki [51] following the lines of Halliburton's technique, carried out exhaustive tests with the cerebro-spinal fluid of calves. He was able to obtain large quantities of fluid, and identified the reducing element as grape sugar. Halliburton finally accepted this view, and authorities have now agreed upon this point.

Mott is of opinion that the cerebro-spinal fluid plays the part of lymph to the brain and that the sugar which it contains is being conveyed from the blood to the neuronic elements.

A glycolitic ferment is probably not present in the fluid, but such a ferment may be produced by the ganglion cells and the sugar thus converted into neural energy.

If this be correct it at once becomes evident that a diminution of the amount of sugar present may be of the gravest import, for the nerve cells would then be unable to obtain a substance necessary for their healthy functioning

The reducing properties of the cerebro-spinal fluid begin to decrease soon after death, and gradually disappear entirely. This decrease is so early remarkable, that the occurrence cannot be relegated to the action of bacteria.

This phenomenon has an analogy in the recognized fact that the sugar-value in blood withdrawn by venesection decreases more or less quickly.

Pascheles and Reichel[52] who, examining two pleuritic exudations 3 and 10 hours post-mortem respectively, could find no sugar; while they always found it in tests undertaken intra-vitam.

I have examined 84 fluids. I have not made an accurate quantitative estimation, but have found that the amount of glucose fluctuates in different conditions.

I found sugar absent in 12 cases including 3 of general paralysis, 3 of imbecility, 2 of dementia praecox, 2 of mania, and 1 of paralysis agitans.

In the remaining cases of general paralysis the reduction was always slight. On the other hand there was a marked

reduction in one case of confusional insanity and one case of epilepsy.

Tension.

In collecting the fluid after lumbar puncture, one is struck by the variations in the rate of flow in different individuals. The usual number of drops per minute is 60; but in a considerable number of cases hyper-tension was present. This hyper-tension varied from a continuous flow of running drops to a regular jet or stream.

In early operations only a small quantity of fluid was withdrawn under 'stream' pressure, since Bramwell [47] warns that fatal results have followed after lumbar puncture in cases of intra-cranial tumour in which there was a great increase of intra-cranial pressure.

Later, it was found that 15 C.C. could be withdrawn under 'stream' tension from cases suffering from general paralysis without any immediate ill-effects.

The tension was recorded at 89 punctures,

and a glance at Table VIII will show that 20 out of 31 cases of general paralysis, 7 out of 8 cases of imbecility, 4 out of 6 cases of epilepsy, 4 out of 7 cases of dementia praecox and one case of brain syphilis show marked hyper-tension.

There was an increase in tension in some of the other diseases, but the proportion was low compared with the above-mentioned.

This increased pressure of fluid in the lumbar region probably indicates a general excess of fluid in the intra-ventricular system, and it may be that some of the symptoms in general paralysis, imbecility, epilepsy and dementia praecox should be assigned to this cause.

Mott suggests that the drowsy stupor and lethargy which come and go in syphilitic basic meningitis are largely due to internal hydrocephalus.

After Effects.

In the first series of my cases from 5 to 7 c.c. of fluid were withdrawn and patients showed no ill-effects.

In the latter series from 10 to 15 c.c. of fluid were taken, and it was these patients that showed pronounced ill-effects.

Twenty-three patients suffered so severely after lumbar puncture as to be worthy of inclusion in the following synopsis :-

No.	Symptoms.
1.	Severe headache and temperature the same evening.
2.	Headache and vomiting three days after.
3.	Headache and temperature the same evening
4.	Headache and nausea coming on three days after.
5.	"Dry Tapping" complained of nausea, pains at back of head and loss of appetite for six days.
6.	Headache and nausea on getting up after being three days in bed.
7.	Headache and temperature.
8.	This patient became brighter and less lethargic.
9.	Spoke day after puncture, previously had not spoken for a year, improvement continued for a fortnight, after which she relapsed

No.	Symptoms.
	into her former stuporose state.
10.	Headache same evening.
11.	Severe headache same evening
12.	Vomited after getting up.
13.	Severe headache 24 hours after, although in bed.
14.	Headache and vomiting after getting up after being 48 hours in bed.
15	do.
16.	do.
17.	Attack of syncope although only a few drops of fluid withdrawn.
18.	Severe headache the same evening. Patient became less lethargic.
19.	"Dry Tapping", vomitted on getting up after being 24 hours in bed.
20.	Severe headache the same evening
21	Vomited 48 hours afterwards although in bed.
22	Temperature the same evening.
23	Nausea and constipation.
24	"Dry Tapping". A lethargic dementia, since operation has been actively excited and restless.
25.	"Dry Tapping". Severe headache and nausea 24 hours afterwards, although kept in bed.

Introduction to Cytology.

Any investigation upon the cells of the cerebro-spinal fluid naturally falls into two divisions, namely the estimation of the total number of cells, and the examination of the various types of cells. Attention has hitherto been mainly directed to the problem of counting the cells, and various methods have been devised for this purpose.

The first of these was that of Widal and Ravaut [57] who published in 1900 a method of counting the cells in the centrifugal deposit from a given quantity of fluid.

The method of Widal consists in centrifuging 5 C. cm. of the fluid until all the cells have been drawn to the bottom of the tube, the time required depending upon the speed of the centrifuge. The supernatant fluid is carefully decanted off, the tube inverted and the bottom scraped with a fine capillary pipette. The drop obtained is transferred to a slide, care being taken not to spread it out, otherwise the concentration is seriously affected. After being allowed to dry, the film may be fixed by heat and then stained by Methyl blue, Jenner's stain or Pappenheim's

pyronin-methyl green, the last having a selective action on the "plasma cells" met with in general paralysis.

This method answered a certain purpose very well; it gave a fair idea as to the presence of a pathological increase in the number of cells, but owing to defective fixation the cells were so very poorly stained that one could not distinguish the various types.

The cells were usually spoken of as lymphocytes, and the increase called a lymphocytosis.

In 1904, Fuchs and Rosenthal[58] with the object of attaining greater accuracy used the ordinary haematological technique, namely the pipette and special counting chamber.

The fluid was drawn directly into the pipette, and a diluent used that would stain the cells; the number of cells present per cubic centimetre was then counted.

With a large number of cells per C.C., the method was fairly accurate, but when only a few cells are present the error varies from 30 to 90 per cent.

Jones[59] has shown how inaccuracies could occur in counting a small number of cells but

his field method yields no better results.

I have employed the method of Widal, partly as a convenient means for determining whether or not a lymphocytosis was present, and partly with the object of comparing the count with that obtained by the Alzheimer method. The Widal method was only used for counting the cells, and not in any way for differential purposes. One of the advantages of this method is that, by means of it, one is able to detect minute traces of blood which are not evident to naked eye observation, but which yet would interfere with the bio-chemical reactions.

In 1907 Alzheimer[60] published a method which bids fair to entirely replace those hitherto in use. This method has been adopted on the Continent and in America, but as far as I am aware there have only been two papers published in this country, one by the American authors, Cotton and Ayer,[61] and, recently, one by Henderson and Muirhead.[62] There can be no doubt to my mind that this method is vastly superior for differential purposes to any hitherto employed. By means of it the various types of cells are fixed and stained in a manner essentially

similar to those of the tissues. The types of cells present in the fluid and the brain can be compared in a way that has never hitherto been possible. In this method the cells are fixed by adding 96 per cent. alcohol to the cerebro-spinal fluid, which precipitates the proteid, and by centrifugalisation are drawn down with the proteid in the form of a coagulum to the bottom of the tube.

<u>In detail the method of Alzheimer as used is as follows</u> :-

1. Lumbar puncture in the usual manner,
2. 96 per cent. alcohol is added drop by drop and well mixed; twice the amount of alcohol to the cerebro-spinal fluid present.
3. Centrifuge the mixture for half an hour at a high speed in a glass tube with conical end. Tube well stoppered to avoid evaporation. (An electric centrifuge was used).
4. The supernatant fluid is poured off, leaving a small coagulum in the bottom of the tube.
5. Add absolute alcohol, alcohol and ether,

each separately for one hour, to dehydrate and harden coagulum.

6. The coagulum is removed from the bottom of the tube by tapping and allowed to drop into thin celloidin where it remains for 12 hours. On several occasions considerable difficulty was experienced in detaching the coagulum from the bottom of the tube. It was found, however, that by previously smearing the conical end of the tube with vaseline before centrifuging the removal of the coagulum was greatly facilitated.

7. Coagulum placed in thick celloidin for 12 hours.

8. Mounted on blocks, hardened in chloroform for half an hour and cut at 8 μ. on a Jung Microtome.

9. Section stained.

Procedure:

1. Sections placed in absolute alcohol for a minute and spread out on a thoroughly cleaned cover glass: as many as eight sections can be accommodated on one cover

glass.

2. Ether vapour is poured over the cover glass, by this means the celloidin is removed and the section fixed to the cover glass.

3. Sections hardened by placing in methylated spirit.

4. Place sections in water.

5. Stain as follows :-

Pappenheim's pyronin-methyl green (Grübler).

The sections are placed in this stain for 5 to 7 minutes in an incubator kept at 37°C.

The sections are then washed in water, differentiated in absolute alcohol, cleared in bergamot oil and mounted in balsam.

Other stains used were Unna's polychrome methyleneblue, Nissl's methylene blue and toluidin blue.

The pyronin-methyl green stain gives excellent nuclear pictures, a slight pink tint to the protoplasm in most cells and is considered specific for plasma cells.

The fluid must be centrifugalised long enough to give a firm coagulum, yet not too long, otherwise the cells are all driven to the

apex of the coagulum. I found by experiment that to have the cells evenly distributed throughout the coagulum centrifugalisation for half an hour at 2000 revolutions per minute proved satisfactory.

The central portion of the coagulum was cut in vertical sections.

Results of Cytological Examination.

Estimation of the number of cells.

For the purpose of estimating the number of cells I have used the methods both of Alzheimer and Widal. Widal's method was used in 61 cases, and Alzheimer's in 52 cases, and for comparative purposes both methods were used in 42 cases.

In forming an opinion as to what constitutes a lymphocytosis, one is met with the difficulty at the outset that no fixed standard has been accepted. Various observers use different standards, and unfortunately in some cases they do not even mention the magnification that they use in making the count.

Purves Stewart [63] using the Widal method states that from 5 c.c. of fluid not more than 4 cells should be seen in the field with a magnification of 450 diameters. Widal considers that a + lymphocytosis is present when there are 6 to 10 cells per field under an oil immersion lens. In making observations, I have used a + magnification of 500 diameters, and have averaged the number of cells counted in 10 fields.

I consider with this magnification any number above 6 per field is pathological.

My results with the Widal method correspond closely with previous observers, that is to say in all of the 15 cases of general paralysis examined I obtained a distinct lymphocytosis and in some cases a marked one.

From an examination of Table V it will be seen that the lowest count in general paralysis was 37, and the highest 558 cells per field. Of the two cases of tabes dorsalis which I examined it will be seen that one showed a well-marked lymphocytosis of 76, but in the other there were only 10 per field. Disregarding five cases which gave a count under 10 per field, we are thus left with seven cases which show a lymphocytosis.

