IMMUNITY-PHENOMENA

IN

CEREBRO-SPINAL MENINGITIS:

OPSONINS AND AGGLUTININS

IN THEIR RELATION TO

CLINICAL FEATURES, PROGNOSIS AND THERAPY

S. M.
ALEXANDER MACGREGOR, M.B., Ch.B.

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INTRODUCTION

The observations which form the basis of this work extend from the summer of 1906, when epidemic cerebro-spinal meningitis appeared in Glasgow. The disease in the following winter reached epidemic proportions, and has continued since in sporadic form. The great majority of the cases studied were under the writer's care in the wards of Belvidere Fever Hospital.

The special object of the investigation was to trace the development of agglutinating and opsonic powers for the meningococcus in the sera of patients with the disease. At the same time an endeavour was made to obtain a clearer view of the disease as a whole by correlating the immunity phenomena with the general toxic manifestations, and with the pathological anatomy in the various phases of illness.

It was hoped in this way to gain information that would prove of value in prognosis, and yield indications for vaccine treatment.

An account is given of some experiments on the injection of vaccines in chronic cases. Incidentally, a point of some interest arose in connection with iso-erythro-agglutinins and opsonins which developed in the sera of certain patients. These investigations are arranged in the following order:-

1 - Agglutinins

2 - Opsonins

3 - Erythro-agglutinins and erythro-opsonins

4 - Note on Vaccine Therapy

5 - General Summary - The toxic phenomena in their relation to the anti-bodies studied

SECTION I

AGGLUTININS

The object of this investigation was to determine to what extent the presence of specific agglutinins in the blood of the patient reflected reaction to infection, and to trace its variation throughout the disease in the hope that some guidance in regard to prognosis and treatment might be obtained. Diagnosis, of course, rests on the certain evidences given by lumbar puncture.

THE ORGANISM

In order to secure results comparable with one another and serially comparable, it was necessary to have some medium which could be depended on to give uniformity of growth. This constituted the chief initial difficulty, and involved considerable study of the organism on various media. Glycerine agar was used in the earlier part of the epidemic. On this, growths could readily be obtained from a severe case where the organism was plentiful, but could not be maintained for more than four or five subcultures at most. Each successive culture became scantier and more degenerate until the organism finally died out. Some did not even survive the first subculture. It was clear that results obtained with a copious early culture could not be compared with those obtained with a later subculture, which, although of the same age, was much more degenerate. This difficulty is commented upon by other observers, the vari-

ability of the organism forming the chief barrier against continuous quantitative comparisons. Accordingly, glycerine agar is of little use except for primary cultures, when the organisms are plentiful and vigorous.

Ordinary coagulated blood serum is also of little use for continuous cultivation. Growths degenerate very easily, and the organisms become viscid and difficult to emulsify. It was later found that blood agar satisfied the conditions required. This is prepared by smearing the surface of a tube of glycerine agar with a drop of fresh blood, and then inoculating the organisms on the surface. It is also by far the most sensitive medium for obtaining primary cultures. It acts well even in chronic cases where the organisms are scanty and of low vitality. Human serum separated from corpuscles by centrifugal rising in a sterile capsule may also be used instead of blood. On this medium a perfectly uniform series of subcultures can be obtained, with organisms in the quantity required for hanging-drops. I have never had the least trouble in keeping cultures alive by this method for long periods. The observations detailed later were carried out chiefly with a single strain which remained perfectly vigorous for over a year, and formed the standard test-organism during the major part of the observations. Cultures on this medium need very little attention; a fortnight or more may intervene between each subculture. The cultures were always kept at 37° C. If the organism is cultivated at regular intervals, the rate of growth of each subculture is remarkably uniform. Colonies appear in 24 hours as discrete dots on the surface, and continue to increase in number and size. As variability in growth is an important cause of variation in agglutinability, on this medium uniform growth and uniform suscepti-

bility of the organism to agglutination are best maintained.

Löffler's alkaline serum with the addition of maltose or glucose also affords good subcultures. It is described more fully when the opsonic index is dealt with. I had not an opportunity of using it till most of my observations on agglutination were completed, but some comparative tests were made with both this medium and nutrose ascitic glucose agar furnished by Dr. Buchanan, City Bacteriologist. On Löffler's serum the organism grows rapidly and copiously as a rule, and subcultures can be maintained for long periods. But at times there is considerable variability, both in rate of growth and quantity of culture, as compared with blood glycerine agar. This fact has an important bearing on the suitability of the organism for opsonic work. On nutrose ascitic agar the organism is much more variable than on Löffler's serum, and usually rapidly dies out. These media were consequently rejected.

Bouillon has been found quite unsuitable. Growth usually fails in this medium, rarely attains sufficient density, and is too uncertain for continuous comparative tests. Some observers have found it very suitable for macroscopic work, the suspensions in Bouillon being more sensitive to agglutination than those made in salt solution (Dunham[7], Park)[20].

Thus, blood glycerine agar could be depended on to give a perfectly uniform series of subcultures for an indefinite period, and was the medium employed.

METHOD OF OBSERVATIONS

A - Hanging-drop preparations were used throughout. The medium used was blood glycerine agar. No cultures older than 48 hours were used. The blood to be tested was collected in capillary tubes, and the serum separated by centrifuge. Blister fluids were never used. The serum was generally obtained on the day of observation, in most cases just before use. The dilutions employed were 1-10, 1-20, 1-40, 1-60, 1-80, 1-100; latterly, 1-5 was added. Normal serum controls were employed in every case at 1-5, 1-10, 1-20 dilutions. Usually several sera were tested at the same time. The observations were made at room temperature for various periods up to twelve hours.

B - Macroscopic. This method was tried in the early part of this investigation and found unsatisfactory for the following reasons:-

(1) The medium used was glycerine agar. Growth could not be maintained in this medium, and comparative results with the same strain were impossible

(2) The cultures used were from two to six days old, and presented so much sedimentation of organisms in salt solution that existence of true agglutination could not be determined. Growths on Löffler's serum would probably be suitable for this method, but no further experiments were made

Dunham[7] made use of this method in testing the agglutinability of various types of diplococci, but it did not yield reliable results.

TABLE showing that agglutinability varies with the age of the culture on the same medium

Cases	Culture	Age	Result	Controls
1	Stock	2 Days	1-20	Nil
		4 "	1-80	1-10
2	"	2 "	Nil	Nil
		4 "	1-10	Nil
3	"	2 "	1-20	Nil
		4 "	1-40	Nil
4	"	2 "	1-20	Nil
		4 "	1-80	1-10
5	"	2 "	1-40	Nil
		4 "	1-100	1-20
6	"	2 "	1-20	Nil
		4 "	1-80	Nil
7	"	2 "	1-10	Nil
		4 "	1-40	Nil
8	"	2 "	1-40	Nil
		4 "	1-80	1-20

This table gives the result of experiments at various times when the agglutinabilities of the stock organisms of two and four days' growth respectively were tested with sera of marked agglutinating power. Control results with normal serum are also given. It will be noted that cultures four days old are much more sensitive to agglutination with normal serum in a 1-10 and even a 1-20 dilution. This tendency to agglutination increases with the age, and may even show itself in comparatively young cultures. It is almost always present if the culture is older than two days.

Spontaneous agglutination of the organism even with young

and vigorous cultures may occur in salt solution. This happened on two occasions during the period when opsonic indices were being estimated; once in the case of the stock organism, which had been in use for some time, clumping was noticed to be occurring rapidly soon after the emulsion was made, and continued till quite a considerable sediment had gathered, but in no case did complete agglutination occur even after a long period. This quality suddenly appeared, lasted for two or three subcultures, and then disappeared. In the second case, the first subcultures of a fresh growth behaved thus; the second subculture was only slightly agglutinated, while the third did not agglutinate at all. The cultures have always been used at the same age - two days -, which may be taken as the standard for these observations. Controls with normal serum have always been made with each series of observation.

Some observers have noted the agglutinability of the organism in normal serum and in salt solution. For instance, Dunham[7], in using the agglutination method for differentiating diplococci, found that the serum of a horse immunized by repeated injections of meningococci gave readings of from 1-200 to 1-4000 even; but the organism was at the same time liable to be agglutinated by normal serum. His cultures were so variable that comparative results could not be made.

Gordon[9] states that the adhesiveness of the organism on solid media prevents proper emulsions being made. This, in my experience, occurs when the medium is dry, and can be avoided to a large extent if the surface is moist. Löffler's serum with a moist surface will usually give a luxuriant growth capable of yielding an emulsion of perfectly discrete organisms. This adhesiveness of growth is most successfully avoided by the

use of the blood medium described.

SUMMARY

It was found that blood agar was the only medium that gave uniform growths. Cultures on this must be of uniform age. The older the individual culture, the more susceptible the organism becomes to the patient's agglutinins: it also tends to become susceptible to normal serum or even to normal saline solution, i.e., specific agglutinability merges into the clumping of degeneration. The period beyond which spontaneous agglutination is liable to occur may be put at 48 hours. The macroscopic method was found unsatisfactory. The hanging-drop method was employed throughout.

A few experiments are quoted to show that the results with various strains on the same medium are approximately the same:-

Strain	Organism	Age	Serum	Normal
I	Stock	2 Days	1-40	Nil
II	Acute case	2 "	1-40	Nil
I	Stock	2 "	1-20	Nil
II	Patient's own organism	2 "	1-20	1-10
I	Stock	2 "	1-80	Nil
II	Acute case	2 "	1-60	Nil
I	Stock	24 Hours	1-40	Nil
II	Acute case	24 "	1-40	Nil

The chief object in this investigation being to trace the presence of agglutinins in the blood at various periods of the

disease, an extensive examination of strains was not attempted. Many comparative tests were made from time to time with cultures on glycerine agar alone of the same age with powerful sera. Such a serum never failed to agglutinate the latter strain as powerfully as it did the stock subculture. Cultures on the latter medium are more liable to auto-agglutination than those on blood glycerine agar, unless they are fresh and vigorous. The objections to this medium have already been mentioned. No strain was ever noticed to resist agglutination in the way that strains may sometimes resist opsonins. Houston and Rankin[11] found that a strain might become resistant to specific opsonins while retaining its agglutinability.

The following measures were thus adopted to ensure, as far as possible, unvarying agglutinability:-

(a) Normal serum controls were used in all cases at dilutions of 1-5, 1-10, 1-20, and occasionally suspensions in normal saline solution. Spontaneous agglutination could be thus detected

(b) Cultures of the same age were used throughout in order to eliminate varying agglutinability due to age

(c) The same strain was used throughout

Agglutinability of the organism was tested from time to time by the use of a serum of previously ascertained agglutinating power. By this means, it was found that the variations that occurred were small. A serum which previously gave a positive result in a dilution of 1-100 might now agglutinate in a dilution of 1-80 as the maximum, or even 1-60; or a serum giving at one time a positive result with a dilution of 1-10 might subsequently give a doubtful result at that dilution, or might show some agglutination in a 1-20 dilution. Within these limits it may be said that the organism remained without any tend-

ency to more marked fluctuation.

The general uniformity in the results from time to time with the same case indicates that the agglutinability remained much the same throughout.

GENERAL DESCRIPTION

In all, 75 cases were examined from time to time throughout the course of the epidemic. Definite results can be given as to the existence of agglutinin in all stages of the disease and in all stages in the same case.

A serum which possesses a maximum amount of agglutinin will clump the organism in all dilutions up to 1-100 in two hours. With a serum obtained from an acute case, such as is described in Class B later, a 1-10 dilution causes an almost immediate result: groups can be seen appearing in a few minutes, and massing may be complete in a quarter of an hour. The more rapid the action, the larger the groups.

This appearance is shown in the Drawing No. 1. With a 1-20 dilution, the result is somewhat slower, and the groups rather smaller. In this case, in half an hour all the organisms are agglutinated. In 1-40 dilutions the action is still slower, and may require one to two hours to become complete. The groups here are still smaller, and agglutination is complete. After a few hours the small groups tend to run together into larger groups, but it is generally noted that very little additional agglutination occurs after four hours. A 1-40 dilution result will never assume the appearance of a 1-10 dilution, no

matter how long time is allowed in the incubator. With a 1-80 dilution and a 1-100 dilution in such a case, definite grouping will be present in two hours and may not be complete; if longer time is allowed, the result will be more evident, so that in four hours or six hours very definite and almost complete agglutination will be present, the groups being small but distinct.

A result such as depicted in Drawing 5 would be considered positive, that is to say, the reading is taken as the lowest concentration which in four hours gives definite agglutination.

In other sera of less agglutinating power, action is slower and less marked in the higher concentrations than the results described. Thus, the time taken and the completeness of the reaction are factors in indicating differences in agglutinating power. Two sera can differ in the rapidity and completeness with which they agglutinate the same organism, and yet both cease to agglutinate at a 1-40 dilution, so that the sera in both cases would be classed as agglutinating at a dilution of 1-20. This is especially the case if a young culture is used.

Considering the marked nature of the result in the higher concentrations, it would appear that agglutinating power is rapidly lost by dilution. I have never, even in the most powerful sera, where the reaction in 1-10 and 1-20 dilutions is rapid and powerful, been able to be satisfied that definite agglutination was present in dilutions of over 1-100. It is this rapidity and intensity of agglutination in the higher concentrations that characterises a serum, rather than its power to clump at high dilutions. (vide plates 1-6)

In those cases in which a higher dilution gave a positive

result, it was noticed that some clumping was taking place in the normal control at 1-5 and 1-10 dilutions, which showed that the limit of specific agglutination had been reached. This usually happened with cultures older than the specified age - two days.

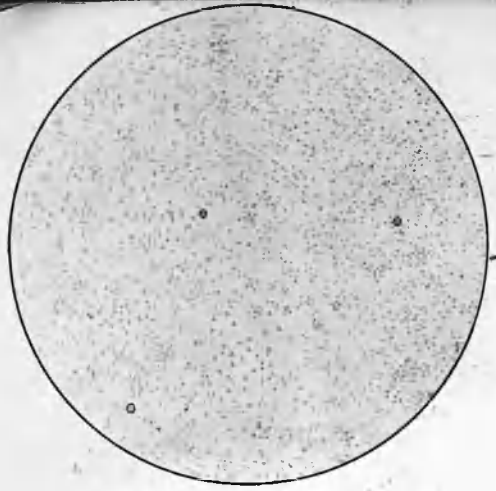
The cases in Group B describe later furnished instances of this powerful action. The drawings were taken from a result with one of these cases.

Control sera were taken from patients with various diseases, and tested against normal sera from time to time:-

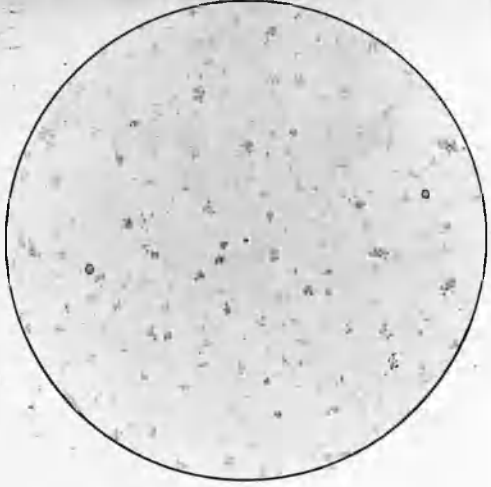
		Result	Control Normal Serum
K. S.	Lobar Pneumonia	Neg. on 5 occasions in all dilutions	Neg.
Mrs. M.	Influenza	Neg.	"
J. J.	Influenza	Doubtful in 1-10	"
- G.	Broncho-Pneumonia	Doubtful in 1-10	"
- G.	Scarlet Fever	Neg. on 2 occasions	"
- R.	Measles	Neg.	"
- C.	Scarlet Fever	Neg.	"

Further corroboration of the specific nature of these agglutinins is to be found in the clumping that often occurs in opsonic index estimations. This feature was entirely confined to sera from severe cases of cerebro-spinal meningitis.

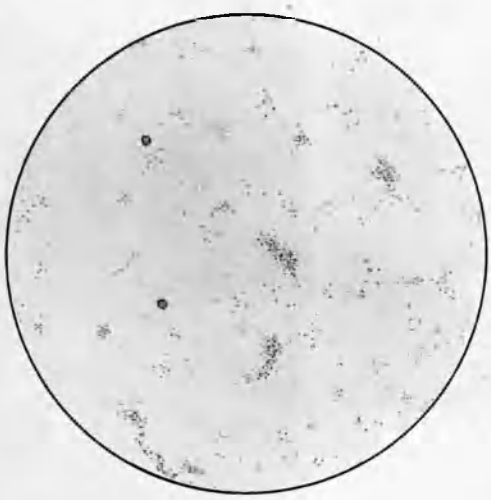
Pl. VI



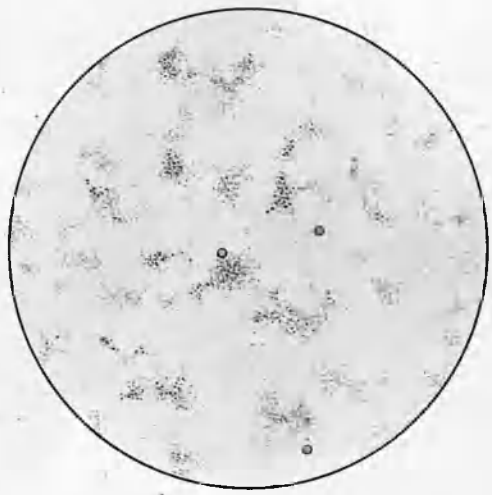
Pl. V



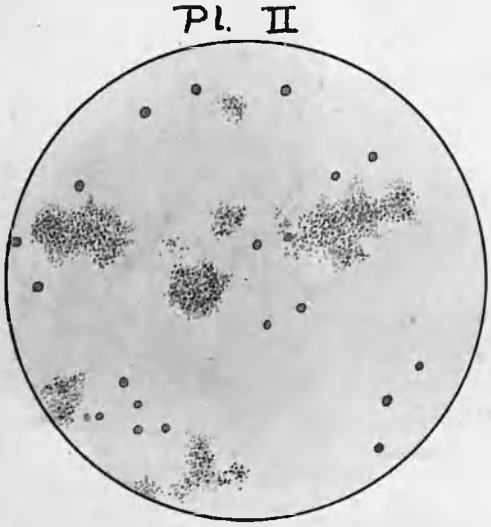
Pl. IV



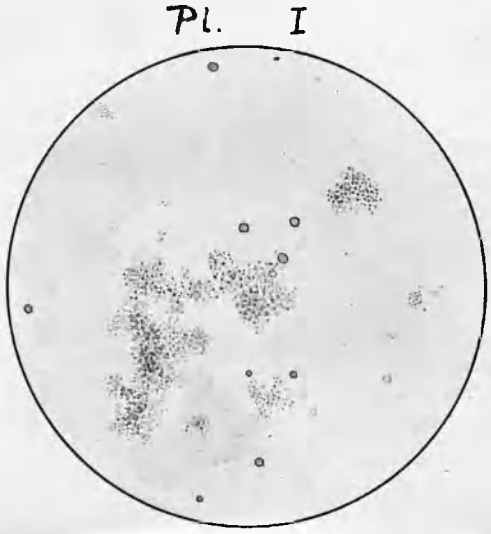
Pl III.



Pl. II



Pl. I



Plates showing appearances in hanging drops at various dilutions when a powerful serum is employed:-

Plate I	1-10	dilution
II	1-20	"
III	1-40	"
IV	1-60	"
V	1-80	"
VI	1-100	"

AGGLUTININS IN CEREBRO-SPINAL MENINGITIS

The greater number of the following cases were under my care, so that the correlation of agglutination results with clinical features was a most interesting and important part of the work. It is from this point of view that the results will be presented. The total cases (75) I have divided in such a way as to bring out this relationship.

GROUP A - Acute cases dying within the first fortnight of the disease. There are 21 cases in all. In 14 of these, agglutination was absent; in seven, some agglutination was present.

GROUP B - Group B is of greatest interest, as it contains those cases whose sera possessed the highest agglutinating power.

As my observations proceeded, I began to notice that there was a certain type of case which could be depended upon invariably to give a powerful and rapid result, and which I was in the habit of using as controls in other experiments. On separating these cases I found that they belonged also to a definite clinical type; 12 cases.

GROUP C - Cases other than those included in Group A, in which no or feeble agglutinative power was present throughout; 21 cases.

GROUP D - Cases intermediate between Groups B and C, in which a moderate degree of agglutination was found; 21 cases.

Group

A. TABLE A.

Days of illness on which observations made

No.	Name.	Age.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	Post Mortem.
1	J.B.	4	0	X												Acute congestion Conjunctival haemorrhages
2	B.M.	8		0	X											—
3	J.M.	3			0											—
4	A.Y.	6		0		X										G.L.
5	J.H.	11			0		X									—
6	W.H.	25				0	X									G.L.
7	J.McC	3				0	X									G.L.
8	J.M.	6				0	X									—
9	Mrs A.	38					0	X								G.L.
10	L.E.	18					0	X								—
11	J.M.	4					0	X								G.L.
12	A.M.	7				0				X						—
13	J.N.	25										0	X			G.L.
14	Mrs T.	44						5					5		X	G.L.
15	J.H.	3½				X 10										G.L.
16	A.L.	3			10		X 80									—
17	M.M.	3					40	X								—
18	W.B.	4							X 60							G.L.
19	J.D.	24					80			X						G.L.
20	W.C.	11.								80	X		X			G.L.
21.	N.F.	9.					20		60			100				

G.L. = General Leptomeningitis

X = Day of Death.

GROUP A

This group comprises all cases examined which died within a fortnight of onset. These are usually classed as acute fatal cases (Massachusett's Report, 1898)[24]. There are 21 cases in all. In 14 of them no agglutinating power was present. In seven cases there was some development of agglutination as shown in the table. Most of these cases were of short duration, 15 of them dying within the first week of illness. In Table A the results are given under the days of illness on which the cases were examined; the "x" indicates the day of death.

The cases in this group have the following clinical features:- the onset is abrupt, and often startlingly sudden, with severe vomiting and headache; in a short time or almost immediately the patient becomes unconscious; he may remain deeply comatose, or have periods of wild restlessness alternating with periods of coma. The facies is flushed or cyanotic, with bloodshot eyes often dotted with haemorrhages. The symptoms are those of a very intense general infection. Those of local infection may be slight, some nuchal stiffness and presence of Kernig's sign being the sole meningeal symptoms, and these often not very definite or even absent.

The temperature remains moderately elevated - 100°-101° as a rule; sometimes, however, it is scarcely raised at all, and furnishes no criterion of severity.

The cerebro-spinal fluid, even within 24 hours of onset, gives definite evidence of abundant cellular exudation, poly-

morphonuclear in character, as seen by a decided milkiness even within the first few hours; it rapidly becomes turbid with flakes of fibrinous deposit, and remains so to the end.

In these cases there was no abatement of symptoms till the day of death, indicating even temporary reaction. Their course was uniformly progressive; all died during the period of acute infection. In this respect they stand in marked contrast to the cases in Group B, in which the disease was cut short by a crisis or semi-crisis in the midst of symptoms almost as severe as those of this group.

While these are the general features which characterise these cases, even where the blood shows that some resistance has been developed, the clinical history of the last two cases in the group gives definite indication of a decided reaction of the nature of a crisis occurring on the sixth and fourth days of illness respectively. The temperature in both cases fell on the days indicated from 103° to 99° in one case, and from 102° to normal in the other case, associated with an abatement of the graver toxic symptoms. High agglutinating power has been found to be associated with a similar though more favourable sudden improvement in other cases. (Case 21, Chart I)

In the rest of the cases there was no clinical evidence of reaction, although agglutinins were present in the blood.

The post-mortem appearances were invariably those of an acute general lepto-meningitis. In the first case which died on the second day of illness, there was simply an acute general congestion with haemorrhages, not only into the brain membranes but throughout the serous membranes and skin, representing an extremely acute and overpowering infection. In the other cases the picture was that of a general invasion of the soft mem-

branes, with copious exudation distributed over vertex, base, cord and ventricles. In some cases of this type, the exudate is found equally distributed over vertex, base and ventricles, or there may become a general vertical exudation with scanty basal exudate. In other cases the basal exudate is much in excess, the basal cisterns filled with thick gelatinous material, while the extension can be seen proceeding up the Sylvian fissures on to the Rolandic area.

To summarise:- There can thus be separated a class of case in which the symptoms are those of an acute infection, progressive and without abatement, in which post-mortem there is found either a generalised or an advancing lepto-meningitis. The duration is usually under 14 days. In most cases there is no formation of agglutinins. In some, however, they develop to a considerable extent. Such cases are not clinically different from those which show no trace of agglutination. They probably indicate that some development of resistance has occurred, but not sufficient to give clinical evidence of its power.

In Group B, Tables I and II, there are 12 cases in which periodic estimations were made from time to time well into convalescence.

The blood of all these cases had a very high agglutinating power, a typical example of which is described in a preceding section. They are representatives of a class in which high agglutinating power is associated with definite clinical features of early and decided reaction.

A high degree of agglutination (dilution 1-80 or 1-100) is invariably found associated with cases of definite clinical type as regards their initial features. These are, acute onset with symptoms of severe general infection, which suddenly abate

Group

B. TABLE I.

Days of Illness on which observations made.

	NO.	NAME.	AGE.	4.	5.	6	7.	8	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	RESULT.	DAY OF CRISIS.
*	1	A.G.	11	40			C	80													R.	7.
*	2	J.K.	9		40		C				100			100							R.	8.
	3	Mrs D.	48			100	C														R.	8.
*	4	A.C.	23			C	100				100				80						D.	7.
*	5	J.H.	10			C		100													Ch.R.	6-7
	6	H.O.	14				C	40													Ch.R.	8
*	7	C.C.	15	C				80													Ch.R.	4
	8	F.A.	4½			C							60								R.	8
*	9	A.F.	4													40					Ch.R.	LYSIS.
	10	J.B.	4			C														80	D.	6-7
	11	A.W.	16				C								100						Ch.R.	8
	12	K.G.	10	1ST OBSERVATION = 28TH DAY. = 1-60.			C														D.	8

R. = RECOVERY. D. = DIED. Ch. R. = CHRONIC, RECOVERY.

C = Day of Crisis.

* = See charts at end of section.

Group B.

TABLE II. Extended cases of table I.

Days of illness on which observations were made.

Corresponding nos. in Table I.	No	NAME	Age	4	6	7	8	9	12	13	14	15	16	17	18	19	20	21	22	24	27	28	30	34	35	37	40	43	45	47	48	49	52	57	68	73	80
2.	1.	J.K.	9	40					100		80		80		80								20										10				
1.	2.	A.G.	11	40			80										100						100						40					10			
8	3.	F.A.	4½						60																												
3	4.	MRS D.	48			100																															
12	5.	K.G.	10																			40			40			20									
9	6.	A.F.	4										40	40					40		60			20	20								10			20	
11	7.	A.W.	16														80																				
6.	8.	H.O.	14					40							20				40								40							5			
7	9.	K.G.	15					80					80						60																		5
4	10.	A.C.	23				100		100		80															60		60									
5	11.	J.H.	10					80					40						40						20								20				
10	12	J.B.	4										100												80				40								

after the manner of a crisis early in the disease (the average day of crisis in these cases was the seventh day), followed either by convalescence or a period of abatement of symptoms before a chronic stage is entered upon. All the cases described were of this type.

In Table I ([redacted]) the results are given under the day of illness on which each observation was made, and represent the maximum agglutinating power of each case. The day of crisis is marked "C". Table II gives the total of observations made on each case, and the day of illness on which the blood was examined. An account of the chief clinical features in each of these cases is given also separately, especially those relating to the period of early infection.

DESCRIPTION OF AN ILLUSTRATIVE CASE

The onset is abrupt as in Group A, with headache and vomiting, and is often startlingly sudden. The patients are usually comatose or stuporose on admission, with a dusky flush and congested conjunctivae, with perhaps haemorrhages. They are unresponsive and resentful of interference. They may remain quiet with periods of restlessness and delirium, or may be actively delirious. Nuchal stiffness and Kernig's sign are usually decided. They appear critically ill, and there is nothing to indicate an approaching crisis. After remaining in this state for a few days, they recover with almost the suddenness of a pneumonic crisis. Those who are restless, querulous and noisy, flushed and unconscious or semi-conscious, fall into a natural sleep, and waken up conscious though somewhat dazed,

Series of Charts illustrative of Course of Agglutination in Group B

Clinical notes are added at end of Section

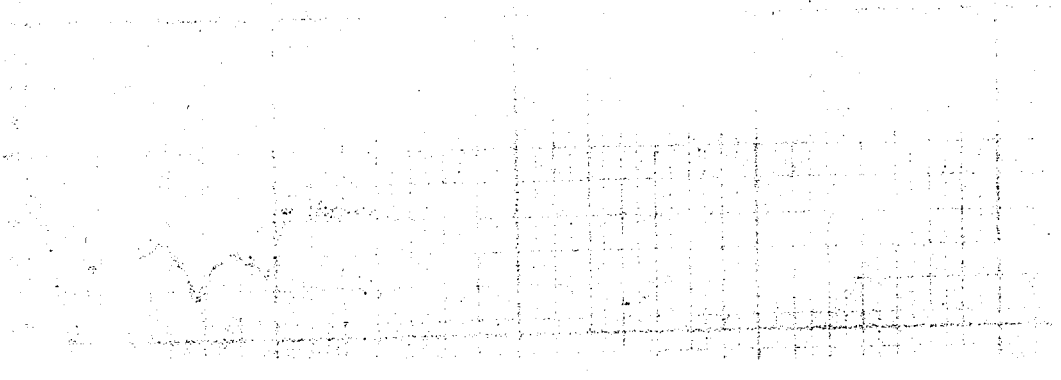


Chart No. I

Group A. Case 21. N. F. 9 yrs.

AGGLUTINATION.

