THE CEREBRO-SPINAL FLUID IN THE INSANE

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THE CEREBRO-SPINAL FLUID IN THE INSANE.

Within recent years it has become a recognised practice to perform lumbar puncture in cases suffering from cerebro-spinal disease; the practical value of the procedure is generally admitted as an aid to diagnosis; and in some diseases, such as tetanus and cerebro-spinal meningitis, the operation is resorted to for therapeutic purposes. It must, however, be admitted that it is only in such diseases of the cerebro-spinal system as are due to the presence of well recognised organisms that the diagnostic value of lumbar puncture approaches scientific accuracy, in such diseases, for example, as tubercular meningitis, cerebro-spinal fever, pneumococcal meningitis, and sleeping sickness. The examination of the cerebro-spinal fluid for the syphilitic reaction must now be recognised as indispensable in a thorough examination of nervous cases in which syphilis is supposed to play a part.

The method employed for obtaining the fluid is as follows:- The patient is placed on the left side, and the knees are drawn up towards the abdomen, and the body bent/

bent slightly forward. A point on a level with the crest of the ilia is chosen, and a long aspirating needle is inserted about 1 c.m. below and to the side of the spinous process; the needle is directed slightly upwards and inwards, the depth to which it is inserted varying with the age of the patient. It is advisable to puncture rapidly so as to avoid the deviating influence of the contracting muscles. Rapid puncture also assures the entrance of the needle into the canal before the venous plexus becomes turgescent from the pain and excitement incident to the operative procedure; the presence of blood in a sample of spinal fluid is not infrequently due to the fact that the patient is restless, and the turgid veins are thus easily pierced by the needle. In excitable cases, and especially in cerebro-spinal fever, it is advisable to give a little chloroform during the operation - just sufficient to dull the senses. In the majority of ordinary cases, however, this is not necessary; local anaesthesia does not appear to be of any use.

The normal spinal fluid is clear, and any departure from this transparency is pathological, unless bleeding has been caused by the needle entering the spinal/

spinal canal in which case a more or less opalescence may occur. It is slightly alkaline with a specific gravity of 1·006 to 1·008, the large amount of fluid in general paralysis being maintained at the normal by the large amount of suspended matter it contains. Normally it contains a very small amount of protein material and practically no corpuscular elements. As regards its chemical composition, the cerebro-spinal fluid contains 99 per cent. water and 1 per cent. solids. Albumin, globulin, fats, cholesterin, sodium lactate, chlorides, phosphates, sulphates are said to be present in very small amounts. The presence of urea has been determined in some cases, and also a substance which reduces Fehling's Solution, and gives rise to a brown colour when boiled with caustic potash; this substance does not, however, undergo fermentation, nor does it form osazone in the presence of phenylhydrazin it is generally looked upon as being pyrocatechin. Glucose is said by some authorities to be present in the fluid at times. Lichtheim reports the presence of glucose in cases of brain tumour determined by the phenylhydrazin test. With regards to albuminous bodies, serum albumin is said to be present only under exceptional conditions, while normally it is possible to demonstrate the presence of globulin and albumoses. With regard to the/

the presence of gases in the fluid Mott has recently found that it contains varying small amounts of oxygen and nitrogen, and that by boiling in vacuo an average amount of 10 per cent. by volume of carbon dioxide can be determined.

It has been found that the amount of albumin present may vary considerably in pathological conditions. In inflammatory conditions such as cerebro-spinal fever and pneumococceal meningitis it may be relatively diminished. Mott and Halliburton (1) found a considerable increase in cases of general paralysis, and they also found that in general paralysis nucleo-albumin may be present; this substance is not present in normal conditions, and these authors attribute its presence to the breaking down of Nissl bodies.

Another substance whose presence in the cerebro-spinal has been determined is cholin. It is said by Gumprecht to be present in traces even in normal conditions. Donath (2) demonstrated its presence in considerable amounts in 15 out of 18 cases of epilepsy, in 3 out of 3 cases of Jacksonian epilepsy, in 2 out of 3 cases of dementia paralytica, and in 10 out of 15 cases of tabes dorsalis; the amount which he demonstrated/

demonstrated varied between 0·021 and 0·046 per cent. Cariat (3) found cholin invariably in general paralysis although also in smaller amounts in other cases, such as alcoholic neuritis, senile dementia and organic dementia. Lecithin was also found by Donath twice in the cerebro-spinal fluid, in a case of tabes and in a case of general paralysis.

The present investigation is concerned with the application of the principles and methods of haemolysis to an examination of the properties of the cerebro-spinal fluid in various types of mental disease. The investigation includes -

1. The intraventricular fluid and the cerebro-spinal fluid
2. An examination of the cerebro-spinal fluid for the syphilitic reaction (Wassermann reaction).
3. An examination for the presence of substances which exercise an activating and inhibitory effect on the haemolytic properties of cobra venom.
4. The relative amount of globulin and substances precipitated by alcohol in cerebro-spinal fluids from various sources; and the relation of the quantity and quality of these substances to the presence of the Wassermann reaction, and to the presence of substances influencing the haemolytic action of cobra venom.

5./

5. Precautions necessary in the separation of the constituents of the cerebro-spinal fluid by filtration.

(1) THE INTRAVENTRICULAR FLUID & THE CEREBRO SPINAL FLUID

It is unlikely that the cerebro-spinal fluid is the product merely of a process of transudation from the blood or lymph vessels, because, while it contains only 0·02 per cent. of protein material the blood plasma and lymph contain 7 per cent. and 4·5 per cent. of protein material respectively. The anti-substances which are present as a rule in the blood as a result of bacterial infection cannot be demonstrated in any appreciable amount in the cerebro-spinal fluid. This holds good even in those infections which primarily involve the central nervous system. McKenzie & Martin (4) have pointed out that in cerebro-spinal fever the specific immune-body which is present in the blood cannot be detected in the spinal fluid, and McGregor (5) has shown that the same holds good as regards the specific opsonins and agglutinin in this disease. In the normal spinal fluid and in the spinal fluid in cerebro-spinal fever as far as can be made out by the usual methods of demonstrating these properties of serum, bacteriolytic and haemolytic complement are also absent. Drugs which

are/

are administered by the mouth or subcutaneously only in exceptional cases reach the spinal-fluid. The tetanus toxin which is believed to travel from the original site of infection by the perineural lymphatics, and to affect ultimately the anterior corneal cells, cannot as a rule be demonstrated in the spinal fluid. Schmorl has recently made some very interesting and important observations on certain properties of the spinal fluid. It is well known that in cases of jaundice the bile pigment is present in considerable amounts in exudates in the serous sacs, such as the peritoneal and pleural cavities. It is also present though in a distinctly lesser degree in the cerebro-spinal fluid. Schmorl (6) has found that in the majority of cases, the intraventricular fluid does not contain a trace of bile pigment even in those cases in which it is present in the spinal fluid. He records the results of the examination of eleven cases, ten adults, and one case of icterus neonatorum. In six of the adult cases the icterus had lasted from 10 to 20 days and in the others for months. In eight cases, including the child, the intraventricular fluid was absolutely clear and did not contain a trace of bile pigment; in one

case, in which the jaundice had been present for eight months, there was a faint yellow colour but no bile reaction, and in two cases the intraventricular fluid was markedly coloured, more so than the spinal fluid. In a case of diabetes Mellitus, Schmorl found in the spinal fluid a reducing substance, which by the same method was not demonstrable in the intraventricular fluid. Seven cases of general paralysis were examined for the Wassermann reaction. In each case both the blood and spinal fluid gave positive results during life. It was subsequently found that while the spinal fluid was positive in each, the intraventricular fluid tested simultaneously was positive only in one. Schmorl suggests, as a result of these observations, that the intraventricular cavities are closed off from the subarachnoid space and that the foramen of Magendie and the foramina of Luschka should be regarded as artefacts. It is the result of his observations in the jaundice cases which specially leads him to this conclusion, since he admits that in the case of general paralysis, it is possible to infer that closure of the foramina which are supposed to afford a communication between the ventricular cavities and the/

the subarachnoid space, might have been produced by the chronic inflammatory processes in the meninges. He suggests further that the presence in the intraventricular fluid of bile pigment and of the substances concerned in the production of the Wassermann reaction, is associated with degenerative changes in the choroid plexus. In the other case with bile stained intraventricular fluid, the patient died from phosphorus poisoning. It was then found that the epithelium of the choroid plexus was fatty and degenerative, and extensively desquamated. In the third case of long standing jaundice with slight yellow discolouration of the intraventricular fluid there was considerable inflammatory infiltration of the plexus with isolated patches denuded of epithelium. In the single case of general paralysis in which a positive Wassermann reaction was obtained with the intraventricular as well as with the spinal fluid, the choroid plexus presented evidence of extensive degeneration. Schmorl therefore found that in the cases where the intraventricular fluid contained no bile pigment and did not give a positive Wassermann reaction, the epithelium of the choroid plexus was/

was intact and where the fluid did contain bile pigment or substances which produced a positive Wassermann reaction, the epithelium of the choroid plexus was degenerate. He also recorded the two following cases in support of his statement that when the intraventricular fluid contains substances which circulate in the blood it is due to disease of the choroid plexus or intraventricular ependyma. In one case a gummatous mass in the brain had extended towards the ventricular cavities and the granulating process involved part of the choroid plexus which had become infiltrated and denuded of its epithelium. In this case the blood and intraventricular fluid gave a positive Wassermann reaction, while the spinal fluid gave a negative reaction. In the other case an abscess about the size of a hen's egg was situated in the right hemisphere, and was separated from the right ventricle by a layer of tissue only 2 m.m. thick. The ventricular wall in the neighbourhood of the abscess was injected and oedematous. Microscopic examination showed that the epithelium of the ependyma was partly necrotic and partly desquamated, and the sub-epithetial tissue was infiltrated with masses of polymorphonucleated cells. The choroid plexus was not affected. In this case/

case, which was not a case of syphilis, the intraventricular fluid contained a considerable amount of albumin while the spinal fluid contained only a trace. The importance of these observations is that they show the local conditions which determine the presence in the spinal and ventricular fluid of substances which circulate in the blood.