These are as follows :-

10 to 20	Dementia Praecox ..	2 cases
	Imbecility	1 case
	Secondary Dementia ..	1 case
20 to 50	Chronic Mania ..	1 case
	Stupor	1 case
	Phychasthenia	1 case

With reference to this last case there was considerable difficulty in the diagnosis, the patient mentally being a neurasthenic with very marked general tremors, but with no evidence of local nervous disease. The Wassermann reaction was, however, positive with the blood serum; a positive Noguchi reaction was obtained in the cerebro-spinal fluid, and the Ross-Jones test was ++ positive with a dilution of 1 in 2. From the pathological side there is therefore a considerable amount of evidence that a ++ syphilitic process was at work in the brain, though it had produced no signs which could be detected clinically.

Abzheimer counting Method.

The standard for comparison used by Cotton and Ayer was the number of cells per 100 fields, but they did not state in their communication the magnification employed.

I communicated with Dr. Ayer privately and he kindly informed me that a magnification of 900 diameters was used. These authors cut their section at 14 μ., but this thickness is not the best suited for differential staining, and I found by experiment that 8 μ. is the most suitable for all-round purposes. Further Cotton and Ayer cut the coagulum in cross section, and averaged the count from about six different levels. This cannot be considered altogether satisfactory, and I have endeavoured to increase the accuracy of the method by cutting the coagulum in vertical section, and using only the sections taken from the deepest part of the coagulum, this being readily noted by the naked eye.

Cotton and Ayer consider a lymphocytosis to be present when the count gives over 100 cells to one field. I have also adopted this

standard, and have been very satisfied with the accuracy by which an increase in the number of cells can be noted in a given quantity of fluid (5 c.c.). My readings, using a magnification of 500 diameters and sections of 8 μ. thickness, correspond very closely with the readings of Cotton and Ayer from sections of 14 μ. thickness counted in fields of 900 diameters.

The cases of general paralysis all gave a distinct lymphocytosis, the lowest reading being 116 cells per 100 fields, and the highest 997.

One case of tabes dorsalis gave a + lymphocytosis of 398 cells per 100 fields, while the other case only showed 87 cells per 100 fields. It will be recalled that by the Widal method this case also gave a very low reading. Nine cases of other forms of insanity also showed a lymphocytosis :- (See Table VI.).

100 to 200	Acute Mania ..	1 case
	Epilepsy	3 cases.
	Alcoholic Insanity ..	1 case
	Stupor	1 case
	Paranoia	1 case
200 to 400	Psychasthenia ..	1 case
	Idiocy (Congenital syphilitic)	1 case

The Method of Widal and Abzheimer compared and contrasted.

The advantages of the Abzheimer method:

1. The cell differentiation is excellent.
2. The cells are evenly distributed throughout the coagulum.
3. Even in the case of a well marked lymphocytosis only a small number of cells are present per field; therefore enumeration is simplified.
4. Naked eye inspection can assure one that the section of the coagulum is entire, whereas in the Widal one cannot be certain that all the cells blown on to the cover glass are still there after staining and washing

Disadvantages:

1. The technique requires at least three days for completion and is one that cannot well be carried out outside a laboratory.
2. 5 C.C. of fluid are used in one operation, and cannot be used for any other purpose.

Advantages of the Widal Method:

1. The simplicity of the method.
2. The supernatant fluid can be used for the bio-chemical tests.

Disadvantages:

1. Cell differentiation is defective, lymphocytes and polumorpho-nuclear leucocytes can be distinguished but no other type of cell.
2. It is impossible to ensure that the emulsion of cells which is drawn up into the pipette is always of the same strength, the dilution of the emulsion varying with the quantity of fluid remaining in the tube after inverting
3. The drop may spread out over the cover glass, thereby affecting the concentration of the cells.
4. The cells are frequently all grouped round the edge of the film so closely that they cannot be counted leaving a clear area with no cells in the centre.
5. In some cases large numbers of pale rounded bodies (probably cells undergoing disintegration), granular deposit of stain, and crystals are present, seriously obscuring the field

and covering up the cells.

6. The film on the cover glass is very thin, and as it is only fixed by heat it is very readily washed away even when extreme care is taken.

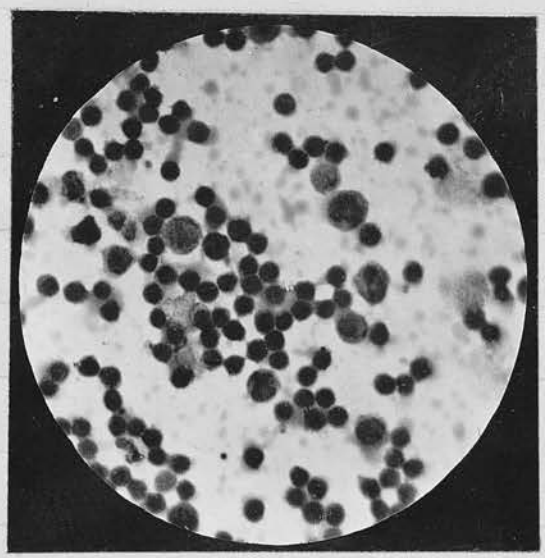
Comparison of the Widal and Abyheimer counts.

In 42 cases 10 C.C. of cerebro-spinal fluid were used, 5 C.C. for each method. On the whole a lymphocytosis indicated by one method was present in the other, and the comparison could be looked upon as very satisfactory.

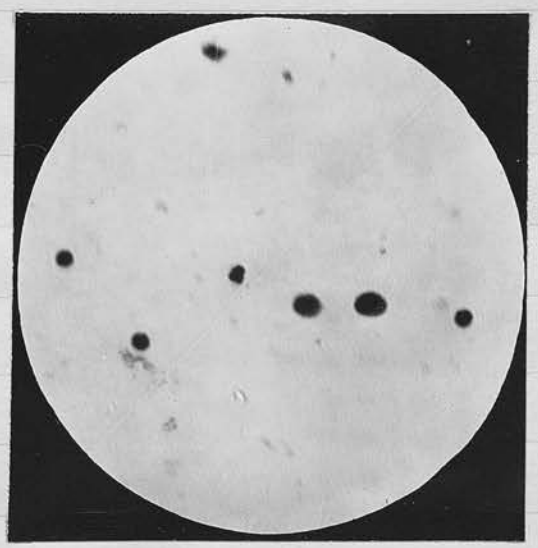
The exception being in two cases of epilepsy and one case of paranoia in which the Widal count showed no increase above the normal, but the Abyheimer indicated a definite lymphocytosis. Again, in one case of tabes, one case of chronic mania, two cases of dementia praecox, one case of imbecility, and one case of secondary dementia the Widal count indicated a lymphocytosis while the Abyheimer count was below the normal.

How is one to reconcile these differences? In every case, with the exception of one of epilepsy, the difference was so slight that it

Plate I.



Lymphocytosis. Widal's Method.
Methylene-blue stain. ×500.



Lymphocytosis. Alzheimer's Method.
Polychrome methylene-blue stain. ×500.

could be explained by the variations which are unavoidable in counting the cells of such a fluid as the cerebro-spinal where the number of cells is so small. In the above mentioned case of epilepsy the Widal count was normal but the Alzheimer count showed 168 cells per 100 fields - it is probable that the latter reading is the more correct one, and that the Widal count can be explained by one of the sources of error mentioned above.

Differentiation of Cells.

Hitherto the diagnosis of General Paralysis by the examination of the cells of the cerebro-spinal fluid has depended entirely on the enumeration of those cells and the determination of whether a lymphocytosis is present or not. Recently, however, it has come to be recognised that a study of the various types of cell is of equal importance.

An example of the importance of a differential as opposed to a total count is afforded by such a disease as spleno-

- medullary leucocythaemia. In this condition, although it is customary to have a large leucocyte count, yet in remissions of the disease, especially under arsenical treatment, the count may fall to normal. If one should encounter such a case in one of these remissions one would be completely misled by a leucocyte count.

When, however, a differential count is made and the characteristic myelocyte discovered, the true character of the disease at once becomes evident. Although Dawin warns us that "analogy is a deceitful guide", yet the same reasoning may be applied to general paralysis.

It was generally recognised that the presence of plasma cells was as characteristic of general paralysis as a lymphocytosis, but this type of cell is common in many chronic inflammations. That plasma cells occur in the cerebral cortex and pia mater in general paralysis is a well known fact, but until Alzheimer's method had been introduced they had never been found in the cerebro-spinal fluid. Indeed Nissl was led to

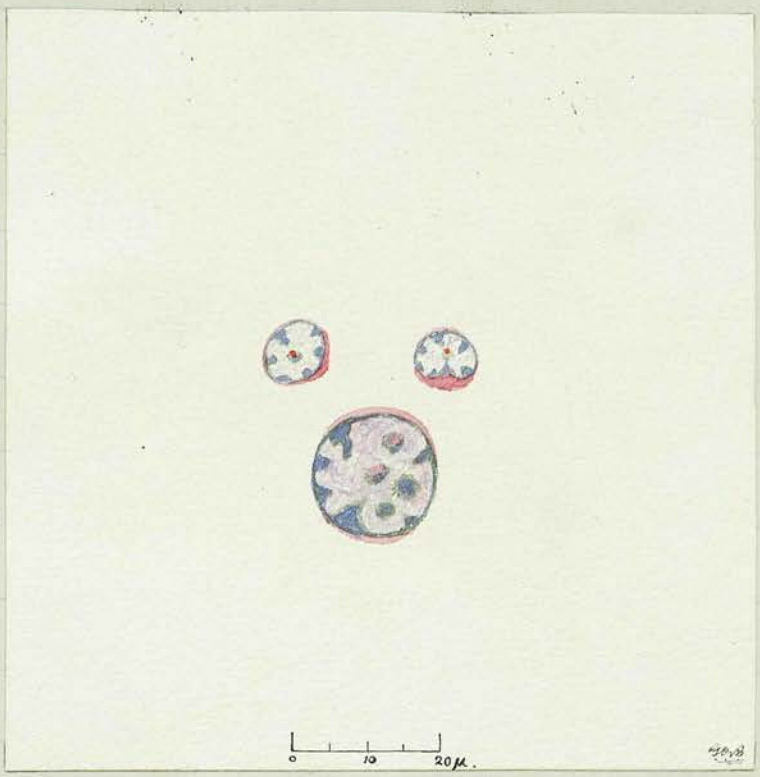
doubt the pial origin of any of the cerebro-spinal cells because of the absence of these characteristic cells in the fluid in cases which showed abundance of them in the pia and cerebral cortex. It is for this reason that Alzheimer's method must be regarded as constituting a marked advantage upon its predecessors for by it alone can plasma cells be demonstrated with certainty.

The description of the cell types closely follows that of Bolton and Ayer,[64] as the appearance in my sections have been in agreement with their findings in fluid withdrawn during life.