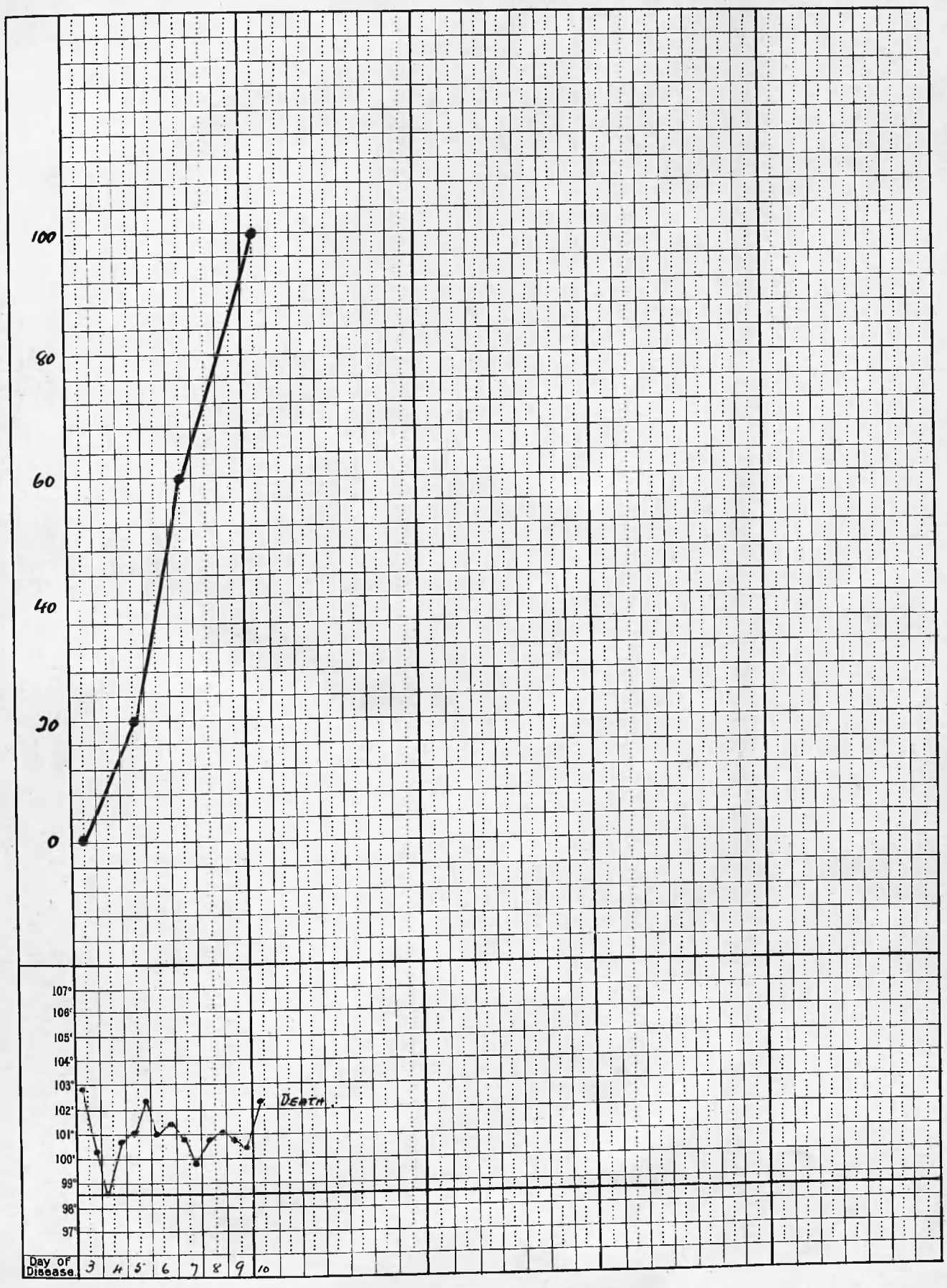
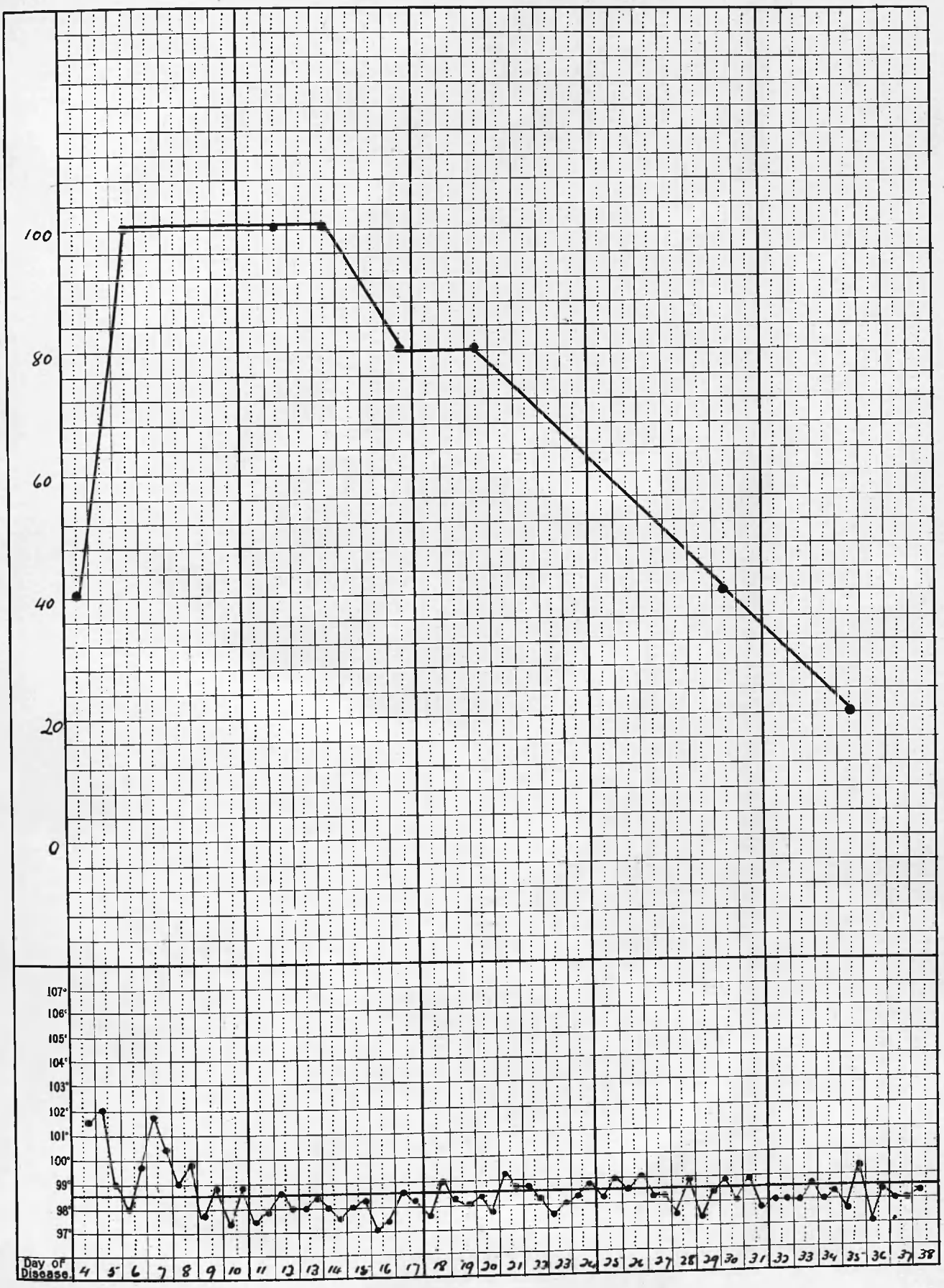


Chart No. 11

Group B. J. K. 9 yrs.

Case 2.

AGGLUTINATION.

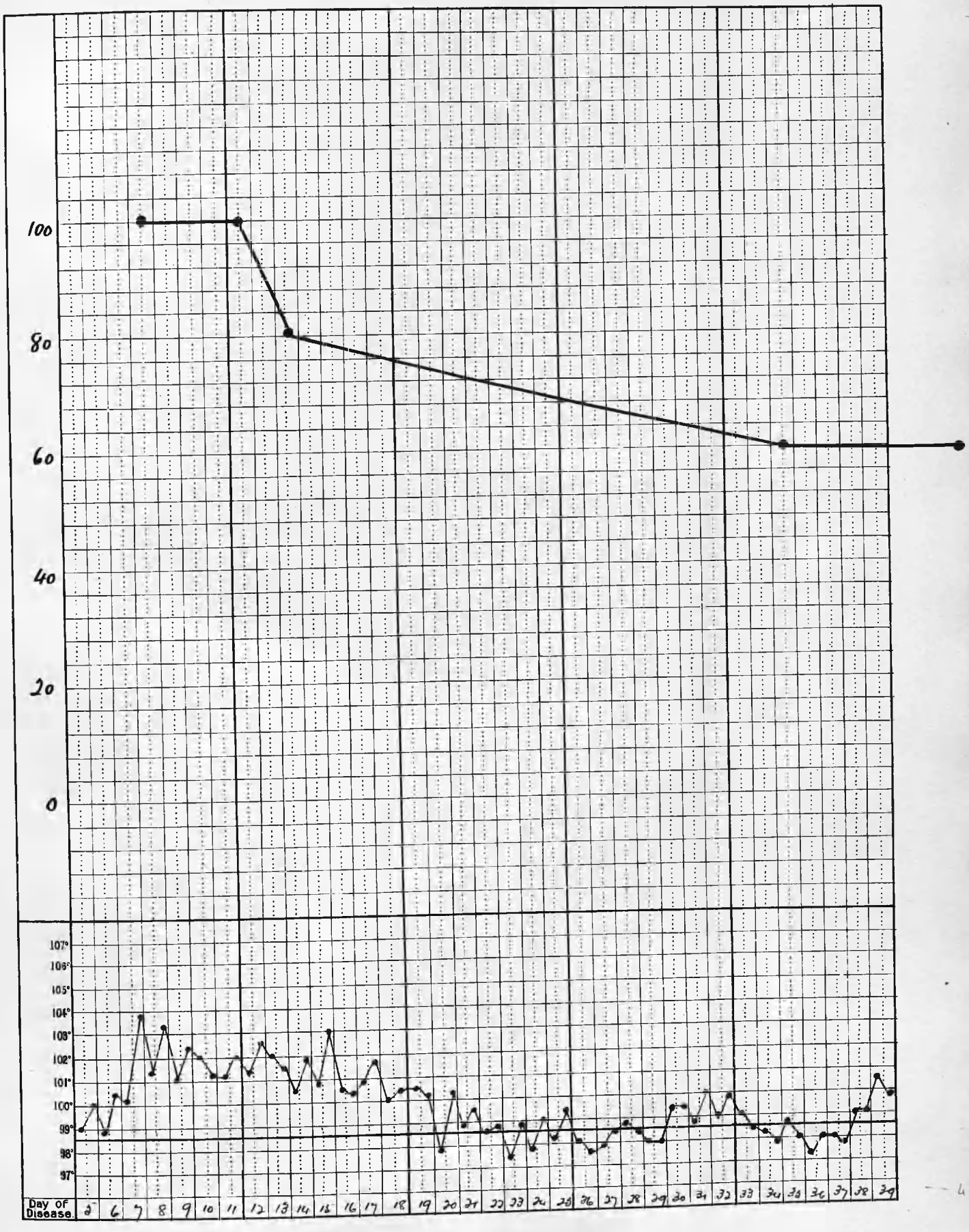


Chart, No. III

Group B. A. C. 23 yrs.

Case 4.

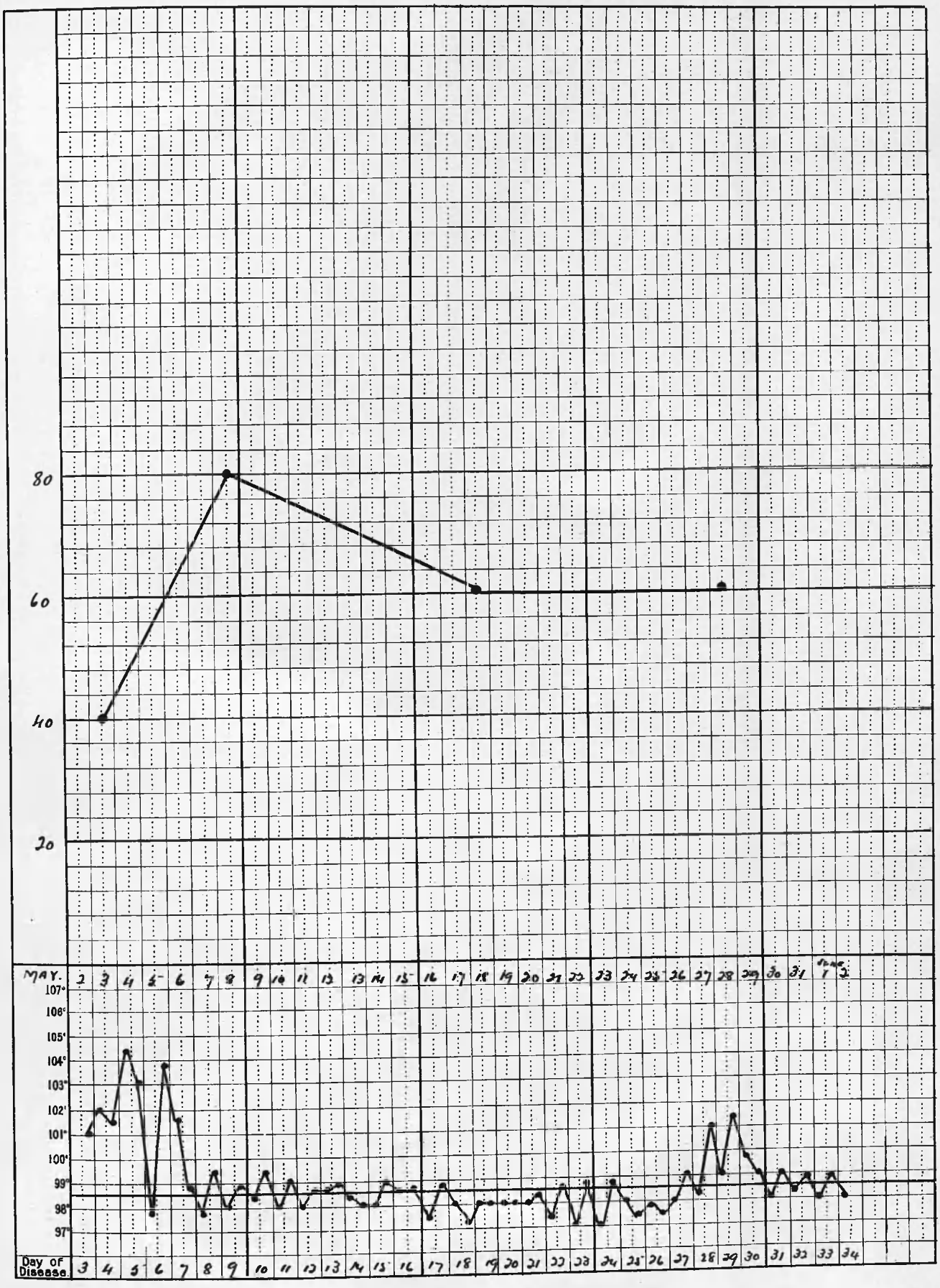
AGGLUTINATION.



Chart, No. IV

Group B. A. G. 11 yrs. AGGLUTINATION.

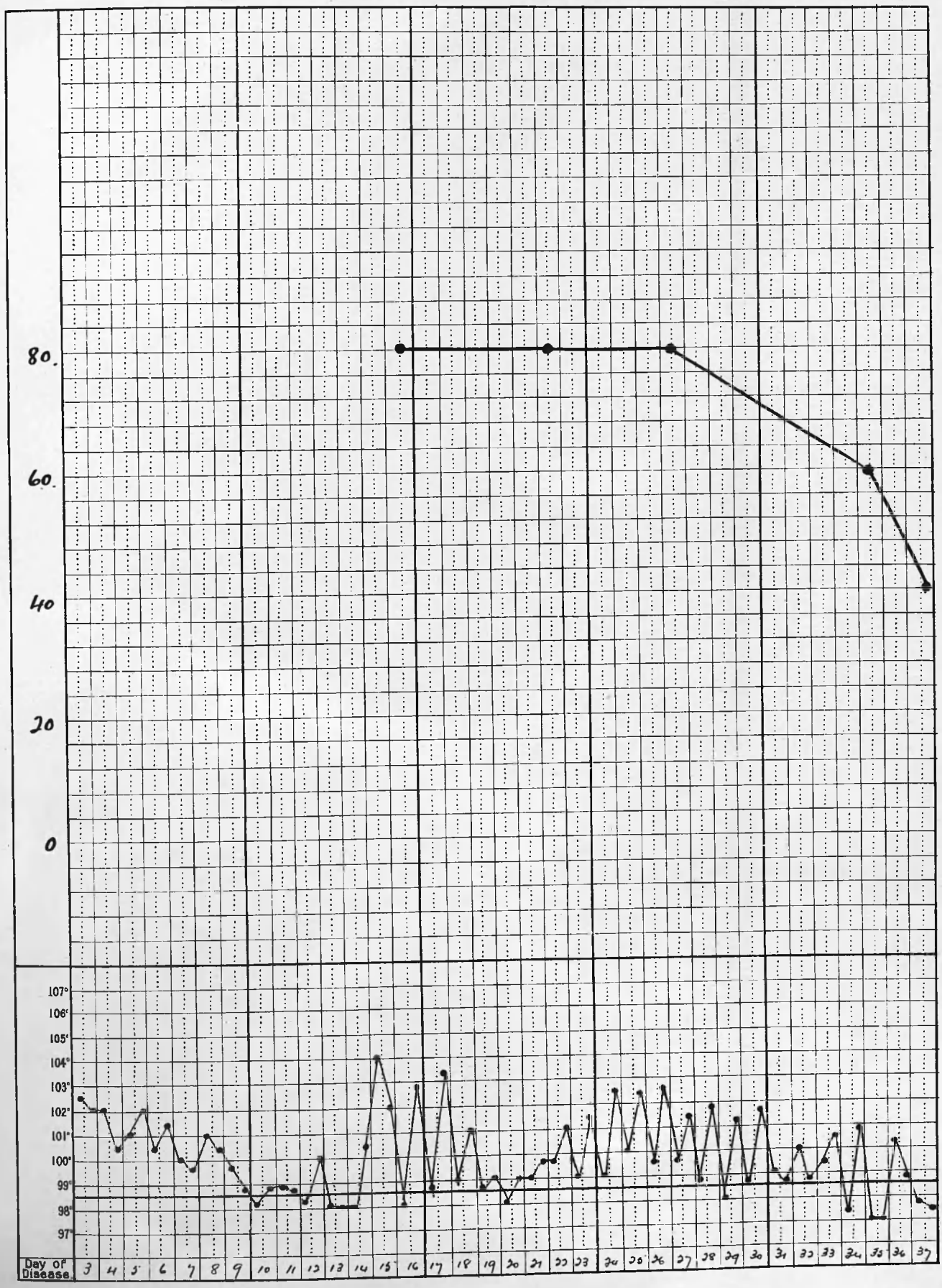
Case 1.



Chart, No. V.

Group B. A. F. 4 yrs. AGGLUTINATION.

Case 9.



become bright, intelligent, having lost all symptoms of irritability. Nuchal stiffness has disappeared or is very slight. Kernig's sign is either absent or remains just appreciable for a day or two: convalescence is established. The temperature falls suddenly by crisis or sometimes by short lysis, and may then remain normal throughout convalescence.

These cases belong to the type known as the abortive type, from the sudden way in which the disease is cut short. Reaction sets in in a very marked manner clinically, and the blood experiments indicate that the evidence of this successful resistance is found in the development of agglutinins in the blood to a high degree.

The cases in this group may be further sub-divided, according to the subsequent course of the case after crisis.

I - Cases which recovered immediately after crisis, temperature remaining normal during convalescence:-

Case		Day of Crisis	
1	J. K.	8	Chart II
2	A. G.	7	" IV
3	M. A.	6*	
4	Mrs. D.	8	

*In this case there were three days further rise of temperature

II - Cases which had a subsequent secondary chronic stage, after a period of normal temperature after crisis:-

Case		Primary Temperature	Normal Period	Secondary Temperature	
5	M. G.	7 Days	10 Days	Variable - Died	
6	A. F.	9 "	4 "	3½ Weeks	Chart V
7	A. W.	9 "	2 "	25 Days	
8	H. O'H.	7 "	2 "	9 "	

III - Cases in which primary pyrexia merged into that of secondary period:-

Case		Day of Crisis	Total Temperature	
9	C. C.	4	25 Days	
10	A. C.	7	21 Days, then variable till death	Chart III
11	J. H.	6	25 Days	
12	J. B.	7	Indefinite, variable till death	

This sub-division of cases whose sera agglutinate most strongly shows that the agglutinating power may be as strong in a case which goes on to secondary fever, as in a case which recovers immediately after the acute infection passes off, and that high agglutinating power is no guarantee that the case will not become chronic. Further, it shows that the intensity of agglutination depends solely on the initial features, and is not augmented during a subsequent chronic stage, should such a period be entered upon.

THE RELATIONSHIP OF AGGLUTINATING POWER TO THE CRISIS IN ACUTE REACTING CASES

This point is difficult to determine, and would require data furnished by daily observation on a number of acute cases. Typical acute cases recovering by crisis are not common; and it is difficult to get an opportunity of observing such cases for some days before the reaction occurs. In none of my cases were daily observations made. In the absence of more accurate data, reference to the table of Group B containing the acute cases will show, broadly speaking, that agglutinating power was at its highest within a few days of the crisis. There are three cases in which tests were made before the crisis:-

	Days of Illness							
	4	5	6	7	8	9	10	11
A. G.	40			C	80			
J. M.			40		C			100
J. F.	C	20		60			100	

Chart I

"C" indicates day on which marked improvement occurred

In the last case it will be seen that the crisis occurred on the fourth day, while on the day following the blood agglutinated at a dilution of 1-20, and did not agglutinate at 1-100 till the tenth day. This patient relapsed, however, on the sixth day of illness, and died on the 10th day. This may account for the subsequent increase in agglutinating power after

the crisis.

RELATION OF DURATION OF CASE TO AGGLUTINATING POWER

The average day of crisis on which definite recovery from the acute symptoms took place in the cases in Group B was the seventh day. The earliest was the fourth day; the latest, the eighth day. In six of the 12 cases it occurred on the eighth day; in three, on the seventh day. There are other cases, however, which run a shorter initial course, and recover suddenly after an acute illness lasting two or three days. Such short cases are difficult to obtain verified by recovery of the organism by lumbar puncture, and most of them do not reach hospital. The relatives of patients admitted to hospital often gave accounts of illnesses in other members of the family, acute in onset and terminating abruptly on the second or third day, the symptoms resembling those of influenza. Again, patients admitted in the chronic stage often give histories of acute onset with sudden abatement of the symptoms of initial infection on an early day. But accurate reliable histories are difficult to obtain.

The following is an account of six cases of this type:-

(a) Two were admitted acutely ill and recovered perfectly on the day of admission, the one on the third day and the other on the evening of the third day or morning of the fourth day. Both were verified by lumbar puncture.

(b) Two cases admitted in the chronic stage whose early histories were accurate. The one case had a short acute ill-

ness of 24 hours' duration; the other had an acute illness lasting for 48 hours. He was then well for some days, but finally lapsed into the chronic stage. In both, the organism was recovered by lumbar puncture.

(c) Two cases from patients who had acute illnesses resembling cerebro-spinal fever of short duration; three days in each case followed by complete recovery. The blood was obtained during convalescence. These cases were not punctured.

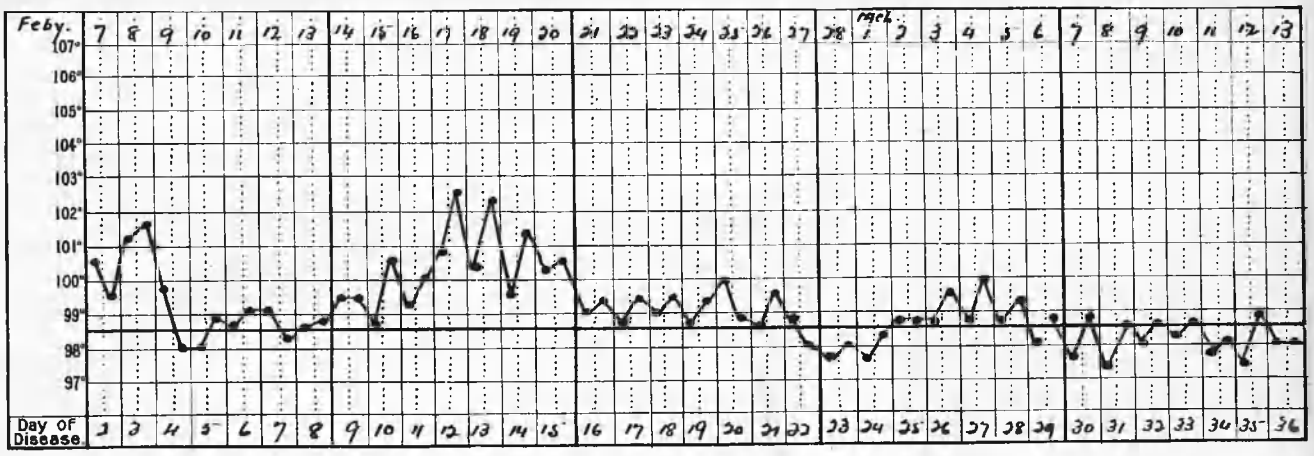
Case	Age	Agg.	Day of Observation	
1 - R. S.	9	1-10	7th Day	Chart VI.
		Nil	A month later	
		Nil	2 "	
2 - L. M'C.	5	1-10	7th Day	Chart VII.
3 - D. D.	14	Nil	30th "	
		Nil	58th "	
4 - J. C.	40	1-5	8th "	Chart. VIII.
		Nil	18th "	
		Nil	30th "	
5 and 6		Nil	Convalescence	

From these data it is evident that the duration of the acute early symptoms has an important relation to the amount of agglutinin in the blood. In those cases with short periods, agglutinins are absent altogether. It will thus be clear that agglutination is of little diagnostic value in those cases in which it might be of most service, which cannot be otherwise definitely diagnosed, unless by lumbar puncture during the acute attack.

The temperature charts of three of these cases are appended, as they show that agglutination may be absent in cases of very dissimilar type. For instance, the chronic stage in the case of J. C. lasting 24 days had no effect in augmenting the

Cases in which agglutinating power of the Blood was absent or feeble. 2/a

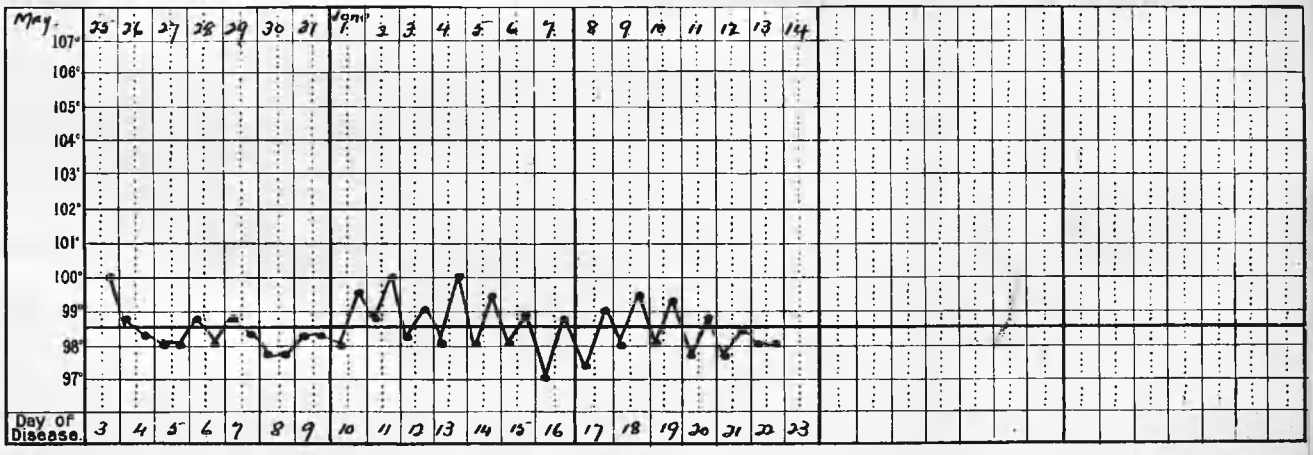
See p. 21.



CASE. I R.S. aet 9. Very acute onset on day before admission: insensible, delirious and screaming next day: deeply comatose on admission. Head stiff, not retracted; flushed facies. On third day more sensible though dazed. On morning of 4th day is bright sensible and intelligent: deaf: muscular condition lax.

Subsequent temp. due to otitis media.

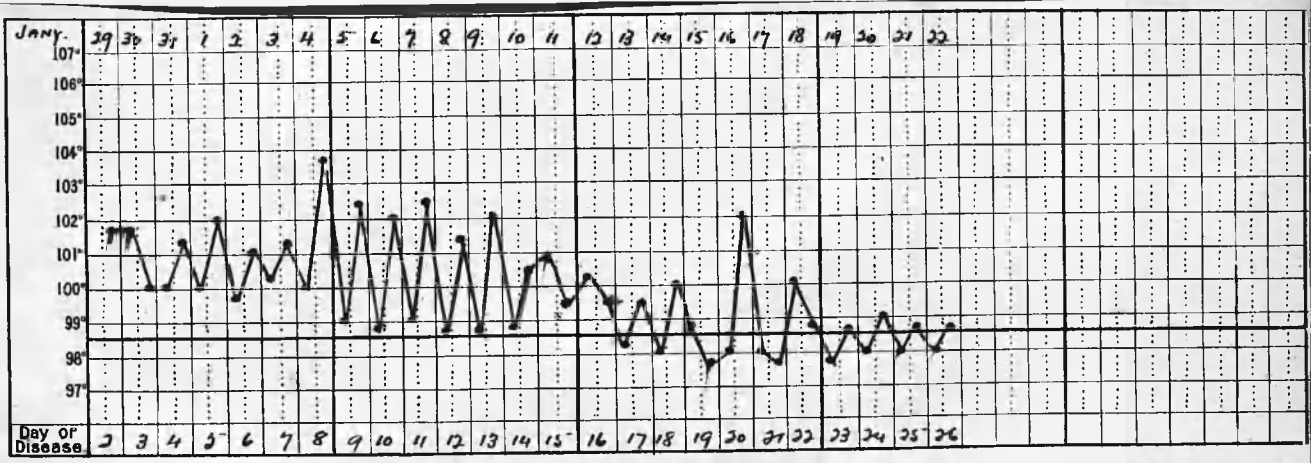
Chart, No. 6



CASE II L. McC. aet 5.

Acute onset, very ill on admission. Crisis on evening of day of admission.

Chart, No. 7.



J. C.

CASE. IV aet 40.

Short acute early infection lasting 24 - 48 hours.

Typical secondary or chronic stage

Chart, No. VIII

agglutinating power of the patient's blood. The case of D. D. is much the same; a prolonged chronic emaciating course was not accompanied by increased agglutinating power.

SUMMARY

(a) Agglutination is related to one special phase of the disease, the acute early infection.

(b) It is proportional to - 1. the severity of the symptoms; 2. the critical nature of the recovery; 3. the duration of the period.

(c) Short sharp infections do not produce agglutinins in the blood, even though followed by long secondary illness.

(d) The agglutinating power is not affected by the subsequent course of the case after the crisis.

(e) Agglutination is of no value in detecting short abortive forms of the disease.

(f) Although high agglutinating power accompanies successful resistance to infection when severe and prolonged, it does not indicate that a further chronic stage will be avoided. In fact, eight out of the 12 cases had subsequent chronic periods; in two of the cases prolonged, and ending fatally.

The difficulties in the way of basing prognoses on blood conditions will be dealt with later.

SHORT ACCOUNT of the Chief Clinical Features of Cases whose Blood possessed High Agglutinating Power

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GROUP B

A. F., aet. 4 - Admitted third day unconscious, flushed, restless; remained acutely ill till ninth day. Temperature falls from 101° to normal during eighth and ninth days; is then normal for five days. Second temperature, 23 days; recovery. (Chart No. V)

A. C., aet. 23 - Admitted fifth day. Sudden onset with vomiting and headache; extremely delirious and unconscious on admission. Rash present sixth day; acute symptoms pass off; patient quiet, conscious, intelligent; head very stiff and painful. Enters chronic stage with 18 days' temperature; death. (Chart No. III)

H. O'H., aet. 14 - Admitted second day. Headache and vomiting at onset; semi-conscious, quiet, flushed, head slightly stiff. Rash; fourth day becomes delirious, restless, herpes. On eighth day is conscious, intelligent, deaf. Occasional rise of temperature; recovery.

A. W., aet. 16 - Admitted fourth day; onset with headache and vomiting; next day delirious. On admission very acutely ill, delirious and noisy; herpes. Crisis eighth day; temperature falls from 101° to normal. Next day much brighter, intelligent, deaf. Enters chronic stage - 24 days; recovery.

J. K., aet. 9 - Admitted fourth day. Onset with headache, vomiting;

drowsy on admission; irritable, very restless, flushed; head very stiff; herpes. Eighth day crisis, temperature falls from 101°6 to 99°; between eighth and ninth days very marked improvement; becomes quiet, composed, intelligent; head less retracted. No further temperature; recovery. Pseudo-crisis fifth day, with no abatement of symptoms. (Chart No. II)

A. G., aet. 11 - Admitted third day; very acute onset with rigors, headache and vomiting. Appears to have been delirious and unconscious for a few hours. On admission, conscious but deaf, very talkative, and at times noisy; herpes. On seventh day very marked change occurred; patient fell asleep; wakened up saying she felt better; head stiffness gone; Kernig absent. Temperature fell from 102° to normal on seventh day. Pseudo-crisis fifth day without abatement of symptoms; temperature remained normal; recovery. (Chart No. IV)

Mrs. D., aet. 48 - Admitted seventh day. Onset with rigor, headache, no loss of consciousness. History somewhat indefinite. Temperature falls to normal on 11th day by slow lysis. Patient had agonizing headaches; temperature remained normal; recovery.

F. A., aet. 4½ - Admitted fifth day; headache and vomiting severe. On day before admission was unconscious and restless. On admission is painfully restless; dusky flush. Suddenly on sixth day temperature falls from 102°4 to normal; recovery. Kernig's sign and some stiffness of neck were present a week later.

