THE WASSERMANN REACTION

IN

THEORY AND PRACTICE.

Thesis for Degree of Doctor of Medicine.

Walter Gilmour, M.B., Ch.B. (Glasgow).

I N T R O D U C T I O N

The following work falls under three main heads:-

I. The elaboration of a method of carrying out the biological syphilis reaction which should yield results of the greatest degree of delicacy and accuracy. Such a method was found in the procedure recommended by Browning, Cruickshank and Mackenzie, in which the patient's serum is tested simultaneously with emulsions of lecithin and lecithin-cholesterin. As will be seen, I was able to establish the practical value of this method for purposes of diagnosis as the result of the examination of a large series of known positive and negative cases.

2. The clinical material of Gartloch Mental Hospital was utilised for the purpose of determining the association of syphilis with mental disease. For this purpose the cases were divided into two groups (I) those presenting the symptoms characteristic of the parasyphilitic affection, general paralysis of the insane, and (2) cases which clinically presented no evidence of general paralysis. In the first place, the blood serum was examined. With a view to determining more precisely the relation of the syphilitic process to the central nervous system, the cerebro-spinal fluids of a large number of cases with symptoms and signs of general paralysis and of those who were not general paralytics were examined. The latter results were considered as important in relation to the elucidation of the still somewhat obscure question of the symptomatology of cerebral syphilis.

3. An analysis of the protein constituents of blood sera was undertaken and the various components were examined separately in order to determine with what constituents the Wassermann reaction was associated.

P A R T I.

The Wassermann Reaction.

So much has now been written with regard to the Wassermann syphilis reaction that I do not intend to go into any details, excepting in so far as they bear directly on the present work.

Definition.

The biological syphilis reactionof Wassermann,Neisser and Bruck depends on the fact that a mixture of syphilitic serum together with certain tissue lipoids leads to the absorption of haemolytic complement whereas,if a non-syphilitic serum is employed,the fixation of complement fails to occur.
This is a broad statement of the facts but it must be remembered that the reaction is strictly a quantitative and not a qualitative one.

Technique.

Briefly,the original technique is as follows:-

> Into a tube are put 0.2c.c. of the patient's serum (previously heated at 55°C. for half an hour),0.1c.c.-0.2cc. of organ extract (watery extract of the liver of a syphilitic foetus),and 0.05cc.-0.1cc. of guinea-pig's serum (complement). Controls are also set up (a) serum plus complement (b) organ extract plus complement (c)a known non-syphilitic serum plus extract plus complement (d) complement alone.
> After incubation of these tubes at 37°C. for one hour, 1cc. of a suspension of sensitised sheep's red blood corpuscles is added to each tube,and all are again incubated for one hour. If no lysis occurs in the tube containing the unknown serum along with organ extract whilst all the controls show complete lysis,the reaction is positive.

The principal objections to this method are that it takes no account of the extreme variability of complement,and,in its controls,makes no proper estimation of the complement-fixing powers of the various reagents.

Modifications of the original technique.

The most common modification consists in the substitution of an alcoholic for the watery tissue extract but practically all neglect the factor of variability of complement. However, as certain of these modifications will again be referred to,it is

essential to describe them here.

Boas' Modification.

Boas sets up a number of tubes and into each, as in the original method, is put a certain amount of tissue extract (alcoholic extract of heart), and of complement (guinea-pig's serum). Then he adds increasing amounts of the patient's serum (previously heated at 55°C. for half an hour). After incubation, 1cc. of the sensitised corpuscles is added as usual.

He claims that by this means he obtains the titre of the serum and so can gauge a patient's progress under treatment according as more or less serum is required at different times to prevent lysis. Further, he maintains that by the use of sheep's instead of ox's corpuscles in the haemolytic system, variability in complement is negligible.

Noguchi's Modification.

This presents several points of difference from the original method. In the first place, the serum is tested either heated or fresh; secondly, instead of tissue extract, an acetone-insoluble lipoid is employed as "antigen", and lastly, the haemolytic system consists of human red blood corpuscles with their homologous immune-body.

The objections to the original method are equally applicable here, but an even graver fault lies in the use of an unheated serum.

Browning & Mackenzie's Modification. (foot-note)

In order to obviate the difficulties arising out of variability in complement, these workers put forward a method in which the actual amount of complement, deviated by serum with extract and by each of these alone, could be calculated in terms of haemolytic doses.

This method will be fully described:-

The patient's serum is heated at 57°C. for half an hour. The "antigen" is an alcoholic extract

(foot-note)
This method will be referred to as the "crude-extract method" and for "crude -extract" the contraction C-E. will be commonly used.

of fresh ox-liver. To prepare it, one part of the minced organ is left in contact with 4 parts of 95% alcohol for 4 days and is then filtered and the filtrate stored in a well stoppered bottle. This extract has been found to remain unchanged for a long period (as much as two years) and is not subject to sudden alterations in its properties. For use in the Wassermann test, the extract is made into an emulsion with NaCl solution (I part of extract to 5 parts of 0.85% NaCl solution). It is a point of importance that this emulsion should be as turbid as possible since it has been shown by Sachs & Rondoni[1] and confirmed by Browning & Mackenzie[2] that the more turbid the emulsion, the greater is the deviation of complement along with syphilitic sera. To obtain this, the salt solution is measured into a test-tube and the requisite amount of extract is gently run from a pipette on to the surface so that no mixing of the fluids results; then, by holding the tube in a slightly slanting position and slowly rotating it, an emulsion of maximum turbidity is obtained (C-E. emulsion).

The complement used is that contained in guinea-pig's serum (I8-24 hours old).

The technique is as follows:-

A number of tubes (generally 6) is set up and into each one is put 0.6cc. of the C-E. emulsion, and 0.05cc. of the heated serum, and lastly, increasing amounts of complement, e.g., 0.0I5cc., 0.025cc., 0.04cc., 0.06cc., 0.09cc., and 0.I3cc.

Controls are now set up by which one measures the amount of complement absorbed by the serum and the emulsion separately.

(a) Two tubes, each containing 0.6cc. of 0.85% NaCl solution, and 0.05cc. of serum, and different amounts of complement, e.g., 0.0I5cc. and 0.025cc.

(b) Three tubes, each containing 0.6cc. of C-E. emulsion, and different amounts of complement, e.g., 0.0I5cc., 0.025cc., and 0.04cc.

Lastly, four tubes are set up for the estimation of the haemolytic dose of complement. Into each of these is measured 0.6cc. of 0.85% NaCl solution, and the following amounts of complement are added, 0.005cc., 0.0075cc., 0.0Icc., and 0.0I5cc. (For greater accuracy, the complement should be diluted four times, i.e., I part of complement plus 3 parts of a 0.85% NaCl solution, so that one adds 0.02cc., 0.03cc., 0.04cc., and 0.06cc.)

All these tubes are then incubated for I½ hours at 37°C.

At the end of this time, Icc. of a 5% suspension of sensitised ox's red blood corpuscles is added to each tube, and the whole again incubated for I½ hours, the tubes being shaken at intervals of 20 minutes.

The reading, taken after removal of the tubes from the incubator, is practically always the same as that taken after the tubes have stood over night at room temperature.

The authors have arbitrarily fixed a positive reaction as resulting when the serum and extract together deviate at least 5 doses of complement more than the sum of the amounts deviated by each alone. But if more than 5 doses of complement, along with organ extract or the patient's serum, are required to cause complete lysis of the test corpuscles, the complement is hyper-sensitive and the results should be discarded.

This method possesses much greater accuracy than the original method of Wassermann in using adequate controls of the variable factors. At the same time, the variations in complement so frequently led to a marked inhibitory effect on the part of the C-E. emulsion, that the results were often vitiated from a diagnostic point of view. Accordingly a method, more independent of the variability in complement, was desirable, and this has been found in the Lecithin-cholesterin method of Browning, Cruickshank & Mackenzie. In this method, a relative criterion of a positive result has replaced the absolute one on the C-E. method, a point of the greatest importance. The accuracy and extreme delicacy of this method have been established by the simultaneous examination of a large number of syphilitic and normal sera by the lecithin-cholesterin and crude extract methods, and as the result of this investigation, the authors finally adopted the method for diagnostic examinations.

References

1. Sachs and Rondoni, Berlin. klin. Woch., 1908.

2. Browning and Mackenzie, Journ. of Path. and Bact. 1909, vol. 13.

The Lecithin-Cholesterin method of performing the Wassermann reaction. (see foot note)

The use of lecithin and cholesterin in performing the Wassermann reaction was described by Browning, Cruickshank and Mackenzie,[1] in 1910. It depends on the fact that a syphilitic serum, in the presence of an emulsion of lecithin plus cholesterin, absorbs much more complement than in the presence of an equivalent amount of an emulsion of lecithin alone, whereas a negative serum absorbs equal amounts with both emulsions.

The lecithin, which is easily obtained from an alcoholic extract of fresh ox liver, is employed in the form of a 0.75% solution in absolute alcohol. The addition to this of 1% cholesterin (Kahlbaum) constitutes the lecithin-cholesterin solution.
The lecithin, for use in the Wassermann reaction, is emulsified by adding 1 part of the 0.75% solution to 7 parts of a 0.85% NaCl solution; the salt solution is first measured into a test-tube and the lecithin solution allowed to run gently on to the surface; then, by slow rotation of the test-tube, an emulsion is obtained of maximum turbidity. The lecithin gives a fairly turbid, uniform emulsion. The lecithin-cholesterin emulsion is prepared in exactly the same way. It is uniform and very dense.

In carrying out the Wassermann reaction by this method, the technique employed is in general similar to that with the crude alcoholic extract, as describe by Browning and Mackenzie[2]

Two series of tubes are arranged:-
(A) contains 0.6c.c. of L.emulsion plus 0.05c.c. of the serum to be tested, previously heated at 57°C. for half an hour.
(B) contains 0.6c.c. of the L-C.emulsion plus 0.05c.c. of the serum.
Four sets of controls are set up:-
(a) containing 0.6c.c. of the L.emulsion alone.
(b) " 0.6c.c. of the L-C. " "
(c) " 0.05c.c. of the serum plus 0.6c.c. of saline.
(d) " 0.6c.c. of saline alone for the estimation of the complement dose.
Increasing quantities of complement (fresh guinea-pig's serum) are added to the tubes of each series.
After incubation at 37°C. for 1½ hours, 1c.c. of a 5% suspension of ox's red blood corpuscles, previously sensitised with 5 minimum haemolytic doses of immune body from the rabbit, is added to each tube. They are again incubated at 37°C., shaken at intervals of 20 minutes, and at the end of 1½ hours the tubes are removed from the incubator.

(foot-note) Throughout, this method will be referred to as the L-C.method. For lecithin, the contraction L. will be used, and for lecithin-cholesterin, L-C.

The reading at this stage is found to be practically always the same as the final reading, taken after the tubes have stood overnight at room-temperature.

In order to establish the suitability of the L-C.method for practical purposes of diagnosis, I examined, in the first place, upwards of 150 sera by this method, and at the same time, by way of control, the sera were tested with the C.E.method as previously advocated by Browning and Mackenzie[2].

In general, 5 or 6 sera were tested at the same time and the importance of having a known negative and positive serum, which had been already examined on a previous occasion, in the series, was borne in mind.

It is well known that an emulsion of lecithin, along with a syphilitic serum, can cause deviation of complement, i.e. give a positive Wassermann reaction (Porges & Meier,[3] and Landsteiner, Müller & Potzl[4]). But this is the case only with powerfully reacting sera. With weaker sera, the lecithin may give no more deviation than with a normal serum (table I). With the L-C.emulsion, in the presence of a normal serum, the deviation was always practically the same as with the L.emulsion (table 2). Thus in only one out of 70 negative reactions, did the L-C.series show the absorption of as much as one dose of complement more than the L.series. On the other hand, in the case of positive sera, the L-C.emulsion always deviated more complement than the L.emulsion (table 2). The amount of this increase varied but with L-C. in the presence of a syphilitic serum there might be five times as much complement absorbed as by L. emulsion; and the increase tended to be well marked where the reaction was weakly positive by the C.E.method (table 3).

Thus the reaction depends on the relative amounts of complement absorbed by L. and L-C.emulsions, and not upon the absolute amount of complement deviated, as in the original method, when a crude alcoholic extract is employed.

This obviates to a great extent a difficulty which very frequently arises in the C.E.method and which is caused by // irregular-

ities in the deviability of complement, depending on individual properties of the complement-containing sera. By the new method, if the absorption of complement by sera in the presence of L. and L-C. emulsions is equal there is an undoubted negative reaction. As before mentioned, an increase of one dose more of complement with the L-C. as compared with the L. emulsion is within the limits of a negative reaction. But even a small increase (2 or 3 doses of complement more absorbed by L-C. than by L.) is in favour of the serum being positive, and a more marked difference (5 doses or more) is conclusive under the conditions stated.

The anticomplementary effect of the emulsions of both L. and L-C. was very constant. In practically all cases, both inhibit complement to the same extent and only rarely are more than 2 or 3 doses of complement required to cause complete lysis of the test blood corpuscles. With only one out of over 100 different complements, have the L. and L-C. emulsions deviated more than 6 doses of complement. The C.E. emulsion compared very unfavourably with this. Constantly one found it deviating 2 to 3 doses more than the L. and L-C. emulsions and, not rarely, much more. (table 4)

This uniformity is a great advantage which the L-C. method possesses. Because of the excessive anticomplementary effect of the C.E. emulsion, about a quarter of the experiments were vitiated from a diagnostic point of view so far as this re-agent was concerned. Under such circumstances, the reaction with an undoubted negative serum may appear positive, whereas the L-C. method shows its certainly negative character. (table 5). But even when the L. and L-C. emulsions were very anticomplementary, the nature of the reaction was in no way in doubt, for even then with negative sera the deviation of complement with both emulsions was equal, and with positive sera, the L-C. emulsion gave the usual increase. (table 6).

A similar difficulty arose in the original method where the serum by itself exerted a marked deviating effect on complement. Here also, the advantage of the L-C. method was apparent.

Accordingly, the new method effects a considerable saving in time since it renders one almost entirely independent of variations in complement.

The superiority of the new method both as regards delicacy and reliability having been thus established, I have relied upon it entirely in the diagnostic examination of sera, of which more than 450 have now been tested, and the results fully bear out the claims made for the method.

Now, only half the quantities previously recommended are employed as the results are equally accurate, a considerable saving both in time and material being thus effected. As the result of many tests, I have adopted the following doses of complement in diagnostic examinations:-

(A) Three tubes, each containing 0.3cc. of L. emulsion and 0.025cc. of the serum (57°C), and increasing amount of complement, namely, 0.02cc., 0.035cc., and 0.055cc.

(B) Five tubes, each containing 0.3cc. of L-C. emulsion and 0.025cc. of the serum, and increasing amounts of complement, namely, 0.02cc., 0.035cc., 0.055cc., 0.08cc. and 0.11cc.

Controls.

(a) Two tubes, each containing 0.3cc. of L. emulsion, with 0.015cc. of complement in one and 0.025cc. in the other tube.

(b) Is exactly similar to (a) except that L-C. emulsion replaces the L. emulsion.

(c) One tube containing 0.3cc. NaCl solution plus 0.025cc. of serum plus 0.02cc. of complement.

(d) Four tubes, containg 0.3cc. NaCl solution with 0.02cc., 0.03cc., 0.04cc., and 0.06cc. of complement (previously diluted eight times with 0.85% NaCl solution).

These are incubated as usual and, at the end of 1½ hours, 0.5cc. of suspension of sensitised corpuscles is added to each tube and all are again incubated.

(foot-note) Throughout this paper, the quantities mentioned in the tables are those used with 1cc. of the suspension of sensitised corpuscles; where only half amounts were used in the experiment the figures have been doubled in the tables, so as to avoid confusion.

As the result of a very extensive experience with the L-C method, a few anomalously reacting sera have been met with, i.e. sera, which with L-C. emulsion deviate not more than 2 to 3 doses of complement more than with L.emulsion, or which even deviate equally with both emulsions, but the total absorption of complement by each emulsion with the serum is much greater than that by a known negative serum, examined as control with a corresponding emulsion and complement. (table 7)

Such anomalously reacting sera behave in the same way on repeated examinations with different specimens of complement, so that the irregularity is obviously a quality inherent in the sera. The fact, that cases, which have yielded markedly positive sera, later on, either as the result of treatment or spontaneously, come to give this anomalous result, points almost conclusively, to the interpretation of such anomalous reactions as real positives.

Appication of the L-C.method to the examination of the spinal fluid.

The method is equally as reliable in the investigation of the reaction with the spinal fluid as with the blood serum. It is not, however, necessary to heat the spinal fluids, since non-specific reactions are not obtained with the unheated fluid from non-syphilitic cases; much larger amounts of this fluid must be employed than of the blood serum. Max Nonne[5] states that as much as Ic.c. of the cerebro spinal fluid can be used with out fear of non-specific cases giving a positive reaction. Candler[6] regularly uses 0.8c.c., and mentions a case of general paralysis in which this amount reacted positively whilst 0.5c.c. gave a negative reaction. Morton adopted the following procedure:-the L.and L-C emulsions are made in the usual proportions but the cerebro-spinal fluid replaces the salt solution; thus, 0.3c.c. of L.and L-C.solution is run on to the surface of 2.Ic.c. of the cerebro-spinal fluid, which must be free of blood or cellular elements, and the emulsions made by gently rotating the tubes so as to obtain the maximum turbidity; for the Wassermann test, 0.3c.c. of the L.emulsion is put into each tube of one series, and an equal amount of the L-Cemulsion into each tube in the second series. Increasing amounts of complement are then added in the usual way,

and after incubation, 0.5c.c. of sensitised corpuscles added. Controls must also be put up, the amount of complement absorbed by 0.4c.c. of spinal fluid being tested for, and also the inhibitory effect of corresponding amounts of L. and L-C. emulsions, made with saline instead of the spinal fluid. The criterion of a positive result is the same as with the blood serum.

I have obtained very satisfactory results by the application of this method to the examination of the spinal fluids in cases of insanity. The spinal fluid reacted positively in 31 out of 33 cases of general paralysis (it is noteworthy that the two negative cases were examined when smaller amounts of the fluid, 0.1c,c, were being used with 1c.c. of the corpuscles suspension)

Apart from general paralysis, the spinal fluid was negative by this method in 51 cases where the serum reacted negatively; and in 18 out of 37 cases, with no clinical evidence of general paralysis, where the serum was positive, the spinal fluid reacted positively.

Of special interest are 3 cases of general paralysis and 1 of cerebro-spinal syphilis, in which the spinal fluid gave a positive reaction while the blood serum reacted negatively. In 3 of these, the serum subsequently reacted positively, but in one, a general paralytic, the reaction of the serum remained persistently negative; in this last case, the post mortem examination confirmed the diagnosis of general paralysis. This case helps to establish the important fact, which has been previously denied, that a case of general paralysis may run its full course without exhibiting a positive reaction of the serum, and affords further evidence of the paramount value of the positive reaction of the cerebro-spinal fluid for making a diagnosis of syphilitic affection of the central nervous system.

REFERENCES.

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6. Kandler, Lancet, Nov. IIth, I9II, p.I320.

T A B L E I

	Doses of Complement required to cause Just Complete Lysis with-						
	0.6cc L. Emulsion + 0.05cc of serum (57°C)	0.6cc L.C. Emulsion +0.05cc of serum (57°C)	0.6cc C.E. Emulsion + 0.05cc of serum (57°C)	0.6cc L. Emulsion alone	0.6cc L.C. Emulsion alone	0.6cc C.E. Emulsion alone	0.05cc serum (57°C) + 0.6cc salt solution
egative serum	IO	IO	IO	3	2	2	3
ositive serum	8	24	24	3	2	2	2

These two sera were examined at the same time. The table shows that a negative serum in the presence of lecithin emulsion may absorb more complement than a positive serum under the same conditions.

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T A B L E 2

	Doses of Complement required to cause Just Complete Lysis with						
	0.6cc L. Emulsion + 0.05cc of serum (57°C)	0.6cc L.C Emulsion + 0.05cc of serum (57°C)	0.6cc C.E. Emulsion + 0.05cc of serum (57°C)	0.6cc L. Emulsion alone	0.6cc L.C. Emulsion alone	0.6cc C.E. Emul-sion alone	0.05cc serum (57°C) + 0.6cc salt solution
Negative serum	2	2	2	I	I	I	4
Positive serum	IO	26	36	I	I	I	2

These two sera were examined at the same time. The negative serum absorbed equal amounts of complement in the presence of both lecithin and lecithin-cholesterin emulsions, whereas the positive serum showed a marked increase in the amount of complement absorbed in the presence of the lecithin-cholesterin emulsion.

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TABLE 3

	Doses of Complement required to cause Just Complete Lysis with						
	0.6cc L. Emulsion + 0.05cc of serum (57°C)	0.6cc L.C. Emulsion + 0.05cc of serum (57°C)	0.6cc C.E. Emulsion + 0.05cc of serum (57°C)	0.6cc L. Emulsion alone	0.6cc L-C. Emulsion alone	0.6cc C.E. Emulsion alone	0.05cc serum (57°C) + 0.6cc salt sol.n
Negative serum	3⅓	3⅓	3⅓	I	I	2	⅔
Positive serum	3⅓	6½	7½	I	I	2	½

This table shows that the Lecithin-cholesterin emulsion in the presence of a weakly positive serum gives a marked increased in the amount of complement absorbed. With the crude extract the reaction is doubtful. The negative serum, examined simultaneously, serves as a control.

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TABLE 4

No. of experiment	Dose of complem.t	Amount of Complement absorbed by Emulsions		
		0.6cc of L. emulsion	0.6cc of L-C. emulsion	0.6cc of C.E. emulsion
I	0.0075cc	0.0Icc just complete lysis	0.0I5cc just complete lysis	0.035cc marked lysis
2	0.005cc	0.025cc just complete lysis	0.025cc Just complete lysis	0.035cc just complete lysis
3	0.005cc	0.0I5cc just complete lysis	0.0Icc just complete lysis	0.025cc just complete lysis
4	0.005cc	0.02cc just complete lysis	0.025cc just complete lysis	0.03cc trace of lysis
5	0.005cc	0.0I5cc just complete lysis	0.0I5cc just complete lysis	0.035cc trace of lysis
6	0.006cc	0.0I5cc Just complete lysis	0.0I75cc just complete lysis	0.03cc distinct lysis

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TABLE 5

	Amount of Complement absorbed by						
	0.6cc L. emulsion + 0.05cc of serum (57°C)	0.6cc L.C. emulsion + 0.05cc of serum (57°C)	0.6cc C.E. emulsion + 0.05cc of serum (57°C)	0.6cc L. emulsion alone	0.6cc L-C. emulsion alone	0.6cc C.E. emulsion alone	0.05cc serum (57°C) + 0.6cc salt solution
Negative SERUM	0.04cc justcomplete	0.04cc complete +	0.085cc just complete	0.0I5cc just complete	0.0I75cc just complete	0.03 cc.distinct lysis	0.0I75 cc. just complete
Positive serum	0.035cc justcomplete	0.065cc just complete	0.09cc just complete	0.0I5cc just complete	0.0I75 cc.just complete	0.03 cc.distinct lysis	0.0I75 cc. just complete

Dose of Complement = 0.006cc

In table 5 a negative serum appears positive in the presence of of an emulsion of Crude Extract because of the marked anticomplementary effact of that re-agent. The lecithin-cholesterin method shows its true character. A positive-serum, examined at the same time, is shown for comparison.

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TABLE 6

Negative serum (57°C).	Emulsions.	Amounts of Guinea-pig's complement			0.6cc Lab. solution + complement
	0.6cc	0.07cc	0.11cc	0.16cc	0.03cc
0.05cc.	L.	Distinct	Marked	Complete	Almost
	L-C.	"	Almost complete	"	complete
Syphilitic serum (57°C)	0.6cc	0.16cc	0.22cc	0.3cc	0.03cc
0.05cc	L.	Very marked	Complete	...	Just
	L-C.	0	Faint trace	Almost complete	complete

Emulsions 0.6cc + Complement 0.05cc = No lysis.

Dose of Complement = 0.015cc

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T A B L E 7

Negative serum (57°C)	Emulsions 0.6cc	Amounts of Guinea-pig's Complement				0.85cc NaCl + solution Complement
		0.04cc	0.07cc	0.11cc	0.16cc	0.04cc
0.05cc.	L.	Complete	...	...	...	Complete
0.05cc	L-C.	"	...	...	...	
Anomalous Serum (57°C)						
0.05cc.	L.	0	Distinct	Marked	Complete	Just
0.05cc	L-C.	0	0	"	Just "	complete

Emulsions 0.6cc + Complement 0.03cc. = Complete.

Dose of Complement = 0.005cc.

Negative serum (57°C)	Emulsions 0.6cc	Amounts of Guinea-pig's Complement				0.6cc NaCl Solution + Complement 0.04cc
		0.04cc	0.07cc	0.11cc	0.16cc	
0.05cc.	L.	Just complete	...	...	...	Complete
0.05cc	L-C.	" "	...	...	...	
Anomalous serum (57°C)						
0.05cc	L.	Faint trace	Distinct	Almost complete	Complete	Complete
0.05cc	L-C.	" "	"	Marked	Just "	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.015cc.

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Constituents employed in the Wassermann Reaction.

In this section, only those points are mentioned in detail which are new or which have not met with general recognition. They will be treated under the following heads:-

1. The complement, especially in reference to its variability.
2. The haemolytic system; an enquiry into the relative values of ox and sheep red blood corpuscles plus their homologous immune-bodies as indicators for complement.
3. The "antigen"; a continuation of the investigation into the efficacy of the lecithin-cholesterin method.
4. The serum; (a) effect of heating sera in the Wassermann reaction.
   (b) effect of using different amounts of serum in the Wassermann reaction.
   (c) Wechselmann's method of converting a negative into a positive reaction.

Complement.

As complement, normal guinea-pig's serum was employed. The animal is bled from the carotid vessels and the blood is received into a sterile vessel; after the serum has separated, the whole is transferred to the ice chest. The complement is used 18 - 24 hours after the blood has been shed since it has been shown by Browning & Mackenzie that quite fresh serum is apt to be hypersensitive to deviating agents of various kinds and that reliable results cannot be obtained by its use.

The complement of serum varies greatly in different members of the same species. Thus guinea-pig's complement shows considerable variations in both its haemolytic power and its deviability. Such widely separated amounts as 0.0025cc. and 0.03cc. of guinea-pig's serum may be required to lyse 1cc. of a 5% suspension of sensitised ox's red blood corpuscles, although the minimum haemolytic dose generally lies between 0.005cc and 0.015cc.

With regard to deviability, similar individual variations are found. Table I. shows the results obtained in the Wassermann reaction with a normal serum tested with four different complements. It will be seen that very different amounts of the individual complements are deviated by the mixture of serum and "antigen", without definite relationship to the haemolytic variations. Thus in (3) the serum plus "antigen" deviates more complement than in (4), although the minimum haemolytic dose of the former is actually less. The results of the examination of a normal serum with two different complements are shown in table 2. Much greater deviation has occurred with complement (2) than with complement (1). These phenomena are due to properties of the individual complement - containing sera and not to the other reagents. But they occur more readily with certain "antigen". As has been previously shown, (p.10) a crude alcoholic extract of ox liver causes marked deviation of complement much more frequently than alcoholic solutions of lecithin and lecithin-cholesterin.

TABLE I.

Showing the amounts of Complement absorbed by a non-syphilitic serum at various times, a different guinea-pig's serum being used each time.

Emulsions 0.6cc.+ serum (57°C) 0.05cc		Amounts of Complement.				Dose of Complement
		0.02cc.	0.04cc.	0.07cc.	0.1cc.	
1.	L.emulsion	Just complete	...	...	...	0.01cc.
	L-C. "	Complete	...	...	...	
2.	L.emulsion	almost complete	Complete	...	...	0.01cc.
	L-C. "	Just "	"	...	...	
3.	L.emulsion	...	...	...	Very marked	0.0125cc.
	L-C. "	...	...	...	" "	
4.	L.emulsion	Marked	Very marked	Complete	...	0.02cc.
	L-C. "	"	" "	"	...	

CONTROLS.

1. Serum 0.05cc + NaCl solution 0.6cc + Complement 0.02cc = Complete
   Emulsions 0.6cc. + Complement 0.02cc. = Complete.

2. Serum 0.05cc + NaCl solution 0.6cc + Complement 0.02cc = Complete
   Emulsions 0.6cc + Complement 0.02cc. = Complete.

3. Serum 0.05cc + NaCl solution 0.6cc + Complement 0.04cc = Marked lysis.
   Emulsions 0.6cc + Complement 0.06cc = Marked lysis.

4. Serum 0.05cc + NaCl solution 0.6cc. + Complement 0.04cc = Just complete.
   Emulsions 0.6cc + Complement 0.04cc = Just complete.

T A B L E 2.

Showing the amounts of Complement absorbed by a non-syphilitic serum with two different complements.

Emulsions 0.6cc.+ Serum (57°C) 0.05cc.	Amounts of Guinea-pig's Complement.			Dose of Complement.
	0.04cc	0.08cc	0.12cc	
I. L.emulsion	Almost complete	Complete	...	0.015cc.
I. L-C. "	" "	"	...	
2. L.emulsion	Marked	Very marked	Almost complete	0.025cc.
2. L-C. "	"	" "	" "	

C O N T R O L S.

I. Serum 0.05cc. + NaCl solution 0.6cc + Complement 0.04cc = Complete
Emulsions 0.6cc + Complement 0.04cc. = Complete.

2. Serum 0.05cc. + NaCl solution 0.6cc + Complement 0.04cc. = Very
Emulsions 0.6cc+Complement 0.06cc =Almost complete. marked

The importance of these points, in their bearing on the Wassermann test, is not sufficiently recognised. In those methods where a fixed amount of complement is used, there are no controls which are capable of revealing the existence of such variations. To obviate this source of error, one must use a method where the deviation of complement, produced by the different reagents, is estimated in doses of complement, as in the Crude-extract method of Browning and Mackenzie and the Lecithin-cholesterin method of Browning, Cruickshank and Mackenzie.

The Haemolytic System.

In the various methods of carrying out the Wassermann test, the most commonly used haemolytic systems are of ox or sheep corpuscles with the homologous immune serum from the rabbit. A few workers, as Noguchi, use human corpuscles with immune-body from the rabbit.

> The corpuscles in the defibrinated blood are washed free from serum three times with 0.85% NaCl solution by centrifugalizing. A 5% suspension is then made by adding 3 parts of the centrifugalized deposit of corpuscles to 97 parts of salt solution; to this, 5 times the minimum haemolytic dose of immune-body is added and the mixture is allowed stand for at least ten minutes. The corpuscles are thus sensitised and act as indicator for complement.

Throughout this work, the ox-rabbit haemolytic system has been employed, but in order to determine whether variations in complement could be obviated by the use of a sheep-rabbit system, as Boas maintains, a large number of sera were tested with seven different complements, the ox-rabbit and sheep-rabbit indicators being used simultaneously; in addition, other sera were tested with four different complements, the sheep-rabbit system being alone employed.