In the course of the present investigation I have had an opportunity of making a comparative examination for the Wassermann reaction with the intraventricular fluid and the spinal fluid in four cases of general paralysis. In two of these the intraventricular fluid was negative and the spinal fluid was positive. In the other two the fluid from both sources reacted positively (See Table I). In one of the latter, however, the reaction of the intraventricular fluid was weak compared with that obtained with the spinal fluid from the same case. In both cases in which the intraventricular fluid gave a positive reaction, the choroid plexus showed evidence of degeneration, whereas in the other two cases degenerative changes were not observed.

TABLE I. /

T A B L E 1.

		Doses of Complement absorbed by s.s.f.+ Ext.
Case 1.	Cerebro-Spinal Fluid	40 -
	Intraventricular fluid	40 -
Case 2.	Cerebro Spinal fluid	30.
	Intraventricular fluid	0.
Case 3.	Cerebra-Spinal fluid	30 +
	Intraventricular fluid	0.
Case 4.	Cerebro-spinal fluid	40
	Intraventricalar fluid	20.

C O N T R O L S.

		Dose of complement.	Amount of complement absorbed by 0.6 c.c. on liver extract emulsion.	Amount of complement absorbed by 0.1c.c. fluid - 0.6c.c. of saline solution.
Case 1.	Cerebro-spinal fluid	.005c.c.	.01c.c. just com. lysis.	.01c.c. marked lysis
	Intraventricular "	.005c.c.	.01c.c. " " "	.01c.c. just com. lysis
Case 2.	Cerebro-spinal fluid	.01.c.c.	.01c.c. " " "	.01c.c. complete lysis
	Intraventricular "	.01.c.c.	.01c.c. " " "	.01c.c. " "
Case 3.	Cerebro-spinal fluid	.005c.c.	.01c.c " " "	.01c.c. just com. lysis
	Intraventricular "	.005c.c.	.01c.c. " " "	.01c.c. " " "
Case 4.	Cerebro-spinal fluid	.005c.c.	.02c.c. " " "	.01c.c. " " "
	Intraventricular "	.005c.c.	.02c.c " " "	.01c.c. " " "

The cerebro-spinal fluid and the intraventricular fluid of each case was examined at the same time. Table I., Case I. shows that the cerebro-spinal fluid and the intraventricular fluid absorb the same amount of complement and are both strongly positive. In cases Nos. 2 and 3 it is seen that the cerebro-spinal fluid both absorb 30 doses of complement while in each case the intraventricular fluid absorbs none. Case 4 shows that while the cerebro-spinal fluid absorbs 40 doses of complement, the intraventricular fluid absorbs only 20 .

(2) THE WASSERMANN REACTION.

The methods of Haemolysis which now play such an important part in forensic and clinical medicine, as well as in physiological chemistry, are founded on the important discovery made by Bordet (7), in 1900, that an immune serum contains a thermostable substance (immune-body) which in the presence of the original immunising agent (blood corpuscles or bacteria) is capable of absorbing haemolytic complement; that is to say, a haemolytic or bacteriolytic serum heated at 57°C. for an hour will lose its power of lysing the homologous corpuscles or bacteria, but it will retain thermostable substances whose activity can be restored by/

by the addition of fresh non-immune serum (complement). The absorption or deviation of complement by an immune body (anti-body) in the presence of the original immunising agent (antigen) is seen when subsequently sensitised red blood corpuscles are added, that is, red blood corpuscles which have already been brought into contact with their specific inactivated immune serum, and only require the addition of complement to complete the haemolytic process. To take a concrete example:- the fresh serum of an animal immunised against the cholera vibrio, if brought into contact with the organisms produces lysis; the same serum heated for an hour at 57°C. fails to do so; if however fresh normal serum be added to the heated serum, then lysis of the organisms does occur; this absorption can be proved by the subsequent addition of sensitised red blood corpuscles which require only the complement of a fresh serum to become lysed; thus if after the addition of the sensitised red blood corpuscles no haemolysis occurs, then a corresponding antigen (cholera vibrio) and antibody must have absorbed the complement: if, on the other hand, haemolysis has occurred, there cannot have been an association of antigen with its specific immune body. The Wassermann reaction for syphilis/

syphilis consists essentially in this that haemolytic complement is absorbed by a mixture of syphilitic serum and organ extract. Wassermann, Neisser and Bruck (8) when they first made the observation believed that the reaction depended on the presence of spirochaetae in the organs from which the extracts were made acting along with homologous antibodies in the syphilitic serum; in other words, that the reaction corresponded exactly with that in the case of the cholera vibrio and its immune serum. It was, however, subsequently demonstrated by Marie and Levaditi that an extract of guinea-pig's liver could serve as "antigen", and it is now recognised that the most serviceable "antigen" is an alcoholic extract of an organ rich in lipoid substances, and the organ mostly employed is the liver. Wassermann and Plaut were the first to examine the cerebro-spinal fluid of general paralysis for a Wassermann reaction. They examined the fluid from advanced cases whose blood reacted positively, and in a certain proportion were able to demonstrate a positive result. Out of 54 samples of spinal fluid from undoubted cases 41 were positive, 5 were negative, and 8 doubtful. Neisser, Bruck and Schucht examined 8 cases and obtained a positive result in/

in 4. Marie and Levaditi got a positive reaction in 29 out of 39 cases, Morgenroth and Stertz in 8 out of 8, Michaelis in 18 out of 20, Braun in 10 out of 12, Fränkel-Heiden in 11 out of 14, Bariart, Brenton and Petit in 67 out of 72, Marie, Levaditi and Yamonouchi in 28 out of 30, Stertz in 40 out of 45, and Chandler and Henderson Smith in 59 out of 64.

In the present investigation 228 cerebro-spinal fluids have been examined for the Wassermann reaction. Three methods were used, as follows:-

I. THE ORIGINAL METHOD.

The extract employed in the present investigation was obtained by reducing ox-liver to a pulp and making a mixture of one part of organ pulp to four parts of 96% alcohol; this was allowed to stand for four days at room temperature and then filtered. For each experiment an emulsion was made up of one part of alcoholic extract to five parts of normal saline solution, and of this emulsion 0·6 c.c. was used for each tube in which emulsion was required. Most important for the purpose of determining comparative results is the estimation of the actual amount of complement absorbed by the various cerebro-spinal fluids; and the complement was in each case measured in terms of "haemolytic doses". Table (2) shows the method adopted in estimating/

ing the differences in the fluids examined.

Three series of tubes are employed: Series A, containing in each tube 0·6 of a cubic centimetre of emulsion of organ-extract; Series B, containing 0·1 of a cubic centimetre of the cerebro-spinal fluid to be tested in 0·6 of a cubic centimetre salt-solution; and Series C, containing the mixture of the test amount of organ-extract (0.6 of a cubic centimetre) and cerebro-spinal fluid (0·1 of a cubic centimetre). The complement-containing serum is then added; to Series A and B, 1, 2, 3, 4, &c., minimum haemolytic doses, and to Series C, 7, 10, 15, 20, 30, 40 doses. In every instance a preliminary estimation of the haemolytic dose of the complement is made, and this is further controlled by incubating amounts of complement in 0·6 of a cubic centimetre salt-solution along with the above series. After one and a half hours at 37°C. the test corpuscles, one cubic centimetre of a 5 per cent. suspension of washed ox-blood sensitised previously with at least five minimum doses of immune body from the rabbit, are added. The mixtures are again incubated and the result is read at the end of one and a quarter hours when the tubes are removed from the incubator. After standing overnight at room temperature/

perature the reading is again taken; both results are almost identical in every case. The accompanying table gives the details of an experiment performed according to this method, showing the comparative deviating properties of a syphilitic and a normal serum. In this experiment the amount of complement necessary to lyse one cubic centimetre of sensitised ox-blood corpuscles was 0·01 cubic centimetre.

Series A shows that, whereas 0·01 cubic centimetre of complement is sufficient to lyse 1 cubic centimetre of sensitised ox red blood corpuscles, 0·02 cubic centimetre of complement is necessary when 0·6 cubic centimetre of alcoholic extract of liver is present; that is to say, 0·6 cubic centimetre of the emulsion of organ-extract is by itself able to absorb one dose of haemolytic complement.

Series B shows that as regards power of complement absorption the two fluids are alike, each being able to absorb one dose of haemolytic complement.

Series C shows that the fluid from the general paralytic in presence of the liver extract has absorbed over 40 doses of complement, whereas that from the case of epilepsy with the same extract has not been able to absorb seven.

TABLE 2.

Showing the comparative Deviating Properties of a positive and a negative Serum.

Series A.	(1)	(2)	(3)	(4)		
Emulsion of extract of liver. . . .	0·6	0·6	0·6	0·6		
Complement.	0·01	0·02	0·03	0·04		
Sensitised ox red blood corpuscles (added after 1½ hours).	1·0	1·0	1·0	1·0		
Series B.	(5)	(6)	(7)	(8)		
Cerebro-spinal fluid (general paralysis) (in 0·6 c.c. normal saline)..	0·1	0·1	0·1	0·1		
Complement.	0·01	0·02	0·03	0·04		
Sensitised ox red blood corpuscles (added after 1½ hours).	1·0	1·0	1·0	1·0		
	(9)	(10)	(11)	(12)		
Cerebro-spinal fluid (epilepsy) (in 0·6 c.c. normal saline) . . .	0·1	0·1	0·1	0·1		
Complement	0·01	0·02	0·03	0·04		
Sensitised ox red blood corpuscles (added after 1½ hours).	1·0	1·0	1·0	1·0		
Series C.	(13)	(14)	(15)	(16)	(17)	(18)
Liver extract emulsion	0·6	0·6	0·6	0·6	0·6	0·6
Cerebro-spinal fluid (general paralysis).	0·1	0·1	0·1	0·1	0·1	0·1
Complement.	0·07	0·1	0·15	0·2	0·3	0·4

TABLE 2. (Contd.)