K. C., aet. 15 - Admitted third day of illness. On admission, conscious, intelligent, restful, but deaf. History of sudden onset; was delirious a few hours after onset. Next morning recovered consciousness, and was deaf. Conjunctival haemorrhages;

enters second stage. Total temperature-24 days; recovery.

J. B., aet. 4 - Admitted fourth day; onset with sickness and vomiting. Next day semi-conscious, very restless and screaming. Admitted drowsy, flushed. Temperature falls by short lysis to normal on eighth day with marked improvement. Child is now bright and lively; has slight stiffness of head, and Kernig slightly present. On 14th day patient enters chronic stage; death.

K. G., aet 10 - Admitted fourth day; onset with headache and vomiting. Next day becomes unconscious, then delirious. On admission is talkative and dazed; remained talkative, drowsy, unsettled, and flushed. Herpes on seventh day. On eighth day temperature falls to normal from 101°6. Child is quiet, bright, and intelligent. On 16th day neck stiffness quite gone; Kernig almost gone. Recurrence on 18th day, secondary temperature; death.

J. H., aet. 10 - Admitted fourth day; onset with rigors and headache; vomiting and general pain. Deeply unconscious on admission; restless and noisy; cyanosed. Haemorrhagic spots on conjunctiva. On sixth day is quieter; on seventh day is bright and intelligent, without pain, but no fall in temperature occurred. Enters second period; total duration, 25 days; recovery.

GROUP C

In this group are included all cases examined other than the acute fatal cases before described (Group A), in which no agglutinins were present on the dates of examination. Cases where the reaction was doubtful in 1-5 or 1-10 dilutions were considered negative.

There are 21 cases, including five children under two years of age. Eleven cases recovered after more or less prolonged periods of chronicity, with variable temperatures. There were 10 deaths, with four post-mortem examinations.

Many of these cases were admitted in the chronic stages, so that in some the histories of initial character of illness is indefinite. A short note of the features as regards onset is given where the cases were observed early and the history definite. These are all characterized by the absence or short duration of acute toxic symptoms at the beginning of their illness. The onset is mild, with symptoms of local irritation chiefly; the symptoms develop gradually, often insidiously, with remissions perhaps for a day or two. There is vomiting, and headache may be severe. Retraction of the head develops, and may become extreme. Wasting commences, and the case assumes a chronic course with resolution after three or four weeks, or prolonged indefinitely. Agglutinating power is thus absent or feeble when the case commences as a chronic one, or has had a short initial acute period.

This table also corroborates what has been already noted, that the chronic or secondary stage does not give rise to ag-

Groups

C. CHRONIC CASES – no agglutination.

Days of illness on which observations made.

NO.	NAME.	AGE.	7.	8	10	11	13	16	18	22	27	28	30	33	35	38	42	57	58	65	79.	RESULT.
1	W.B.	4						5⁻	5⁻													R.
2	P.C.	6								0				0			0					D.
3.	A.D.	14									0											D.
4.	H.P.	8	5⁻				0					0										R.
5	J.D.	3 1/3		0							5⁻				5							R.
6	D.D.	14											0									R.
7.	T.M.	14			0		0		0													D.
8.	M.M.	3					0								0							D.
9	J.J.	6								0												R.
10	A.S.	8	5⁻													5⁻						D.
11	J.C.	40		5⁻					0													R.
12	T.C.	3			0			0														R.
13	T.P.	16				0					0											R.
14	M.C.	5⁻							0				0									R.
15	M.C.	7									0											R.
16.	B.E.	2															0			0		D.
17.	A.M.	1 1/2									0	0		0			0	0				D.
18.	V.M.	2																	10	0	0	R.
19.	F.R.	11/12																		0	0	D.
20	H.H.	2		0																		D.
21	J.M.	1 3/12			0		0															D.

R. = RECOVERED. D. = DIED.

glutinins in the blood.

SHORT ACCOUNT OF CASES IN GROUP C

Case

1 Onset with headache, pain in legs; sickness a fortnight before admission. Return of headache and retraction five days before admission. Temperature = 30 days; recovery.

2 Admitted on third day of illness; drowsy, quiet, semi-comatose; enters chronic stage without definite reaction. Temperature = 24 days; died 53rd day.

3 Mild onset four weeks before admission; got well; recurrence; chronic stage on admission. Died in seventh week.

4 Admitted fifth day, not very ill; quite conscious and intelligent; enters chronic stage. Temperature = 24 days; recovery.

5 Took ill 10 days before admission with headache; no acute symptoms in chronic stage; recovery.

6 Admitted fourth week. History of acute onset with unconsciousness; sudden recovery; deaf; chronic stage. Recovery.

7 Onset with headache and vomiting; no history of coma or delirium. On admission, retraction and great headache. Died 22nd day.

8 Admitted on second day of illness. Onset sudden, with vomiting and screaming; head retracted, squint. Died.

9 Onset with headache and sickness. No acute toxic symptoms; local only. Recovered.

10 Onset with headache, vomiting, pain in back; no very severe symptoms; conscious and intelligent on admission. Died 32nd day.

11 Acute onset; coma for 24 hours, then enters chronic wasting stage. Temperature = 24 days; recovery.

13 Admitted in fourth week; indefinite.

14 Acute onset; rash; very prolonged course.

16 Admitted fifth day. Symptoms of onset local only; no general symptoms

GROUP D

This group comprises those cases examined which ran a more or less chronic course, and whose agglutinating power varies from 1-10 to 1-80. There are 21 cases - 11 recoveries and 10 deaths. There were five post-mortem examinations. In one case there was persistent basal exudation and hydrocephalus; in two cases, organised basal exudation with hydrocephalus; in two cases vertical extension had occurred, and was the cause of death.

This list includes the more prolonged cases in which there was definite evidence of agglutination, ranging from 1-10 to 1-80. They were all cases which had prolonged secondary periods of temperature of varying duration.

Here also it is found that agglutinating power of the serum tends to be proportional to the initial severity, being present in those cases which commence with symptoms of an acute

Group.

D.

Days of illness on which observations were made.

NO.	NAME.	AGE.	4	5⁻	6	7	8	9	10	11	12	13	14	15⁻	17	20	22	23	27	28	30	32	33	34	35⁻	38	39	40	41	42	44	45⁻	46	48	66	67	68	69	70	71	77	79	84	92	RESULT.
1	D.M.	8											60							40																								D.
2	K.M.	6																											100				80	60					20					D.
3	S.M.	28														60								40																		10		R.
4	J.M.	15⁻	40												20																													R.
5⁻	B.S.	16																									40			20				40	40		40		40				D.
6	A.T.	16								10			10																														D.
7	A.W.	3½			40			40						40					20			20							20		40										10	R.
8	A.D.	15⁻		20		40			40	20								10																									D.
9	M.K.	5⁻					20																																				R.
10	W.K.	3					10		10					10																														D.
11	A.M.	7									20																																	R.
12	W.M.	25⁻																		20																								D
13	T.C.	10				20																				10																	R.
14	A.H.	3⅓															20																											R.
15⁻	J.W.	6												20													10																	D.
16	W.M.	16																					10																					R.
17	A.M.	10															20	20																										D.
18	M.R.	7			10																																							R.
19	D.M.	10											60															40																D.
20	S.M.	6						20			20																																	R.

D.= DIED. R. = RECOVERED.

infection, and show evidence of a marked improvement early in the disease; for instance, the first case quoted had a definite crisis on the fifth day of illness.

This table also shows that, if agglutination is low in the early phase of the disease, it remains low throughout in spite of prolonged pyrexia and profound wasting.

SHORT ACCOUNT OF CASES IN GROUP D

Case

1 Onset acute; headache, vomiting, unconsciousness and restlessness. Recovery from acute symptoms on the fifth day; restlessness and irritability remain. Recurrence on sixth day. Died on 34th day.

2 History of acute onset but very indefinite. Admitted on 11th day of illness in secondary stage. Temperature variable. Died on 58th day.

3 Admitted on second day of illness; actively delirious. On fourth day was much quieter and composed; on sixth day irritability returns; chronic wasting stage. Recovery.

4 Admitted on third day of illness; comatose, quiet, head retracted. On fourth day recovered his senses; very well and restful for two days; return of irritable symptoms on sixth day. Temperature = 26 days. Recovered.

5 History of this case very indefinite. Admitted on fifth day of illness; chronic stage. Died.

6 Onset slow and gradual, with headache and vomiting, and periods

of remission of symptoms. Admitted on fifth day of illness; quite conscious and in great pain and very restless; herpes on ninth day. Died on 24th day.

7 Onset acute; headache and vomiting. Admitted on third day of illness; very flushed, semi-unconscious; head retracted. Temperature = 28 days. Recovery.

8 On admission was unconscious, flushed and quiet. Remained very ill till 10th day, then become more sensible. Temperature indefinite. Died on 40th day.

9 Admitted on fifth day of illness; quite comatose; no definite critical improvement. Temperature = 28 days. Recovery.

10 Admitted on fifth day of illness; flushed, drowsy, insensible, and very irritable; decided improvement on ninth day; merges into chronic stage. Indefinite pyrexia. Died on 73rd day.

11 Admitted on eighth day of illness. History of acute onset with semi-unconsciousness. Conscious on admission; in chronic stage with much head retraction. Temperature = 26 days. Recovery.

12 Admitted on fifth day of illness. Conscious, but with marked symptoms of acute spinal irritation; onset with rigor and headache; enters second stage with great irritability, restlessness and emaciation. Died on 50th day.

13 Symptoms on admission, those of marked cerebro-spinal irritation; head retraction and opisthotonos. Early history indefinite of delirium and restlessness; onset acute; chronic stage, 30 days. Recovery.

14 Onset sharp, with headache and vomiting; said to have been delir-

ious at intervals; sharply ill on admission; some haemorrhages. Temperature = 24 days; chronic stage. Recovery.

15 Admitted on sixth day of illness; semi-unconscious with head retracted; flushed facies. On eighth day, temperature falls and consciousness returns; enters chronic stage. Died on 65th day.

16 Admitted on 15th day. History indefinite. Recovery.

17 Admitted on fifth day. History indefinite. Gradual but decided improvement in general condition; was very restless and noisy on admission, but by ninth day was quiet, bright and intelligent; cessation of acute symptoms. Died on 71st day.

18 Admitted on fourth day; no loss of consciousness or delirium in history; symptoms local from onset; headache and vomiting; chronic stage. Temperature = 44 days. Recovery.

19 Admitted second day; comatose and delirious; quiet delirium. On fifth day sudden return of consciousness; is bright and intelligent. No pain; is generally comfortable. No crisis in temperature, or a very transient one. Died 36th day.

20 Admitted in chronic stage with head retraction; quite conscious; no general symptoms. History very indefinite. Temperature indefinite. Recovery.

Bettencourt and Franca[4] found that sera from patients with cerebro-spinal meningitis agglutinated the meningococcus in dilution from 1-10 to 1-100. Weichselbaum and Ghon[23] obtained similar results. Von Lingelsheim[16] obtained positive results in 32% of all cases examined, in dilutions of 1-10 to 1-200, and sometimes 1-400. Normal sera, and sera from patients with other diseases, did not agglutinate the meningococcus. He concluded

that agglutinating power had no definite relationship to the onset of the disease. In my cases this was the most decided feature elicited. Goodwin and Von Sholly[10] found specific agglutinins in a few cases, the maximum dilution being 1-200. Davis[6] examined seven cases; in one the organism was agglutinated at 1-500 dilution; in two cases, at a dilution of 1 in 100; the others agglutinating at varying strengths up to 1 in 100 dilution.

The differences in agglutinating strength here noted may be due to the effect of different media employed, and to the age of the culture on agglutinability.

GENERAL RESULTS

1. In very acute infections where toxaemic symptoms are severe and the case ends fatally, there is an entire absence of agglutinins from the blood. These cases formed the chief epidemic type.

2. On the other hand, the blood in very chronic cases contains no agglutinins. The organism settles down locally at the base of the brain without causing general acute symptoms, and a chronic infection results. These cases may have prolonged pyrexial courses, but the specific toxins absorbed ~~are~~ do not ~~such as~~ cause the formation of agglutinins.

3. Maximum agglutinating power in any given case depends on the initial features of the case, being proportional to (a) acuteness of onset, (b) duration of primary toxaemia, and (c) degree of reaction. The typical case is that which commences with symptoms of severe toxaemia, and terminates by crisis on the

seventh or eighth day without any secondary period.

4. In short acute cases lasting from two to three days, agglutinins are usually absent or of feeble power; hence they are of little value in the diagnosis of such short, often indefinite, cases. Some method of diagnosing such cases would be of great value from an epidemiological standpoint.

5. Agglutinins are produced only in antagonism to the early toxins; they appear to be independent of the subsequent course of the case.

6. The disease in infants presents the typical chronic case. In five cases under aetat two there were no agglutinins in the blood.

SECTION II

O P S O N I C I N D E X

THE ORGANISM

The chief difficulties in this work centre round the cultivation of the organism. The organism is so delicate and so liable to degeneration that the conditions to be fulfilled for successful work are very exacting. These are, adequate amount of growth with absence or at least a minimum of degeneration. It was only after much trial and close attention to detail that the latter condition was successfully fulfilled.

Various media were tried:-

1. Glycerine Agar - This was found to be useless, as the growth was rarely rapid enough to prevent the presence of degeneration forms, and subcultures rapidly died out.

2. Nutrose Ascitic Glucose Agar - This was used for a time. It gave somewhat better growths, but had the same disadvantages.

3. Coagulated Blood Serum - This medium proved useless for the same reasons.

4. Blood Agar. It was thought that this medium, having

been so suitable for agglutination purposes, would yield satisfactory results. But it was found that the chief mode of growth was by formation of tetrads, which interfered with accurate estimations. Further, here also, degeneration forms were often considerable in amount in 24 hours, which was the shortest time in which a proper quantity of growth could be obtained. (a) Growth is not sufficiently rapid to avoid the presence of a percentage of small and disrupted cocci. (b) Tetrad formation is a common mode of growth.

5. Löffler's Alkaline Serum - This was tried alone, and with the addition of glucose or maltose; on this medium, very rapid growths can be obtained which approximate to the desired conditions. The addition of maltose gives even better results than glucose. By careful attention to detail, subcultures can be obtained, in which a sufficient quantity of organisms are present, in six to eight hours or even earlier, to form the necessary emulsion. The great majority of organisms will be found in a condition of perfect preservation with practically no degeneration forms. There is, however, even on this medium, some uncertainty of growth, but this can often be avoided with care. It may happen that successive subcultures grow at varying rates, some taking 12 hours, others 24 hours, to appear, the growths being more or less scanty. This is likely to occur if too great an interval of time supervenes between the subcultures. Thus, in order to preserve the organism in good cultural condition, it must be subcultivated at short and regular intervals.

Absence of degeneration is directly proportional to rate of growth; so that, from the results obtained it may be laid

down that, if a definite growth is obtained in six or eight hours, the resulting organism will be vigorous, free from degeneration forms, the diplococci often large, well formed and staining deeply; and on this medium there is a complete absence of tetrads. Growth will be sufficient for a large number of observations. It is not safe to use cultures that have taken longer than this to appear without a preliminary examination to see if they are suitable. According to my experience, if growth is delayed longer than this time, the organism will be small, with tendency to degeneration. A culture which has yielded a copious growth in six hours may be used at any hour up to about 12 hours. Thus, growth must be of a definite rapidity in order that degeneration of the cocci be avoided.

To summarize - the following conditions must be observed:-

(a) Medium - Löffler's alkaline serum with addition of glucose or maltose (preferably the latter)

(b) Daily subculture is advisable, or at most every second day

(c) Growth must be sufficient in six hours; the quicker the growth, the better the result

By means of repeated subculture at intervals of one or two days, the organism can be kept in a uniformly vigorous condition for months. If a longer time is allowed to elapse between the subcultures, they will grow too slowly and be useless. In fact, the culture which is to be used for observation must be made from a culture not more than 48 hours old. If the culture to be used is older than two or three days, a subculture should be made on the day prior to the proposed experiment.

Other conditions aid in obtaining a satisfactory organism.

The surface of the medium should be moist; should it be dry, a loopful of bouillon may be smeared over it before inoculation. There often appears to be some variable delay in the commencement of growth. I have often found that, where growth is delayed, it may be stimulated by re-spreading the surface of the medium with a platinum wire. Where this has been practised as a routine, I have occasionally been able to obtain an excellent culture in four hours. This re-spreading may be done one to two hours after inoculation.

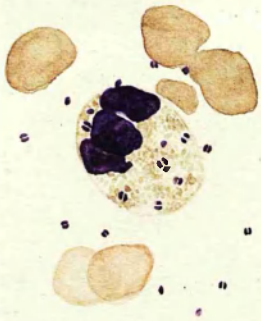
The temperature of the incubator was kept at 38-40° C. By observing these conditions, stock cultures may be preserved in a uniformly active condition. If cultivated in an erratic fashion, the organisms are likely to become small and degenerate. The relationship between this and its suitability for opsonic index is described in a later section.

Sometimes even with Löffler's serum, cultures fail to grow in an unexpected way. They may not grow with sufficient rapidity in spite of the above conditions being observed. This may sometimes be related to the use of a fresh supply of serum. A culture which was yielding an organism of large size and of rapid growth will suddenly yield cocci smaller form, and with degeneration forms appearing early. This is likely to happen if (a) too long a time elapses since last culture, (b) with a fresh supply of serum tubes, before the organism becomes accustomed to the fresh medium.

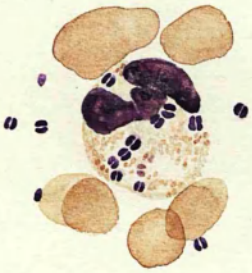
MODE OF OBTAINING GROWTHS FROM THE CEREBRO-SPINAL FLUID

When the case is in the early stages with acute symptoms, and the cerebro-spinal fluid turbid and organisms plentiful and vigorous, cultures are readily obtained on any of the media described. The one in most common use in these cases was Löffler's alkaline serum. It was occasionally possible to obtain on this medium a primary culture with the organisms in good enough condition for use in opsonic index estimations. But as a rule, growth was not evident for 12 to 24 hours. It was quite possible, however, to obtain the first subculture in suitable condition.

Later, when the chronic stage has set in with the cerebro-spinal fluid containing a small amount of cellular exudate and very few organisms, the blood agar medium is by far the most satisfactory. The cerebro-spinal fluid being allowed to stand, a fine coagulum forms, which contracts and retains whatever organism may be present. This clot is smeared over the surface of a blood agar tube, and incubated at 37° C. Growth will be slow if the case is a very chronic one, a colony or two gradually appearing as starlike growths on the second or often not till the third day. The organism can then be subcultured on blood agar, from which it may be transferred to Löffler's serum and used for opsonic index estimations. Direct transference to Löffler's serum from the primary growth may not be successful. In this way it is almost always possible to obtain the patient's own organism for use in any stage



Pl. 7. To shew small form of meningococcus.



Pl. 8. To shew large form of meningococcus.

of the disease. In one case which remained in a chronic hydrocephalic condition for almost a year and ultimately recovered, it was possible to obtain growths six months after the onset of the illness.

The organism in culture varies very much in size; a vigorous growth may be found to yield organisms uniformly large or uniformly small - mixed types do not readily occur. The relative sizes of cocci are shown in the drawings annexed.

These variations in size, in my experience, have nothing to do with the particular strain of organism used. They are a variable feature of its cultural life. The size of the organism may vary from culture to culture in the same strain. In general, it may be said that the optimum conditions described tend to produce a large form of organism, while adverse conditions produce small forms. When growth is slow, the forms are usually small. It has also been observed that the smaller forms are more liable to become degenerate. The size of the organism as it exists in the cerebro-spinal fluid does not appear to have any relation to the severity of the case. In fact, in the same fluid diplococci of all sizes may be met with. Thus (a) a vigorous culture produced under the best conditions may yield either a large organism or a small one. (b) The smaller size, however, tends to show evidences of early degeneration more readily than the larger size.

The relative behaviour of organisms of various sizes towards opsonins is of interest. There is a general tendency for the larger type to resist phagocytosis. I cannot say that this is an invariable rule, but there is an undoubted general tendency for the organism to behave in this manner. As an example, two strains of organism used for comparison, both of

the same age, six hours, yield vigorous growths, but the organisms in growth A are large, while those in B are small. Emulsions are used of approximately the same concentration. In the resulting films with the pool or normal serum, it may be found that organism A (the large form) is scarcely taken up at all, so that the number in 50 cells may be estimated at 12. In the films made with culture B (the small form), the number in 50 leucocytes may read 60. The indices of the cases under examination will be the same, however, with both cultures showing that the two forms of organism have the same relative susceptibility to phagocytosis. These results cannot be due to variation in the leucocytes, as leucocytes from the same person were used in these comparative trials. Where the organism is large and resistant to phagocytosis, it is necessary to count the organisms in at least 100 leucocytes.

TECHNIQUE

The method followed was that of Wright and Douglas.[22] The corpuscles were washed in a solution of Sod. Citrate 1%, in Sod. Chloride .8%, and then passed through a solution of Sod. Chloride .8%. The sera to be examined were always collected from the patients just before the observations were made, as were also the control sera. The leucocytes employed were throughout those of the observer. The organisms were cultivated as described in the foregoing section, a six to eight hours' growth on Löffler's serum being always employed. The emulsion of cocci was made in .8% Sod. Chloride solution. The

quantities used in the actual experiments were:-

1 Vol. Leucocyte emulsion
1 " Serum to be tested
1 " Emulsion of organisms

The period of contact allowed was 15 minutes at 37° C. Leishman's stain was used throughout.

GENERAL DESCRIPTION OF THE ORGANISM AS SEEN IN THE FILMS

When the meningococcus is grown on the medium described, it is possible to obtain a perfect emulsion in salt solution. The cocci are found practically all in diplococcal forms, and only rarely do tetrads occur. Very occasionally spontaneous agglutination in salt solution takes place (vide supra). This was noted with two cultures, and in both cases this peculiarity disappeared in subsequent subcultures. In no instance was clumping present in any of the films with normal sera.

The variation in size of the organism in culture has already been noted. If the culture has been satisfactory, the cocci, whether large or small, will be of typical shape, will stain deeply and equally, will be of uniform size, and clear and distinct in outline; a small number of small degenerate forms may be present. Grouping or agglutination of the organism only occurs under the action of a powerful serum; the most marked agglutination in the films has been found where the phagocytosis is very great and vice versa. Extreme phagocytosis is always accompanied by agglutination of the organism.

The number of organisms ingested by each leucocyte on the

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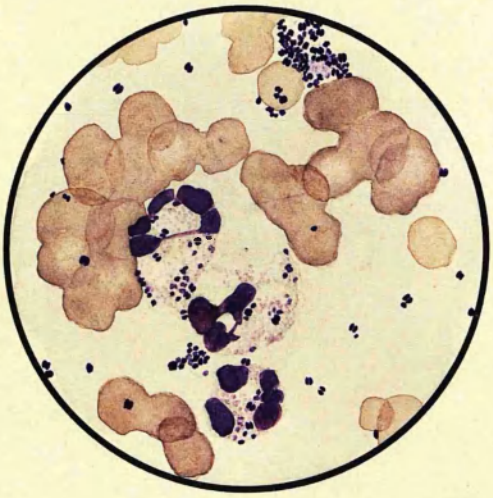

Pl. 9. Showing agglutination and phagocytosis.

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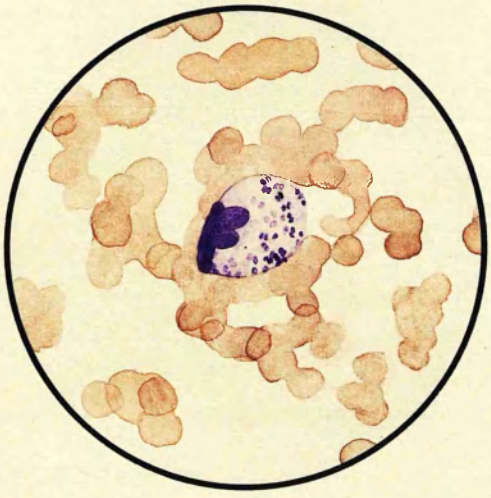


Pl. 10. Intraleucocytic digestion.

with eosinophiles.

In many instances no attempt was made to give a numerical value to phagocytosis, the result being simply noted as "very high". As many as 50% of the leucocytes may contain an almost uncountable number of cocci in an extreme case. Intra leucocytic digestion may render some of the cocci swollen and faint. This is the more likely to appear, the greater the phagocytosis; it is slight in films with normal sera. This may occur even when young and vigorous organisms are used. It appears also that old organisms which are known to be in a state of autolysis are also most actively digested within the leucocytes. The diplococci (a) become swollen, and stain faintly; (b) the components of the diplococci separate from one another. A single phagocyte may show large healthy, evidently recently ingested, diplococci along with others in various degrees of digestion. (Plate 10)

The effect due to the occasional accompanying occurrence of erythro-agglutination and erythro-phagocytosis will be described later.

In the case of sera containing a considerable amount of opsonin, it becomes evident that the phagocytic power of the individual leucocyte varies. That is to say, marked phagocytic activity may be shown by a few leucocytes, while others are taking no part in the process. It is rare to find all the leucocytes containing even approximately the same number of cocci. This is also strikingly the case in the phagocytosis of diplococci in the cerebro-spinal fluid itself. Here, too, even when organisms are abundant and phagocytosis marked, the leucocyte content is very unequal, a few being full of cocci, the great majority containing none.

Chart. No 9.

CASE V

CASE IV

CASE III

CASE II

CASE I

Control cases.

I = M.I. aet. 6 Chronic case. see also Chart-10

II = W.R. aet. 17 Chronic case. " " " 11.

III = Mas D aet. 28 Acute case

IV = P.O. aet. 16 Acute case

V = Mas G. aet. 26 Sub acute case

Day of Disease

97° 98° 99° 100° 101° 102° 103° 104° 105° 106° 107°

May. 1908.

15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 Feb 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 1 2

6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53

Illness

Comparison of strains of meningococcus with charts of control cases.

Each colour represents a different strain.

All results in any vertical line were estimated at the same time.

For full description see text.

VARIATION IN STRAIN OF ORGANISM

EFFECT ON OPSONIC INDEX

In the foregoing sections, the conditions necessary to produce an organism that will give reliable results in opsonic index estimations are described. This involved a prolonged study of the organism before it became possible to obtain results that could be depended upon.

As the observations accumulated, it became clear that the index in cases of similar character was extremely inconstant, and that remarkable fluctuations occurred in the same case. For instance, the case of M. I. illustrates this feature. A reference to the chart nos. 9, 10 will show that a sudden fall of index took place early in the disease, and that the opsonic index remained low for three weeks, during which time there was definite evidence that resolution of the exudation was actively proceeding. The fact that the case was proceeding to cure is shown by a study of the cerebro-spinal fluid, which was drawn off by lumbar puncture at intervals of a few days. Chart no. 10 shews these features. The fluid remained highly turbid for about three weeks and then began to clear up; it became less and less turbid until, on the 27th day, it was almost clear, and no growth was obtained. Organisms during the earlier part of the disease were very numerous, and growths readily obtained. Thus, in spite of the fact that a process of cure was evidently proceeding with phagocytosis active in the cerebro-spinal system, the opsonic index fell almost to normal and remained so during the greater part of the

resolution process.

This having happened in other cases in similar circumstances, it was thought that a sudden variation in the susceptibility of the organism might account for the fall. It was at least necessary to see whether the organism was at fault. To ascertain this, a comparative study of the behaviour of various strains to the opsonic action of various sera was begun. Two chronic cases were selected, the one M. I., and the other W. R., whose index was also being estimated serially at the time, was used as a continuous control. (Chart 9.) Various other cases, both acute and chronic as opportunity offered, were used as controls; also in a number of the observations, the index of a normal person was estimated in addition to the pool count. Thus the same two, M. I. and W. R., were used throughout with different strains, and from time to time other cases were introduced as further controls as occasion offered. In this way two or more strains couldbe compared with reference to their susceptibility to the opsonins of two or more sera.

From these data it could be decided to which factor, serum or organism, any particular variation was due. The results obtained are represented graphically in the chart submitted, in which five different strains of organism are compared from time to time (see also Tables I and II) (page 49). The sera from the two Cases, I and II, were used throughout; other cases were introduced as represented in the chart. In the majority of these observations, 100 leucocytes were counted, and in no case less than 50.

The observations were commenced with a stock culture (blue in the chart) which had been in use for some time, giving good results with other cases. The opsonic index was at first high.

The subsequent sudden fall which took place being difficult to account for, another strain (black) was introduced, recently isolated from an acute case; this strain was about a fortnight old when first used. For four successive observations the indices with this strain were practically the same, and remained low. At the third observation on the 24th day of illness, a third strain (red) isolated from the patient herself was added. This strain had been isolated three days previously, and the first sub-culture was now employed. The index was still low. Thus the period of low opsonic index in this patient was verified with three different strains of organism which were reacting to the sera from other cases. On the 34th day the index suddenly rose to 6.3 in the case of the red strain, and to 7.3 in the case of the black strain. The original blue strain unfortunately became contaminated at this period, and was discarded. The index to the red strain remained high throughout the succeeding observations. The organism was sub-cultured every second day, so that the red culture had reached its fifth sub-culture when the second observation was made. As it was thought that increasing age might have rendered it more susceptible to opsonic action, a fresh strain (green) was now obtained from the same case, and the result with the first sub-culture of the latter organism compared with the sixth sub-culture of the red strain. The index to the red strain or sixth sub-culture was now 7.5, and to the green strain or first sub-culture from the same case, 5.5. In order to verify this difference, a second culture was made from this patient, and two days later a first sub-culture was again compared with, this time, the seventh sub-culture of the original red strain. The index with the former was now 7; with the latter, 8.7. These two

strains, red and green, were then compared from time to time, being sub-cultured every second day, sometimes daily. (A description of this investigation is given in the next section.)

The behaviour of the black strain is, however, peculiar. Comparing Cases I and II in the chart, it will be noted that the black strain and the blue one gave similar indices for the first two observations in both cases. In Case II at the third observation, the index with the black strain fell to 1.2, while that with the blue strain remained at 3.5. This is put into the form of the following table:-

Case	Strains		
	Black	Red	Blue
I, M. I.	1.6	1.9	
II, W. R.	1.2	4.3	3.5
III, Mrs. D.	2.5	6	

Later it will be seen that the indices to the black strain in both cases rose simultaneously to points close to the indices with the red strain in both cases, a suspicious circumstance, as it appeared to indicate that the organism had exhibited latterly low affinity to opsonin. The next variation is still more striking. At the following observation, both indices with the black strain fell almost to normal again - 1.6 in both cases. This fall was controlled by observations with two other strains, red and green, which were found to yield high indices, within 20% of one another. The result with control case III also indicated that the red strain was able to take up its necessary complement of opsonin. Further, two

other control cases which gave high indices with the red and green strains were found to give low ones with the black organism. At the next series of observations, two days later, the black strain was again found to have lost its susceptibility to the opsonin of both cases and a control case. It was then discarded. These facts are arranged in the following table:-

Case	Date Feb.	Strains		
		Black	Red	Green
I, M. I.	17th	1.6	8.7	7
	19th	1.5	6.9	6.3
II, W. R.	17th	1.5	4.5	3.5
	19th	1.6	8.5	3.8
IV, P. O., Control	17th	-	7.8	-
	19th	1.8	8.5	6.2
F. T., Control	17th	1.6	2.5	-
	19th	-	-	-

A fifth strain (brown in the chart) was afterwards introduced from an acute case. The result with this strain agreed fairly well with those yielded by the red and green strains with which it was compared. The relation between the results as regards (a) the sera and (b) the strains is given in tabular form.