Table I. shows the minimum haemolytic doses of complement in the different experiments. Where the two indicators were used together, the doses of complement were alike in three cases; in other three, very little difference existed, whilst in the seventh

the greatest difference occurred, the dose of complement with ox corpuscles being 0.0I75cc. and with sheep corpuscles 0.0Icc. The second part of the table gives the four complements tested with sheep corpuscles alone, and it is seen that the haemolytic values vary considerably.

As regards the effect in the Wassermann reaction, it was found that, as a rule, very little difference existed between the two indicators. This is well shown in table 2; two normal and two syphilitic sera, examined with the same complement, gave almost similar results with both indicators, although the doses of complement were different. Table 3 shows a normal serum deviating somewhat more complement with the sheep-rabbit than with the ox-rabbit system, the dose of complement being the same in both cases.

Again, variations in deviability of complement were found to occur with the sheep indicator. Thus, in table 4, are represented three normal sera which, with sheep corpuscles, are all deviating alike in the presence of "antigen". On the other hand, a different complement, with the same indicator, shows much greater and very variable deviability with other three normal sera (table 5).

Conclusions.

I. Variations in (a) dosage and (b) deviability of guinea-pig's complement occur when sheep's red blood corpuscles plus immune-body are used as indicator in the Wassermann reaction just as when ox's red blood corpuscles plus immune body are used.

2. There is almost complete parallelism between the behaviour of the two indicators with the same specimen of complement. Hence there is no special advantage in using sheep's red blood corpuscles as indicator.

3. The individual variations in complement are independent of the haemolytic system employed.

Reference.

Boas, Die Wassermannsche Reaktion, Berlin, I9II.

TABLE I

The following minimum haemolytic doses were obtained by testing complement with ox-rabbit and sheep-rabbit haemolytic systems simultaneously.

	Doses of Complement with	
	Ox's blood corpuscles	Sheep's blood corpuscles
I.	0.0I75cc.	0.0I75cc.
2.	0.0I25cc.	0.0Icc.
3.	0.0I75cc.	0.0I25cc.
4.	0.0I5cc.	0.0I5cc.
5.	0.0Icc.	0.0Icc.
6.	0.0I75cc.	0.0I5cc.
7.	0.0I75cc.	0.0Icc.

The following minimum haemolytic doses of complement were obtained with sheep-rabbit haemolytic system, used alone.

I. 0.0Icc.
2. 0.0I75cc.
3. 0.0Icc.
4. 0.02cc.

TABLE 2.

Four sera, tested simultaneously in the Wassermann reaction with ox-rabbit and sheep-rabbit haemolytic systems.

Negative Serum (57°C) 0.05cc	Emulsions 0.6cc.	Amounts of Guinea-pig's complement 0.04cc	0.07cc	0.11cc	0.16cc	0.22cc	0.6cc NaCl. solution + Complement. 0.05cc
Ox	L.	Complete.	...	...	...	...	Almost complete
	L-C.	"	...	...	...	...	
Sheep	L.	Just complete	...	...	...	...	Complete
	L-C.	Complete	...	...	...	...	
Negative Serum							
Ox	L.	Complete	...	...	...	...	Complete
	L-C.	Just "	...	...	...	...	
Sheep	L.	Complete	...	...	...	...	Complete
	L-C.	"	...	...	...	...	
Syphilitic Serum							
Ox	L.	Distinct	Marked	Complete	...	...	Very marked
	L-C.	0	0	0	Distinct	Very marked	
Sheep	L.	0	0	Complete	...	...	Very marked
	L-C.	0	0	0	0	Marked	
Syphilitic Serum							
Ox	L.	Almost complete	Complete	...	...	...	Almost complete
	L-C.	Faint trace	Distinct	Just complete	Complete	...	
Sheep	L.	Complete	...	...	...	...	Just complete
	L-C.	Trace	Marked	Almost complete	Complete	...	

Ox. Emulsions 0.6cc + Complement 0.02cc = Just complete

Sheep. " 0.6cc + " 0.02cc = " "

Ox. Dose of complement = 0.0175cc.

Sheep. " " " = 0.01cc

.....

TABLE 3

Non-syphilitic serum tested in the Wassermann reaction with ox-rabbit and sheep-rabbit haemolytic systems simultaneously.

Serum 57°C. 0.05cc.	Emulsions. 0.6cc	Amounts of Guinea-pig's Complement.		
		0.02cc.	0.04cc	0.07cc
Ox's corpuscles.	L.	Almost complete	Just complete	Complete
" "	L-C.	" "	Complete	"
Sheep's corpuscles.	L.	Very marked	Almost complete	Just complete
" "	L-C.	" "	Just "	Complete

CONTROLS.

Ox. Serum 0.05cc.+ NaCl solution 0.6cc.+ Complement 0.03cc = Just complete.

Sheep. " 0.05cc.+ " " 0.6cc.+ " 0.03cc = " "

Ox. Emulsions 0.6cc. + Complement 0.05cc. = Almost complete.

Sheep. " 0.6cc, + " 0.05cc. = " "

Doses of Complement.

Ox = 0.015cc.

Sheep = 0.015cc.

TABLE 4

Sheep-rabbit haemolytic system. To show variability in complement. Three non-syphilitic sera tested simultaneously with the same complement.

Negative serum (57°C) 0.05cc.	Emulsion 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.6cc. NaCl solution + Complement 0.03cc.
Case I.	L.	Complete	Just complete
	L-C.	"	
Case 2.	L.	Complete	Marked.
	L-C.	"	
Case 3	L.	Complete	Just complete.
	L-C.	"	

Emulsions 0.6cc+Complement 0.02cc. = Complete.

Dose of Complement = 0.0175cc.

This table shows the results obtained with a non-deviable complement.

TABLE 5

Sheep-rabbit haemolytic system. To show variability in complement. Three non-syphilitic sera tested simultaneously with the same complement.

Negative Sera (57°C)	Emulsions 0.6cc.	Amounts of guinea-pig's complement				0.6cc Lipoid solution + Complement
		0.04cc.	0.07cc.	0.11cc.	0.16cc.	0.04cc.
Case I. 0.05cc.	L.	Very marked	Complete	...	...	Complete
	L-C.	" "	"	...	...	
Case 2. 0.05cc.	L.	Marked	Almost complete	Complete	...	Very marked
	L-C.	"	" "	"	...	
Case 3. 0.05cc.	L.	Trace	Marked	Almost complete	Complete	Marked
	L-C.	"	"	" "	"	

Emulsions 0.6cc + Complement 0.05cc. = Almost complete.

Dose of Complement = 0.02cc.

This table shows the results with a deviable complement.

"Antigen"

Under this heading, I intend merely to deal with lecithin and cholesterin in their relation to the Lecithin-cholesterin method of performing the Wassermann reaction. In the course of the investigation into the efficiency of the Lecithin-cholesterin method, the effect of using different amounts of lecithin, and the comparative actionof lecithins from different sources was studied.

Preparation of lecithin.

A crude extract of ox liver (obtained by macerating I part of tissue with 4 parts of 95% alcohol) was evaporated on a water-bath at 60°C. The residue was rubbed up with quartz sand and rapidly extracted with ethyl acetate at 60°C. The portion, which precipitated out of the ethyl acetate on standing in the ice chest, was again dissolved in ethyl acetate at 60°C., and again precipitated as before, this process being repeated until the supernatant fluid was no longer coloured. The precipitate was then dissolved in water-free ether and precipitated with acetone, the treatment with ether and acetone being repeated three times. Finally, the acetone precipitate was rubbed up with quartz sand and the soluble portion, lecithin, taken up in absolute alcohol at room temperature. The alcoholic solution was then titrated.

Different amounts of lecithin.

The alcoholic ox liver lecithin solution was diluted with alcohol invarying proportions, and, to a portion of each, I% cholesterin was added. Emulsions were then made (I part of lecithin solution and of lecithin-cholesterin solution to 7 parts of 0.85% NaCl solution) so as to obtain the maximum turbidity; thus the amount of alcohol was the same throughout.

The general result was that the larger amounts of lecithin, within certain limits, caused an increased deviation of complement along with syphilitic sera. The same increase occurred when cholesterin was added to the lecithin solution (table I). In some cases, however, the same amount of complement was absorbed by the different amounts of lecithin plus cholesterin along with syphilitic serum (table 2). Occasionally, a zone phenomenon occurred; thus, in table 3 is shown a syphilitic serum deviating less complement along with 0.I% lecithin plus I% cholesterin than with 0.0I% and 0.75% lecithin plus I% cholesterin.

For all practical purposes, a 0.75% alcoholic solution of lecithin and a 0.75% alcoholic solution of lecithin plus 1% cholesterin were found to be suitable. The point of importance isthatthe lecithin-cholesterin solution should deviate more complement than the lecithin solution along with syphilitic serum, whilst deviating the same amount along with normal serum; with these proportions, this phenomenon practically always occurs.

It should be noted that the alcohol, present in these solutions, aids the deviation of complement. By increasing the amount of alcohol (within certain limits) a greater deviation of complement is produced; at the same time, the inhibitory effect of the various emulsions on complement is the same (table 4).

Lecithin from various sources.

Lecithins, prepared from ox's liver and heart, pig's liver, heart and brain, and egg yolk have been tested in the Wassermann reaction. These were all prepared from fresh organs of healthy adult animals by the process already described, and were tested simultaneously with a stock ox liver lecithin solution. A 0.75% solution of each in alcohol, with and without 1% cholesterin, were used.

It was found that heart lecithins gave the greatest deviation of complement along with syphilitic sera; brain and egg yolk lecithins gave least, whilst liver lecithins occupied an intermediate position. Specimens of commercial ovo-lecithin (Poulenc Frères and Riedel 1&2) gave similar results to the egg yolk lecithin. The inhibitory effect on complement of the various lecithins by themselves was slight and practically equal (table 5).

The heart lecithin, although leading to the greatest deviation of complement, was found to be unsuitable in practice, since it sometimes led to a positive Wassermann effect with known negative sera, i.e., the heart lecithin plus cholesterin can deviate more complement than the lecithin alone in the presence of a non-syphilitic serum (table 6).

From the above results,it is clear that the most suitable lecithin for use in the Wassermann test is one prepared from a fresh healthy liver. Next to heart lecithin,(a) it fixes most complement witha syphilitic serum; (b) it leads to the greatest increase in complement deviation with cholesterin along with syphilitic serum; (c) unlike heart lecithin,it does not give a greater deviation of complement with cholesterin than without it in the presence of a non-syphilitic serum.

TABLE I.

0.6cc of Emulsion of	Syphilitic Serum 57°C	Amounts of Guinea-pig's Complement		
		0.04c.c.	0.07c.c.	
Lecithin 0.0I5%	0.05c.c.	Just complete	Complete	
Lecithin 0.I%	0.05c.c.	Almost complete	Complete	
Lecithin 0.75%	0.05c.c.	Marked	Complete	
		0.Ic.c.	0.I4c.c.	0.2c.c.
Lecithin 0.0I5% + Cholesterin I%	0.05c.c.	Trace	Very marked	Complete
Lecithin 0.I% + Cholesterin I%	0.05c.c.	Trace	Marked	Very marked
Lecithin 0.75% + Cholesterin I%	0.05c.c.	Faint trace	Trace	Marked

CONTROLS

Serum 0.05c.c. + NaCl solution 0.6cc + Complement 0.02cc = Complete

Emulsions 0.6c.c.

Lecithin 0.0I5% + Complement 0.03c.c. = Complete.
" 0.I% + " 0.03c.c. = "
" 0.75% + " 0.04c.c. = "
" 0.0I5% + Cholesterin I% + Complement 0.03c.c. = Complete
" 0.I% + " I% + " 0.03c.c. = "
" 0.75% + " I% + " 0.04c.c. = "

Dose of Complement = 0.0Ic.c.

.....

TABLE 2.

0.6c.c. of Emulsion of	Syphilitic Serum 56°C	Amounts of Guinea-pig's Complement		
		0.07c.c.	0.1c.c.	0.14c.c.
Lecithin 0.375% + Cholesterin 1%	0.05c.c.	Trace	Distinct	Just complete
Lecithin 0.75% + Cholesterin 1%	0.05c.c.	"	"	" "
Lecithin 2% + Cholesterin 1%	0.05c.c.	"	"	" "

CONTROLS.

Serum 0.05cc + NaCl Solution 0.6cc + Complement 0.015cc = Complete.

Emulsions 0.6c.c.
Lecithin 0.375% + Cholesterin 1% + Complement 0.015cc = Complete
" 0.75% + " 1% + " 0.015cc = "
" 2% + " 1% + " 0.015cc = "

Dose of Complement = 0.01c.c.

.....

TABLE 3.

0.6cc of Emulsion of	Syphilitic Serum 57°C	Amounts of Guinea-pig's Complement		
		0.07c.c.	0.1c.c.	0.14c.c.
Lecithin 0.015% + Cholesterin 1%	0.05c.c.	Trace	Marked	Just complete
Lecithin 0.1% + Cholesterin 1%	0.05c.c.	Marked	Just complete	Complete
Lecithin 0.75% + Cholesterin 1%	0.05c.c.	Trace	Very marked	Just complete

CONTROLS.

Serum 0.05cc + NaCl solution 0.6cc + Complement 0.02cc = Complete

Emulsions 0.6c.c.
Lecithin 0.015% + Cholesterin 1% + Complement 0.03cc = Complete
" 0.1% + " 1% + " 0.03cc = "
" 0.75% + " 1% + " 0.04cc = "

Dose of Complement = 0.01cc.

.....

TABLE 4.

2.37% solution of ox heart lecithin in alcohol, I part emulsionised with 7 parts of saline, so as to produce the maximum turbidity = stock emulsion.

A. 2.5c.c. of stock emulsion + 5.5c.c. saline.

B. 2.5c.c. " " " + 5.2625c.c. " + 0.2375c.c. alcohol.

C. 2.5c.c. " " " + 4.8125c.c. " + 0.6875c.c. "

Thus each emulsion contained 0.75% lecithin but different amounts of alcohol.

Syphilitic Serum (57°C) 0.05c.c. + Lecithin Emulsion 0.6c.c.	Amounts of Guinea-pig's Complement.			
	0.09c.c.	0.13c.c.	0.17c.c.	0.22c.c.
Emulsion A.	Trace	Distinct	Very marked	Complete
Emulsion B.	Very faint trace	Faint trace	Trace	Very marked
Emulsion C.	0	Very faint trace	Faint trace	Trace.

CONTROLS

Serum 0.05c.c. + NaCl solution 0.6c.c. + Complement 0.02 c.c. = Complete

Emulsions 0.6c.c. + Complement 0.02c.c. = Complete.

Dose of Complement = 0.01c.c.

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TABLE 5.

0.6cc Emulsion of 0.75% alcoholic solution of Lecithin + I% Cholesterin	Syphilitic serum 57°C	Amounts of Guinea-pig's Complement			
		0.I4cc	0.2cc	0.28cc	0.38cc
Ox's liver Lecithin	0.05c.c.	0	Faint trace	Marked	Complete
Pig's liver Lecithin	0.05c.c.	0	0	Distinct	Complete
Pig's heart Lecithin	0.05c.c.	0	0	Trace	Almost complete
Pig's brain Lecithin	0.05c.c.	Just complete	...	...	...
Yolk Lecithin	0.05c.c.	Complete	...	...	...

CONTROLS.

Serum 0.05c.c. + 0.6c.c. NaCl solution + Complement 0.0I5cc =Complete

Emulsions 0.6c.c.

Ox's Liver lecithin	+	cholesterin	+	complement	0.0I5cc = Complete
Pig's " "	+	"	+	"	0.0I5cc = "
Pig's heart "	+	"	+	"	0.0I5cc = "
Pig's brain "	+	"	+	"	0.02cc = "
Yolk "	+	"	+	"	0.0I5cc = "

Dose of Complement = 0.0Ic.c.

.....

TABLE 6.

Emulsion 0.6cc + Normal Serum (57°C) 0.05cc	Amounts of Guinea-pig's Complement			
	0.02cc.	0.04cc.	0.07cc.	0.1cc.
Ox liver Lecithin	Very marked	Complete	...	...
" " " + Cholestein	Almost complete	"	...	...
Ox heart Lecithin	...	Just complete	...	...
" " " + Cholestein	...	Faint trace	Distinct	Marked

CONTROLS.

Serum 0.05c.c. + NaCl Solution 0.6c.c. + Complement 0.02cc = Complete

Emulsions 0.6c.c.

Ox liver Lecithin	+ Complement	0.02c.c.	= Complete
" " " + Cholestein	+ "	0.02c.c.	= "
Ox heart Lecithin	+ "	0.02c.c.	= "
" " " + Cholestein	+ "	0.03c.c.	= "

Dose of Complement = 0.008 c.c.

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Influence of heat on the blood serum in the Wassermann Reaction.

In the original Wassermann test and in most of its modifications, the sera to be tested are first of all exposed to a temperature of 55°C. - 57°C. for half an hour. There are two reasons for this ; firstly, to destroy any complement contained in the sera and secondly, because certain negative sera in the unheated state, along with "antigen", can deviate a large amount of complement, i.e., give a positive reaction. Heating to 55°C. has a marked effect in diminishing the power of all sera, normal and syphilitic, to deviate complement along with "antigen".

Noguchi[1] exposed the serum from a case of secondary syphilis to temperatures of 45°C., 50°C., 55°C., and 60°C. for 20 minutes, and then determined by fixation ~~of~~ tests the amount of antibody available. He found that it was greatly reduced at 45°C.; at 50°C. it was reduced to one half, and at 55°C. to one quarter of the original amount, and at 60°C. it was still further diminished. He also studied the rate of destruction at 55°C. at 5, 10, 20, 30 and 60 minute periods. During the first 5 minutes, the antibody was reduced to one third of the original amount, after 30 minutes to one fourth, and after 1 hour to one tenth of the original; thus, he found the rate of destruction to be greatest during the first 5 minutes.

Sachs[2] states that exposure to 62°C. for 30 minutes destroys the reaction of a syphilitic serum.

The principal reason for heating sera to 55°C. previous to examination in the Wassermann test is, that certain normal sera, in the unheated state can give a positive reaction, a property destroyed by heating (Sachs).

Browning and Mackenzie[3] obtained positive results in a number of cases of scarlet, enteric and typhus fevers, in eclampsia and early dementia, when the sera were used fresh, whereas control experiments with the same sera, after heating to 57°C. for half an hour, all gave negative results. They used an emulsion of crude alcoholic extract of ox liver as "antigen". Boas[4] found 45 positive results out of 230 normal cases when the sera were tested unheated, whereas all were negative after heating.

On the other hand, Noguchi[1] maintains that, in his modification of the Wassermann test, sera can be used either heated or unheated since he has found that, when the "antigen" is in the form of acetone-insoluble lipoids, a non-specific reaction is not obtained with the unheated serum.

Syphilitic sera do not lose their power to give a positive reaction as the result of heating, although in all cases there is a greatly diminished deviation of complement as compared with the fresh serum.

The following experiments were undertaken to determine more precisely what influence heating had on sera in relation to their Wassermann effect. The L-C. method of Browning, Cruickshank and Mackenzie has been employed throughout, although a few cases have been tested at the same time by the method of Browning and Mackenzie using a crude alcoholic extract of ox liver.

First of all, a number of sera, both normal and syphilitic, were tested in the fresh state and after heating to 57°C. for half an hour. In all cases, heating produced a marked diminution in the amount of complement deviated along with L. and L-C. emulsions (table I). On the L-C. criterion of a positive result, three unheated normal sera gave positive reactions (table 2A, L & C). In all of these, more complement is fixed in the L-C. than in the L. series, the difference being 5 haemolytic doses of complement in A., 6 doses in B., and 9 doses in C.; this last case also gave a positive reaction with crude extract emulsion.

The experiment shown in table 2D. is very interesting; it shows an unheated normal serum which has deviated 28 doses of complement along with emulsions of lecithin, lecithin-cholesterin and crude liver extract. By the L-C. method, this result must be considered negative since exactly the same of amount of complement is deviated by the serum witheach emulsion. However, on the absolute basis of a positive reaction of the C-E. method, the serum is positive with all three emulsions.

In the original Wassermann reaction and practically all its modifications, a positive result depends on the fact that serum and "antigen" together can deviate a fixed amount of complement (0.1cc and more recently 0.05cc), whilst neither of them alone can do so.

Table I A.&B. and table 2 B.,C.and D. show that an unheated normal serum along with "antigen" can deviate this amount of complement, and therefore,by these methods,many unheated negative sera would give a positive result. With the L-C. method,however,this happens less frequently since the increased deviation of complement by the fresh serum is generally equal in amount with both L. and L-C. emulsions.

With regard to Noguchi's statement,that such non-specific reactions with unheated sera do not occur with acetone-insoluble lipoids as "antigen",these results are not in agreement. Such "antigen" is used in the L-C. method,and yet with it,as has been seen,positive results with unheated normal sera can be obtained. In addition,Noguchi uses a fixed amount of guinea-pig's complement (usually 0.04cc. plus complement in fresh human serum,C.02cc.), and as the above experiments show,an unheated normal serum with an acetone-insoluble lipoid can deviate a much larger amount than that. Other methods,such as Hecht's,Stern's,Tschernogubow's, Fleming's and Emery's,in which the complement used is that present in the unheated human serum to be tested are all clearly open to the danger of recording positive results from negative sera.

Results of heating sera at different temperatures.

A further series of experiments was conducted to determine the effect of heating sera for half an hour at different temperatures, namely,37°C.,42°C.,47°C.,52°C.,57°C.,and 62°C. In this way,17 sera have been examined with 10 different complements.

The results are striking. In practically all cases,both normal and syphilitic,no alteration occurs in the deviating power of the serum in the presence of "antigen" up to a temperature of 47°C.; In a few cases,a slight diminution occurs. But invariably,there is a marked fall at 52°C., and no further loss of deviating power, or very little,is recorded at 57°C. At 62°C., the power of a syphilitic serum to give a positive reaction is practically gone, (tables 3 & 4).

Further experiments were undertaken to determine at what temperature between 47°C. and 52°C. the loss of deviating power occurred, but the results were variable. It would appear that in different instances, it is more marked at different temperatures.

Thus in table 5A., the results are shown in the case of a normal serum which was heated at 46°C., 48°C., 50°C., and 52°C for half an hour. At 48°C., rather less complement is deviated than at 46°C., whilst at 50°C., very little further change is recorded, but at 52°C., a marked diminution has occurred in the amount of complement absorbed.

Table 5B. shows a syphilitic serum examined at the same time as the above. In this case, practically no difference exists in the reactions at 46°C., 48°C., and 50°C., but at 52°C., there is a considerable decrease in the deviating power of the serum.

In table 6 in the case of a syphilitic serum, it is seen that the greatest alteration occurs between 50°C. and 52°C, whilst at 46°C., 48°C. and 50°C., the reactions are almost identical; the unheated serum deviates a little more complement than after heating to 46°C.

A number of charts are appended which graphically represent the effect of heating sera at different temperatures, as regards the action in the Wassermann test.

Along the base lines are marked the temperatures to which the sera have been heated, whilst, on the vertical lines, are represented the doses of complement deviated. The amounts of complement absorbed by the sera along with L. and L-C. emulsions at the different temperatures are thus shown.

Charts I and 2 show clearly how the diminution in deviating power of both normal and syphilitic sera occurs between 47°C. and 52°C.; in the former case, the lines representing the L. and L-C. emulsions are identical, whilst in the syphilitic cases, they run almost parallel. In chart 3, however, in which a syphilitic serum is represented, the L. emulsion shows a slight diminution in complement absorption up to 47°C., whereas the L-C. emulsion shows none; in both cases, a marked fall occurs between 47°C. and 52°C. and a still further diminution at 57°C. and at 62°C. It is noteworthy, however, that at 62°C., the L-C. emulsion is deviating 5 doses of complement more than the L. emulsion, a positive reaction, so that even at this temperature the syphilitic character of the serum is not wholly destroyed. In chart 2, it will be seen that at 62°C., a difference in the two series also persists in the case of the syphilitic serum but it is smaller in amount.
Chart 4 represents a normal serum and it shows that, whereas no alteration occurs in the deviation of complement by the L. emulsion up to 47°C., the L-C. emulsion is fixing less complement at 42°C. than with the fresh serum.
Charts 5, 6 and 7 represent the results obtained by heating sera to temperatures from 46°C. to 52°C.
Chart 5A. shows a normal serum. One line represents both the L. and L-C. series; a fall in deviating power occurs between 46°C. and 48°C. and again between 50°C. and 52°C., whilst between 48°C. and 50°C., no alteration has occurred.

On the other hand in chart 5B.in the case of a syphilitic serum,the only fall occurs between 50°C. and 52°C. A different condition is shown in chart 6 with a negative serum, the greatest fall being recorded between 48°C. and 50°c., whilst further alteration occurs at 52°C. Again in chart 7, an unheated syphilitic serum with both L. and L-C. emulsions deviated more complement than at 46°C.,no further diminution in deviating power occurring until after 50°C.

These results,obtained by heating sera to different temperatures,are not altogether in agreement with Noguchi's results. He records a marked loss of antibody at 45°C.,whereas I find that the diminution usually occurs between 47°C. and 52°C.,and in those cases,where a loss has occurred at less temperatures than 47°C., it has been only small in amount;however,this discrepancy may possibly be due to the different methods employed,for whereas Noguchi uses a fixed amount of complement with increasing amounts of serum,in the L-C. method,the amount of serum is constant and the dosage of complement varies.

As will be shown later,this same phenomenon is obtained by heating the globulins of normal and syphilitic sera to different temperatures.

Effect of heating sera for various periods of time.

Two sera,a normal and a syphilitic,were examined in the Wassermann reaction,unheated and after heating at 55°C. for 5,10,20,30 and 60 minutes. The loss of deviating power was as great after 5 minutes as after 60 (table 7). The serum from another case of syphilis was heated at 48°C. for 5,10,20,30,60 and 120 minutes, and here also practically no difference was found in the amounts of complement deviated with both L. and L-C. emulsions at these various periods.

Anticomplementary effect of heated and unheated sera.

As a general rule,heating has no effect on the amount of complement absorbed by the serum alone. In only two instances did the fresh serum absorb more complement by itself than the serum heated to 57°C. for half an hour,and as the same complement was used in both cases,the result was probably due to some peculiarity of that complement. Thus the differences obtained in the Wassermann

reaction by unheated and heated sera cannot be explained by alterations in the anticomplementary effect of the sera by themselves.

Conclusions.

I. Both normal and syphilitic sera, along with "antigen", deviate much more complement in the fresh state than after heating.

2. This property is destroyed by exposure for 5 minutes to a temperature of 55°C.

3. Certain unheated normal sera, in virtue of this property (v.I), are capable of giving a positive Wassermann reaction, irrespective of the particular "antigen" used.

4. In the case of syphilitic sera, heating to 52°C. causes a diminution in their power to absorb complement but does not destroy their power to give a positive Wassermann reaction.

5. The property of syphilitic sera, in respect of which a positive Wassermann effect is obtained, is almost destroyed at 62°C., but even then the sera lead to absorption of more complement with lecithin-cholesterin emulsion than with lecithin emulsion.

6. As a rule, a fresh serum, normal or syphilitic, deviates no more complement by itself than after heating at 52°C.

7. For diagnostic purposes, it is absolutely essential to heat all sera at 52°C. - 57°C., in order to avoid fallacious results, no matter what method may be employed.

References.

I. Noguchi, Serum Diagnosis of Syphilis, 1910, p.84.

2. Sachs, Semaine Med., June 24th, 1908.

3. Browning and Mackenzie, Recent Methods in the Diagnosis and Treatment of Syphilis, 1911, p.80.

4. Boas, Die Wassermannsche Reaktion, Berlin, 1911.

TABLE I.

— A —

Serum 0.05cc. Negative	Emulsions 0.6c.c.	Amount of Guinea-pig's Complement				NaCl Solutn. 0.6c.c. + Complement
		0.04c.c.	0.07c.c.	0.I C.c.	0.I4c.c.	0.0I5c.c.
57°C	L.	Complete	Complete	Complete	Complete	Marked
"	L-C.	"	"	"	"	...
"	C.E.	"	"	"	"	...
Unheated	L.	0	0	Very faint trace	...	Complete
"	L.C.	0	0	" "	Faint trace	...
"	C.E.	0	0	" "	" "	...

— B —

57°C	L.	Complete	Complete	Complete	Complete	Just complete
"	L-C.	Just "	"	"	"	...
"	C.E.	" "	"	"	"	...
Unheated	L.	0	0	0	...	Complete
"	L-C.	0	0	0	Very faint trace	...
"	C.E.	0	0	0	" "	...

Emulsions 0.6 c.c. (L / L-C) + Complement 0.035 c.c. = Very marked

" 0.6 c.c. (Crude Extract) + " 0.035 c.c. = No lysis.

Dose of Complement = 0.0I25 c.c.

— C —

Serum 0.05c.c. Positive	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement.					NaCl Solutn. 0.6 c.c. + Complement
		0.07cc	0.II 6c.	0.I6 cc.	0.22 cc	0.3cc	0.04c.c.
57°C ½ hr.	L.	Complete	Complete	Complete	Complete	Complete	Just complete
" "	L-C.	Distinct	Marked	Just complete	"	"	...
Unheated	L.	Trace	Distinct	Marked	Very marked	...	Just complete
"	L-C.	0	0	0	0	Distinct	...

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.0I c.c.

TABLE 2

1

Negative Serum 0.05 c.c.	Emulsions 0.6 c.c..	Amounts of Guinea-pig's Complement				NaCL Solution 0.6 c.c. + Complement
		0.04cc.	0.07 cc.	0.II cc	0.I6cc	0.04 c.c.
57°C	L..	Just complete	Complete	...	...	Complete
"	L-C.	" "	"	...	...	...
Unheated	L..	0	Marked	Very marked	Just complete	Almost complete
"	L-C.	0	0	Distinct	Very marked	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.0I c.c.

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2

Negative Serum 0.05 c.c.	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement			NaCl Solution 0.6 c.c. + Complement
		0.04 c.c.	0.07 c.c.	0.II c.c.	0.03 c.c.
57°C	L.	Complete	...	...	Complete
"	L-C.	"	...	...	...
Unheated	L.	...	Complete	Complete	Complete
"	L-C.	...	Very marked	"	...

Emulsions 0.6 c.c. + Complement 0.02 c.c. = Complete

Dose of Complement = 0.0065 c.c.

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TABLE 2

C

Negative Serum 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement					NaCl Solut 0.6 c.c. + Complement
		0.02 cc	0.04 cc	0.06 cc	0.I0c	0.I4cc	0.0I5 c.c.
57°C	L.	Marked	Complete	...	...	...	Complete
"	L-C.	Complete	"	...	...	...	...
"	C.E.	Marked	"	...	...	...	...
Unheated	L.	0	0	Faint trace	Just complete	Complete	Complete
"	L-C.	0	0	Very " "	Marked	Almost "	...
"	C.E.	0	0	0	Distinct	Marked	...