In the normal cerebro-spinal fluid one has practically only one type of cell to consider, namely, the lymphocyte.

It is doubtful whether the polymorpho-nuclear leucocyte can enter the fluid during health, and its presence always indicates the existence of some irritative process. As a rule, this is acute and the condition in which the greatest polymorpho-nuclear leucocytosis occurs is acute suppurative meningitis. This cell is, however, also found in the chronic

Plate II.



Large and small Lymphocytes.

Transitional Lymphocytes.

inflammatory conditions of which the best example is general paralysis.

In general paralysis and tabes dorsalis several other varieties of cells occur, and in these diseases the cells were sufficiently numerous for a fair differential count to be made. A differential count was made in cases in which at least two hundred cells could be distinguished. In some cases this necessitated a search through six to eight sections. The percentage cell counts will be found in Table IX. In conditions in which the cells were too scarce for differential purposes, the existence of each type of cell encountered has been denoted in Table X.

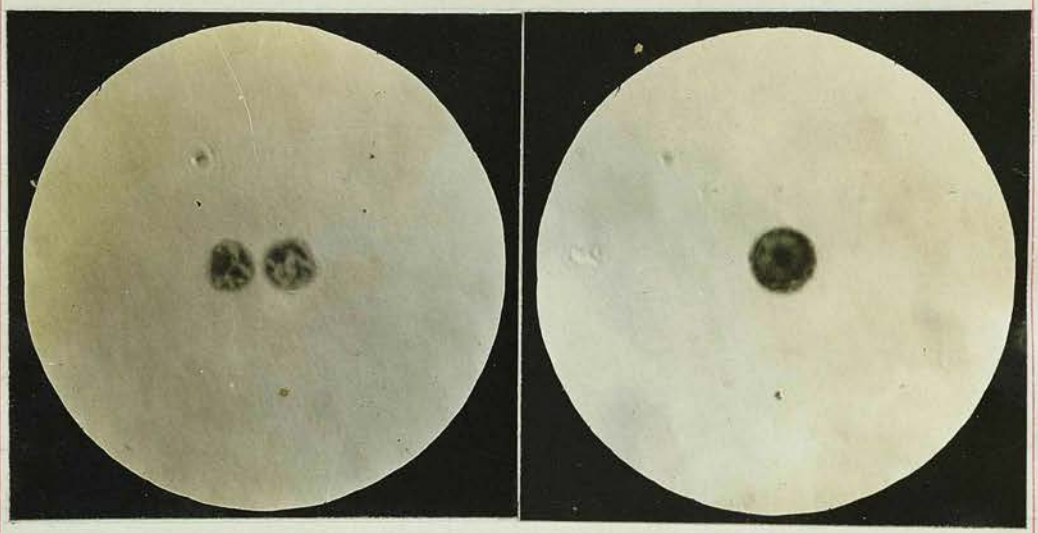
The following is a brief description of the various types of cells.

Lymphocytes.

Lymphocytes are found in all fluids, but apart from fluids of parasyphilitic conditions they occur in very small numbers.

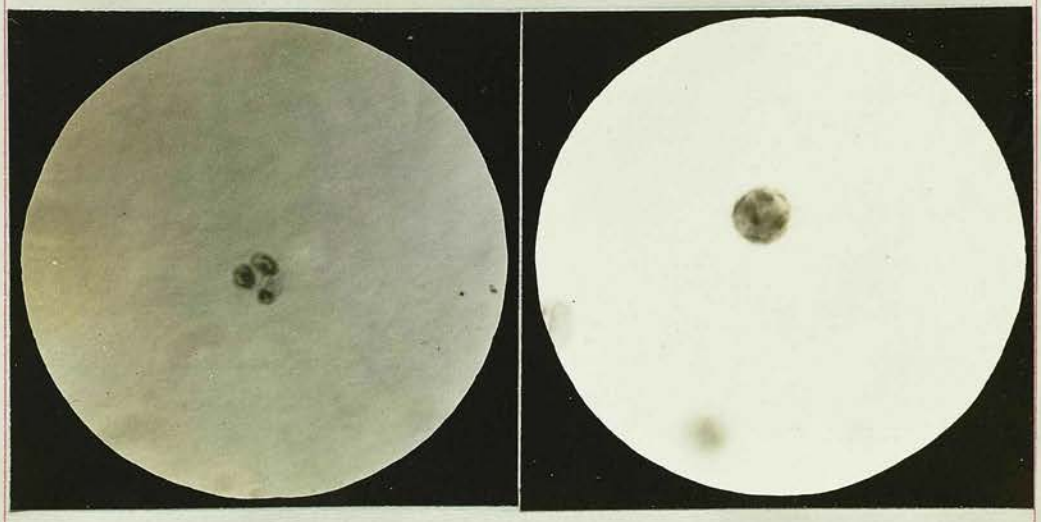
The nucleus is small and round, sometimes oval and slightly indented and

Plate III.



Small Lymphocytes. Large Lymphocyte.

N.B. Plates III, V, VII and VIII are microphotographs of cells in Alzheimer sections. Pyronin methyl-green stain. Magnification – 1,200.



Polymorphonuclear Leucocyte. Polyblast.

and contains as a rule a single bright red nucleolus. The chromophilic granules lie round the periphery giving a "clock face" appearance, and take on a deep blue green stain with Pappenheim's stain.

The protoplasm is found as a thin line round the nucleus, stains a faint pink, and it is usually a little wider on the indented side of the nucleus.

Lymphocytes show altered and transitional forms. The nucleus is similar but there is an increase in the protoplasm. Occasionally the nucleus takes up a deeper stain. Another type of lymphocyte, classed as the "large", is one in which the nucleus, with its granules, is much increased in size, and has a thin ring of protoplasm around it.

In general paralysis the differential count shows that the lymphocytes are the principal cells increased, varying from 39 to 78 per cent., the transitional forms ranging from 2 to 19 per cent. Including all types of lymphocytes together in one class the average in general paralysis is 71 per cent. The total cell count in general paralysis averages 458 to 100 fields.

Plate IV.

Plasma Cells.

1 Fibroblast. 2+3 Polymorphs. 4 Polyblast.

Polymorphonuclear Leucocytes.

Polymorphonuclear Leucocytes were present in 15 out of the 17 cases of general paralysis, and in only 6 of the remaining 48 cases of insanity.

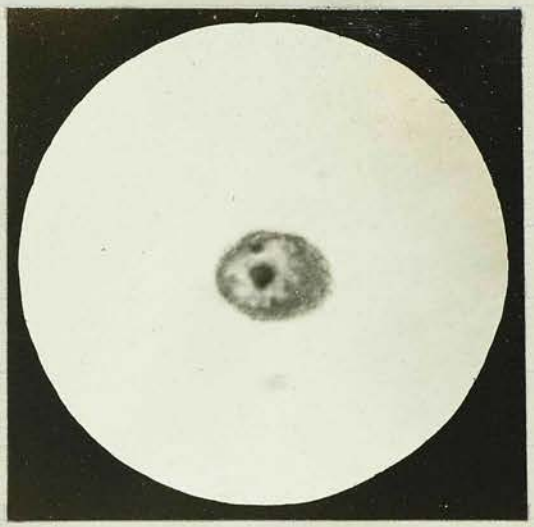
Case No. 12 is worthy of note, as the leucocyte count reached the high figure of 29 per cent. Blood contamination could be fairly excluded in this case both by the clearness of the centrifuge deposit, and the absence of red blood corpuscles in the Widal film from the same fluid as the Hzyheimer. Further the withdrawal of the fluid had no relation in point of time with any seizure or expected seizure, or change in the patients' physical or mental state.

With the pyronin-methyl green stain the nuclei only are stained, no protoplasm is visible, and the cells are distinctive.

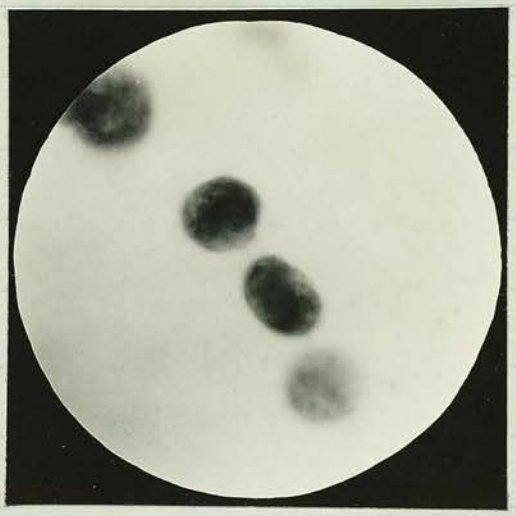
Plasma cells.

These cells are comparable to that of a lymphocyte with its protoplasm greatly increased in amount.

Plate V.



Plasma cell.

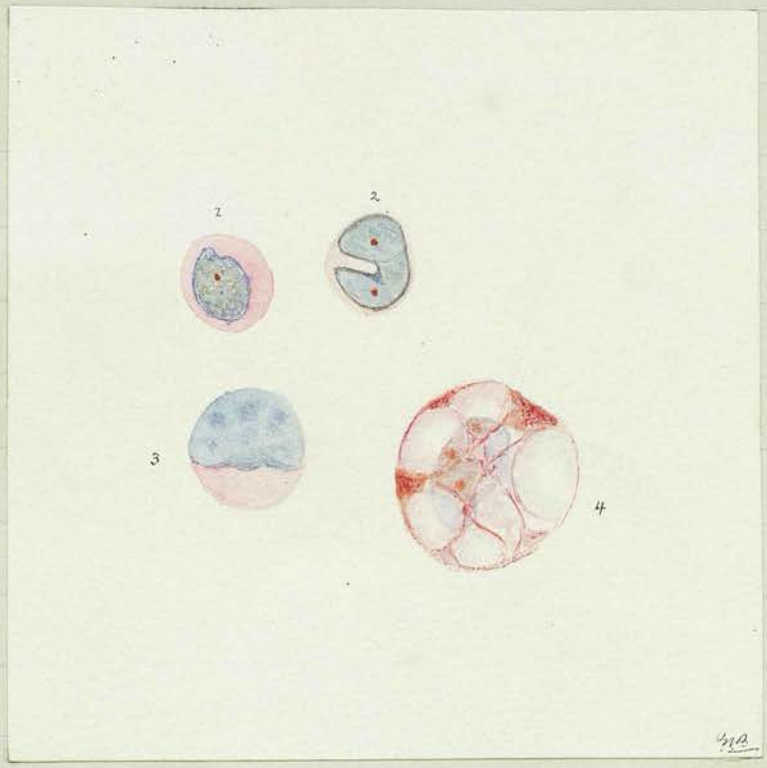


Plasma cells.

The nucleus is about the size of that of a lymphocyte, but the chromatin granules are more distinct, take on a deeper blue-green stain, and there is a bright red nucleclus. The protoplasm is two to three times the size of the nucleus, takes on a deep red pyronin stain, and occasionally has a lighter area round the nucleus. The nucleus is always placed eccentrically in this deep-red protoplasm. Double nuclei are not uncommon. The protoplasm is of oval outline, and in only a few cells could the protoplasm be described as polygonal. This description corresponds with the plasma cells as originally described by Waldeyer and Unna[74]. In well stained sections the plasma cell is distinctive and of easy differentiation.