TABLE I - THE DIFFERENT STRAINS COMPARED

Date	CASE I				CASE II				CASE III			
	Strains				Strains				Strains			
	Blue	Black	Red	Green	Blue	Black	Red	Green	Blue	Black	Red	Green
Jan. 14	7.8				4.2							
16	8.6				3							
19					2.2							
20	3.6				2.2							
22	2				2.4							
23	2.6	2.8				2.2						
26	2.2	2.6			3.4	2.6						
Feb. 2	1.6		1.8		3.4	1.2	4.4			2.7	6	
7	1.8	2				1.2			2.6	2.6		
12		7.2	6.4			3.2	3.6			2		
15			7.4	5.6			3.8	3.4				
17		1.6	8.6	7		1.6	4.4	3.6				
19		1.6	6.8			1.6	4.4	4				
21			7	5.6								
24			7	7.8			4.2	4.8				
29			9.4	9.2								

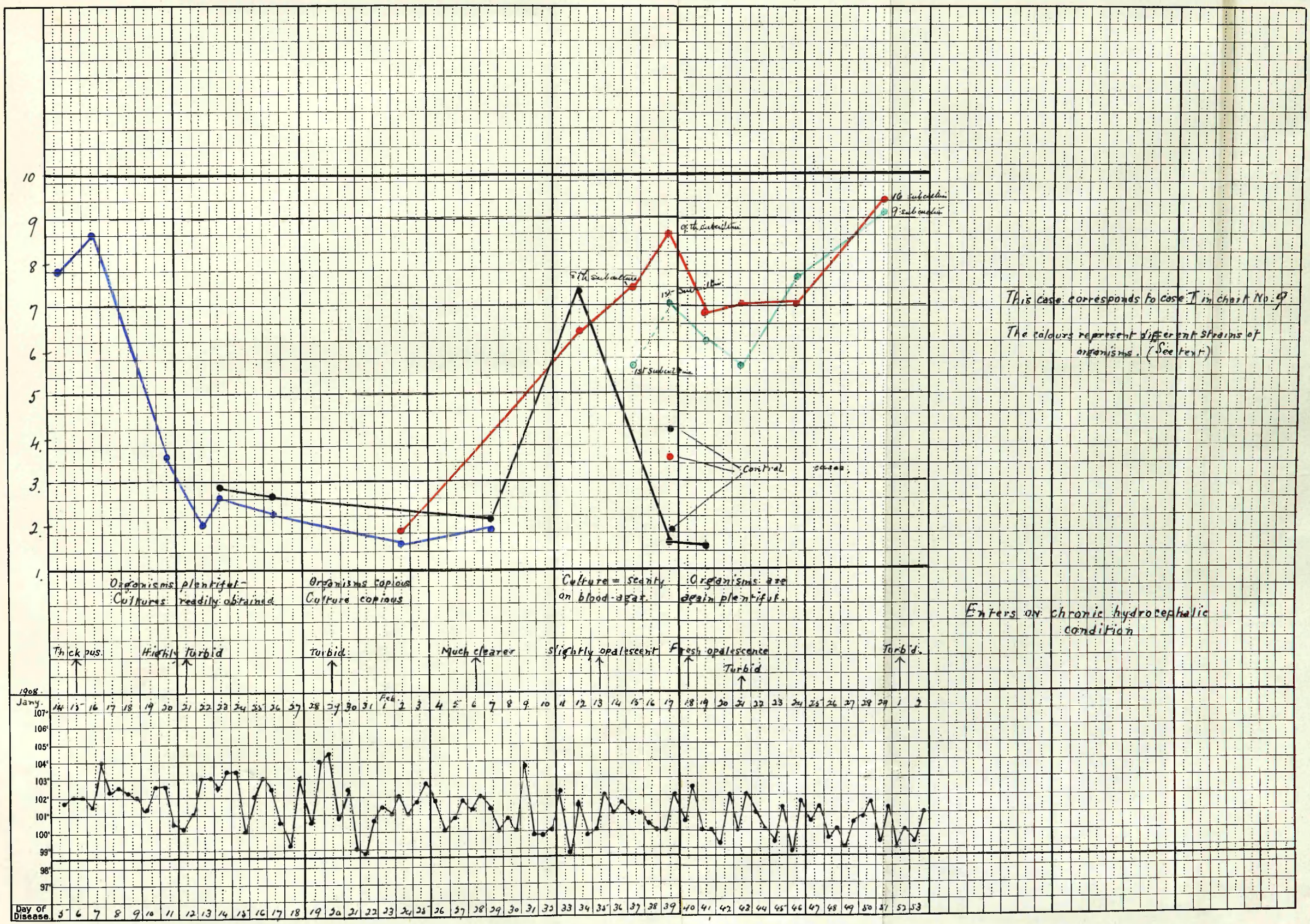
Date	CASE IV				CASE V			
	Strains				Strains			
	Blue	Black	Red	Green	Blue	Black	Red	Green
1.8		7.8						
		8.5						
		7.4			5.4			
		4.6			6		5	
		4			3		4.6	

TABLE II - THE CASES COMPARED

Date	BLUE STRAIN					BLACK STRAIN					RED STRAIN					GREEN STRAIN				
	Cases					Cases					Cases					Cases				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Jan. 14	7.8	4.2																		
16	8.6	3																		
19		2.2																		
20	3.6	2.2																		
22	2	2.4																		
23	2.6					2.8	2.2													
26	2.2	3.4				2.6	2.6													
Feb. 2	1.6	3.4					1.2	2.7			1.8	4	.4	6						
7	1.8		2.6			2	1.2	2.6												
12						7.2	3.2	2			6.4	3	.6							
15											7.4	3	.8			5.6	3.4			
17						1.6	1.6				8.6	4	.4	7.8	3.5	7	3.6			
19				1.8		1.8	1.6				6.8	4	.4	8.5			4			
21											7			7.4	5.4	5.6				
24											7	4	.2	4.6	6	7.8	4.8			5
29											9.4			4	3	9.2			3.8	4.6

Chart, No. 10

M. I. 7 yrs. = Case I in chart 9



This case corresponds to case I in chart No. 9

The colours represent different strains of organisms. (See text)

Enters on chronic hydrocephalic condition

The variation in the condition of the Cerebro-spinal fluids indicate a preliminary attempt at gradual resolution - the fluid being merely opalescent and cultures negative on the 37th day. A reinfection occurred after this, the fluid became again turbid, and organisms plentiful and easily cultivated.

Chart 10.

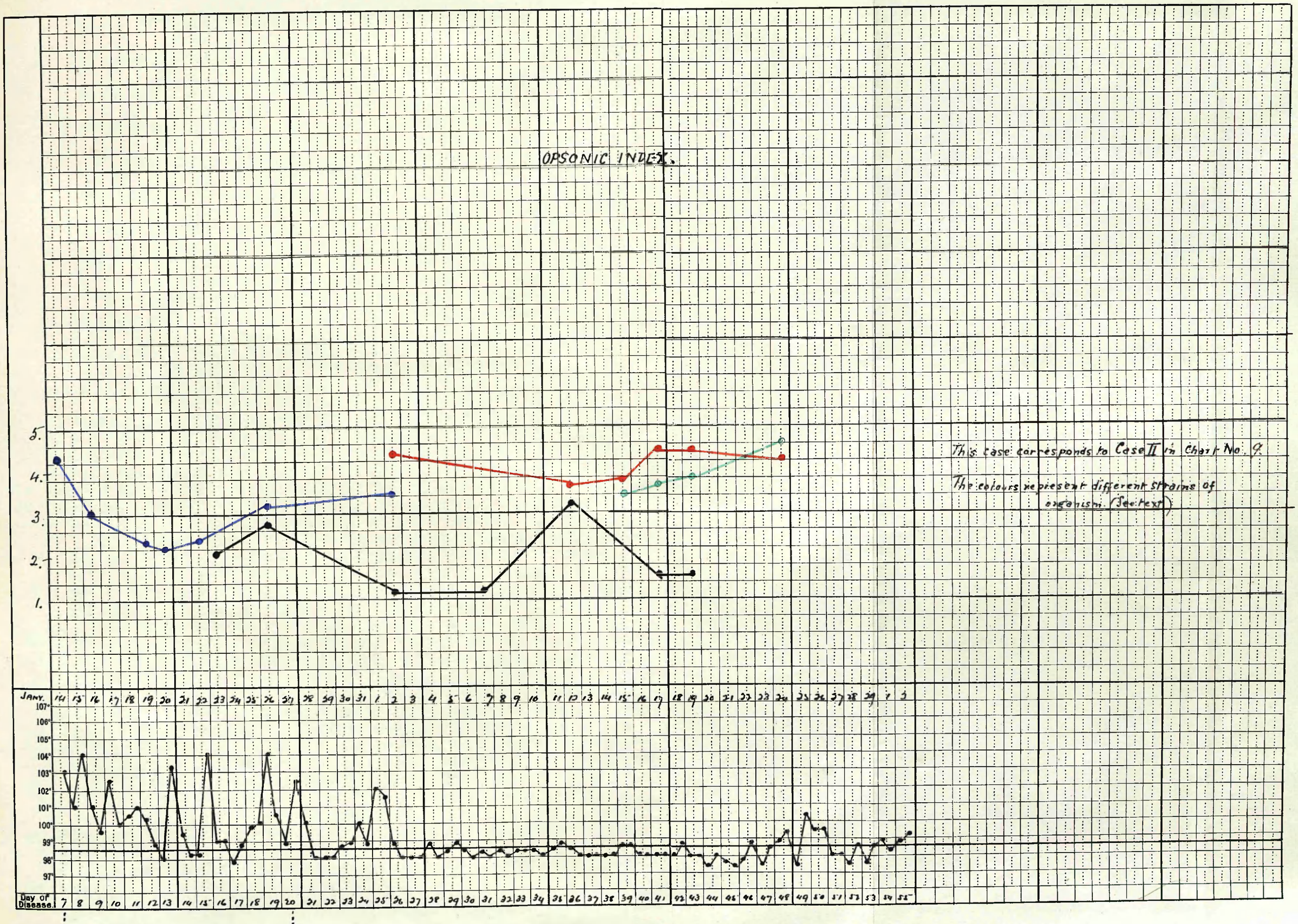
A series of experiments is here detailed in which the patient's own serum is tested with his own organism at different ages, isolated at different periods of his illness. The first culture was obtained from the patient's cerebro-spinal fluid on the 20th day of illness; the fluid was moderately turbid, and the organisms numerous. Culture on blood agar gave a copious growth of discrete colonies in 24 hours. A sub-culture was then made on Löffler's alkaline serum, and used after eight hours' growth. The organism was thus in vigorous state, the forms large and non-degenerating. This culture was then sub-cultured every second day until the 37th day of illness. It was then compared with an organism isolated from the same case 17 days after the first culture. The fluid at this time was much clearer, and the organisms more scanty. Growth on blood agar was slow, a few stellate colonies appearing on the third day, but a sub-culture on Löffler's serum yielded a copious healthy growth. This fresh organism was compared with the older organism, which had now reached its eighth sub-culture, and was 17 days old. On the 39th day, a further fresh culture was isolated in the same way and tested against the original culture. After this, two strains, the first and the third, were sub-cultured successively, and compared in regard to the opsonic index of the patient's own blood. Though in the first culture the original organism was much more vigorous, both in sub-culture yielded copious growths of large undegenerate diplococci.

p. 61.

From the table it will be seen that the index of the younger culture was at first lower than that of the older one, and both latterly tended to approach one another in value after about a week. This is shown also in the curves on the charts;

Chart, No. 11

W. R. 17 yrs. = Case II in chart 9.



CONDITION OF CEREBRO-SPINAL FLUID FROM TIME TO TIME.

the younger organism is in green ink, the older in red ink. A table of control cases is also added, as also are estimations of normal sera as a control of the pool serum. There are also included a few observations of the index of the blood under examination with other strains.

The general results with the control cases indicate that the index is, as a rule, higher with an older strain than with a more recent culture. But this is not always the case. The culture marked brown (Chart 9.) was a recently isolated one from an acute case; at the first observation with it in Case I, the index was practically the same as that with the red and green strains, while five days later the index with it was lower than the corresponding results with those strains. In Case V (Chart 9.) the same thing happened.

PATIENT - M. I. Chart 10

Date 1908	Day of Illness	A.	B.	C.	D.
			1st subcult.	2-3 mths. cult.	4 weeks cult.
Feb. 2	24		1.8	1.4	
7	29			1.5	1.8
12	34		6th subcult. 6.3		7.2
15	37	1st subcult. 5	8th subcult. 7.5		
17	39	1st subcult. 7	9th subcult. 8.7		
19	41	3rd subcult. 6.3	10th subcult. 7.2		
21	43	4th subcult. 5.6	11th subcult. 6.9		
24	46	6th subcult. 7.7	13th subcult. 7		Cult. from acute case 8
29	51	9th subcult. 9.3	16th subcult. 9.5		7

This table compares the indices of the patient to his own organism isolated at different ~~ages of growth~~ times and in successive subcultures.

CONTROL CASES IN ABOVE EXPERIMENTS

Date 1908	Name		
		1st sub-culture	8th sub-culture
Feb.15	W. R.	2.8	3.7
	Normal	1.3	1
		1st sub-culture	9th sub-culture
17	P. O.	6.1	7.8
	Normal	1.1	.9
		1st sub-culture	9th sub-culture
17	W. R.	3.5	4.6
	Normal	1.1	1.4
		6th sub-culture	13th sub-culture
24	W. R.	4.4	4.3
	Normal	1	1.1

TABLE giving comparison of Index with Patient's own Organism and with a Control Organism

	Name		Patient's Own Organism	Control Organism
Case	1 Mrs. G.	(1)	5.1	6
	"	(2)	4.5	3
"	2 M. I.	(1)	7	8
	"	(2)	9.3	7.5
"	3 J. G.	(1)	2.2	3.2
	"	(2)	2.2	2.8
"	4 J. G., Cerebro-spinal fluid	(3)	.47	.44
"	5 B. C.	(1)	.6	.66
"	6 B. C.	(2)	.6	1.2

These results show that the index with the patient's own organism is sometimes greater by 20%, and sometimes less by 30%, than that with the second or control organism. Reference to table and charts showing the relationship of results with different strains will show that the differences there also are much the same in amount; e.g., in the case of M. I., one strain gave results at first 35% higher than did a corresponding strain from the same case. Thus, there appears to be no special relationship between the patient's serum and his own organism as regards opsonic index. The differences are not such as to modify the general results obtained with a standard culture.

These prolonged comparative investigations were carried out with a view to determining the relation of culture peculiarity to sensitiveness to opsonin. The fluctuations in the index with the black culture, and its subsequent loss of susceptibility, show that great care is necessary in interpreting the results obtained. They suggest that some unexpected variations, met with in the opsonic index, may be due to changes in the properties of the organism. As far as possible, such variations have been checked by comparison with other strains of the organism.

CONCLUSIONS

(a) Different strains may show fairly accurate correspondence in their sensitiveness to the action of the serum of a series of cases in repeated observations; but a particular strain may show erratic variations in its susceptibility, and

may become quite insensitive to the action of powerful sera.

(b) There is no notable difference in the sensibility of recently isolated strains as compared with those which have been frequently sub-cultured; nor does a recently isolated strain behave differently towards the serum of the patient from which it was isolated than towards the sera of other cases.

(c) Any variation in the opsonic content of a particular serum can only be accepted as due to a change in the serum when the susceptibility of the particular strain of organism has been tested with several other sera and found to re-act, as the clinical condition of these cases would suggest.

(d) To avoid all chance of error, it would be necessary to control the results further by the use of several strains, both with the serum in question and with control cases. In this way alone might a conclusion be drawn that a sudden and clinically unexpected variation in the index was due to some alteration in the serum. Where observations, however, are made at the same time with several sera, the results afford a sufficient practical control. A sudden alteration in the index of one case, accompanied by a similar alteration in the others in the same direction, would draw attention to the organism as the cause of the change.

In my observations on the general course of the index in the disease, I have particularly watched for any such variations, common to all the cases which were being examined at the same time. Most of them were estimations with the same strain throughout, and rarely less than four separate sera were examined at one time. The organism then in use appears to have preserved much the same relative susceptibility throughout the

observations, which may consequently be taken as accurate estimations of the general course of the index, sufficiently so to enable a broad general view to be taken of its relationship to the stages of the disease.

RELATION OF TYPE OF CASE YIELDING THE CULTURE TO THE BEHAVIOUR OF THE ORGANISM TOWARDS OPSONINS

Two cases were selected, the one (Case A) in the third day of illness and very acute, the other (Case B) in the 14th day and in the chronic stage. Their sera were tested against one another's organisms as recently isolated as possible.

The primary culture on Löffler's serum in Case A was not vigorous enough to yield a satisfactory organism. The primary culture from Case B was made on blood agar. The first sub-culture on Löffler's serum was in both cases vigorous, and was employed after eight hours' growth.

Case		Culture from	Index	Cult. from Chronic Case
A	Acute	A	.6	-
"		B	1.2	.6
B	Chronic	A	2.8	-
"		B	2.2	3.4

The last column gives result of estimation on previous day with a culture from a chronic case

A glance at this table will show that there is no evident

difference in the behaviour of the recent organism from an acute and from a chronic case towards sera from the respective cases.

Houston and Rankin[11] found that occasionally a particular strain might cease to respond to the specific opsonins of the patients. This happened with one of their own stock cultures which had been sub-cultured for four or five months. Much difference was noted between the susceptibility of their own strain and those from other laboratories. Davis[6] found that the varying susceptibility of different strain was slight and could be neglected; that the organism recently isolated behaves similarly to the specific serum as one cultivated for some time.

STUDY OF OPSONINS IN CEREBRO-SPINAL MENINGITIS

By the estimation of the opsonic index it was hoped that information might be obtained with regard to (a) a means of estimating reaction to infection in a manner more definite than could be yielded by the clinical symptoms, which are quite unreliable in guiding prognosis; (b) its possible diagnostic value; and (c) the basis for a vaccine treatment of such cases as do not rapidly undergo spontaneous cure but become more or less chronic, a character possessed by 50% of all cases, a large majority of whom in the normal course die in this period. Many cases which enter the chronic stage ultimately recover after many weeks of illness in an unexpected way. It was thought that, if suitable vaccine treatment were adopted early in all such cases, the percentage of recoveries might be increased.

It appears from the observations that no figures, however carefully counts have been made, can represent more than approximately the number of ingested organisms. That this is the case will be seen from the following considerations, which apply especially to very active sera yielding high phagocytic counts.

(1) Agglutination occurs prior to phagocytosis, and large clumps appear to be ingested en masse; wherever possible, leucocytes which had ingested such agglutinated masses of cocci were not counted, so that the highest figures in the table may be taken as under, rather than over, the true estimate. (2) The occurrence of intracellular digestion often renders an exact estimation of the ingested cocci impossible. This phenomenon has also been most marked with sera which were highly active in preparing the cocci for phagocytosis. (3) It is, of course, impossible to count accurately upwards of 20 to 25 diplococci within a leucocyte, and even with sera which yield a moderate average count (5-10), a considerable proportion of the leucocytes may be very full of organisms. With low and moderate phagocytosis, however, quite accurate estimations are possible.

In the actual enumerations, a cell that contains over 20 definite organisms is put down as 20+, and where the index in such a case comes to over 10, it is put down as 10+, so that 10+ represents a high and uncountable figure.

The cerebro-spinal fluids in all of these cases were examined at least weekly by Dr. Connal, so that all were repeatedly verified by lumbar puncture.

A few examples are quoted from the various control estimations from time to time:-

Table I

M. H.	1.05	Tubercular Meningitis
J. S.	.7	"
J. D.	.7	"
- S.	1.2	Lobar Pneumonia
J. B.	0.87	Tubercular Meningitis
"	1.3	"
J. McM.	1.1	Normal
W. M.	1.2	Convalescent Erysipelas
J. S.	1.3	Tubercular Meningitis
J. McK.	1.3	"
J. A.	.8	Normal
J. McC.	1.1, 1.3	"
A. C.	1.1, .8	"
A. M.	1.1, .9, 1.3, 1, .9, 1.1, 1.3	"

These figures indicate variations from .8 to 1.3 in the case of normal sera, and from .7 to 1.3 in case of sera from the sources noted.

TABLE showing Course of Index in the early days of the Acute Disease

TABLE II.

No.	Name	Age	Days of Illness on which Observations were made															P.M.
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	18	20	21	
1	L. M.	10			1.1	1.4	x													No P.M.
2	B. C.	5			6.6	.6	x													No P.M.
3	I. M.	5			.5	.6		x		(chart 12)										No P.M.
4	A. P.	15	.6		.7		2.7	x		(" 14)										G.L.
5	R. C.	17							x											G.L.
6	J. M.	4					1.3		x											G.L.
7	J. M.	13½			.9		1.8		1.1x	(chart 13)										No P.M.
8	R. T.	40					3.5		4x											No P.M.
9	J. S.	45						1.9		x										G.L.
10	T. N.	21				.8						3.	x							G.L.
11	W. H.	40				1.5		(chart 14)					7	x						No P.M.
12	Mrs. T.	44						4.8			3.5				x					G.L.
13	Mrs. D.	25								2.7					2.5	x				No P.M.
14	H. S.	1								1.				1.5		x				No P.M.
15	A. T.	17						(Chart 16)				8.4						x		G.L.
16	C. M.	2 4/12								1.				1.5					x	G.L.
17	J. G.	3½														3.4			x	No P.M.
18	W. R.	15							4.3		3.2			2.3						R.
19	M. I.	8					7.8		8.6				3.5							R.
20	J. M.	15				1.1			2.8											R.
21	J. A.	17				3.1	4.4		3.2					3.4						Died 27th day
22	J. B.	4					1.				2.6									Died 92nd day

x = Day of Death

R. = Recovered

G.L. = General Lepto-Meningitis

BEHAVIOUR OF THE OPSONIC INDEX IN THE ACUTE EARLY STAGES OF THE DISEASE

In 17 out of the 22 cases here included, death occurred within the first three weeks. This is to be accounted for by the fact that those cases received early in the disease are very severe in onset, and many are clinically hopeless from the beginning. There are eight post-mortem examinations. The pathological appearances in all of these correspond closely with those described in a group of similar cases under agglutination (Group A). A more or less generalized lepto-meningitis with exudate was present in all; details need not be given.

The letters "G.L." in the tables indicate general lepto-meningitis. The figures representing the indices are arranged under the days of illness on which the estimations were made; the "x" indicates the day of death.

From the table it will be seen that the index tends to be under one in the early days of the acute disease. In two cases the index was below one throughout.

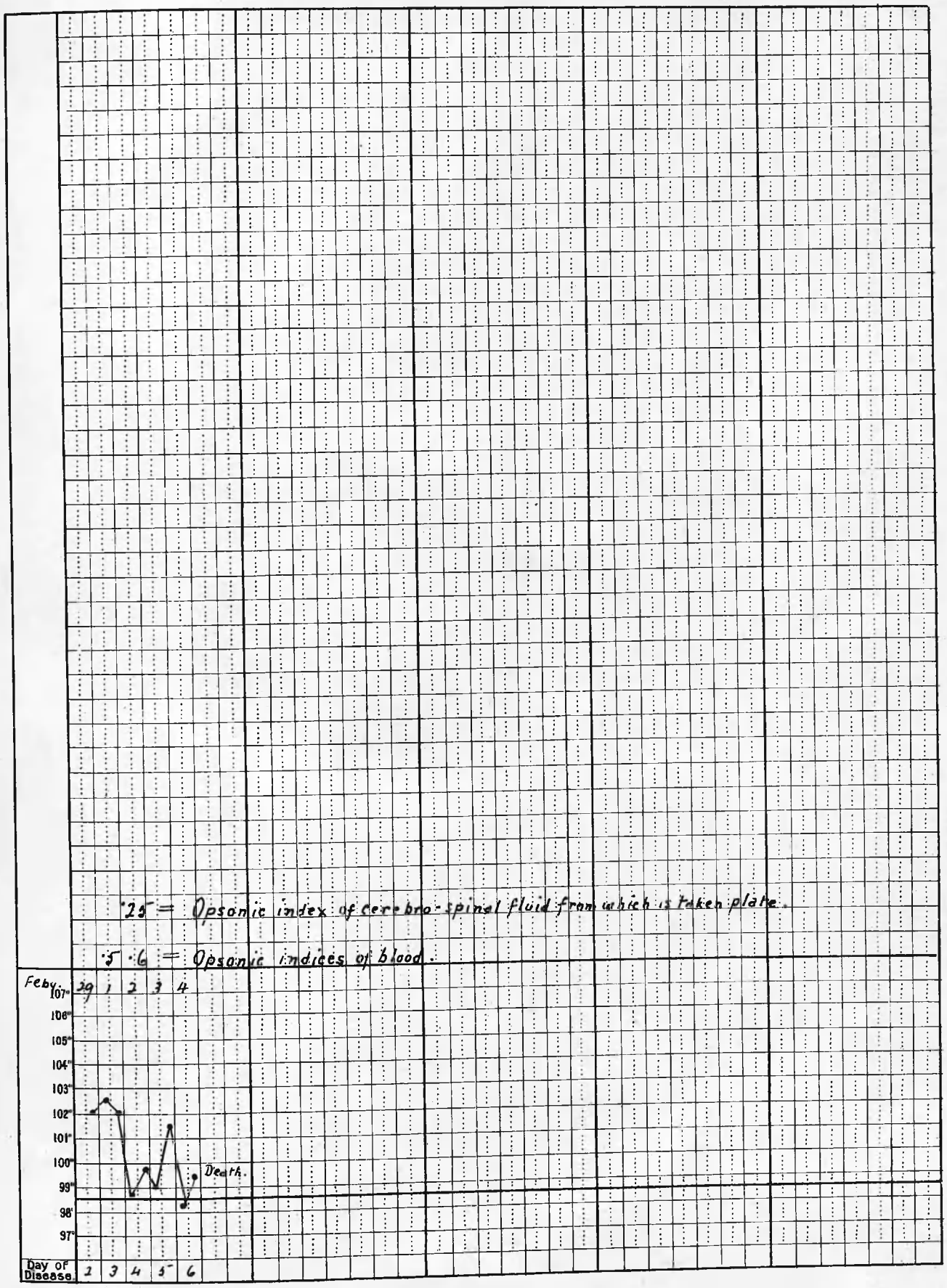
NUMBER OF CASES EXAMINED

ON THE SEVERAL DAYS OF THE EARLY PERIOD OF THE DISEASE

Day Examined	No. of Cases	Index Above 1	Indices vary from
3	5	1	1.1
4	7	1	3.1
5	7	6	1.3 to 7.8
6	2	2	1.9 and 4.8
7	6	6	1.1 to 8.6
8	2	1	2.7
9 and 10	5	5	3. to 8.4

Chart No. 12

I. Mc P. aet. 3½. Opsonic Index in a very acute fatal case.



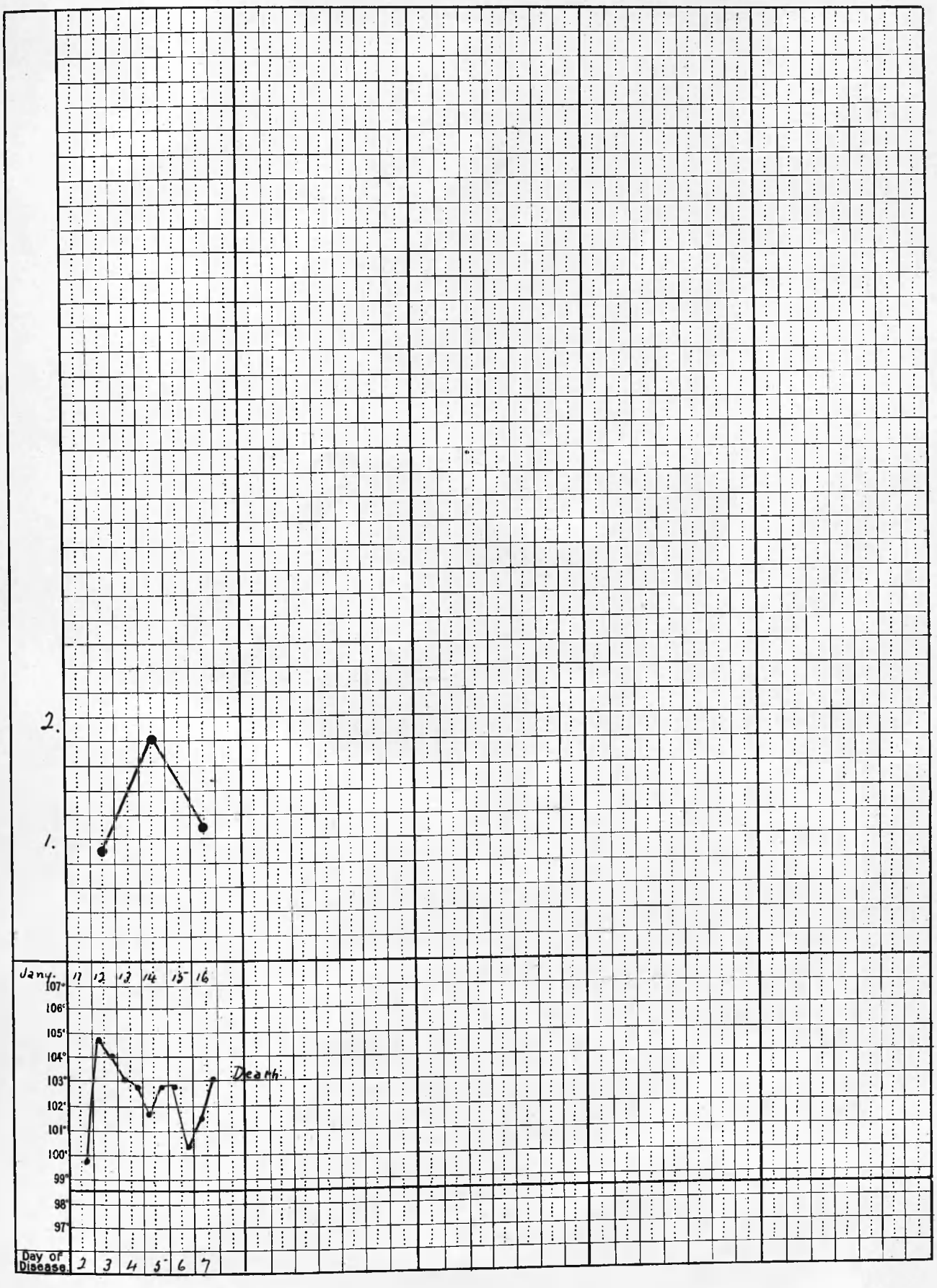
Cerebro-spinal fluid turbid.

Chart No. 13

OPSONIC INDEX.

13

J.A. McN. aet. 13½



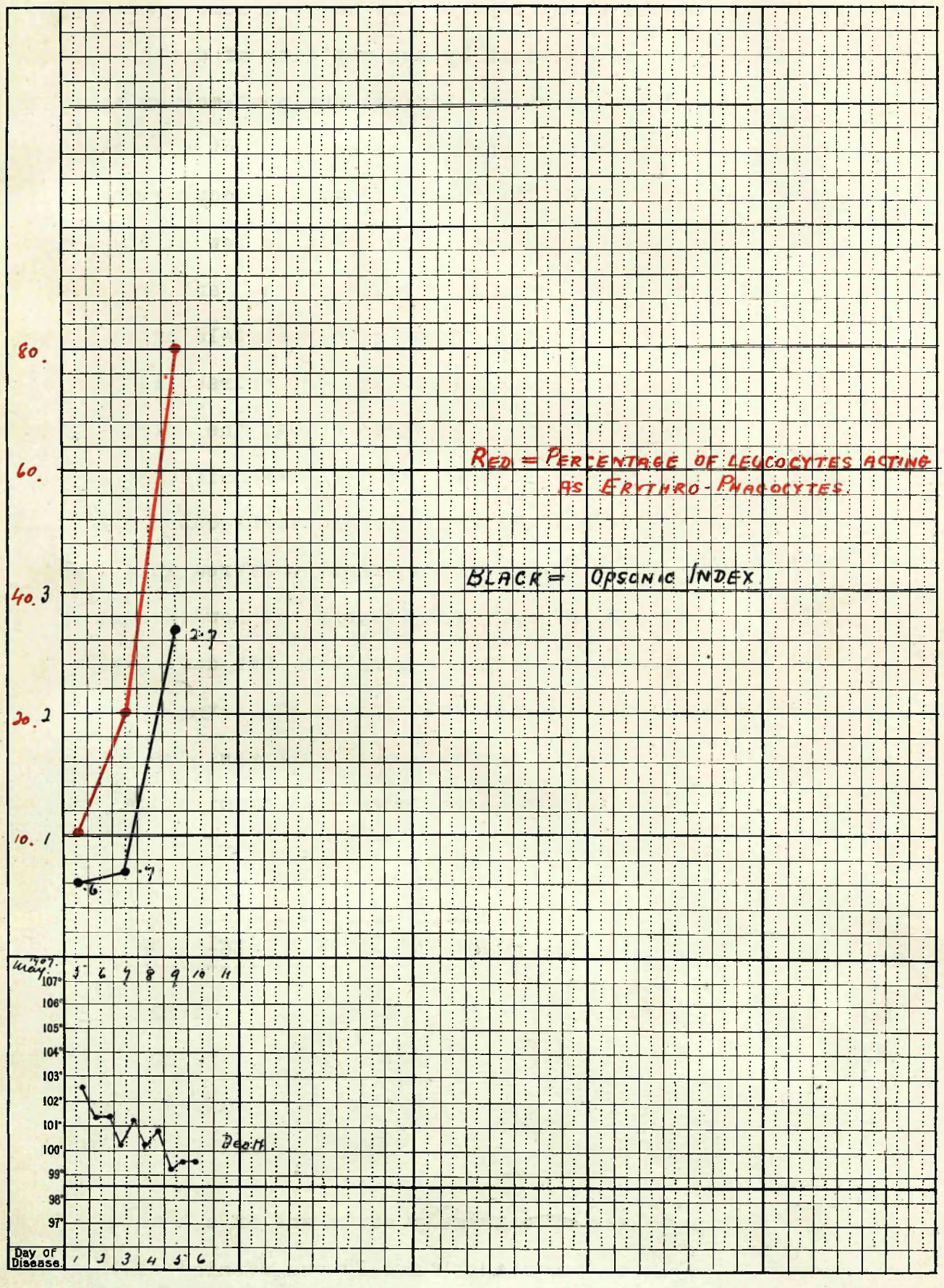
Cerebro-spinal fluid opalescent.