Emulsions 0.6 c.c. + Complement 0.0I5 c.c. = Complete

Dose of Complement = 0.0065 c.c.

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D

Negative Serum 0.05 c.c.	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement					Serum 0.05cc + NaCL Soln 0.6 cc + Complement
		0.02 cc	0.04 cc	0.07 cc	0.I cc	0.I4cc	0.0I5 c.c
57°C	L.	Complete	...	...	...	...	Complete
"	L-C.	"	...	...	...	...	
"	C.E.	"	...	...	...	...	
Unheated	L.	0	0	Trace	Marked	Complete	Complete
"	L-C.	0	0	0	"	"	
"	C.E	0	0	0	Distinct	"	

Emulsions 0.6 c.c. + Complement 0.0I5 c.c. = Complete

Dose of Complement = 0.005 c.c.

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TABLE /3/

Negative Serum 0.05 c.c.	Emulsions 0.6c.c.	Amounts of Guinea-Pig's Complement					NaCL Soluti 0.6c.c. + Complement
		0.02c.c.	0.04cc.	0.07cc.	0.I cc.	0.I4cc	0.03 c.c.
62°C	L.	Complete	...	...	...	...	Complete
"	L-C.	"	...	...	...	...	
57°C	L.	Very marked	Complete	Complete	...	...	Just Complete
"	L-C.	" "	Just "	"	...	...	
52°C	L.	Almost complete	Just complete	Complete	...	...	Complete
"	L-C.	Marked	" "	"	...	...	
47°C	L.	0	Faint trace	Trace	Marked	Just complete	Just complete
"	L-C.	0	Trace	Distinct	"	Complete	
Unheated	L.	0	Faint trace	Trace	Marked	Just complete	Complete
"	L-C.	0	Trace	Distinct	Very "	Complete	
Positive Serum 0.05 c.c.	Emulsions 0.6c.c.	0.02c.c.	0.04cc.	0.07cc.	0.I cc.	0.I4cc	0.03 c.c.
62°C	L.	Complete	...	...	...	...	Complete
"	L-C.	Very marked	Complete	...	...	...	
57°C	L.	...	Marked	Complete	...	...	Complete
"	L-C.	...	...	Marked	Almost complete	Complete	
52°C	L.	...	Almost complete	Complete	...	...	Just complete
"	L-C.	...	...	Marked	Just complete	Complete	
47°C	L.	...	...	Faint trace	Trace	Marked	Just complete
"	L-C.	...	...	...	Very ft. trace	Trace	
Unheated	L.	...	...	0	Trace	Marked	Complete
"	L-C.	...	...	...	Very ft. trace	Faint trace	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Just Complete

Dose of Complement = 0.015 c.c.

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TABLE 4

Negative Serum 0.05 c.c	Emulsions 0.6c.c.	Amount of Guinea-pig's Complement				NaCl Solut 0.6 c.c. + Complement
		0.04 c.c	0.07 c.c	0.I c.c.	0.I4 c.c	0.02 c.c.
57°C	L.	Verymarked	Complete	...	...	Almost complete
"	L-C.	"	"	...	...	
52°C	L.	Marked	Complete	...	...	Very marked
"	L-C.	"	"	...	...	
47°C	L.	0	Faint trace	Marked	Complete	Marked
"	L-C.	0	" "	"	"	
42°C	L.	0	Faint trace	Marked	Complete	Marked
"	L-C.	0	" "	"	"	
Unheated	L.	0	Faint trace	Trace	Almost complete	Marked
"	L-C.	0	" "	"	Just "	

Syphilitic Serum 0.05 c.c.	Emulsions 0.6c.c.	0.I c.c.	0.I4 c.c.	0.2 c.c.	0.28cc	0.02 c.c.
57°C	L.	Verymarked	Complete	...	...	Almost complete
"	L-C.	Faint trace	Trace	Marked	Complete	
52°C	L.	Verymarked	Complete	...	...	Complete
"	L-C.	0	Very faint trace	Very marked	Complete	
47°C	L.	...	0	0	Very faint trace	Marked
"	L-C.	...	0	0	0	
42°C	L.	...	0	0	Faint trace	Marked
"	L-C	...	0	0	0	
Unheated	L.	...	0	0	0	Marked
"	L-C.	...	0	0	0	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.005 c.c.

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TABLE 8

A

Negative Serum 0.05 c.c.	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement					NaCl sol. 0.6 c.c. +Complement
		0.04cc.	0.07 cc.	0.I cc	0.I4 cc	0.2 cc.	0.05 cc
52°C	L.	Complete	...	...	...	...	Complete
"	L-C	"	...	...	...	...	
50°C	L.	0	Distinct	Almost complete	Complete	...	Almost complete
"	L-C.	0	"	" "	"	...	
48°C	L.	0	Trace	Marked	Complete	...	Almost complete
"	L-C.	0	"	Distinct	"	...	
46°C	L.	0	Faint trace	Trace	Marked	Complete	Just complete
"	L-C.	0	Very " "	Faint "	"	"	

B

Syphilitic Serum 0.05 c.c.	////	0.07cc	0.I cc.	0.I4 cc	0.2 cc.	0.28cc	
52°C	L.	Distinct	Very marked	Just complete	...	...	Almost complete
"	L-C.	0	0	Marked	Complete	...	
50°C	L.	0	0	Faint trace	Trace	Very marked	Very marked
"	L-C.	0	0	Very " "	Faint "	Marked	
48°C	L.	0	0	0	Trace	Very marked	Very marked
"	L-C.	0	0	0	Very ft. trace	Marked	
46°C	L.	0	0	0	Trace	Very marked	Very marked
"	L-C.	0	0	0	0	Marked	

Emulsions 0.6 c.c. + Complement 0.02 c.c. = Just Complete.

Dose of Complement = 0.015 c.c.

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TABLE 6

Syphilitic Serum 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement				NaCl Soluth. 0.6 c.c. + Complement
		0.Ic.c.	0.I4 c.c	0.2 c.c.	0.28 c.c.	0.05 c.c.
52°C	L.	Complete	...	...	...	Complete
"	L-C.	Distinct	Very marked	Complete	...	
50°C	L.	0	Distinct	Complete	...	Complete
"	L-C.	0	Very faint trace	Marked	Complete	
48°C	L.	Trace	Distinct	Almost complete	Complete	Just complete
"	L-C.	0	Trace	Distinct	"	
46°C	L.	0	Distinct	Just complete	Complete	Just complete
"	L-C.	0	0	Marked	"	
Unheated	L.	Very ft. trace	Trace	Marked	Just complete	Complete
"	L-C.	0	0	Trace	Very marked	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.0I5 c.c.

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TABLE 7

A

Normal Serum 55°C 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement 0.04 c.c	0.07 c.c	0.11 c.c	0.16 cc	0.6cc NaCl Solution + Complement 0.04 c.c.
Unheated	L.	0	Faint trace	Trace	Complete	Complete
"	L-C.	0	0	"	"	
5 Minutes	L.	Very marked	Just complete	...	...	Complete
"	L-C.	Marked	" "	...	...	
10 Minutes	L.	Very marked	Just complete	...	...	Just complete
"	L-C.	" "	" "	...	...	
20 Minutes	L.	Very marked	Just complete	...	...	Complete
"	L-C.	" "	" "	...	...	
30 Minutes	L.	Very marked	Complete	...	...	Almost complete
"	L-C.	Almost complete	"	...	...	
60 Minutes	L.	Almost complete	Complete	...	...	Complete
"	L-C.	" "	"	...	...	

B

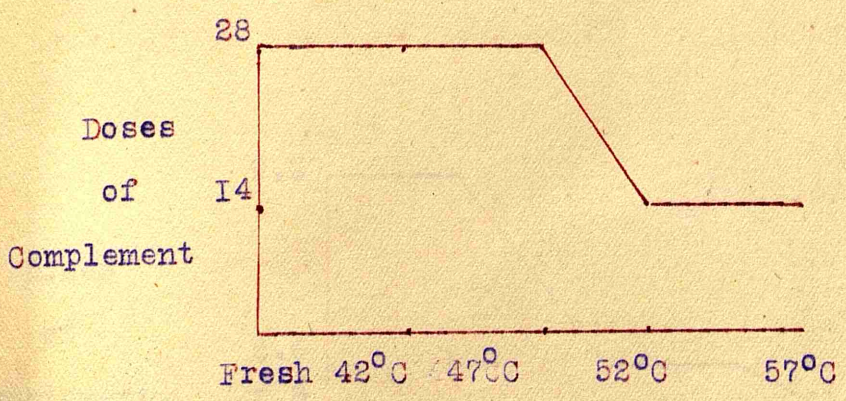
Syphilitic Serum 55°C 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement 0.07 c.c	0.11 cc	0.16 cc	0.22cc	0.6cc NaCl Solution + Complement 0.04 cc
Unheated	L.	0	0	0	...	Complete
"	L-C.	0	0	0	0	
5 Minutes	L.	Very marked	Complete	...	...	Almost complete
"	L-C.	0	0	Trace	Very marked	
10 Minutes	L.	Very marked	Complete	...	...	Almost complete
"	L-C.	0	0	Trace	Just complete	
20 Minutes	L.	Very marked	Complete	...	...	Almost complete
"	L-C.	0	Faint trace	Trace	Just complete	
30 Minutes	L.	Very marked	Just complete	...	...	Almost complete
"	L-C.	0	Trace	Distinct	Almost complete	
60 Minutes	L.	Almost complete	Complete	...	...	Complete
"	L-C.	0	0	Trace	Very marked	

Emulsions 0.6 c.c. + Complement 0.05 c.c. = Almost Complete

Dosr of Complement = 0.0175 c.c.

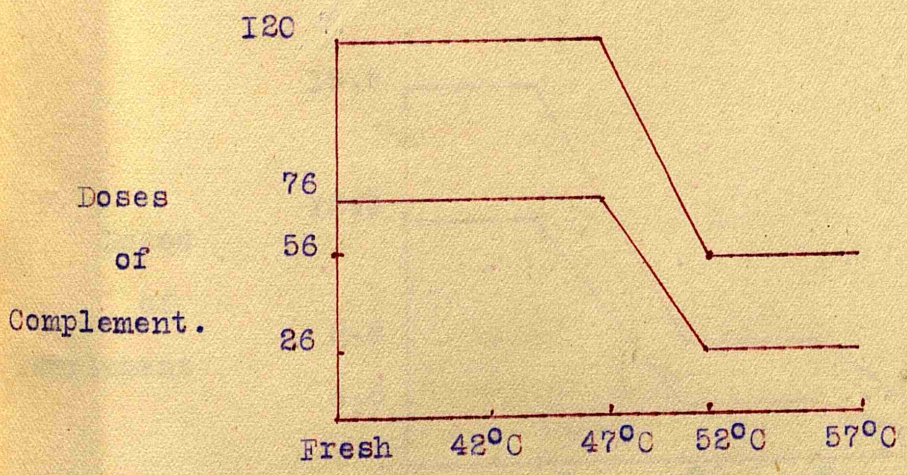
C H A R T I.

Negative Serum.



The one line represents both the L. and L-C. series.

Syphilitic Serum.

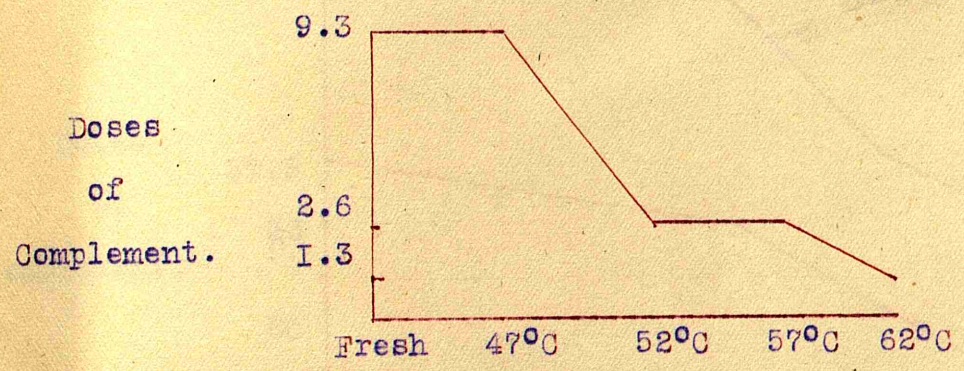


The upper line represents the L-C. series.

The lower line represents the L. series.

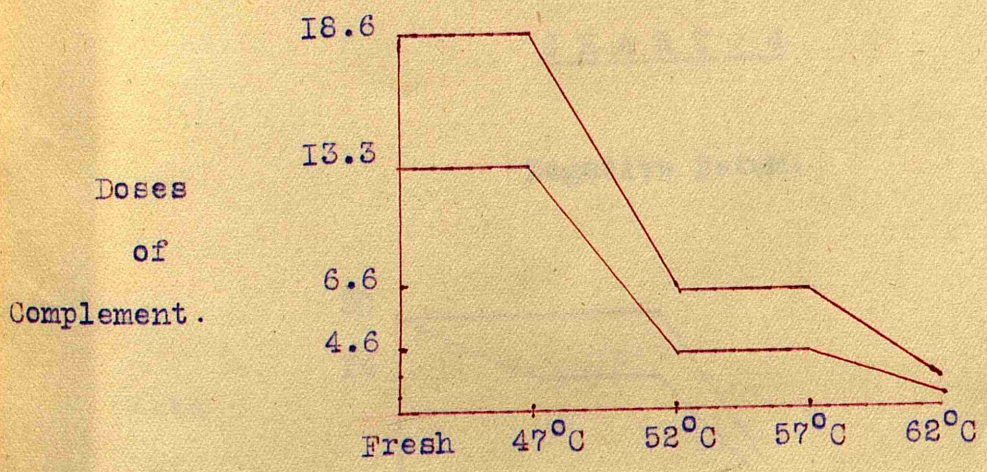
C H A R T 2

Negative Serum.



The one line represents both the L.and L-C. series.

Syphilitic Serum.

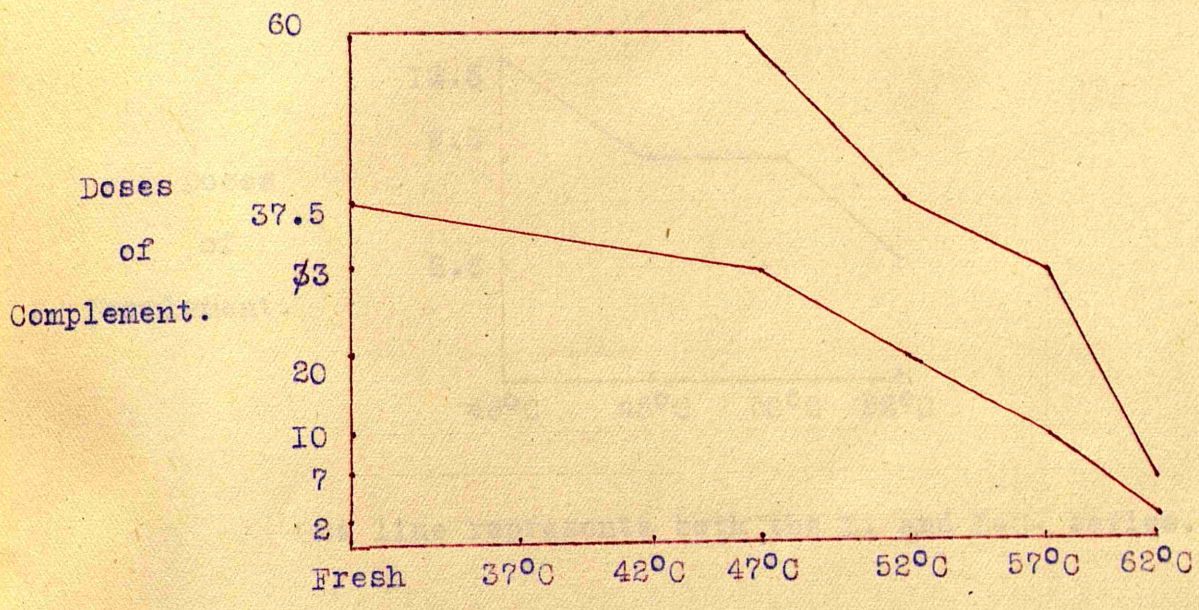


The upper line represents the L-C. series.

The lower line represents the L. series.

CHART 3

Syphilitic Serum.

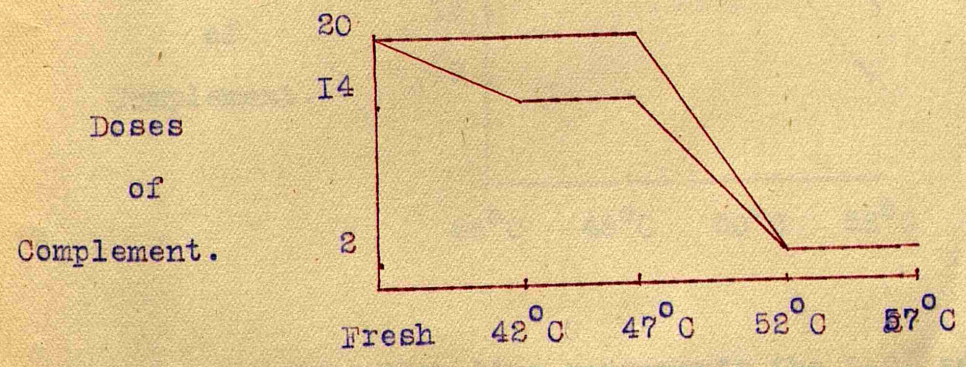


The upper line represents the L-C. series.

The lower line respresents the L. series.

CHART 4

Negative Serum.



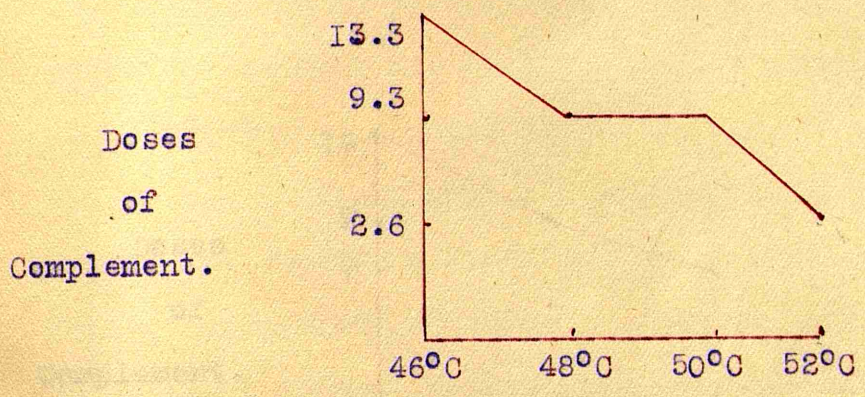
The upper line represents the L. series.

The lower line represents the L-C. series.

C H A R T 5.

A.

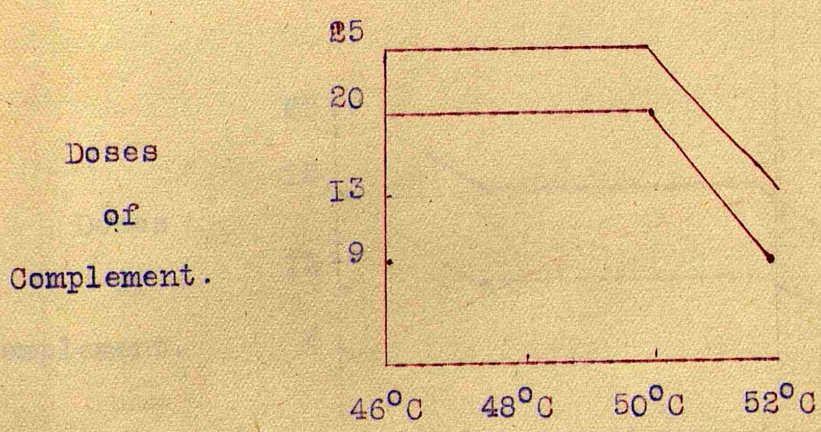
Negative Serum.



One line represents both the L. and L-C. series.

B.

Syphilitic serum.

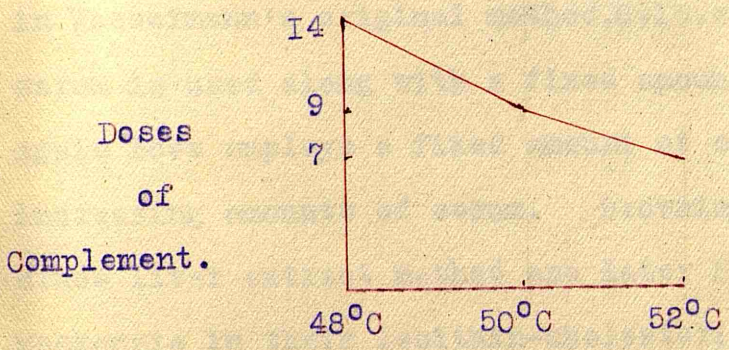


The upper line represents the L-C. series.

The lower line represents the L. series.

C H A R T 6.

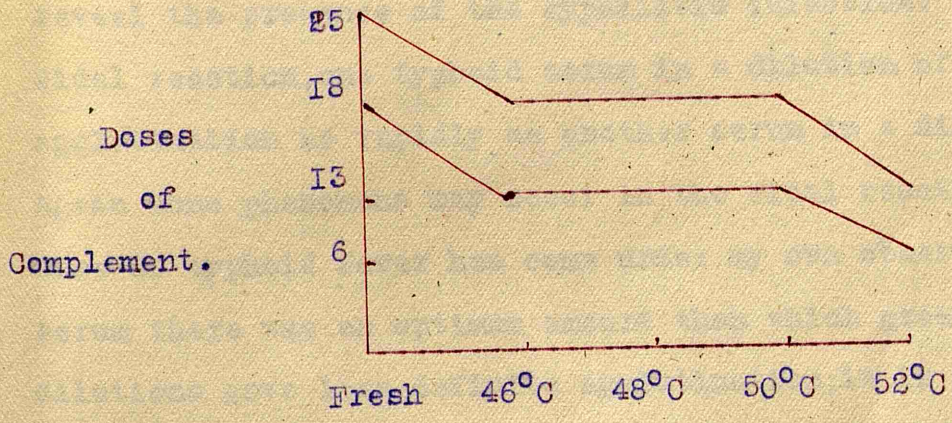
Negative Serum.



One line represents both the L. and L-C. series.

C H A R T 7.

Syphilitic Serum.



The upper line represents the L-C. series.

The lower line respresents the L. series.

Investigation into the effect of varying the amounts of serum in the Wassermann syphilis reaction.

It has seemed to me a point of considerable importance that the effect of using varying amounts of serum in the Wassermann test should be investigated in view of the fact that markedly different amounts are employed in the methods in common use. Thus in Wassermann's original method, 0.1c.c. to 0.2c.c. of inactivated serum is used along with a fixed amount of complement and "antigen". Again Boas employs a fixed amount of complement and "antigen" with increasing amounts of serum. Browning and Mackenzie in their crude liver extract method and later Browning, Cruickshank and Mackenzie in their lecithin-cholesterin method employ 0.05c.c. of inactivated serum in the test.

Again syphilitic sera vary enormously in their power to fix complement in the presence of "antigen", i.e. the number of syphilitic antibody units per cubic centimetre of serum is widely different in different cases. Hence it might be assumed that where the antibody content was small, a larger amount of serum would be required to react with "antigen" in fixing complement so as to reveal the presence of the syphilitic infection. Thus in the Widal reaction, one typhoid serum in a dilution of 1-100 may cause agglutination as rapidly as another serum in a dilution of 1-20; Again zone phenomena may occur in the Widal reaction; thus a case of typhoid fever has come under my own observation, with whose serum there was an optimum amount than which greater or less dilutions gave less definite agglutination; it is possible that a similar phenomenon may occur in the Wassermann reaction.

A number of sera have been examined in amounts ranging from 0.01c.c. to 0.5c.c., the lecithin-cholesterin method being employed in all cases except one, where the crude liver extract method was alone employed. However, the results obtained in the lecithin-cholesterin series are comparable with those obtained with crude

extract emulsion. The deviation of complement by a syphilitic serum along with crude extract is generally somewhat greater than with lecithin-cholesterin, but the difference is small, and a syphilitic serum which reacts weakly or strongly with crude extract always shows the same behaviour with lecithin-cholesterin.

The two most important points that arise out of this investigation are (I) Can a negative serum give a positive reaction when used in larger amounts in the test?; (2) Can a weak syphilitic serum give a positive reaction by the use of a larger quantity whilst giving a negative or doubtful reaction in the amounts generally employed?. These are obviously points of great practical importance, especially in their bearing on those methods in which increasing amounts of serum are employed, e.g. Boas' method.

In my series of cases, including 8 negative and 9 positive sera, no negative has been converted into a positive by the use of larger amounts. With negative sera, if any increase in complement deviation occurs with the use of larger amounts of serum, this increase is equal in both the lecithin and lecithin-cholesterin series, so that the negative character of the reaction is maintained. Such increase only occurred with one serum out of the series. (Table I A & B). At the same time, the use of larger amounts of negative sera has never led to such an increase in complement-fixation with the lecithin-cholesterin emulsion as would give a positive result on the absolute basis of the crude extract method; for where such increase occurs, it is accompanied by an increased anticomplementary effect on the part of the serum by itself, which fully accounts for the increased deviation of complement in the presence of "antigen".

In regard to the second point, viz., the conversion of a weak positive into a strong positive reaction by the employment of larger amounts of serum, 3 very weakly reacting syphilitic sera have been examined, one of them on two different occasions.

Case I J.M. (vide table 2)

Clinically this patient is an undouted case of general paralysis. On the first occasion on which his serum was tested, it proved to be negative, whilst the cerebro-spinal fluid reacted positively.
On this occasion the serum was tested in the following amounts:-0.02c.c., 0.05c.c., 0.I5c.c., and 0.3c.c., and 0.5c.c. At the same time the spinal fluid was examined in the usual way.
The table shows the following points:-
(I) With the smallest amount of serum (0.02c.c.) the reaction is negative.

(2) With 0.05c.c. of serum, the amount usually employed in the test, there is a very doubtful positive result, this amount deviating 2 doses of complement more along with L-C. emulsion than L. emulusion.

(3) With 0.I5c.c. of serum, there is a greater total deviation of complement but with the same difference of 2 doses between the lecithin and lecithin-cholesterin series. In this amount, the serum by itself is deviating more complement than in (I) and (2).

(4) 0.3c.c. and 0.5c.c. of serum given still greater deviation of complement along with emulsion but there is now no difference between the lecithin and lecithin-cholesterin series and therefore the reaction is negative; at the same time the reaction with the L-C. emulsion is negative on the absolute basis adopted by Browning and Mackenzie in their crude extract method. The anticomplementary effect of these amounts of serum is very marked.

From the above results, it is obvious that little or no advantage is gained by using an amount of serum greater than 0.05c.c. Certainly there is increased deviation of complement by the larger amounts but that is entirely explained by the increase anticomplementary effect of the serum in these amounts.

CASE 2 H.S. Manic-depressive Insanity. (vide table 3)

This patient's serum reacts anomalously which, inconjunction with her history; points to a probable syphilitic infection.
History:- This patient was a prostitute and has had three pregnancies; the first ended in the birth of a child about whom nothing further is known; the second pregnancy terminated either in a miscarriage or in the birth of a child who died very shortly afterwards; the third child died shortly after birth.
The serum was tested in the following amounts:- 0.05c.c., 0.I5c.c., 0.3c.c., and 0.5c.c. A negative serum, examined at the same time in different amounts, is included in the table.

(I) With 0.05c.c. of serum, there is not much total deviation of complement and the difference between the lecithin and lecithin-cholesterin series is small (about $2\frac{1}{2}$ doses of complement)

(2) With 0.I5c.c. of serum, there is a greater difference between the two series (about 4 doses), but this is due to the L. emulsion causing less deviation with

0.I5c.c. of serum than with 0.05c.c.,whilst with the L-C. emulsion the amount of complement deviated ~~y~~ ~~i~~ ~~t~~ ~~h~~ ~~p~~ ~~y~~ is practically the same in both cases.

(3) With a larger amount of serum (0.3c.c. and 0.5c.c.) the amount of complement deviated is equal in both the lecithin and lecithin-cholesterin series so that the result is negative. At the same time the total complement deviation is much less,so that the reaction given by these amounts of serum is little different from that of the negative control.
The table shows the value of having a negative control in the diagnostic examination of sera. It will be seen that,although the total complement deviation given by the smaller amounts of serum is not great, yet it is considerably more than that given by the negative serum,and also in the former case more complement is deviated along with the L-C. emulsion than with the L. emulsion.
This case shows that whereas the use of the usual amounts of serum causes one to suspect the presence of syphilis,the larger amounts would suggest its absence.

CASE 3 A.F. (vide table 4 A & B)

A case of general paralysis,confirmed by post mortem examination. The serum from this case was examined in different amounts on two occasions. (Mention of my first experiment appears in Browning & Mackenzie's book "Recent Methods in the Diagnosis and Treatment of Syphilis" pages 85 and 86).
The cerebro-spinal fluid which gave a positive reaction was tested at the same time by way of control.
The serum was examined in the following amounts:-
0.05c.c., 0.2c.c.,and 0.5c.c.

Table 4a shows that 0.2c.c. of serum gave a somewhat more definitely positive reaction than the lesser amount, whilst 0.5c.c. gave a less definite effect than either of the other two.

At a later date,this experiment was repeated with the same amounts of serum but in addition to the L-C. method the Crude Extract method was also employed.(Table 4b) By the L-C. method,the reaction is practically identical with 0.05c.c. and 0.2c.c. of serum and is a doubtful positive,whilst with 0.5c.c. it is quite negative and the larger amount of serum at the same time deviates more complement by itself. With the C.E. emulsion,0.05c.c. of serum causes considerable deviation of complement,yet there is not a deviation of 5 doses of complement more than the sum deviated by the serum and emulsion alone,and therefore the reaction is negative. With 0.2c.c. and 0.5c.c.of serum plus C.E. emulsion,less complement is deviated and at the same time the inhibitory effect on complement by the serum alone is greater with these amounts than with the small amount of serum.

The general results are that the use of increased amounts of serum in the L-C.method of performing the Wassermann reaction leads in different cases to different effects.

With negative sera:-

(I) In a number of instances, the larger amounts of serum along with emulsion cause deviation of more complement than do the smaller amounts. This, however, is associated with an increased anticomplementary effect on the part of the serum itself that fully accounts for the greater fixation of complement in presence of emulsion. The use of the larger amounts of serum does not lead to a positive reaction either on the relative basis of the L-C.method or the absolute basis of the C.E.method. (table I A & B)

(2) Again, a number of negative sera give exactly the same degree of complement deviation when employed in different amounts and in all there is no increased anticomplementary effect with the larger amounts of the serum alone. (table 3B & 5 A,B,& C)

(3) In a third group of negative cases, the use of larger amounts of serum beyond a certain point leads to a diminution in complement fixation with both L. and L-C. emulsions. (table 6 A & B).