Plasma cells were found in 16 out of the 17 cases of general paralysis, the average being 2 per cent., in two cases of tabes dorsalis, the average being 2 per cent, and in one case of a congenital syphilitic idiot boy.

Plate VI.



1, 2, 3, – Endothelial cells.

4 – Lattice cell.

Phagocytic cells.

Inclusions :- 1 Lymphocyte. 2 Blood pigment.

Endothelial Cells.

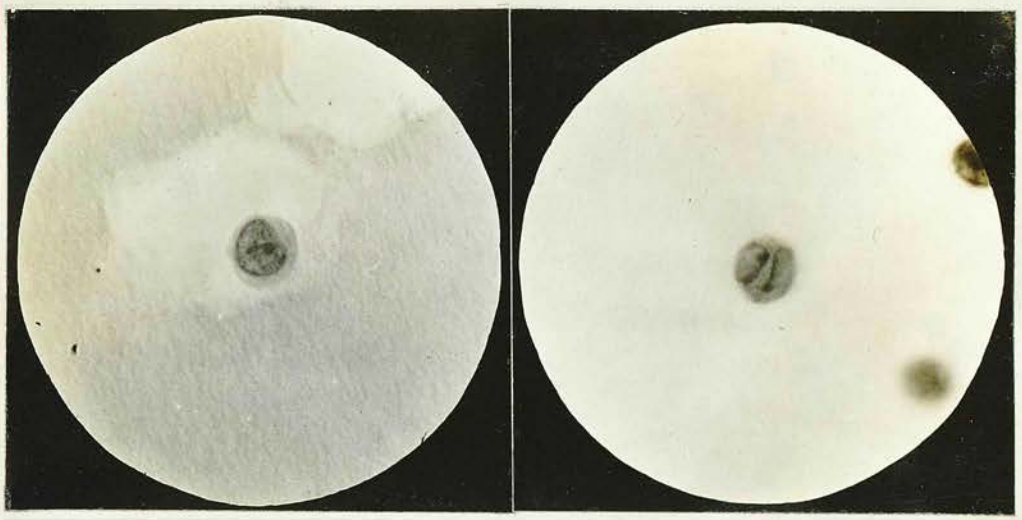
These cells were always present in the fluids of my series of cases. They vary considerably in size and shape, and are the largest cells found in the fluid.

The nucleus is as a rule kidney-shaped, sometimes oval, and is usually lying at the periphery of the cell.

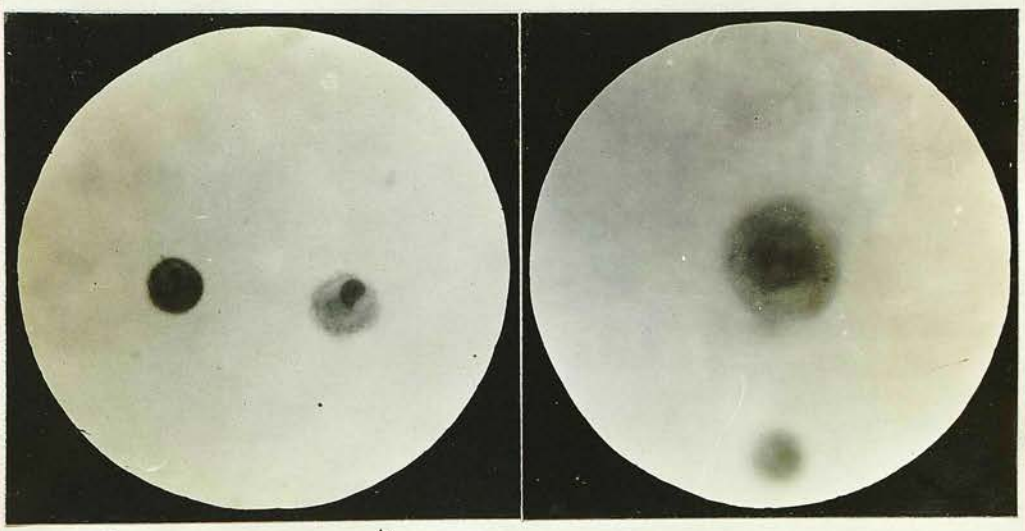
The nucleus stains a faint blue-green with pyronin-methyl green stain, contains few chromatin granules and has from one to three bright red nucleoli. The protoplasm stains a faint pink, and shows marked variation in amount even in the same fluid.

There is a distinct form of endothelial cell, designated as "Gitter" cell by Rehm,[65] in which the protoplasm presents a fenestrated or latticed appearance. These clear areas suggest that these cells are probably of a phagocytic character. These latticed cells were found in 15 out of 22 cases of general paralysis, in 3 cases of epilepsy, in 2 cases of melancholia, and in one case of each of the following:- Tabes dorsalis, imbecility and idiocy.

Plate VII.



Endothelial Cells.



Transitional. Endothelial Endothelial Cell.

The average endothelial cell count in general paralysis was 22 per cent., and showed little variation from the average, the highest counts being 43 and 39 per cent. in two general paralytics who were in a dying condition.

Phagocytes.

The most distinctive phagocytic cell encountered was an endothelial cell which had engulfed a lymphocyte.

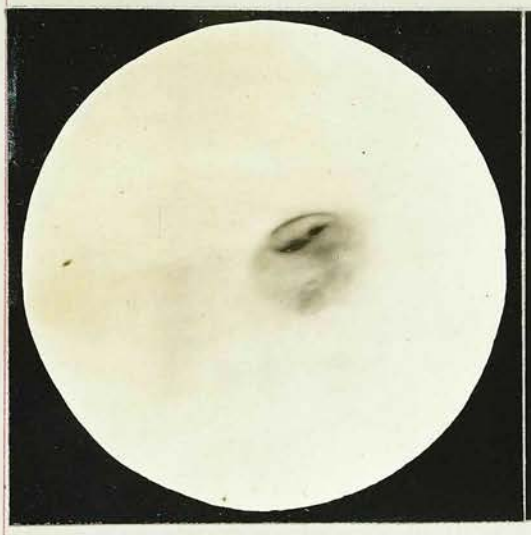
The endothelial nucleus was horse-shoe shaped and devoid of chromatin and stained a pale blue.

The lymphocyte nucleus was sharp and the chromatin elements deeply stained a dark blue-green.

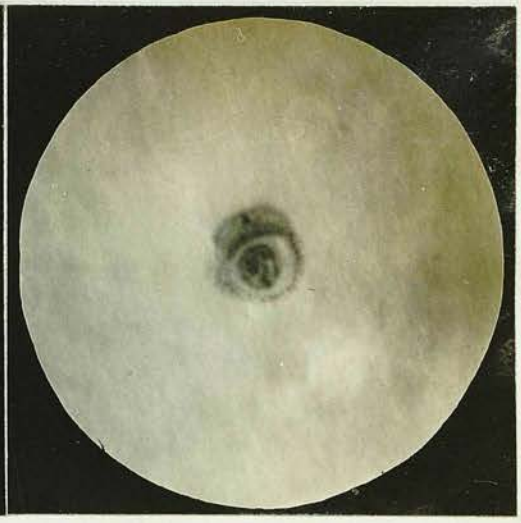
The endothelial nucleus occupied one segment at the border of the cell, and the lymphocyte nucleus occupied a central position.

The protoplasm of the cells was faintly stained pink except the area round the lymphocyte nucleus which was quite colourless.

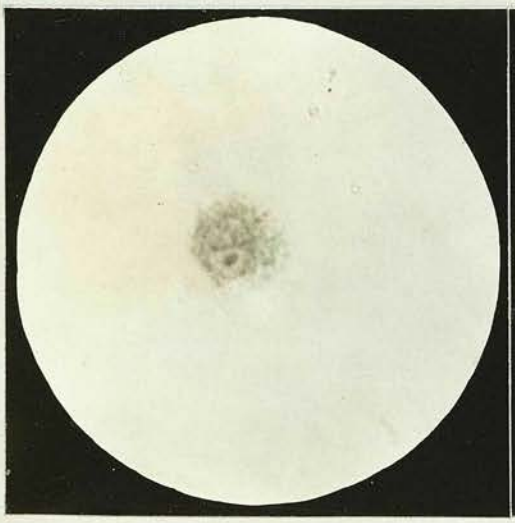
Plate VIII.



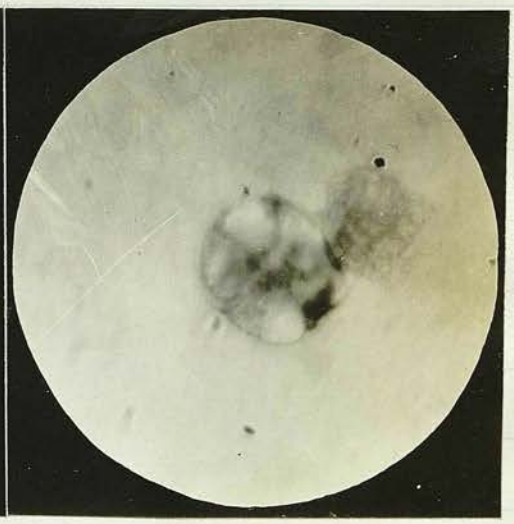
Phagocyte (blood pigment).



Phagocyte (lymphocyte).



Körnchen cell.



Lattice cell.

This type of phagocyte was found in three of the 17 cases of general paralysis, but as a rule only nuclear remnants were present in the protoplasm.

A second type of phagocytic endothelial cell was found in comparatively large numbers (9%) in the fluid of case 5. At the time of withdrawal the fluid was found to be tinged yellowish-red, and the colour remained even after centrifuging, thus proving that the colour was due to blood originally present in the fluid, and not to contamination at the time of puncture. Further, no red blood corpuscles were found in the Widal film.

The cells were endothelial in type, as they contained eccentric oval nuclei, and a large amount of protoplasm. In the protoplasm could be seen fine yellowish granules occupying the greater part of the cell protoplasm, and these were considered to be composed of altered blood pigment.

The clinical history of the case is of interest. The man was an excited general paralytic, who had a slight seizure on February 26th 1913 followed by a severe seizure on March 2nd which left him with a right-sided hemiplegia.

Lumbar puncture was performed on 11th. March. The patient died on 17th. March, and post-mortem examination revealed an extensive meningeal haemorrhage over the left motor area. It can fairly be considered, therefore, that the endothelial cells of the cerebro-spinal fluid were acting as phagocytes for the blood pigment derived from the haemorrhage.

Mitotic Cells.

Only two cells showing mitotic figures were encountered, and these were found in sections of two different general paralytic fluids. Both cells showed well-marked mitotic figures, and one appeared to be under-going sub-division.

Unclassified Cells.

This class has been necessary, as there are a few cells that could not be included among the types above-mentioned.

The Fibroblast.