Unconscious and acutely delirious. Diagnosis made only by lumbar puncture.
Kernig's sign only slight and rigidity slight. No retraction.
Picture of an acute general infection. Organisms sparse.

Chart. No. 14

A. P. 15 yrs.

(14)



From this it will be seen that (1) the index tends to be under one in the early days of the acute disease. The more acute the case as measured by the rapidity of death in cases uncomplicated (as shown by clinical and post-mortem conditions), the less likely is the index to be much, if at all, above unity. (2) A definite rise in the index may occur within the first seven days of illness. The index is low at the commencement of the disease, normal or below normal, and from the fourth to the 7th day the number of cases yielding an index of over one tends to increase steadily. Of course, it must be noted that in the majority of these cases, the intoxication is very severe, a condition which is well recognised as adverse to the production of anti-bodies. In the eight cases which lived longer than a week, a decided increase occurred in all with the exception of Case 14, in which it remained low throughout. In one case, No. 15, it reached 8.4 on the 10th day. Between these extremes it remained moderate (i.e., 8 to 4); in others it was high during the second week of illness (Cases 11, 15).

Four of these acute cases were also examined for agglutinins:- (Case 4, Chart 14)

A. P. Case 4. Chart 14.

Day of Illness	Opsonic Index	Agglutination
2	.6	1-10
3	.7	1-20
6	2.7	1-80

This case was an extremely acute one in a healthy young adult. The onset was abrupt, and by the evening of the first day he was delirious, and was admitted wildly resistive and

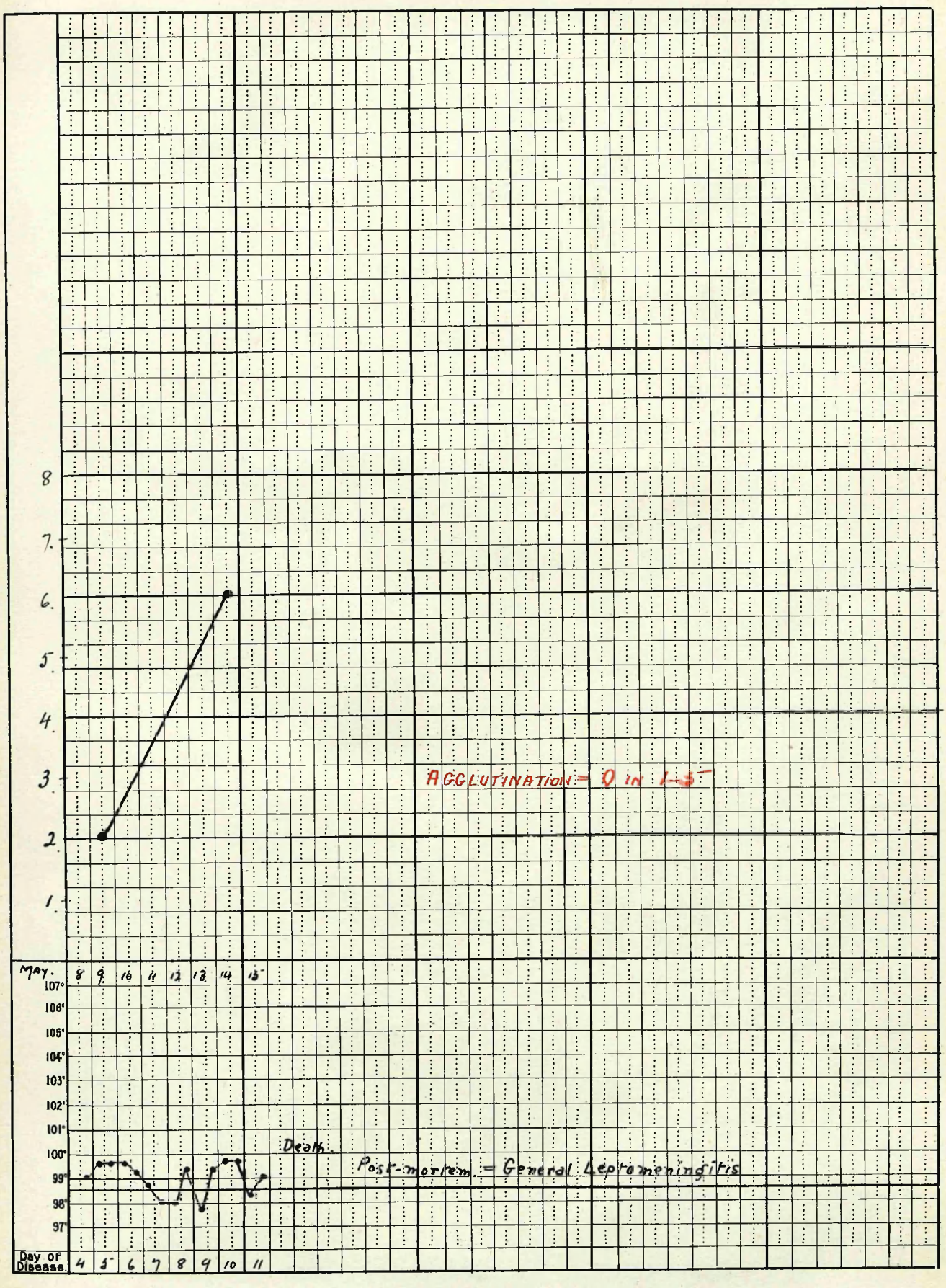
Chart No. 15 (B.)

Case 10.

15

T.N. 21 yrs.

OPSONIC INDEX.



Chart, No 16. (C)

Case 15.

A.T. 16 yrs.

16

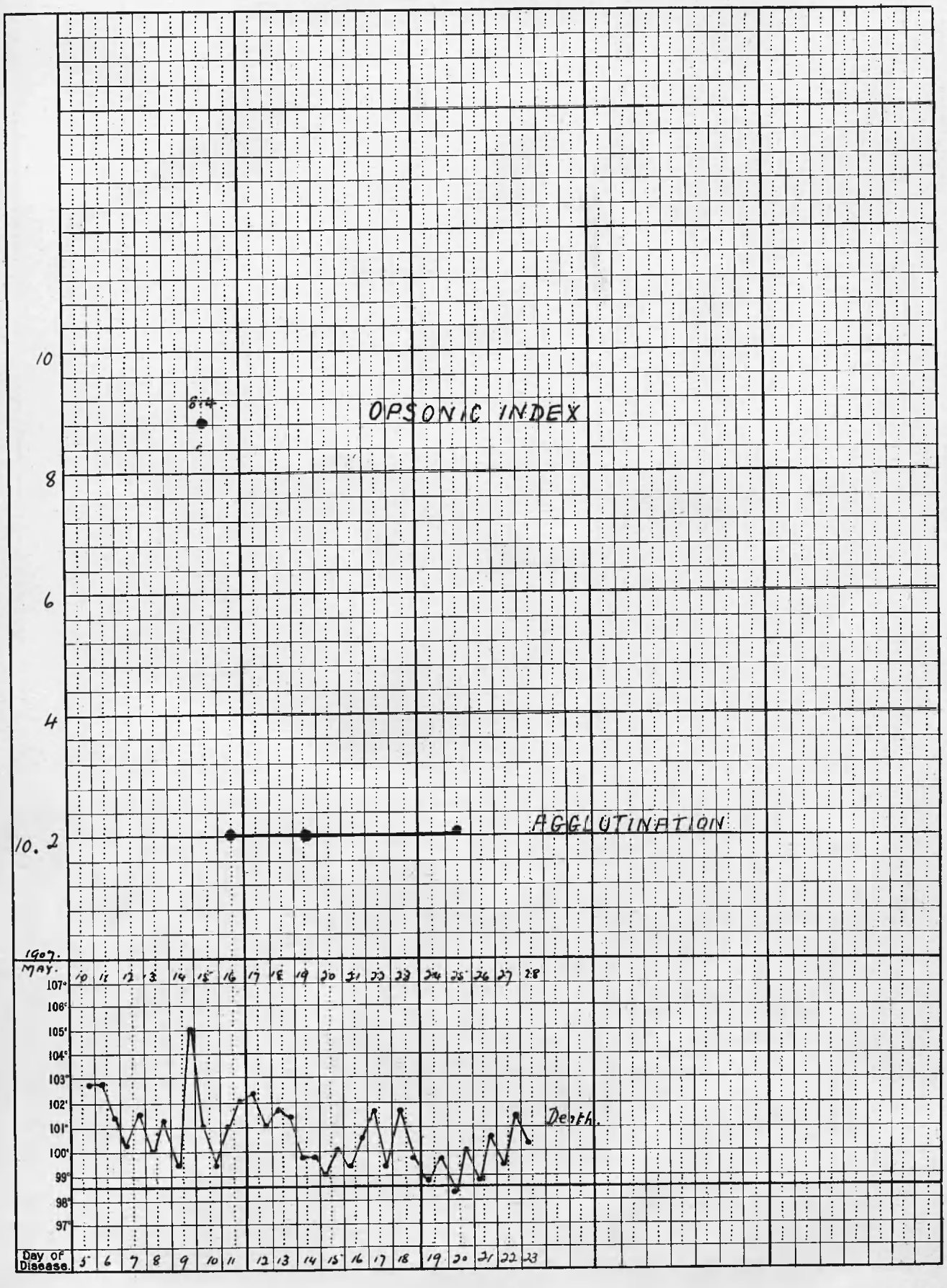
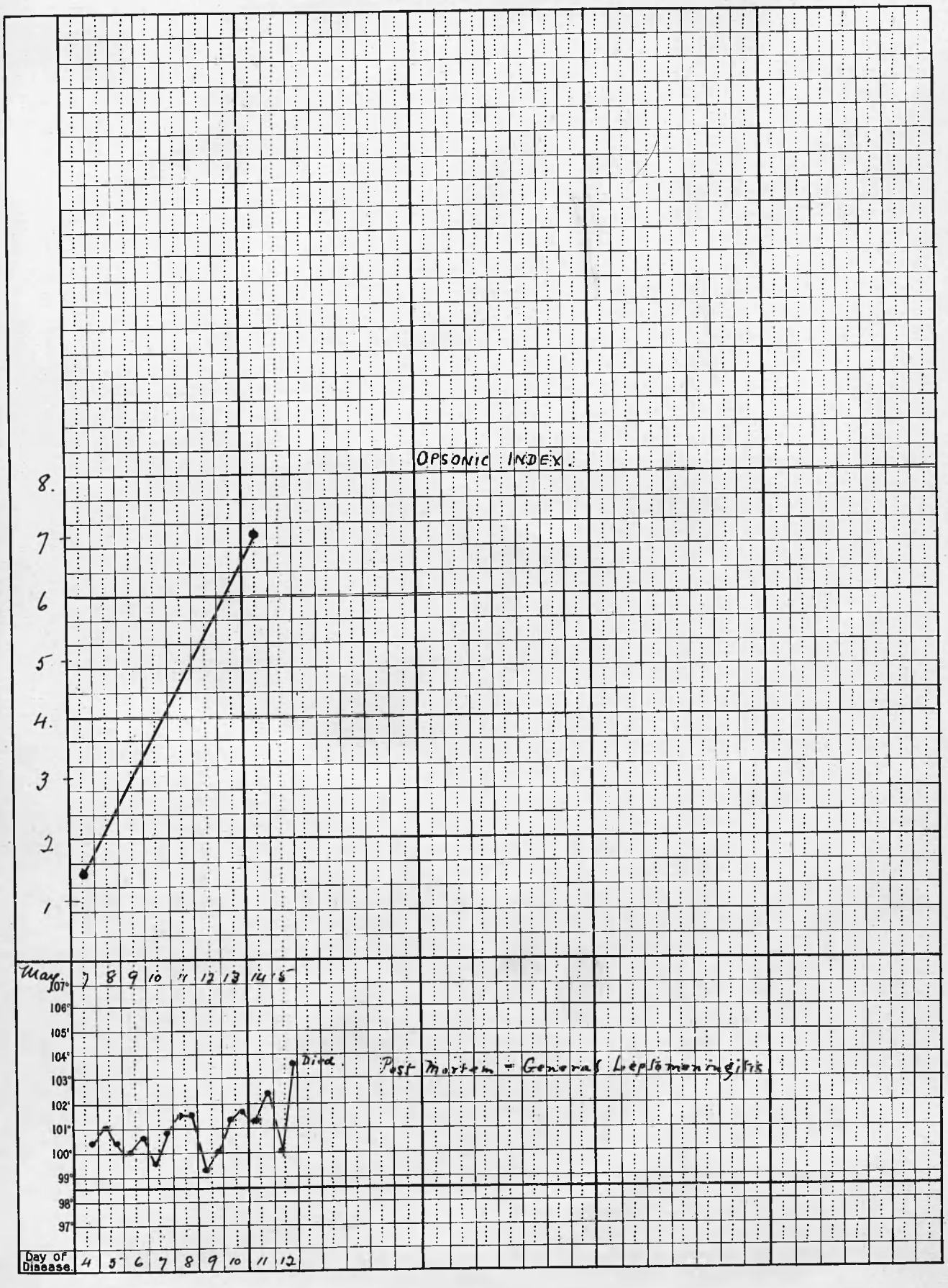


Chart No. 17

W. H. 40 yrs.

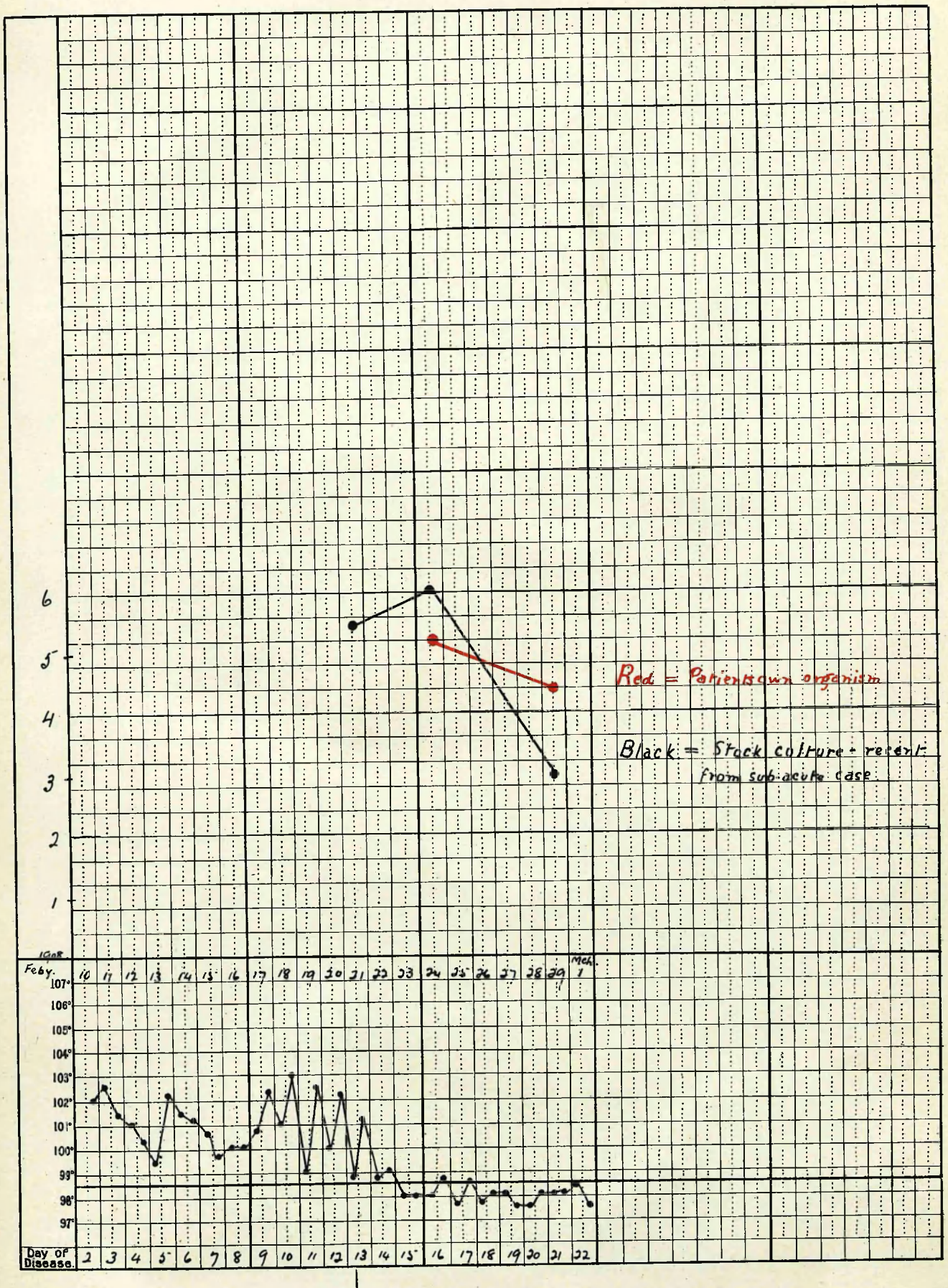


Chart, No. 18

Case 4 p. 63

OPSONIC INDEX.

MRS G. 28 yrs.



Cerebro-spinal fluid turbid.

Culture on blood agar.

Chart, No. 19

Case 3. p.63

MRS S. 45 yrs.

OPSONIC INDEX.

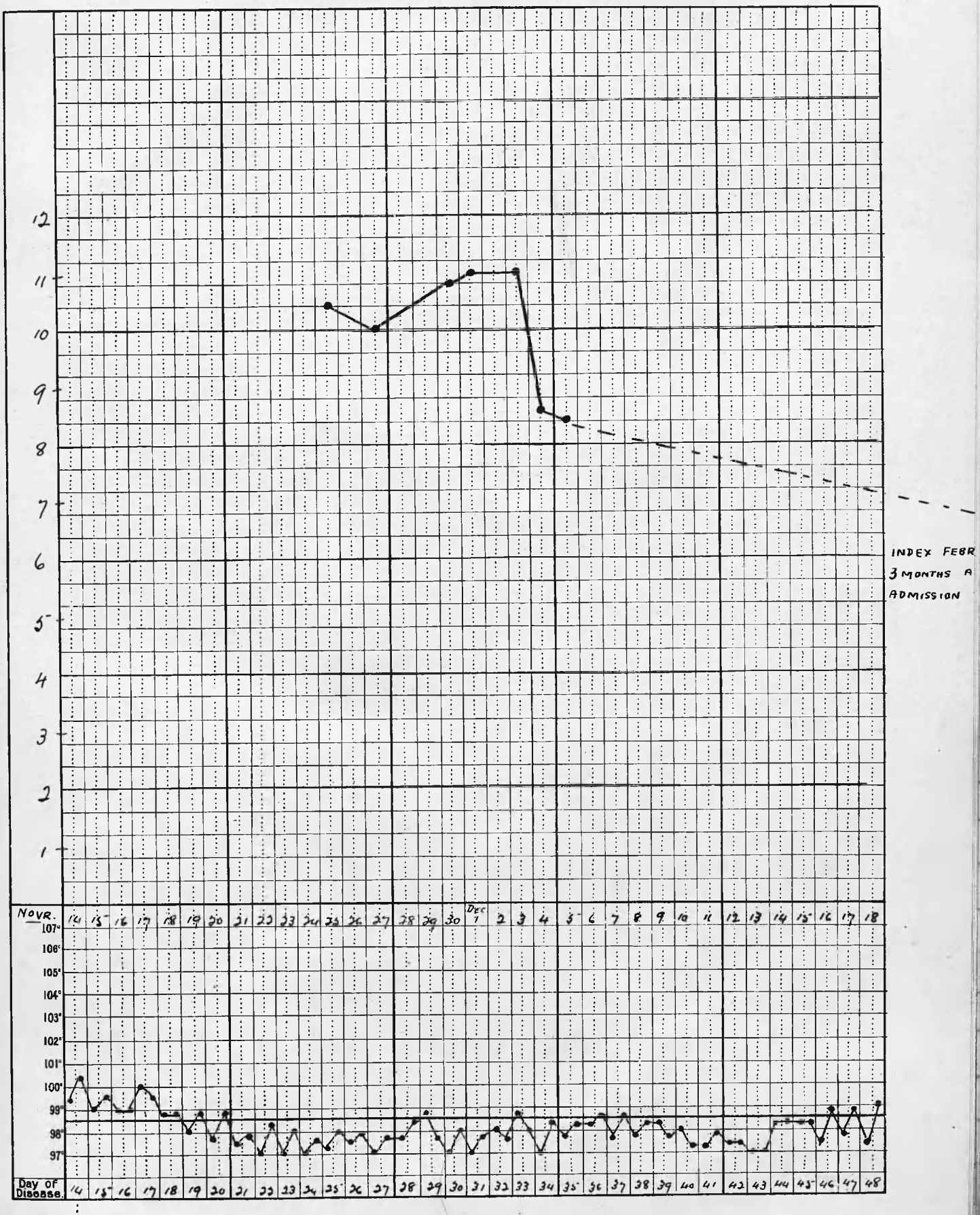
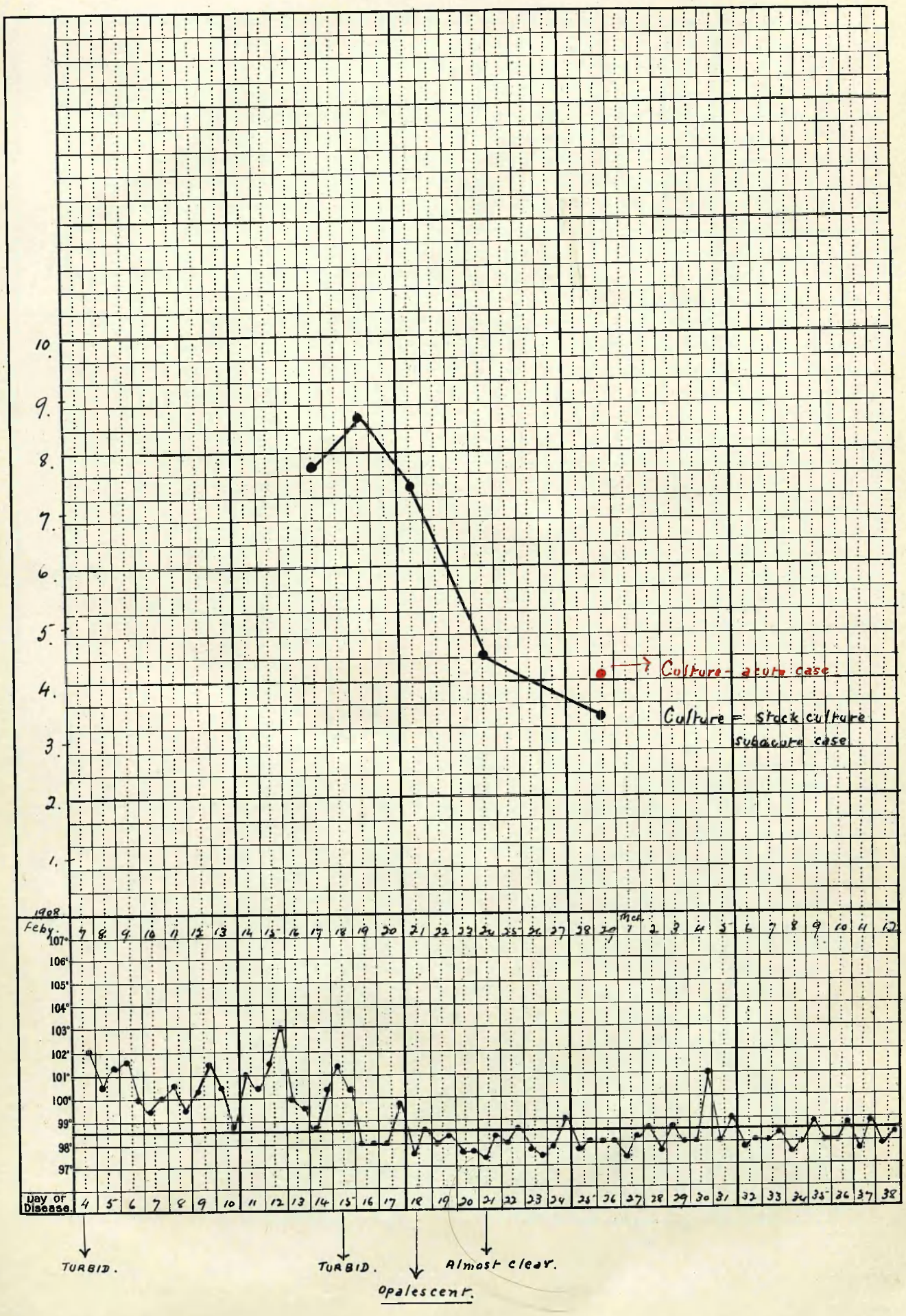


Chart. No. 20

Case 5. J.63

P.O. 16 yrs.

OPSONIC INDEX.

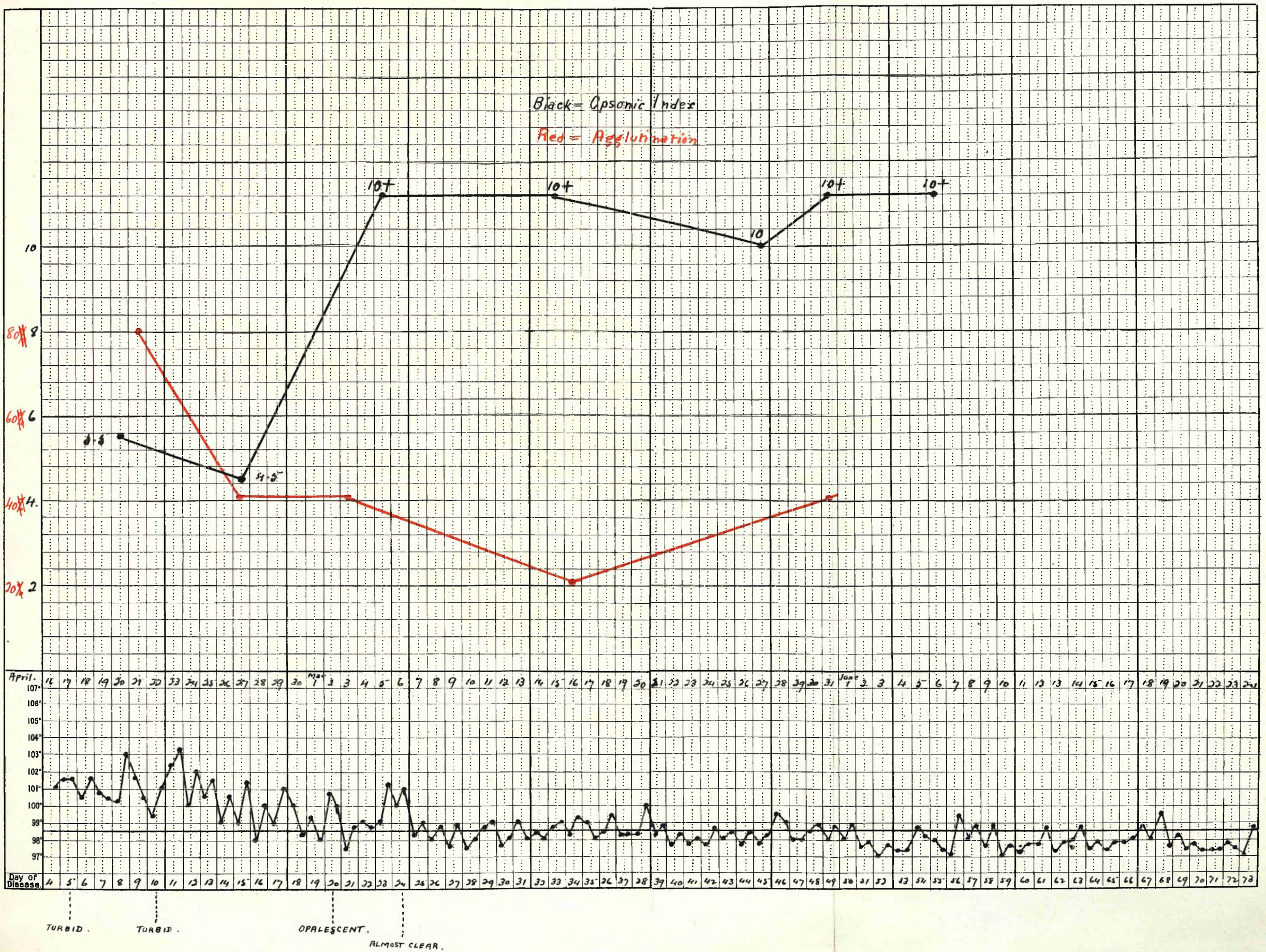


CONDITION OF CEREBRO-SPINAL FLUID.

