[1]THE ACTION OF THE COMPLEMENT-FRACTIONS ON A TROPIN-*B. TYPHOSUS* SYSTEM WITH COMPARATIVE HAEMOLYTIC EXPERIMENTS.

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CONTENTS.

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Introductory.

THIS communication contains a record of a series of experiments undertaken primarily to elucidate the action of complement and its fractions on a Tropin-*B. typhosus* system as estimated by the phagocytic index. Haemolytic experiments were also undertaken with the combined object of testing the activity of the complement-fractions and of investigating by the use of another system, certain points which arose in the course of the phagocytosis experiments. The haemolytic experiments are recorded in Part II of this communication. The term "Tropin" employed throughout the paper to designate the antibody in an inactivated immune serum which, in the absence of complement, is able to effect sensitisation of micro-organisms for phagocytosis, has been adopted solely for the sake of convenience and entirely without prejudice to the conception we have formed of its relation to the immune opsonin so-called, or to the general antigen-antibody reaction which, under certain conditions of experiment, involves the fixation of complement.

We are the more reluctant to use specific terms for anti-substances which under certain systems perform certain effects, in view of the fact that the experiences we have had in the course of this research, have impressed on us the necessity of reviewing our conception of antigen-antibody reactions in the light of the more recent work on the application of the principles of colloidal chemistry to immunity reactions. It appears probable at least that many of the inhibition and acceleration phenomena occurring in immunity reactions, will find a reasonable explanation in the laws which govern the mutual interaction of colloids to which all antigen and antibody solutions belong.

In 1907 Ferrata showed that if fresh guinea-pig serum was dialysed for 24 hours against running water, the haemolytic complement could be separated into two fractions. The one fraction was contained in the precipitated globulin, the other in the supernatant fluid. Complementing was obtained by the united action of the two fractions, but not by either fraction acting independently. Since the publication of Ferrata's paper, two other methods for the separation of the complement-fractions have been devised, that of Sachs (1909), and that of Liefmann (1909). Sachs' method consists in the partial precipitation of guinea-pig serum by the addition of very dilute hydrochloric acid. Liefmann effected a similar separation by saturation with carbon dioxide. These three

methods depend on the production of a precipitate of euglobulin. One fraction of the complement is carried down with this precipitate while the other fraction remains in the supernatant fluid. The precipitate is separated in the centrifuge, washed and dissolved or suspended in 0·85 % saline solution. Such a solution contains one of the fractions of the complement. The other fraction is contained in the supernatant fluid which is made isotonic by the addition of a suitable quantity of sodium chloride. If the separation has been successful, neither fraction has by itself any action as complement, while excellent complementing is obtained if the two fractions are mixed together.

References to later work will be found under the appropriate heading in the body of the paper.

Preparation of materials employed in the experiments.

Preparation of the Complement-Fractions.

The method used in preparing the fractions of the complement was slightly modified from that of Liefmann (1909). This method carried out in the manner which is to be described, yielded very satisfactory results.

To one volume of fresh guinea-pig serum is added nine volumes of ice-cold distilled water. The mixture is placed in a bottle of a capacity equal to about twice the volume of the contained mixture. The bottle is provided with a rubber cork which should not be too small. The bottle containing the diluted serum is placed in a can containing ice-cold water. A brisk stream of CO_2 is allowed to flow into the bottle for a few seconds. The bottle is then corked and briskly shaken. The bottle is repeatedly filled with CO_2 and shaken until the fluid is completely saturated. The mixture is then placed in the cold room or ice safe for an hour, when it is usually found that the precipitate of globulin has begun to flocculate and settle to the bottom of the bottle. The mixture is then centrifugalised. The supernatant fluid is poured off and should be quite clear. The exact volume of the fluid is measured and sufficient sodium chloride is dissolved in it to obtain a concentration of 0·85 %. This represents a 1 in 10 dilution of the "end-piece" of the complement.

The deposit is twice washed in ice-cold distilled water (the deposit is suspended in ice-cold distilled water and centrifugalised). The washed deposit is then dissolved in a quantity of cold 0·85 % salt

solution equal to the volume of the original 1 in 10 dilution of the serum. The result is a 1 in 10 dilution of mid-piece. The globulin deposit should dissolve readily and the resulting solution in saline should be nearly clear. It is usually necessary to filter the mid-piece solution through filter paper to remove the particles of foreign matter which get into the centrifugalised deposit. It is necessary to keep the mixture and the precipitated globulin as cold as possible during the entire process. Unless this is done, great difficulty is experienced in attempting to re-dissolve or re-suspend the globulin precipitate in saline. The only other point which requires special attention is concerned with the length of time which it is advisable to allow, after the mixture has been saturated with carbon dioxide and before it is centrifugalised. If the interval allowed be too short, it will be found that the separation has been incomplete and that the supernatant fluid still contains a demonstrable quantity of mid-piece. If too long a time is allowed, the globulin precipitate is very difficult to dissolve. After many trials it was found best to allow an interval of one hour after saturation with CO_2 and before the mixture was centrifugalised. This procedure gave in nearly every case, a satisfactory separation of mid- and end-piece.

If the supernatant fluid is allowed to stand for a few hours before the sodium chloride is added, a further deposit of globulin occurs. This readily dissolves when the salt is added. In practice the salt was always added immediately after the supernatant fluid had been separated by the centrifuge. On two occasions the supernatant fluid was intentionallly kept for 24 hours without addition of salt. The second deposit of globulin was collected, washed in distilled water and re-suspended in saline. It was not found to contain any demonstrable quantity of either mid- or end-piece.

Preparation of Blood Corpuscles and Haemolytic Serum.

These were prepared in the usual manner. Sheep's blood was collected from the jugular vein and defibrinated with glass beads. The corpuscles were separated by the centrifuge and thoroughly washed.

The haemolytic serum was prepared by injecting the ear vein of a rabbit with washed sheep corpuscles. The serum obtained from the rabbit was inactivated in the usual way by heating at 56° C. for half an hour.

Immune Typhoid Serum.

This was prepared by immunisation of the rabbit with killed typhoid bacilli, the inoculation being made intravenously. Apart from the few experiments dealing with the splitting of the fresh immune serum itself by the CO_2 method, the same immune serum was used throughout. It was kept after inactivation in the cold room at a temperature of 1°–2° C.

Bacillary Emulsion.

The bacillary emulsion was prepared from an emulsion in saline of *B. typhosus* grown on ordinary agar for 18 hours at a temperature of 20° C. The advantage of using for phagocytic experiments bacilli grown at this temperature is considerable. The bacilli are of more uniform size, longer, stouter and more readily counted inside leucocytes than bacilli grown at 37° C.

Part I.

Technique of the Phagocytosis Experiments.

In a few of the earlier experiments the constituents entering into each test were mixed together directly without previous digestion of the heated immune serum with the bacillary emulsion. This technique, however, did not yield satisfactory results and recourse was had to the method of previous digestion of the immune serum + mid-piece with the bacilli—a method which has been shown to give the best results in corresponding haemolytic experiments.

The following may be taken as a sample of technique most frequently followed in the course of the experiments:

Inactivated immune serum was diluted to a concentration of, *e.g.* 1 in 500. Equal volumes of this diluted serum and of the bacillary suspension and salt solution (1 c.c. of each, *i.e.* 3 c.c. in all) were mixed together in a large test tube and placed in the incubator at 37° C. for a period varying from 30 mins. to one hour.

Similarly, equal volumes of the mid-piece solution (corresponding to fresh complement in dilution of 1 in 10), immune serum and bacillary emulsion (1 c.c. of each, *i.e.* 3 c.c. in all) were digested together for the same time. Corresponding volumes of bacillary emulsion and salt solution alone were treated similarly. At the end of the period of

sensitisation the tubes in which immune serum was present showed as a rule a greater or less degree of agglutination. Any detrimental effect of such agglutination in the subsequent phagocytosis experiments was, however, almost entirely avoided by rolling the tubes between the hands for some time when it was found that the clumps practically disappeared. Shaking was sometimes resorted to and in fact acted in the same way and more quickly but had the disadvantage of causing considerable frothing.

When all the constituents were ready the phagocytosis test proceeded as follows (in a typical experiment). By means of capillary pipettes the essential constituents were taken up one by one into capillary pipettes with rubber teats from which they were immediately blown into small test tubes and thoroughly mixed together. Immediately after mixing each test tube was placed in a to-and-fro shaker working in an incubator kept at 37° C. The period of incubation was as a rule 10, 12, 14, minutes and most frequently 12 minutes. No intra- or extracellular lysis occurred with this short period of incubation and with the materials employed. The following indicates the constituents of a typical experiment:

Previous sensitisation. "A" immune serum (1 in 500) 1 c.c. + bacillary emulsion 1 c.c. + 0·85 % saline 1 c.c.

"B" immune serum (1 in 500) 1 c.c. + bacillary emulsion 1 c.c. + mid-piece solution (corresponding to 1 in 10 fresh serum) 1 c.c.

"C" bacillary emulsion 1 c.c. + salt solution 2 c.c.

Phagocytosis test.

(1)	3 vols. salt solution	+ 2 vols.	Leucocytes	+ 1 vol. "A."
(2)	3 vols. „ „	+ 2 vols.	„	+ 1 vol. "B."
(3)	3 vols. end-piece (1 in 10)	+ 2 vols.	„	+ 1 vol. "A."
(4)	3 vols. „ („)	+ 2 vols.	„	+ 1 vol. "B."
(5)	3 vols. complement (1 in 10)	+ 2 vols.	„	+ 1 vol. "A."
(6)	3 vols. „ („)	+ 2 vols.	„	+ 1 vol. "C."
(7)	3 vols. end-piece	+ 2 vols.	„	+ 1 vol. "C."