This cell is distinguished by its spindle-shaped nucleus which contains faintly stained chromatin filaments, and has apparently only a small amount of protoplasm at the poles of the cell.

The Polyblast.

This cell has been described by Wickman,[66] and by McIntosh and Turnbull[67] as occurring in the infiltration of the meninges in poliomyelitis, and has not been described so far as I am aware as occurring in the cerebro-spinal fluid. The cell occupies on an average an area equal to that of three red corpuscles, is round in shape and the nucleus closely resembles that of a polymorphonuclear leucocyte. The nucleus is stained darkly with pyronin methyl-green, with a paler area in the centre which may show a red nucleolus. The protoplasm takes on a fairly red tint with a pinker stain round the nucleus - thus it is distinguished from the polymorphonuclear leucocyte. An occasional cell was found in general paralysis (9 out of 17 cases), in two cases of tabes dorsalis, in one case of dementia praecox, idiocy, and neurasthenia.

Discussion of Cytology.

The main features of interest have already been mentioned under the cell types.

Plasma cells, phagocytes, and lattice cells call for a brief discussion.

Plasma cells were present with one exception in all the general paralytic fluids. The exception was that of a slightly demented patient in whom the disease was slowly progressing.

My findings in tabes dorsalis agree with those of Henderson and Mourhead,[68] who found plasma cells present in two out of three cases of this disease. I also found these cells present in the fluid of a congenital syphilitic idiot, but in no other form of insanity.

Plasma cells cannot, therefore, be considered pathognemonic of general paralysis, but it may be taken that their presence in a case of mental disease is strong evidence of a parasyphilitic lesion.

Phagocytic cells were only found in four cases, viz., three of general paralysis and one of paranoia (no. 27). This latter case is a querulant, impulsive patient, who has so far exhibited no symptoms of general paralysis. The cerebro-

- spinal fluid showed no protein increase but the glycyl-tryptophane was positive. A moderate lymphocytosis, the indication of a ferment and the presence of phagocytic cells in the fluid of this case are at least suggestive of some irritative cause. Stoddart [69] states that there is no discoverable pathological basis for the mental change in paranoia. The future progress of this case will be of interest

Lattice cells. Cotton and Ayer [70] describe, under the name of Körnchen, a type of phagocyte cell filled with numerous fat droplets or fatty pigment, which they only found in ventricular fluids. Henderson and Muirhead [71] consider these cells to be an early stage of the lattice, and I am inclined to agree with their suggestion.

All endothelial cells, which appear to have a granular or vacuolated protoplasm, are included under the one class — lattice.

As mentioned on page 63, these cells were found in a number of conditions, and I cannot substantiate the view taken by Henderson and Muirhead, who considered that the absence of lattice cells in their cases of tabes dorsalis might

be a point of value in the differential diagnosis between general paralysis and tabes.

Jelly Method.

In 1909 H.C. Ross [72] invented a method whereby he could observe ameboid movement in leucocytes. C.H. Ross [73], using this method, made the noteworthy announcement that he had found a protozoal parasite always present in the lesions of secondary syphilis, and his findings have been corroborated by other investigators.

Method.

The Jelly method of H.C. Ross is as follows:- 3 c.cm. of a 2 per cent. solution of agar in water, boiled and filtered; 1 c.cm. of Unna's polychrome methylene blue, which has been previously diluted with two volumes of water - that is, 1 in 3; and 2 c.cm. of a solution containing 4·5 per cent. sodium citrate, 1·5 per cent. sodium chloride, and 0·225 per cent. atropine sulphate. This is boiled up together in a test tube and 0·3 c.cm. of a 5 per cent.

solution of sodium bicarbonate added. Then a drop of this mixture when molten is poured on to a microscope slide and allowed to spread thereon, to cool, and to set. A drop of blood is placed upon a cover-glass, and inverted on to the set jelly.

Having familiarised myself with the technique and observed the behaviour of the cells of freshly drawn blood, I considered that it would be of interest to observe the cells of the cerebro-spinal fluid when subjected to similar conditions.

One of the first difficulties met with was due to the scattering of the cells to the edge of the cover-glass, where they whirled round and round in an endless stream when an oil-immersion lens was lowered. Fortunately I found that, if the set jelly were cut into a square smaller in area than the cover-glass, the cells remained stationary.

Only cells from fluids of general paralytics were watched, and the following is a short account of my observations :-

Within the first two or three minutes, the cells appear as pale colourless discs, discrete for the most part, but here and there occuring

in clumps. More cells lay along the edges of the cover-glass than in the centre.

After two or three minutes the cells begin to take up the stain and can be differentiated. Many of them at the same time show ameboid movements, putting out processes of varying size, and altering their shape in the most curious manner. The question as to the exact part of the cell which participates in these movements will be referred to later.

By far the commonest cell is the lymphocyte. But the lymphocyte of the vital method is very different from the lymphocyte of the Widal or Abgheimer method. In the first place it is considerably larger, 7 μ to 8 μ in diameter, and stands out with a clearness which is almost startling.

More striking than the difference in size, however, is the difference in internal structure. The nucleus, as before, occupies the greater part of the cell, but in the centre of the nucleus is a body which is never shown by the ordinary methods of staining. This body is annular in shape, and in some cases a thickening can be seen at one side, giving the whole the

appearance of a signet ring. This body is invariably present in lymphocytes, but it can also be seen in larger cells to be described hereafter, which are not themselves lymphocytes, but which may be derived from lymphocytes.

Outside the nucleus, which is of a pale blue tint, there is a ring of granules which stain red with varying degrees of intensity in different cells, a ring which is usually thicker at one part than at another, but which may be of uniform thickness throughout.

On careful focussing, one can see a clear unstained zone outside the granules, in most cases of about the same thickness as the granular zone, but sometimes a good deal wider.

This clear zone, which is probably quite unstained by the ordinary methods, is of peculiar interest in connexion with the ameboid movements already referred to, for in most cases it is it and it alone which participates in these movements. Sometimes, however, the granular area is also involved in these protrusions and prolongations which the cell throws out under the stimulating influence of the atropine.

Gradually, dependent upon the temperature of the room, the composition of the jelly, etc., the processes are withdrawn, the nucleus takes up a deep red stain and the cell dies, when it may either burst through liquefaction of its cytoplasm or it remains as an oval mauve-red blotch of stain. Brownian movement was occasionally observed in the granules after death of the cell.

Numerous varieties of cells were encountered, and their behaviour corresponded more or less to the above detailed description of the lymphocyte.

Large lymphocytes, 10 to 14 μ. in diameter, always showed more active ameboid movement than any other cells.

Polymorphonuclear cells could be distinguished. In many cases the polymorph bears a strong resemblance to the same cell fixed and stained in the ordinary way. The nucleus, however, does not take up the stain at all, and the granules in the cytoplasm are larger, more distinct and more deeply stained. Coarsely granular polynuclear or eosinophile cells were observed on more than one occasion

in a fluid in which no blood was present in the specimen.

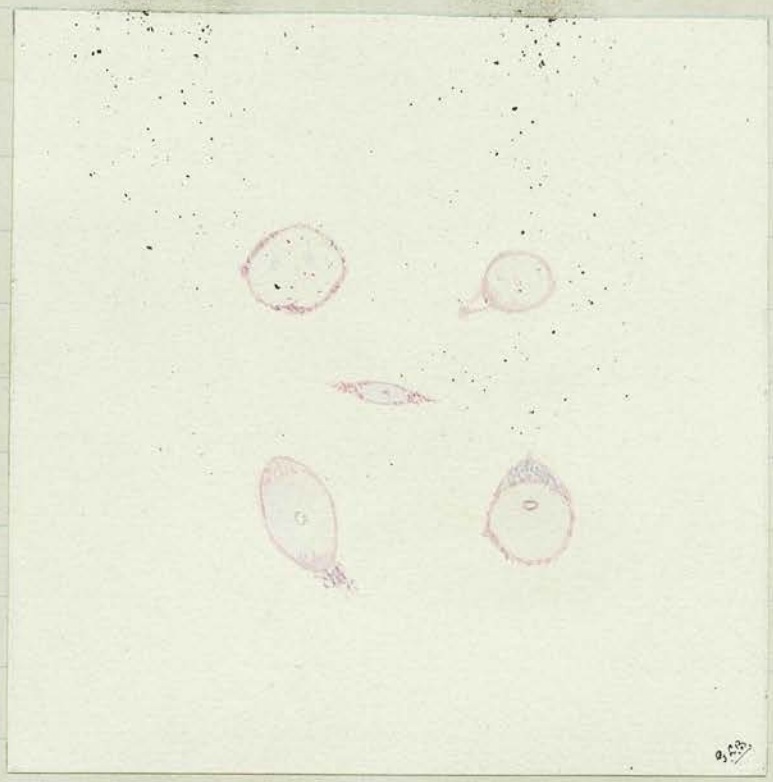
The remainder of the cells are much more difficult to classify and do not closely resemble any of the cells seen in a specimen prepared in the usual way.

Large cells, with round or oval eccentric nucleus, abundant deeply stained protoplasm with a clear area near the nucleus, resembled plasma cells. Other large cells, irregular and ill-defined in outline, with course granules in the protoplasm, showed active ameboid movements. It is difficult to say if these cells were mono- or polynuclear.

Sketches were made at each examination, and the main features in the cycle of changes have been reproduced in the accompanying plates, with the object of illustrating the description in the text.

Finally, I wish to acknowledge my indebtedness to Dr. Simpson for giving every laboratory facility for the carrying out of this research, and to my colleagues, Drs. Boyd, Hopwood and Rogers for their valued criticism, interest and advice extended towards me during the progress of my task.

Plate IX.



Vital Jelly Method.

Lymphocytes taking up stain after being placed on jelly for about two minutes. Note pale nucleus with its ring-like dot. The granular zone is not yet distinct, and there is no evidence of a clear zone with pseudopodia.

Plate X.

Vital Jelly Method.

Group of cells showing three living lymphocytes with two ghost or dead cells. The central cell is enclosed by four cells, its nucleus is circular but the granular zone appears to accommodate its shape to its surroundings.

Plate XI.

Vital Jelly Method.

Group of cells, two lymphocytes and a large cell of irregular outline. The latter cell lost its stain after being on the jelly for five minutes.

Plate XII.

Vital Jelly Method.

Cells throwing out pseudopodia.

1. Large lymphocyte and
2. Small lymphocyte

show three zones

a. Clear nuclear

b. Mauve-red granular

c. Clear pseudopodic.

3. Remarkable for number of processes.

4. Curious gemmular projections from a cell which has two pseudopodia thrown out.

Plate XIII.

Vital Jelly Method.

Cells of irregular outline.
The balloon-shaped cell, which
is not uncommon, is developing
a yellowish colour in the
granular zone — an indication
of approaching death.

Plate XIV.

Vital Jelly Method.

Cells of irregular outline.