Chart No. 22

Case II p.65

J. H. 10 yrs.

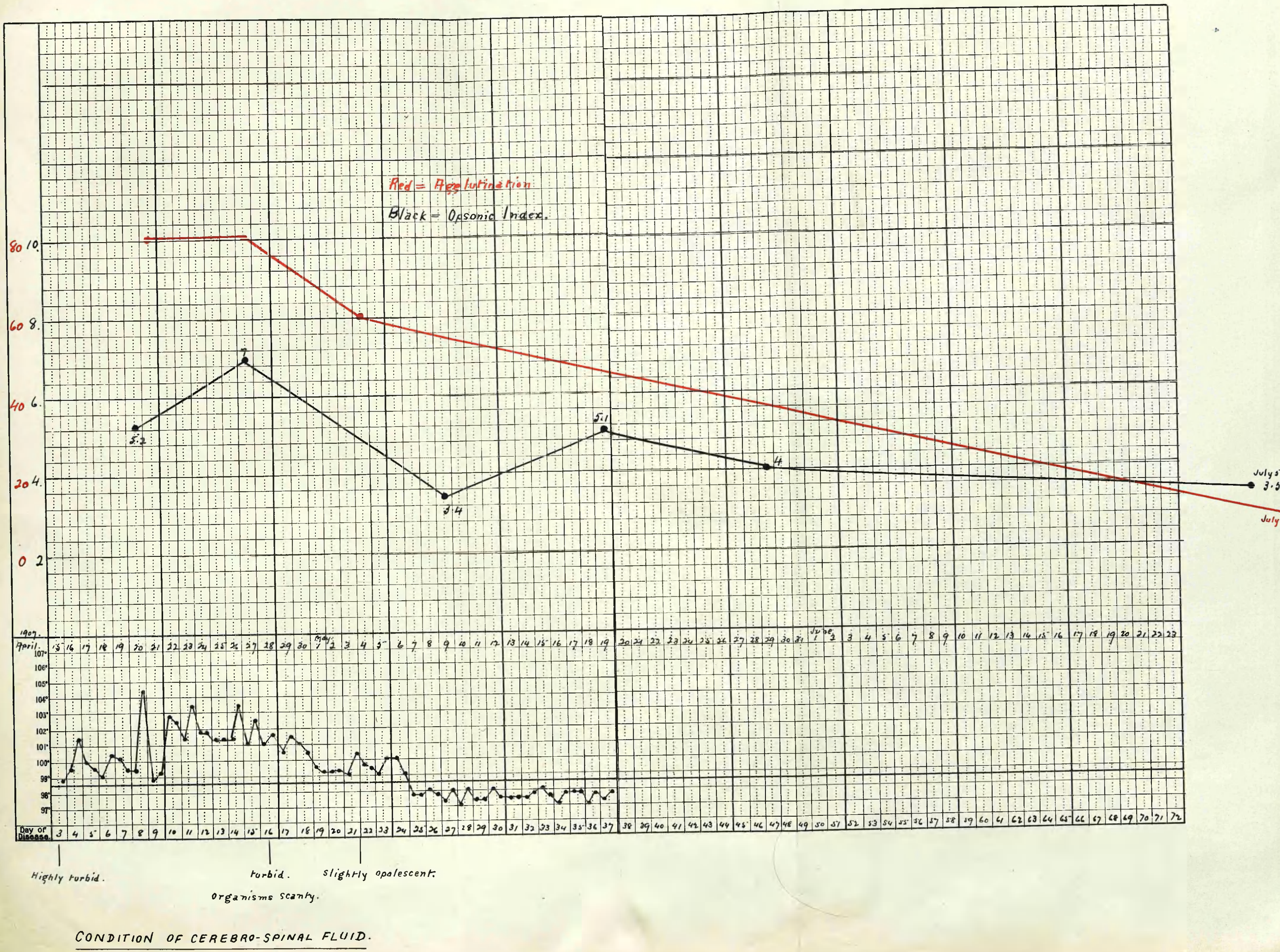


CONDITION OF CEREBRO-SPINAL FLUID FROM TIME TO TIME.

Chart No. 21.

Case 10 p. 63

C. C. 15 yrs.



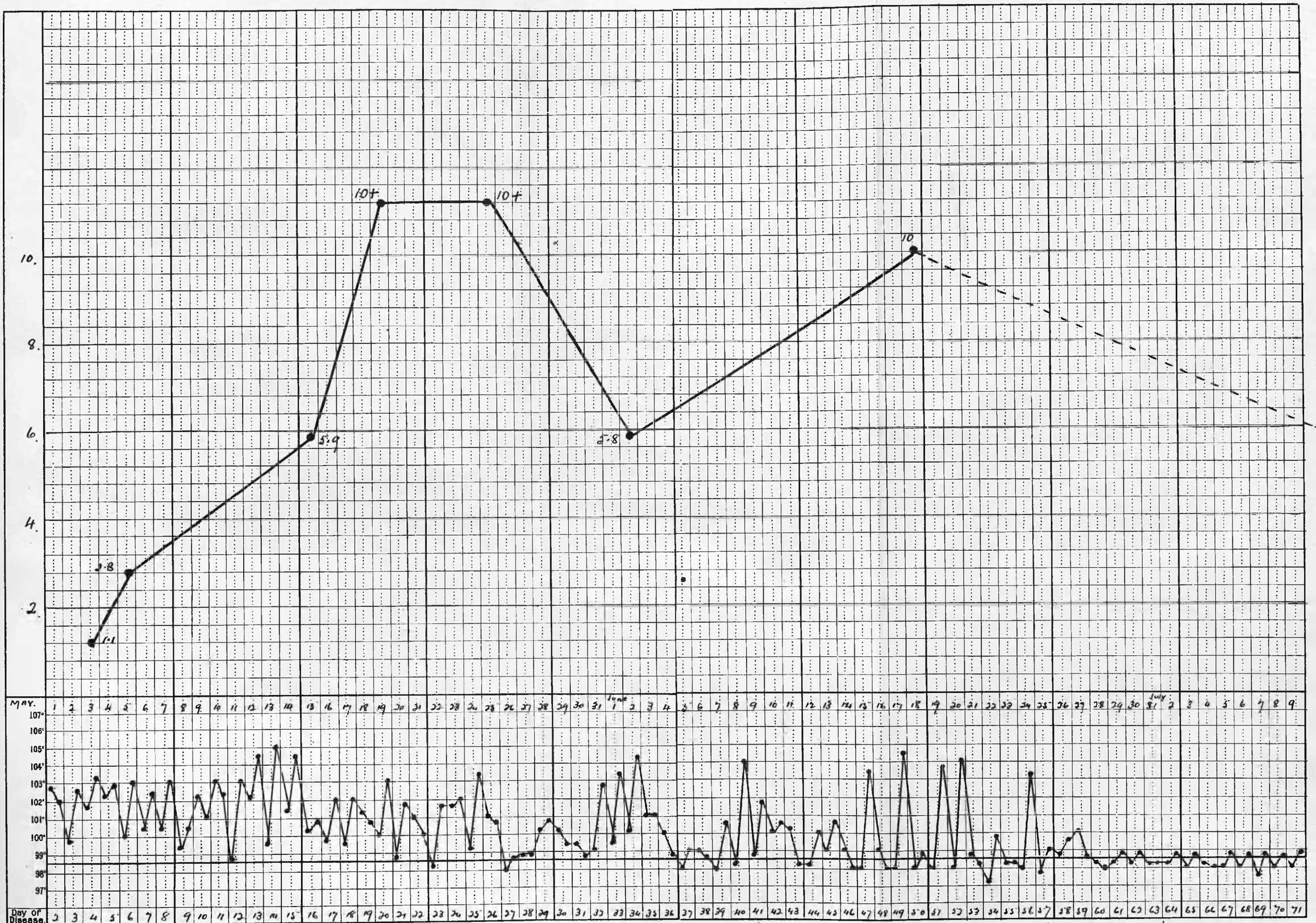
CONDITION OF CEREBRO-SPINAL FLUID.

Chart, No. 23.

Case 25 - p. 63.

J. M. 16 yrs.

OPSONIC INDEX.



CONDITION OF CEREBRO-SPINAL FLUID FROM TIME TO TIME.

cyanotic. On the third day he recovered consciousness and was quiet and composed, but on the following day he relapsed, and became again drowsy, flushed, and restless; finally he sank into a deep coma and died on the sixth day. The agglutinating power of the blood in this case rose rapidly to a high degree, while the opsonic power was elevated moderately.

(Case 10, Chart 15 B) In this case, agglutinin was absent on the 10th day, while the opsonic index had risen to 3. (Case 15, Chart 16 C) Agglutination never occurred at a higher dilution than 1-10. On the 10th day the index was 8.4. (Case 12) Opsonic index = 3.5, agglutination = 1:5 on ninth day.

From these examples it will be seen that the agglutinating and opsonic powers do not necessarily correspond; in fact, high agglutinating power may exist with low opsonic effect, and vice versa. Assuming that both these phenomena are indicative of an immune reaction, it is obvious that such anomalous behaviour makes it impossible to draw clinical deductions either from an estimation of the one or other condition, or from both combined.

The last three cases are typical examples of onset with "local" symptoms, and without any marked symptoms of general infection such as was present in Case 4. There was an absence of the conditions necessary to produce marked agglutinating power in the blood.

TABLE OF INDICES OF THE MORE PROLONGED CASES TABLE III

No.	Name	Age	Week of Illness on which Observation was made													Dur. of Temp.	Max. Agg.	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1	A. G.	11				6.2											1-100	R.
2	J. D.	23			10.5												11	Died
3	Mrs. G.	28		5.5		4											14	R.
4	Mrs. S.	45			10	10.9	8.5										17	R.
5	P. O.	16			7.7	4.2	3.3										1-40	R.
6	Mrs. M.	30		5.9	10+												18	R.
7	P. H.	25			12.5												20	R.
8	M. M.	3		8.9													22	R.
9	E. G.	1		4.3													Nil	R.
10	C. C.	10		5.2	7	3.4		5.1	4					3.5			23	R.
11	J. H.	10		5.5	4.5	10+	10		10+	10+					9		28	R.
12	H. P.	8				2.5											25	R.
13	T. C.	10				2	2.5										1-80	R.
14	W. R.	17	4.3	3.2	4.3		3.5	4.3									25	R.
15	S. M.	27												7.7			1-80	R.
16	J. C.	40												5			23	R.
17	W. D.	10			2.6	2	1.7										Nil	R.
18	A. M.	7				2.7						2.1					27	R.
19	P. C.	6			2.1												Nil	R.
20	W. M.	5							.9					.8			26	R.
21	A. H.	3½			2.4	2.1		6		5	8.3	3.5					1-20	R.
22	A. M.	10					3	3.7									28	R.
23	C. S.	2		1.2			1.4	3									1-60	R.
24	K. G.	10							3.7	2							24	R.
25	J. M.	16	2.8		5.9	10+	10+		10+				5				34	R.
26	M. I.	6	7.8	3.6		1.8	6.3	6.9		9.5							25	R.
27	J. B.	4	1	2.6	10	3.9		.9									1-20	R.
28	J. B.	29				1.2	1.2										24	Nil
29	W. M.	25			3.4	1.5	1											Died 8th week
30	J. A.	17	4.4	3.4	1.6													16 wks.' indef. temperature R.
																		9 " R.
																	1-20	6 " R.
																		9 " R.
																		13 " Died
																	1-40	8 " R.
																		14 " Died
																		15 " Died
																		R.
																	1-20	7 " Died
																		4 " Died

R. = recovered

Cases 3, 4, 5, 10, 11, 25 - See separate Charts (18 to 23)

TABLE IV.

Days of illness on which observations made - exclusion of previous table.

No.	Name	Age	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	43	45	46	47	48	50	51	52	57	58	66	68	77	81	83	84	89	90	92	94	107	119
1	A. G.	11																											6.2																																				
2	J. D.	23												10.5																																																						
3	Mrs. G.	28									5.5				6				3																																																
4	Mrs. S.	45																						10.4		10			10.8	11		11	8.7	8.6																														4.1
5	P. O.	16											7.7		8.6		4.4								3.4																																										
6	Mrs. McC.	47															5.9							10																																									
7	P. H.	30																						12.5																																								
8	M. McP.	3										8.9													1																																								
9	E. G.	11/12											4.3																																																			
10	C. C.	15						5.2					7													3.4										5.1											4										3.5						
11	J. H.	10						5.5						4.2								10									10																																
12	F. P.	8																										2.5																																			
13	T. C.	10																										1.8		2.7											2.7																							
14	W. R.	17					4.3		3.2		2.2	2.2		2.4				3.2							3.4										3.6		3.7		4.6	4.6			4.2																			
15	S. M.	27																																																													
16	J. C.	40																																																												
17	W. D.	10																	2.6								2							.7																												
18	A. McB.	7																	2.7																																											
19	P. C.	6																		2.																						.9																	.8	.7			
20	W. McC.	5																																					6									5		8.3		3										3.6		
21	A. H.	3½																		2.							2.1			3						3.7																										
22	A. McL.	10																												1.4				3																												
23	C. S.	2							1.2																																						3.7			2										2			
24	K. G.	10																															5.9														10						5									
25	J. M.	16		1.1		2.8									5.9				10						10								6.3			7.4		8.6		6.8	6.9		7			9.4													
26	M. I.	6			7.8		8.6			3.6		2	2.6			2.2							1.6					1.8							3.9																												
27	J. B.	4		1					2.6					2.7			5.7										2.6			1.2		1.2				.9																				2.2					
28	J. B.	9																								1.2								1		4									8															
29	W. McL.	25													3.4						2.5							1.5																																				
30	J. A.	17		3.1	4.4		3.2				3.6		.9	2.5	1.8		1.6		1.6							1.2																																								

BEHAVIOUR OF THE OPSONIC INDEX IN CASES WHICH RUN A MORE PROLONGED COURSE

The behaviour of the opsonic content of the blood has been examined in 30 cases which belong to this group. The general results are shown in Table III p.63. Since, in general, the index has not been liable to sudden and irregular variation, it has been judged sufficient to divide the duration of the cases into weeks, and to state the particular index as holding for the particular week. In many cases the figure indicates merely one of several closely corresponding estimations made in the particular week. These detailed serial estimations are given in Table IV but need not be further referred to. In particular instances, the course of the case as regards the opsonic indices is indicated in charts.

A red vertical line indicates the point at which resolution of the exudate is complete, as indicated by gradual clarification of the cerebro-spinal fluid, and permanent return of the temperature to normal. By this means it is possible to compare the behaviour of the index in a number of cases at a period when resolution was proceeding in all. The behaviour of the index during and after the resolution process might be expected to give indications which would have a prognostic value, by affording evidence of the probable course of events in cases where resolution was delayed, and no indication of their ultimate fate could be obtained from clinical observation alone.

Cases 1 to 19 Table III p.63 form a group of cases which had an acute on-

set, and proceeded to resolution by lysis within four weeks. Case 1, which recovered by crisis at the end of the first week, is the only exception. The index shows marked variation in the individual instances.

In general, however, from the end of the first week till convalescence, the index is high, but the elevation is within wide limits - 4.3 in Case 10 and 12.5 in Case 8 -, while in others it is noted as uncountable. An exception is to be noted in Case 13, which had a low index (2) prior to the completion of resolution and before the end of the febrile period.

The behaviour of the index after resolution is very variable; a fall usually occurs, but is neither very marked nor very rapid. Case 10 had a marked fall at this period from 7 to 3.4 (see Chart 21) but subsequently rose somewhat, and eight weeks after resolution was definitely elevated (3.5). See also Case 5, in which a fall occurred shortly after the temperature became normal.

In contrast to these cases is No. 11, which in clinical course was practically identical with Case 10, but the index rose from 4.2 to 10+ just before the period of resolution was complete, and remained high on repeated observations, being 9 on the 11th week of recovery. In Case 14 the index remained moderately elevated (3.2 to 4.3) throughout the period of observation, which extended from the first week of illness to a fortnight after resolution was complete in the fourth week of illness. Cases 17, 18 and 19 constitute a group in which the onset of the disease was subacute but in which resolution occurred within three to five weeks, so that their duration corresponds with those in the above series. The notable feature in these is the uniformly low index. Cases 17 and 18 recovered.

Case 19 became hydrocephalic, and died. Case 17 is interesting as having had a haemorrhagic eruption. This case had a low index prior to completion of resolution, and a sub-normal index subsequent to resolution, but ultimately recovered. Case 2 died suddenly in the fourth week; post-mortem, an extreme hydrocephalus was found. Patient had had no symptoms, and a normal temperature for over a fortnight.

Cases 20-25 belong to a class which pursue a long chronic course; six to 16 weeks temperature. The onset was moderate and mild, with the exception of Nos. 24 and 25 which had an acute onset. The indices in these cases were all moderate or low except in Case 21, where it was high during the latter part of the temperature. In four of these cases the major part of the exudate was resolved within the first four weeks, and the temperature approached normal by the usual lysis but a very chronic infection persisted, the symptoms and course of the temperature during this subsequent mild course being very characteristic; little attacks of headache and sickness, with restlessness and irritability, coming on at intervals of a few days, with quiescent periods between. The temperature rises, remains moderately elevated for a few hours or days, the whole cycle resembling a miniature attack of meningitis (see Chart 23. Case 25 is given as an example). These repeated infections do not appear to maintain the index at a high level.

Case 26 showed a very decided fall in the index early in the case, with subsequent secondary rise when recurrence took place, as indicated by renewed turbidity of the cerebro-spinal fluid. Cases 27-30 are those in which erythro-phagocytosis was present. They were all fatal with the exception of Case 30. The course of these cases is described (with charts) in Section

3. It is notable that No. 30, which alone recovered, shows nothing in the index which distinguishes it from the others.

The behaviour of the index may be summarised as follows:-

(1) The highest indices are met with in those cases with acute onset, which also, as has been noted, are those showing most marked agglutination (see Charts 22; 23 Cases 11, 25). Where agglutinating power has been low, the index has usually been low.

(2) Accordingly, a high index is a sign of a forcible reaction to a fairly severe infection, that is, where there has been a notable "ictus immunisatorius"; and is of no prognostic value, as cases with low index may recover and cases with a high index have died.

(3) The indices tend to be highest during the second and third weeks of illness in an average case.

(4) The grade of immunity after recovery as measured by the opsonic index is very variable. There may be an active opsonin in the blood for many weeks after resolution, or it may occasionally disappear comparatively rapidly. The disappearance has not in this series been so rapid as the statements of Houston and Rankin[11] seem to suggest.

(5) It is scarcely worth while to refer to its diagnostic value. In a typical severe case the opsonic films are no doubt extremely characteristic, but examination of the cerebro-spinal fluid furnishes all the diagnostic evidences required.

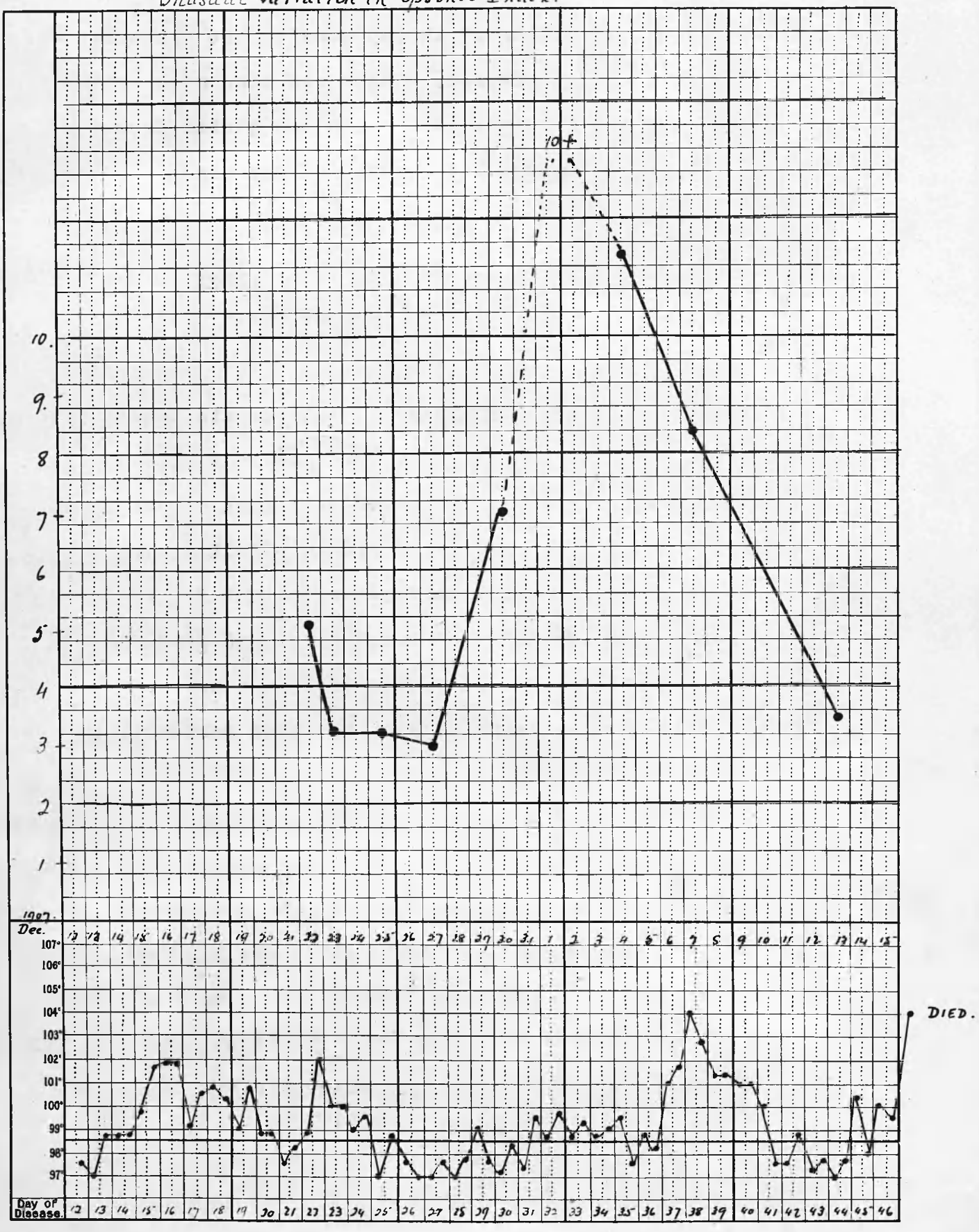
Chart, No. 24

OPSONIC INDEX.

24

G. W. aet 5 mths. IN CHRONIC STAGE.

Unusual variation in Opsonic Index.



Houston and Rankin[11] examined the blood of 114 cases for the opsonic index. From the sixth day onwards the opsonic power of the serum was much increased. There was also much variation in the extent of the increase, and cases are quoted from whose sera there was an unexpected absence of opsonins.

UNUSUAL VARIATION IN THE INDEX (CHART 24.)

This case is that of an infant in the late phase of the disease. The index for the first few observations was low; it then suddenly rose, and remained very high for three successive observations, when it as suddenly fell again.

There was nothing in the case to account for the rise in the index. Some elevation of temperature and some signs of restlessness and irritability were present a week later. The child died some days later, and at the post-mortem examination there was found an extreme hydrocephalus, with clear fluid. There was some basal organisation, but absolutely no trace of recent extension such as might have given rise to the rapid increase in the index described.

Further, the strain which yielded these high results was in use for other simultaneous observations. None of these cases exhibited a corresponding rise, as might have been expected had there occurred an altered susceptibility in the culture.

TABLE SHOWING FLUCTUATION IN INDEX OF G. W.

See also Chart

Date	G. W.	Control Cases	
		J. A.	Mrs. G.
22-12-07	5.1	3.1	
23-12-07	3.2	4.6	
25-12-07	2.8	3.2	
27-12-07	2.6		10
30-12-07	7.0	3.3	10.9
3- 1-08	V.H.	2.1	V.H.
5- 1-08	11.5	1.8	11
8- 1-08	8.2	1.6	6.5
12- 1-08	1.3	1.2	8.4

V.H. = Index very high, or almost uncountable, and means that the index is certainly over 10

From the table and chart it will be seen that there occurred a very marked and sudden rise in the index, with an apparently quite inadequate clinical reason. The collateral tests indicate that the fluctuating factor was the serum and not the organism. I have met with no other examples of variation so extreme as this.

ESTIMATIONS of Opsonic ~~Index~~ Power of the Cerebro-spinal Fluid in Various Stages of the Disease

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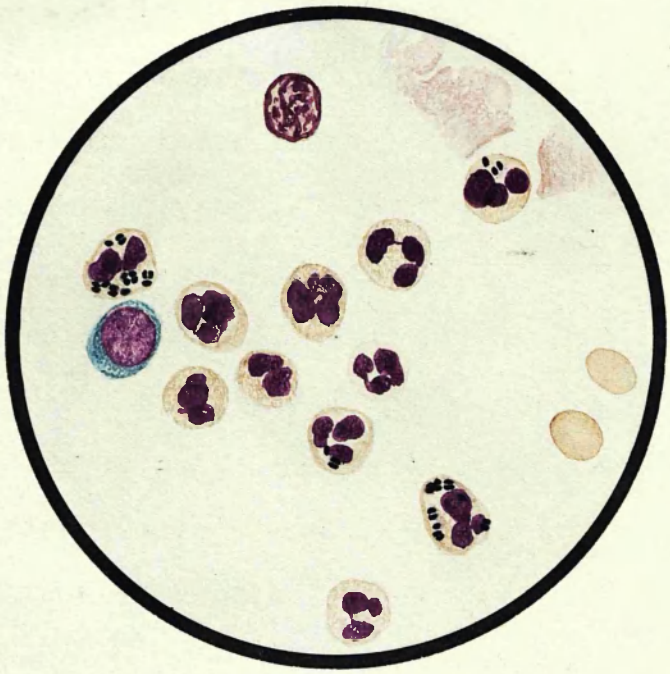
TABLE I - Opsonic Index ~~of~~ with Cerebro-spinal Fluids - Effect of Removal of Fluid by Lumbar Puncture on the Index

Case	Day of Illness	Nature of Fluid	Index of C.-S. Fluid	Blood Index	Lumbar Puncture	
I B.C. aet. 5	3rd	Turbid	.3	.66	1st	24 hrs. between the punctures
	4th	"	.4	.6	3rd	
	5th died					
II - McP. aet. 5	3rd 10 a.m.) 12	Turbid	.27		2nd	
	10 p.m.) hrs.		.25	.5	3rd	This fluid centrifugalised immediately on withdrawal
	4th			.6		
	6th died					
III J.G. aet. 3½	13th	Opalescent	.44		1st	
	14th 10 a.m.) 12	"	.3	3.5	2nd	
	10 p.m.) hrs.	"	.3		3rd	Used immediately after puncture
	15th 10 p.m. 24 hrs.	"	.44	3	4th	"
	15th, Own organism	"	.47	2.2		Patient's own organism
	21st died					

NOTE: At each puncture, 4 to 6 drams of fluid were withdrawn

Table I comprises a group of three cases in which the observations were carried out, in conjunction with the estimation of the opsonic index of the blood. Cases I and II were acute, and were examined on the third day of illness. Case I died on



Pl. 11. Phagocytosis in cerebrospinal fluid on third day of illness.

the fifth day of illness, and Case II died on the sixth day of illness. In both cases the blood index was reduced, as it usually is in the early days of the acute disease.

Although there is thus no trace of free opsonin in either blood or cerebro-spinal fluid, evidence of its action is seen in the early phagocytosis, which can be demonstrated to occur in the cerebro-spinal system very early in the disease, and increasingly as the case proceeds. This would indicate a very low concentration of opsonin. It may even be absent, as Löhlein[15] has shown that, when leucocytes are suspended in salt solution, phagocytosis will be as complete as in serum, provided the time of contact be long enough. In his experiments two hours were sufficient to produce phagocytosis to an equal extent in both salt solution and serum.

In the first case the cerebro-spinal fluid was turbid on the third day; there was great exudation of polymorphonuclear leucocytes; organisms were numerous, and many leucocytes could be found containing a group of organisms in their substance. A drawing is given of a film from the fluid of this patient taken on the third day of illness. (Plate 11)

It may be noted that phagocytosis as seen naturally occurring resembles that seen in opsonin tests, in the fact that the phagocytic action seems to be exhibited by a few cells to an extreme degree rather than equally by all. The same feature is noticed in the case of erythro-phagocytosis described elsewhere. A study of the opsonic films reveals no apparent difference between the active and inactive cells, either in staining qualities or amount of convolution of the nucleus. If the latter point is to be accepted as a criterion of the age of the cell, then age is an indifferent factor in phagocytic action.

(Arneth)[1].

The third patient was commencing the third week of illness, and the blood index was raised. There was still an entire absence of opsonin from the cerebro-spinal fluid.

It was considered important to determine whether drawing off fluid by lumbar puncture produced any influence on the index, as increase in the latter might form an argument in favour of the periodic use of lumbar puncture as a means of treatment. Cases II and III, acute and sub-acute cases respectively, were submitted to periodic lumbar puncture. Case III was examined on four successive occasions, the first three at intervals of 12 hours, and the fourth 24 hours later. The patient had had no puncture performed on him prior to admission. It will be seen that there was no appreciable alteration in the index either of the fluid or of the blood. With Case II, an acute case, the result was similar.

These results, as far as they go, show that removal of cerebro-spinal fluid by lumbar puncture does not stimulate the formation of opsonins either in acute or in chronic cases, where such action is most desirable, nor does it determine accumulation of opsonins in the cerebro-spinal fluid even when they are abundant in the blood. One might have expected opsonins to appear in the cerebro-spinal fluid after repeated punctures, on the analogy of Bordet's[3] experiments, where anti-substances appeared in the aqueous humour on repeated puncture.

A further table (II) is given showing that the cerebro-spinal fluid of Case III contains no opsonin either for the patient's own organism or for two other strains; the one an old culture, and the second a recent one from an acute case.

TABLE II

Culture	Index	
A	.4	Recently isolated from an acute case
B	.3	Old culture from chronic case
C	.47	Patient's own organism recently isolated

A further list, Table III, is given of the indices of cerebro-spinal fluids estimated from time to time in various cases in different stages of disease, the blood index being estimated at the same time. It will be seen that no matter how high the blood index may be, there is no free opsonin present in the cerebro-spinal fluid. It may be inferred that it is absent throughout the disease.

TABLE III

Case	Day of Illness	Index of Cerebro-spinal Fluid	Blood Index
I	20	0.8 1.1	9 6.9
II	25	.6	12.5
III	Latechronic	.5	
IV	13	.75	5.5
V	Latechronic	.4	1.4
VI	16	.5	8.5

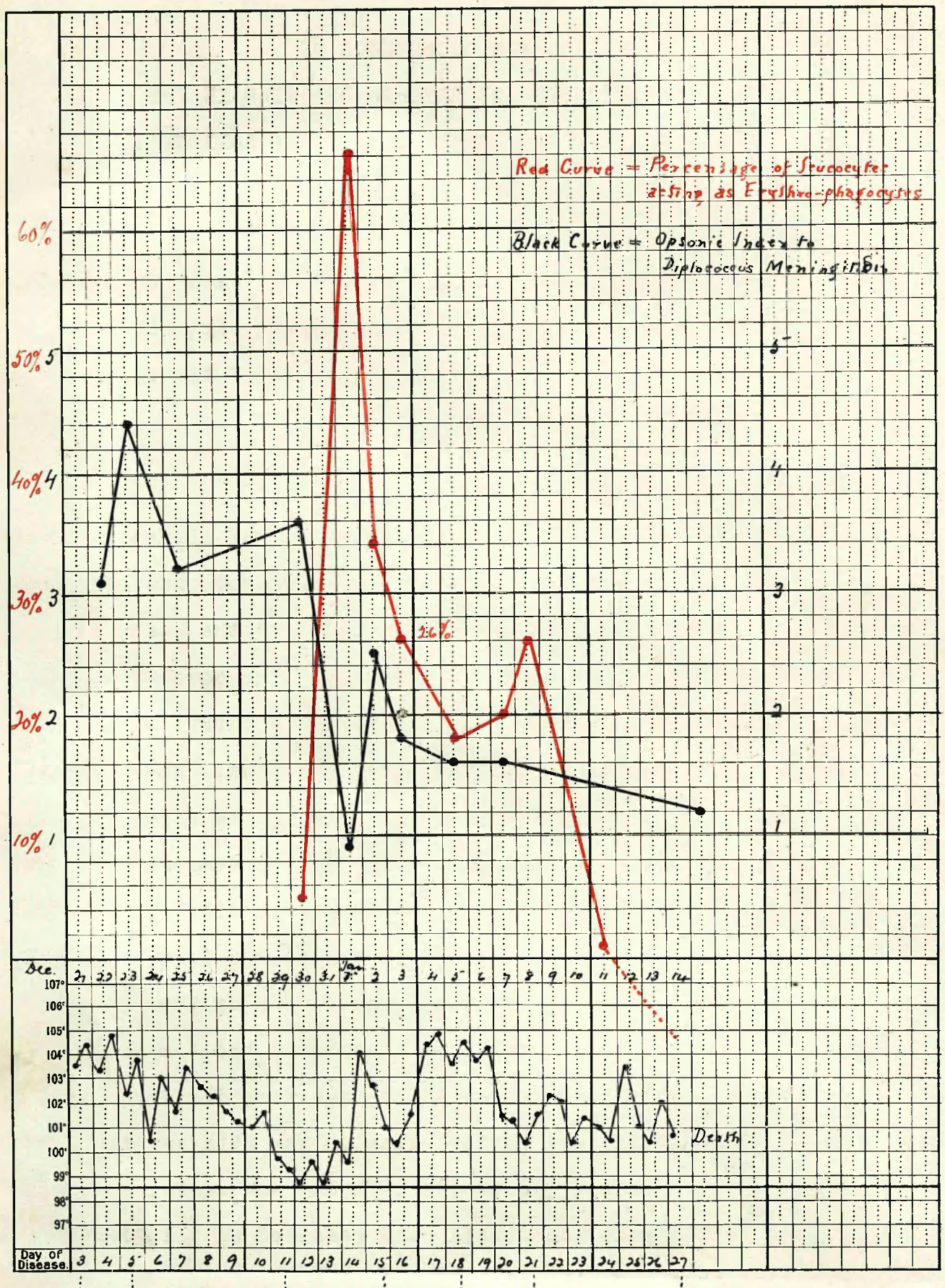
This corresponds with an absence of agglutinins from the

cerebro-spinal fluid noted by Davis[5]. A lack of bactericidal action due to an absence of immune body, as well as of complement from the fluid of cases whose sera were actively bactericidal, was demonstrated by Mackenzie and Martin.[17]

Note on effect of Heat - p. 94.