In the tables only the essential constituents whose action is being investigated, are inserted.

It will be seen, therefore, that the fresh diluted complement or the end-piece came into action with the tropin and bacilli, or bacilli only, during the incubation stage of 10–12 minutes.

At the end of the period of incubation a small drop from each tube was taken up in a capillary pipette and placed on a slide from which films were made. The films were fixed in methyl alcohol and stained with Giemsa solution.

The number of cells counted varied from 100–300, no constant number being counted for the obvious reason that in films which presented numerous leucocytes containing no included bacilli a very much larger number had to be counted in comparison with films in which practically every leucocyte had been active.

It may be well to indicate here that T (1 in 500) or tropin (1 in 200) denotes the dilution of immune serum employed for sensitisation purposes. In the final phagocytosis experiment the concentration of immune serum in the total mixture would be (when six volumes in all were employed) 1 in 9000. Similarly the concentration of mid-piece in the total mixture would be (supposing the pure mid-piece (= 1 in 10) were digested in equal volumes with immune serum and bacilli originally) 1 in 18, or = 1 in 180 of pure original serum. Similarly where three volumes of end-piece were employed, as proved most satisfactory, the concentration in the total mixture of six volumes would be 1 in 2 (= 1 in 20 of original guinea-pig serum) and so on.

Analysis of the Experimental Results.

Phagocytosis Experiments.

For the following reason we have decided to tabulate the great majority of the experimental results in chronological order.

At the commencement of the work it was anticipated from experience with haemolytic systems, that the demonstration of the complementing effect of a mid-piece-end-piece complex on a Tropin-*B. typhosus* system would be a comparatively easy task. This did not, however, prove to be the case and although it would have been a simple matter to choose from the mass of experiments only those which showed these typical and more or less expected effects, we felt assured that, behind what appeared at the time to be anomalous or inconsistent results, there lay the basis of problems which further experimentation would, and did, go far to elucidate. Moreover, by investigation of such problems as they arose in the course of the phagocytosis experiments, by means of a haemolytic system (Part II), we were enabled to throw a further light on the mechanism at work in producing the results in question. There can be little doubt, however, that it will only be possible to obtain a clear understanding of the various effects, when they are considered in the light of the chemistry of colloidal solutions where very similar inhibition phenomena are met with. The difficulties we

have encountered in the course of the work, mainly from persistent inhibition effects, have opened up in fact a most interesting field for further research and we hope that the complete record of the experiments made will form a guide for other workers in this field.

Explanation of Abbreviations used in Tables.

T = Tropin-containing serum, *i.e.* inactivated immune typhoid serum.
C = Complement from the guinea-pig.
M = Mid-piece from guinea-pig complement, corresponding to complement 1 in 10.
E = End-piece from guinea-pig complement, and corresponding to complement diluted 1 in 10.

Mid-piece and End-piece acting independently.

(Illustrative Experiments: See Table I, Exps. 1, 2, 7, 13, 14, 17, 19, 20, 21, 24 and 27.)

The investigation of the normal opsonic amboceptor in guinea-pig serum or in its mid-piece and end-piece fractions did not come specially within the scope of the present research. In the course of our experiments, however, and especially in the earlier ones, it was essential for control purposes to determine the independent action of these fractions.

It will be seen from the experiments in which separate estimations of mid-piece and end-piece are made and compared with complement diluted 1 in 10 that (1) the mid-piece has practically no sensitising action by itself, and (2) the end-piece though possessing a slight though definite action comes very short of that obtained with the diluted complement.

In Exp. 17, it appears that three volumes of end-piece are required to give the same phagocytic index as one volume of complement diluted 1 in 10.

In the two earlier experiments (1 and 2), the mid-piece appears to have a slight action by itself but the exact relation of the mid-piece concentration to that of the complement from which it was precipitated was not known. In all the later experiments the precipitated mid-piece was dissolved in a bulk of saline equivalent to the amount of diluted serum used.

In Exp. 21, the action of four different end-pieces of various ages are compared and on the whole there appears to be little difference between them as estimated by the phagocytic index.

TABLE I. *Phagocytosis Experiments I—XXIX.*

Exp. I. 11/5/11.

No previous sensitisation. Age of fractions 2 days. Incubation 15 mins.

	P. I.
T (1 in 5)	1·3
Do. + C (1 in 1)	3·8
Do. + C (1 in 10) ...	2·3
Do. + M (concen[a]) ...	0·14
Do. + M (1 in 10) ...	0·4
Do. + E (=1 in 10) ...	0·8
C (1 in 1) ...	1·4
C (1 in 10) ...	0·8
M (concen[a]) ...	0·1
M (1 in 10) ...	0·06
E (=1 in 10) ...	0·03
Heated normal rabbit ser.	0·03

Exp. II. 12/5/11.

No previous sensitisation. Mid-piece prepared 24/3/11 in powder form. Strength of M relative to C unknown.

	P. I.
T (1 in 5)	3·0
Do. + C (1 in 1)	9·1
Do. + M (1 in 5)	3·7
Do. + E (1 in 10) ...	3·6
C (1 in 1) ...	3·5
M (1 in 5)	0·04
E (=1 in 10) ...	0·058
Heated normal rabbit ser.	0·03
Fractions active haemolytically.	

Exp. III. 15/5/11.

No previous sensitisation. Same fractions as in Exp. II. Incubation 10 mins.

	P. I.
T (1 in 5)	0·3
Do. + C (1 in 1)	1·4
Do. + M (1 in 5)	0·4
Do. + E (=1 in 10) ...	0·2
C (1 in 1)	0·5
Do. + (M_1 + E_4)	0·4

Exp. IV. 17/5/11.

Previous sensitisation. Fractions freshly prepared. Incubation 10 mins.

	P. I.
T (1 in 1)	0·4
Do. + C (1 in 10) ...	1·9
Do. + M	0·4
Do. + E	0·33
Do. + M + E	0·53
C (1 in 10) ...	0·009
Fractions active haemolytically.	

Exp. V. 23/5/11.

Previous sensitisation. New E 7 days old. Old E 2 months old. M 7 days old. Incubation 12 mins.

	P. I.
T (1 in 20)	0·3
Do. + C (1 in 10) ...	2·1
Do. + M	0·53
Do. + E (new)	0·55
Do. + E (old)	0·58
Do. + M + E (new) ...	0·64
Do. + M + E (old) ...	1·3
C (1 in 10) ...	0·07
Fractions inactive haemolytically.	

Exp. VI. 24/5/11.

Previous sensitisation. M from powder of 23/3/11. E also of 23/3/11. Incubation 12 mins.

	P. I.
T (1 in 500)	3·8
Do. + C (1 in 10)	10·8
Do. + M + 1 E	5·8
Do. + M + 2 E	7·8
Do. + M + 3 E	8·0
Fractions inactive haemolytically.	

Exp. VII. 29/5/11.

Previous sensitisation. Fractions freshly prepared. Incubation 12 mins.

	P. I.
T (1 in 500)	1·7
Do. + 1 C (1 in 10) ...	5·2
Do. + 3 C (1 in 10) ...	5·2
Do. + M	1·78
Do. + 1 E...	2·3
Do. + 3 E	4·0
Do. + M. + 1 E.	6·1
Do. + M + 3 E	7·1
1 C (1 in 10) ...	?
3 C (1 in 10) ...	1·0
1 E	0·01
3 E	0·6
M	0·0
M + 1 E	0·09
M + 3 E	1·2
Haemolytically E was not pure.	

Exp. VIII. 30/5/11.

Previous sensitisation. M and E 1 day old. Incubation 12 mins.

	P. I.
T (1 in 500)	2·3
Do. + 1 C (1 in 10) ...	12·5
Do. + 3 C (1 in 10) ...	13·1
Do. + 3 E...	5·3
Do. + M	1·3
Do. + M + 3 E	8·5
E now haemolytically pure.	

Exp. IX. 1/6/11.

Previous sensitisation. Fractions fresh. Incubation 12 mins.

	P. I.
T (1 in 500)	3·5
Do. + 1 C (1 in 10)	6·3
Do. + 3 C (1 in 10)	9·3
Do. + 1 E	3·3
Do. + 3 E	4·7
Do. + M (1 in 10)	2·2
Do. + do. + 1 E	4·9
Do. + do. + 3 E	9·4
Do. + M (1 in 40)	4·8
Do. + do. + 1 E	7·0
Do. + do. + 3 E	8·9
Do. + M (1 in 160)	2·2
Do. + do. + 1 E	3·6
Do. + do. + 3 E	5·6
E haemolytically pure.	

TABLE I (*continued*).

EXP. X. 6/6/11.

Previous sensitisation. Same fractions as in Exp. IX, *i.e.* 5 days old. Incubation 12 mins.

	P. I.
T (1 in 5000)	0·01
Do. + 1 C (1 in 10) ...	1·16
Do. + 3 C (1 in 10) ...	3·7
Do. + M + 1 E	0·03
Do. + M + 3 E.	0·31
1 C (1 in 10) ...	0·009
3 C (1 in 10) ...	1·10

EXP. XI. 3/7/11.

Previous sensitisation. Fractions freshly prepared. Incubation 15 mins.