With positive sera:-

Positive sera react in much the same way as negative sera.

(I) Larger amounts may deviate more complement than smaller amounts; this is always accompanied by an increased anticomplementary effect which may or may not account for the total increase with emulsion. (table 2 & 7).

(2) In other cases there is less complement absorbed by the larger amounts of serum with both L.& L-C. emulsions (table 3 A & 5 D). This may be associated with an increased anticomplementary effect. (table 4 B).

(3) Certain syphilitic sera show a decrease in complement deviation when larger amounts are employed with the L-C. emulsion whereas the deviation in the presence of L. emulsion is increased. (table 8 A & B). This table gives the results of two examinations of a syphilitic serum (L). On both occasions the larger amounts of serum lead to a greater deviation of complement with the L. emulsion whilst showing less deviation with the lecithin-cholesterin emulsion. At the same time, there is an increased anticomplementary effect with the larger amounts alone. This serum was on another occasion examined by the C.E. method and the result obtained is comparable to that given by the L-C. emulsion. (table 8 C).

With the C.E.emulsion, the greatest deviation occurs with 0.05c.c. of serum; with 0.2c.c., there is a striking diminution in the complement fixation, although a positive result is still obtained; but with 0.5c.c. of serum, the result is absolutely negative. Thus the similarity only exists in the dimished deviation of complement and not on the actual result, for with L-C. emulsion in the two former experiments the result would be positive on the crude extract criterion of a positive reaction.

It has been stated that the diminution of complement fixation, caused by the larger amounts of serum as compared with the smaller amounts in the presence of the emulsion of alcoholic tissue extract, is due to the greater quantity of natural immune body for the test corpuscles, thus introduced. But, obviously, this explanation cannot hold in the case of a serum which shows this phenomenon with L-C. emulsion, whilst giving the opposite result with the L. emulsion.

Examination of Boas' method of performing the Wassermann test.

In the method of performing the Wassermann test, as desribed by Boas, fixed amounts of complement and "antigen" are used along with increasing amounts of serum, and a positive result depends on the amount of serum required to cause fixation of the complement used. He claims that by this method, a patient's progress under treatment can be determined according to whether greater or less amounts of serum are required to inhibit lysis at various times.

This method, like others in which a fixed amount of complement is used, is fundamentally defective because it takes no account of the extreme variability of complement. Boas contends, however, that by the usew of a sheep-rabbit haemolytic system, this variability does not occur. But numerous experiments, which I have carried out, disprove this statement. Again, it is obvious from the foregoing results, that zone phenomena may occur with the use of varying amounts of serum, that is, there is an optimum amount in the case of each individual serum and lesser or greater amounts than this may give either a less definite or a quite erroneous result. Again, the method does not take into account that, in the larger amounts, the serum may have a very strong anticomplementary action by itself, which results in large deviation of complement by the serum and emulsion together.

In view of these facts, a number of sera were examined by Boas' method. It was most probable that with a weak syphilitic serum in

the presence of an active complement, an increase in the amount of serum would not lead to deviation of the complement used; and at the same time, the experiments were devised so as to demonstrate that erroneous conclusions regarding improvement in the patient's state, as shown by decrease in the "antibody" content of the patient's serum, might be arrived at owing to variability in the complements used. A few experiments by Boas' method have been sufficient to uphold these contentions.

Experiments I. (table 9).

The serum of a known syphilitic patient was tested with two different complements of the same age. Two series were set up. In each, 0.6c.c. of L. and L-C. emulsions, according to Browning, Cruickshank and Mackenzie's method, was used, 0.2c.c. of complement and increasing amounts of serum.
The table shows that, in the case of the L. emulsion, none of the various amounts of serum is capable of deviating either of the complements. But with the L-C. emulsion, the result is very different. Whereas with complement I, it requires 0.4cc of serum to completely inhibit 0.2c.c. of the complement, with complement 2, only 0.1c.c. of serum is necessary to give the same results with an equal amount of complement.
No more conclusive evidence could be afforded of variability in complement, and of the extreme importance of this variability in the Wassermann reaction. This serum gives 2 totally different results on the same day. Obviously, this method is useless for estimating a patient's condition as regards degree of infection.

Experiemnt 2. (table IO)

This is similar to the above, but, in addition to the L. and L-C. emulsions, a C.E. emulsion was used. Again the two complements were of the same age. The results here are not so striking, but they show another point, i.e. a zone phenomenon in the case of the L. emulsion. With both complements it will be seen that, along with the L. emulsion, 0.2c.c. of serum deviates more complement than does 0.1c.c. or 0.4c.c. The L-C. series are practically indentical but the C.E. series show a difference.

Experiment 3. (table II).

Three syphilitic sera were examined, each with three complements all of the same age. L. and L-C. emulsions were alone used.
M'G.'s serum shows complete lysis throughout, i.e. is quite negative
K's serum shows a complete deviation of complement throughout.
L's serum gives complete lysis with L. emulsion with all three complements. With L-C. emulsion, the reactions with complement I and complement 3 are almost identical, whilst with complement 2 a somewhat less amount of serum is required to cause deviation of the complement. But the chief point of interest in this table is the case of M'G. This same sample of serum was examined on the following day by the L-C. method and a very definitely positive result was got. (table II B).

It cannot be too strongly affirmed that many weak syphilitic sera are bound to be missed by methods in which a fixed amount of complement is used, and this danger cannot be obviated by the use of varying amounts of serum.

C O N C L U S I O N S.

I. A negative serum never gives a positive reaction by the use of larger amounts of serum.

2. With syphilitic sera, there is an optimum amount. As a rule, 0.05c.c. of serum serves excellently for the test. But where this amount gives a doubtful reaction, it is advisable to test the serum in greater amounts, although not much information is likely to be gained by this procedure.

3. The use of larger amounts of serum, normal or syphilitic, may lead)a) to an increased deviation of complement, (b) to no alteration in the amount of complement fixed, (c) to an actual decrease which, in the case of a positive serum, may result in a negative reaction.

4. Where larger amounts of serum, normal or syphilitic, cause increased deviation of complement, this is directly explained by the increased anticomplementary effect of the serum in negative cases and in some positive cases.

5. Any method, employing varying amounts of serum with a fixed amount of complement, gives less accurate results than one which estimates, in haemolytic doses of complement, the actual amount which is deviated by a fixed mixture of patient's serum and "antigen".

TABLE I.

A.

Serum 57°c ½ hour	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement 0.02 c.c.	0.04 c.c.	0.07c.c.	0.1c.c.
(Normal 0.01cc	L.	Just complete	Complete	...	...
(" 0.01cc	L-C.	Complete	"	...	...
(Normal 0.05cc	L.	Very Marked	Just complete	Complete	...
(" 0.05cc	L-C.	Almost complete	Complete	"	...
(Normal 0.5 cc	L.	0	Trace	Marked	Complete
(" 0.5 cc	L-C.	0	"	"	"

C O N T R O L S

Serum,normal 0.01cc + NaCL solution 0.6cc + Complement 0.03cc= Complete

" " 0.05cc + " " 0.6cc + " 0.03cc= "

" " 0.5 cc + " " 0.6cc + " 0.08cc= Very Marked

Emulsions 0.6cc + Complement 0.02cc = Complete

Dose of Complement = 0.01cc

B.

Serum 57°c. ½hour	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement 0.02 c.c.	0.04 c.c.	0.07 c.c.
(Normal 0.01cc	L.	Very Marked	Just complete	Complete
(" 0.01cc	L-C.	" "	Complete	"
(" 0.05cc	L	Marked	Complete	Complete
(" 0.05cc	L-C.	Almost complete	"	"
(" 0.5 cc	L	...	Marked	Complete
(" 0.5 cc	L-C.	...	"	"

C O N T R O L S.

Serum,normal 0.01cc + NaCL solution 0.6cc + Complement 0.04cc= Complete

" " 0.05cc + " " 0.6cc + " 0.04cc= "

" " 0.5 cc + " " 0.6cc + " 0.04cc= Trace

Emulsions 0.6cc + Complement 0.04 cc = Complete

Dose of Complement = 0.015 cc

TABLE 2

Serum 57°C.	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement. 0.02 c.c.	0.04 c.c.	0.07 c.c.	0.I c.c.
(0.02 c.c.	L.	Very marked	Complete	...	...
(0.02 c.c.	L-C	Marked	Just complete	Complete	...
(0.05 c.c.	L.	Very marked	Complete	...	...
(0.05 c.c.	L-C.	Trace	Marked	Complete	...
(0.I5 c.c.	L.	Distinct	Almostcomplete	Complete	...
(0.I5 c.c.	L-C.	Trace	Marked	Almost complete	Complete
(0.3 c.c.	L.	Trace	Marked	Very marked	Complete
(0.3 c.c.	L-C.	0	Trace	" "	Just "
(0.5 c.c.	L.	0	Trace	Marked	Very marked
(0.5 c.c.	L-C	0	Very faint Trace	"	" "

C O N T R O L S.

Serum 0.02 c.c.+ NaCL solution 0.6 c.c. + Complement 0.02cc = Complete

" 0.05 c.c.+ " " 0.6 c.c. + " 0.02cc = Just "

" 0.I5 c.c.+ " " 0.6 c.c. + " 0.03cc = Very marked

" 0.3 c.c.+ " " 0.6 c.c. + " 0.03cc = Marked

" 0.5 c.c.+ " " 0.6 c.c. + " 0.03cc = 0

Emulsions 0.6 c.c. + Complement 0.02 c.c. = Just Complete

Dose of Complement = 0.0I5 c.c.

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TABLE 3.

A.

Serum 55°C. ½ hour	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement 0.04 c.c.	0.07 c.c.	0.11 c.c.	0.16 c.c.
0.05 c.c.	L.	Very faint trace	Distinct	Almost complete	...
0.05 c.c.	L-C	" " "	"	Marked	Just Complete
0.15 c.c.	L.	Faint trace	Very Marked	Complete	...
0.15 c.c.	L-C	" "	Trace	Very marked	Just complete
0.3 c.c.	L.	Marked	Complete	...	...
0.3 c.c.	L-C	"	"	...	...
0.5 c.c.	L.	Just complete	Complete	...	...
0.5 c.c.	L-C	Very Marked	Just complete	...	...

CONTROLS.

Serum 0.05c.c. + NaCL solution 0.6c.c. + Complement 0.04c.c. = Complete
" 0.15c.c. + " " 0.6c.c. + " 0.04c.c. = "
" 0.3 c.c. + " " 0.6c.c. + " 0.04c.c. = "
" 0.5 c.c. + " " 0.6c.c. + " 0.04c.c. = "
Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete
Dose of Complement = 0.015 c.c.

B.

Normal Serum 55°C., ½ hour	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement 0.04 c.c.
0.05 c.c.	L.	Just complete
0.05 c.c.	L-C	" "
0.15 c.c.	L	" "
0.15 c.c.	L-C	" "
0.3 c.c.	L	" "
0.3 c.c.	L-C	" "

CONTROLS

are same as above.

TABLE 4.

A.

Serum 57°C	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement. 0.02 c.c.	0.04 c.c.	0.07 c.c.	0.I c.c.	0.I4 c.c.
0.05cc	L.	Very Marked	Complete	Complete	Complete	Complete
0.05cc	L-C.	Marked	Very marked	Almost complete	Just complete	"
0.2 cc	L.	Marked	Almost complete	Complete	Complete	Complete
0.2 cc	L-C.	Distinct	Marked	Very Marked	Almost "	"
0.5 cc	L.	...	Very marked	Complete	Complete	Complete
0.5 cc	L-C.	...	Marked	Almost "	"	"
C.S.Fluid 0.Icc	L.	Faint trace	Marked	Almost complete	Complete	Complete
" 0.Icc	L.C.	0	Very faint trace	Trace	M arked	Almost complete

CONTROLS.

Serum 0.05c.c. + NaCL solution 0.6c.c. + Complement 0.03c,c.=Just complete
" 0.2 c.c. + " " 0.6c.c. + " 0.03c.c.=Very marked
" 0.5 c.c. + " " 0.6c.c. + " 0.04c.c.= " "
C.S.Fluid 0.Ic.c. + " " 0.6c.c. + " 0.02c.c.=Complete
Emulsions 0.6 c.c. + Complement 0.06 c.c. = Complete
Dose of Complement = 0.0I5 C.C.

B.

Serum 57°C	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement. 0.04 c.c.	0.08 c.c.	0.I2 c.c.	0.I7 c.c.
0.05cc	L.	Very marked	Just complete	Complete	Complete
0.05cc	L-C.	Distinct	Very marked	"	"
0.05cc	C.E.	Trace	Marked	Very marked	"
0.2 cc	L.	Marked	Just complete	Complete	Complete
0.2 cc	L-C.	"	Almost "	Just complete	"
0.2 cc	C.E.	Trace	Marked	Complete	"
0.5 cc	L.	Marked	Complete	Complete	Complete
0.5 cc	L-C.	"	"	"	"
0.5 cc	C.E.	Trace	Marked	"	"

CONTROLS.

Serum 0.05c.c. + NaCL solution 0.6c.c. + Complement 0.04 c.c. = Complete
" 0.2 c.c. + " " 0.6c.c. + " 0.04 c.c. = Just "
" 0.5 c.c. + " " 0.6c.c. + " 0.04 c.c. = Trace
Emulsions (L & L-C-) 0.6 c.c. + Complement 0.04 c.c. = Complete
(Crude Extract) 0.6 c.c. + " 0.07 c.c. = Almost "
Complement dose = 0.02 c.c.

TABLE 5

Normal Serum 57°C	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement.					NaCl Solution 0.6c.c. + Complem.
		0.04 c.c.	0.07 c.c.				0.04c.c.
(0.05cc	L	Just complete	Complete				Complete
(0.05cc	L-C	Almost "	"				Complete
(0.2 cc	L	Just complete	Complete				Complete
(0.2 cc	L-C	" "	"				"
(0.5 cc	L	" "	"				"
(0.5 cc	L-C	Almost "	"				"
(0.05cc	L	Almost complete	Complete				Complete
(0.05cc	L-C	Very marked	"				"
(0.2 cc	L	Almost complete	"				"
(0.2 cc	L-C	Just "	"				"
(0.5 cc	L	Almost "	"				"
(0.5 cc	L-C	" "	"				"
(0.05cc	L	Almost complete	Complete				Complete
(0.05cc	L-C	" "	"				"
(0.2 cc	L	" "	Just "				"
(0.2 cc	L-C	" "	" "				"
(0.5 cc	L	" "	Complete				"
(0.5 cc	L-C	" "	"				"
Syphilitic Serum		0.04 c.c.	0.07 c.c.	0.II c.c.	0.I8 c.c.	0.26 c.c.	0.04c.c.
(0.05cc	L	Marked	Just Complete	Complete			Complete
(0.05cc	L-C	0	Trace	Distinct	Marked	Almost complete	"
(0.2 cc	L	Marked	Just complete	Complete			"
(0.2 cc	L-C	Trace	Distinct	Distinct+	Marked	" "	"
(0.5 cc	L	Almost complete	Complete				"
(0.5 cc	L-C	Distinct	Marked	Marked+	Very "	Complete	"

Emulsions 0.6 c.c. + Complement 0.04 c.c. = Complete

Dose of Complement = 0.0I5 c.c.

TABLE 6.

Normal Serum 57°C.	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement.			NaCl solution 0.6 c.c. + Complement
		0.04 c.c.	0.07 c.c.	0.II c.c.	0.04 c.c.
A (0.05c.c.	L.	Marked	Almost complete	Complete	Complete
(0.05c.c.	L-C.	Distinct	" "	"	"
(0.I5c.c.	L.	Almost complete	Complete	Complete	Complete
(0.I5c.c.	L-C.	Just "	"	"	
(0.3 c.c.	L.	Just "	Complete	Complete	Complete
(0.3 c.c.	L-C.	" "	"	"	
B (0.05c.c.	L.	Trace	Marked	Just complete	Complete
(0.05c.c.	L-C.	Very faint "	"	" "	
(0.I5c.c.	L.	Trace	Marked	Just complete	Complete
(0.I5c.c.	L-C.	0	Trace	Complete	
(0.3 c.c.	L.	Very marked	Complete	Complete	Complete
(0.3 c.c.	L-C.	Distinct	Very marked	"	
(0.5 c.c.	L.	Marked	Complete	Complete	Complete
(0.5 c.c.	L-C.	"	"	"	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete.

Dose of Complement = 0.0I25 c.c.

TABLE 7.

Syphilitic Serum 47°C.	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement. 0.04 cc	0.07 cc	0.1 cc	0.14 cc	0.2 cc
0.01 c.c.	L	Distinct	Very marked	Just complete	Complete	Complete
0.01 c.c.	L-C	Faint trace	Distinct	Marked	Almost complete	"
		0.1 cc	0.14 cc	0.17 cc	0.23 cc	0.31 cc
0.05 c.c.	L	Almost complete	Complete	...	...	...
0.05 c.c.	L-C		Trace	Distinct	Just complete	Complete
0.5 c.c.	L	Distinct	Marked	...	...	...
0.5 c.c.	L-C		Trace	Distinct	Very marked	Complete

CONTROLS.

Serum 0.01c.c. + NaCL solution 0.6c.c. + Complement 0.04c.c. = Complete

" 0.05c.c. + " " 0.6c.c. + " 0.04c.c. = Very marked

" 0.5 c.c. + " " 0.6c.c. + " 0.08c.c. = Marked

Emulsions 0.6 c.c. + Complement 0.05 c.c. = Complete

Dose of Complement = 0.02 c.c.

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TABLE 8

A

Syphilitic Serum. 57°C	Emulsions 0.6 c.c	Amount of Guinea-pig's Complement					NaCL solution 0.6cc + complement
		0.08 c.c.	0.I2cc	0.I7 cc	0.23 cc	0.3Icc	0.03 c.c.
0.0I cc	L.	Complete	...	...	...	...	Complete
0.0I cc	L-C.	...	Very marked	Complete	...	...	
0.05 cc	L.	Complete	...	...	...	...	Complete
0.05 cc	L-C.	...	0	Trace	Marked	Almost complete	
0.5 cc	L.	Almost complete	Complete	...	...	...	Trace
0.5 cc	L-C.	...	...	Marked	Almost complete	Complete	

Emulsions 0.06 c.c. + Complement 0.02 c.c. = Complete
Dose of Complement = 0.0I c.c.

B

Syphilitic serum 57°C	Emulsions 0.6 c.c.	Amount of Guinea-pig's Complement.					
		0.04 cc	0.07 cc	0.I cc	0.I4 cc	0.2 cc.	
0.0I cc	L.	Marked	Just complete	Complete	...	...	
0.0I cc	L-C	0	0	Veryfaint trace	Faint trace	Marked	
		0.04 cc	0.07 cc	0.I cc	0.I4 cc	0.I7cc	0.23 cc
0.05 cc	L.	Distinct	Very marked	Complete	...	...	...
0.05 cc	L-C.	0	0	0	0	0	Very faint trace
0.5 cc	L.	0	Very faint trace	Faint trace	Marked	...	...
0.5 cc	L-C.	0	0	0	0	Faint trace	Very/ Trace

C O N T R O L S.

Serum 0.0I cc.+ NaCL solution 0.6cc + Complement 0.04 cc = Complete.
" 0.05 cc.+ " " 0.6cc + " 0.04 cc = Very marked.
" 0.5 cc.+ " " 0.6cc + " 0.08 cc = Faint trace.

C

Syphil.c Serum 57° C	Emulsions 0.6 c.c	Amount of Guinea-pig's Complement						NaCl Sol. 0.6cc + Complement
		0.07 cc	0.I cc	0.I4 cc	0.2 cc	0.28cc	0.38cc	0.05 cc
0.05 cc	C. E.	Trace	Distinct	Marked	Marked+	Very marked	Complete	Complete
0.2 cc	C. E.	"	"	"	Very marked	Complete	"	"
0.5 cc	C. E.	Very marked	Almost complete	Complete	...	...	...	Very marked

Emulsion 06 c.c + Complement 0.07 C.c. = Marked
Complement dose = 0.0I5 c.c.

TABLE 9

Guinea pig's Complement 0.2c.c.	Emulsions 0.6 c.c.	Amounts of Syphilitic Serum (57°C)				
		0.025cc.	0.05 cc.	0.I cc.	0.2 cc.	0.4c.c.
Compl. I	L.	Complete	Complete	Complete	Complete	Complete
	L-C.	"	"	Very marked	Marked	0
Compl.II	L.	Complete	Complete	Complete	Complete	Complete
	L-C.	Just "	Marked	0	0	0
Complement 0.04 c.c	NaCL Solution 0.6 c.c.		Serum as	above		
Compl. I	NaCL Solution 0.6 c.c.	Almost complete	Just complete	Almost complete	Very marked	Distinct
Compl.II	" "	Complete	Complete	Just complete	Just complete	Very marked

Emulsions 0.6 c.c. + Complement I 0.03 c.c. = Complete.

" 0.6 c.c. + " II 0.03 c.c. = Complete.

//////////////////////

T A B L E IO

Guinea pig's Complement 0.2 c.c.	Emulsions 0.6c.c.	Amount of Syphilitic Serum (57°C) 0.025cc.	0.05 cc.	0.I c.c.	0.2 c.c.	0.4 c.c.
Compl. I	L.	...	...	Almost complete	Marked	Complete
	L-C.	Just complete	Trace	0	0	0
	C.E.	" "	Distinct	Trace	0	0
Compl. II	L.	...	...	Distinct	Trace	Complete
	L- C.	Just complete	Faint trace	0	0	0
	C.E.	Marked	Distinct	Very faint trace	Faint trace	0
Complement 0.04 c.c.	NaCL sol^{n} 0.6c.c.	Serum as above.				
Compl. I	NaCL sol^{n} 0.6c.c.	Complete	Complete	Complete	Complete	Complete
Compl. II	" "	"	"	"	"	"

Emulsions (L. / L-C.) 0.6 c.c. + Complement 0.03 c.c. I Complete, II Complete

Emulsions (Crude Extract) 0.6c.c. + Complement 0.07c.c. I Almost complete. II Almost "

Dose of Complement I 0.0I25 c.c. II 0.0I5 c.c.

//////////////////////

T A B L E XI

A.

Guinea-pig's Complement 0.2 c.c.	Emulsions 0.6 cc	Amount of syphilitic Serum (57°C) 0.025cc	0.05cc	0.1cc	0.2 cc	0.4cc	Serum Control NaCL Soln. 0.6cc. Serum 0.2cc Compl.t 0.04cc	Serum 0.4cc Compl.t 0.05cc
M'G.								
Compl. I	L.	...	...	Just Complete	Complete	Complete	Complete	Just complete
	L-C.	Complete	Complete	Comple.	"	"	...	...
Compl.II	L.	...	...	"	"	"	Complete	Complete
	L-C.	Complete	Complete	"	"	"	...	...
Compl.III	L.	...	...	"	"	"	Compl.te	Complete
	L-C.	Complete	Complete	"	"	"	...	...
K.								
Compl. I	L.	...	...	0	0	0	Complete	Marked
	L-C.	0	0	0	0	0	...	...
Compl. II	L.	...	...	Trace	0	0	Complete	Marked
	L-C.	0	0	0	0	0	...	...
Compl.III	L.	...	...	0	0	0	Complete	Marked
	L-C.	0	0	0	0	0	...	...
L.								
Compl. I	L.	...	...	Complete	Complete	Compl.te	Complete	Complete
	L-C.	Complete	Complete	"	Faint trace	0	...	...
Compl. II	L.	...	...	"	Complete	Compl.te	Complete	Complete
	L-C.	Complete	Complete	Marked	0	0	...	...
Compl.III	L.	...	...	Complete	Compl.te	Compl.te	Complete	Almost complete
	L-C	Complete	Complete	"	Marked	Trace		

Emulsions 0.6c.c. + Complement 0.03 c.c. = Complete

Doses of Complement I = 0.01c.c. II = 0.01c.c. III = 0.01c.c.

B.

Serum 57°C	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement 0.04c.c.	0.07 c.c.	0.11 c.c.	0.16c.c.	NaCl Solution 0.6 c.c. + Complement 0.04 c.c.
M'G						
0.05cc	L.	Almost complete	Complete	...	...	Complete
0.05cc	L-C	0	Distinct	Marked	Just complete	...

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.01 C.c.

Wechselmann's Method of converting a negative into a positive reaction.

Wechselmann claims that, in cases of secondary syphilis, where the serum reacts negatively, a positive result may be obtained by previously treating the serum with freshly precipitated barium sulphate. His method is as follows:-

> 0.9cc. of the heated serum plus 3cc. of 0.85% NaCl solution plus 0.5cc. of a 7% suspension of freshly precipitated barium sulphate are shaken up and kept for one hour at 37°C. Thereafter the whole is centrifugalized and the serum pipetted off, ready for testing. He states that the phenomenon depends on the removal by the barium sulphate of inhibitory substances. Noguchi and Bronfenbrenner find that larger amounts of barium sulphate may remove the syphilitic antibody also from the serum.

Several sera from cases of general paralysis have been examined by this method. The barium suphate was precipitated from a solution of barium chloride by sulphuric acid and afterwards washed free of acid. Numerous washings were essential before this could be accomplished but the total absence of acidity is essential since it has been shown by Sachs and Altmann that the addition of acid ($\frac{I}{1000}$ to $\frac{I}{2000}$ $\frac{N}{I}$ HCl) to serum increases it power to absorb complement along with "antigen". However, with the sera tested, it was not found that this treatment caused any alteration in their Wassermann effect. Table I. shows the results obtained from a serum treated with barium sulphate. It was examined in different amounts by both the L-C. and C-E. methods and was controlled by the untreated serum, similarly examined. The case was one of general paralysis whose serum at various times gave either a negative or very weak positive result. Practically no difference is shown in the results by the treated and untreated portions of the serum.

References.

Wechselmann, Zeitschr.f.Immunitats forsch., Bd., 3 1909, p.525.

Noguchi & Bronfenbrenner, Journ. of Exper.Med., vol 13, 1911, p.217.

Sachs & Altmann, Berlin.klin.Woch., 1908 p.699.

TABLE I

Serum 57°C untreated	Emulsion 0.6cc	Amounts of Guinea-pig's Complement 0.02cc	0.04cc	0.08cc	0.12cc	NaCl Solution 0.6cc + Complement 0.04cc
0.05cc	L.	Marked	Very marked	Just complete	Complete	Complete
0.05cc	L-C.	Trace	Distinct	Very marked	Complete	
0.05cc	C.E.	0	Trace	Marked	Very marked	
0.2cc	L.	Distinct	Marked	Just complete	Complete	Complete
0.2cc	L-C.	Trace	Marked	Almost complete	Just complete	
0.2cc	C.E.	0	Trace	Marked	Complete	
0.5cc	L.	Trace	Marked	Complete	Complete	Complete
0.5cc	L-C.	Faint trace	Marked	Complete	Complete	
0.5cc	C.E.	0	Trace	Marked	Complete	
Serum 57°C treated						
0.05cc	L.	Marked	Very marked	Complete	Complete	Complete
0.05cc	L-C.	Trace	Marked	Almost complete	Complete	
0.05cc	C.E.	0	Trace	Marked	Just complete	
Equivalent of 0.2cc	L.	Trace	Marked	Complete	Complete	Almost complete
0.2cc	L-C.	Trace	Distinct	Almost complete	Complete	
0.2cc	C.E.	Faint trace	Distinct	Just complete	Complete	
0.5cc	L.	0	Distinct	Complete	Complete	Very marked
0.5cc	L-C.	0	Trace	Almost complete	Complete	
0.5cc	C.E.	0	Trace	Just complete	Complete	

Emulsions 0.6cc (L.&) (L-C) + Complement 0.04cc = Complete

" 0.6cc (C.E) + " 0.07cc = Almost complete

Dose of Complement - 0.02cc.

PART 2

CLINICAL APPLICATION OF THE TEST

In the course of an investigation into the Wassermann reaction in General Paralysis of the Insane, I was struck by the large percentage of positive results amongst control cases from other forms of insanity. These gave 16% positive results out of 35 cases, although no evidence of syphilis could be detected in them. Accordingly it was decided to continue the investigation with a large number of asylum patients. Altogether the blood serum in 423 cases has now been examined and these include all the various psychoses met with in asylum work.

In the first series the cases were all examined by both the lecithin-cholesterin and crude alcoholic liver extract methods, but in the 2nd series the lecithin-cholesterin method had alone been used, the delicacy and accuracy of this method having been fully established.

GENERAL PARALYSIS.

The sera of 94 general paralytics (77 males and 17 females, mostly from Gartloch Asylum, but partly from other Glasgow Asylums) have been examined and have, with the exception of one male, reacted positively = practically 99%. This is higher than on the previous occasion and is to be explained by the fact that certain sera react anomalously, i.e., are negative at one time and subsequently positive and again one case (H.D.) previously included is not now considered to be a general paralytic.

H.D. Male aet.54. First admitted in July, 1908.

History.

He had been having "fits" for about 6 months prior to admission; at times he was violent and dangerous. He had been drinking heavily for some time.

On Admission

He was in a state of confusion succeeding a series of "fits", from which he gradually emerged. His speech was slow and hesitating, there was tremours of tongue and facial muscles. Pupils small and equal, reacted to accommodation, but very sluggishly to light. There were no fits during his residence in hospital. Discharged recovered 4/5/09

He/

He was re-admitted,2/IO/IO,with a history that he had delusions concerning his wife and daughter,to whom he threatened violence. He was sleepless and very irritable. He had not had more fits. On admission,very little couldbe detected pointing to insanity. There was blurring of the finer judgment,failure to appreciate the full significance of his circumstances. No deficiency of memory.

There is still slight tremor of the lips on speaking but none of the tongue. The speech is distinct but somewhat slow,not,however,hesitating. There is no difficulty in pronouncing the usual test words. Knee jerks present and not exaggerated,gait normal,pupils small but react well to light and accommodation. Blood serum negative to Wassermann reaction;spinal fluid not examined. He admits having been a heavy drinker previous to first admission,but very frankly denied ever having been exposed to syphilitic infection. There is no doubt,both from his own statements and from the statements of his relatives,that he has strong cause for resentment against his wife.

The serum of the case of general paralysis which reacted negatively,was repeatedly examined but always with the same result,but the spinal fluid never failed to give a positive reaction though tested on many occasions. About a fortnight before death, this patient's cerebro-spinal fluid was straw-coloured and contained a large amount of albumen,the appearances suggesting an admixture with blood plasma,but even then it gave a powerful reaction whereas the blood serum,tested at the same time,was negative. In this case the diagnosis was confirmed by the post-mortem examination,the following lesions being found.