Sensitised ox red blood corpuscles (added after 1½ hours).	1·0	1·0	1·0	1·0	1·0	1·0
	(19)	(20)	(21)	(22)	(23)	(24)
Liver extract emulsion.	0·6	0·6	0·6	0·6	0·6	0·6
Cerebro-spinal fluid (epilepsy) . .	0·1	0·1	0·1	0·1	0·1	0·1
Complement.	0·07	0·1	0·15	0·2	0·3	0·4
Sensitised ox red blood corpuscles (added after 1½ hours).	1·0	1·0	1·0	1·0	1·0	1·0

Red lines mean that lysis has occurred in this tube.

Following this method sixty cases of general paralysis and 30 cases of mental disease, other than general paralysis, were examined. The reaction was considered positive when lysis was incomplete with five haemolytic doses of complement in addition to the sum of the amounts inhibited by cerebro-spinal fluid and by emulsion alone. With regard to the results of the experiments it was found that 52 out of the 60 cases of general paralysis reacted positively, while the remaining 8 were negative.

In/

In none of the 30 other cases was there a positive reaction. As regards the intensity of the reaction, as measured in amounts of complement absorbed, it was found that less complement was on the whole absorbed by fluid from early cases than by fluid from late cases.

METHOD NO. 2. is in reality only a modification of the above.

In the method just described an emulsion was made with ox-liver extract and 0·85 per cent. sodium chloride solution and of this 0·6 c.c. was put into each tube and subsequently 0·1 c.c. of cerebro-spinal fluid was added. In the present method the emulsion is made as follows:- three and a half cubic centimetres of fluid to be examined are poured into a test tube (4 in. by ½ in.). Half a cubic centimetre of the extract of ox-liver is carefully pipetted on to the surface of the fluid and an emulsion made by slowly rotating the tube. Five tubes are then taken and 0·5 c.c. of the emulsion is placed in each. Increasing amounts of complement are then added and the mixture is incubated for an hour and a half at 37°C. The sensitised ox corpuscles (1 c.c.) are then added, and/

and after a further incubation for an hour and a quarter at 37°C. the tubes are read. A control experiment is made to estimate the amount of complement absorbed by the spinal fluid alone; To two tubes each containing .5 c.c. of cerebro-spinal fluid 0·01 c.c. and 0·02 c.c. of complement is added and the tubes treated in the same way as the above. The amount of complement absorbed by the extract alone is estimated by making the emulsion with ox-liver and normal saline solution instead of spinal fluid in the proportion of one to seven.

T A B L E 3.

Wassermann Reaction done Method 1.

	0.6 Emulsion of organ ex. 0.1 cerebro-spinal fluid. Complement. 0.025,0.05,0.075,0.1,0.15					0.1 cerebro-spinal fl. 0.5 Nacl. Complement. 0.01,0.02.		0.6 Emulsion of organ ext. Complement. 0.01,0.02,0.03.		
Gen.Paralysis.	0	0	0	0	0	0	c	m	c	c
" "	0	0	c	c	c	m	c	t	c	c
" "	0	c	c	c	c	0	c	0	c	c
" "	0	0	o	o	o	c	c	ac	c	c
Dementia Praecox	c	c	c	c	c	ac	c	ac	c	c
" "	ac	c	c	c	c	c	c	jc	c	c
Epilepsy	c	c	c	c	c	t	c	c	c	c
Tubercular Meningitis	c	c	c	c	c	c	c	ac	c	c

T A B L E 4.

Wassermann Reaction done on same fluids on the same day as above.

	0.5 Emulsion made with 7 cerebro-spinal fluid to 1cc.organ extract. Complement 0.025,0.05,0.075,0.1,0.2.					.5 cerebro spinal fluid. Complement .01,.02.		0.5 Emulsion of organ ext. in normal saline in pro.1 to 7. Complement. 0.01,0.02,0.03,		
Gen. Paralysis	0	0	0	0	0	c	c	m	c	c
" "	0	0	0	0	0	c	c	t	c	c
" "	0	0	0	0	c	c	c	t	c	c
" "	0	0	0	0	0	c	c	ac	c	c
Dementia Praecox	c	c	c	c	c	c	c	jc	c	c
" "	c	c	c	c	c	c	c	jc	c	c
Epilepsy	c	c	c	c	c	c	c	c	c	c
Tubercular Meningitis	c	c	c	c	c	c	c	ac	c	c

Dose of complement .01cc.

c - complete.
ac - almost complete
m - marked
t - trace
jc- just complete
0 - no lysis has occurred in these tubes.

Following this method thirty cases of general paralysis and twenty of mental cases other than general paralysis were examined. This method was found to be more satisfactory than the first method described. It was found that a positive result was obtained in the whole of the 30 cases of general paralysis examined, which included two that had a negative reaction with the first method, while the remaining 20 cases, other than general paralysis gave a negative reaction. Included in those 20 cases were two cases of tubercular meningitis with a large amount of globulin and notwithstanding that they gave a negative reaction.

METHOD NO. 3. The Lecithin, Lecithin-cholesterin method (Browning, Cruickshank and McKenzie).

The organ extract employed in the above two methods was prepared by reducing ox-liver to a pulp and making a mixture of one part of organ pulp to four parts of 96 per cent alcohol, but the extract originally employed by the discoverers of the reaction was prepared by macerating the liver from a case of congenital syphilis in salt solution, and it was considered that in this way an extract containing the specific receptors of the spirochetae pallida was obtained/

obtained (Antigen), which combined with corresponding antibodies in the syphilitic serum and caused fixation of complement. Porges and Meier 1907, 1908 (10); Landsteiner, Müller, and Pötzl, 1907, 1908 (11); Levaditi and Yamanouchi 1907 (12), found that extract of normal as well as syphilitic tissues had the property of deviating complement in the presence of syphilitic serum. The fact that the "antigenic" property is possessed by alcoholic extracts led to the supposition that the active agents are of lipoid nature, and a large number of lipoids and allied compounds, more or less pure chemically, have been investigated.

Browning, Cruickshank and McKenzie (9) described a new method of performing the Wassermann reaction. It depends on the fact that a syphilitic serum in the presence of an emulsion of lecithin and cholesterin absorbs more complement than in the presence of an equivalent amount of an emulsion of lecithin alone, whereas a non-syphilitic serum absorbs equal amounts of complement with both emulsions.

The lecithin, which is easily obtained from an alcoholic extract of fresh ox-liver, is dissolved in sufficient absolute alcohol to make a 0·75 per cent/

cent. solution. Saturation of this with cholesterin gives the lecithin-cholesterin solution.

The lecithin emulsion is prepared by adding one part of the 0·75 per cent. solution to seven parts of a 0·85 per cent. Na cl solution. It has been definitely shown by Sachs and Roudoni (13) and confirmed by Browning and McKenzie (14) that the more turbid the emulsion is, the better is the reaction with a syphilitic serum.

In carrying out the Wassermann reaction by the lecithin-cholesterin method, the technique employed was similar to that described in Method II. Two test tubes (4 in. by $\frac{1}{2}$ in) are taken and into each there are poured three and a half cubic centimetres of spinal fluid. Half a cubic centimetre of alcoholic lecithin solution is poured carefully with a pipette on to the surface of one, and an equal amount of the lecithin-cholesterin solution is poured on to the surface of the other; the fluids are mixed slowly by rotating the tubes. Two series of tubes are now taken, and half a cubic centimetre of the lecithin and spinal fluid mixture is placed in each tube of the one series, and half a cubic centimetre of the lecithin-cholesterin and spinal fluid mixture is placed/

placed in each tube of the second series. Increasing amounts of complement are put into each series, and the mixture is incubated for an hour and a half at 37°C The sensitised corpuscles (1 c.c.) are then added and after a further incubation for one hour and a quarter at 37°C. the results are read. If the amount of complement absorbed by the lecithin-cholesterin mixture and cerebro-spinal fluid be greater than that absorbed by the mixture of lecithin alone and cerebro-spinal fluid then the result is positive.

It will be seen that by this method, almost half a cubic centimetre of spinal fluid is used for each tube in the test. A control experiment is made to estimate the amount of complement absorbed by the spinal fluid alone. The amount of complement absorbed by the lecithin and the lecithin-cholesterin emulsion respectively in the proportion used in the test is also estimated. These control mixtures of lecithin and lecithin-cholesterin are prepared by using normal saline solution instead of spinal fluid in the proportion of one to seven. The spinal fluid should be tested as soon as possible after withdrawal, it should be centrifugalised to free it of its cellular elements, and it should not be heated before use. This method gives the most/

most satisfactory results. By adopting it the fallacies which arise as a result of variations in the individual properties of the extracts and haemolytic complement are obviated. There is no disadvantage in using the large amount of spinal fluid required.

T A B L E 5.

Shows the result of an experiment by the Lecithin - Cholesterin method.

	Lecithin-cholesterin - cerebro-spinal fluid (1.7)						Lecithin & cerebro-spinal fluid (1.7)					
Complement	.02cc,	.04cc,	.06cc,	.08cc,	.1cc,	.15cc.	.02cc,	.04cc,	.06cc,	.08cc,	.1cc,	.15cc.
General Paralysis.	o	o	O	o	o	T	o	o	T	c	c	c
Dementia Praecox	c	c	c	c	c	c	c	c	c	c	c	c

Controls

Lecithin-Cholesterin & Nacl solution (1.7)			Lecithin Nacl Solution (.7)		
Complement	.01cc,	.02cc, .03cc.	Complement	.01cc,	.02cc, .03cc
m	c	c	m	c	c

General paralysis, Cerebro-spinal fluid .5 c.c.		Dementia praecox Cerebro-spinal fluid .5c.c.	
Complement	.01c.c. .02c.c.	Complement	.01c.c. .02c.c.
c	c	c	c

Dose of complement .005cc.

The cerebro-spinal fluid of forty five cases of general paralysis at various stages has been examined by this method, and in every case the result has been positive. Twenty control cases, including fluids from cases of tubercular meningitis, cerebro-spinal fever, epilepsy, and dementia praecox, have also been examined, and in every case a negative result was obtained. Among the non syphilitic fluids examined by this method were two cases of cerebro-spinal meningitis and one of tubercular meningitis; each of which contained a considerable amount of protein material and their unheated fluids gave absolutely negative results.