	P. I.
T (1 in 500)	3·9
Do. + C (1 in 10) ...	5·6
Do. + M	0·77
Do. + E	6·7
Do. + M + E	2·7
Do. + M + C	5·8
C (1 in 10) ...	0·0

E pure haemolytically.

EXP. XII. 4/7/11.

Previous sensitisation. M of Exp. XI. E 6 days old. Incubation 12 mins.

	P. I.
T (1 in 500)	2·6
Do. + C (1 in 10)	1·7
Do. + M	0·5
Do. + E	1·5
Do. + M + E	0·3
Do. + M + C	1·4
Do. + heated M	1·9
Do. + do. + E	0·6

EXP. XIII. 18/7/11.

Previous sensitisation. M and E, 8 days old. Incubation 13 mins.

	P. I.
T (1 in 500)	1·8
Do. + C (1 in 5)	2·5
Do. + M	0·12
Do. + 3 E...	4·3
Do. + M + 3 E	4·4
Do. + M + 3 heated E ...	1·8
Do. + M + C (1 in 5) ...	5·8
C (1 in 5)	0·12
3 E	0·01

EXP. XIV. 19/7/11.

Previous sensitisation. Fractions same as in Exp. XIII, *i.e.* 9 days old. Incubation 14 mins.

	P. I.
T (1 in 500)	1·7
Do. + C (1 in 5)	5·0
Do. + M	0·01
Do. + 3 E...	2·4
Do. + M + 3 E	0·6
Do. + M + 3 heated E ...	2·5
Do. + M + C (1 in 5) ...	5·4
C (1 in 5)	0·12
3 E...	0·009

EXP. XV. 20/7/11.

Previous sensitisation. Fractions same as in Exp. XIII, *i.e.* 10 days old. Incubation 13 mins.

	P. I.
T (1 in 500)	2·5
Do. + C (1 in 5)	0·67
Do. + M	0·18
Do. + 3 E	4·2
Do. + M + 3 E	0·59
Do. + M + 3 heated E ...	0·34
Do. + M + C (1 in 5) ...	1·1

EXP. XVI. 21/7/11.

Previous sensitisation. Fractions same as in Exp. XIII, *i.e.* 11 days old. Incubation 13 mins.

	P. I.
T (1 in 500)	1·2
Do. + 1 C (1 in 5) ...	4·7
Do. + 3 C (1 in 5) ...	5·8
Do. + 3 E	4·1
Do. + 3 heated E ...	2·9
Do. + M + 3 E	1·0
Do. + M + 3 heated E ...	0·87
Do. + M + C (1 in 5) ...	6·8

EXP. XVII. 21/9/11.

Previous sensitisation. Fractions freshly prepared. E heated ½ hour at 56° C. Incubation 12 mins.

	P. I.
T (1 in 500)	0·5
Do. + 2 C (1 in 10) ...	0·54
Do. + M	0·26
Do. + 3 E...	0·87
Do. + 3 heated E ...	1·01
Do. + M + 3 E	1·3
Do. + M + 3 heated E ...	1·09
1 C (1 in 10) ...	0·14
3 C (1 in 10) ...	1·06
3 E...	0·10
3 heated E ...	0·01

EXP. XVIII. 22/9/11.

Previous sensitisation. Same fractions, *i.e.* 1 day old. Incubation 12 mins.

	P. I.
T (1 in 500)	2·2
T (1 in 10)	0·77
T (1 in 500) + M	0·05
T (1 in 10) + M	0·39
T (1 in 500) + 3 E ...	2·1
T (1 in 500) + 3 heated E	1·4
T (1 in 10) + 3 E... ...	0·55
T (1 in 10) + 3 heated E...	1·3
T (1 in 500) + M + 3 E ...	0·14
T (1 in 500) + M + 3 heated E	0·04
T (1 in 10) + M + 3 E ...	0·72
T (1 in 10) + M + 3 heated E	0·77

EXP. XIX. 26/9/11.

Previous sensitisation. Same fractions, *i.e.* 5 days old.

	P. I.
T (1 in 500)	3·0
Do. + M	0·22
Do. + 3 E...	1·5
Do. + M + 3 E	1·6
3 E	0·09

EXP. XX. 27/9/11.

Previous sensitisation. Same fractions, *i.e.* 6 days old. Incubation 12 mins.

	P. I.
T (1 in 500)	5·0
Do. + M	0·28
Do. + 3 E...	4·9
Do. + M + 3 E	4·8
3 E	0·0

EXP. XXI. 29/7/11.

Previous sensitisation. End-pieces of various ages. Incubation 12 mins.

	P. I.
T (1 in 500)	1·7
Do. + 3 E "α" (1 day old)	3·5
Do. + 3 E "β" (Fresh) ...	2·7
Do. + 3 E "γ" (Fresh) ...	1·3
Do. + 3 E "δ" (10 days)...	1·8
3 E "α"	0·007
3 E "β"	0·02
3 E "γ"	0·03
3 E "δ"	0·03

TABLE I (*continued*).

Exp. XXII. 4/10/11.

Previous sensitisation. Effect of filtered mid-pieces of various ages. Incubation 12 mins.

	P. I.
T (1 in 500)	0·30
Do. + M "*a*" (16 days)...	0·29
Do. + M "*b*" (14 days)...	0·21
Do. + M "*c*" (6 days) ...	0·24
Do. + M "*d*" (6 days) ...	0·22
Do. + M "*e*" (2 days) ...	0·34
Do. + M "*f*" (Fresh) ...	0·10
Do. + 3 E...	0·77
Do. + M "*a*" + 3 E ...	2·2
Do. + M "*b*" + 3 E ...	1·1
Do. + M "*c*" + 3 E ...	1·3
Do. + M "*d*" + 3 E ...	2·7
Do. + M "*e*" + 3 E ...	2·7
Do. + M "*f*" + 3 E ...	3·3

Exp. XXIII. 9/10/11.

Previous sensitisation. Fractions freshly prepared. Mid-piece filtered through paper. Incubation 12 mins.

	P. I.
T (1 in 500)	3·3
Do. + M	0·4
Do. + 3 E...	5·9
Do. + M + 3 E	5·4

Fractions active haemolytically.

Exp. XXIV. 20/10/11.

Previous sensitisation. Fractions. freshly prepared. Incubation 14 mins.

	P.I.
T (1 in 200)	9·5
Do. + M	1·9
Do. + 3 C (1 in 10)... ...	11·6
Do. + 3 E	7·2
Do. + 3 heated E	7·3
Do. + M + 3 E	9·8
Do. + M + 3 heated E ...	1·8
Do. + M + 3 C (1 in 10) ...	8·3
Do. + heated M	7·4
Do. + heated M + 3 E ...	7·3
Do. + heated M + 3 heated E	8·8
Do. + heated M + 3 C (1 in 10)	8·5
3 C (1 in 10) ...	0·12
3 E	0·0
3 heated E	0·0

Exp. XXV. 21/10/11.

Previous sensitisation. Same fractions, *i.e.* 1 day old. Incubation 12 mins.

	P. I.
T (1 in 500)	5·4
Do. + M (1 in 10) ...	0·3
Do. + M (1 in 20) ...	1·1
Do. + M (1 in 40) ...	1·3
Do. + heated M	4·9
Do. + 3 E	4·3
Do. + M (1 in 10) + 3 E...	0·7
Do. + M (1 in 20) + 3 E...	0·38
Do. + M (1 in 40) + 3 E...	0·5
Do. + heated M + 3 E ...	1·1

Exp. XXVI. 22/10/11.

Previous sensitisation. Effect of filtered, unfiltered and heated mid-pieces.

	P. I.
T (1 in 500)	4·7
Do. + filtered M "*a*" ...	0·52
Do. + unfiltered M "*a*"...	0·14
Do. + heated M "*a*" ...	3·7
Do. + 3 E...	3·4
Do. + filtered M "*a*" + 3 E	0·6
Do. + unfiltd. M "*a*" + 3 E	2·6
Do. + heated M "*a*" + 3 E	1·9
Do. + filtered M "*b*" ...	4·6
Do. + unfiltered M "*b*"...	2·1
Do. + heated M "*b*" ...	5·9
Do. + filtered M "*b*" + 3 E	2·0
Do. + unfiltd. M "*b*" + 3 E	2·9
Do. + heated M "*b*" + 3 E	2·4

Exp. XXVII. 6/11/11.

Fractions freshly prepared. Incubation 14 mins.

	P. I.
T (1 in 500)	4·1
Do. + M (1 in 10) ...	5·2
Do. + M (1 in 20) ...	4·2
Do. + M (1 in 40) ...	4·1
Do. + M (1 in 80) ...	3·0
Do. + 3 E	5·2
Do. + M (1 in 10) + 3 E...	8·1
Do. + M (1 in 20) + 3 E...	7·6
Do. + M (1 in 40) + 3 E...	6·9
Do. + M (1 in 80) + 3 E...	5·1
Do. + 3 C (1 in 10) ...	8·6
3 C (1 in 10) ...	1·1
3 E	0·008

Exp. XXVIII. 8/11/11.

Same fractions, *i.e.* 2 days old.

	P. I.
T (1 in 500)	5·8
Do. + M (1 in 10) ...	2·9
Do. + M (1 in 20) ...	5·3
Do. + 3 E...	6·7
Do. + 3 heated E ...	8·1
Do. + M (1 in 10) + 3 E...	11·0
Do. + M (1 in 20) + 3 E...	12·3
Do. + M (1 in 10) + 3 htd. E	7·4

Exp. XXIX. 10/11/11.

Previous sensitisation. Same fractions, *i.e.* 4 days old.