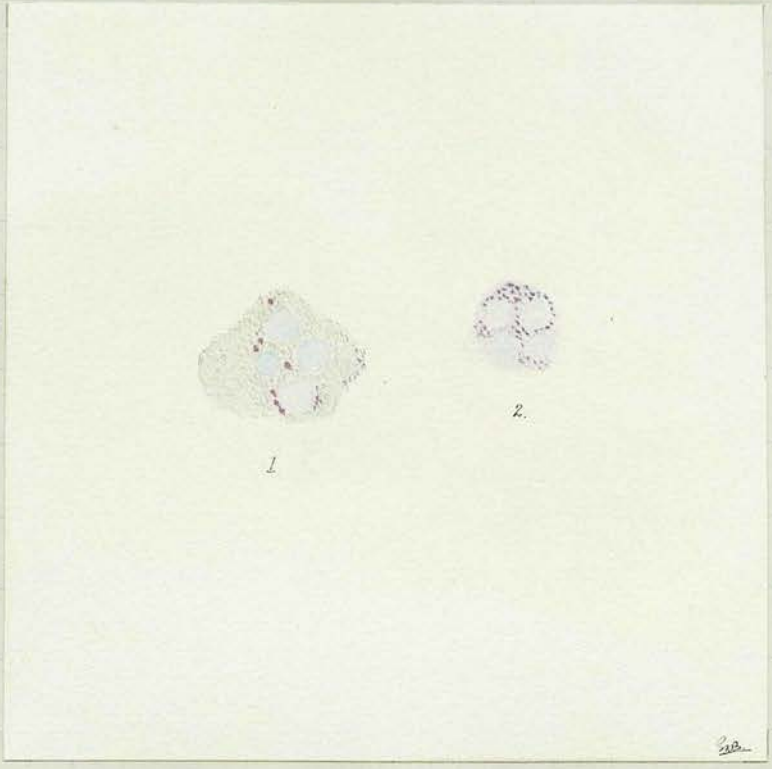
1. The pseudopodia in this cell, instead of being clear, appear to have taken up stain and contain granules.

Plate XV.

Vital Jelly Method.

1. Large cell which shows a clear area round nucleus, and appears to correspond to a plasma cell.
2. Cell showing vacuolation of the cytoplasm.

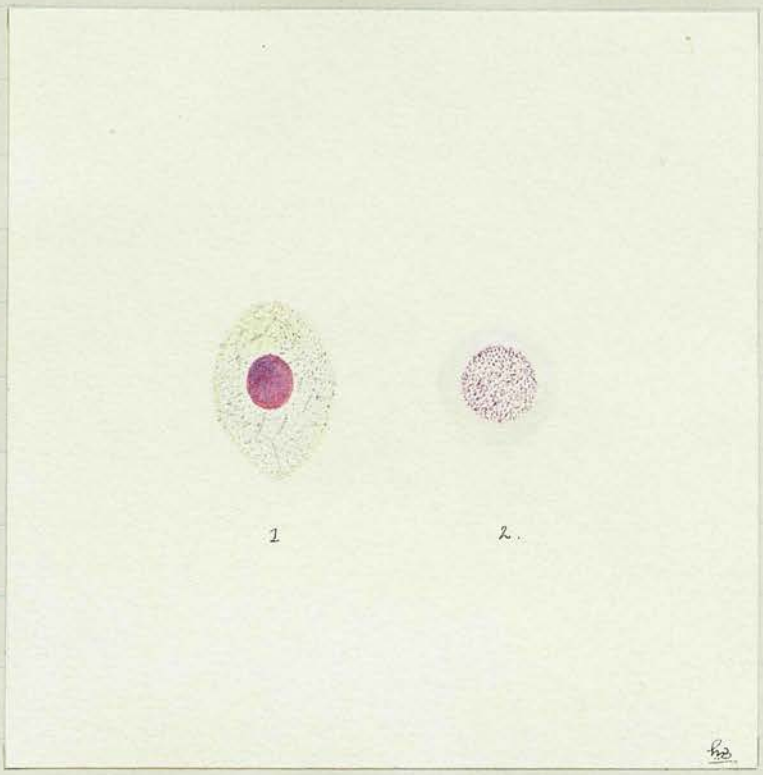
Plate XVI.



Vital Jelly Method.

1. Eosinophile cell with pale nuclei, and faintly speckled cytoplasm with a few coarse granules.
2. Polymorphonuclear cell with small distinct granules in cytoplasm. The pseudopodium indicates that the cell is alive.

Plate XVII.

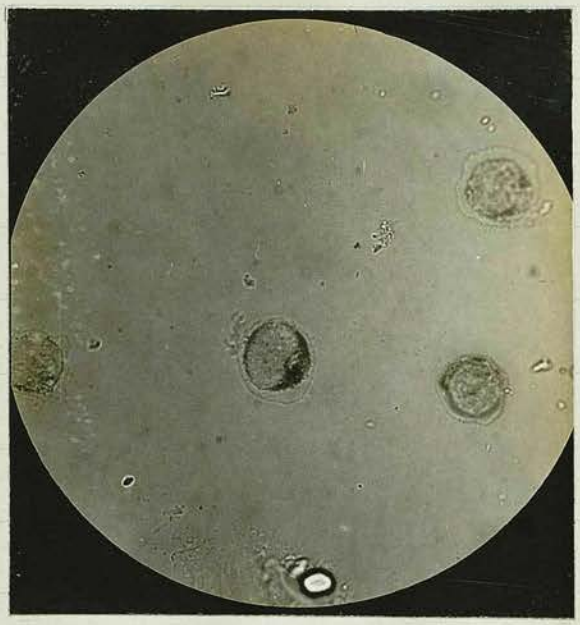


Vital Jelly Method.

Signs of approaching death of the cell.

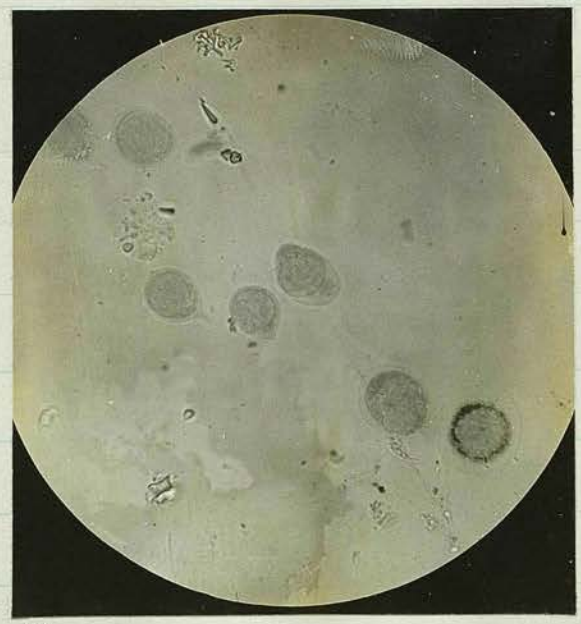
1. Staining of the nucleus.
2. Brownian movement of the granules followed almost immediately by bursting of the cell.

Plate XVIII



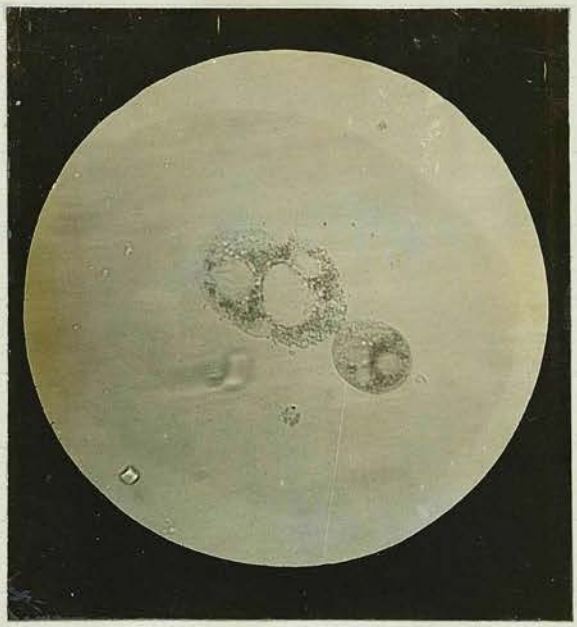
Central cell shows three distinct zones.

Microphotographs of living cells — Jelly method.



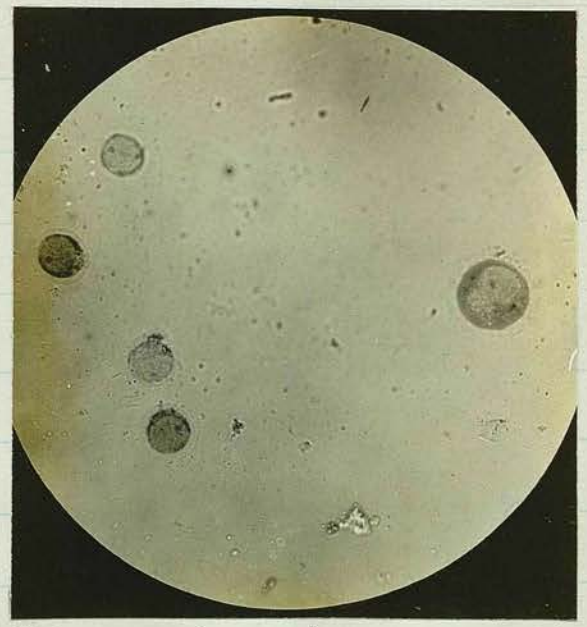
Living cells taking up stain.

Plate XIX



Liquefaction of cytoplasm and rupture of a polynuclear cell.

Microphotographs of living cells – Jelly method.



Four small lymphocytes and a plasma? cell showing signet-ring dot in nuclei.

Conclusions.

Examination of the cerebro-spinal fluid is of great importance and a valuable aid in the diagnosis of mental disease.

An exudation of serum, due to local or general lesions of the central nervous system, may be indicated by the presence in the cerebro-spinal fluid of a ferment capable of splitting the dipeptide, glycyl-tryptophane, into its two component parts.

An increase in the number of lymphocytes and in the protein content is the almost invariable rule in General Paralysis and Tabes Dorsalis, but such an increase may occasionally occur in other forms of mental disease.

The Butyric Acid reaction of Noguchi is strong presumptive evidence of a parasyphilitic lesion.

The quantitative modification of the

Conclusions (continued).

Butyric Acid test is of value in deciding whether a doubtful reaction is positive or negative.

The ammonium sulphate ring test gives a positive reaction in conditions other than parasyphilitic, but when the dilution method is used it is of greater diagnostic accuracy, and is a simple quantitative method of estimating the protein content.

The Fehling-reducing substance tends to be decreased in general paralysis, imbecility and dementia praecox.

Abzheimer's method is the best for the cytological examination of the cerebro-spinal fluid, cells can be differentiated in a way never hitherto possible, and a fair quantitative count can be made.

The cells of the greatest diagnostic importance are the plasma cell, the phagocytic endothelial cell, and the lymphocyte in excess.

Conclusions (continued).

A high cell count with excess of lymphocytes together with the presence of plasma cells is strong evidence of a para-syphilitic lesion.