There was thickening and opacity of the pia-arachnoid which was adherent to the cerebral cortex. The vertebral artery on each side was thickened and a patch of atheroma occurred on the right middle cerebral near its origin. The other vessels of the base were healthy. The brain showed considerable wasting. The grey matter was thinned and slightly congested and its markings were very indistinct. There was a small softening of old standing in the white matter close to the cortex in the right parietal lobe. The ependyma of the ventricles was thickened but not granular. Sections of the cerebral cortex showed distinct subpial felting and all the vessel walls were markedly infiltrated with lymphocytes and plasma cells. No syphilitic lesions were found in any of the other organs. This case is of particular interest as showing that the spinal fluid may react positively when the blood serum is negative in the Wassermann reaction.

The cerebro-spinal fluids of 33 general paralytics have been tested with a resulting positive reaction in 31 cases = practically 94%. Of these cases both the blood and spinal fluid were positive in 30 instances, the blood serum alone in 2 and the spinal fluid alone ~~fifty~~ in I. Thus out of 93 cases of general paralysis, if the reaction with both blood and spinal fluid be considered, 100% have given a positive Wassermann reaction. It should be noted in the cases whose spinal fluids reacted negatively that each of them was examined on only one occasion and for the test 0.1cc. was used instead of 0.5c.c. as is now recommended.

Various opinions are expressed as to whether or not the Wassermann reaction in general paralysis is stronger with the blood or the spinal fluid. I do not think that any definite opinion can be expressed on this point; in many cases the spinal fluid deviates more complement than does the serum, whereas in other cases the reverse is found; again the serum may react positively when the spinal fluid is negative.

One case has already been referred to where the serum was negative throughout whilst the spinal fluid was strongly positive; in addition other 2 cases have behaved somewhat similarly, for in both of them the blood serum at first was quite negative but later gave a weak though quite definitely positive reaction, whereas the spinal fluid was positive from the beginning. These 2 cases were undoubtedly general paralytics and in one of them a post mortem examination revealed the lesions characteristic of that disease. But no true comparison can be made between the blood and spinal fluid as regards their behaviour in the Wassermann test, since the blood is heated to 57°C for ½ an hour, whereas the other fluid is used unheated and again only 0.05c.c. of blood serum is used as against 0.5c.c. of spinal fluid.

The above results in general paralysis are pretty much in agreement with those of other workers. Thus Boas (2) reports 100% positive reactions with the sera of 139 cases; Plaut (3) and Lesser (4) give a similar percentage whilst practically all workers now report over 90% of positive results with the serum in cases of

general paralysis. As regards the spinal fluid, Wassermann and Plaut (5) obtained 41 positive reactions out of 54 cases; Marie and Levaditi (6) in 29 cases out of 39 cases; Marie, Levaditi and Yamanouchi (7) in 28 out of 30 cases; Henderson, Smith and Candler (8) in 59 out of 64 cases; Boas (2) 61 out of 67 cases; Plaut (3) in 95% out of 200 cases; Henderson (9) gives 90 % positive results from 53 cases; Candler (10) in 67 out of 69 cases.

In General Paralysis of the Insane one looks for a positive Wassermann reaction with the blood and spinal fluid in practically all cases and thus it comes to have a very high value as a diagnostic agent in this disease. The occurrence of a positive reaction with the spinal fluid is now accepted as strong evidence in favour of the case being one of paralytic dementia and in this respect I have found it invaluable. Several cases whose fluids I examined and found positive were then for the first time suspected to be general paralytics and further clinical observation confirmed this suspicion.

But the reaction in the serum and spinal fluids is equally valuable in the reverse direction and has shown conclusively that a diagnosis of general paralysis cannot be made on mental symptoms alone in the absence of physical signs.

I have met 4 such cases, all exhibiting the grandiose delusions of facile manner and other mental phenomena so typical of general paralysis, but not giving any of the physical signs, - pupillary phenomena, tremours and alterations of reflexes - in which the diagnosis of general paralysis was only finally dismissed when the Wassermann test proved to be negative in both blood and spinal fluid. The absence of progressive deterioration has confirmed this conclusion.

At the same time I cannot agree with the general opinion that a positive reaction on the part of the cerebro-spinal fluid is strong evidence in favour of general paralysis as opposed as to cerebral syphilis. I am firmly convinced that cases of insanity other than general paralysis, in which syphilis is the direct cause give/

give a positive reaction to the Wassermann test with the spinal fluid in the majority of cases; but this will be more fully referred to later.

Insanity other than General Paralysis.

Apart from general paralysis the serum has been examined in 329 cases of insanity, comprising 156 females and 173 males and of these 54 have reacted positively = 16.4%. Of the females, 23 are positive = 14.74% and of the males 31 = 18%.

The spinal fluid was examined in 88 cases. These are divided into 2 groups. (1) Those in which the serum reacted negatively, numbering 51 cases; in all of these the spinal fluid was negative. (2) Those in which the serum was positive, 37 in number; the spinal fluid was positive in 18 of these = 48.6%.

From the serum reaction in these cases of insanity other than general paralysis, no deduction can be drawn as regards the incidence of syphilis in the asylum population generally, because the various psychoses are too unequally represented; thus, practically all the epileptics in the asylum were examined and a large proportion of the dementia praecox cases. More accurate information on this point is available from examination of a large number of admissions taken in sequence.

From October 1911 till May 1912, 76 females were admitted and in 75 of these the blood was tested as to its effect in the Wassermann reaction. From November, 1911 till May 1912, of 56 males admitted these sera of 54 cases were tested.

Of the 75 females, 16 gave a positive reaction = 21.3%. These include 3 cases of general paralysis all of whom reacted positively, of the remainder (72 cases) 18% were positive. 35.2% of the males were according to the Wassermann test syphilitic. Of these 6 were general paralytics, all positive; 27% of the remaining 48 cases were positive.

Thus altogether 129 persons had been examined as they were admitted/

Results obtained in the Wassermann Reaction by the Blood Sera of Cases of Insanity, other than General Paralysis.

	Females		Males		Total	
	Negat.e	Posit.e	Negat.e	Posit.e	Negat.e	Posit.e
Epilepsy.	I3	3	40	5	53	8 (I3.I%)
Dementia Praecox,	I7	0	26	I	43	I (2.27%)
Delusional Insanity.	I5	2	I7	5	32	7 (I8.0%)
Manic Depressive Insanity.	I7	5	I2	4	29	9 (23.7%)
Imbeciles sine Epilepsy.	I0	2	I9	5	29	7 (I9.4%)
Imbeciles cum Epilepsy.	8	0	3	I	II	I (8.3%)
Secondary Dementia.	8	I	I0	7	I8	8 (30.8%)
Senile Insanity.	I0	I	3	0	I3	I (7.0%)
Melancholia.	II	2	3	I	I4	3 (I7.7%)
Alcoholic Insanity.	7	5	0	0	7	5 (4I.6%)
Confusional Insanity.	9	I	3	I	I2	2 (I4.3%)
Mania.	4	I	3	I	7	2 (22.2%)
Hysteria.	2	0	I	0	3	0 (0.0%)
Paranoia.	0	0	2	0	2	0 (0.0%)
Puerperal Insanity.	I	0	0	0	I	0 (0 .0%)
Hypochondriacal Insanity.	I	0	0	0	I	0 (0.0%)

admitted to the asylum; 9 of these were cases of general paralysis; all gave a positive Wassermann reaction; of the remaining 120 cases, 22.6% were positive.

These results in cases of mental disorder, other than general paralysis, show a proportion of syphilitic cases very much greater than what is generally supposed to exist.

But I find than Dr Stansfield in the annual report of the Bexley Asylum 1907, states that in 37% of the males and in 11% of the female admissions, syphilis was the principal etiological factor; according to the Cheddleton Asylum (Staffordshire) annual report for 1903, Dr Menzies found evidence of syphilis in 28% out of 106 male admissions. In both cases these results were arrived at altogether apart from the biological serum test.

Practically all other asylum reports, however, give a very much lower percentage of luetic cases.

The incidence of syphilis amongst mental patients may of course differ widely in different asylums according to the class of people from whom the inmates are principally drawn, especially as regards women. Thus in Gartloch and Bexley and to a large extent in Cheddleton, the patients are drawn from urban districts where syphilis is known to be prevalent. At the same time the figures quoted above reveal a proportion of syphilis presumably much higher than what obtains amongst the general inhabitants of such urban districts and suggests that in insanity, syphilis plays a part the importance of which has not hitherto been fully appreciated. The occurrence of syphilis in more than one third of the male and one fifth of the female admissions places it in the forefront as an etiological factor, either primary or secondary in the causation of ~~syphilis~~.

As regards the incidence of syphilis as determined by the Wassermann reaction, in the individual psychoses, the following results have been reported.

Boas and Neve (11) got negative results in all of 20 cases of epilepsy.

Roubinovitch and Levaditi (12) examined 15 cases of dementia

praecox and obtained a positive result in 3 with the blood serum. The spinal fluids of all reacted negatively.

Marie (I3) found IO cases of epilepsy and I4 of dementia praecox all negative.

Morton (I4) with the spinal fluid got negative results in 30 cases of epilepsy and dementia praecox.

Dean (I5) obtained I5.4% of positive result with the sera of 330 cases of idiocy and got I positive spinal fluid out of I2 examined.

Henderson (9) gives the following results;
I6 cases of cerebral syphilis,94% positive with the serum and 50% with the spinal fluid.
I4 cases of alcoholic psychosis,3 reacted positively with the spinal fluid.
II cases,comprising cases of dementia praecox,manic-depressive insanity and constitutional inferiority,were all negative.

Scott Williamson (I6) examined the spinal fluids in 22 cases of insanity other than general paralysis and found all negative.

W.Muirhead (I7) with one exception got the serum and spinal fluid negative in 77 cases of insanity other than general paralysis.

Schölberg and Goodall (I8) have examined a large number of cases but so many of these have given variable reactions on different occasions that their results need not be quoted.

As regards my own results,it is apparent that in dementia praecox syphilis is exceedingly rare; thus,out of a series of 44 cases only one reacted positively,the reaction being given with blood and spinal fluid.

D.A. This was a typical case of dementia praecox and a careful examination after the result of the Wassermann reaction was known revealed no reason to alter the diagnosis. Death resulted from a cardiac collapse but no post mortem was granted.

In 6I cases of epilepsy,8 sera reacted positively (I3.I%). In 6 of these 8 positive cases,the spinal fluid was examined and found positive in 3 and negative in 3. The 3 cases of epilepsy which react positively with both blood and spinal fluid present the following clinical features.

K.C. Female,aet.44 years. Admitted to Hartwood Asylum in November,I908 and transferred to Gartloch in March,I909.

On admission. Mentation slow but memory and judgment fair. Free from hallucinations but knows that she has heard voices, and acknowledges she is an epileptic.

Physical condition. Gait somewhat ataxic, slight Rombergism, deep reflexes cannot be elicited, speech slow but distinct, no tremors of tongue or face, looks much older than 44 years. At the time of fits she becomes hallucinated, quarrelsome and dangerous to others; also her motor power becomes much impaired. She herself states that she got syphilis from her second husband and that the fits commenced after her third marriage. At times she has had gastric crises.
This case is considered to be one of late epilepsy with locomotor ataxia.
Blood and spinal fluid react positively.

A.L. Male, aet. 50 years. Admitted from Barnhill Poorhouse November, 1904.

No history obtainable.
He suffers from typical epileptic fits and is now very demented. He is quite memoryless and unable to understand the simplest questions.
His pupillary and deep reflexes are normal.
Blood and spinal fluid react positively.

The wife of this patient is also an inmate of this asylum. She was admitted to Hawkhead Asylum in December 1906 and transferred to Gartloch in February, 1907.
On admission, she was an excited, restless maniac, but has now passed into a state of apathy.
Physically she presents no signs of disease but her face is slightly asymmetrical, palate narrow, teeth crowded and forehead flat.
Blood reacts positively and the spinal fluid negatively.

J.G. Male, aet. 32 years. Admitted August, 1910.

Mental condition. He takes typical grand-mal fits with succeeding confusion. Hallucinations and delusions were prominent before the onset of dementia.
Physical condition. There is a marked atrophy of the deltoid, flexors of forearm, and interossei on the right side; on the left side, the extensors of the forearm, the interossei and muscles of the thenar eminence are atrophied. There is wrist drop on both sides but more marked on the left. Fibrillary tremors have been noted in the atrophied muscles of the hands.
This patient's blood reacted negatively on the first three examinations extending over a period of 2 months, whereas the spinal fluid was positive each time. The blood and spinal fluid were both positive when tested again 8 months later. Previous to the third examination of the blood and spinal fluid, he received an intramuscular injection of Salvarsan, and a second injection (intravenous) before the fourth examination. The dementia and muscular atrophy, however, are still progressing.

Delusional Insanity. Out of 39 cases, 7 gave positive results with the blood serum (18%). The spinal fluid, examined in 4 of the 7 cases, was negative in all.

Manic-Depressive Insanity. Out of 38 cases, 9 gave a positive result with the blood serum (23.7%). The spinal fluid, examined in 6 of these cases, reacted positively in 3 of them and negatively in 3

H.P. Female, aet. 20 years. Admitted December, 1911.

History. She had become unsettled over three weeks before admission. She had not done well at school but afterwards had earned her trade's wages. Said to have been always a good, healthy, and moral girl.
Mental condition. She mistakes identities and cannot give any account of herself. At times she is restless and appears exalted.
Physical condition. Healthy looking but features are not intellectual. Looks younger than age stated. Palate deformed anteriorly, face somewhat asymmetrical, nose somewhat thickened and flattened. Reflexes all present; no clinical evidence of syphilis.
Wassermann reaction positive in the blood and spinal fluid.
She is now well mentally except at menstrual periods.

W.F. Male, aet. 40 years. Admitted December, 1911.

Mental condition. He is at times depressed and morbid, with delusions of persecution and at other times excited, talkative and cheerful.
Physical condition. Pupils react normally; deep reflexes normal; no tremors of face or tongue.
Wassermann reaction positive with blood and spinal fluid.

H.M'B. Male, aet. 38 years. Admitted December, 1911.

Mentally, he varies between states of depression and exaltation. Has very vivid hallucinations and delusions.
Physically no abnormal signs are noted.
The Wassermann reaction is positive with both blood and spinal fluid.

Imbeciles without epilepsy. 36 cases of which 7 were positive with the blood serum (19.4%). The spinal fluids were examined in 5 of the positive cases and of these 2 were negative and 3 positive.

M.W. Male, aet. 26 years. Admitted March, 1909.

Mental condition. This patient is a mental defect; he is childish, foolish in manner and speech, and unable to apply himself to any kind of work.
Physical condition. Apart from a squint in the left eye, he presents no abnormal physical signs.
Blood and spinal fluid react positively in the Wassermann test.

A.M'I. Female. aet 41 years. Admitted March, 1911.

Mental condition. She appears to be an imbecile; she is excited, restless, and cannot appreciate her surroundings; memory is fair.
Physical condition. Her pupils are irregular in outline but react well to light and accommodation; knee jerks active ankle clonus elicited.

History. She did not walk till the age of 7 years and was never at school. Her mother died at the age of 54 years, supposed cause phthisis. Her father died age 33 years, cause unknown; he had been a heavy drinker. Three of patient's brothers died in infancy. One brother, grown up, was a heavy drinker and died of pneumonia. A sister drinks heavily.
Blood and spinal fluid react positively.

F.S. Male, aet 22 years. Admitted February, 1912.

Mentally defective.
Physical condition. There is marked tremor of the tongue with hesitating speech. Knee jerks are active; there is no ataxia. Pupils are equal and react slowly to light and accommodation; consensual reflex present. The Wassermann reaction is positive with the blood and spinal fluid. The diagnosis of juvenile general paralysis in this case is not definitely excluded.

Imbeciles with epilepsy. 11 cases of which one only reacted positively with the blood serum. His spinal fluid was also positive.

D.M'N. Male, aet 19 years. Admitted November, 1910.

Mental condition. This patient is deficient mentally and is subject to epileptic fits.
Physical condition. He has a saddle-shaped nose but no scars about the corners of the mouth nor Hutchinsonian teeth. There is paresis of the left arm and leg.
The blood serum and spinal fluid react positively.

Secondary dementia. Out of 26 cases, 8 gave a positive serum reaction (30.8%); 3 of these gave a positive result with the spinal fluid, one was negative and 4 were not examined.

J.L. Male, aet 46 years. Admitted August, 1911.

This patient was admitted in a condition of marked dementia due to organic brain disease.
Blood and spinal fluid reacted positively.
The diagnosis was confirmed by post mortem examination; the brain was much atrophied and there was extensive syphilitic disease of the cerebral arteries; there was nothing pointing to general paralysis.

W.W. Male, aet. 66 years. Admitted March, 1911.

Mental Condition. He has delusions of grandeur. His memory for old events is fairly good but for recent events is almost entirely lost. He is simple, facile, and in many respects childish.
Physical condition. The pupils are equal and react normally. His knee jerks are active. Speech is good and there is no tremor of the face or tongue.
This patient is now bedridden and there is considerable dementia.
The blood and spinal fluid react positively.

G.F. Male, aet. 60 years. Admitted October, 1911.

A restless, resistive and demented old man. He has been blind for the past five years from optic atrophy. There are no muscular tremors, and the deep reflexes are active.
The Wassermann reaction is positive with the blood serum and spinal fluid.

Senile Insanity.

Out of 14 cases, one gave a positive reaction with the blood, whilst the spinal fluid reacted negatively.

Melancholia.

17 cases were examined, of which 3 reacted positively with the serum. Only one spinal fluid was examined and that proved negative.

Alcoholic Insanity.

In this class 12 females were examined. Five sera reacted positively, whilst in 3 spinal fluid was negative and in the other 2 positive.

L.B. Female, aet. 49 years. Admitted January, 1912.

On admission. She was restless, excitable with tendency to chatter and scold. Her memory seemed good but could not be properly estimated. Refused to give any information regarding herself.
Physical condition. Her tongue was tremulous, pupils equal and reacted to light and accommodation. Deep reflexes exaggerated; speech good; no ataxia.
Progress. Has become more grandiose, mixes identities and is very fanciful and often noisy.
History. She was in Duke Street Hospital for 12 days prior to her admission to Gartloch and was regarded as a case of delirium tremens. She is reported by the police to be alcoholic. She has no friends.
Wassermann reaction is positive with both blood serum and spinal fluid.

H.G. Female, aet. 32 years. Admitted August, 1911.

On admission. She was childish but erotic, noisy and excited.
Physical condition. She was clean; her face suggested poor mentation; She was hyperaesthetic to abdominal palpation. The eye reflexes are present, the knee jerks active and there are no tremors nor slurring of speech.
Progress. She became destructive, dirty, and irresponsible, resembling a case of toxic insanity. The Wassermann reaction was positive with the blood and spinal fluid. On December 1st, 1911, she received an intravenous injection of 0.6 grammes of Salvarsan, after which her mental condition gradually improved. She became helpful in the ward and was no longer incoherent but still mixed identities. On May 1st, 1912, the Wassermann

reaction was still positive and there was no further mental improvement.

Confusional Insanity.

2 out of I4 cases gave a positive reaction with the blood serum. One of these also gave a positive result with the spinal fluid. This case is of particular interest in view of the post mortem findings and warrants a full desription.

M.N. Female, aet.43 years. Admitted August, I9II, to Murthly Asylum, and transferred to Gartloch Asylum in September, I9II.

On admission. She was excited and suspicious. Her memory was fair. There were no glandular enlargements; the pupils reacted normally; there were no tremors of the tongue and facial muscles; speech was umimpaired and the patellar reflexes were active.

Progress. Five days after admission, she became confused and memoryless. Afterwards she varied between states of confused torpor, restless semi-delirium and agitated suspicion. The Wassermann reaction was positive in both blood and spinal fluid. She gradually became more and more emaciated and died February, I9I2, despite artificial feeding.

History. She was married in I895 and has had no children. Her husband gives a frank denial of syphilis; her sister hints at some disease which the patient had before marriage, about which the husband could get no information.

Post Mortem Examination. The membranes, sinuses and vessels at the base were normal. The brain weighed I,298 grms. It was of normal size and complexity, firm and showed no wasting. The cortex was of natural colour, slightly congested and not narrowed; Its striations were readily distinguished. No abnormality could be detected in other parts of the brain on naked eye examination. Microscopic sections revealed nothing but alterations in the cortical pyramidal cells.
In the aorta there were a few patches of early atheroma. There was a subacute nephritis. The other organs were healhty.
Death was due practically to inanition.

Mania

9 cases of which one reacted positively with the blood serum and negatively with the spinal fluid, whilst another was positive with both fluids.

J.K. Male, aet.46 years. Admitted August, I9II.

Chronic mania. The presence of a double optic atrophy suggested syphilis as the cause of the mania. The knee jerks were active and there was no affection of the speech or tremors of the tongue and facial muscles. The blood and spinal fluid reacted positively.

A.H.A. Female, aet.41 years.

She was first admitted in September,1901,and was regarded as a case of alcoholic mania on admission. She was known to have led an immoral life. In October,1901 she developed a secondary syphilitic rash and received very thorough treatment with mercury and potassium iodide,and improved so markedly mentally that she was discharged in January,1902.

Re-admitted September,1904, She was treated for a month with large doses of potassium iodide and again improved so rapidly that she was discharged recovered in December,1904.

Last admission,July,1909. She was excited,violent in speech and manner,and very delusional. She now varies between periods of excitement,discontent,and comparative well-being,but is easily upset mentally. Reflexes are all normal,there is no tremor nor ataxia. During this last residence in Hospital,antisyphilitic remedies (mercury,potassium iodide,and Salvarsan) have in no way affected her mental state. The blood serum reacted positively in the Wassermann test whilst the spinal fluid (examined for the first time on 4/5/12) reacted negatively.

The appearance of the syphilitic rash during her first mental breakdown and the rapid recovery with antisyphilitic treatment suggested that this was probably a case of cerebral syphilis. At the same time it must be remembered that this patient was addicted to abuse of alcoholandenforced abstinence in hospital may also have accounted for the rapid recovery.

Lastly,a few cases of hysteria,paranoia,puerperal and hypochondriacal insanity have been examined,the blood sera of all being negative. Only one spinal fluid,from a case of hysteria,was tested and it also was negative.

Insanity associated with Cerbral Syphilis.

The relationship of syphilis to mental disease is still obscure as regards many points. On the one hand there is the parasyphilitic affection, general paralysis, a disease characterised by marked and constant anatomical lesions, and the signs and symptoms constitute a definite clinical entity.

The fact that the blood sera of general paralytics give a postive Wassermann reaction in practical 100% of cases can scarely bear any otherinterpretation than that they are subjects of active syphilitic infection and this is in agreement with Krafft-Ebing's [19] observations in which he failed to infect general paralytics with syphilitic material; for it has been repeatedly shown by experiments that reinfection with syphilis fails only so long as the body is still infected

The occurrence of a positive Wassermann reaction with the spinal fluid of practically every case of general paralysis as shown recently by perfected methods of examination, indicates further, that the cerebro-spinal system and the tissues adjacent are actively participating in the disease.

On the other hand, there is a great variety of mental conditions associated with syphilis which are included under the term cerebral syphilis; it is said that there is not a single psychosis that syphilis cannot produce. In one class of cases, gross lesions of certain structures of the brain are present and during life there are clear indications of their presence. In another class, there is no characteristic sign or symptom of syphilis clinically, and the post-mortem examination may not afford any further evidence of the existence of a syphilitic infection of the brain. Hence in this class, in the total absence of such evidence of syphilis, important information may be gained from the behaviour of the blood serum and of the cerebro-spinal fluid in the Wassermann test, as in the case M.M. described on p. 88 in which the Wassermann test alone indicated the syphilitic basis of the condition, evidence on this point being negative both clinically and at post-mortem.

Value of a Positive Reaction with the Blood Serum.

Inasmuch as a positive Wassermann reaction on the part of the blood serum merely indicates the presence of a syphilitic infection, one is not justified in assuming that there is necessarily any causal relationship between the infection and the mental state.

In the first place, the syphilitic infection may be merely coincident and play no part as an etiological factor in the existing insanity. Secondly, syphilis may act as a determining cause in a patient with a psychopathic tendency just as alcohol and physical hardship do; in this respect syphilis is probably a powerful agent in view of its wide-spread activity. Such cases, however, do not come into the category of cerebral syphilis. Lastly, syphilis may be the actual cause of the insanity, producing a true cerebral syphilis. A definite proof of the intimate relationship must be arrived at from further evidence than the serum reaction.

Value of the Wassermann Reaction in the Cerebro-spinal fluid.

The cerebro-spinal fluid is a product peculiar to the central nervous system, while the blood plasma obviously is not. It has been shown that the cerebro-spinal fluid is definitely of the nature of a specific secretion, and therefore, more information is likely to be gained from its reaction in the Wassermann test.

Plaut[3] and Boas and Lind2[0] have examined the spinal fluids in a number of cases of secondary and tertiary syphilis in which there was no evidence of involvement of the central nervous system. In all cases the spinal fluid reached negatively, although the blood serum was positive in almost every one. Hence a positive reaction with the spinal fluid may be taken as a certain indication of cerebro-spinal syphilis and its presence in an insane person would be almost conclusive evidence in favour of a diagnosis of syphilitic insanity.

However, practically all recent workers find that, in marked contrast to general paralysis, the spinal fluid in cerebral syphilis gives a positive Wassermann reaction in only a small proportion of cases. Thus Henderson reports 50% positive results with the

spinal fluid in I6 cases of cerebral syphilis,the blood serum being positive in 94%,whilst many observers (Plaut,Nonne,Gandler) state that,in this condition it is rare to find a positively reacting spinal fluid.

In view of my own results,I cannot agree with these statements.

The spinal fluid was examined in 88 cases of insanity,other than general paralysis. In 5I cases,the blood serum,previously tested,was negative and in all of these,the spinal fluid also gave a negative reaction;in 37 patients,the blood serum was positive and of these,I8 gave a positive reaction with the spinal fluid equal to 48.6%. It is noteworthy that all the cases,giving clinical evidence,apart from mental symptoms,of cerebro-spinal affection, reacted positively with both fluids; these comprised two with optic atrophy (vide pp.87,88),one with progressive muscular atrophy (vide p.84),one with locomotor ataxia (vide p.83),one with muscular palsy (vide p.86)and one with strabismus (vide p.85). The other cases,in which both blood serum and spinal fluid were positive,presented only mental symptoms,in no way characteristic of any etiological agent. On a synptomatic classification,the I9 cases,whose blood sera are positive and spinal fluids negative, represent a wide variety of psychoses. In only one of them is there any suggestion that syphilis was the direct cause of the insanity (Case A.H.A. vide p.89) and,in this case,the diagnosis is based on the fact,that symptoms of secondary syphilis developed during her first attack of mania,and the mental symptoms subsided under antisyphilitic treatment (mercury and potassium iodide). However,this patient was also an alcoholic,and the complete stoppage of the alcohol may also have been a factor in her rapid recovery. Latterly,antisyphilitic remedies (potasium iodide and 0.6 grms. salvarsan intramuscularly) have had no influence in cutting short the out-bursts of mania. The remaining I8 cases of this group present absolutely no clinical evidence of syphilitic lesions in the nervous system,and their mental

symptoms represent nine different psychoses. Now, since a positive reaction in the blood serum merely indicates the presence of a syphilitic infection, and as these cases, quoted above, were chosen at random, it seems to me there is no rational basis for considering that they are all cases of syphilitic insanity. It is evident from these statistics that, unless every case of insanity with a positively reacting serum is cerebral syphilis, then the proportion of cases, other than general paralysis, with syphilitic affection of the central nervous system which have a positive spinal fluid, must amount to much over 50%.

The fact that the spinal fluid may react positively whilst the blood serum is negative lends additional support to the view that the cerebro-spinal fluid gives a positive Wassermann reaction in a much larger proportion of cases in which the central nervous system is the seat of active syphilitic infection, than has been generally stated. Mott[21] denies that this phenomenon can occur, but it has been met with in 4 cases of my series, 3 of general paralysis and I of epilepsy with progressive muscular atrophy, (J.G. vide p.84). In 3 of these the blood serum ultimately became positive, although it only reacted weakly on repeated examination, whilst, in the fourth, a general paralytic, it remained negative throughout.

With regard to post-mortem evidence, examinations have been held in two cases in whom the blood and spinal fluid reacted positively, namely, M.N. (vide p.88) in whom no lesion at all suggestive of syphilis could be detected, and J.L. (vide p. 86) in whom syphilitic lesions of the cerebral arteries were found. None of the cases, however, which gave a positive serum and negative spinal fluid reaction, have come to post-mortem.

C O N C L U S I O N S.

1. A positive Wassermann reaction is given by both the blood and cerebro - spinal fluid in practically all cases of general paralysis.
2. Syphilis of the central nervous system, as shown by the positive Wassermann reaction of the cerebro-spinal fluid, can produce the mental symptoms of all the classified psychoses; the high proportion of syphilitics amongst asylum patients, other than general paralytics, as shown by the postive serum reaction, would indicate that syphilis is one of the most important etiological factors in the production of insanity.
3. A postive Wassermann reaction is found with the cerebro-spinal fluid in more, and probably many more, than 50% of cases of cerebral syphilis. In the majority of these cases, the diagnosis is based entirely on the result obtained in the Wassermann reaction with the cerebro - spinal fluid.
4. The post-mortem examination in one case has shown that syphiltic infection of the central nervous system may exist without the occurrence of any gross lesions of the brain (such as, gummata, syphilitic arteritis and meningitis), the diagnosis of the condition resting on the fact that the cerebro-spinal fluid reacted positively in the Wassermann test.
5. The occurrence of a positive Wassermann reaction with the cerebro-spinal fluid is of the greatest diagnostic value in differentiating general paralysis and cerebral syphilis from other froms of insanity, but is of little value in the differential diagnosis between general paralysis and other mental conditions associated with cerebral syphilis.
6. In cases with mental symptoms, a positive Wassermann reaction has been obtained with the cerebro-spinal fluid when the blood serum was negative. The reaction of this fluid is of more importance than that of the blood serum as an indication that syphilis is the causal factor of the mental disease.

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PART 3

Proteid contents of Blood Serum and their relation to the Wassermann Reaction.

Much work has been done to determine what alterations occur in the proteid contents of the blood serum as the result of a syphilitic infection and whether these alterations in any way elucidate the nature of the Wassermann Reaction. With regard to the cerebro-spinal fluid, it has been proved that, in syphilitic infections of the cerebro-spinal system such as general paralysis of the insane, there is a marked increase of the globulin content and this globulin can replace the cerebro-spinal fluid in the Wassermann test. However, no such definite statements can be made in relation to the blood serum in cases of syphilis, since different results have been obtained by different observers, although most are agreed that the globulin portion of syphilitic serum is capable of giving a positive Wassermann reaction. Thus Friedemann,[1] Noguchi,[2] Gross and Volk,[3] Landsteiner and Muller[4] & Bauer & Hirsch[5] all agree that the globulin precipitate of a syphilitic serum contains the reacting substances. However the publications of these workers suggest that they have taken little account of the precautions necessary to obtain even comparatively pure specimens of the separated protein constituents.