	P. I.
T (1 in 500)	4·3
Do. + M (1 in 10) ...	0·46
Do. + M (1 in 20) ...	2·02
Do. + M (1 in 10) + 3 E ...	1·4
Do. + M (1 in 20) + 3 E ...	3·7

The end-piece when inactivated still possesses a very slight action (see Exp. 17). The independent action of end-piece though admittedly slight is best explained on the assumption that small amounts of active mid-piece are still present in the filtrate after removal of the deposit, and although in the haemolytic system (Part II) these fractions appeared to be pure, it is possible that, in a phagocytosis system, minute traces of mid-piece in the end-piece may be put in evidence. Moreover from certain results to be detailed later it would appear that the removal of the mid-piece may in certain cases enhance markedly the value of the end-piece relatively to the untreated serum.

The Action of Mid-piece and End-piece in concert.

On this point Exp. 7, may be consulted. In the latter part of that experiment, values of mid-piece and end-piece and combinations of such are compared with those of the diluted complement. It will be seen that three volumes of end-piece in combination with mid-piece give practically the same value as three volumes of complement diluted 1 in 10. Without the addition of mid-piece the three volumes of end-piece give only half the value of the diluted complement. It has to be noted, however, that this particular experiment was one of the few in which the mid-piece exerted no inhibitory action throughout and consequently excellent complementing effects ensued.

Hata (1908) in a communication on the structure of the normal opsonin performed a few opsonic experiments with staphylococci and the fractions of normal guinea-pig serum obtained by the method of dialysis. Neither of the fractions appeared to have any considerable sensitising power when acting independently, but when acting in concert, values were obtained which approached far more closely to those given by the original serum than we were able to obtain in our experiments.

The Action of Complement on Tropin.

It is unnecessary to discuss this question in any detail as it has been shown by various workers that an inactivated immune typhoid serum which of itself effects sensitisation of organisms for phagocytosis (so-called tropin-content) can have this action greatly enhanced by the addition of guinea-pig complement. In the great majority of the experiments recorded in the tables, such complementing effects are very clearly seen, the total effect of the mixture of heated immune serum (T)

and complement being considerably greater than the sum of the two separate effects. In a few experiments, however (*e.g.* Exps. 12, 15, 17), no complementing effect occurred. In fact the value of the mixture was (in two of these experiments) considerably less than that of the tropin acting separately. In some of these experiments the complement though inactive or inhibitory in the phagocytosis system was found to be active in a haemolytic system.

This peculiar action of complement on tropin cannot be readily explained without examining a large series of guinea-pig complements. Out of over 20 complements tested it occurred only three times. From the frequent inhibitory effects obtained with various mid-pieces and presently to be described, it might be suggested that in a particular complement the ratio of mid-piece to end-piece may be altered, with the result that inhibition of the tropic effect occurs owing to excess of mid-piece. The fact that in the haemolytic system such complements proved active is of great interest, but it has to be remembered that in the latter case the time factor may be of importance. In the phagocytosis experiments the period of incubation could not be extended much beyond 12 minutes owing to the onset of intracellular lysis which affected considerably the accuracy of the count. It is also not unlikely that the inactivity of the complement in these few cases may have been due to a slight cloudiness not observed at the time of experiment.

Remarks on Tropins and Immune Opsonins.

Our experiments were not directly devised with a view to obtaining evidence on the identity or non-identity of the tropins and immune opsonins, as we are of opinion that the evidence drawn simply from their mode of action cannot be employed in support of the view that these substances are essentially different. In Exp. 10 an attempt was made to determine whether the addition of complement to an inactivated immune serum diluted to a point where tropic action was in abeyance, would still give a definite complementing result. It will be seen from this experiment that a very definite complementing effect was obtained, a result which we are inclined to regard as analogous to the marked fixation of complement which occurs in mixtures of antigen and antibody which yield no visible precipitate. In this connection the recent experiments of Dean (1911) may be consulted.

By adding together the separate values of tropin and complement and subtracting the sum from that obtained with the mixture of tropin

and complement, the percentage increase so calculated may give some measure of the fixation of complement in each case. This varies very greatly as might be expected in the different experiments. In Exps. 1, 2, 3, 4, 5, 7, 10, 11, 13, 14, 24 and 27 the percentage increases are respectively: 40·7 %, 40 %, 75 %, 364 %, 467 %, 92 %, 233 %, 43 %, 30·2 %, 174 %, 20·5 %, and 65·3 %.

We do not attach much importance to these figures as the conditions in the different experiments were not strictly comparable. It is of interest, however, that Exp. 24, in which the mixtures of antigen and antibody employed gave rise to a very high tropin effect, showed the least complementing effect—a result which may be compared with the insignificant fixation of complement which occurs in mixtures of antigen and antibody which give rise to large visible precipitates. We put forward these analogies here solely with the view of directing attention to the possibility that the union of antigen and antibody may give rise to similar redistribution of the constituent colloid particles of the system, the effects of which will vary according to the particular system of investigation employed (precipitating, tropic, agglutinative, etc.).

The Action of Mid-piece on Tropin and the Effect of final addition of End-piece.

Practically all the experiments recorded in the tables contain evidence on this question, but attention will be directed to particular groups of these in which the addition of mid-piece to tropin resulted uniformly in an inhibition of the latter or was indifferent.

We shall consider in the first place those experiments in which the addition of mid-piece (corresponding to complement diluted 1 in 10) depresses the tropic action (see Table I, Exps. 1, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 20, 22, 23, 24, 25, 26 and 29). The percentage degrees of inhibition calculated from these figures are respectively:

(1) 69 %, (8) 43 %, (9) 37 %, 37 %, (11) 80 %, (12) 80 %, (13) 93 %, (14) 99 %, (15) 92 %, (17) 48 %, (18) 97 % (T 1 in 500), 49 % (T 1 in 10), (19) 92 %, (20) 94 %, 22) 66 %, (23) 87 %, (24) 80 %, (25) 92 %, 79 %, 75 %, (26) 88 %, 97 %, 2·1 %, 55 %, (29) 89 %, 53 %.

(Degrees of depression under 25 % have been reckoned under the category of "Indifferent" (see Table II)).

It will be seen that the addition of mid-piece causes in a very large proportion of the experiments a marked depression of the tropic value as estimated by the phagocytic index. In the annexed table (Table II) data bearing on this point have been extracted from the various

TABLE II.

		Effect of mid-piece		Effect of final addition of end-piece		
	Exp.	Depressant	Indifferent	Depressant	Indifferent	Complementing
	5	...	+	...	...	+
	7	...	+	...	...	+
	9 (2)	...	+	...	...	+
	22 (*a*)	...	+	...	...	+
	22 (*c*)	...	+	...	...	+
	22 (*d*)	...	+	...	...	+
	22 (*e*)	...	+	...	...	+
	27 (1)	...	+	...	...	+
	27 (2)	...	+	...	...	+
	27 (3)	...	+	...	...	+
	28 (2)	...	+	...	...	+
	27 (4)	...	+	...	+	...
	3	...	+	...	+	...
	4	...	+	...	+	...
	26 (3)	...	+	+	...	...
	22 (6)	32 %	...	...	...	+
	9 (1)	37	...	...	...	+
	9 (3)	37	...	...	+	...
	8	43	...	...	...	+
	17	48	...	...	...	+
T (1 in 10)	18	49	...	...	...	+
	28 (1)	50	...	...	...	+
	29 (2)	53	...	...	+	...
	26 (4)	55	...	...	+	...
	22 (*f*)	66	...	...	...	+
M (1 in 40)	25	75	...	+	...	...
M (1 in 20)	25	79	...	+	...	...
	24	80	...	...	+	...
	11	80	...	+	...	...
	12	80	...	+	...	...
	23	87	...	...	+	...
	26 (1)	88	...	+	...	...
	29 (1)	89	...	+	...	...
	15	92	...	+	...	...
	19	92	...	+	...	...
M (1 in 10)	25	94	...	+	...	...
	13	93	...	...	+	...
	20	94	...	...	+	...
T (1 in 500)	18	97	...	+	...	...
	26 (2)	97	...	+	...	...
	14	99	...	+	...	...

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experiments and arranged in such a manner as to show in order the effects of the various mid-pieces tested, from "indifferent" action to ascending degrees of inhibition. In this table the effects of final addition of the end-piece are also included and it will be apparent that a definite relationship exists between these two effects.

If the mid-piece is "indifferent," *i.e.* if the tropic value remains practically unaltered by addition of mid-piece, the subsequent addition of end-piece gives, as a rule, a marked complementing effect, *i.e.* the value of the mixture T + M + E is very much greater than the value of T + E. Even in those instances in which the mid-piece depresses the tropic value by amounts up to 50 % the addition of end-piece is still able to cause definite complementing action. Greater degrees of inhibition by mid-piece cannot, however, be overcome by addition of end-piece, and the final value of T + M + E is either "indifferent," *i.e.* not greater than T + E, or is considerably less than T + E ("depressant"). The fact that the end-piece can complement a system in which the persensitised organisms possess a marked degree of negative tropism towards the leucocyte, raises the question of the exact nature of persensitisation from the colloidal point of view. Leaving aside for the moment the consideration of the factors which determine the inhibitory or non-inhibitory character of a certain mid-piece solution, let us examine the probable action of mid-piece when in contact with a tropin solution and bacilli. The tropic substances of the immune serum are, we presume, associated with the end-piece fraction of that immune serum. That this is so, will be shown later.