The in-vitro staining of the cells of the cerebro-spinal fluid by the jelly-method advances our knowledge of active cell-life, and of the changes occurring during cellular death.

Rest in bed after lumbar puncture is desirable to avert the after effects. No permanent ill effects have followed the operation in my series.

Geo. R. Boulton

Tables and References.

Table 1.

Showing results of Noguchi Test.

Condition	No. of Cases	Positive	Negative
General Paralysis	30	30	
Tabes Dorsalis	2	2	
Brain Syphilis	1		1
Mania Delirious	2		2
" Mono	2		2
" Acute	1		1
" Recurrent	2		2
" Chronic	2		2
" Puerperal	1		1
" Senile	1		1
Melancholia	5		5
" Climacteric	2		2
Alcoholic Insanity	7	1	6
Epilepsy	10		10
Delusional Insanity	3		3
Dementia Praecox	8		8
Imbecility	6		6
Idiocy	2		2
Stupor	1		1
Confusional Insanity	1		1
Parancia	1		1
Secondary Dementia	5		5
Paralysis Agitans	1		1
Neurasthenia	1		1
Psychasthenia	1	1	
Totals	98	34	64

Table II.

Shewing the Protein content expressed in terms of the dilution of the Cerebro-spinal fluid which gave a positive reaction with the Ammonium Sulphate test.

Condition	No. of Cases.	Negative Result	Undiluted Fluid	Dilution of Cerebro-spinal Fluid				
				1 in 2	1 in 4	1 in 6	1 in 8	1 in 10 to 18
General Paralysis	30		2	6	12	5	3	2
Tabes Dorsalis	2			1		1		
Brain Syphilis	1		1					
Mania Delirious	2	1	1					
" Mono.	2	2.						
" Acute.	1		1					
" Recurrent	2	1	1					
" Chronic	2	1		1				
" Puerperal	1	1						
" Senile	1	1						
Melancholia	4	4						
" climacteric	2	1	1					
Alcoholic Insanity	6	3	1	1	1			
Epilepsy	11	8	3					
Delusional Insanity	3	2	1					
Dementia Praecox	8	6	2					
Imbecility	5	4	1					
Idiocy	2	1	1					
Stupor	1	1						
Confusional Insanity	1	1						
Paranoia	1	1						
Secondary Dementia	5	4	1					
Paralysis Agitans	1	1						
Neurasthenia	1	1						
Psychasthenia	1			1				
Totals	96	45	17	10	13	6	3	2

Table III.

Quantitative estimation of Protein Content by Noguchi's Butyric Acid Test.

No.	Condition	Precipitate.	No.	Condition	Precipitate.
1	General Paralysis	0.2	20	* General Paralysis	0.15
2	* do.	0.6	21	do.	0.15
3	do.	0.8	22	* Tabes Dorsalis	0.3
4	* do.	0.2	23	Alcoholic Dement	0.15
5	do.	0.3	24	do.	0.05
6	do.	0.3	25	Secondary Dementia	0.08
7	do.	0.4	26	Idiocy	0.1
8	* do.	0.25	27	do.	0.1.
9	* do.	0.25	28	do.	0.08
10	do.	0.25	29	Imbecility	0.05
11	do.	0.3	30	do.	0.05
12	* do.	0.15	31	Acute Mania	0.05
13	do.	0.15	32	Chronic Mania	0.01
14	* do.	0.35	33	Melancholia	0.1
15	do.	0.3	34	Delusional Insanity	0.02
16	* do.	0.15	35	do.	0.1
17	* do.	0.32	36	Neurasthenia	0.04
18	do.	0.22	37	* Psychasthenia	0.15
19	do.	0.3			

* The asterisk denotes that the Wassermann Reaction was positive with the blood serum.

Table IV.

Showing result of Glycyl-tryptophane test.

Condition	No. of cases	Positive	Negative
General Paralysis	12	6	6
Tabes Dorsalis	1	0	1
Mania Delirious	1	0	1
" Acute	1	0	1
" Recurrent	2	1	1
" Chronic	1	1	0
Melancholia	2	0	2
Alcoholic Insanity	2	1	1
Dementia Praecox	4	1	3
Epilepsy	7	2	5
Imbecility	3	0	3
Idiocy	2	0	2
Paranoia	1	1	0
Secondary Dementia	4	2	2
Paralysis Agitans	1	1	0
Psychasthenia	1	0	1
Totals	45	16	29

Table V.

Shewing the Cell-Count by Widal's Method in Sixty Cases.

Condition	No. of cases	Cell-Counts.					
		Normal	Pathological.				
		1–6	7–20	20–50	50–100	100–200	200–600
General Paralysis	15			2	4	3	6
Tabes Dorsalis	2		1		1		
Brain Syphilis	1	1					
Mania	6	4	1	1			
Melancholia	5	4	1				
Epilepsy	9	8	1				
Delusional Insanity	1	1					
Paranoia	2	2					
Dementia Praecox	7	4	3				
Stupor	1			1			
Dementia	5	4	1				
Neurasthenia	1	1					
Psychasthenia	1			1			
Imbecility	4	2	2				
Totals	60	31	10	5	5	3	6

Table VI.

Showing the Cell-count by Alzheimer's Method in 52 cases.

Condition.	No. of Cases	Cell-counts.					
		Normal			Pathological		
		1-20	20-50	50-100	100-200	200-400	Over 400
General Paralysis	16				3	3	10
Tabes Dorsalis	2			1		1	
Mania.	5	3		1	1		
Melancholia	6	2	3	1			
Epilepsy	5		2		3		
Delusional Insanity	1		1				
Paranoia	1				1		
Dementia Praecox	4	1	2	1			
Stupor	1				1		
Dementia	4	1	1	2			
Neurasthenia	1		1				
Psychasthenia	1					1	
Imbecility	2	1		1			
Idiocy	1					1	
Alcoholic Insanity	2			1	1		
Totals	52	8	10	8	10	6	10

Table VII.

Comparison of Cell-counts by Widal's and Abzheimer's Methods in 43 cases.

Widal - Cells per field.
Abzheimer - Cells per 100 fields.

No.	Condition	Widal	Abzheimer
1	General Paralysis	167	400
2	do.	250	676
3	do.	37	264
4	do.	99	221
5	do.	129	116
6	do.	558	146
7	do.	99	340
8	do.	88	880
9	do.	124	418
10	do.	250	722
11	do.	558	568
12	Tabes Dorsalis	76	398
13	do.	10	87
14	Mania Acute	20	150
15	" Puerperal	1	13
16	" Recurrent	1	10
17	" do.	1	8
18	" Chronic	23	81
19	Melancholia	3	32
20	do.	6	30
21	do. Chronic	1	56
22	do.	8	23
23	do.	3	20
24	Epilepsy	9	120
25	do	2	105
26	do.	1	28
27	do.	2	25
28	do.	5	168
29	Delusional	3	26
30	Dementia Praecox	12	36
31	do.	17	74
32	do.	1	1
33	do.	3	31
34	Imbecility	11	60
35	do.	3	14
36	Stupor	21	135
37	Parancia	6	125
38	Dementia	3	72
39	do.	1	29
40	do.	12	61
41	do.	1	10
42	Neurasthenia	6	32
43	Psychasthenia	33	290

Table VIII.

Showing rate of flow in 89 lumbar punctures.

Condition	No. of Cases	Normal 1 drop per sec.	Running drops	Stream
General Paralysis	31	11	11	9
Tabes Dorsalis	1	1	0	0
Brain Syphilis	1	0	0	1
Imbecility	8	1	3	4
Epilepsy	6	2	1	3
Mania	11	8	1	2
Melancholia	8	5	1	2
Dementia	7	6	1	0
Dementia Praecox	7	3	2	2
Delusional Insanity	2	1	1	0
Alcoholic Insanity	3	2	1	0
Confusional Insanity	1	1	0	0
Stupor	1	1	0	0
Paralysis Agitans	1	1	0	0
Neurasthenia	1	1	0	0
Totals	89	44	22	23.

Table IX.

Showing the analysis of the cells in 30 cases.

No.	Name	Sex	Diagnosis	Fluid	Cells per 100 Fields	Small lympho-cyte	Transition-al	Large Lympho-cyte	Endothelial	Plasma	Poly-morph	Phago-cyte	Unclassified
1	J.B.	F.	General Paralysis	Clear	414	35	13	21·5	18	5	7		·5.
2	M.C.	F.	do.	..	400	62·5	10·5	3·5	21·5	1	1		
3	T.C.	M.	do.	..	146	51	19	7·5	19	1·5	2·5	·5	
4	W.H.	M.	do.	..	676	44	4	28	21·5		2·5		
5	A.H.	M.	do.	Yellow	264	50		1	39	1		9	
6	R.H.	F.	do.	Clear	340	59·5	10·5	4·5	19	2·5	1·5	1	1·5
7	S.J.	F.	do.	Sl. bloody	221	72·5	2·5	1·5	21	1	·5		1
8	A.K.	M.	do.	Clear	530	73·5	6·5	2·5	15	·5	1		1
9	J.M.	M.	do.	..	997	68·5	11·5	6·5	7·5	4	·5		1·5
10	F.M.	M.	do.	..	460	75		1	17	2	5		
11	C.G.	F.	do.	..	880	74	2·5	4·5	17·5	1·5			
12	J.M.	F.	do.	..	418	23·5	8	18	19	1	29		1·5
13	T.N.	M.	do.	..	177	32·5	14·5	10	33·5	2·5	3·5		3·5
14	A.C.	M.	do.	..	722	59·5	7	16	12·5	3·5	1		·5
15	A.S.	M.	do.	..	234	31·5	8·5	7·5	43	4·5	2·5		2·5

Table IX continued.

No.	Name	Sex	Diagnosis	Fluid	Cells per 100 Fields	Small lympho-cyte	Transition-al	Large lympho-cyte	Endothelial	Plasma	Polymorph	Phagocyte	Unclassified
16	J.W.	m.	General Paralysis	Clear	116	71·5	5	2	17·5	3			1
17	J.H.	m.	do.	..	260	63	9		20·5	2·5	2·5		2·5
18	A.M.	m.	Tabes Dorsalis	..	87	60·5	4	2	31·5	1	·5		·5
19	W.C.	m.	do.	..	398	34	21	6	33	3			3
20	F.W.	m.	Acute Mania	..	150	73		10	16		1		
21	L.R.	m.	Epilepsy	..	168	54		5	41				
22	B.H.	m.	Delusion. Insan.	..	70	75	3·5	1·5	20				
23	W.C.	m.	Dement. Praecox	..	38	50	2	3	41		1		3
24	W.N.	m.	Imbecility	..	60	22	6	4	68				
25	W.F.	m.	Idiocy (cong. syph.)	..	219	23	16	8	51·5	·5			1
26	E.C.	f.	Stupor	..	135	34	16·5	7·5	42				
27	J.G.	m.	Paranoia	..	125	30·5	19	10	40			·5	
28	J.P.	m.	Dementia	..	131	19	10	9	62				
29	J.J.	m.	Neurasthenia	..	32	57	13·5	1·5	27				1
30	C.M.	f.	Psychasthenia	..	290	89			11				

Table X.