This method of examining the fluids should be applied in cases of locomotor ataxy where the substances which produce a Wassermann reaction would seem to be present in greater dilution than in the fluid from general paralysis. I have not had the opportunity of examining the fluids from cases of locomotor ataxy since the introduction of this method, but McKenzie in an earlier series of cases, out of six samples of spinal fluid from patients suffering from this disease, found that only two reacted positively. In these cases only 0.1 c.c. of spinal fluid was used for each tube. The relation of the occurrence of the Wassermann reaction and

of/

of its intensity when present to constituents of the cerebro-spinal fluid will be referred to later.

THE SIGNIFICENCE OF THE WASSERMANN REACTION IN NERVOUS DISEASES.

If, in a nervous disease a positive reaction be obtained with the blood serum, the disease may be syphilitic in character or it may be a non-syphilitic disease occurring in the later stages of syphilis or concomitantly with a syphilitic lesion outside the nervous system. If in addition to a positive blood reaction, the cerebro-spinal fluid is positive, then one is justified in diagnosing a syphilitic affection of the central nervous system. The nature of the syphilitic affection must be determined by further clinical evidence although speaking generally, it is only in general paralysis and locomotor ataxy that the spinal fluid, as a rule, gives a positive Wassermann reaction. Some differences of opinion exist as to whether the reaction with the spinal fluid is more intense than it is with the blood serum from the same case, Marie, Levaditi and Yamanouchi, also Raviart, Breton and Petit contend that in general paralysis the reaction is more marked than with the spinal fluid. Plaut and Boas on the other hand hold the opposite view. To decide this question is difficult. The blood/

blood serum must be heated at 57°C for half an hour to deprive it of the deviating properties, which are sometimes present in the sera of non-syphilitics. It is doubtful whether such heating produces a fluid which is exactly comparable with the spinal fluid. A series of cases has been examined estimating in terms of haemolytic doses the comparative amounts absorbed by the spinal fluid and the heated serum from the same cases. The results of such examination showed that in some cases the spinal fluid, and in others the blood serum produced the greater deviation. In two cases Gilmour found that the spinal fluid gave a positive result whereas the blood serum after half an hour's exposure to a temperature of 57°C. gave a negative result. It is so far impossible to say where the substances which give a positive reaction are formed. It may be that they are formed in various parts of the body and only present themselves in the cerebro-spinal fluid when there is some anatomical lesion in the structures immediately surrounding the Thecal space, or it may be that when there is a syphilitic lesion in these structures that the substances are formed locally and pass directly from the local lesion into the surrounding spinal fluid.

SPECIAL POINTS TO BE NOTED IN CARRYING OUT THE WASSER: :MANN REACTION.

In carrying out the test attention should be paid to the following - (1) The Haemolytic System, (2) The Serum Complement, (3) The Cerebro-Spinal Fluid to be tested, (4) The tissue extract or "antigen".

(1) THE HAEMOLYTIC SYSTEM. The haemolytic system used is that in which ox corpuscles are sensitised with immune serum of the rabbit, fresh guinea-pig serum being used as complement. The ox blood is whipped to remove the fibrin and 5 c.c. of the defibrinated blood is washed with 10 to 15 c.c. of a 0·85 per cent. sodium chloride solution and centrifugalised; the supernatent fluid is then pipetted off and saline again added. This is repeated three times and then 3 c.c. of the corpuscles is made up to 100 c.c. with 0·85 per cent. Sodium chloride solution, five minimum haemolytic doses of immune body for each cubic centimetre of blood is than added.

The immune body is the serum of a rabbit which has received several intraperitoneal injections of washed ox-blood, 4, 5, and 6 c.c. injected at intervals of a week usually yields 10 days after the last injection a serum of which 0·001 c.c. is the minimum haemolytic dose, that is, 0·001 c.c. of the treated rabbit's serum is/

is sufficient to lyse 1 c.c. of a 5 per cent. suspension of ox-blood corpuscles after 1 hour at 37°C, in the presence of excess of complement. It has been found that the minimum haemolytic dose of the same immune serum may vary slightly for different samples of ox-corpuscles so when estimating the dose the serum should be tested against ox-corpuscles from five different sources and an average taken: it should be tested when the rabbit is bled and a subsequent examination made a week later. The average minimum haemolytic dose of the immune serum where the reaction has been successful is about 0·001 c.c. It is then sealed in tubes and heated at 57°C. for 1 hour on 3 or 4 successive days to sterilize it and destroy the complement.

THE SERUM COMPLEMENT.

The complement is contained in the fresh serum of the guinea-pig. It should be used within twenty-four hours of killing the animal as after standing for upwards of twenty-four hours in the ice chest, although there is little or no diminution in the amount of haemolytic dose, the complement becomes less deviable, both by/

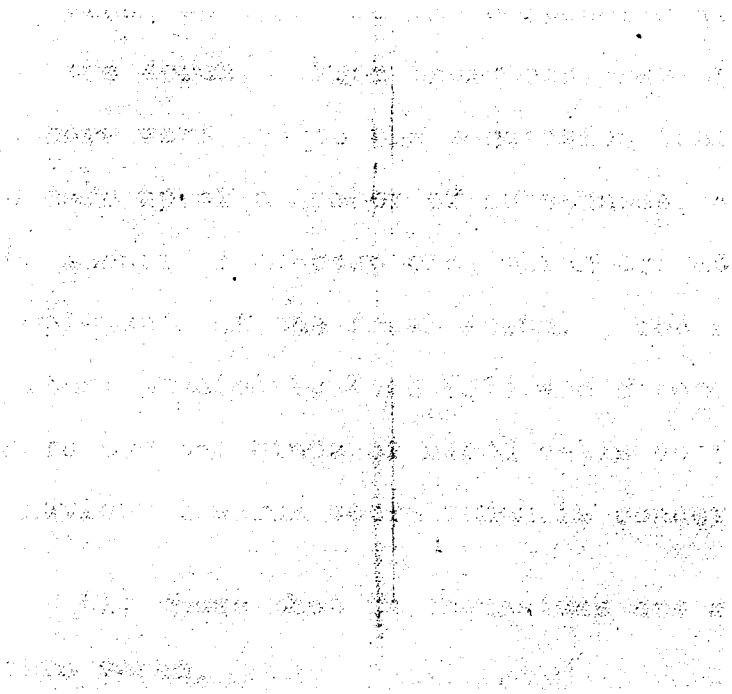
by the emulsion of organ extract alone, and by the emulsion along with syphilitic cerebro-spinal fluid. It is difficult to find a satisfactory explanation for such individual variations in complements. The possibility of conversion of complement into complementoid on standing naturally suggests itself, but the fact that there is not a corresponding fall in haemolytic value makes such an explanation improbable. It might also be supposed that when a complement was only slightly absorbed by an organ extract or a mixture of organ extract and syphilitic cerebro-spinal fluid this was due to the presence in the guinea-pig's serum of a natural immune body for ox corpuscles which was specially suited to act in conjunction with the guinea-pig complement. Browning & McKenzie have, however, shown that that is not so.

(3) THE CEREBRO-SPINAL FLUID should be tested immediately after withdrawal as on standing it tends to lose its power of giving the syphilitic reaction.

(4) <u>THE TISSUE EXTRACT</u>.

When an alcoholic extract of liver is used care should be taken that it is not lytic for the test corpuscles in the amount used. If it were lytic it would be/

be impossible to test the anticomplement effedt which it has by itself and thus one of the necessary controls in the test could not be made. When the suitable reagents can be procured the lecithin and lecithin-cholesterin form of "antigen" should be employed.



(3). COBRA VENOM REACTION.

In 1902 Flexner and Noguchi (16) published the results of a series of experiemnts on the nature of the haemolytic properties of cobra venom. These authors found that although red blood corpuscles whose serum has been completely removed by washing with salt solution are agglutinated by snake venom, they are not dissolved. Haemolysis, however, occurs if serum be added to the corpuscles and venom, or if the venom be added to the corpuscles without washing out the serum. From these observations, these authors were led to the conclusion that snake venom is made up of a number of substances, acting after the manner of amboceptors, which are activated by the complements of the fresh serum. The subject was further studied by Kyes (17) who discovered that there are two kinds of blood cells so far as their behaviour towards cobra venom is concerned:-

(1) Those that in themselves are destroyed by cobra venom.

(2) Those that are lysed by cobra venom only after the addition of other substances (complements etc.)

Those belonging to the first class are the red/

red blood cells of the guinea-pig, dog, rabbit and man, while the second class is represented by the corpuscles of the ox, sheep and goat. It was further determined that certain sera which activate cobra venom lose this property when heated for an hour at 57°C.; this applies for example to guinea-pig serum when placed along with cobra venom and ox corpuscles; from this it has been concluded that the activation is due to serum complement in the restricted sense of the term. Other sera, however, e.g. human serum, do not lose their activating power when heated at 57°C., and by extraction with alcohol it was found that the lecithin in blood serum possesses strong activating properties; it was thus concluded that lecithin is probably the substance in such sera which produces the activation; and in the case of blood corpuscles which are lysed without the addition of serum it was suggested that a "disponible lecithin" in the blood corpuscles commenced the activation, and that it was completed by the lecithin set free from the degenerating stromata. Another advance in the study of the subject was accomplished by Kyes and Sachs when they discovered that as regards their effect on the haemolytic action of cobra venom lecithin and cholesterin possess antagonstic properties, the activating power of lecithin being/

being inhibited by cholesterin; and again in the case of an alcoholic extract of blood serum it was found that while the alcohol extracted activating substances, the precipitate possessed inhibitory properties.