The mid-piece may be inhibitory in three ways:

(1) by partial absorption of the immune end-piece thus preventing the bacilli from being efficiently sensitised by the tropin;

(2) by acting on the bacilli forming a protective coating on them and so preventing the action of the tropic substances;

(3) by some inhibitory action on the leucocytes.

We have tested both the second and third hypotheses and found that it makes no difference whether the mid-piece be added in the first instance to the bacilli and digested for a time with them, the tropin being added later, or whether the mid-piece is added subsequently to the tropin. Also, so far as we have observed the leucocytes do not appear to have their phagocytic powers affected by being digested in a mid-piece solution. There remains the first hypothesis which we think will largely explain the facts. We have observed that when the tropin is used in high concentration, the addition of mid-piece does not have

so great an inhibitory effect as when great dilution of tropin is used (see Exp. 18). Other experiments bearing on this point will be described later. It seems most probable, therefore, that *inhibition by mid-piece* when it does occur, is due to partial absorption of the immune end-piece, thus preventing the primary sensitisation of the organisms by the tropin. It will be shown in the haemolytic experiments (see Part II) that an old mid-piece has a distinct inhibitory action on the end-piece of the normal complement. This action in the case of the phagocytosis experiments may contribute to the depressant effect in those cases in which final addition of end-piece was made to a mixture of tropin and mid-piece which was itself inhibitory.

Action of End-piece on Tropin.

As we have already mentioned, the end-piece as separated by the CO_2 method has a slight though definite action by itself, doubtless owing to the presence of small quantities of mid-piece. When added to tropin, this end-piece had, in a large number of cases, a complementing action on tropin in the absence of a further supply of mid-piece. In several cases, haemolytic experiments were performed on the same day with the same materials and not infrequently it was found that a marked complementing action by the end-piece itself in the phagocytosis system coincided with a similar independent though slight lytic action of the end-piece in the haemolytic system. In other cases the end-piece for lytic purposes was quite inactive while still possessing considerable complementing properties in the phagocytosis system.

Of 32 experiments bearing on this question the end-piece had a complementing action in 16 cases (+ 25 % to + 240 %), a depressant action in six cases (− 25 % to − 50 %) and an indifferent action in ten cases (− 25 % to + 25 %).

Of 13 experiments in which the end-piece was complementary by itself, further complementing was obtained in the system T + M + E in eight cases. In the remaining five cases the mid-piece was so inhibitory as to overcome any complementing action that the end-piece may have possessed in the absence of mid-piece.

The conclusion is clear from the analysis of the various experiments that definite complementing effects are to be obtained in the system T + M + E only when M possesses by itself an indifferent or a slightly depressant action on T. With higher degrees of depression, the end-piece is unable to convert the system T + M from a "negatively tropic" to a "positively tropic" state.

Action of Mid-piece on a Mixture of Tropin and Complement.

(Table I, Exps. 11, 12, 13, 14, 15, 16, 24.)

It is of some interest that the complementing action of complement on tropin is often markedly enhanced when mid-piece is present in the mixture, even though the particular mid-piece in combination with tropin causes great inhibition. (Compare haemolytic experiments on this point, Part II.)

Effect of keeping on the Mid-piece and End-piece and suggested explanation of inhibitory phenomena, with the comparative haemolytic experiments.

It will be seen from the tables that there is a tendency for a mid-piece, which when fresh exerts no inhibitory action on tropin, to become very markedly inhibitory on keeping (see particularly Exps. 27, 28 and 29 in which the same materials were employed on different dates). On the first day the mid-piece was not inhibitory in a dilution of 1 in 10. On the second day this dilution had a depressant action of 50%, while on the third day the depressant action was as high as 89% and the dilution of 1 in 20 was also inhibitory to the extent of 53%. It was observed that in the cold room the mid-piece solution tended to become cloudier on standing, and this, as we have seen, was accompanied by increased inhibitory powers. None the less it frequently happened that a freshly prepared and apparently clear mid-piece possessed definite inhibitory properties in dilution of 1 in 10.

It is obvious that the physical state of the colloid particles of the mid-piece solution plays a great part in determining its indifferent action on tropin and its action on the joint system T + M + E (see Exp. 26). It is of interest to compare with this inhibition effect the inhibitions got by Schmidt (1911) in cloudy globulin solutions in elucidating the Wassermann reaction on colloidal principles.

The end-piece on the other hand does not appear to depreciate on standing and excellent results have been obtained with end-pieces which have been kept for over two months in the cold room. Mid-piece solutions which have been passed through a Berkefeld filter lose their inhibitory power as well as their capacity for complementing in conjunction with end-piece. (Compare haemolytic experiments on this point, Part II.)

Effect of Heated Mid-piece and End-piece on Tropin.

Heating the mid-piece for one hour at 55° C. entirely removes any inhibitory action it may have had on tropin independently (see Exps. 12, 24, 25 and 26). In two of these experiments the following values were obtained:

T	= 5·4	T	= 9·5
T + M (1 in 10)	= 0·3	T + M	= 1·9
T + M (1 in 20)	= 1·1	*T + heated mid-piece*	= 7·4
T + M (1 in 40)	= 1·3	T + heated M + E	= 7·3
T + heated mid-piece	= 4·9		
T + heated M + E	= 1·1		

In none of these experiments with heated mid-piece however was a complementing effect obtained on subsequent addition of end-piece. In fact the value of the combination T + heated M + E was in two cases much less than that of T + E in spite of the indifferent action of the heated mid-piece on tropin. It is suggested therefore that the heated mid-piece has no absorbing action on the immune rabbit end-piece containing the tropin but a very marked absorbent action on the guinea-pig end-piece.

The end-piece does not appear to be so greatly influenced by heat (see Exps. 13, 14, 15, 16, 17, 18, 24, and 28) as the mid-piece when its action on tropin is investigated.

It would appear that the single addition of heated end-piece to tropin may in some cases increase the tropin value, but as a rule not to the same degree as the unheated end-piece. Occasionally however the heated end-piece has even exceeded the unheated end-piece in this action. In the presence of an inhibitory mid-piece the heated end-piece has in nearly all cases less power than the unheated end-piece has, of converting the "negatively tropic" system T + M. Experiment 24 is an interesting illustration of this point. The mid-piece in this case was very markedly inhibitory and the value of T compared with that of T + M was as 9·5 to 1·9 (5 : 1). The addition of end-piece to the system T + M had just the effect of restoring the initial conditions (T + M + E = 9·8), whereas the addition of heated end-piece to the system T + M brought about no increase (T + M + heated E = 1·8).

Further experiments however will require to be performed before we can draw any definite conclusions regarding the thermo-resistance of the end-piece in relation to a phagocytic system.

Experiments with Mid-piece and End-piece obtained from Rabbit Complement.

In order to ascertain whether similar effects were to be obtained with the complement-fractions obtained from the rabbit the following experiments were performed.

Experiment I. 27. x. 11.

R.E. = Rabbit end-piece. R.M. = Rabbit mid-piece. G.M. = Guinea-pig mid-piece.
G.E. = Guinea-pig end-piece. R.C. = Rabbit complement.

Fractions freshly prepared.

	Phagocytic index
T (1 in 500)	9·6
Do. + R.C.	22·7
Do. + R.E.	10·9
Do. + R.M.	2·4
Do. + heated R.M.	10·9
Do. + G.E.	10·3
Do. + heated R.E.	9·5
Do. + R.M. + R.E.	16·8
Do. + R.M. + G.E.	7·4
Do. + heated R.M. + R.E.	17·0
R.C.	1·8
R.E.	0·01
G.E.	0·0

It will be seen that the rabbit mid-piece was inhibitory to tropin but that the heated rabbit mid-piece was quite indifferent.

Good complementing was obtained on final addition of rabbit end-piece, but the effect of guinea-pig end-piece was only to neutralise the inhibitory effect of the rabbit mid-piece.

The rabbit complement gave excellent complementing with tropin.

Good complementing was also obtained with rabbit end-piece in the presence of heated rabbit mid-piece so that from this experiment one might assume a considerable resistance to heat on the part of the rabbit mid-piece.

A similar experiment on 28th October, 1911, with the same materials gave the following result:

	Phagocytic index
T (1 in 500)	5·0
Do. + R.E.	7·6
Do. + R.M.	0·15
Do. + R.M. + R.E.	8·2

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It will be seen that the inhibition by rabbit mid-piece amounted to 97 % (75 % on previous day) on this occasion and that little or no complementing ensued on addition of the rabbit end-piece.

A later experiment on 31st October, 1911, was made with the same fractions (now five days old).

	Phagocytic index
T (1 in 1000)	2·2
Do. + R.E.	3·3
Do. + R.M.	0·16
Do. + R.M. + R.E.	2·0
Do. + heated R.M.	3·4

Again the rabbit mid-piece was very inhibitory (92 %) and no complementing occurred with rabbit end-piece.

Experiment.

2. XI. 11. On this date an attempt was made to compare the efficiency of complement-fractions from the guinea-pig with those from the rabbit. Fresh rabbit and guinea-pig sera were split on the same day. The tropin was used in a dilution of 1 in 1000 and 14 minutes incubation were given.