Chart No. ~~24~~ 25

J. A. 17 yrs.



CONDITION OF CEREBRO-SPINAL FLUIDS FROM TIME TO TIME.

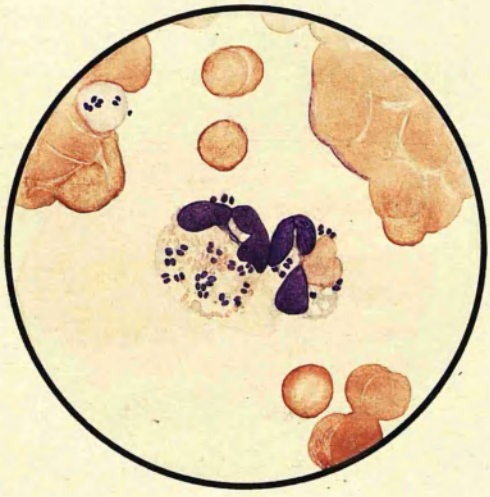
SECTION III

ON THE OCCURRENCE OF ERYTHRO-AGGLUTININS AND ERYTHRO-OPSONINS IN THE BLOOD OF CERTAIN CASES OF CEREBRO-SPINAL MENINGITIS

The following is a description of four cases whose blood was found accidentally to possess an erythro-agglutinating and erythro-phagocytic action on the red cells of the observer during estimations of the opsonic index.

CASE I - J. A., male, aet. 17 years *chart no. 25*

Patient was admitted on his third day of illness. He was acutely ill; he was semi-unconscious, cyanosed, and the respirations rapid. The neck was very stiff, and the head somewhat retracted. Kernig's sign was definite. The cerebro-spinal fluid obtained by lumbar puncture was very turbid and under considerable pressure; meningo-cocci numerous. The patient showed signs of gradual improvement during the first fortnight, corresponding with a gradual decline in the temperature. The cerebro-spinal fluid on the sixth day was decidedly less turbid than on admission, and on the 11th day it was described as opalescent, indicating advancing resolution of the exudate. On the 15th day he became dull and stuporose; the temperature began to rise, and head retraction became more decided; rapid emaciation set in, and patient died on the 27th day of illness. The cerebro-spinal fluid on the 15th day was again less clear. On the 18th day it was turbid, and remained so till death, indicating that a further increase in the inflammatory process was taking place, corresponding with the rise of temperature



Pl. 13. i. Agglutination of red cells.
ii. Phagocytosis of meningococci.
iii An example of erythrophagocytosis.

and renewal of symptoms. This was corroborated by the conditions found post-mortem.

Post-mortem Examination. A great excess of turbid basal fluid escapes when the brain is removed. A large collection of gelatinous purulent material is present in the arachnoid cistern underneath the cerebellum, extending forward over the interpeduncular space. The pons varolii and optic chiasma are embedded in thicker, more solid and adherent organizing exudate corresponding to the early infection. Extension is found proceeding along the Sylvian fissures. The ventricles are not distended but are full of turbid fluid.

Phenomena of erythro-agglutination and erythro-phagocytosis occurring during estimation of the Opsonic Index of this patient. During the first thirteen days of illness, the Opsonic Index remained between three and four. On the 13th day it was noticed that the red cells clumped almost instantaneously when the patient's serum was added to the observer's blood, and that they were still in agglutinated masses when the films were made and examined. A few red cells were found inside the leucocytes to the extent of 3% to 4%,* while the index for the meningo-coccus was apparently not interfered with. Two days later this feature was repeated, but with a very much increased phagocytosis of red cells, fully 66% of the leucocytes containing one or more red cells. The Opsonic Index fell to .9, possibly owing to the preponderating action of the leucocytes on the red cells interfering mechanically with the ingestion of organisms. On the 17th day the percentage of erythro-cytosis

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*The erythro-opsonic value is calculated throughout as the percentage of leucocytes taking part in phagocytosis of red cells

fell to 34%, and there was a corresponding rise in the opsonic index to 2.5. The red cells and leucocytes of a second normal person (A. C.) were now used and found to resist agglutination and phagocytosis, and the opsonic index was estimated with the blood of the latter person.

Leucocytes and Red Cells from	Agglutination	Erythro-Phag.	Opsonic Index for Meningococcus
1 - Observer	Agg. = +	26%	1.8
2 - A. C., normal person	Agg. = 0	0	2.0
3 - J. A., normal person	Agg. = 0	0	-

The blood of a third normal person (J. A.) was also negative as regards erythro-phagocytosis and agglutination. Thus the agglutinating and phagocytic power of the patient's serum was manifested only towards the observer's red cells, and not towards those of two other normal persons. (For a further analysis of the factors, see later.)

The agglutinin and opsonin were never found dissociated, or rather there was never any phagocytosis of red cells without previous agglutination of so marked a character as to be easily detected as soon as the serum was added to the cells. As the case proceeded, the agglutinin remained, but the opsonin disappeared rapidly and for some days before death was only manifested by a few cells. The agglutinin also appeared to diminish in intensity before death, but no direct observations on this were made. On day before death -

Leucocytes and Red Cells	Erythro-Agg.	Erythro-Phag.	Opsonic Index
Observer	Agg. = +	Very occasional	1.1
A. C., normal person	Agg. = 0	0	1.2

Course of the phenomenon in this case:-

1 - Appearance of agglutination

2 - Appearance of opsonin from 4% to 66% in three days

3 - Opsonic power maintained between 20% and 30% for about eight days

4 - Fall to less than 1% four days before death; agglutinating power remains till end, but diminished

Chart A gives diagrammatically the course of the erythro-phagocytic index and the opsonic index for meningo-coccus. Character of cerebro-spinal fluid is also detailed from time to time.

SOME EXPERIMENTS ON THE SERUM OF THE PATIENT

Leucocytes	Red Cells	Serum	Result		Time
1 - Observer	Observer	Patient	Agg. = +	Phag. = 66%	15 mins.
2 - Patient	Observer	Patient	Agg. = +	Phag. = 51%	15 mins.
3 - Patient	Patient	Patient	Agg. = 0	Phag. = 0	1 hr.

No. 2 of this experiment indicates that the leucocytes of the patient take up the agglutinated red cells of the observer in the presence of patient's own serum, but not to quite the same extent as the observer's own leucocytes do.

Total number of intracellular red cells in 1 = 111

" " in 2 = 74

No. 3 indicates the non-susceptibility of the patient's own red cells. There is no auto-agglutination or auto-phagocytosis.

The corpuscles of the normal person (A. C.) were resistant to the patient's serum. The leucocytes of A. C. were now tested with a mixture of the patient's serum and the observer's red cells. These were freed from leucocytes by repeated centrifugalising in citrate solution, and pipetting off the upper layers each time so as to remove the leucocytes. The observer's red cells were agglutinated as before but only a stray example of erythro-phagocytosis could be found, although the control experiment with the observer's leucocytes indicated this at 26%.

Leucocytes	Red Cells	Serum	Result
1 - Observer	Observer	Patient	Agg. = + Phag. = 26%
2 - A. C.	Observer	Patient	Agg. = + Phag. = ?

The occasional example of phagocytosis found in (2) might be accounted for by a few of the observer's leucocytes being present also.

These results may be grouped as follows:-

No.	Serum	Leucocytes	Red Cells	Result	
				Agg.	Phag.
1	P	P	P	0	0
2	P	A. M.	A. M.	+	+
3	P	A. C.	A. C.	0	0
4	P	J. A.	J. A.	0	0
5	P	P	A. M.	+	+
6	P	A. C.	A. M.	+	0
7	P. & A. C. 1 vol. of each	A. M.	A. M.	+	0

A. M. = Observer; A. C. and J. A. = Normal persons
P = Patient

Experiments 2, 5 and 6 of the above table show that the leucocyte is not an invariable factor. The leucocytes of the patient and of the susceptible blood act phagocytically towards the red cells of the susceptible blood, but those of the resistant blood (A. C.) do so very slightly, if at all. Experiments Nos. 1, 3, 4 and 5 show that the red cell plays an important role, those of the patient and of two other persons being resistant to agglutination and phagocytosis by the patient's serum. Experiment No. 7 demonstrates the anti-opsonic effect of the serum (A. C.), whose red cells were resistant. When 1 vol. of the serum of this person was mixed with 1 vol. of that of the patient, the mixture was found to have lost its opsonic power although agglutination occurred as before - a single doubtful example more could be found in the film. The control experiment with patient's serum alone and observer's red cells gave erythro-phagocytosis = 26%. Thus in the case of the re-

sistant blood (A. C.), the leucocytes are doubtfully phagocytic as compared with those of the observer and of the patient, and the serum neutralises the erythro-opsonin in the patient's blood towards susceptible red cells, but does not alter the agglutinating power.

All the results of the above experiments were verified several times with the exception of Nos. 6 and 7. These are single observations, as subsequently (ante-mortem) the serum had lost its opsonic power.

EFFECT OF HEAT

Serum	Leucocytes	Red Cells	Result	
1 vol. P	A. M.	A. M.	Agg. = + Phag. = 18%	Control
1 vol. P (57° C. 40 mims)	A. M.	A. M.	Agg. = + Phag. = 0	
1 vol. P (51° C. 40 mims) 1 vol. A. M.	A. M.	A. M.	Agg. = + Phag. = 0	

Results:-

1 - At 57° C. phagocytosis is lost, agglutination is retained

2 - Opsonic power lost at 57° C. is not restored by reactivation with normal serum

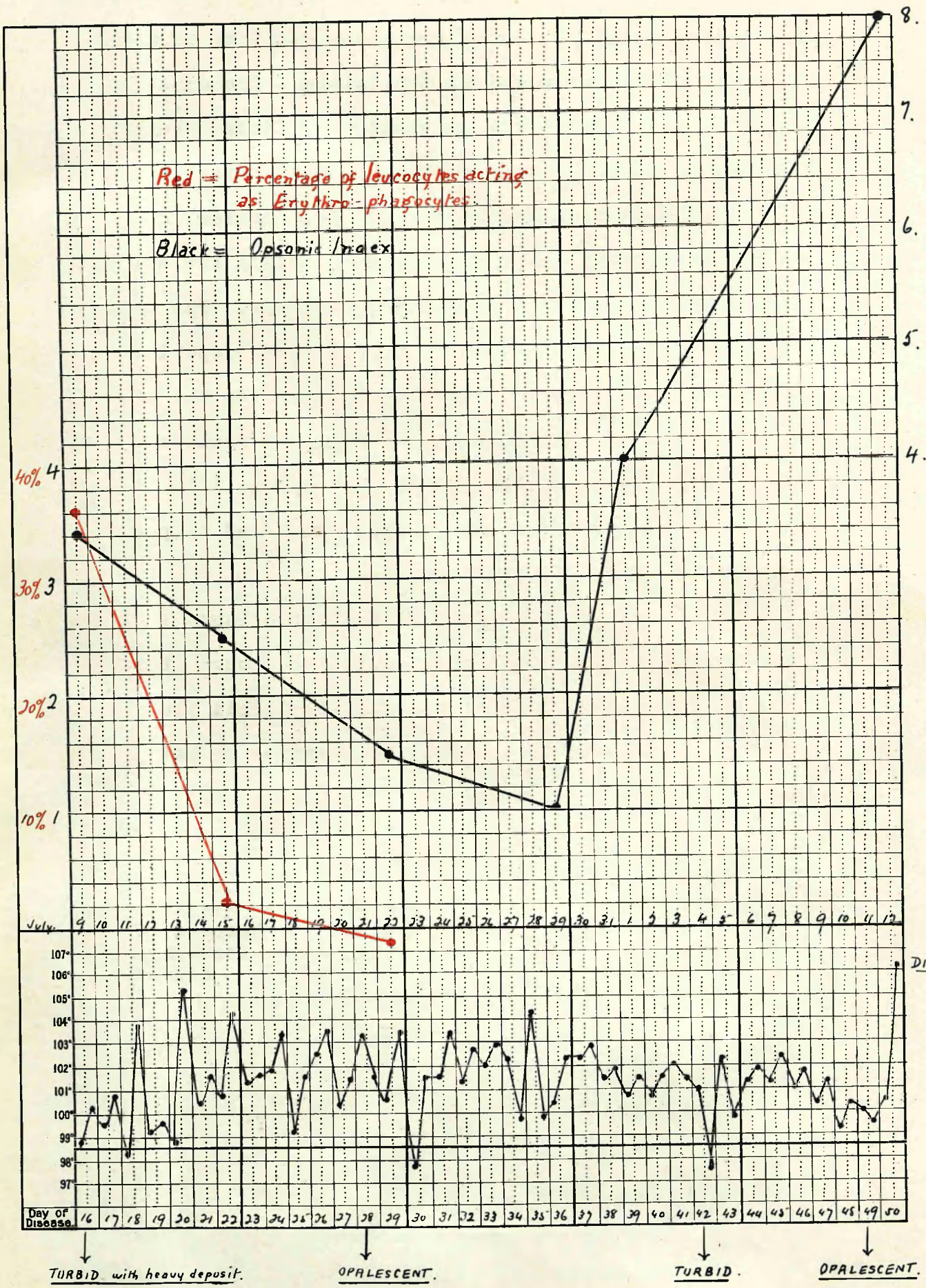
These features are dealt with later.

A short description follows of the other cases in which this phenomenon occurred, with charts of the erythro-phagocytic index and opsonic index for meningo-coccus.

Chart, No. 26

(26)

W. McL 25 yrs.



CONDITION OF CEREBRO-SPINAL FLUID

CASE II - W. M., male, aet. 25 years *Chart 26.*

This patient presented the usual symptoms of a moderately acute case of cerebro-spinal meningitis. The onset was not very abrupt, nor were the symptoms very acute. Persistent and variable headache, with periodic attacks of restlessness, were the chief symptoms. Emaciation was very marked and rapid throughout. The patient finally sank into a low muttering delirium, and died on his 50th day of illness. The cerebro-spinal fluid on admission was turbid, with a heavy deposit. On the 21st day it was very considerably clearer; on the 42nd day it was turbid again, and then became slightly opalescent before death. This secondary turbidity corresponds with a fresh extension of the inflammatory process, verified by post-mortem examination as in the previous case.

Post-mortem examination. The optic chiasma and pons varolii were buried in organizing exudation, tough, thick and adherent. The cisterna magna was full of turbid flaky fluid, and there was extension up the Sylvian fissures on both sides. Vertex was free from exudation.

COURSE OF CASE

Day of Illness	Erythro-Agg.	Erythro-Phag.	Opsonic Index for Meningococcus
16th	+	36%	3.4
22nd	+	2%	2.5
29th	+		1.5
36th	+	0	1
39th	+	0	4.5
44th	+	0	
50th	+	0	8.5

Serum	Leucocytes	Red Cells	Result	Time
W. M.	A. M.	A. M.	Ph. = 0 Agg. = +	1 hr.
W. M.	W. M.	W. M.	Agg. = 0 Ph. = 0	1 hr.
A. M.	A. M.	A. M.	0	1 hr.
A. M.	W. M.	W. M.	0	1 hr.

The mixtures were - 1 vol. Serum
1 vol. Leucocytes and red cells
1 vol. Normalsaline solution

A. M. = Person yielding the cells susceptible to agglutination and phagocytosis

This table shows that the serum of the person whose cells were agglutinated and phagocytosed by the action of the serum of W. M. (patient) did not agglutinate the cells of the patient, nor submit them to phagocytosis by leucocytes known to be actively phagocytic towards opsonised red cells.

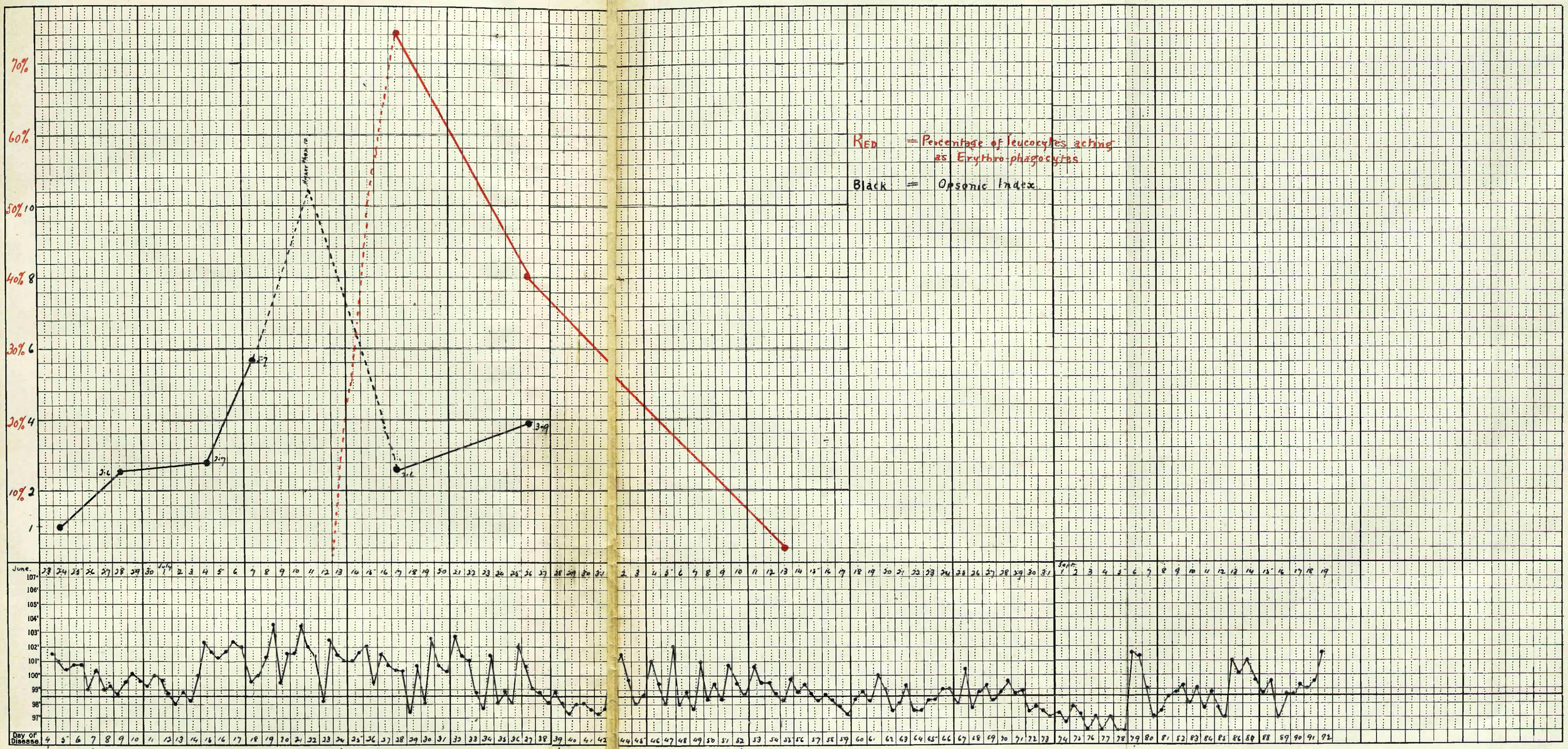
The ventricles contained turbid fluid and were little distended. There was no exudation on the cord. Emaciation was extreme.

The blood in this case was first examined on the 16th day of illness and found to have both erythro-agglutinating and phagocytic properties in a high degree. The agglutinating power remained high till death.

The features of this case are very similar to those of J. A., and need not be detailed. Reference to Chart 26 and table will illustrate the course of the phenomenon. The haem-opsonic power disappeared about the 29th day, and was present at first observation on the 16th day.

Chart, No. 24.

J. B. 4 yrs. female.



CONDITION OF CEREBRO-SPINAL FLUID FROM TIME TO TIME.

Chart 27.

CASE III - J. B., female, aet. 4 years (Chart 27.)

This child on admission was acutely ill and comatose. The facies had a dusky flush; restful; the neck was stiff, but the head was not retracted. The cerebro-spinal fluid was opalescent on the fourth day, and was so thick and turbid on the seventh day that it would scarcely flow through the needle. On the seventh day the child recovered her senses, was bright, lively and intelligent, and could sit up in bed without aid. After a few days of apparent convalescence, she lapsed into the chronic stage with recurring headache and vomiting, and an irregular oscillating temperature. Wasting was now noticeable, and continued rapid and to an extreme degree. Throughout this period the cerebro-spinal fluid remained turbid. On the 38th day it was much clearer, and it varied between turbidity and opalescence up till death. These facts indicate that resolution was delayed, or that fresh infection had occurred.

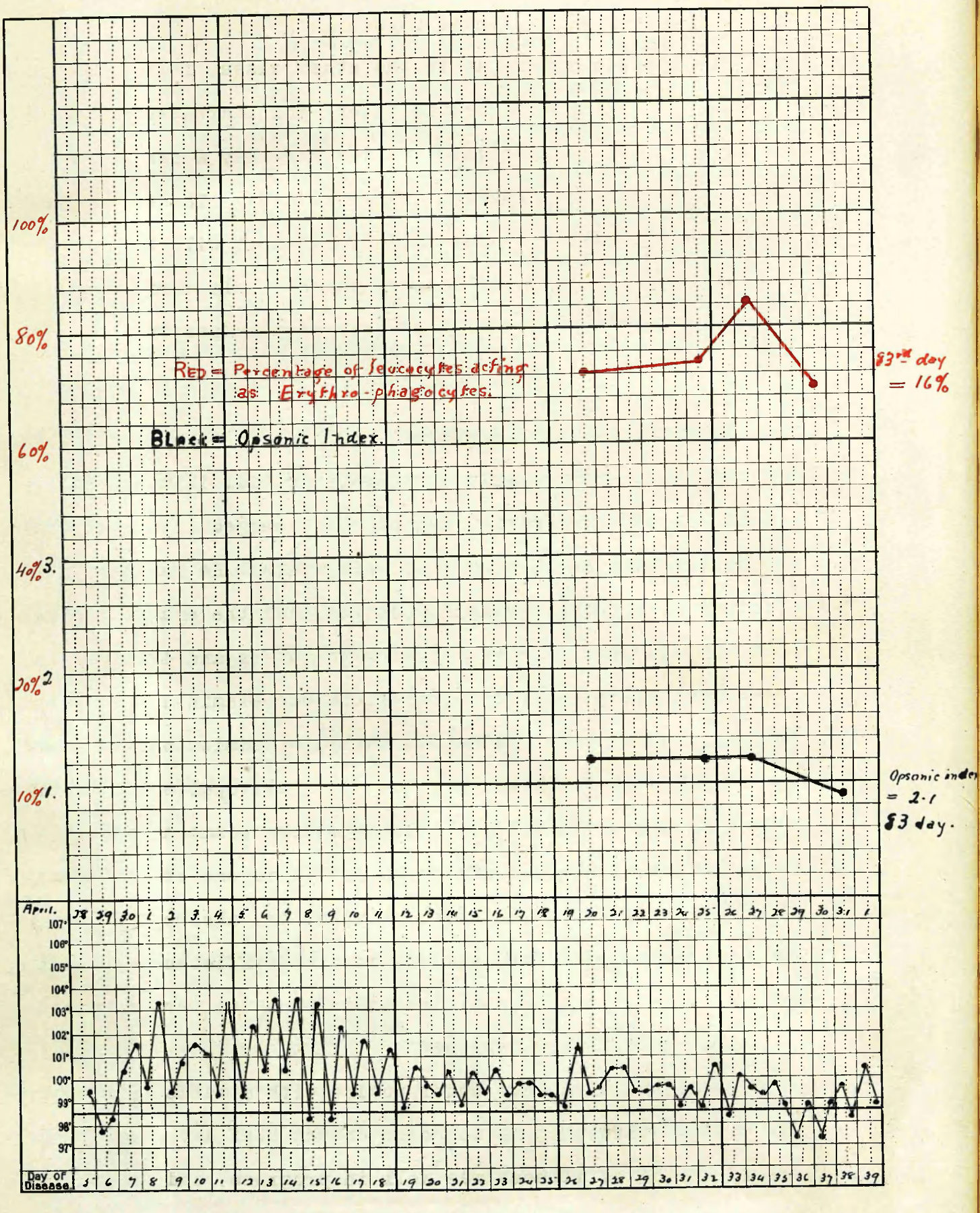
There was no post-mortem examination in this case, but, judging from similar cases, a collection of unresolved exudation at the base of the brain and an advanced degree of hydrocephalus, would probably have been found. On 6th September the fluid became clearer, indicating that resolution was going on, but symptoms of hydrocephalus continued to develop and the fluid remained under high pressure. Patient died on the 102nd day.

I am unable to say when the erythro-agglutinating power began to manifest itself, but on the 22nd day of illness, when first observation was made, it was very striking. It remained marked and immediate, and was still present on the 53rd day of illness but much diminished in intensity.

The erythro-phagocytic power was here most marked of all

Chart, No. 28

J. B. 9 yrs. male.



the cases; it reached 74% on the 28th day from nil on the 25th day, developing within three days.

The opsonic index, as in the other cases, fell with the rise in the erythro-phagocytic index, and increased rapidly when the latter disappeared. On the 53rd day a few cells, showing erythro-phagocytosis, could be seen. The total duration of this phenomenon was 25 days; thus it will be seen that the period of evidence of erythro-phagocytosis corresponds here also roughly with the period of secondary temperature, coming on after the first acute symptoms have passed off.

CASE IV - J. B., male, aet. 9 years (chart-29)

This patient had only a moderate initial illness, with typical secondary pyrexial period which merged into a chronic variable condition lasting about six weeks. He had the usual features of a rather prolonged course, and the emaciation was very considerable. He recovered with senses and intelligence unimpaired. The cerebro-spinal fluid was examined weekly by lumbar puncture. The fluid was turbid on admission; became opalescent a month later; remained variably opalescent, slightly opalescent and turbid till temperature became normal, two and a half months after admission. This is one of those cases in which basal exudation has continued, and absorption has occurred after a prolonged period, without sufficient organisation changes at the base of the brain to cause permanent hydrocephalus as so easily happens.

This case also came under notice with a marked agglutinatory and erythro-phagocytic power on the 22nd day when the secondary period of illness was well in progress, and was still present on the 83rd day, when the observations were interrupted,

and convalescence with clear cerebro-spinal fluid and a normal temperature was present. Thus the phenomenon was very intense, 74%, 72%, 84%, and 70% at its height, and very prolonged (probably well over the period of observation, i.e., 61 days).

This was the only one of the four cases which recovered. It will be also noted that the opsonic index was never above two.

COMPARISON OF CASES OF ERYTHRO-PHAGOCYTOSIS

Case	Erythro-Phagocytosis commences	Erythro-Phagocytosis disappears	Total Duration of Erythro-Phagocytosis	Duration of Illness	
I	13th day	23rd day	10 days	27 days	Death
II	28th day	53rd day	25 days	104 days	Death
III	First noted 16th day	29th day	13 days	50 days	Death
IV	First noted 22nd day	Last observ. 83rd day	61 days	78 days	Recovery

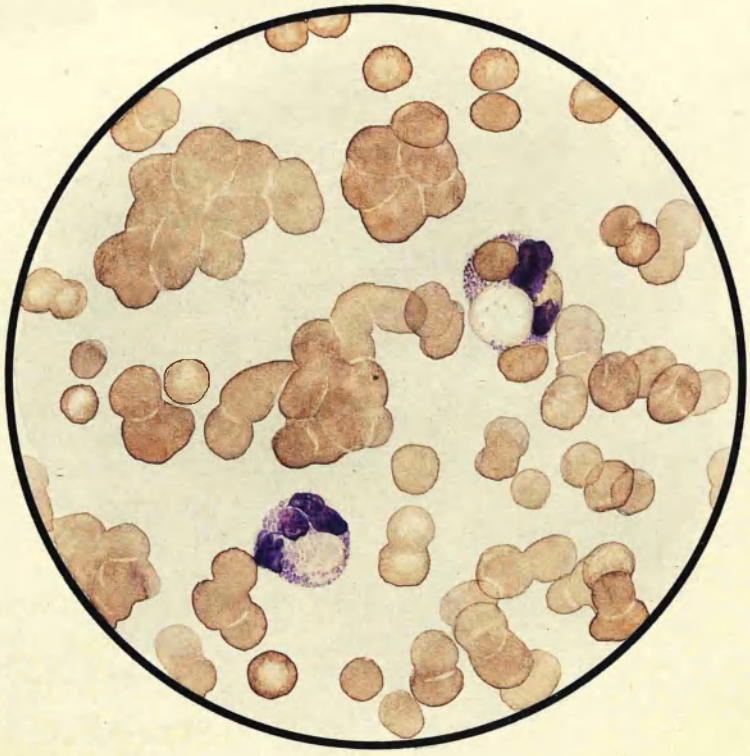
NOTE: In the three cases that died, the phenomenon of erythro-phagocytosis disappeared ante-mortem. In the one case that recovered, it persisted well into convalescence. Of these four cases, two, III and IV, came under observation with the phenomenon well marked at the first observation. The agglutination in the first three cases remained throughout, but was more intense during the periods of erythro-phagocytosis, although no definite observations of its strength were made. In Case II it can be said definitely that agglutination was ob-

served microscopically a week before the appearance of erythro-phagocytosis, as at that time its appearance was being watched for. In Case I this was noted at times, but when the films were examined for the opsonic index (as examined) it was found to be present before there was any evidence of erythro-phagocytosis. Its presence was not noted at the time. The action at its height is sometimes so marked that it cannot escape notice as immediate agglutination occurs; but in the early stages when it is weaker, it may readily be missed.

In Cases III and IV the commencement of erythro-phagocytosis must date from at least some days earlier, as the percentage of cells containing erythrocytes in both cases was over 70 when observations were first made. Allowing seven days for this development, the day of occurrence in three of the cases would be about the 12th day. In Case II, however, it did not show itself till the 28th day.

SOME GENERAL FEATURES OF THE PHENOMENON

The agglutination of red cells which occurs in these cases is very rapid and complete; it is noticeable almost at the moment of mixing. When incubated for 15 minutes and spread out in films, these masses do not separate out into their units as ordinarily massed red cells do. An ordinary serum will cause clumping of the red cells of another normal person in greater or less degree as is well known, but the agglutination is not a firm one, and in spreading the films they become easily separated, or else lie in rouleaux or clusters. In agglutination as

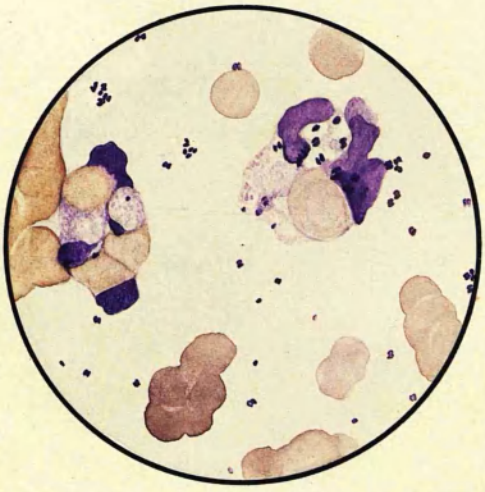


Pl. 14. i. Close agglutination of red cells. (Note contour lines)
ii. Erythrophagocytosis.