The application of the principles involved in these experiemnts to the examination of the cerebro-spinal fluid were carried out as follows:-

Ox corpuscles were washed free of the serum by repeated centrifugalising with normal saline solution; 3 c.c. of washed sediment were made up to 100 c.c.with normal saline, this giving approximately a 5% suspension of ox red blood corpuscles; to the corpuscles a solution of cobra venom in the proportion of 1 c.c. of a 1 : 1000 solution to 10 c.c.of blood suspension was added; this suspension was used as a reagent for the detention of activating substances in the cerebro-spinal fluid. In the first place fresh cerebro-spinal fluid was added to the corpuscles and the mixture incubated at 37°C. for 2 hours and the results examined on the following day. On no occasion, however, was it possible to demonstrate the presence of activating substances in the fresh fluid. This applies to specimens of cerebro-spinal fluid from cases of general/

general paralysis, dementia praecox, cerebro-spinal meningitis, epilepsy etc. The fluids were, in the second place, extracted with alcohol, in the proportion of one part of fluid to four parts of alcohol (96%). This was allowed to stand at room temperature for 3 or 4 days. The amount of the precepitate was registered in each case; this will be referred to later on. The precipitate was allowed to settle or the mixture was centrifugalised and the extract pipetted off. The alcoholic extract was then diluted with normal saline in the proportion of one part of extract to four parts of normal saline; the lytic power of the mixture on ox corpuscles sensitised with cobra venom was then tested. It was found that the mixture thus prepared had no activating effect on the cobra venom. On filtering with filter paper (No.595 Schleicher and Schulz) slight activating properties manifested themselves with comparatively large doses of the mixture. If, however, the mixtures were first boiled and then filtered, the activating properties were demonstrable with comparatively small doses. Table (6) shows an experiemnt of this kind:-

T A B L E 6.

Mixture made without heating or filtering alcoholic extract.

Cerebro-Spinal Fluid.	Alcohol ppt.	*Amount of Extract which activated cobra venom.				
		0.3cc.	0.5cc.	0.75cc.	1cc.	1.5cc.
No. 6	0.1	0	0	0	0	0
" 7	0.1	0	0	0	0	0
" 8	1.4	0	0	0	0	0
" 9	1.2	0	0	0	0	0
Mixture made without heating but after filtering alcoholic Extract.						
No. 6	0.1	0	0	0	m	c
" 7	0.1	0	0	T	ac	c
" 8	1.4	0	0	0	0	c
" 9	1.2	0	0	T	c	c
Mixture made after both heating and filtering alcoholic Extract.						
No. 6	0.1	0	c	c	c	c
" 7	0.1	0	c	c	c	c
" 8	1.4	0	c	c	c	c
" 9	1.2	c	c	c	c	c

Cases 6 and 7 were dementia precox.

Cases 8 and 9 were general paralytics.

* In the various Tables the extent of haemolysis is designated by the following signs:-

0 = no lysis.
T = trace of lysis.
m = marked lysis.
ac. = almost complete lysis.
c. = complete lysis.

Here the four fluids are examined under different conditions. In the first series no lysis occurs; in the second series, where the extracts had been filtered there was lysis with the larger doses whereas where the extracts were heated before filtering the lytic dose is much smaller. A considerable variation is noticeable in the amount of lytic substance present in each case, and it is also evident that the amount of lytic substance does not bear any relation to the amount of precipate present in the original mixture of cerebro-spinal fluid and alcohol. It was also obvious from an examination of a large number of cases that the fluids from cases of general paralysis did not, on the whole, contain these activating substances in greater amount than the fluids from other cases of mental disease. The extent to which the process of filtration affects the result will be referred to later. The filter paper which was used in separating the extract does undoubtedly contain substances which have haemolytic properties, but not in sufficient amount to account for the results of the experiments referred to.

Experiments were also performed with a view to demonstrating the presence of inhibitory substances in the cerebro/

cerebro-spinal fluid. The method adopted was as follows:-

It was found that the emulsion made from alcoholic extract of ox liver and used for carrying out the Wassermann reaction, possessed an activating power for cobra venom with ox corpuscles in a dosage of 0·075 c.c. for 1 c.c. of sensitised ox corpuscles. To a series of tubes, increasing doses of fresh cerebro-spinal fluid were added (0·25, 0·5, 0·75, 1 c.c.), and to each tube was added 0·1 c.c. of emulsion. This was allowed to incubate at 37°C. for an hour and then the ox corpuscles sensitised with cobra venom were added, and the mixture again allowed to incubate for two hours. It was found that in few cases inhibition did occur; these, however, were cases in which there was a considerable amount of cellular elements in the fluid, and the inhibitory effect disappeared on centrifugalising. The mixture of alcoholic extract of cerebro-spinal fluid and saline did not exhibit inhibitory properties, nor did the precipitate formed by adding alcohol to the cerebro-spinal fluid. It must, however, be concluded from the results of experiments of which that shown on Table VI. is an example, that a mixture of cerebro-spinal fluid/

fluid and alcohol does contain both activating and inhibitory substances; the activating substances are more manifest after heating and filtration, and it is probable that in the process of filtration absorption of inhabitory substances takes place, thus leaving the activating substances free.

4. CHEMICAL EXAMINATION.

It has long been recognised that the cerebro-spinal fluid in cases of general paralysis contains an abnormal amount of proteid substance. To this increase in proteid content considerable importance has been attached from the diagnostic point of view, and various methods have been devised for determining the presence or absence of this condition. Nonne and Apelt introduced the method of precipitation by means of half saturation with ammonium sulphate. This procedure gives an indication of the globulin content of the spinal fluid, although it fails to differentiate between an increase in globulin due to general paralysis and an increase due to other diseases such as tubercular meningitis.
Noguchi (15) has also introduced a procedure which gives very delicate results, so far as indicating the presence/

presence of protein material is concerned. According to this method, one or two parts of the spinal fluid are mixed with five parts of a 10 per cent dilution of butyric acid in physiological salt solution, and the mixture is heated and boiled for a few seconds over a flame. One part of normal Na O H. Solution is then added quickly to the heated mixture and the whole mixture is again boiled for a few seconds. The actual quantities recommended and used in the present investigation are 0.2 ccm. of spinal fluid, 0.5 ccm. of butyric acid solution and 0.1 ccm. of Normal Na.O.H. solution. It is necessary to have the spinal fluid absolutely free from blood. The presence of an increased protein content of the spinal fluid is indicated by the occurrence of a granular or floccular precipitate which gradually settles on standing. The rapidity with which the precipitate falls is proportional to the amount of protein present, and Noguchi considers that a positive result has been obtained when the precipitate settles within two hours. This method, however, simply gives an indication of the protein contents of the fluid examined and does not afford a basis for differentiation between/

between the various conditions in which a high protein content of the spinal fluid is present. Noguchi himself does not claim any more for the method.

In the present investigation 96 per cent alcohol was used as the precipitant in the proportion of one volume of fluid to one of alcohol. An arbitary standard was made by taking the turbidity of a volume of fluid of an advanced case of general paralysis in an equal volume of alcohol as 1, and with this as the standard the turbidity of the others was compared and measured. It was found that although by this method the lipoids remain in solution, still comparing the results with the precipitates from the butyric acid and ammonium sulphate, the precipitates with alcohol were generally more dense; in a series of cases, however, the comparative results were parallel; and the examination of the alcoholic extracts of the fluids showed that lipoids were present only in small amounts even in those cases where there was an abundant alcoholic precipitate. The amount of lipoids present were estimated by their effect on cobra venom, and the experiments carried out as described in the preceding chapter. The Wassermann reaction was also done in all the cases and its intensity measured by the amount of haemolytic doses absorbed. The intensity/

sity was found to be independent of the precipitable substances and also independent of the lipoids present as measured by the degree of activation with cobra-venom. The cases examined included:
Cases without mental disease or meningitis, general paralysis early and late, toxic stupor, dementia praecox in the acute and terminal phases, dementia, tubercular meningitis, cerebro-spinal meningitis, epilepsy one of the latter being examined during the seizures when he was in the state of status epilepticus and again in the quiescent stage.

It was found that a great variation existed in the amount of precipitable substances with the alcoholic precipitant in the different cerebro-spinal fluids. Table (7) shows the results of a series of cases examined on the same day. These figures, representing the varying amounts of precipitate in different mixtures, do not represent absolute amounts, only the amount of precipitate in one case compared with the amount in another.

TABLE 7.

Cerebro-spinal fluid.	Alcohol precipitate.	Wassermann reaction, doses of complement absorbed.	Amount of emulsion which activated cobra venom.
No. 21	1.0	30	1 c.c.
" 22	1.4	40-	0.75 "
" 23	0.8	10	0.75 "
" 24	1.0	20	1 "
" 25	0.6	10	0.75 "
" 26	1.2	10	0.75 "
" 27	0.8	7	0.75 "
" 28	0.8	24	0.5 "
" 29	1.2	12	0.5 "
" 30	0.3	30	0.5 "
" 31	0.8	12	0.75 "
" 32	0.2	40	0.6 "
" 33	1.0	24	0.75 "
" 34	0.4	40	0.3 "
" 35	0.2	0	0.75 "
" 36	0.1	0	0.75 "
" 37	0.2	0	0.5 "
" 38	0.2	0	0.75 "
" 39	0.2	0.	1 "
" 40	1.0	0	0.75 "

Cases Nos. 21-34 suffered from general paralysis, while Nos. 35-39 were cases of dementia praecox and melancholia. All these fluids were examined on the same day.

Cases without mental disease or meningitis showed only a very faint opalescence and the case of chronic dementia showed practically no more turbidity than those that had no evidence of mental or meningeal trouble.

On reviewing the results recorded in this table, it is obvious that a great variation exists in the amount of precipitable substances in different cerebro-spinal fluids. On the whole the precipitate is more abundant in cases of general paralysis although there are instances, e.g., No. 40, where the amount of precipitate was considerable, yet the Wassermann reaction was negative.

When one compares the amounts of precipitate in the various cases with the intensity of the Wassermann reaction, as estimated by the number of doses of haemolytic complement which has been absorbed, one finds that there is no direct relationship. A fluid such as No. 26 yields a precipitate with alcohol represented by 1.2 and in the Wassermann reaction absorbs ten doses of haemolytic complement, whereas No. 34 yields a precipitate represented by .4, and in the Wassermann test deviates over forty doses of complement. Again, Nos. 27 and 28 contain each an amount of precipitable substance represented/

represented by 0.8; while in the Wassermann test No. 27 absorbs seven doses of complement and No. 28 absorbs twenty-four doses. There is thus no relationship between the intensity of the Wassermann reaction and the fluid content of substances precipitable by alcohol.