Result:

		Phagocytic index	
	T (1 in 1000)	3·1	
A.	Do. + R.E.	2·4	(Indifferent < 25 %).
	Do. + G.E.	4·5	(Compl. 45 %).
B.	Do. + R.M.	1·0	(Both inhibitory).
	Do. + G.M.	1·5	
C.	Do. + R.C.	6·6	(Complementing slight).
	Do. + G.C.	4·6	(No complementing).
D.	R.C.	2·0	
	G.C.	1·7	
E.	Do. + R.M. + R.E.	1·9	(Indifferent < 25 %).
	Do. + R.M. + G.E.	3·1	(Slight depression 31 %).
F.	Do. + G.M. + G.E.	4·0	(Indifferent < 25 %).
	Do. + G.M. + R.E.	3·8	(Complementing 58 %).
G.	Do. + R.M. + R.C.	7·2	(Indifferent < 25 %).
	Do. + R.M. + G.C.	3·8	(,, ,,).
H.	Do. + G.M. + G.C.	6·2	(Slight complementing 34 %).
	Do. + G.M. + R.C.	10·6	(Good complementing 60 %).

Guinea-pig mid-piece appears on the whole to act better than rabbit mid-piece in the presence both of guinea-pig end-piece and rabbit end-piece, *i.e.* the rabbit end-piece goes better with the guinea-pig mid-piece than with the homologous mid-piece. Also as in the previous experiments the rabbit mid-piece went better with the homologous end-piece than with the guinea-pig end-piece, *i.e.*

$$\text{R.E.} + \text{G.M.} > \text{R.E.} + \text{R.M.} > \text{G.E.} + \text{R.M.}$$

As in previous experiments the addition of mid-piece in the system T + C increases the complementing action, and in this respect the guinea-pig mid-piece is again more powerful than the rabbit mid-piece.

It cannot be said, however, that either the rabbit end-piece or the rabbit complement is at all inferior to guinea-pig end-piece or guinea-pig complement when a suitable mid-piece (guinea-pig) is supplied.

The haemolytic experiments performed in this connection (see Part II) do not bear out this result. In fact the latter show very clearly that rabbit complement contains as efficient a mid-piece as guinea-pig complement but that its end-piece has practically no lytic action with either mid-piece. It would be necessary to extend the phagocytosis experiments considerably before concluding that this difference in the effects got by the two systems is a valid one. It may be that the proportions of mid-piece and end-piece necessary for complementing a tropin system differ considerably from those necessary for lysis. The suggestion has occurred to us that the concentration of mid-piece employed by us in the majority of the experiments may have been too high, and that in fact the amount of mid-piece left in the supernatant fluid with the end-piece, after saturation with CO_2, may be sufficient in many cases for complementing purposes. We hope to make further experiments in this connection.

Effect of splitting the Immune Tropin-containing Serum with CO_2.

For this purpose an immune serum (to *B. typhosus*) was prepared from another rabbit and immediately after bleeding, saturated with CO_2 in the usual way. Care was taken to render the end-piece filtrate as free of mid-piece as possible by prolonged sedimentation after centrifugalisation.

Our object was to determine whether some explanation could be afforded of the *lessened inhibitory action* of mid-piece on tropin in high concentration than on highly diluted tropin. It was hoped also that some light might be thrown on the inhibition zone phenomenon so frequently noticed in highly concentrated immune sera.

Experiment I.

Comparison of the tropic values of the ordinary heated immune serum and of the same serum freed of mid-piece. The latter will be called in this and subsequent experiments the "immune end-piece."

Both fluids were digested as usual with the bacillary emulsion for 1 hour at 37° C. before the phagocytosis experiment was made.

Result:

Ordinary immune serum	Phagocytic index	Immune end-piece	Phagocytic index
1 in 10	6·4	1 in 10	18·7
1 in 100	4·6	1 in 100	5·0
1 in 1000	0·12	1 in 1000	0·37
1 in 5000	0·11	1 in 5000	0·12

It will be seen that removal of the mid-piece has increased enormously the tropic value of the immune serum in dilution of 1 in 10. In lower dilutions the effects are not obvious.

Experiment II.

Similar materials employed. Phagocytosis 12 minutes.

Ordinary immune serum	Phagocytic index	Immune end-piece	Phagocytic index
1 in 10	1·8	1 in 10	4·4
1 in 50	5·1	1 in 50	4·9
1 in 100	3·2	1 in 100	5·6

The same phenomenon is again seen in the 1 in 10 dilutions.

Experiment III.

In this experiment the same materials were employed but a comparison was also made with immune end-piece, to various dilutions of which guinea-pig mid-piece was added.

Result:

Ordinary immune serum	Phagocytic index	Immune end-piece	Phagocytic index	Immune end-piece +g.-pig mid-piece	Phagocytic index
1 in 10	8·7	1 in 10	16·1	1 in 10 + M	5·5
1 in 50	11·2	1 in 50	13·5	1 in 50 + M	2·4
1 in 100	8·1	1 in 100	7·6	1 in 100 + M	2·4

Again the immune end-piece in dilution of 1 in 10 is much more powerful than the ordinary immune serum in the same dilution. In lower dilutions the values are equalised. The effect of adding guinea-pig mid-piece to the various dilutions of immune end-piece causes very marked inhibition in all. It would appear, therefore, that to get the full effect of an immune serum in high concentration it is an advantage to get rid of the mid-piece (or the greater part of it).

Experiment IV.

Attempt to ascertain whether the inhibitory effect of the mid-piece was exerted better by keeping it first in contact for a time with the bacilli and then later adding the immune end-piece. Bacilli and mid-piece were digested together for half an hour at 37° C. The immune end-piece was then added (Fluid A). Bacilli and mid-piece and immune end-piece were added together simultaneously and digested for the same time (Fluid B). Bacilli and immune end-piece were digested together (Fluid C). Bacilli and immune end-piece and heated mid-piece were digested together (Fluid D). The dilution of immune end-piece employed was 1 in 50, and the mid-piece was the ordinary guinea-pig mid-piece (=1 in 10).

Result:

	Phagocytic index
Fluid A	0·26
,, B	0·21
,, C	2·3
,, D	0·84

Apparently therefore there is no difference between A and C. The mid-piece is very inhibitory (88 % reduction). The heated mid-piece has as usual much less inhibitory action (63 %).

Experiments on this subject are being continued.

PART II.

HAEMOLYTIC EXPERIMENTS.

On the Properties of the Separated Fractions of the Complement.

The effect of heat on the two fractions of the complement has been investigated by numerous workers. Ferrata's original communication contains the statement that the globulin fraction withstands a temperature of 55° C. while the so-called albumen fraction is destroyed.

Brand (1907) found that both fractions were thermolabile and this conclusion has been confirmed by Tsurusaki (1908), Sachs and Bolkowska (1910) and Fränkel (1911). Mutermilch (1911) heated fresh guinea-pig serum for half an hour at temperatures varying from 53° C. to 57° C. The heated serum was then dialysed as in Ferrata's method. The time necessary to obtain precipitation of the euglobulin increased with the degree of temperature which had been employed. Serum heated to 53° C. or 54° C. required several hours, serum heated to 55° C. 24 to 48 hours and serum heated to 56° C. or 57° C. commenced to precipitate after seven or eight days. A mixture of the globulin obtained from heated serum with the supernatant fluid obtained by the dialysis

of fresh serum produced haemolysis. Haemolysis was frequently obtained by mixing the supernatant fluid from heated serum with the globulin of fresh serum. Occasionally (in four cases out of twelve) Mutermilch obtained haemolysis by mixing the two fractions obtained from heated serum.

Similar results were obtained by H. R. Marks (1911) who was able to reactivate heated guinea-pig serum by the addition of fresh end-piece. On one occasion a guinea-pig serum which had stood for 17 days at room temperature was reactivated with fresh end-piece. Marks also separated the globulin from heated serum both by Sachs and Altmann's and by Liefmann's method. He was able to reactivate the precipitate so obtained with fresh end-piece.

In considering the properties of preparations of mid-piece and end-piece it is necessary to remember that we are dealing with suspensions of serum proteids in salt solution, and that the properties which can be demonstrated are largely dependent on the somewhat artificial experimental conditions.

The mid-piece preparations obtained in the method described in the first part of the paper were immediately after their preparation perfectly clear. They were kept in the cold room, and after a period varying from 24 hours to six days they began to get turbid and eventually a definite deposit formed at the bottom of the bottles in which they were kept. They remained active for variable periods, usually at least a week and often for as long as six or seven weeks. During this time there was a very gradual deterioration in their haemolytic properties. The activity of an old mid-piece preparation could only be demonstrated by allowing the mid-piece to act for about 20 minutes on a mixture of corpuscles and haemolysin and then adding the end-piece. Fresh mid-piece solutions produced haemolysis when added together with the end-piece to sensitised corpuscles. Better results were, however, obtained even with fresh mid-piece by the preliminary incubation method. This property of the mid-piece will be referred to at length in a subsequent paragraph. If the preliminary incubation method was employed it was often possible to demonstrate the activity of mid-piece solutions many weeks after their preparation.

During the course of these experiments the effect of heat on three different samples was examined. Both fractions were in each case found to be thermolabile. The mid-piece was, however, distinctly more thermolabile than the end-piece.

The phagocytic, haemolytic and inhibitory properties of the mid-piece were readily destroyed by heat. The haemolytic action of the end-piece was destroyed more easily than its phagocytic action.

The results of a typical haemolysis experiment are shown in Table III.