Indicating that cells were present in the following 35 conditions, but in such a small number that percentages were valueless.

No.	Name	Sex	Diagnosis	Fluid	Cells per 100 Fields	Lympho-cyte	Endo-thel-ial	Plas-ma	Poly-morph
31	H.C.	f.	General Paralysis	Clear		+	+	+	
32	S.B.	m.	do.	..		+	+	+	+
33	J.H.	m.	do.	..		+	+	+	
34	E.L.	f	do.	..		+	+	+	+
35	A.M.	f	do.	..		+	+	+	
36	S.P.	m.	Mania Mono	..		+	+		
37	M.R.	f	„ Recurrent	..		+	+		
38	T.T.	m.	„ „	..	10	+	+		
39	W.A.	m.	„ Chronic	..	81	+	+		
40	M.D.	f	„ „	..	64	+	+		
41	E.D.	f	„ Puerperal	..	13	+			
42	J.H.	m.	„ Senile	..		+	+		
43	M.C.	f.	Melancholia	..	32	+	+		
44	S.C.	m.	do.	..	4	+	+		
45	S.F.	m.	do.	..	30	+	+		
46	M.H.	f.	do.	..	20	+	+		
47	M.R.	f.	do.	..		+	+		
48	P.R.	m.	do.	..	56	+	+		

Table X continued.

No.	Name	Sex	Diagnosis	Fluid	Cells per 100 fields	Lympho-cyte	Endo-thelial	Plasma	Poly-morph
49	W.W.	m.	Melancholia	Clear	36	+	+		
50	W.B.	m.	Epilepsy	..	120	+	+		
51	S.C.	f.	do.	..	105	+	+		+
52	J.D.	m.	do.	Sl. bloody		+	+		
53	J.F.	m.	do.	Clear	25	+	+		
54	M.M.	f.	do.	..	28	+	+		
55	J.L.	m.	Delusion. Insan.	..	26	+	+		
56	H.M.	m.	do.	..	21	+	+		
57	E.B.	f.	Dement. Praecox.	..	36	+	+		
58	G.M.	f.	do.	..	74	+	+		
59	C.K.	f.	do.	..		+	+		
60	M.M.	f.	do.	..	2	+			
61	A.R.	f.	Imbecility	..	14	+	+		
62	R.G.	m.	Dementia	..	61	+	+		
63	J.W.	m.	do.	..	29	+	+		
64	W.T.	m.	do	..	72	+	+		
65.	R.T.	f.	do	..	10	+			

References.

1. Alzheimer : Centrablatt f. Nervenheil. u. Psych., June 15th 1907.
2. Pacchioni : Quoted by Trolard. "Les Granulations de Pacchioni." Journ. de l'Anat. et de la Physiol., 1892, p. 209.
3. Cotugnuo : Quoted by Dircksen: "Etude sur la Composition Chimique et la Concentration Moléculaire du Liquide Céphalo-rachidien," These de Paris, 1901.
4. Majendie : Recherches sur le Liquide Céphalo-rachidien, 1825.
5. Conquest. : "Results of paracentesis of the head in 19 cases of Hydrocephalus." Lancet. vol. 1, 1838.
6. Malgaigne : "Ueber die Punktion des Schädels bei dem Chron. Hydrocephalus." Bullet de Thérap. T. XIX.
7. Percy : "Analysis of the liquid of Spina Bifida." Lancet, p. 851, vol. 37 - 1846.
8. Landerer : "Examination of the liquid of Spina Bifida". Chemical Gazette, p. 10, Vol. 1.

9. Battersby : "On chronic, acquired, sanguineous and congenital Hydrocephalus." Edin. Journ., July, 1850.

10. Schlossberger : "Analyse hydrocephalischer Flüssigkeiten". Archiv. f. phys. Heilk. X. 1851.

11. Schmidt : "Charakteristik der epidemischen Cholera, Leipzig und Milan 1850".

12. Bussy : "Analyse d'un liquide qui s'est écoulé de l'oreille d'un homme qui avait une fracture du crâne". Bull. de l'Acad. de Méd. de Paris, 7. Dec. 1852.

13. Claude Bernard: "Leçons de Physiologie experimentale appliquée á la Médicine." Paris 1855 + 1856.

14. Quincke : "Die Lumbalpunktion des Hydrocephalus". Berliner Klin. Wochenschrift, 1891, No. 39.

15 Widal : Sicard et Ravaut: "cytologie du Liquide céphalo-rachidien." Bull. de la Soc. Méd. des Hop. de Paris, 1901.

16. Abadie : "Resultats de l'examen cytologique de quelques Liquides

Céphalo-rachidiens." Compt. Rend. de la Soc. de Biol., p. 946. Vol. V, 1902.

17. Castellani : "Researches on the Etiology of Sleeping Sickness." Journ. Trop. Med., June, 1903.

18. Wassermann, Neisser and Bruck : Deut. Med., Woch. 1906.

19. G. M. Robertson: The Morrison Lectures, 1913. Journ. Ment. Sc., April 1913.

20. H. C. Ross : "On a combination of substances which excites ameboid movement in leucocytes by which living can be differentiated from dead cells". Lancet. p. 152, 1909.

21. Mott : Oliver Sharpey Lectures, 1910 Lancet, July 2nd and 9th, 1910.

22. Greenfield : "On the value of a quantitative albumen estimate of the cerebro-spinal fluid." Lancet, Sept. 1912. p. 685.

23. Ravaut : Loc. cit.

24. Cavazzani : "Contributions à la Physiologie du Liquide Cérébro-Spinal". Archiv. Ital. de Biolog., 105.

Vol. XXXVII., p. 30.

25. Mott : Loc. cit..

26. Geohegan : Zeitschrift für Physiologische Chemie. Band 1., page 330.

27. Mott and Halliburton: "The Physiological Action of Choline and Neurine". Trans. of the Roy. Soc. Vol. CXC., p. 211.

28. Hebb. : Quoted by Purves Stewart: The Diagnosis of nervous Diseases, 1908, p. 391.

29. Halliburton and Rosenheim: Brit. Med. Journ., 1907. p. 1043.

30. Pighini : Quoted by Mott: "The Pathology of the cerebro-spinal fluid," Lancet, July 9. 1910.

31. Mott. : Loc. cit.

32. Purves Stewart: The Diagnosis of Nervous Diseases, 1908, p. 393.

33. Guillain : Rev. Neurolog., April 30th. 1903.

34. Nissl Centrabl. f. Nerv. u. Psychol., April, 1904.

35. Nonne and Apelt: Quoted by Jones and Ross Brit. Med. Journ., May 8, 1909.

36. Cimbal : Ibid.

37. Noguchi : Lancet Vol. 1. 1909, p. 1666.

38. Jones and Ross: "On the use of certain new chemical tests in the diagnosis of general paralysis and tabes." Bull. of the Ontario Hosp. for Insane, Vol. II. November, 1909.

39. Boyd : "The cerebro-spinal fluid in certain mental conditions." Journ. of Ment. Sc., April 1912

40 Connell : "The cerebro-spinal fluid in paresis." Amer. Journ. of Insanity, July, 1907. p. 73.

41. Marr : "The examination of the cerebro-spinal fluid in General Paralysis for purposes of Diagnosis." Rev. of Neurol + Psych. Vol. VI. p. 635.

42. Jones + Ross : Loc. cit.

43. Winifred Muirhead: "The examination of the cerebro-spinal fluid in general paralysis and other forms of Insanity." Journ. of Ment. Sc., Oct. 1910, p. 649.

44. Williamson : "The cerebro-spinal fluid in General Paralysis and the

Nervous Lues." Journ. of Ment. Sc. Oct., 1909, p. 662.

45. Boyd : Loc. cit., p. 17.

46. Turner : "Examination of the cerebro-spinal Fluid as an aid to Diagnosis in certain cases of Insanity, etc.." Journ. of Mental Sc., July, 1910, p. 486.

47. Bramwell : "The diagnostic and therapeutic value of Lumbar puncture." Clinical Studies, Vol. VII, Part II., p. 180.

48. Williamson : Loc. cit., p. 659.

49. Muirhead : Loc. cit..

50. Hallibuton : "The cerebro-spinal fluid". Journ. of Physiol., Vol. X, 1889.

51. Nawratzki : "Zur Kentniss der cerebro-spinal flüssigkeit." Zeitschr. f. Physiol. Chemie, 1897, Part VI, p. 532.

52. Pascheles and Reichel: "Ueber den Zuckergehalt pathologischer Flüssigkeiten". Wien. Klin. Woch., No. 17, 1896.

53. Neubauer and Fischer: Deutch. Archiv. f. Klin

Med., 1909, Band XCVII, p. 499.

54. W. Hall and G. S. Williamson: "The diagnosis of gastric carcinoma by the cleavage of polypeptides." Lancet 1, 1911, p. 731.

55. G. S. Williamson: "Further investigations on the cerebro-spinal fluid in Insanities". Journ. of Ment. Sc., Jan., 1912, p. 84.

56. G. S. Williamson: Ibid.

57. Widal and Ravaut: compt. rend. de la Soc. de Biol. de Paris, 1900, p. 648.

58. Fuchs and Rosenthal: Wien Med. Pr., Nos. 44-7, 1904.

59. E. Jones : Rev. of Neur. and Psych., July, 1907.

60. Abzheimer : "Einige Methoden zur Fixierung der zelligen Elemente der Zerebrospinalflüssigkeit." Zentr. f. Nerv. u. Psychiatr., 1907, No. 239

61 Cotton and Ayer: "The bytological Study of the Cerebro-spinal fluid by Abzheimer's Method, and its Diagnostic Value in Psychiatry." Rev. of Neur. and Psychiatry, Vol. 6, 1907, p. 207.

62 Henderson and Muirhead: "The Differentiation of Cells in the Cerebro-spinal Fluid by Abzheimer's Method." Rev. of Neur. and Psych., Vol. XI, April 1913.

63. Purves Stewart: Loc. cit., p. 393.

64. Cotton and Ayer: Loc. cit.

65. Rehm : Münch. Med. Woch., Aug. 4, 1908, p. 1636.

66. Wickman : "Die Akute Poliomyelitis, Berlin, 1911. Springer.

67 McIntosh and Turnbull: "Transmission to Monkeys of a virus obtained from English cases of poliomyelitis." Lancet, Feb. 22nd, 1913.

68. Henderson and Muirhead: Loc. cit.

69. Stoddart : Mind and its Disorders. 1908. p. 350.

70. Cotton and Ayer: Loc. cit.

71. Henderson and Muirhead: Loc. cit.

72. H. C. Ross : Loc. cit.

73. E. H. Ross : "An intracellular parasite developing into Spirochaetes." Brit. Med. Journ., Dec. 14, 1912, p. 1651.

74. Walldeyer and Unna: "On the plasma cell," See Councilman, Journ. of Exper. Med., Vol. I, 1897.