Table 7 shows, further, a comparison of the content in precipitable substances with the activating power of the alcoholic extract of the various serebro-spinal fluids, and also a comparison of the intensity of the Wassermann reaction with the activating power of the same extracts. It is obvious here also that no relationship exists between these phenomena. Cases 22 and 23 deviate forty and ten doses of complement respectively, yet when extracts of these fluids are made their activating effect for cobra-venom is exactly the same, viz., 0.75 c.c.; and with regard to the relationship of the amount of precipitate to the activating power of the alcoholic extract, Case 21, with a precipitate represented by 1.0, has an activating power in doses of 1 c.c.; while Case 29, with a precipitate of 1.2, has activating power in doses of 0.5 c.c.; and Case 34, with a precipitate of 0.4, has an activating power in doses of 0.3 c.c. With regard to Cases 35 to 40, the same variation with absence of relationship between the phenomena compared seems to exist.

The/

The Wassermann reaction is negative in all these cases; there is a variation in precipitable content between 0.1 and 1.0, and in the activating power of their alcoholic extracts between 0.5 c.c. and 1.0 c.c.; Cases 38 and 40 have the same activating dose of cobra venom, still the former has an alcoholic precipitate represented by 0.2, while the latter has a precipitate represented by 1.0. The result which transpires from the experiments just described is that the cerebrospinalnfluids from general paralytics can be distinguished from that of other cases by a positive Wassermann reaction. The fluids which give a positive Wassermann reaction have a high content of substances precipitable by alcohol, but there is no relationship between the intensity of the Wassermann reaction and the amount of this content; there is, further, no relationship between the amount of the precipitable content, the intensity of the Wassermann reaction, and the presence of extractable substances which activate cobra venom.

Cases of Dementia Praecox in the acute phases showed a precipitate ranging from .4 to 1.6., gave a negative Wassermann reaction, and showed the same want of cpnnection between the precipitate and the activating power of/

of cobra venom.

Cases of Toxic Stupor and Epilepsy showed the same phenomenon and in the latter disease it was found that not only was there a considerable difference in the amount of alcoholic precipitate in various cases but in one case which was examined during the seizures when he was bordering on the state of Status Epilepticus he had an alcoholic precipitate represented by .8 in our arbitary scale and in the quiescent state ten days after his last fit he was again punctured and it was found that the amount of precipitate had fallen from .8 to .1., thus leading one to suspect that during the acute stages there is a definite anatomical and chemical process going on which varies with the acuteness of the symptoms manifested by the patient. This conclusion is supported on looking over the results of the large number of cases examined. In acute diseases such as general paralysis, catatonia, and cerebro-spinal meningitis a large precipitate with alcohol is obtained, whereas in chronic dementia, dementia praecox in its terminal stages and also in cases where there is no mental disease or meningitis, the amount diminishes almost to vanishing point.

In/

In the second place the protein content of the various fluids was estimated by Noguchi's method described above, a positive result was recorded when a coarse granular or floculent precipitate appeared; when the precipitate was only in the form of a slight uniform opalescence the result was regarded as negative. At the same time the Wassermann reaction was done, the alcoholic precipitation was recorded, the precipitation of Nonne and Apelt and the whole compared and contrasted.

Table 8 shows the results of such an experiment, all the fluids being examined at the same time.

TABLE 8.

Cerebro-spinal fluid	Wassermann reaction	Boiling and dilute acetic acid precipitate	Half satur'd ammon. sulph. precipitate.	Noguchi's test.	Alcohol precipitate
No. 50	-	trace	1·4	+	1·2
" 51	-	0	0	-	0·1
" 52	-	0	0	-	0·1
" 53	-	0	0·1	-	0·2
" 54	+	0	0·1	-	0·2
" 55	+	trace	0·6	+	0·8
" 56	+	trace	0·6	+	0·8
" 57	+	0	0·3	+	0·4
" 58	+	0	0·1	-	0·2
" 59	+	trace	1·2	+	1·6
" 60	-	0	0·1	-	0·2
" 61	-	trace	1·2	+	1·4

In Table 8 the Noguchi test for protein content is compared in the first instance with the Wassermann reaction. No 50 which gives a negative Wassermann reaction, is distinctly positive to the Noguchi test.

This was a particularly interesting and instructive case. He was 31 years of age and was said to have enjoyed good health, mentally and physically, till about a year prior to admission. He was regarded as a dementia praecox of the acute catatonic type: dull, apathetic, refused his food, showed stereotypty, and had diarrhoea with very foul smelling stools. His cerebro-spinal fluid gave a negative Wassermann, but a positive Noguchi test. A precipitate with alcohol represented by 1·2 and positive Nonne and Apelt represented by 1·4 on our arbitary scale, but it is to be noted at the same time that on boiling and acidulating with dilute acetic acid there was a slight cloudiness present showing that the fluid in this case contained albumen. Albumose was also present. He remained in this state of acute catatonia for some months during which time he lost flesh and became quite emeciated. The change came suddenly; the catatonia disappeared, he began to take his food and in a very short time ate ravinously and became very stout. He, however/

however, is demented and though quiet as a rule yet is subject to impulsive attacks. His cerebro-spinal fluid was again examined six months after the improvement set in with the following result. Wassermann reaction negative, precipitate with alcohol 0·2, precipitate with ammonium sulphate 0.1, Noguchi test negative, no albumen, and no albumose.

CASE 61., was also very interesting. It was that of a man aged forty-two years whose illness was of six weeks' duration. He was regarded as a case of toxic stupor. He too showed a tendency to catatonia, refused his food and like the case mentioned above had very foul smelling stools. There was a history of syphilis 20 years ago, but his cerebro-spinal fluid gave a negative Wassermann, a positive Noguchi test, and cloudiness on boiling and acidulating with dilute acetic acid. Albumose was however absent in this case. Nos. 54 to 59 give a positive syphilitic reaction, while two out of the same six give a negative butyric acid reaction, the remaining four being positive. The same spinal fluids were boiled and acidulated with dilute acetic acid; Nos. 50, 55, 56, 59 and 61 showed a trace of precipitate, the others did not. The globulin content of the fluids was then determined by mixing equal parts of spinal fluid and saturated ammonium sulphate/

sulphate. It is noticeable that the precipitation obtained by this method is strictly comparable with that observed with the Noguchi test. The results of the precipitation by ammonium sulphate as detailed in the table is based on the standard used for estimating the amount of precipitation by alcohol; this is also detailed in the last column in Table 8. It will be seen on comparison that there is a very definite correspondence between the butyric acid precipitation and that of ammonium sulphate, and also that by alcohol. On the whole the density of the precipitate is greater with alcohol than with ammonium sulphate; an exception to this is seen in case 50 where the ammonium sulphate precipitate is recorded at 1·4 and that by alcohol as 1·2 . Again some fluids failed to show any precipitation or cloudiness on semi-saturation with ammonium sulphate, but no sample of spinal fluid was examined which did not show at least a turbidity on the addition of alcohol. It was seen from Table 8 that no relation existed between the amount of alcoholic precipitate and the intensity of the Wassermann reaction or the deviating power of the extract of spinal fluid; and in as much as a close correspondence exists between the various precipitates produced by the different methods, it follows that/

that there is no relationship between the intensity of the Wassermann reaction and the density of the precipitate thrown down by ammonium sulphate or the Noguchi method. Although the amount of precipitate obtained by half saturation with ammonium sulphate is no indication of the relative amount of complement absorbed in the Wassermann reaction, yet the substance indispensable to the production of deviation of complement in the presence of an emulsion of organ extract are present in the precipitate obtained by half saturation with ammonium sulphate.

THE NATURE OF THE WASSERMANN REACTION.

There seemed at one time to be some doubt as to whether the substances in the blood serum or cerebro-spinal fluid which determined a positive Wassermann reaction, was of the nature of lipoid (Levaditi & Yamanouchi, Mott & Pighini) or a protein (Sachs, Noguchi). I have already given details of experiments that I have carried out on a number of cerebro-spinal fluids from various sources, with a view to determining their lipoid content and the possible relationship between the lipoid content and the Wassermann reaction. The spinal fluids were extracted with alcohol, and the lecithin content of the extract was estimated by its lytic effect on ox red/

red blood corpuscles to which a suitable amount of cobra venom had been previously added. It appeared that the lytic effect along with venom, of extracts of the various specimens had no relation to the production of a Wassermann reaction in a series of positive cases. Of course it must be remembered that such alcoholic extracts contain not merely substances which are lytic with cobra venom (lecithin) but also antilytic bodies (cholesterin) and Pighini has found comparatively large amounts of cholesterin in the cerebro-spinal fluids of cases of general paralysis, epilepsy and dementia praecox especially in the acute phase. It was also found impossible to extract with alcohol the substances in the fluids ("Antibody") which were concerned in the syphilitic reaction.

The following experiments show that the substances concerned in the production of a Wassermann reaction is of the nature of a globulin or is associated with the globulin precipitate by Ammonium Sulphate. Two specimens of spinal fluid were examined. One from an advanced case of general paralysis and the other a pooled specimen from two cases of severe epilepsy. The globulin fractions were precipitated and tested as shown in the following table.

TABLE 9.

	.2cc.cerebro-spinal fluid - .7cc.extract of ox liver. Of above emulsion 0.5cc. Complement, .025cc.	.05cc.	.1cc.	.15cc.	.2cc.	Cerebro-spinal fluid alone 0.5cc. complement .01cc.	.02cc.
Advanced general paralytic	0	0	0	T	c	c	c
Mixture of cerebro-spinal fluids from cases of Epilepsy.	c	c	c	c	c	c	c

	10cc.cerebro-spinal fluid - 10cc. Ammonium sulphate - centrifugalise and to precipitate add 10cc.saline; of this 6cc. were taken and an Emulsion made with 0.6cc.of ox liver extract. of above emulsion 0.5cc. Complement .025cc.	.05cc,	.1cc,	.15cc,	.2cc.	0.5cc. saline solution of ammonium sulphate, without ox liver extract Complement .01cc.	.02cc.
Advanced general paralytic	0	0	0	0	0	t.	c.