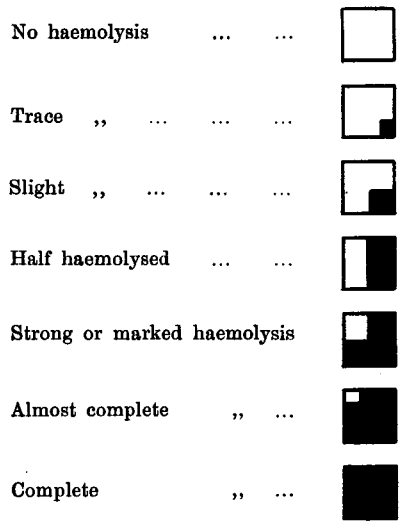
The end-piece preparations in fact showed themselves to be in every way more stable than those of the mid-piece. Preparations were kept for as long as six months in the cold room without apparent loss of haemolytic power.

Another difference between the two fractions was demonstrated by filtration. The mid-piece was inactivated by filtering it through a Berkefeld filter. The end-piece preparation passed through a Berkefeld filter without any loss.

An attempt was made to filter the end-piece solution through a gelatin filter. The filtrate was found to be inactive.

These experiments suggested the possibility of effecting a separation of the complement-fractions by passing guinea-pig serum through a Berkefeld filter. Several specimens of guinea-pig serum were accordingly passed through a Berkefeld filter, but the filtered serum showed no loss of complement.

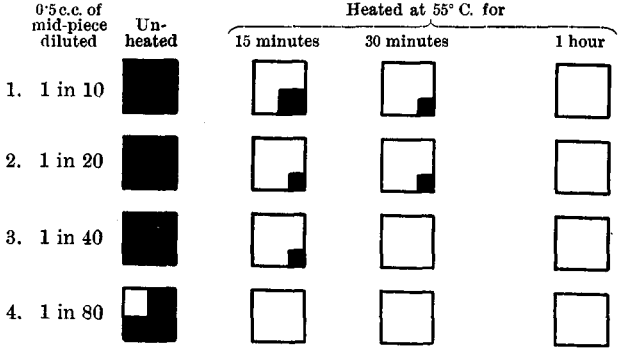
Explanation of scheme used to illustrate the complement fixation experiments.



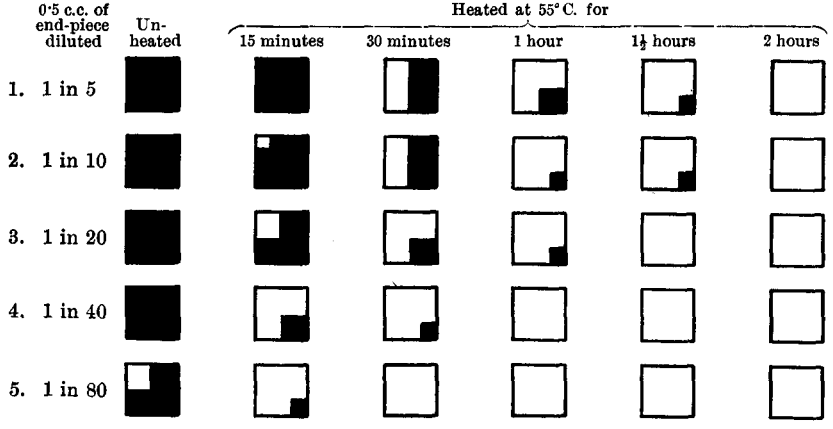
Each tube in the original experiment is represented by a square in the Table. A black square represents total haemolysis. A white square represents a tube in which no haemolysis has occurred.

TABLE III.

To show the effect of heating the separated mid- and end-piece at a temperature of 55° C.



To the various dilutions of mid-piece were added 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles, and 0·5 c.c. of a 1 in 300 dilution of inactivated haemolytic serum. After preliminary incubation for half an hour were added 0·5 c.c. of a 1 in 10 dilution of unheated end-piece and 0·5 c.c. of saline solution. Haemolysis was then allowed to proceed for two hours.



A mixture was prepared of corpuscles 1 in 20, haemolysin 1 in 300, fresh mid-piece 1 in 20 and saline solution in equal parts. This mixture was incubated for half an hour and 2 c.c. of it were added to each dilution of end-piece. Haemolysis was allowed to proceed for two hours. Control tubes which showed that neither mid- nor end-piece was able, when acting alone, to produce haemolysis, were also set up.

It will be remembered that Muir and Browning (1909) published experiments in which it was found that guinea-pig complement would not pass through a Berkefeld filter. The filters used by us may have been more permeable than those used by Muir and Browning, but the experiments performed by us with whole serum were too few to enable us to reach a definite conclusion on this point. Our experiments did, however, show that whole guinea-pig serum could pass through the filter which we used, without appreciable loss in the value of the complement, while a salt solution preparation of the mid-piece was completely inactivated by passing through the same filter. The fact that the mid-piece was retained by the filter must probably be attributed to the size of the particles of the globulin suspension.

The state of the globulin in a salt solution suspension is probably quite different from its state in a saline dilution of normal serum, and some of the results obtained with the use of the mid-piece preparations are quite possibly due to the physical state of the globulin and not to any specific action of the mid-piece fraction of the complement.

On the Relative Proportions of Mid-piece and End-piece necessary for Haemolysis.

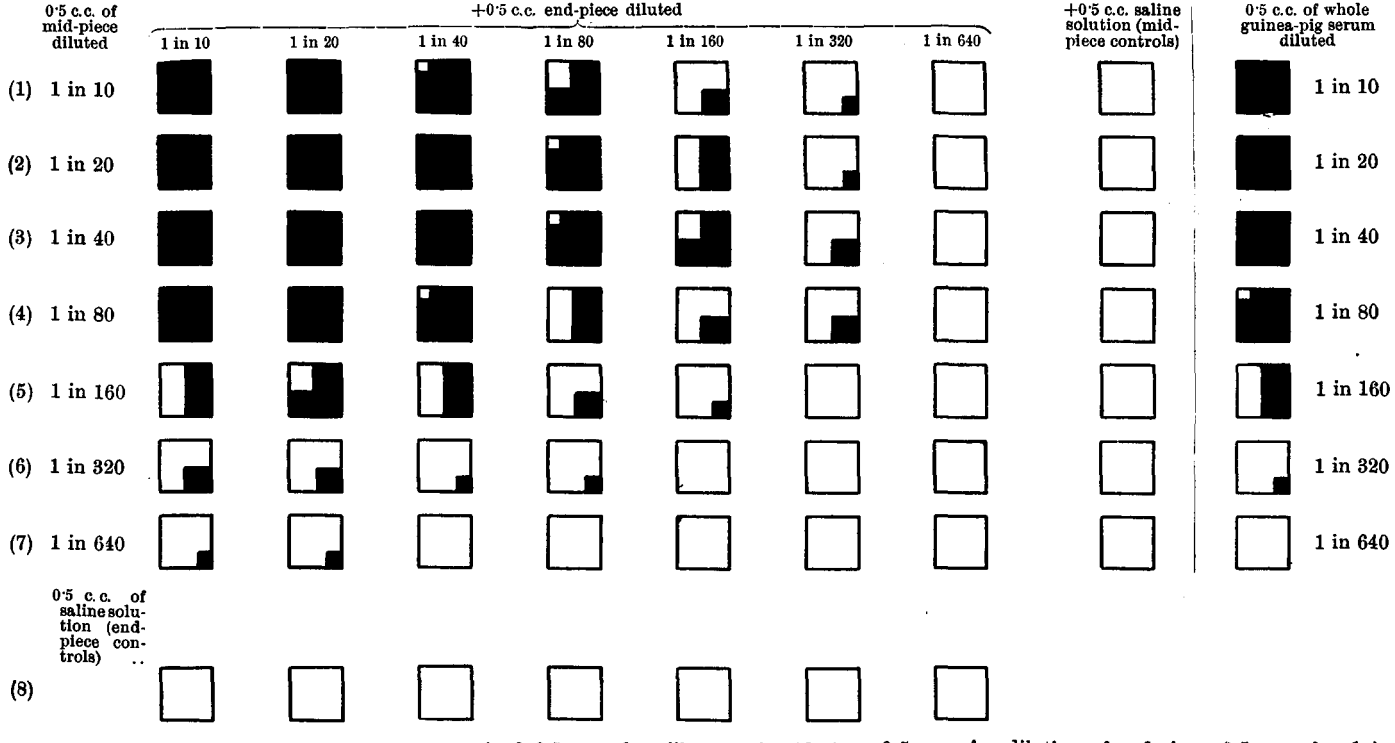
Fränkel (1911) made several experiments with mid- and end-piece in varied proportions. He concluded that within a narrow range decrease of one fraction could be compensated by increase of the other. Occasionally, however, when rather small amounts of end-piece were used, haemolysis was inhibited by an increase of the amount of mid-piece in the mixture. Fränkel also noted that mid-piece could be diluted further than end-piece.

The inhibitory action of relative mid-piece excess is also recorded by Marks (1911), who concluded that it was possible that complement underwent physico-chemical alterations during the process of separation whereby a new proportion between mid- and end-piece became necessary for the attainment of the best results. Marks also stated that the inhibitory influence of mid-piece excess might be abolished by the use of preliminary persensitisation.

Table IV gives a typical result of an experiment in which various quantities of mid-piece were titrated with various quantities of end-piece. The haemolytic value of the whole guinea-pig serum from which the fractions were prepared is given in the right hand column. It will be seen that the mid-piece could be diluted to a greater extent than the

TABLE IV.

To show the proportions in which mid-piece and end-piece produce haemolysis.



All the tubes of the experiment proper contained 0·5 c.c. of a dilution of mid-piece, 0·5 c.c. of a dilution of end-piece, 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles, 0·5 c.c. of a 1 in 400 dilution of a haemolytic serum (rabbit v. sheep cells) and 0·5 c.c. of normal saline solution. The corpuscles, haemolytic serum and mid-piece were mixed and incubated for half an hour at 37° C. End-piece was then added and after a further period of incubation for two hours at 37° C., the results were read and recorded.