Normally the blood serum contains two proteids viz: serum albumin and serum globulin, and these have certain well defined characteristics which are given below in tabular form.

Serum Albumin	Serum Globulin
Soluble in distilled water	Insoluble in distilled water
Precipitated by saturation with Ammonium Sulphate	Precipitated by half-saturation with Ammonium Sulphate
Not precipitated by complete saturation with Magnesium Sulphate	Precipitated by complete saturation with Magnesium Sulphate
Not precipitated by dialysis	Precipitated by dialysis
Not precipitated by CO_2 gas.	Precipitated by CO_2 gas.

However, by none of these methods can one ensure a proper separation of the globulins from the albumins. It always happens that when an attempt is made to precipitate the globulin, some of it is retained in solution whilst at the same time some of the albumin is mechanically brought down with the globulin. Hence, it is impossible to obtain unmixed products by simple precipitation. Of these various methods of separating proteids, the most satisfactory is the process of "salting out" by means of Ammonium Sulphate, different degrees of saturation with this salt causing precipitation of different proteids; but, as already stated, the precipitated globulin contains a considerable amount of albumin while some globulin is still retained in solution. However, by redissolving the precipitate in water and again precipitating by the addition of 50% ammonium sulphate a still purer globulin is obtained, and repetition of this process will result in almost but not quite pure products.

By means of Ammonium Sulphate, serum globulin can be separated into three different constituents. Kauder[6] has shown that globulin precipitation commences when ammonium sulphate is present in 24 - 29% of complete saturation and ends when the saturation reaches 36 - 46%.

The various globulins obtained are as follows:-

(1) Fibrinoglobulin precipitated by 25% Ammonium Sulphate
(2) Euglobulin " " 33% " "
(3) Pseudoglobulin " " 46% " "

After 46% saturation, no further precipitation occurs until 64% is reached when the albumins commence to come down and this/

this ceases when 90% Ammonium Sulphate is present.

For this "salting-out" process, it is better that the serum should be well diluted since by this means the precipitate from any particular degree of saturation with ammonium sulphate is less mixed with other proteid constituents.

Technique

For the purposes of this investigation the "salting-out" process by Ammonium Sulphate has been principally utilised. A certain number of sera have also been subjected to precipitation of their globulin contents by carbonic acid gas and by dialysis.

(1) "Salting-out" by Ammonium Sulphate.

A saturated solution of ammonium sulphate was prepared by adding the salt in excess to boiling distilled water which was then allowed to cool; thereafter it was filtered to remove the ammonium sulphate which had been thrown out of solution, and finally sterilized in the Koch sterilizer for one hour or more. Kahlbaum's ammonium sulphate (guaranteed for analysis) was used, a solution of this salt being neutral to litmus.

Generally, two sera, a known negative and a known syphilitic were investigated at the same time. As a rule they were fresh, but sometimes they were heated at 57°C. for half an hour, prior to the addition of the ammonium sulphate. A small amount of serum was taken (2 or 3c.c.) and made up to 7.5c.c. with distilled water. With this diluted serum the requisite amount of saturated solution of ammonium sulphate was mixed. Usually these were mixed in bulk rapidly, the ammonium sulphate solution being put into a beaker and the serum poured into it whilst the beaker was shaken.

A number of sera were examined for fibrinoglobulin by the addition of 25% ammonium sulphate but in allcases either no precipitate or so little was obtained that the attempt to isolate this fraction was consequently given up.

3.75c.c. of the saturated solution of ammonium sulphate were placed in a beaker; with this the diluted serum was rapidly mixed and a precipitate resulted consisting mostly of euglobulin. This was centrifugalised and the supernatant fluid was decanted. The precipitate was then dissolved in 7.5 c.c. of distilled water and again precipitated and rapidly mixed with 3.75c.c. of saturated ammonium sulphate solution (33% saturation), this process being again repeated after centrifugalisation. The supernatant fluid, after each centrifugalisation, was made up to 46% saturation with ammonium sulphate by rapidly mixing it with 2.65c.c. of the saturated solution, and it was generally found that the supernatant fluid, obtained

after/

after the third centrifugalisation of the euglobulin, gave only a very faint precipitate with 46% of ammonium sulphate. But if, in any case, this third supernatant fluid was found to contain a marked amount of pseudoglobulin, then the euglobulin was again dissolved in 7.5c.c. of distilled water and again precipitated. In no case was it necessary to repeat this process a fifth time.

The supernatant fluids, obtained from centrifugalisation of the euglobulin, were added together after being made up to 46% saturation with ammonium sulphate and then centrifugalised. The pseudoglobulin thus obtained was then dissolved in 7.5c.c. of distilled water and rapidly mixed with 6.4c.c. of the saturated solution of ammonium sulphate, and again centrifugalised; then this process was once again repeated. The supernatant fluids thus obtained after removal of the pseudoglobulin were saturated by the addition of finely powdered ammonium sulphate, and, as with the euglobulin, it was generally found that the third supernatant fluid gave only a faint precipitate; but if necessary the pseudoglobulin was redissolved and again precipitated.

Throughout this treatment of the sera it was constantly observed that the euglobulin and pseudoglobulin obtained on the first addition of the requisite quantity of ammonium sulphate, were much greater in amount than on the subsequent precipitations; in other words the first products were always extremely impure and the necessity for repeated precipitation of the products was clearly indicated.

As already stated, the supernatant fluids which resulted after the removal of the pseudoglobulin were saturated with finely powdered ammonium sulphate. It was found, however, impossible to separate the precipitated albumin by means of the centrifuge on account of the lightness of the precipitate and the density of the fluid. Sometimes the greater part of it would be deposited by centrifugalisation and this could be secured, but more often, the albumin remained in the form of a fine emulsion or even rose to the surface of the fluid; therefore it had to be separated by filtration and since it could pass through filter paper, a Massen filter, measuring about four inches in length and 5/8 inch in bore, was utilised. The saturated solution was poured into this and the fluid drawn through by aspiration. Thereafter distilled water was poured into the filter, shaken to dissolve the albumin, and finally poured off. In this way, most of the albumin was recovered although naturally in all cases, some was lost.

By this method of "salting out" one obtained fairly uniform results as regards the appearances and relative bulk of the different fractions.

To make certain that the euglobulin and pseudoglobulin were two distinct substances, on several occasions ammonium sulphate was gradually added to the diluted serum, and it was found that precipitation did not occur until 25% saturation had been reached and ceased at 33%.

Thereafter no precipitation occurred until about 40% saturation and again ceased at 46% saturation. The same result followed with the pure substances. But it must be clearly understood that these limits of precipitation are not absolute, they merely refer to the majority of sera. Thus in some cases precipitation may continue to occur between 33% and 40% saturation but it is never abundant until the 40% is reached; and again it may continue after 46% saturation, but, as before, it is only a small amount that comes down after 46% is exceeded.

On/

On two occasions, however, results totally different from the usual were obtained; on each of these occasions, 2 sera, a negative and a positive, were examined at the same time; no precipitation occurred with 33% saturation, but a fairly abundant precipitate was obtained with 50% saturation after removal of that caused by 46% saturation, whereas in other instances, where the eugolobulin and pseudoglobulin were present as usual, no precipitate or only a very faint one was obtained on the subsequent addition of ammonium sulphate to make 50% saturation. From these results, it will be observed that the different constituents of the globulin contents of sera are somewhat variable. The irregular behavior with 2 sera at the same time suggests that the technique was at fault; however, the two experiments were not consecutive, the ammonium sulphate solution used gave the customary result with other sera and there was no error in the amounts added.

In a number of instances the diluted sera were mixed with equal volumes of saturated ammonium sulphate solution so as to obtain precipitation of the whole globulin content; this was freed from albumin in the usual way. In a few cases one portion of the serum was subjected to this treatment whilst another portion was subjected to fractional precipitation.

So far, no mention has been made as to the recovery of those portions which were not precipitated but remained in solution in the supernatant fluids e.g. the euglobulin remaining in solution after precipitation with 33% saturation with ammonium sulphate. In the case of the euglobulin, this was done on several occasions by dissolving the precipitated pseudoglobulin in distilled water and then adding 33% ammonium sulphate.

However this necessitated considerable expenditure of time, thereby prolonging the process of "salting out" to such an extent as to lead to the danger of alterations occurring in the various products. The fact that the behavior of the two globulin fractions in the Wassermann reaction was so widely different excluded the possibility of erroneous conclusions being drawn from neglecting this part of the process.

In the case of the pseudoglobulin, this purification could not be carried out on account of the difficulty in separating the albumin precipitate. But here again the results have shown that any pseudoglobulin, remaining in the albumin portion, led to no fallacy, since pseudoglobulin and albumin behaved very differently in the syphilis reaction.

After these various products had been obtained in as pure a form as possible, they were suspended or dissolved in a small quantity of distilled water and dialysed in running water until all the ammonium sulphate was removed. This generally occupied 36 hours or even longer. They were then made up with distilled water to the original bulk of the serum and 0.85% NaCl added. In a few cases, ammonium sulphate could be detected after dialysis by the addition of barium chloride but this was never present in sufficient amount to alter the effect in the Wassermann test.

Parchment dialysers were at first used but later dialysers made from fish's swim bladder were substituted for these, as they gave more rapid and more efficient results.

Characters of Various Proteins obtained by precipitation with ammonium sulphate.

Certain points were noted as to the character of these various products. The globulin fractions were white products, giving a firm deposit after centrifugalisation; generally, a considerable time was required in the centrifuge to bring them down. They were insoluble in distilled water but the addition of 0.85% NaCl generally caused a partial solution and sometimes complete solution of both euglobulin and pseudo-globulin, but different specimens varied considerably in this respect. It is, of course, well known that many proteids, which are insoluble in distilled water, are soluble in water containing a salt in less amount than that required to cause precipitation. They both reacted neutral to litmus. In one or two cases, where some ammonium sulphate was still present after dialysis, a faintly acid reaction was obtained.

The pseudoglobulin was always much more abundant than the euglobulin, and neither was increased or diminished in a syphilitic as compared with a normal serum so far as one could judge by comparison of the volume in the centrifuge tubes after centrifugalising. It is possible, of course, that some difference might be found by weighing the dried products, but here the impossibility of obtaining absolutely pure substances would prove a serious barrier to accurate results. Noguchi (2) states that the globulin content is increased in the sera of untreated and slightly treated cases of primary and secondary syphilis. His method consisted in weighing the precipitate obtained by half saturation of the sera with ammonium sulphate (or one part of serum plus four parts of a half saturated

solution/

solution of ammonium sulphate). Precipitates so obtained must contain variable amounts of albumin, and most of, but not all, the globulin; and as the products were not dried, prior to weighing, the results were only of relative and not absolute values.

The albumin was a soft fleecy material which always retained the colouring matter of the serum, none of which ever came down in the globulin fraction. The albumin was completely soluble in water, which became coloured like the serum, though not so highly. The albumin formed a much less dense precipitate than the globulin. It was always neutral to litmus.

Precipitation of Globulin by carbonic acid gas.

A number of sera were subjected to precipitation by CO_2 gas which brings down a portion of the globulin constituents. The gas was obtained from a cylinder of liquid CO_2. The method adopted was as follows:

3c.c. of serum were diluted 10 times with distilled water and CO_2 gas allowed to bubble slowly through it for 10-15 minutes. Sometimes the diluted serum was kept at room temperature, sometimes immersed in ice, but the result was the same in either case. Although 10-15 minutes were allowed for the process, yet precipitation was probably complete within less than 5 minutes. If the gas is allowed to bubble only very slowly through thesolution, then little or no alteration occurs in the temperature of the diluted serum, hence there is no fallacy from the occurrence of cooling.

After precipitation was complete, the serum was centrifugalized, the supernatant fluid decanted and the globulin dissolved in a volume of saline solution equal to the original bulk of the serum. It was only partially soluble in saline, an opaque solution always resulting.

The supernatant fluid was always examined by the addition of increasing amounts of ammonium sulphate. On only one occasion was a precipitate obtained with the 33% saturation with ammonium sulphate and that was very slight. But precipitation usually commecned with 36% saturation and continued up to 46% saturation, after which no further precipitation occurred until the albumin commenced to come down. However, the amount of precipitate thus obtained with 46% saturation was always less than that obtained from another portion of the same serum treated throughout with ammonium sulphate, so that the "globulin" precipitated by CO_2 gas

was/

was composed of the whole fibrino-and euglobulin content and a portion of the pseudo-globulin.

The globulin obtained by CO_2 gas, resembled that precipitated by ammonium sulphate, in colour, density and solubility and in being neutral to litmus.

Precipitation of Globulin by Dialysis.

The globulins of two sera, a positive and a negative, were obtained by dialysis.

2.5c.c. of each were dialysed for forty hours in running water. The globulins were separated by centrifugalisation, and then dissolved in 0.85% salt solution (2.5c.c.). The precipitates, thus obtained, were less abundant than those obtained by half saturation with ammonium sulphate from the same sera and, as the Wassermann reaction showed, some globulin remained in solution in the case of the positive serum.

Finger shaped dialysers, made from fish's swim bladders, were used in this experiment.

Action of the Various Proteids in the Wassermann Reaction.

It is more or less generally admitted that the Wassermann syphilis reaction is given by the globulin fraction of a syphilitic serum. (Elias, Neubauer, Porges & Salomon (7) Landsteiner & Müller (4), Gross & Volk (3) Noguchi (2); Bauer & Hirsch (5)).

But these workers differ considerably in details. Thus Gross & Volk find that the euglobulin of human sera, whether normal or syphilitic, has by itself, in the heated or unheated state, an inhibitory effect on complement; Noguchi finds that the active principles of syphilitic sera are precipitable with the globulin and chiefly with the euglobulin; he makes no mention of anticomplementary effect. At the same time his method of isolating the euglobulin by dialysis is fallacious. Landsteiner & Müller find that globulin, precipitated by CO_2 gas from syphilitic serum, gives the Wassermann reaction after heating to 56°C., but normal globulin reacted only weakly after heating.

Friedemann[1] has recently carried out extensive experiments on the constituents of serum concerned in the Wassermann reaction and has come to the following conclusions.

(1) The globulin fraction of many unheated normal sera (obtained by ½ saturation with ammonium sulphate) deviates complement along with alcoholic organ extract, but, by itself, does not inhibit complement, i.e. it gives a positive Wassermann reaction. The globulins of some normal sera, however, exert by themselves an anticomplementary effect. The globulin of syphilitic serum reacts positively; it has no anticomplementary effect by itself.

(2) The euglobulin of all sera, both normal and syphilitic (obtained by 1/3 saturation with ammonium sulphate of the unheated serum) has by itself an anticomplementary effect.

(3)/

(3) The globulin, precipitated from heated normal sera, does not give a positive Wassermann reaction and is not anticomplementary. But a positively reacting globulin after precipitation from unheated normal serum continues to give a positive Wassermann reaction after heating at 56°C., although the complement deviation is more marked with the unheated globulin; on the other hand, the globulin from a heated syphilitic serum gives the Wassermann reaction.
(4) pseudo-albumin of both normal and syphilitic serum (i.e. the constituents of serum which remain in solution after 1/3 saturation) when mixed with normal globulin, - both being in concentrated solution,- deprives the latter of its power to give a positive Wassermann reaction. Syphilitic globulin, however, is not affected by the addition of pseudo-albumin. Heating at 56°C. deprives the pseudo-albumin of its "anti-globulin" effect.
(5) The anti_globulin action of albumin and the anticomplementary action of globulin are not dependent on complement components, - end piece or middle piece respectively.

Friedemann's criterion of a positive result is discussed later.

The various proteids obtained from sera by the above detailed methods have been examined in the Wassermann reaction, the Lecithin-cholesterin method being employed throughout. In almost every case, the products from a negative and a positive case have been examined at the same time; when the globulins have been separated by different methods, these also have generally been examined together, and in practically all cases, the serum has been tested by way of control. As a rule, the proteids have been obtained from fresh unheated sera and have then been divided into two portions, one of which was kept unheated whilst the other was heated at 57°C., for half an hour; in some instances, these have been compared with the same products separated from previously heated samples of the same sera. It will be shown that the same result is obtained in the Wassermann reaction with the proteids from a heated serum and the subsequently heated proteid from an unheated portion of the same serum.

Before considering the results given by the serum proteid constituents/

constituents in the Wassermann reaction it is essential to consider the criterion of a negative or a positive result by different methods. Two standards exist, the absolute and the relative.

In the former, a positive result depends on the fact that a certain fixed amount of "antibody" in the presence of "antigen" is capable of deviating an arbitrary amount of complement. The Crude Extract method of Browning & Mackenzie is an improvement on this, in that, it enables one to measure the actual amount of complement absorbed, in terms of haemolytic doses, and when this amount represents at least 5 doses of complement more than the sum of the amounts absorbed by "antibody" and "antigen" alone, a positive result is said to be obtained.

The Lecithin-cholesterin method of Browning, Cruickshank & Mackenzie is an example of a relative method. A positive result depends on the fact that the "antibody" along with lecithin cholesterin emulsion deviates more complement than along with lecithin emulsion; whereas a negative result is obtained when equal amounts of complement are absorbed with both emulsions. At the same time the lecithin-cholesterin solution is comparable to a crude alcoholic liver extract, so that it can be used for estimating a positive result on the absolute basis as in Browning & Mackenzie's method.

Friedemann's method represents another example of a relative criterion; if less "antibody" is required to deviate a certain amount of complement along with "antigen" than without it, the result is considered positive. However, as he does not appear to have estimated how much complement is absorbed by the "antigen" alone, his deductions cannot be accepted without question.

Globulins obtained by half saturation with Ammonium sulphate.

The globulins from 10 fresh normal sera and 9 fresh syphilitic sera have been examined. From the normal sera, the globulins all reacted negatively in the Wassermann test, both unheated and after heating to 57°C., for half an hour, on both the relative and absolute bases. (Tables, 1, 2, & 3.)

The globulins from 6 of the fresh syphilitic sera reacted positively, both in the heated and unheated state (Table 1, 2, & 3.). From one syphilitic serum, the globulin, heated and unheated, was negative; (Table 4.). From the remaining two syphilitic cases the reaction with the globulins was positive in the unheated and negative in the heated state (Table 5 & 6.); at a subsequent date the globulin from a fresh

sample of serum from one of these cases was examined with the same result (Table 7.); the serum from this case, examined on the first occasion, gave a strong positive Wassermann reaction (Table 6.).

Effect of Heating Globulins.

The unheated globulin from the fresh sera of both normal and syphilitic cases always caused more deviation of complement along with "antigen" than did the heated globulin, and, in this respect, resembled the results previously obtained with the sera themselves. This greater deviation on the part of the unheated globulins generally occurred with both lecithin and lecithin-cholesterin emulsions, but in one case, with lecithin emulsion, the unheated globulin deviated less complement than after heating whilst with the lecithin-cholesterin emulsion, the usual result was obtained (V Table 7b).

Anticomplementary effect of Globulin.

In practically all cases, the unheated globulin by itself deviated much more complement than the heated globulin; (Table 8); this property is rarely shown by the sera which generally have the same slight anticomplementary effect in the heated and unheated states. (P. 44) No doubt this marked anticomplementary effect on the part of the unheated globulins was, to some extent, responsible for producing the greater deviation of complement with "antigen" but, on the other hand, more complement was absorbed by the unheated than by the heated globulin, with "antigen", even when the former showed no greater anticomplementary effect than the latter (Table 2, & 7b).

The inhibitory effect on the part of the unheated globulins was altogether independent of the reaction with the sera from/

from which they were obtained. It was shown as frequently by the globulins of normal as of syphilitic sera.

That this property is not dependent on the method of obtaining the globulins is shown by the fact that the globulins isolated from different sera at the same time, and afterwards examined simultaneously, may give different results. Thus the unheated globulin from a normal serum was very anticomplementary as compared with the heated globulin , whilst the globulin from a positive serum, examined at the same time, was equally and only weakly inhibitory in both heated and unheated states (Table 9A). An exactly opposite result is shown in Table 9C. Again the anticomplementary effect of a globulin, obtained at different times from the same case,may be variable, i.e. on one occasion it may be very inhibitory unheated and at another time show the same small absorption of complement as the heated globulin (McG. Table 9 A & B).

Properties of Globulins from Heated Sera.

No difference has been found between the properties of heated globulins from fresh sera and the unheated globulins from heated samples of the same sera. They always react negatively if obtained from normal sera and are equally anticomplementary; from positive sera, the same reaction is to be looked for in each case and here also the anticomplementary effect is the same (Tables 10, 11 & 12).

These results do not agree with those of Friedemann who states:-

(1) that the globulins of fresh syphilitic sera are not anti-complementary whereas the globulins of some fresh normal sera are;

(2)/

(2). the globulin fractions of many unheated normal sera give a positive Wassermann reaction.
(3). the globulins of heated normal sera do not give a positive reaction; but a positively reacting globulin, after precipitation from an unheated normal serum, continues to give a positive Wassermann effect after heating at 56°C., although the complement deviation is more marked with the unheated globulin.

As previously stated, Friedemann made no mention of attempts at purifying the globulin precipitates; also his criterion of a positive reaction cannot be accepted. At the same time, these facts do not explain the different results obtained in the anti-complementary effects of the globulins nor the fact that he gets different reactions with the globulins from heated normal sera and heated globulins from fresh sera.

Effect of heating Globulin to Different Temperatures.

An investigation was made into the effect of heating Globulins to various temperatures, and the results are exactly similar to those previously obtained with the blood serum (Vide. p. 42). On each occasion a negative and a positive serum and their respective globulins were tested fresh and after heating to 47°C., 52°C., & 57°C., for half an hour and a marked fall in the amount of complement absorbed occurred between 47°C., and 52°C., and at the same temperature the globulins lost their anticomplementary effect (Tables 13 A & B and 14 A & B).

Different Amounts of Globulin (Tables 15 & 16 A &B).

The globulin, obtained from a syphilitic serum previously heated at 57°C. for half an hour, was tested in the following amounts, 0.05c.c., 0.1c.c. and 0.2c.c. The larger amounts led to a greater deviation of complement with both the lecithin and lecithin-cholesterin emulsions but did not show any increase in the anticomplementary effect of the globulin itself. Thus, with larger amounts, the reaction was more definite, although,

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even with the smallest amount (0.05c.c.), the positive nature of the result was clearly shown (Table 15).

In Table 16 A & B the results of two other experiments are given. Globulins were obtained from an unheated normal and syphilitic serum, and were then tested unheated and after heating at 57°C. for half an hour in amounts of 0.05c.c. and 0.1cc.c. The sera were examined at the same time 0.05c.c. being used.

In the negative case (Table 16A), 0.05c.c. and 0.1c.c. of the heated globulin gave an almost identical reaction. The unheated globulin as usual deviated more complement than the heated portion, but the difference was somewhat greater with the larger amount of globulin (0.1c.c.). A similar result was obtained with the globulin from the syphilitic serum (Table 16B); 0.1c.c. of the heated globulin deviated practically the same as 0.05c.c. with both lecithin and lecithin-cholesterin emulsions; in the unheated state, however, 0.1c.c. of globulin with both emulsions gave greater deviation of complement than did 0.05c.c. of globulin.

Comparison of the Properties of Globulin and Serum.

It would thus appear that, in the Wassermann reaction, the total globulin content of a serum behaves in much the same way as the serum itself; the globulins from normal sera react negatively, whilst those from syphilitic sera frequently give a positive Wassermann reaction; in other words, the substances, peculiar to positively reacting sera, which, with lipoid emulsion, have the power of deviating complement are generally precipitated with the globulin. When the globulin is removed from a positively reacting serum, the remaining fraction (Albumin) always fails

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to give a positive reaction. In certain cases, the globulin, separated from a positive serum, fails to give a reaction, and in these cases the albumin remainder is, of course, also negative. Hence it appears that, in some cases, in the process of separation the property of giving a positive reaction is lost. It is at present impossible to give an explanation of this phenomenon.

Unheated sera, in the presence of "antigen", deviate more complement than heated sera; the same has been found to be the case with the globulin of both positive and negative sera. In a few instances, unheated normal sera deviate more complement along with lecithin-cholesterin emulsion than with lecithin emulsion i.e. react positively, and it is interesting that, in one such case, the pseudoglobulin showed the same phenomenon (To be referred to later p.119).

Heating to different temperatures has the same effect on the globulin as on the native serum (Vide. p.42) as regards the Wassermann reaction. Thus the marked diminution in deviating power occurs between 47°C. and 52°C. in both cases.

The effect obtained by using larger amounts of globulin in the Wassermann test shows the same variation as in the case of the serum (Vide. p.54).

One notable difference which exists between the actions of serum and globulin is the marked inhibitory effect on complement exerted by some unheated globulins. Such anticomplementary effect is rarely met with in sera but it occurs frequently with the globulins and must be inherent in the globulins since sera examined at the same time and with the same complement do not show the phenomenon.

This anticomplementary effect is always destroyed

by/

by heating to between 47°C. and 52°C. but it must not be confused with that property of unheated globulin which enables it to deviate a large amount of complement in the presence of "antigen", since certain globulins, which show the latter phenomenon, exert only a slight anticomplementary effect by themselves.

Fractional Precipitation of Serum by Ammonium Sulphate.

Euglobulin (obtained by 1/3 saturation).

Gross and Volk[3] have found that the euglobulin of human sera, whether normal or syphilitic, had by itself in the heated and unheated state an inhibitory effect on complement. Friedemann[1] agrees with this statement. However, no attempt was made by these authors to obtain a pure euglobulin by repeated precipitation. Noguchi[2] states that the euglobulin from syphilitic sera posesses the property of giving a positive Wassermann reaction, whilst that from normal sera does not. He makes no mention of the anticomplementary effect, nor of the effect of heating. His results are based on the examination of 3 samples of syphilitic sera and 2 of normal sera. The euglobulin was precipitated by dialysis of the sera in celloidin sacs, and, as no further procedure was adopted, it follows that a proportion of pseudoglobulin must also have been present.

The euglobulin has been isolated from 6 fresh normal sera and from 8 fresh syphilitic sera. On two occasions, as previously mentioned, no precipitation was obtained with 33% saturation with ammonium sulphate.

Effect of Euglobulin in the Wassermann Reaction.

(1) From Normal Sera. The euglobulins from 5 fresh normal sera were examined, heated and unheated, and all reacted negatively (Table 17A). Another specimen, which was examined unheated only, was also negative.

(2) From Syphilitic Sera. The euglobulin fraction from 5 fresh syphilitic sera reacted negatively; one of these was examined unheated only, the others both heated and unheated. (Table 17B). One euglobulin fraction reacted positively both heated and unheated (Table 18B). Two euglobulin fractions, obtained at/

at different times from separate samples of a patient's serum and tested with different complements, were negative heated, but, unheated, the results were inconclusive though probably negative (Table 19).

From another case, the euglobulin was isolated on 3 different occasions; at the first examination it reacted negatively heated and unheated; at the two subsequent examinations, the heated euglobulins gave a positive reaction whilst the result with the unheated portions was inconclusive both times (Table 20).

Effect of heating Euglobulin.

In the majority of instances, the unheated euglobulin, along with "antigen" deviated either no more complement or very little more than did the heated euglobulins.

Anticomplementary effect of Euglobulins

As a rule the inhibitory effect on complement was very slight and was equal with both heated and unheated euglobulins

However, certain unheated euglobulins gave a very marked deviation of complement with "antigen" and, at the same time, were very inhibitory by themselves; these results occurred with the euglobulins of 4 sera, one normal (Table 18A) and 3 syphilitic (Tables 18B & 19 & 20).

From the above results, it is obvious that the effect of euglobulin in the Wassermann test bears a close relationship to the anticomplementary effect of the euglobulin by itself; where an unheated euglobulin absorbed much more complement in the Wassermann reaction than the heated euglobulin, this was always associated with a strong inhibitory effect on the part of the former/

former. The different results of those authors already quoted are probably partly explained by their imperfect technique in separating the euglobulin so that they had a large admixture with pseudoglobulin.

Pseudoglobulin (precipitated by 46% Saturation with Ammonium Sulphate of the Serum after removal of the Euglobulin.

The pseudoglobulin has been isolated from 8 fresh normal sera and 10 fresh syphilitic sera.

Effect in Wassermann Reaction.

(1) Normal cases.

All the pseudoglobulin fractions obtained from negative sera have, without exception, reacted negatively both heated and unheated (Table 21). The exception is of particular interest; the unheated serum and unheated pseudoglobulin both caused greater deviation of complement with the lecithin-cholesterin emulsion than with the lecithin emulsion, i.e. gave a positive Wassermann reaction, but, after heating, both reacted negatively (Tables 32A)

Syphilitic cases.

One pseudoglobulin, tested unheated only, was positive. Four were positive, heated and unheated (Table 22). Two were negative heated and unheated (Table 23). One was negative heated and positive unheated (Table 24). The remaining two were examined, each on two occasions, the pseudoglobulin being obtained from different samples of serum each time, and tested with a different complement. Of these two, one on first examination was negative heated and positive unheated; (Table 30A); on the second examination, a doubtful result was obtained with both heated and unheated pseudoglobulin, (Table 30B). The other, at first, gave

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a positive reaction with the heated product but an inconclusive result with the unheated. (Table 31A). On the secomd occasion, the heated pseudoglobulin reacted negatively whilst, unheated, it gave a very weak positive result (Table 31B).

Effect of Heating Pseudoglobulin.

All the pseudoglobulin fractions, whether from normal or syphilitic sera, absorbed more complement in the Wassermann reaction in the unheated than in the heated state.

Anticomplementary effect of Pseudoglobulin.

After heating, no pseudoglobulin showed a strong anticomplementary effect, but unheated, it was generally more inhibitory and sometimes markedly so.

Comparison of Euglobulin and Pseudoglobulin.

No definite relationship can be traced between the reaction given by the euglobulin and the pseudoglobulin of positively reacting sera. Thus, in one case, where the pseudoglobulin reacted negatively, both heated and unheated, the euglobulin did likewise, (Table 25), whereas in another case, where the former was negative, the euglobulin was positive in both the heated and unheated states (Table 26).

The same statement applies to the anticomplementary effect of these two globulin fractions. Thus the unheated euglobulin and pseudoglobulin of a syphilitic serum gave a strong anticomplementary effect on one occasion; but om a subsequent examination an anticomplementary effect was obtained with the unheated euglobulin only (Table 27A & B). However, it would appear, that the active substances of syphilitic sera, in respect of which deviation of complement occurs with "lipoid" emulsion, appear more frequently in/

in the pseudoglobulin than in the euglobulin fraction. Again pseudoglobulin more closely resembles serum than euglobulin in that, in the unheated state, it deviates more complement with emulsion than after heating. This phenomenon is the rule with pseudoglobulin, but it is only rare with euglobulin. This similarity between the effects of the serum and the pseudoglobulin is further borne out by the result obtained with the pseudoglobulin from a normal serum where both, when unheated, reacted positively, whilst the unheated euglobulin was negative.