	30cc. cerebro-spinal fluid - 30cc. saturated solution of ammonium sulphate centrifugalise and to precipitate add 10cc. saline solution; of this 6cc. were taken and an emulsion made with 0.6cc.of ox liver extract. Of above emulsion 0.5cc. Complement .025cc,	.05cc,	.1cc,	.15cc,	.2cc.	.5cc.saline solution of Ammonium sulphate, without ox liver ext. Complement .01cc,	.02cc.
Mixture of cerebro-spinal fluids from cases of Epilepsy.	c	c	c	c	c	c	c

Controls

Extract 6cc Nacl - 0.6cc.extract of Emulsion 0.5cc.
Complement 0.01cc, 0.02cc, 0.03cc, 0.04cc.

a.c. c c c

Complement dose .005.

Further light is thrown on the nature of the Wassermann reaction by the following experiment - Ten cubic centimetres of spinal fluid from an advanced case of general paralysis were placed in a test tube and one cubic centimetre of alcoholic extract of ox-liver was floated on the surface of the spinal fluid. Mixing by rotation of the tube produced a dense precipitate which could be brought down by centrifugalising. This fluid inhibited the action of haemolytic complement whereas the clear fluid did not. It was found that the alcoholic extract of liver had precipitated the proteid content of the cerebro-spinal fluid. Again, the alcoholic Extract of liver possessed the power of activating cobra venom to this extent, that of a mixture of one part of extract with ten parts of normal saline solution 0·015 c.c. sufficed to produce complete lysis of 1 c.c. of a 5 per cent. suspension of ox red blood corpuscles with cobra venom (1 c.c. of a $\frac{1}{1000}$ solution of cobra venom to 10 c.c. of a 5 per cent. ox blood suspension). It was found that 3 c.c. of the clear supernatent fluid obtained after centrifugalising the mixture of liver extract and cerebro-spinal fluid did not lyse 1 c.c. of a 5 per cent. suspension of ox red blood corpuscles with cobra venom. On the other hand the mixture of

the/

the precipitate and normal saline solution possessed activating properties for cobra venom in an amount equal to 0·015 c.cm. for 1 c.c. of ox red blood corpuscles. It was found that the separation of the protein content of spinal fluid and the lipoid content of the liver extract can be effected only when the fluid from very advanced cases of general paralysis is employed. It was also found that it is only particular alcoholic extracts which produce a precipitate dense enough to admit of complete separation by centrifugalisation.

EXPERIMENTS.

a. Ten cubic centimetres of spinal-fluid from a case of general paralysis were mixed with ten cubic centimetres of a saturated solution of ammonium sulphate. The mixture was centrifugalised and the precipitate dissolved in ten cubic centimetres of normal saline. Care must be taken to remove the ammonium sulphate as thoroughly as possible from the precipitate, because it exercises an anti-complement effect in minute amounts.

b./

b. To the ten cubic centimetres of solution of precipitate in normal saline, one cubic centimetre of alcoholic extract of ox-liver was added. This was done by floating the extract on to the top of the fluid in an ordinary test tube. By gradual rotation of the tube and slow mixing, the resultant mixture formed a dense emulsion which was centrifugalised. The supernatent fluid was put into a clean test tube, to be subsequently tested as Fluid A. To the precipitate obtained by centrifugalising, ten cubic centimetres of normal saline were added, and this was subsequently tested as Fluid B.

FLUID A. obtained by taking cerebro-spinal fluid and half saturating with ammonium sulphate, then dissolving the precipitate in normal saline solution and adding to this a tenth of its volume of an alcoholic extract of liver and mixing gradually so as to produce a dense emulsion, and on centrifugalising this emulsion, the clear supernatent fluid is Fluid A.

FLUID B. is the mixture obtained by taking the precipitate, which is left after removing Fluid A. and adding to it an amount of normal saline solution equal/

equal to the amount of cerebro-spinal fluid originally used.

These two fluids were tested and compared (1) as regards their power of inhibiting the action of haemolytic complement (Wassermann reaction) and (2) as regards their power of activating the haemolytic properties of cobra venom for ox red blood corpuscles.

TABLE 10.

Fluid A.	Complement (guinea-pig)	5 per cent. suspension of ox red blood corpuscles with haemologous Immune-body.	result.
0·5 c.c.	0·025 c.c.	1 c.c.	lysis complete
0·5 c.c.	0·05 c.c.	1 c.c.	" "
0·5 c.c.	0·075 c.c.	1 c.c.	" "
0·5 c.c.	0·1 c.c.	1 c.c.	" "
0·5 c.c.	0·15 c.c.	1 c.c.	" "
0·5 c.c.	0·2 c.c.	1 c.c.	" "

TABLE /

TABLE 11.

Fluid B.	Complement (guinea-pig)	5 per cent. suspension of ox red blood corpuscles with homologous immune-body.	Result
0·5 c.c.	0·025 c.c.	1 c.c.	no lysis
0·5 c.c.	0·05 c.c.	1 c.c.	" "
0·5 c.c.	0·75 c.c.	1 c.c.	" "
0·5 c.c.	0·1 c.c.	1 c.c.	" "
0·5 c.c.	0·15 c.c.	1 c.c.	" "
0.5 c.c.	0·2 c.c.	1 c.c.	trace of lysis

Controls for Tables 10 and 11. (a) complement dose 0·01 c.c., (b) 0·5 c.c. of the solution of ammonium sulphate precipitate in normal saline solution did not deviate more than two doses by itself, that is, haemolysis was complete with 0·03 c.c. of complement, (c) an emulsion of one part of alcoholic extract of liver to ten parts of normal saline solution was made, and it was found that 0·5 c.c. of this emulsion did not deviate more than one dose of complement, that is haemolysis was complete with 0·02 c.c. of complement.

TABLE /

TABLE 12.

Fluid A.	5 per cent. Suspension of ox red blood corpuscles with cobra venom - 0·1 m.g. : 1 c.c.	Result
0.01 c.c.	1 c.c.	no lysis
0·015 c.c.	1 c.c.	" "
0·03 c.c.	1 c.c.	" "
0·06 c.c.	1 c.c.	" "
0·1 c.c.	1 c.c.	" "
0.2 c.c.	1 c.c.	" "
0·3 c.c.	1 c.c.	" "

TABLE 13.

Fluid B.	5 per cent. Suspension of ox red blood corpuscles with cobra venom - 0·1 m.g. : 1 c.c.	Result
0·1 c.c.	1 c.c.	trace of lysis
0.015 c.c.	1 c.c.	complete lysis
0·03 c.c.	1 c.c.	" "
0·06 c.c.	1 c.c.	" "
0·1 c.c.	1 c.c.	" "
0.2 c.c.	1 c.c.	" "
0·3 c.c.	1 c.c.	" "

Controls for tables 12 and 13 - (a) of an emulsion made up/

up of alcoholic extract of liver to ten parts of normal saline solution, 0·015 c.c. sufficed to produce complete lysis of 1 c.c. of the ox corpuscle suspension and cobra venom, (b) The amount of cerebro-spinal fluid used in the experiment did not have any activating power by itself.

The conclusions which may be drawn from these experiments on the nature of the Wassermann reaction and the activation with cobra-venom are:-

1. The Wassermann reaction is related to the presence of a substance associated with the globulin of the cerebro-spinal fluid, and to the interaction of this substance with lipoid bodies such as are present in an alcoholic extract of liver. On the other hand a cerebro-spinal fluid may contain abundant albumen and globulin yet react negatively in the syphilitic test.
2. The specific substances ("syphilitic antibody") in the cerebro-spinal fluid can be removed by precipitation on half saturation with ammonium sulphate, i.e. they are carried down with the globulin fraction; they are also precipitated by a suitable amount of a suitable alcoholic extract of ox's liver.
3. In the removal of the Specific substances ("Syphilitic antibodies") by precipitation with alcoholic extract/

extract of liver, the substance in the extract which possesses the power of activating cobra-venom (lecithin) also passes into the precipitate.

4. The lipoid substances which are precipitated from the alcoholic extract of liver by the cerebro-spinal fluid do not lose their power of activating cobra venom.

COMPARISON OF THE WASSERMANN TEST AND CYTOLOGICAL EXAMINATION.

In the differential diagnosis of diseases of the central nervous system, importance has been attached to the results of cytological examination of the spinal fluid. While, it is the case that general paralysis is as a rule associated with the presence of a high cellular content in the spinal fluid (Hamilton Marr), there are other diseases apart from the well recognised infections of the central nervous system in which a high cellular content is also present. For example in the case of catatonic stupor, , No. 50 in Table 8, where the protein content of the fluid was high and the Wassermann reaction was negative, about 350 cells of mononuclear type were present in each cubic centimetre. Plaut made a cytological examination of the spinal fluids from 56 cases of nervous diseases, which he considered free from syphilis. He regards/

regards the result of a cytological examination as negative when the number of cells in 1 c.c. is less than six, as doubtful when less than ten and as positive when more than ten. In 48 out of the 56 cases the cytological examination gave a negative result, in 4 it was doubtful and in 4 it was positive. The spinal fluid of the four cases in which the cytological result was positive gave a negative Wassermann reaction. These cases included one of pachy meningitis, one of epilepsy, one which had been anaesthetised by the intraspinal injection of cocaine, and a case in which the diagnosis was uncertain. Cases of tubercular meningitis with lymphocytosis of the spinal fluid and a negative Wassermann reaction were also observed.

In the next place a series of recognised syphilitics in different stages of the disease were examined by Plaut. The blood serum and the spinal fluid were examined for the Wassermann reaction and a cytological examination of the spinal fluid was also made. In a series of ten cases with symptoms of the secondary stage the serum was positive in each, and the spinal fluid was negative in all of the cases examined (the spinal fluid of one case was not examined). Cytological examination showed a positive result (over 10 cells in 1 c.c)/

1 c.c.) in three cases, and in one case there were 77 cells in 1 c.c. of the fluid. A doubtful result (6-8 cells) was obtained in four cases and a negative result (2-3 cells) was obtained in three. Twelve cases in the latent tertiary period were examined in the same way. In eleven the cerebro-spinal fluid gave a negative Wassermann reaction; in the twelfth it was not examined. The blood serum was examined in eleven cases and was positive in nine. In two cases the cytological examination gave a positive result (22 and 23 cells in 1 c.c.) in one case the result was doubtful (6 cells in 1 c.c.) and in nine cases it was negative.