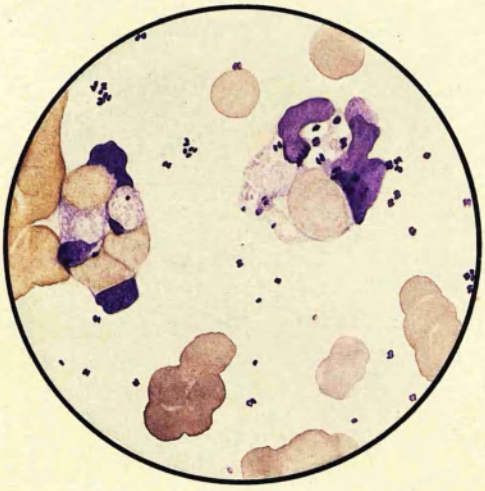
occurring above, the union of cells is a close and forcible one with characteristic microscopic appearances. (Plate 14)

The extent to which phagocytosis occurs in quarter of an hour is remarkable. Where the number of leucocytes acting as phagocytes is large, many have their protoplasm swollen and nuclei distorted to include five or six red cells, a vacuole, and a few diplococci. In some films the leucocytes containing two or three red cells are in a majority. The ingested red cells are often found larger or smaller than those outside. (Pl. 14) Both swollen and shrunken forms are characterised by the weakness of their affinity for eosin as compared with normal red cells.

In view of the theory that eosin granules represent ingested haemoglobin, it is of interest to note that in these experiments no appearance of increase of eosinophile granules in cells was observed even after incubation for one hour. It was never possible to mistake fragments of ingested and partially digested red cells for eosinophile granules; the leucocytes phagocytosing red cells also phagocytose cocci; eosinophiles do not phagocytose cocci. Mononuclear leucocyte cells also take part in the general phagocytosis, and can be seen with their nuclei and protoplasm distorted so as to enclose two or three red cells. Phagocytosis of organisms can be seen going on at the same time. In fact, those cells which take up most red cells are those in which most organisms are found, while other leucocytes are taking part in neither action. This feature, so uniformly noticeable in opsonic work with the organism, appears to hold good here also. The phagocytic action is manifested by a few cells to an extreme degree, rather than distributed over all more or less equally. It is common to see two or three red



Pl. 12. Phagocytosis of
Erythrocytes & meningococci.



Pl. 12. Phagocytosis of
Erythrocytes & meningococci.

cells and six or 12 organisms in a single leucocyte, while others around contain nothing at all. In some films, all the cells containing red cells also contain organisms. (Plate 12)

Various sera, both in health and disease, have been found by other observers casually to possess an erythro-agglutinin, and an erythro-opsonin for the cells of another normal person's blood. Agglutinins occur much more frequently than opsonins, and have been studied by various observers. Any relationship which they were formerly thought to possess towards pathological states has been disproved (Hektoen[13], Gay[8]). In opsonic index estimations it is often found that marked clumping of the red cells occurs and interferes with accuracy, but the fact that the rise and disappearance of the phenomena in these cases can be traced, removes them from that category. This property of the serum develops during and has some relationship to the course of the disease. It was present in only four of the 58 cases examined in various stages for the opsonic index, although it is probable that these sera might have had analogous properties towards another person's corpuscles, had a search been made.

In the case of J. A., Case I, the red cells of two normal persons were found to be non-susceptible, and a further search with the sera of four other cerebro-spinal cases tested against the cells of three normal individuals failed to give any result. Thus its occurrence appears to be purely accidental, and its interest only as a casual manifestation of the nature of an accident of metabolism. However, in the cases described, its presence can be reconciled with definite features of the disease. It develops and reaches a maximum during the second or chronic stage of the disease, the associated features being

continued fever of an oscillating type characteristic of this stage, continued turbidity of the cerebro-spinal fluid, continuance of symptoms, and marked wasting. In Case IV it seemed to outlast the period of infection, being still present when patient was definitely convalescent; that is to say, it belongs to a period when toxic absorption is at its height. The chief clinical feature in these cases has been emaciation, rapid and to extreme degree, although in other cases equally extreme emaciation has been present without the development of erythro-agglutinin and erythro-opsonin, as far as the observer's blood is concerned. It will be noted that the opsonic index fell with the establishment of this feature. In the case of W. M., Case II, it was also low throughout when tested with a blood whose cells were resistant to agglutination and phagocytosis.

RESUME

Points of experimental interest are given along with the descriptions of the cases yielding the phenomena. No attempt has been made to analyse fully the mechanism on which erythro-phagocytosis depends. I made an attempt to obtain more cases in order to make some further experiments, but without success. Four patients' sera were tested against three other normal persons' cells, but yielded negative results. However, the following points appear evident:-

1 Although phagocytosis was never noticed without agglutination, yet agglutination occurred without phagocytosis, as seen where agglutinin appeared first and opsonin later, and also

where the latter disappeared first. In the example mentioned by Hektoen[13], the two phenomena are associated, though, he remarks, that rarely opsonin may exist without agglutinin. Davis[6] noted two cases of agglutination without phagocytosis in cerebro-spinal meningitis.

Further, heating to 57° C. destroyed the opsonin but not the agglutinin.

2 The exact nature of the erythro-opsonin among anti-bodies has not been determined; like normal opsonins in general, it is destroyed by heat, but this might be due merely to the destruction of a complement element with the persistence of an immune body, which by itself has no opsonic effect. In this respect, therefore, the opsonin would appear to depend for its action upon complements, just as most normal opsonins do, as established by Muir[18] and Martin.[18,19] It may be noted that the immune haemopsonins investigated by Barratt[2] and Hektoen[13] were notably stable, resisting 70° C. for one hour.

A positive result with the reactivation experiment would have been conclusive; but the negative result obtained does not permit of any definite conclusion since, as was seen in the case of another normal serum whose cells were resistant to the opsonic action, the mixture of his serum with the opsonic serum inhibited the opsonic action. Further experiments in the direction of absorption in the cold would be desirable, but further cases have not been forthcoming.

3 The individual origin of the red corpuscle is an essential feature. The patient's leucocytes and those of the observer were phagocytic towards the opsonized red cells of the susceptible blood (the observer's), and the patient's serum contained

active agglutinating and opsonic substances for those red cells, but there was no auto-agglutinin or auto-haemopsonin in the patient's serum in regard to his own red cells. The existence of auto-agglutinins and auto-opsonins is noted by Hektoen[13] in some acute infections.

Wright[21] describes two cases in which the patient's leucocytes submitted his own red cells to phagocytosis; this action was found in a case of pneumococcal cellulitis of the neck and in a pneumococcal cystitis. He suggests that some toxic damage may have been done to the red cells of the patient leading to auto-phagocytosis without the intervention of opsonin. Auto-agglutination and auto-phagocytosis could never be demonstrated in vitro.

In his study of the blood, Dr. Dow examined a large number of films from marantic cases without noticing any example of erythro-phagocytosis. This process does not appear to occur at least in the circulating blood. Accordingly, there is no evidence that a similar process of erythro-phagocytosis occurs within the tissues, and is responsible for the marantic condition.

4 The leucocyte is not an indifferent factor, for, given red corpuscles which, under the influence of the serum, were phagocytosed by the leucocytes of one individual, were not taken up by the white cells of two other subjects. The variable phagocytic activity of the leucocytes to opsonized corpuscles is a significant feature, in view of the theory held of the neutral role played by the leucocytes in phagocytosis in general. This individual variability in human leucocytes is also remarked upon by Hektoen[13], who mentions this feature as also well established in animal experiments.

5 Erythro-agglutination and phagocytosis, as far as they have been studied, occur in those cases where toxic phenomena are marked, e.g., extensive emaciation, excessive nitrogenous excretion in the urine, etc., and during that phase of the case where this is greatest, the chronic stage.

6 The apparent effect on the opsonic index is to be noted. Where haemopsonin appears in the blood, the opsonic index falls markedly, and rises when the former disappears. Where the percentage of cells ingesting red corpuscles is high (60% or 80%), as in two of the cases, it might be supposed that the cocci are crowded out although opsonized. But there is an absolute deficiency in bacteriopsonin because, on employing leucocytes and red cells with which erythro-agglutination and phagocytosis did not occur, the index was also comparatively low, just as when erythro-phagocytosis was present. This, of course, presupposes that the leucocytes of the second normal person were of similar phagocytic activity to those of the observer.

Agglutination of human corpuscles by human serum is a well recognised phenomenon. The occurrence of haemopsonins is, however, much rarer.

The chief account of this phenomenon is by Hektoen.[13] He[14] quotes a number of cases of acute infections in man, e.g., typhoid fever, pneumonia, cellulitis, and scarlet fever, in which haemagglutinins and haemopsonins were present for the cells of some individuals but not for others. Its course in any particular case has not been traced, and no attempt made to analyse its significance. Auto-agglutinins and auto-opsonins were also present in a few of these cases. It is suggested that such substances may be the causal agents in grave anaemias.

Davis[6] found auto-agglutinin and auto-opsonin in two cases of cerebro-spinal meningitis. In the one case it was noticed on the seventh day of illness, and in the other case, in the second week. The phenomenon was not traced. These are the only references to the existence of these substances in cerebro-spinal meningitis which have been found.

The existence of haemopsonins is well established by animal experiments. Rabbits rendered immune to goats' blood furnish a serum that submits the red cells of the goat to phagocytosis. The spontaneous occurrence of such bodies in disease has merely been noted and never studied.

So far, no facts have been adduced which explain the presence of these substances in the blood. Their occurrence is purely accidental, and their significance unknown. The subject is, however, worthy of further study, as investigation may help to throw some light on the nature of agglutinins and opsonins in general. The frequent occurrence of erythro-agglutinins and opsonins in acute disease suggests that they may be found to play some part in the phenomena that accompany acute infections.

NOTE ON EFFECT OF HEAT ON THE OPSONIC POWER OF THE SERUM FOR THE MENINGO-COCCUS

The facts that normal human serum has only a very slight opsonic effect on the meningo-coccus, and that in the course of an infection with that organism the patient's serum may become very active in causing phagocytosis, are suggestive of the development of a specific anti-substance. Accordingly, it ap-

peared of interest to observe the effect of heating on sera which possessed a fairly active opsonin.

The results of a number of such are shown in Table-p.96 It will be noted that the slight opsonic power of normal serum is still further reduced as the result of heating at 57° C. for 40 minutes. With the patient's sera, in four out of the six cases a very marked fall in opsonic effect occurred. In one other (M. I.), a moderate amount of opsonin remained over in one experiment, but later on in the disease during a relapse, although the index was higher than previously (9.3), it fell to 1. on heating. The serum of another patient (Mrs. S.) evidently contained a large amount of thermostable opsonin (10+) before heating. In this last instance, heating at 65° C. for 40 minutes completely destroyed the opsonin as well as the agglutinin. This variable behaviour with respect to heating has also been noted by Houston and Rankin.[11]

It is a generally accepted fact that complements are not increased by experimental immunization, and that the specific bodies which develop are of a tolerably stable nature. It is possible that here we have to deal with an opsonic effect due to a specific immune body, which in many instances has by itself a comparatively small opsonic effect but which is greatly augmented in its power when complement acts in combination with it. Reactivation experiments would in all likelihood have given definite information on this point.

Name	Index	Index after heating at 57° C. for 40 minutes	
Normal	1	.3	
P. O.	4.5	.4	
M. I.	7	2.5	
Normal	1	.6	
M. I.	9.3	1	
P. O.	4	.5	
P. H.	4	.4	
Mrs. S.	10*	5.9	At 65° C. Agg. and Phag. = 0
J. C.	4.5	1.1	
A. G.	6.2	.7	

SECTION IV

NOTE ON VACCINES

The effect of injecting an emulsion of organisms killed by heat was studied in 12 cases, a few of these receiving inoculations on two or three occasions at intervals of a week. The doses varied within wide limits from amounts which would correspond roughly to from half a culture on Löffler's serum to three or four cultures. Two or three cases usually received the same dose so as to contrast the effects produced. The cases were all in the chronic or late chronic stages, either with oscillating temperatures or with temperatures practically

normal. Seven of the 12 cases ultimately recovered.

It was found that, in general, it required large doses to produce an appreciable clinical reaction, some cases failing to show any disturbance even with amounts corresponding to three or four cultures, while others would react moderately to an injection of one culture. None, however, reacted so markedly as did a normal person to an injection of half a 24 hours' culture. Where a sufficient dose has been given to a chronic case to produce a clinical reaction, a rapidly produced and transient disturbance results. Chart 29. is characteristic; e.g., in the case of J. B., aetat nine, an amount corresponding to one culture of five days' growth on glucose ascitic agar was injected at 4 p.m. on the 58th day of illness. The culture was killed by heat at 60° C. for half an hour. This patient was in the late chronic stage; the temperature as a rule normal, but showing occasional oscillations every few days. The cerebro-spinal fluid was opalescent, and contained organisms. At 6 p.m. the temperature was 101° C., and the pulse had risen from 104 to 144. There was some vomiting and patient was somewhat restless; at 10 p.m. temperature was normal, and at 2 a.m. the pulse was again normal. In other cases headache was present after injection, but the febrile reaction was never more decided than this, even with considerably larger doses. Where the temperature is an oscillating one, the injection may cause a higher oscillation than usual, or determine a rise in temperature where it would not have been expected. The increase in pulse and temperature, and the headache, if present, usually follow within two to four hours of the injection. These injections were given into the groin, and were usually followed by some (often considerable) local irritation.

A dose of vaccine which was borne without disturbance by two patients in the chronic stage of cerebro-spinal meningitis caused decided illness in a normal subject. The effect of injection of vaccine was compared in three cases:-

Case I, aet. 7, 87th day of illness, temperature continuing normal, hydrocephalus; organism present in cerebro-spinal fluid.

Case II, aet. 10, 45th day of illness; temperature in general normal, with infrequent small oscillations.

Case III, aet. 3, normal subject.

An amount equal to half a culture of a laboratory strain on Löffler's serum of 24 hours' growth was injected into each patient at the same time. In Cases I and II no reaction occurred. In Case III the result is shown in Chart *30*

The temperature rose to 100.6 four hours after the injection, and there was a simultaneous increase in both pulse and respirations. Next day the temperature remained between 101° and 102° C., and patient was somewhat restless, but there was no headache. The temperature reached normal rapidly 30 hours after injection. There was some tenderness round the site of injection, and the inguinal glands remained palpable for a day or two.

The behaviour of the opsonic index in this case is shown in the chart.

Date	Index	
24th July	1.3	Before injection
25th "	1.0	During febrile period
26th "	2.1	Temperature normal
29th "	0.8	
1st Aug.	1.2	
6th "	-	Agglut. = 0

There appears to be with the dose employed either no negative phase, or at most a very fugitive one followed by a distinct positive phase one day after temperature has reacted normal and two days after injection. The positive phase was very transient, however, and three days later the effect of the inoculation seemed to have passed off.

In general these results correspond with the findings of Davis[6], who treated himself by injection of a 24 hours' culture killed by exposure to 65° C. for 30 minutes. In his case also the maximum temperature occurred within a few hours of the injection but declined somewhat more gradually, reaching normal on the fourth day. The opsonic index rose on the second day to 2.3, then falling till the fifth day, when it was slightly subnormal. There is a striking general resemblance between these results and those of the writer, which were obtained independently in July, 1907, before the publication of Davis' paper. Further, the effects caused by injection of dead organisms in the case of patients with cerebro-spinal meningitis are in accord with those of Davis, who describes two cases both in the chronic stage. It was found that injections of, in the one

Chart-29.

June 1907

DATE.	17	18	19	20	21	22						
Day of illness.	55	56	57	58	59	60						
	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.	A.M. P.M.
	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10	2 6 10 2 6 10

TEMPERATURE—FAHRENHEIT.

107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97° 96°

Pulse 130 110 100 90 80

J.B 9 yrs Showing rise in temp. and pulse.

INJECTION OF VACCINE

PULSE,

RESPN.,

Chart 30

July. 24 25 August

DATE.	6-10	2-6	10-2	6-10	26	27	28	29	30	31	1																			
DAY OF ILLNESS.	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME

TEMPERATURE—FAHRENHEIT.

107°	
106°	
105°	
104°	
2 103°	
1·8 102°	
1·6 101°	
1·4 100°	
1·2 99°	
1 98°	
97°	

J. R. act 3

Red = Opsonic Index.

Black = Temperature.

Pulse.	
Resp.,	
Bowels.	
Urine. Oz.,	
Urine. Alb.,	

Chart 31

MAY 1907.

DATE.	28		29		30		31		1		2		3		4		5		6	
Day of Illness.	43		44		45		46		47		48		49		50		51		52	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10	2 6 10

TEMPERATURE—FAHRENHEIT.

107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97° 96°

Pulse 120 110 100 90 80 70

A. McB. 7 YRS. showing rise in temp. and pulse

INJECTION OF VACCINE

PULSE.

RESPN.,

case, several cultures, and in the other of a single culture of the same strain as used in the control experiment on himself, produced only a mild reaction. The former case recovered, the latter died. It is not possible to say that the injections had any notable effect.

It is impossible to draw conclusions regarding any benefit resulting from such injections, as the number of cases was too small. But it has been definitely noted that, after the reaction (if any), patients may be brighter and freer from symptoms for a longer time than previously. This was corroborated by independent observers.

As none of the injections were controlled by opsonic estimations, nothing can be said regarding their effect on the opsonic content of the blood. In this connection the fact may be recalled that the opsonic index has been found to bear little relationship to the ultimate course of the disease. As already noted, a high index may exist in a case that ultimately dies, or a low one in a case that ultimately recovers.

SECTION V

GENERAL CONSIDERATIONS

THE CLINICAL APPLICATION OF THE PRECEDING OBSERVATIONS

An effort has been made to correlate immunity phenomena, i.e., agglutinating and opsonic power of the serum with the clinical aspect of the disease and its pathological anatomy. A study of the literature made this seem desirable, since the accounts met with are written from a more or less one-sided point of view. Thus, while the picture of the natural history of the disease would be completed, some aid towards a specific

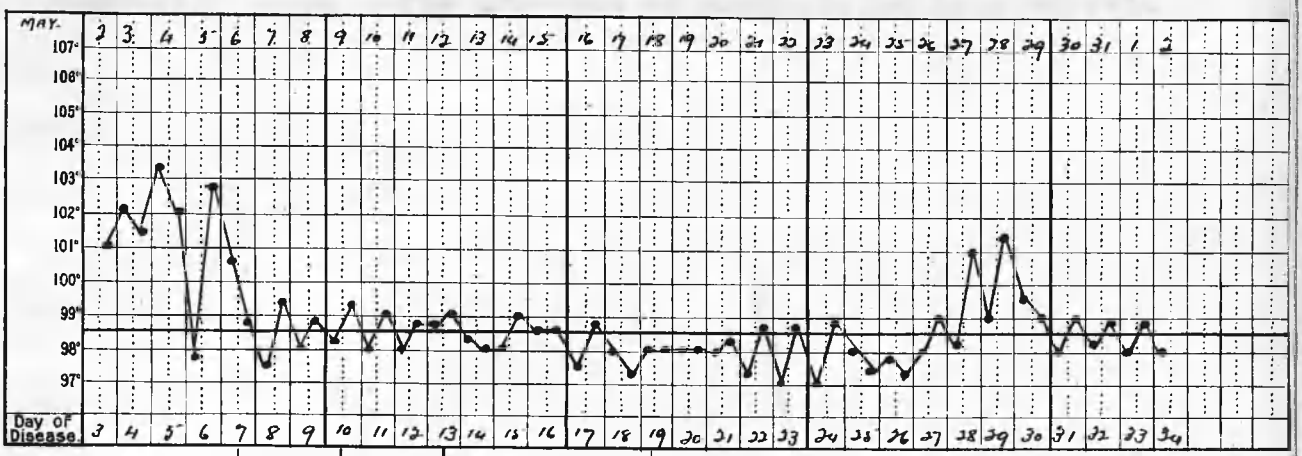
treatment by means of vaccines might be obtained from the opsonic results. In the following paragraphs the results bearing on these points are combined as far as possible, and the prognostic and therapeutic indications discussed.

THE TOXIC PHENOMENA THAT ARISE IN THE COURSE of the DISEASE AND THEIR RELATIONSHIP TO THE ANTI-BODIES STUDIED

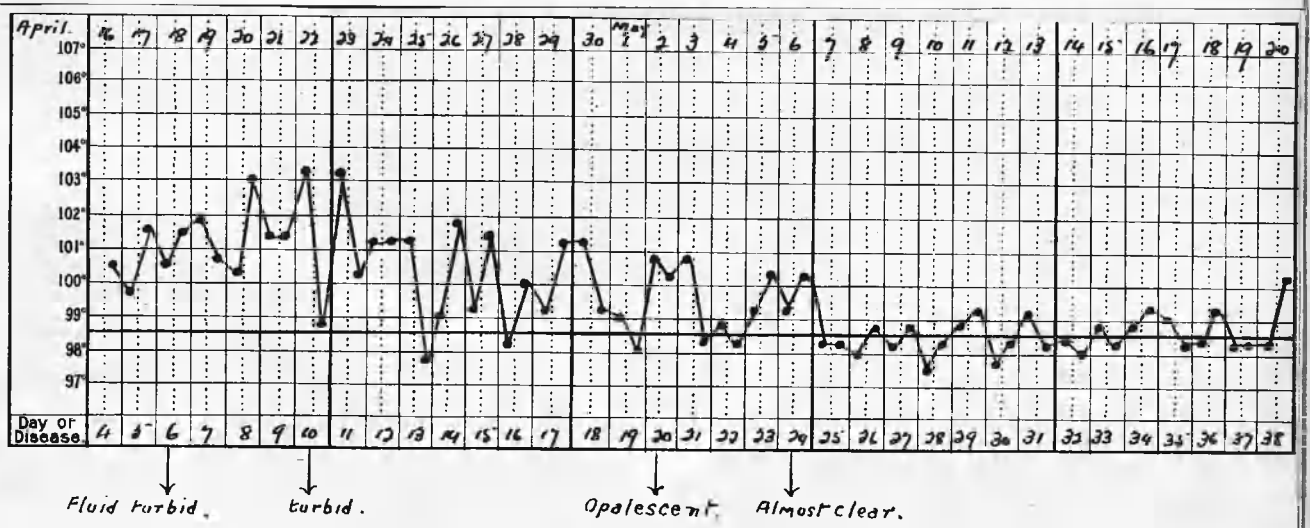
The onset in cerebro-spinal meningitis is extremely variable. On the one hand the patient may die of toxaemia before even clinical diagnosis of the nature or site of infection is possible. On the other hand the initial symptoms may be mild, the disease simply declaring itself as a chronic local infection. Between these extremes all variety of initial symptoms of toxaemia occur. But the typical acute meningo-coccal infection is acute in onset, severe in course, has gravely toxaemic symptoms, and terminates by crisis on any day from the fourth to the ninth. In these respects it resembles an acute lobar pneumonia due to a pneumococcal infection. These cases are of a very prominent class clinically. Chart 32 Cases of shorter duration and earlier crisis probably occur, but do not reach hospital. Agglutination reaches its highest in cases which conform to this type. It has been shown to be proportional to the severity, duration, and amount of reaction to the early infective symptoms which accompany the onset of the acute disease. Agglutinins fail to develop on the one hand where the symptoms of toxaemia are extreme and the case is rapidly fatal, or on the

Examples of natural modes of recovery:-
Charts showing characteristic course of cases.

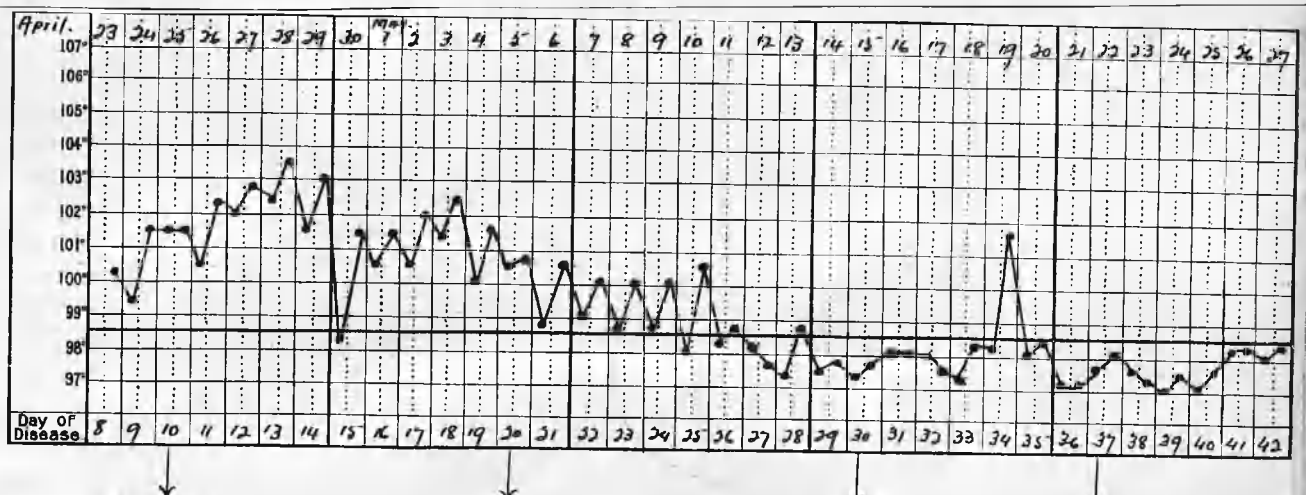
CASE I. Recovery by crisis on the 7th. day.
CASE II. Recovery by lysis within 4 weeks (acute onset)
CASE III. Recovery by lysis within 4 weeks (mild onset)



CASE I. A.G. Chart, No. 32



CASE II. J.H. Chart No. 33.



CASE III. A.M. Chart, No. 34

other hand where the symptoms of onset are sub-acute or only transiently acute. The presence of agglutinins in a chronic case enables us to say that the onset was acute, and the reaction was more or less vigorous.

Similarly, the development of opsonins appears to depend on the same processes as regulate the formation of agglutinins. There are, however, some striking exceptions in which opsonin is very marked and agglutinin practically absent, and vice versa.

Granting that the patient survive the acute stage, which rarely lasts longer than a week, he may enter upon a second period more or less prolonged, during the latter part of which the exudate, previously laid down, is absorbed with greater or less rapidity. Various factors come into play which determine the length of this period and the final result. One may mention here 1. Amount of exudate; 2. Site of exudate; and 3. Fate of exudate, i.e., resolution or organisation. Which of the two latter processes takes place depends on factors not yet ascertained. The estimation of agglutinins and opsonins fails to give any information as to the duration and ultimate event of this phase.

The variability in duration is remarkable, but as experience of cases accumulates, one is struck by the fact that very many of the cases which recover in this stage commence to convalesce within about four weeks' time from the onset of the disease. These cases always terminate by a lysis which lasts from seven to 14 days, and during this time the temperature shows considerable remissions. (Charts 33-34)

The charts of these patients show a striking similarity, the temperature during the early period of the acute illness

remaining moderately elevated, then commencing to oscillate during the secondary stage, falling to normal about the middle or end of the fourth week. The course of the temperature accurately reflects the underlying pathological processes. The process of infection can be readily studied by successive lumbar punctures, and correlated with the temperature.

For instance, in the acute critical variety, the temperature falls by crisis and remains normal in the most favourable case. In many of these cases the cerebro-spinal fluid is distinctly turbid, and is under considerable pressure. Whatever exudate is present is removed without further pyrexia, as in cases of pneumonia. Chart 32 Again, in cases which have entered the secondary stage where recovery is by lysis, the cerebro-spinal fluid begins to become less turbid synchronously with the fall of temperature, and continues to clear as the temperature declines, becoming normal some days after the pyrexia has ceased. This may be called the second natural mode of favourable termination. The gradual clarification of the fluid associated with the decline in pyrexia is one of the best guarantees that resolution is proceeding satisfactorily. This course of events has been established in a large series of cases by Dr. Connal, whose results are in course of publication.

These points are emphasized, as the general condition of the patient gives no indication of the progress of the case.

It was for these reasons that the study of the opsonic index was combined with that of the progress of resolution in the hope that some relationship might be established between them. The opsonic content during this period is usually very high, and may remain high or continue to increase during this period.

During the secondary period, fresh symptoms and fresh phenomena make their appearance. In spite of the ingestion of large quantities of food and of apparent efficient absorption from the alimentary canal, extreme emaciation may rapidly supervene. The pyrexia by itself is not sufficient to account for this, as is evident on comparison with enteric fever cases. Recovery may occur even in cases with extreme emaciation. In this stage, nitrogenous excretion in the urine is excessive. As the results of Dr. McCall (not yet published) show, there is a very high excretion of urea during this period.

Apart from these examples of extreme emaciation, marked general loss of weight is a characteristic feature of this stage. Erythro-agglutinins and erythro-opsonins were seen in certain cases at this time. Some accident or perversion of metabolism is most probably responsible for these phenomena.

From the above considerations it would appear that the natural course of the disease has two definite phases, either of which may be practically suppressed, and that the average case is a combination of two separate broad toxic processes succeeding one another.

A brief summary of these facts is thus given:-

1st Period - Acute general infection; marked production of agglutinins in proportion to the severity, length and reaction. The typical case recovers by crisis. Rarely lasts more than a week. Opsonic index definitely established within first week

2nd Period - Symptoms of local irritation. Toxic phenomena are metabolic, wasting, urea excretion much raised; erythro-phagocytosis; recovery by lysis with gradual

Pl. 15. Persistent exudate at base of cerebellum. (None elsewhere)

resolution usually within four weeks. Agglutinins diminish rather than increase. Opsonins may increase or continue high

see chart no. 23.

3rd Period - Very chronic, variable temperature, persistence of organism, scanty exudate as a rule. Opsonins remain as in second stage. Agglutinins absent; opsonin remains as in second stage

THERAPEUTIC INDICATIONS

In many of these cases which are fatal after some weeks' duration, the post-mortem conditions simply show persistence of exudate at the base of the brain, and continued presence of organisms. The analogy between such cases and other long continued chronic infections suggests treatment by vaccines. Vaccine treatment might be controlled by opsonic estimations in the long standing cases where the index is low or moderate; but it has been shown that a persistently low index is quite compatible with ultimate recovery. On the other hand, cases may succumb to the disease although having a powerfully acting serum. (Plate 15 shows condition described)

Accordingly, it appears that the course of events in the cerebro-spinal system cannot be gauged by the opsonic content of the blood serum. If vaccine treatment is to be successful, it would have to be employed early in the second stage of the disease in order to prevent (a) hydrocephalus; (b) delayed resolution; and (c) recurrences due to secondary extension, which carry off a considerable percentage of patients.

LUMBAR PUNCTURE

It has been shown that there is throughout the disease an absence of opsonin from the cerebro-spinal fluid even though the blood content is high, and that lumbar puncture and withdrawal of a considerable quantity of fluid from the spine (6 drs. to 1 oz.), even when repeated at frequent intervals, does not determine the accumulation of anti-bodies in the cerebro-spinal system. This procedure does not appear to be harmful, as the removal of considerable quantities does not seem to cause extension of the disease. In only one case during the epidemic did renewal of the disease appear to occur after this operation.

The patient, a girl, aetat six, was progressing satisfactorily after a long illness. Lumbar puncture was performed in order to ascertain the condition of the fluid during early apparent convalescence. This was followed by a renewal of symptoms, renewed turbidity of the cerebro-spinal fluid, and a fatal result. Of course, it is not positively asserted that the relapse was due to the particular lumbar puncture. Some observations were made on the opsonic content of the blood before and after lumbar puncture (see Table I *p. 70*). These observations indicated that no increase in opsonin occurred 12 or 24 hours after lumbar puncture. They were carried out, however, on early acute fatal cases.

The value of Mackenzie and Martin's[17] mode of treatment by intra-meningeal injection of the patient's own serum would rest on the fact that, in this way, they artifically alter the dis-

tribution of anti-substances in the body, and determine their presence in the cerebro-spinal system where it appears to be most necessary.

The recognition of phases in the natural course of the disease is important when it comes to estimating the effects of treatment. The acute phenomena of infection may terminate abruptly, while in the secondary stage there is a natural tendency to resolution within four weeks. The underlying pathological condition is best studied by successive lumbar puncture. The possibility of the spontaneous occurrence of such favourable alterations must render an accurate and critical judgment of the effects of treatment very difficult, except in a very extensive series of cases. It has been seen that, unfortunately, observations on agglutinin and opsonins are thus far of uncertain value in regard either to prognosis or therapy. Symptomatology also tells little regarding the underlying pathological conditions.

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