Globulin (obtained by 50% Saturation with Ammonium Sulphate after removal of Pseudoglobulin).

As before mentioned this globulin fraction was obtained from 4 sera only and, in these cases, no precipitate was obtained with 1/3 saturation with ammonium sulphate. In all other cases, a precipitate of euglobulin and pseudoglobulin was got, and either no precipitate or only a faint haze resulted from bringing the remaining fluid up to ½ saturation.

These globulins were obtained from 2 negative and from 2 positive sera; the former, (46% & 50% saturation) all gave negative Wassermann reactions, whilst, of the latter, the ½ saturation fraction was negative in one and positive in the other, both heated and unheated, whilst the 46% fraction was positive in both. No anticomplementary effect was found in the unheated state in any case.

Albumin (obtained by Saturation of the Serum with Ammonium Sulphate after removal of the Globulin.

The Albumin of all sera, whether negative or syphilitic gave a negative Wassermann reaction and never showed any marked anticomplementary effect. In the unheated state, it sometimes deviated/

deviated more complement along with "antigen" but the increase was usually slight. In other cases, the deviation was alike with both heated and unheated Albumin. (Tables 28 & 29). Thus the active substances of syphilitic sera do not appear in the Albumin.

Globulin (Precipitated by Carbonic Acid Gas).

Globulin was isolated in this manner from 3 fresh normal and 5 fresh syphilitic sera; it was also separated from one heated positive serum.

Effect in Wassermann Reaction.

The globulins, obtained from the normal sera, all reacted negatively, both heated and unheated. From the syphilitic sera, unheated, they reacted positively but, heated, 4 were positive and one negative. One of those which reacted positively both heated and unheated, was examined again later from a different sample of the serum, and was found to be negative after heating, though it was again positive unheated. The globulin which was precipitated from the heated syphilitic serum reacted positively (Tables 30A & B, 31A & B, 32A & B, 33, & 34A & B).

The unheated globulin always deviated much more complement in the Wassermann test than did the heated globulin but was never more inhibitory by itself.

The diluted fluids, remaining after removal of the globulin, from one normal and one syphilitic case, were, examined, heated and unheated. The fluid from the normal serum reacted negatively, deviating no more complement unheated than after heating; whilst the fluid from the syphilitic serum reacted positively both heated and unheated, thereby indicating that all the globulin had not been precipitated from it. Also the unheated fluid in this case was somewhat more inhibitory by itself than the heated portion (Table 33).

Globulin/

Globulin precipitated by dialysis.

This method was employed with one fresh normal and one fresh syphilitic serum. In the Wassermann test, the former reacted negatively whilst the latter was positive both heated and unheated.

With both negative and positive globulins, the unheated portion deviated much more complement than the heated portion in the Wassermann test but by itself was no more inhibitory. The fluids, which remained after removal of the globulins were also examined. That from normal serum reacted negatively, and showed no greater deviation of complement in the unheated state; the other was positive heated and unheated, the complement - fixation being almost alike with both portions. Also, the unheated fluids were no more anticomplementary than the heated ones.
The positive result obtained with the fluid from the syphilitic serum shows that, by this method also, all the globulin is not precipitated. (Tables 34A & B).
A number of tables are appended, giving the results of simultaneous examination of globulins precipitated by different methods (Tables35 - 39).

Globulin of Cerebro-spinal Fluids obtained by ½ saturation with ammonium sulphate.

A large amount of work has been recorded on the subject and it has been definitely shown that the globulin content of the spinal fluid of general paralytics is greatly in excess of normal, and this fact has been utilized in the diagnosis of general paralysis. Noguchi's[8] butyric acid reaction depends on excess of euglobulin and Ross and Jones[9] and Nonne and Apelt's[10] reactions with ammonium sulphate are simply indications of the presence of globulin in excess of normal.

Morton[11] has examined a large number of spinal fluids, however, and has concluded that these methods of diagnosis are less accurate than the Wassermann reaction since he obtained a marked excess of globulin in cases which were non-syphilitic and which gave a negative Wassermann reaction.

Only a small number of spinal fluids, 2 negative and 7 syphilitic, were analysed. Of these 7 syphilitic cases, 6 were general paralytics and the seventh was a case of alcoholic insanity.

The globulin was obtained by adding to a quantity of spinal fluid (5 or 10c.c.) an equal volume of saturated solution of ammonium sulphate; the fluid was then centrifugalized, the supernatant fluid removed and the deposit dissolved in a volume of normal saline solution equal to that of the spinal fluid originally used.

In all the syphilitic cases an abundant precipitate was obtained whilst in the 2 normal cases, the globulin was very scanty.

The globulin was only partially soluble in the saline solution; it was always neutral to litmus. In the Wassermann test, 0.2c.c. of the globulin solution was generally used.

From the non-syphilitic cases, the globulin reacted negatively and in one case was anticomplementary by itself.

The globulin from all the syphilitic cases reacted positively and one only was anticomplementary (Table 35).

In one case, the globulin was tested heated and unheated, a positive result being obtained with each, but the complement - deviation was greater with the unheated globulin along with "antigen", whilst the effect by themselves on complement was the same.

Conclusions.

1. The various proteids of blood serum, obtained by fractional precipitation by ammonium sulphate, must be redissolved and reprecipitated several times before they can be obtained in pure form.

2. The pseudoglobulin of serum is always more abundant than euglobulin, and neither was increased in a syphilitic as compared with a non-syphilitic serum, as judged by the volume of the centrifugalised precipitate.

3. Globulin, obtained by 50% saturation of the fresh serum by ammonium sulphate, resembled the serum in ~~many of~~ its ~~proportions~~ behaviour.

The globulins from normal sera always react negatively in the Wassermann test, whilst from syphilitic sera, they frequently react positively. Unheated globulins from unheated negative sera do not give a positive reaction, either on a relative or an absolute basis; those from unheated syphilitic sera give a positive reaction in most cases. Unheated globulins from unheated sera, whether normal or syphilitic, deviate more complement along with "antigen" than the heated globulins. This property is destroyed by heating to between 47°C. and 52°C. Unlike sera, the unheated globulins frequently exert a marked anticomplementary effect by themselves; this is also destroyed by heating to between 47°C. and 52°C. Unheated globulin from serum, previously heated at 57°C., behaves in the same way as the heated globulin from the unheated serum; this applies to both negative and positive cases.

4. Euglobulin (obtained by 1/3 saturation of the unheated serum with ammonium sulphate) always reacts negatively, whether heated or unheated, if obtained from negative sera; obtained from syphilitic sera, it reacts negatively in most cases. As a rule, the unheated euglobulin deviates either no more or very little more complement than the heated euglobulin in the Wassermann reaction, and is rarely anticomplementary by itself.

5. Pseudoglobulin (obtained by 46% saturation of the unheated serum with ammonium sulphate after removal of the euglobulin) generally reacts negatively, whether heated or unheated if obtained from normal sera. But in one case, where the unheated normal serum reacted positively, the unheated pseudoglobulin was also positive; both the serum and the pseudoglobulin were negative after heating. From positive sera, the unheated pseudoglobulin reacts positively in most cases, but after heating, it sometimes loses its power to give a positive reaction.

The unheated pseudoglobulin deviates more complement along with "antigen" than the heated pseudoglobulin, whether obtained from normal or syphilitic sera. The unheated pseudoglobulin, from normal and syphilitic sera, is frequently anticomplementary by itself.

Pseudoglobulin, in its action, resembles serum more closely than euglobulin does.

6. Globulin, precipitated by carbonic acid gas, consists of all the euglobulin and some of the pseudoglobulin. From negative sera, it reacts negatively, heated and unheated; from positive sera, it always reacted positively unheated, and all, with one exception, were positive after heating.

The unheated globulin always deviated more complement with "antigen" than the heated globulin but was never more anti-complementary by itself.

7. Globulin, precipitated by dialysis from 2 unheated sera, consisted of only a portion of the total globulin content. From the negative serum, it reacted negatively, heated and unheated; from the positive serum, it reacted positively, heated and unheated. In the Wassermann test, the unheated portions deviated more complement than the heated, but were no more inhibitory by themselves.

8. Albumin (obtained by saturation of the serum with ammonium sulphate after removal of the globulin) always reacted negatively whether heated or unheated, and the latter only occasionally deviated more complement than the former along with "antigen". It never showed any marked anticomplementary effect.

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TABLE I

Negative case	Emulsions	Amounts of Guinea-pig's Complement				0.6cc.NaCl Solution + Complement
0.05 c.c.	0.6 c.c.	0.04c.c.	0.07c.c.	0.II c.c.	0.I6 c.c.	0.05 c.c.
Serum 57°C	L.	Complete	...	...	...	Complete
" "	L-C.	Just "	...	...	...	
Globulin 57°C	L.	Almost complete	Just complete	Complete	...	Complete
" "	L-C.	Just "	Complete	...	...	
Globulin Unheated	L.	...	Marked	Almost complete	Complete	Very marked
" "	L-C.	...	"	" "	"	
Syphil.tc case	0.6 c.c.	0.04c.c.	0.07c.c.	0.II c.c.	0.I6 c.c.	0.05 c.c.
Serum 57°C	L.	Faint trace	Trace	Just complete	Complete	Complete
" "	L-C.	0	Very faint "	Faint trace	Marked	
Globulin 57°C	L.	Trace	Marked	Complete	...	Complete
" "	L-C.	0	Trace	Marked	Almost complete	
Globulin) Unheated)	L.	0	0	0	Marked	Almost complete
	L-C	0	0	0	0	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Just Complete

Dose of Complement = 0.015 c.c.

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TABLE 2

Negative Case	Emulsions	Amount of Guinea-pig's Complement				0.6c.c.NaCl solution + Complement
0.05 c.c.	0.6 c.c.	0.04 cc	0.07 c.c	0.II c.c		0.03 c.c.
Serum 57°C	L.	Complete	...	...	...	Complete
" "	L-C.	"	...	...	...	
Globulin 57°C	L.	Complete	...	...	...	Complete
" "	L-C.	Just "	Complete	...	...	
Globulin) Unheated)	L.	...	Complete	...	...	Complete
	L-C.	...	"	...	...	
Syphilitic Case						
0.05 c.c.	0.6 c.c.	0.04 cc	0.07 c.c.	0.II c.c.	0.I6 c.c.	0.03 c.c
Serum 57°C	L.	0	Very marked	Complete	...	Complete
" "	L-C.	0	0	0	Marked	
Globulin 57°C	L.	Just complete	Complete	...	...	Just complete
" "	L-C.	0	Distinct	Just complete	Complete	
Globulin unheated	L.	0	Faint trace	Very marked	Complete	Complete
" "	L-C.	0	0	Marked	Very marked	

Emulsions 0.6 c.c. + Complement 0.02 c.c. = Just Complete

Dose of Complement = 0.0065 c.c.

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TABLE 3.

Negative Case	Emulsions	Amounts of Guinea-pig's Complement.				0.6c.c.NaCl Solution + Complement
0.05 c.c.	0.6 c.c.	0.04 cc.	0.07 cc.	0.II cc.	0.I6cc.	0.04 c.c.
Serum 57°C	L.	Complete	...	...	...	Complete
" "	L-C.	"	...	...	...	
Globulin 57°C	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	Verymarked	"	...	...	
Globulin) unheated)	L.	0	0	0	Distinct	0
	L-C.	0	0	0	"	
Syphil.tc Case						
0.05 c.c.	0.6 c.c.	0.07 cc	0.II cc	0.I6 cc	0.22cc.	0.04 c.c.
Serum °57 C	L.	0	Distinct	Almost complete	Complete	Just complete
" "	L-C.	0	0	0	0	
Globulin 57°C	L.	Marked	Just complete	Complete	...	Complete
" "	L-C.	0	0	Marked	Just complete	
Globulin unheated	L.	0	Trace	Almost complete	Complete	Trace
" "	L-C.	0	0	0	Distinct	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.0I5 c.c.

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TABLE 4.

Syphilitic Case 0.05 c.c.	Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement				0.6 cc.NaCl Solution + Complement
		0.04 c.c.	0.07 c.c.	0.11 cc.	0.16 cc.	0.03 c.c
Globulin 57°C	L.	Distinct	Complete	...	...	Just
" "	L-C.	"	Just "	...	...	complete
Globulin unheated	L.	0	0	Faint trace	Trace	0
" "	L-C.	0	0	" "	Marked	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.015 c.c.

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TABLE 5

Negative Case 0.05 cc	Emulsions 0.6c.c.	Amounts of Guinea-pig's Complement. 0.07 c.c	0.1 c.c.	0.14 c.c	0.2 c.c.	0.28 c.c.
Globulin 57°C	L.	Just complete	...	...	...	...
" "	L-C.	" "	....	...	...	...
Globulin unheated	L.	Faint trace	Trace	Distinct	Very marked	Complete
" "	L-C.	0	Faint "	"	" "	"
Syphilitic Case 0.05 cc						
Serum 57°C	L.	Very marked	Just complete	...	...	...
" "	L-C.	Trace	Very marked	Just complete	Complete	...
Globulin 57°C	L.	Distinct	Very marked	Complete	...	...
" "	L-C.	Trace	Marked	"	...	...
Globulin) unheated)	L.	0	0	Trace	Distinct	Complete
	L-C.	0	0	0	Trace	Very marked

CONTROLS.

Serum 0.05 c.c. + NaCl Solution 0.6c.c. + Complement 0.03c.c. = Complete

Globulin (heated) 0.05c.c. + " " 0.6c.c. + " 0.03c.c. = "

" (unheated) 0.05c.c. + " " 0.6c.c. + " 0.08 c.c.= Trace of Lysis

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete.

Dose of Complement = 0.01[illegible]5c.c.

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TABLE 6

Negative Case	Emulsions	Amounts of Guinea-pig's Complements					0.6ccNaCl Solution+ Complement
0.05 c.c.	0.6 c.c	0.04cc.	0.07cc.	0.11cc	0.16cc	0.22cc	0.05cc
Serum 57°C	L.	Marked	Almost complete	Complete	...	...	Just complete
" "	L-C.	Very "	Just "	"	...	...	
Serum unheated	L.	0	0	0	0	Faint trace	Just Complete
" "	L-C.	0	0	0	0	" "	
Globulin 57°C	L.	Marked	Almost complete	Complete	...	...	Almost complete
" "	L-C.	Very "	Just "	"	...	...	
Globulin) unheated)	L. L-C	0 0	0 0	0 0	0 0	0 0	0
Syphilitic Case							
0.05 c.c.	0.6 c.c	0.11cc	0.16cc	0.22cc	0.3cc	0.4cc	0.05cc.
Serum 57°C	L.	0	Marked	Very marked	Just complete	Complete	Marked
" "	L-C.	0	0	0	Trace	Marked	
Serum-- unheated	L.	0	0	Trace	Marked	Almost complete	Distinct
" "	L-C.	0	0	0	0	0	
Globulin 57°C	L	Just complete	Complete	...	...	...	Almost complete
" "	L-C.	Complete	"	...	...	...	
Globulin) unheated)	L. L-C.	0 0	0 0	0 0	0 0	Distinct 0	0

Emulsions 0.6 c.c. + Complement 0.05 c.c. = Marked Lysis

Dose of Complement = 0.03 c.c.

TABLE 7

A

Negative Case 0.05 c.c.	Emulsions 0.6c.c.	Amount of Guinea-pig's Complement 0.04cc.	0.07 cc	0.11 cc	0.16 cc	0.22 cc	0.6 ccNaCl Solution + Complement 0.05 cc
Globulin 57°C	L.	Marked	Very marked	Almost complete	...	...	Very marked
" "	L-C.	Complete	...	...	...	...	
Globulin) unheated)	L.	0	0	0	0	Trace	Distinct
	L-C.	0	0	0	0	"	
Syphilitic Case 0.05 c.c.			B				
Globulin 57°C	L.	Distinct	Marked	Very marked	Almost complete	...	Almost complete
" "	L-C.	Almost complete	Complete	...	...	...	
Globulin unheated	L.	Very marked	Almost complete	Just complete	...	...	Almost complete
" "	L-C	Distinct	Marked	Very marked	Almost complete	Complete	

Emulsions 0.6 c.c. + Complement 0.05 c.c. = No lysis

Dose of Complement = 0.02 c.c.

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TABLE 8

Anticomplementary Effect of Globulins, obtained from Fresh Sera

Globulins	0.6 c.c. NaCl Solution + Complement		Dose of Complement
0.05 c.c.	0.03 c.c.	0.07 c.c.	
Negative Heated	Almost complete	Complete	0.015 c.c.
" Unheated	0	0	
Positive Heated	Just complete	Complete	
" Unheated	0	0	
Negative Heated	Just complete	Complete	0.015 c.c.
" Unheated	Trace	Marked	
Positive Heated	Almost complete	Complete	
" Unheated	0	Distinct	
	0.03 c.c.	0.08 c.c.	0.0175 c.c.
Negative Heated	Complete	...	
" Unheated	0	Trace	
Positive Heated	Just complete	...	
" Unheated	0	Trace	
	0.03 c.c.	0.05 c.c.	0.015 c.c.
Negative Heated	Just complete	...	
" Unheated	0	0	
Positive Heated	Just complete	...	
" Unheated	0	Marked	
	0.05 c.c.	0.08 c.c.	0.015 c.c.
Negative Heated	Complete	...	
" Unheated	Very marked	Just complete	
Positive Heated	Complete	...	
" Unheated	Almost complete	Complete	

TABLE 9

Anticomplementary Effect of Globulins, obtained from Fresh Sera.

Globulins.	0.6 c.c. NaCl Solution + Complement.		Dose of Complement.
	A		
0.05 c.c.	0.03 c.c.	0.05 c.c.	
(S) Negative Heated	Marked	Very marked	0.02 c.c.
" Unheated	0	Distinct	
(M'G) Positive Heated	Very marked	Almost complete	
" Unheated	" "	" "	
	B		
0.05 c.c.	...	0.05 c.c.	
(S) Negative Heated	...	Almost complete	0.03 c.c.
" Unheated	...	0	
(M'G) Positive Heated	...	Almost complete	
" Unheated	...	0	
	C		
0.05 c.c.	0.04 c.c.	0.05 c.c.	
Negative Heated	Complete	...	0.01 c.c.
" Unheated	"	...	
Positive Heated	Complete	...	
" Unheated	0	Trace	

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TABLE IO

Negative Case 0.05 c.c.	Emulsions 0.6c.c.	Amounts of Guinea-pig's Complement 0.04 cc	0.07 cc	0.I cc	0.I4 cc	0.6 cc NaCl Solution + Complement 0.02 cc
Serum 57°C	L.	Complete	...	...	...	Almost complete
" "	L-C.	"	...	...	...	
Globulin from heated serum	L.	Almost complete	Complete	...	...	Complete
" " "	L-C.	Just "	"	...	...	
Globulin (57°C) from fresh serum	L.	Almost complete	Complete	...	...	Almost complete
	L-C.	" "	"	...	...	
Globulin (unheated) from fresh serum	L.	0	Faint trace	Trace	Distinct	0
	L-C.	0	" "	"	"	
Syphilitic Case 0.05 c.c.						
Serum 57°C	L.	Marked	Almost complete	Complete	...	Just complete
" "	L-C.	0	0	0	0	
Globulin from heated serum..	L.	Distinct	Marked	Very marked	...	Almost complete
" "	L-C.	0	0	Faint trace	Trace	
Globulin (57°C) from fresh serum	L.	Distinct	Marked	Very marked	...	Just complete
	L-C.	0	0	Faint trace	Trace	
Globulin (unheated) from fresh serum	L.	0	0	0	0	0
	L-C.	0	0	0	0	

Emulsions 0.6 c.c. + Complement 0.03 c.c. = Complete

Dose of Complement = 0.0I c.c.

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TABLE II

Negative Case 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement 0.07 c.c.	0.II cc	0.I6 cc	0.22 cc	0.6 cc NaCl solution + Complement 0.03 c.c.
Serum 57°C	L.	Complete	...	...	...	Complete
" "	L-C.	"	...	...	...	
Globulin from heated serum	L.	Distinct	Marked	Complete	...	Just complete
	L-C.	"	"	"	...	
Globulin(57°C) from fresh serum	L.	Distinct	Marked	Complete	...	Just complete
	L-C.	"	"	"	...	
Globulin (unheated) from fresh serum	L.	0	Faint trace	Distinct	Marked	0
	L-C.	0	" "	"	"	
Syphilitic Case 0.05 c.c.	0.6 c.c.	0.I6 cc	0.22 cc	0.3 cc	0.4 cc	0.03 cc
Serum 57°C	L.	Very marked	Complete	...	...	Almost complete
" "	L-C.	0	0	Trace	Almost complete	
Globulin from heated serum	L.	Very marked	Complete	...	...	Just complete
" "	L-C.	0	Faint trace	Almost complete	...	
Globulin (57°C) from fresh serum	L.	Marked	Just complete	...	...	Very marked
	L-C.	Faint trace	Marked	Complete	...	
Globulin (unheated) from fresh serum	L.	0	Trace	...	...	0
	L-C.	0	0	0	Distinct	

Emulsions 0.6 c.c. + Complement 0.05 c.c. = No lysis

Dose of Complement = 0.0I 5 c.c.

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TABLE I2

Negative Case 0.05 c.c.	Emulsions 0.6 c.c	Amounts of Guinea-pig's Complement 0.04 cc	0.07 cc			0.6cc NaCl Solution + Complement 0.04 cc
Serum(57°C)	L.	Very marked	Complete			Just complete
" "	L-C.	" "	"			
Globulin from heated serum	L.	Very marked	Complete			Complete
" "	L-C.	Almost complete	"			
Syphilitic case 0.05 cc	Emulsions 0.6 cc	0.07 cc	0.I2 cc	0.I8 cc	0.26 cc	0.04 cc
Serum (57°C)	L.	0	Marked	Almost complete	Complete	Just complete
" "	L-C.	0	0	Faint trace	Just "	
Globulin from heated serum	L.	Just complete	...	...	...	Just complete
" "	L-C.	Marked	Just complete	...	...	

Emulsions 0.6 c.c. + Complement 0.04 cc = Complete.

Dose of Complement=0.0I5 cc

TABLE I3

A

Negative case 0.05 cc	Emulsions 0.6 cc	Amounts of Guinea-pig's Complement 0.04 cc	0.07 cc	0.II cc	0.I6 cc	0.6 cc NaCl Solution + Complement 0.03 cc
Serum (57°C)	L.	Complete	...	...	...	Just complete
" "	L-C.	"	...	...	...	
Serum (52°C)	L.	Just complete	...	...	...	Complete
" "	L-C.	" "	...	...	...	
Serum (47°C)	L.	0	Very marked	Just complete	Complete	Just complete
" "	L-C.	0	Distinct	Almost "	"	
Serum (unheated)	L.	0	Distinct	Just complete	Complete	Just complete
" "	L-C.	0	"	" "	"	
Globulin (57°C)	L.	Almost complete	Complete	...	...	Just complete
" "	L-C.	Very marked	Just "	Complete	...	
Globulin (52° C)	L.	Very marked	Complete	...	...	Just complete
" "	L-C.	Complete	...	...	...	
Globulin (47°C)	L.	0	0	0	Trace	0
	L-C.	0	0	0	"	
Globulin (unheated)	L.	0	0	0	Distinct	0
	L-C.	0	0	0	"	

Emulsions 0.6 cc + Complement // 0.03 cc = Complete

Dose of Complement = 0.015 cc

TABLE I3

B

Syphilitic Case 0.05 cc	Emulsions 0.6 cc	Amounts of Guinea-pig's Complement 0.II cc	0.I6 cc	0.22 cc	0.3 cc	0.6 cc NaCl Solution + Complement 0.05 cc
Serum (57°C)	L.	Distinct	Almost complete	Complete	...	Complete
" "	L-C	0	0	0	Just complete	
Serum (52°C)	L.	Trace	Marked	Complete	...	Marked
" "	L-C.	0	0	Faint trace	Just complete	
Serum (47°C)	L.	0	Marked	Complete	...	Very marked
" "	L-C.	0	0	0	Almost complete	
Serum (unheated)	L.	0	Distinct	Complete	....	Very marked
	L-C.	0	0	0	0	
Globulin (57°C)	L.	Just complete	...	...	...	Complete
" "	L-C.	0	Marked	Just complete	...	
Globulin (52°C)	L.	Complete	...	...	...	Complete
" "	L-C.	0	Marked	Complete	...	
Globulin (47°C)	L.	Trace	Very marked	Complete	...	Marked
" "	L-C.	0	0	Marked	Just complete	
Globulin (unheated)	L.	Trace	Almost complete	Complete	...	Marked
" "	L-C.	0	0	Distinct	Just complete	

Emulsions 0.6 cc + Complement 0.03 cc = Complete

Dose of Complement = 0.0I5 cc

TABLE I4

A

Negative case 0.05 cc	Emulsions 0.6 cc	Amounts of Guinea-pig's Complement				0.6 cc NaCl solution + Complement.
		0.07 cc	0.II cc	0.I6 cc	0.22 cc	0.03 cc
Serum (57°C)	L.	Complete	...	...	...	Complete
" "	L-C.	"	...	...	...	
Serum (52°C)	L.	Complete	...	...	...	Complete
" "	L-C.	"	...	...	...	
Serum (47°C)	L.	0	Faint trace	Distinct	....	Complete
" "	L-C.	0	Trace	"	...	
Serum (unheated)	L.	0	Faint trace	Trace	...	Complete
" "	L-C.	0	" "	"	...	
Globulin (57°C)	L.	Distinct	Marked	...	...	Just complete
	L-C.	"	"	...	...	
Globulin (52°C)	L.	Distinct	Marked	...	...	Just complete
	L-C.	"	"	...	...	
Globulin (47°C)	L.	0	Distinct	Marked	Very marked	0
" "	L-C.	0	Trace	Distinct	" "	
Globulin (unheated)	L.	0	Faint trace	Distinct	Marked	0
" "	L-C.	0	" "	"	"	

Emulsions 0.6 cc + Complement 0.05 cc = No lysis.

Dose of Complement = 0.015 cc.

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TABLE 14

B

Syphilitic Case 0.05 cc	Emulsions 0.6 cc	Amounts of Guinea-pig's Complement 0.16 cc	0.22 cc	0.3 cc	0.4 cc	0.6 cc NaCl solution + Complement 0.03 cc
Serum (57°C)	L.	Very marked	Complete	...	...	Almost complete
	L-C.	0	0	Trace	Almost complete	
Serum (52°C)	L.	Marked	Complete	...	...	Very marked
	L-C.	0	0	Distinct	Almost complete	
Serum (47°C)	L.	0	Distinct	...	...	Distinct
	L-C.	0	0	0	Trace	
Serum (unheated)	L.	Faint trace	Just complete	...	...	Marked
" "	L-C.	0	0	0	0	
Globulin (57°C)	L.	Marked	...	...	...	Very marked
	L-C.	Faint trace	Marked	Complete	...	
Globulin (52°C)	L.	Almost complete	...	...	...	Marked
" "	L-C.	Trace	Distinct	Almost complete	...	
Globulin (47°C)	L.	0	0	...	...	0
	L-C.	0	0	0	Trace	
Globulin (unheated)	L.	0	Trace	...	...	0
	L-C.	0	0	0	Distinct	

Emulsions 0.6 cc + Complement 0.05 cc = No lysis.

Dose of Complement = 0.015 cc

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TABLE I5

Syphilitic Case	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.IIcc	0.I6cc	0.6cc NaCl Solution + Complement 0.04cc
Serum (57°C) 0.05cc	L.	Marked	Just complete	...	...	Complete
	L-C.	0	0	0	Faint trace	
Globulin from heated serum 0.05cc	L.	Very marked	Complete	...	...	Complete
	L-C.	0	Very marked	Just complete	Complete	
Globulin from heated serum 0.Icc	L.	Trace	Almost complete	Complete	...	Complete
	L-C.	0	Faint trace	Very marked	Just complete	
Globulin from heated serum D.2cc	L.	0	Trace	Almost complete	Complete	Just complete
	L-C.	0	0	Faint trace	Trace	

Emulsions 0.6cc + Complement 0.03cc = Just complete

Dose of Complement = 0.0Icc

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TABLE 16

A

Negative Case	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.1cc	0.14cc	0.2cc
Serum 57°C 0.05cc	L.	Very marked	Just complete	...	...	...
	L-C.	" "	Complete	...	...	...
Globulin 57°C 0.05cc	L.	0	Marked	Very marked	...	...
	L-C.	Trace	"	" "	...	...
Globulin 57°C 0.1cc	L.	Distinct	Marked	Very marked	...	...
	L-C.	"	"	" "	...	...
Globulin unheated 0.05cc	L.	0	0	0	Trace	Marked
	L-C.	0	0	0	"	"
Globulin unheated 0.1cc	L.	0	0	0	Faint trace	Trace
	L-C.	0	0	0	"	"

CONTROLS

Serum 0.05cc + NaCl solution 0.6cc + Complement 0.03cc = Complete

Globulin (57°C) 0.05cc + NaCl sol^{n} 0.6cc + " 0.03cc = Just complete

" " 0.1cc + " " 0.6cc + " 0.05cc = " "

" (unheated) 0.05cc + " " 0.6cc + " 0.08cc = Marked

" " 0.1cc + " " 0.6cc + " 0.08cc = Faint trace

0.6cc (L + Complement 0.05cc = Distinct
Emulsions (L-C) + " " = Very marked

Dose of Complement = 0.015cc

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TABLE 16

B

Positive Case	Emulsions 0.6cc	Amounts of Guinea-pig's Complement. 0.16cc	0.14cc	0.2cc	0.28cc	0.38cc
Serum 57°C 0.05cc	L.	Marked	Very marked	Almost complete	...	...
	L-C.	Very faint trace	Faint trace	Trace	Very marked	Complete
Globulin 57°C 0.05cc	L.	Faint trace	Trace	...	...	...
	L-C.	" "	"	...	...	...
Globulin 57°C 0.1cc	L.	Trace	Distinct	...	...	...
	L-C.	Faint "	Trace	...	...	...
Globulin unheated 0.05cc	L.	0	0	Faint trace	Distinct	Marked
	L-C.	0	0	0	Faint trace	Distinct
Globulin unheated 0.1cc	L.	0	0	0	Faint trace	Trace
	L-C.	0	0	0	0	Faint trace

CONTROLS

Serum 0.05cc + NaCl solution 0.6cc + Complement 0.03cc = Just complete

Globulin(57°C) 0.05cc + NaCl Soln. 0.6cc + " 0.05cc = " "

" " 0.1cc + " " 0.6cc + " 0.05cc = Almost "

"(unheated) 0.05cc + " " 0.6cc + " 0.08cc = Distinct

" " 0.01cc + " " 0.6cc + " 0.08cc = Faint trace

Emulsions 0.6cc (L + Complement 0.05cc = Distinct
(L-C + " 0.05cc = Very marked

Dose of Complement = 0.015cc

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TABLE I 7

A

Negative Cases 0.05 cc.	Emulsions 0.6 cc	Amount of Guinea-pig's Complement 0.04 cc	0.07 cc	0.6 cc NaCl solution + Complement 0.02 cc	Dose of Complement
Serum (57°C)	L. L-C.	Complete "	... ...	Complete	0.0075 cc
Euglobulin (57°C)	L. L-C.	Complete "	... ...	Complete	
Euglobulin (unheated)	L. L-C.	Complete "	... ...	Complete	
Serum (57°C) " "	L. L-C.	Almost complete Marked	Complete "	Marked	0.0075cc
Euglobulin (57°C)	L. L-C.	Complete "	Complete "	Complete	
Euglobulin (unheated) " "	L. L-C.	Just complete Complete	Complete "	Complete	
Serum (57°C)	L. L-C.	Complete Just "	Complete "	Almost complete	0.015cc
Euglobulin (57°C) " "	L. L-C.	Just complete " "	Complete "	Almost complete	
Euglobulin (unheated) " "	L. L-C.	Almost complete " "	Complete "	Very marked	
Serum (57°C)	L. L-C.	Complete "	Complete "	Complete	0.0065cc
Euglobulin (57°C)	L. L-C.	Complete "	Complete "	Complete	
Euglobulin (unheated)	L. L-C.	Complete "	Complete "	Complete	

Emulsions 0.6 cc + Complement 0.03 cc = All complete

TABLE I7

B

Syphilitic Cases 0.05 cc	Emulsions 0.6 cc	Amount of Guinea-pig's Complement 0.04 cc	0.07 cc	0.II cc	0.6 cc NaCl solution + Complement 0.02 cc	Dose of Complement
Serum (57°C)	L.	Complete	...	...	Complete	
	L-C.	0	Marked	Just complete		
Euglobulin (57°C)	L.	Complete	...	...	Complete	0.0075cc
	L-C.	"	...	...		
Euglobulin (unheated)	L.	Complete	...	...	Complete	
	L-C.	Just "	Complete	...		
Serum (57°C)	L.	Distinct	Complete	...	Almost complete	
	L-C.	Trace	Marked	Just complete		
Euglobulin (57°C)	L.	Just complete	Complete	...	Complete	0.0075cc
" "	L-C.	Complete	"	...		
Euglobulin (unheated)	L.	Just complete	Complete	...	Complete	
" "	L-C.	" "	"	...		
Serum (57°C)	L.	Trace	Distinct	Complete	Very marked	
	L-C.	0	0	Trace		
Euglobulin (57°C)	L.	Very marked	Complete	...	Almost complete	0.0I5cc
" "	L-C.	Trace	Almost complete	Complete		
Euglobulin (unheated)	L.	Marked	Almost complete	Complete	Almost complete	
" "	L-C.	"	" "	"		
Serum (57°C)	L.	0	Very marked	Complete	Complete	
" "	L-C.	0	0	0		
Euglobulin (57°C)	L.	Just complete	Complete	...	Complete	0.0065cc
" "	L-C.	Marked	"	...		
Euglobulin (unheated)	L.	Complete	...	...	Complete	
	L-C.	Marked	Complete	...		