In the cases of general paralysis it was found that in the great majority of cases both a positive Wassermann reaction and a positive cytological result could be determined. A few cases, however, were encountered in which the results were divergent; a positive cytological result was found in eight cases where the spinal fluid gave a negative Wassermann reaction; in each case the blood gave a positive reaction. Plaut, however, considered it possible that these cases might have been suffering from cerebral syphilis, and not general paralysis. In eight cases he found that the spinal fluid reacted positively in the Wassermann test without there being any accompanying lymphocytosis. Five of these cases were in the very/

very early stages of general paralysis, and it is possible that in two other cases the disease was cerebral syphilis and not general paralysis. Corresponding results were obtained in a few cases of locomotor ataxy, which were examined. A positive Wassermann reaction was associated with lymphocytosis in most cases, although three cases were met with in which the cells were few in number and the Wassermann reaction strongly positive, and in one case there was a lymphocytosis with a negative Wassermann reaction.

Of special interest and importance were the results obtained by Plaut in cases of cerebral syphilis. He examined 21 cases of cerebral syphilis of congenital origin, and only in one case was the cytological result negative. In three cases the result was doubtful (5 to 9 cells in 1 c.c.) In the remaining 17 cases there was a distinct leucocytosis, in one instance the number of cells representing 424 in 1 c.c. Only one spinal fluid gave a strongly positive Wassermann reaction three gave a weak action, and the remaining seventeen were negative. The blood serum was positive in every case. The four cases which gave positive Wassermann reactions showed a lymphocytosis of varying degrees. The fluid which reacted strongly contained 17 cells in 1 c.c., while the three which reacted weakly contained 10, 103, and 345 in/

in 1 c.c. respectively. These cases show definitely that lymphocytosis of the spinal fluid in syphilis and the presence in the fluid of substances which give a positive Wassermann reaction, are indepedent manifestations of the disease. In the course of the present investigation two cases of Plumbism, presenting clinically symptoms resembling those of general paralysis were examined. The cerebro-spinal fluid was found to contain 200 and 250 cells per cubic centimetre respectively. They each gave a negative Wassermann reaction, but a positive Nonne and Apelt and a positive Ross and Jones.

5. ADDENDUM ON THE FILTRATION OF EXTRACTS OF SPINAL FLUID.

It has already been pointed out that the presence of activating substances in the alcoholic extracts of cerebro-spinal fluids can be determined only after filtration. If a mixture were allowed to sediment, and the supernatant fluid pipetted off, this fluid did not, as a rule, show activating properties; when filtered, however, or still better, when heated and filtered, the presence of activating substances in the filtrate could easily be shown. It was, however, necessary to control such an experiment by an examination of the filter paper used, in order to determine whether the paper itself might/

might not contain substances which might pass into the filtrate. It was actually found that alcohol did extract from the filter paper (Schleicher and Schülz No. 595) a small quantity of lytic substances. The amount of lytic substances extracted, however, by the small quantity of alcohol used in these experiments does not invalidate the general results, although the absolute amount of activating substances in the alcoholic extract of spinal fluid must be less than that shown in the experiment after the heated extract has been filtered. A series of experiments were performed in order to determine to what extent substances possessing lytic properties could be extracted from the filter paper employed. Table 14 gives the result of such an experiment.

T A B L E 14.

	1cc.5% ox corpuscles and cobra venom.					Corpuscles without cobra venom	
Amount of mixture of extract & saline in ea.tube.	0.3	0.5	0.75	1.0	1.5	1.0	1.5
1. Paper - water - ether - alcohol - Emulsion c saline line	0	m	c	c	c	0	c
2. Paper - ether - alcohol -" " "	0	0	c	c	c	0	c
3. Paper - alcohol " " "	t	c	c	c	c	c	c
4. Alcohol - - " " "	0	0	0	0	0	0	0
5. Alcohol - filtered through paper boiled in alcohol - " "	0	0	0	0	0	0	0
6. Alcohol - filtered through paper boiled in ether - " "	0	0	0	0	0	0	0
7. Alcohol - filtered through paper boiled in water - " "	0	0	0	0	0	0	0
8. Alcohol - boiled filtered through ordinary filter paper " "	0	0	0	0	0	0	0

In series I, 20 pieces of filter paper were boiled in 100 c.c. of water for one minute; the water was evaporated and the residue taken up in ether; this was again evaporated and the residue taken up in 20 c.c. of alcohol: a mixture was made of one part of the alcoholic solution to 5 parts of normal saline, and it was found that 0·5 c.c. of this mixture was sufficient to lyse 1 c.c. of a 5% suspension of ox corpuscles to which cobra venom had been added; 1 c.c. of the mixture had no effect no ox corpuscles without cobra venom. While this may not be a case of real activation of cobra venom, still it proves that certain substances may be extracted from filter paper by hot water, that these substances might influence cobra venom activation.

Series 2 and 3 show that similar substances are extracted from the filter paper by means of ether and alcohol.

Series 4 shows that the alcohol alone in the amount used does not influence the corpuscles.

Series 5, 6, and 7 show that by treating the filter paper first with alcohol or ether it is possible to get rid of the lytic substances.

SUMMARY /

SUMMARY OF RESULTS.

1. The Wassermann Reaction was done in the intraventricular and the spinal fluid in four cases of general paralysis; in two the intraventricular fluid was negative and the spinal fluid positive. In the other two cases the fluid from both sources reacted positively; but in one the reaction of the intraventricular fluid was weak compared with that obtained with the spinal fluid from the same case. In both cases in which the intraventricular fluid gave a positive reaction, the choroid plexus showed evidence of degeneration, whereas in the other two cases degenerative changes were not observed.

2. A positive Wassermann reaction was present (with the spinal fluid) in fifty-two out of sixty cases of general paralysis (by the original method); the spinal fluid of thirty cases of epilepsy and dementia praecox, examined by the same method, gave a negative reaction.

   Using the cerebro-spinal fluid alone as a diluent for the alcoholic extract a positive reaction was got in thirty out of thirty cases of general paralysis, including two which gave a negative reaction with the original method. Twenty cases of mental disease other/

other than general paralysis were also examined by this method and a negative reaction got in each. Forty-five cases of general paralysis at various stages and twenty cases of mental disease other than general paralysis were examined by the Lecithin, Lecithin-cholesterin method, again using the cerebro-spinal fluid as a diluent for the alcoholic extract. The general paralytics all reacted positively and the others negatively. This was found to be the most delicate method.

3. A quantitative examination of the intensity of the reaction, in terms of haemolytic doses of complement, showed that great variation exists from case to case; speaking generally the more advanced the case the greater the amount of complement absorbed,

4. Fresh cerebro-spinal fluid possesses no activating properties for cobra venom; certain fluids rich in cellular content were found to inhibit the activating power of alcoholic extract of liver; this inhibitory action disappeared when the cellular elements were centrifugalised.
Alcoholic extract of cerebro-spinal fluid possesses to a slight extent and in a very varying degree the power/

power of activating the lytic action of cobra venom for ox red blood corpuscles. This power of activation has no relation to the occurrence of substances which produce a Wassermann reaction, and is present in spinal fluids from various sources, such as epilepsy, dementia praecox and maniac depressive insanity as well as in the fluids from cases of general paralysis.
(In filtering alcoholic or etherial extracts allowance must be made for the fact that certain lytic substances in the filter paper may be extracted, but this point does not invalidate the general conclusions stated here).

5. Mixtures of cerebro-spinal fluid and alcohol present varying degrees of turbidity; as a rule the greatest degree of turbidity is seen in cases of general paralysis, but equally turbid mixtures were observed with fluids from cases of epilepsy and dementia praecox in their acute phases.
There is no relationship between the degree of turbidity and the amount of complement absorbed in the Wassermann reaction; a fluid which, with an equal volume of alcohol, gives rise to an opalescent mixture may deviate more haemolytic complement in the Wassermann/

Wassermann test, than a fluid which, with alcohol, gives rise to a markedly cloudy precipitate. Examination of the protein content of the spinal fluid by precipitation with ammonium sulphate, and also by the Noguchi method, showed that there is a clear correspondence between these methods of precipitation and the precipitation by alcohol. A few cases of dementia praecox were found with a high protein content, just as they sometimes show a considerable precipitate with alcohol. These is, however, no relationship between the protein content of the spinal fluid and the intensity of the Wassermann reaction. Most cases of general paralysis show a high protein content in the spinal fluid, but a fluid with a high protein content may give a weak reaction. Cases of dementia praecox with a high protein content do not give a Wassermann reaction.

6. The Wassermann reaction is related to the presence of a substance associated with the globulin of the cerebro-spinal fluid, and to the interaction of this substance with lipoid bodies such as are present in an alcoholic extract of liver.

The specific substances ("syphilitic antibody") in the/

the cerebro-spinal fluid can be removed by precipitation on half saturation with ammonium sulphate, i.e. they are carried down with the globulin fraction; they are also precipitated by a suitable amount of a suitable alcoholic extract of ox liver.
In the removal of the specific substances ("syphilitic antibodies") by precipitation with alcoholic extract of liver, the substances in the extract which possess the power of activating cobra venom (lecithin) also pass into the precipitate, and do not lose their power of activating cobra venom.

7. In general paralysis as a rule there is a high cellular content in the spinal fluid, but there are other diseases apart from general paralysis in which a high cellular content is also present. In a case of Catatonic Stupor about 350 cells of a mononuclear type were present in each cubic centimetre, and in two cases of Plumbism the cerebro-spinal fluid was found to contain 200 and 250 cells per cubic centimetre respectively. These cases had a high protein content in their cerebro-spinal fluid, but in each case the Wassermann reaction was negative.

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