Emulsions 0.6cc + Complement 0.03cc = All complete

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TABLE I8

A

Negative case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04 cc	0.07cc	0.IIcc	0.I6cc	0.6cc NaCl Solution + Complement 0.04cc
Serum (57°C)	L.	Just complete	Complete	...	...	Complete
" "	L-C.	" "	"	...	...	
Euglobulin (57°C)	L.	Marked	Just complete	Complete	...	Just complete
" "	L-C.	Very "	" "	"	...	
Euglobulin (unheated)	L.	0	Faint trace	Trace	Distinct	Faint trace
" "	L-C.	0	" "	"	"	

B

Syphilitic case 0.05cc	0.6cc	0.07cc	0.IIcc	0.I6cc	0.22cc	0.04cc
Serum (57°C)	L.	Just complete	...	...	...	Complete
" "	L-C.	0	0	Distinct	Just complete	
Euglobulin (57°C)	L.	Complete	...	...	...	Complete
" "	L-C.	Very marked	Complete	...	...	
Euglobulin (unheated)	L.	0	Distinct	Very marked	...	Faint trace
" "	L-C.	0	0	0	Marked	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.0Icc

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TABLE I9

Syphilitic Case (W.B.) 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.07cc	0.IIcc	0.I6cc	0.22cc	0.6cc NaCl solution + Complement 0.04cc
I4/3/I2.						
Serum(57°C)	L.	Almost complete	Complete	...	...	Just complete
" "	L-C	Faint trace	Distinct	Very marked	Almost complete	
Euglobulin (57°C)	L.	Complete	...	...	...	Complete
	L-C.	Just "	Complete	...	...	
Euglobulin (unheated)	L.	0	0	0	0	0
	L-C.	0	0	0	Trace	
20/3/I2.						
Serum (57°C)	L.	Just complete	Complete	...	...	Complete
" "	L-C.	Marked	Just "	Complete	...	
Euglobulin (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Euglobulin (unheated)	L.	0	0	0	Faint trace	0
" "	L-C.	0	0	0	" "	

Emulsions 0.6cc + Complement 0.04cc = Complete

Doses of Complement (I4/3/I2) = 0.0I5cc
(20/3/I2) = 0.0I25cc

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TABLE 20

Syphilitic Case (J.H.) 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.22cc	0.6cc NaCl solution + Complement 0.04cc
5/3/12.							
Serum (57°C)	L.	Almost complete	Complete	...	...	...	
" "	L-C.	0	Distinct	Marked	Just complete	Complete	Just complete
Euglobulin (57°C)	L.	Complete	...	...	...	...	
	L-C.	"	...	...	...	...	Complete
Euglobulin (unheated)	L.	Complete	...	...	...	...	
	L-C.	"	...	...	...	...	Complete
14/3/12							
Serum (57°C)	L.	Very marked	Complete	...	...	...	
" "	L-C.	0	Distinct	Very marked	Just complete	Complete	Complete
Euglobulin (57°C)	L.	Almost complete	Complete	...	...	...	
" "	L-C.	Faint trace	Marked	Almost complete	...	...	Complete
Euglobulin (unheated)	L.	0	0	0	0	...	
	L-C.	0	0	0	0	.....	0
20/3/12							
Serum (57°C)	L.	Very marked	Just complete	Complete	...	...	
" "	L-C.	0	Faint trace	Very marked	Complete	...	Complete
Euglobulin (57°C)	L.	Marked	Just complete	Complete	...	...	
" "	L-C.	Faint trace	Very marked	Just "	Complete	...	Complete
Euglobulin (unheated)	L.	0	0	0	0	Faint trace	
" "	L-C.	0	0	0	0	Very" "	0

Emulsions 0.6cc + Complement 0.04cc = All complete.

Doses of Complement 5/3/12 = 0.01cc
14/3/12 = 0.015cc
20/3/12 = 0.0125cc

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TABLE 2I

Negative Cases 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.02cc	0.04cc	0.07cc	0.1cc	0.6cc NaCl solution + Complement 0.02cc
A						
Serum (57°C)	L.	Marked	Almost complete	Complete	...	Marked
" "	L-C.	Distinct	Very marked	"	...	
Pseudoglob^{n}. (57°C)	L.	Just complete	Complete	...	...	Complete
" "	L-C.	" "	"	...	...	
Pseudoglob^{n}. (unheated)	L.	0	0	Trace	Marked	0
	L-C.	0	0	Distinct	"	
B						
Serum (57°C)	L.	Marked	Complete	...	...	Just compl?
" "	L-C.	Distinct	"	...	...	
Pseudoglob^{n}. (57°C)	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	" "	"	...	...	
Pseudoglob^{n}. (unheated)	L.	...	...	Just complete	Complete	Complete
" "	L-C.	...	...	Almost complete	"	

A. Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.0075cc

B. Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.0075cc

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TABLE 22

Syphilitic Cases 0.05cc	Emulsions 0.6cc	Amount of Guinea-pig's Complement 0.04cc	0.07cc	0.1cc	0.14cc	0.6cc NaCl solution + Complement 0.02cc
A						
Serum (57°C)	L.	0	Trace	Marked	Complete	Complete
" "	L-C.	0	0	0	Faint trace	
Pseudoglob^{n}. (57°C)	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	0	Faint trace	Almost complete	Complete	
Pseudoglob^{n}. (unheated)	L.	0	Marked	Complete	...	Complete
	L-C.	0	0	Marked	Complete	
B	0.6cc	0.04cc	0.07cc	0.11cc	0.16cc	0.03cc
Serum(57°C)	L.	0	Very marked	Complete	...	Complete
" "	L-C.	0	0	0	Marked	
Pseudoglob^{n}. (57°C)	L.	Just complete	Complete	...	...	Complete
" "	L-C.	Marked	Very marked	Complete	...	
Pseudoglob^{n}. (unheated)	L.	...	Marked	Complete	...	Complete
	L-C.	...	0	0	Just complete	

A. Emulsions 0.6cc + Complement 0.04cc = Complete

Dose of Complement = 0.01cc

B. Emulsions 0.6cc + Complement 0.02cc = Complete

Dose of Complement = 0.0065cc

?.....

TABLE 23

Syphilitic Cases 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.02cc	0.04cc	0.07cc	0.1cc	0.6cc NaCl solution + Complement 0.02cc
A						
Serum (57°C)	L.	Trace	Distinct	Just complete	...	Almost complete
" "	L-C.	0	Trace	Marked	Almost complete	
Pseudoglobn (57°C)	L.	Marked	Almost complete	Complete	...	Complete
" "	L.C.	"	" "	"	...	
Pseudoglobn (unheated)	L.	0	Trace	Marked	Very marked	Distinct
" "	L-C.	0	"	"	" "	
B	0.6cc	0.04cc	0.07cc	0.11cc	0.16cc	0.04cc
Serum (57°C)	L.	...	Just complete	Complete	...	Complete
" "	L-C.	...	0	0	Distinct	
Pseudoglobn (57°C)	L.	Marked	Almost complete	Complete	...	Just complete
" "	L.C.	"	Just "	"	...	
Pseudoglobn (unheated)	L.	0	0	Marked	Just complete	0
" "	L-C.	0	0	Distinct	" "	

A. Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.0075cc

B. Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.01cc

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TABLE 24

Syphilitic Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement				0.6cc NaCl Solution + Complement 0.05cc
		0.04cc	0.07cc	0.11cc	0.16cc	
Serum (57°C)	L.	Trace	Distinct	Complete	...	Complete
" "	L-C.	0	Faint trace	Trace	Marked	
Pseudoglob.n (57°C)	L.	Almost complete	Just complete	Complete	...	Just complete
" "	L-C.	" "	" "	"	...	
Pseudoglob.n. (unheated)	L.	...	Trace	Very marked	Just complete	Marked
" "	L-C.	...	...	Trace	Marked	

Emulsions 0.6cc + Complement 0.03cc = Just Complete.

Dose of Complement = 0.015cc

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TABLE 25

Syphilitic Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement				0.6cc NaCl solution + Complement
		0.02cc	0.04cc	0.07cc	0.1cc	0.03cc
Serum (57°C)	L.	Distinct	Marked	Just complete	...	Complete
" "	L-C.	Faint trace	Trace	Marked	Very marked	
Euglobulin (57°C)	L.	Very marked	Just complete	...	...	Complete
" "	L-C.	" "	Complete	...	...	
Euglobulin (unheated)	L.	Very marked	Just complete	...	...	Complete
" "	L-C.	" "	" "	...	...	
Pseudoglob^{n} (57°C)	L.	Marked	Almost complete	Complete	...	Complete
" "	L-C.	"	" "	"	...	
Pseudoglob^{n} (unheated)	L.	Very Ft. trace	Faint trace	Marked	Very marked	Marke d
" "	L-C.	0	Trace	Distinct	" "	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement ≧ 0.0075cc

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TABLE 26

Syphilitic Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	...	Just complete	Complete	...	Complete
" "	L-C.	...	0	0	Distinct	
Euglobulin (57°C)	L.	Very marked	Complete	...	...	Complete
" "	L-C.	0	Very marked	Complete	...	
Euglobulin (unheated)	L.	0	0	Distinct	Very marked	Faint trace
" "	L-C.	0	0	0	0	
Pseudoglobn. (57°C)	L.	Marked	Just complete	Complete	...	Just complete
" "	L-C.	"	" "	"	...	
Pseudoglobn. (unheated)	L.	0	0	Marked	Just complete	0
" "	L-C.	0	0	"	" "	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.01cc

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TABLE 27

Syphilitic Case (J.H.) 0.05cc	NaCl solution 0.6cc	Amount of Complement 0.04cc	Dose of Complement
A			
Euglobulin (57°C)	"	Complete	0.0I5cc
" (unheated)	"	0	
Pseudoglobulin (57°C)	"	Very marked	
" (unheated)	"	0	
B			
Euglobulin (57°C)	"	Complete	0.0I25cc
" (unheated)	"	0	
Pseudoglobulin (57°C)	"	Complete	
" (unheated)	"	Just Complete	

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TABLE 28

Negative Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.02cc	0.04cc	0.07cc	0.1cc	NaCl Solution 0.6cc + Complement 0.03cc
Albumin (57°C)	L.	Marked	Very marked	Just complete	...	Just complete
" "	L-C.	"	" "	Complete	...	
Albumin (unheated(	L.	Faint trace	Distinct	Very marked	Just complete	Very marked
" "	L-C.	" "	"	" "	" "	
Positive Case 0.05cc						
Serum (57°C)	L.	Marked	Very marked	Just complete	...	Almost complete
" "	L-C.	Trace	Distinct	Marked	Almost complete	
Albumin (57°C)	L.	Very marked	Just complete	...	...	Complete
" "	L-C.	" "	" "	..,	...	
Albumin (unheated)	L.	Faint trace	Marked	Very marked	Almost complete	Almost complete
" "	L-C.	Very " "	"	" "	Complete	

Emulsions 0.6cc + Complement 0.05cc = Just complete.

Dose of Complement = 0.015 cc

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TABLE 29.

Negative case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.02cc.	0.04cc.	0.07cc.	NaCl Solution 0.6cc + Complement 0.02cc.
Serum (57°C)	L. L-C.	Very marked " "	Complete "	... ...	Complete
Albumin (57°C)	L. L-C.	Complete "	... ...	... ...	Just complete
Albumin (unheated)	L. L-C.	... ...	Complete "	... ...	Complete
Syphilitic Case 0.05cc.	**0.6cc**	**0.04cc**	**0.07cc**	**0.1cc**	**0.02cc**
Serum (57°C)	L. L-C.	Just complete ...	... Distinct	... Very marked	Almost complete
Albumin (57°C)	L. L-C.	Complete "	... ...	... ...	Complete
Albumin (unheated) " "	L. L-C.	Almost complete " "	Complete "	... ...	Just complete

Emulsions 0.6cc + Complement 0.02cc = Complete

Dose of Complement = 0.005cc.

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TABLE 30

A

Syphilitic Case (W.B.) 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl Solution + Complement 0.04cc
Serum (57°C)	L.	Very marked	Almost complete	Complete	...	Just complete
" "	L-C.	0	Faint trace	Distinct	Very marked	
Euglobulin (57°C)	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	Marked	Just "	...	...	
Euglobulin (unheated)	L.	0	0	0	0	0
	L-C.	0	0	0	Trace	
Pseudoglobn. (57°C)	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	Very marked	"	...	...	
Pseudoglobn. (unheated)	L.	0	0	Faint trace	Very marked	Marked
" "	L-C.	0	0	0	Distinct	
Globulin (ppt. by CO_2 gas) (57°C)	L.	Very marked	Just complete	Complete	...	Complete
	L-C.	Marked	Very marked	Just "	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	0	Trace	Marked	Complete
	L-C.	0	0	0	Distinct	

Emulsions 0.6cc + Complement 0.05cc = Complete

Dose of Complement = 0.015cc

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TABLE 30

B

Syphilitic case (W.B.) 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.22cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	Very marked	Just complete	Complete	...	...	Complete
" "	L-C.	...	Marked	Just "	Complete	...	
Serum (unheated)	L.	0	0	Trace	Very marked	...	Just complete
" "	L-C.	0	0	0	Trace	Just compl.e	
Euglobulin (57°C)	L.	Just compl.e	Complete	...	...	...	Complete
" "	L-C.	" "	"	...	...	...	
Euglobulin (unheated)	L.	0	0	0	0	Faint trace	0
" "	L-C.	0	0	0	0	" "	
Pseudoglob.n (57°C)	L.	Compl.e	...	...	...	...	Complete
	L-C.	Very marked	Just complete	Complete	...	...	
Pseudoglobn. (unheated)	L.	...	Distinct	Just complete	Complete	...	Complete
" "	L-C.	...	0	Almost "	"	...	
Globulin (ppt. by CO_2 gas) (57°C)	L.	Very marked	Just complete	Complete	...	...	Complete
	L-C.	Trace	" "	"	...	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	0	0	Very marked	...	Just complete
	L-C.	0	0	0	Faint trace	Trace	

Emulsions 0.6cc + Complement 0.03cc = Complete

Dose of Complement = 0.0125cc

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TABLE 31

A

Syphilitic case (J.H.) 0.05cc.	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	Very marked	Complete	...	...	Complete
" "	L-C.	...	Distinct	Very marked	Just complete	
Euglobulin (57°C)	L.	Almost complete	Complete	...	...	Complete
" "	L-C.	Faint trace	Marked	Almost complete	Complete	
Euglobulin (unheated)	L.	0	0	0	0	0
	L-C.	0	0	0	0	
Pseudoglobn. (57°C)	L.	0	Trace	Very marked	...	Very marked
" "	L-C.	0	Faint "	Marked	Almost complete	
Pseudoglobn. (unheated)	L.	0	0	0	0	0
	L-C.	0	0	0	0	
Globulin (ppt. by CO gas) (57°C)	L.	Very marked	Just complete	Complete	...	Complete
	L-C.	Marked	Almost complete	"	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	0	0	Faint trace	Complete
	L-C.	0	0	0	0	

Emulsions 0.6cc + Complement 0.05cc = Complete

Dose of Complement = 0.015cc

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TABLE 3I

B

Syphilitic case (J.H.) 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.22cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	Very marked	Just complete	Complete	...	...	Complete
" "	L-C.	0	Faint trace	Very marked	Complete	...	
Serum (unheated)	L.	0	0	Almost complete	Just complete	Complete	Complete
" "	L-C.	0	0	0	0	Marked	
Euglobulin (57°C)	L.	Marked	Just complete	Complete	...	...	Complete
" "	L-C.	Faint trace	Very marked	Just complete	Complete	...	
Euglobulin (unheated)	L.	0	0	0	0	Faint trace	0
" "	L-C.	0	0	0	0	Very ft. trace	
Pseudoglob^{n}. (57°C)	L.	Just complete	Complete	...	...	...	Complete
" "	L-C.	" "	"	...	...	...	
Pseudoglob^{n}. (unheated)	L.	0	0	Almost complete	Complete	...	Just complete
" "	L-C.	0	0	Marked	Just "	...	
Globulin (ppt. by CO_2 gas) (57°C)	L.	Almost complete	Complete	...	...	...	Complete
	L-C.	Very marked	Just complete	Complete	...	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	0	0	Very marked	...	Complete
	L-C.	0	0	0	0	Just complete	

Emulsions 0.6cc + Complement 0.03cc = Complete

Dose of Complement = 0.0125cc

.....

TABLE 32

A

Negative Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.22cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	Just complete	Complete	...	...	...	Complete
" "	L-C.	" "	"	...	...	...	
Serum (unheated)	L.	0	Marked	Very marked	Just complete	...	Almost complete
" "	L-C.	0	0	Distinct	Very marked	...	
Euglobulin (57°C)	L.	Marked	Just complete	Complete	...	...	Just complete
" "	L-C.	Very "	" "	"	...	...	
Euglobulin (unheated)	L.	0	Faint trace	Trace	Distinct	Very marked	Faint trace
" "	L-C.	0	" "	"	"	" "	
Pseudoglobn. (57°C)	L.	Marked	Complete	...	...	...	Complete
	L-C.	Almost complete	"	...	...	...	
Pseudoglobn. (unheated)	L.	0	0	Trace	Almost complete	Complete	Trace
" "	L-C.	0	0	0	Distinct	Just "	
Globulin (ppt. by CO gas) (57°C)	L.	Almost complete	Complete	...	...	...	Complete
	L-C.	" "	"	...	...	...	
Globulin (ppt. by CO gas) (unheated)	L.	0	0	Faint trace	Distinct	...	Just complete
	L-C.	0	0	" "	Very marked	...	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.01cc

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TABLE 32

B

Syphilitic Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	...	Just complete	Complete	...	Complete
" "	L-C.	...	0	0	Distinct	
Serum (unheated)	L.	...	0	0	Faint trace	Complete
" "	L-C.	...	0	0	0	
Euglobulin (57°C)	L.	Very marked	Complete	...	...	Complete
" "	L-C.	0	Very marked	Complete	...	
Euglobulin (unheated)	L.	0	0	Distinct	Very marked	Faint trace
" "	L-C.	0	0	0	0	
Pseudoglob^{n}. (57°C)	L.	Marked	Just complete	Complete	...	Just complete
" "	L-C.	"	" "	"	...	
Pseudoglob^{n}. (unheated)	L.	0	0	Marked	Just complete	0
" "	L-C.	0	0	Distinct	" "	
Globulin (ppt.) by CO gas) (57°C)	L.	Faint trace	Very marked	Complete	...	Complete
	L-C.	0	0	Almost "	Complete	
Globulin)ppt. by CO gas) (unheated)	L.	0	0	0	Trace	Almost complete
	L-C.	0	0	0	0	

Emulsions 0.6cc - Complement 0.03cc = Complete

Dose of Complement = 0.01cc

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TABLE 33

Negative Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement. 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl solution + Complement 0.04cc
A						
Serum (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Globulin (ppt. by CO_2 gas) (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	Very faint trace	Almost complete	Complete	Just complete
	L-C.	Very faint trace	Trace	Almost complete	Complete	
Supernatant fluid (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Supernatant fluid (unheated)	L.	Complete	...	...	...	Just Complete
	L-C.	"	...	...	...	
B						
Syphilitic Case 0.05cc	0.6cc	0.07cc	0.11cc	0.16cc	0.22cc	0.04cc
Serum (57°C)	L.	0	0	Marked	...	Complete
	L-C.	0	0	0	0	
Globulin (ppt. by CO_2 gas) (57°C)	L.	0	Marked	...	...	Complete
	L-C.	0	0	0	Complete	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	0	0	...	Complete
	L-C.	0	0	0	0	
Supernatant fluid (57°C)	L.	Faint trace	Just complete	Complete	...	Complete
	L-C.	0	0	0	Trace	
Supernatant fluid (unheated)	L.	0	Trace	Complete	...	Very marked
	L-C.	0	0	0	0	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.005cc

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T A B L E 34

A

Normal Case 0.05cc	Emulsions 0.6cc	Amounts of Guinea-pig's Complement 0.04cc	0.07cc	0.11cc	0.16cc	0.6cc NaCl solution + Complement 0.04cc
Serum (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Serum (unheated)	L.	0	0	Almost complete	Complete	Just complete
" "	L-C.	0	Complete	...	...	
Supernatant fluid (57°C)	L.	Complete	...	...	...	Complete
	L-C.	Just complete	Complete	...	...	
Supernatant fluid (unheated)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
Globulin (ppt. by dialysis) (57°C)	L.	Just complete	Complete	...	...	Complete
	L-C.	" "	"	...	...	
Globulin (ppt. by dialysis) (unheated)	L.	0	Distinct	Marked	Very marked	Complete
	L-C.	0	"	"	Complete	
CO_2- Globulin (57°C)	L.	Complete	...	...	...	Complete
	L-C.	"	...	...	...	
CO_2- Globulin (unheated)	L.	Trace	Very marked	Complete	...	Complete
" "	L-C.	Trace	" "	"	...	
Globulin (ppt. by Ammonium Sulphate) (57°C)	L.	Just complete	Complete	...	...	Complete
	L-C.	" "	"	...	...	
Globulin (ppt. by Ammonium Sulphate) (unheated)	L.	Faint trace	Marked	Just complete	Complete	Complete
	L-C.	Trace	"	" "	"	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.01cc.

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TABLE 34

B

Positive Case	Emulsions	Amounts of Guinea-pig's Complement				Antibody + NaCl Sol^{n} 0.6cc + Complement
0.05cc	0.6cc	0.04cc	0.07cc	0.11cc	0.16cc	0.04cc
Serum (57°C)	L.	Almost compl^{e}	Complete	...	...	Complete
" "	L-C.	0	Distinct	Marked	Just complete	
Supernatant fluid after dialysis (57°C))	L.	Just complete	...	...	...	Complete
	L-C.	0	Almost complete	Complete	...	
Supernatant fluid after dialysis (unheated)	L.	Marked	Complete	...	...	Complete
	L-C.	Trace	Almost "	Complete	...	
Globulin (ppt. by dialysis) (57°C)	L.	Marked	Complete	...	...	Complete
	L-C.	0	Distinct	Just complete	...	
Globulin (ppt. by dialysis) (unheated)	L.	0	Marked	Very marked	...	Almost complete
	L-C.	0	0	0	Trace	
Globulin (ppt. by CO_2 gas) (57°C)	L.	Marked	Just complete	Complete	...	Complete
	L-C.	Faint trace	Marked	Just complete	...	
Globulin (ppt. by CO_2 gas) (unheated)	L.	0	Trace	Marked	...	Complete
	L-C.	0	0	0	Marked	
Globulin (ppt. by 50% Ammon. Sulphate) (57°C)	L.	Very marked	Complete	...	...	Complete
	L-C.	0	Very marked	Complete	...	
Globulin (ppt. by 50% Ammon. Sulphate) (unheated)	L.	0	Faint trace	Distinct	...	Trace
	L-C.	0	0	Faint trace	Trace	

Emulsions 0.6cc + Complement 0.03cc = Complete.

Dose of Complement = 0.01cc

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TABLE 35

Normal Case	Emulsions	Amounts of Guinea-pig's Complement			NaCl Sol^{n} 0.6cc + Complement
	0.6cc	0.04cc			0.04cc
Spinal fluid 0.2cc	L.	Complete			Complete
	L-C.	"			
Globulin 0.2cc	L.	Complete			Complete
	L-C.	"			
Syphilitic case	0.6cc	0.18cc	0.26cc	0.38cc	0.04cc
Spinal fluid 0.2cc	L.	Trace	Complete	...	Complete
	L-C.	0	0	Trace	
Globulin 0.2cc	L.	Marked	Complete	....	Complete
	L-C.	0	0	Trace	

Emulsions 0.6cc + Complement 0.04cc = Complete.

Dose of Complement = 0.01[illegible]

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TABLE 36

Case of General Paralysis	Doses of Complement causing just complete lysis		
	With L.emulsion	With L-C.emulsion	Without emulsion
Serum heated	5	I6	2.6
Euglobulin heated	4	4.6	2.6
" unheated	I0.6 (no lysis)	I0.6 (trace of lysis)	2.6 (no lysis)
Pseudoglob^{n} heated	4	4	2.6
" unheated	I0.6 (very marked lysis)	I0.6 (distinct Lysis)	2.6 (marked lysis)
CO_{γ} globulinheated	4.6	7.3	2.6
" " unheated	I0.6 (marked lysis)	I0.6 (distinct lysis)	2.6
Case of General Paralysis			
Serum heated	4	I0.6	2.6
Euglobulin heated	4	8.6	2.6
" unheated	I0.6 (no lysis)	I0.6 (no lysis)	2.6 (no lysis)
Pseudoglob^{n} heated	I0.6	I3.3	4
" unheated	I0.6 (no lysis)	I0.6 (no lysis)	2.6 (no lysis)
CO_{γ} globulinheated	4.6	7.3	2.6
" " unheated	I0.6 (trace of lysis)	I0.6 (no lysis)	2.6

TABLE 37

Case of General Paralysis	Doses of Complement causing Just Complete Lysis.		
	With L.Emulsion	With L-C.Emulsion	Without Emulsion
Serum heated	5.6	8.8	3
" unheated	I4	I7.6	3
Euglobulin heated	3	3	3
" unheated	I7.6 (trace of Lysis)	I7.6 (trace of Lysis)	5.6 (trace of Lysis)
Pseudoglobn heated	2.5	5.5	3
" unheated	8.8	II.5	3
CO_2 Globulin heated	5.6	5.6	3
" " unheated	I4	28	3
Case of General Paralysis			
Serum heated	5.6	I2	3
" unheated	I2	24	3
Euglobulin heated	5.6	8.8	3
" unheated	I7.6 (trace of Lysis)	I7.6 (trace of Lysis)	5.6 (no Lysis)
Pseudoglobn heated	3.5	3.5	3
" unheated	8.8	I2.8	3
CO_2 Globn heated	5	5.6	3
" "unheated	I4	I7.6	3

TABLE 38

Negative Serum from case of Dementia Precox	Doses of Complement causing Just Complete Lysis		
	With L. Emulsion	With L-C.Emulsion	Without Emulsion
Serum heated	4	4	4
" unheated	I6	22	5
Euglobulin heated	7	7	4
" unheated	26	26	7 (Distinct Lysis)
Pseudoglob^{n} heated	7	7	4
" unheated	I8	24	7 (marked Lysis)
CO_{2} Globulinheated	6	6	4
" " unheated	26	22	4
Case of General Paralysis			
Serum heated	7	22	4
"unheated	I6 (trace of Lysis)	40	4
Euglobulin heated	6	I0	4
" unheated	22	35	7 (marked Lysis)
Pseudoglob^{n} heated	8	7	4
" unheated	I6	I6	7 (trace of Lysis)
CO_{2} Glob^{n} heated	I0	I4	4
" " unheated	I6 (trace of Lysis)	22 (trace of Lysis)	5

TABLE 39

Negative Serum from Imbecile	Doses of Complement causing Just Complete Lysis.		
	With L.Emulsion	With L-C.Emulsion	Without Emulsion
Serum heated	4	4	4
"unheated	II	7	4
Globulin ppt. by dialysis heated	4	4	4
" " "unheated	I8	I6	4
Serum after dialysis heated	4	4	4
" " unheated	4	4	4
Globulin ppt. by CO_2 Gas heated	4	4	4
" " unheated	I0	I0	4
Globulin ppt. by 50% Ammon. Sulphate heated	4	4	4
" " " unheated	II	II	4
Case of General Paralysis.			
Serum heated	6	I6	4
Globulin ppt. by dialysis heated	7	II	4
" " " unheated	I5	30	5
Serum after dialysis heated	4	9	4
" " " unheated	7	I0	4
Globulin ppt. by CO_2 gas heated	7	II	4
" " " unheated	I7	34	4
Globulin ppt. by 50% Ammon. Sulphate heated	6	I0	4
" " " unheated	I8	34	5 (trace of Lysis)