The mid-piece and end-piece control tubes contained an extra 0·5 c.c. of saline solution. On the right hand side of the Table are given the corresponding dilutions of the whole guinea-pig serum from which the fractions were prepared.

end-piece. This result was often more marked than in the experiment here recorded. It was, as a rule, found that the end-piece could be diluted to about the same extent as the whole complement, but that the mid-piece could be diluted considerably further provided that a sufficient quantity of end-piece was added. The action of the smaller quantities of mid-piece was, as a rule, improved by increasing the quantity of end-piece in the mixture. Great relative excess of end-piece seemed in some of the experiments to exercise a slight inhibitory action. The inhibitory effect of great relative excess of end-piece was not a constant phenomenon in these experiments and was never well marked. On the other hand relative mid-piece excess had a very pronounced inhibitory action. This inhibitory action was much more marked when mid-piece and end-piece were added simultaneously. When mid-piece, corpuscles and haemolysin were submitted to a period of incubation before the addition of end-piece, the inhibitory action was less marked.

We have made numerous experiments with different samples of mid- and end-piece and with results similar to those recorded in Table IV.

We do not agree with Fränkel's conclusion that decrease of one fraction can be compensated by increase of the other. It is quite true that the action of relatively small amounts of mid-piece can be increased by increasing the amount of end-piece. If, however, a relatively small amount of end-piece is used, haemolysis is inhibited and not favoured by increasing the quantity of mid-piece in the mixture.

Experiments dealing with the inhibitory action of the mid-piece fraction will be described in a subsequent paragraph.

Effect of adding fresh Mid-piece Preparation to whole Complement.

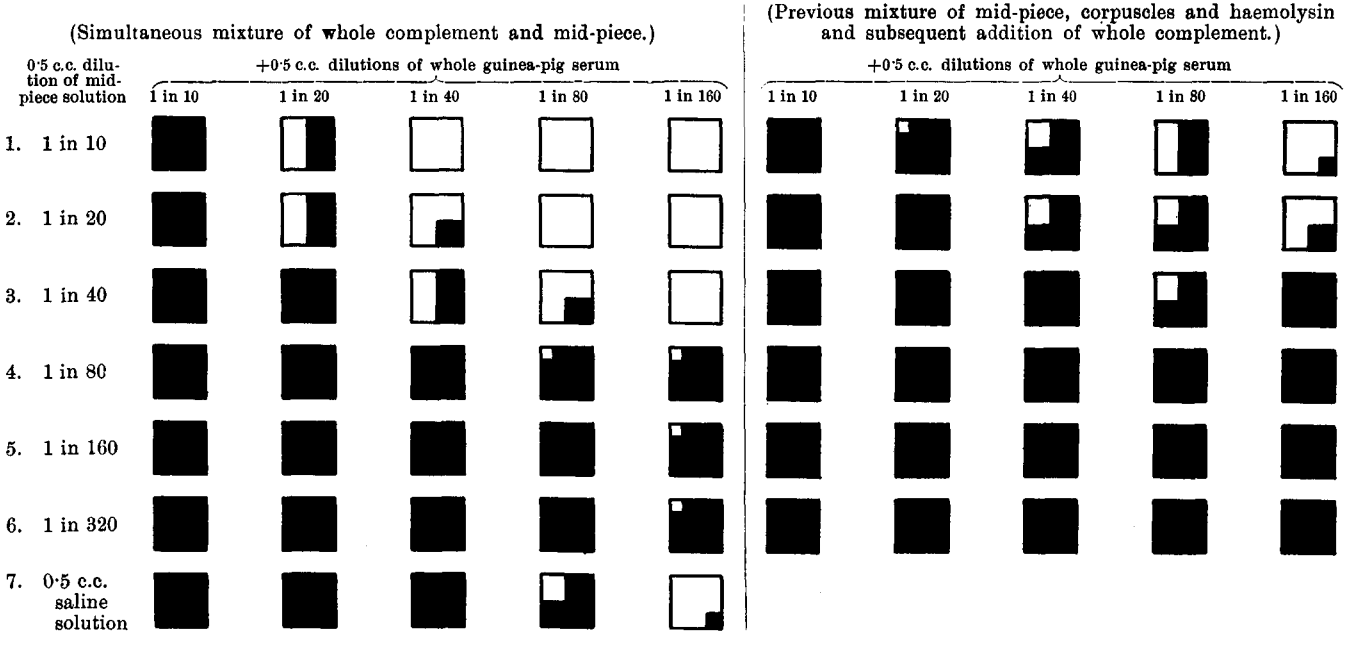
The effect of adding the separated fractions to whole complement was investigated by Fränkel who found that both fresh and old preparations of mid-piece as a rule had an inhibitory action on the whole complement. Occasionally, however, haemolysis was improved by the addition of mid-piece. The addition of end-piece either fresh or old always improved the action of complement.

Hecker (1907) also stated that the action of either whole complement or of an active mixture of mid- and end-piece could be inhibited by the addition of the mid-piece.

The following table shows the results obtained by adding mid-piece in various amounts to various amounts of whole complement.

TABLE V.

Effect of adding fresh mid-piece solution to whole complement.



Each tube (excepting the tubes in row 7) contained 0·5 c.c. of corpuscles (1 in 20), 0·5 c.c. of haemolytic serum (1 in 400), 0·5 c.c. of diluted whole guinea-pig serum, 0·5 c.c. of mid-piece dilution and 0·5 c.c. of saline solution. In one series all the ingredients were mixed at the same time, in the second series the corpuscles, haemolysin and mid-piece were mixed and incubated for $\frac{3}{4}$ hour. The complement was then added. Row 7 shows the haemolytic value of the whole complement without the addition of mid-piece. In the case of each series haemolysis was allowed to proceed for two hours at 37° C.

It will be seen that the smaller quantities of the mid-piece preparation greatly increased the haemolytic action of the smaller, and by themselves insufficient, quantities of whole complement. The larger quantities of the mid-piece had a pronounced inhibitory action on the smaller quantities of whole complement.

When the corpuscles, haemolysin, whole complement, and mid-piece were simultaneously mixed together, this inhibitory action was very marked. When the corpuscles, haemolysin and mid-piece were allowed a preliminary period of incubation before the addition of the whole complement this inhibitory action was diminished, while the supplementary action of the smaller amounts of mid-piece was enhanced.

A suspension of euglobulin if present in sufficient amount acts by interfering with the action of the end-piece fraction of the complement whether the end-piece is added as a preparation of pure end-piece or in a dilution of whole complement. These results are in accord with the results obtained in the experiments made with a view of determining the optimal proportions of the two fractions. The inhibitory action of relative mid-piece excess, was more evident in those experiments in which there was a simultaneous mixture of mid-piece, end-piece, corpuscles and haemolysin.

The changes which occur, on keeping, in the separated fractions of the complement.

If freshly prepared mid-piece solution is added to freshly prepared end-piece solution the mixture can be used in the same way as a corresponding dilution of the whole complement. That is to say if it is added to a suitable mixture of corpuscles and inactivated haemolytic serum, lysis of the corpuscles rapidly takes place.

If an interval is allowed to elapse after the separation of the two fractions a change is found to have taken place, in that very little haemolysis follows the addition of a mixture of the two fractions to sensitised corpuscles. The degree of lysis produced under these conditions is very slight and only becomes apparent after a considerable latent period. If, however, corpuscles, haemolytic serum and mid-piece solution be mixed and incubated for about fifteen minutes and if end-piece is then added to the mixture lysis is rapid and complete.

Brand (1907) who was the first to note the occurrence of this change explained the results by a further elaboration of Ehrlich's theory. Brand gave the name mid-piece (Mittelstück) to that fraction of the

complement which is contained in the globulin solution on the assumption that it fitted directly on to the complementophile group of the amboceptor. The second fraction of the complement, which remains in the supernatant fluid, was called the end-piece (Endstück) on the assumption that it completed the chain by becoming attached to the free end of the mid-piece.

The various components of the haemolytic reaction were supposed to form a chain linked in the following order: corpuscle, amboceptor, mid-piece, end-piece.

Hecker (1907) explained the change which takes place in the separated fractions by assuming an increased avidity of the mid-piece for the end-piece whereby it effects a union with the end-piece, but fails to couple up with the amboceptor.

Experiments intended to illustrate and explain the changes which occur, on keeping, in the fractions of the complement.

The first question to answer is whether the change occurs in the mid-piece or in the end-piece. To settle this point the following experiments were carried out:

(1) Two mid-piece preparations (see Table VI) were selected. The one of these was quite fresh and had been prepared on the day of the experiment, the other had been kept in the cold room for 13 days. These two mid-piece preparations were tested with six end-piece preparations, one of which had been prepared on the day of the experiment, while the others were 3 (two preparations), 4, 11 and 13 days old respectively. That is to say the two preparations of mid-piece were each tested with six different preparations of end-piece. The mid- and end-piece preparations were mixed in each tube and sheep corpuscles and haemolytic serum were then added. The result shows that complete haemolysis took place in all the tubes which contained the fresh mid-piece, while in the tubes which contained the old mid-piece no haemolysis took place. The mixture of 13-day old end-piece with fresh mid-piece produced haemolysis, while the 13-day old mid-piece produced no trace of haemolysis with end-piece prepared on the day of the experiment.

(2) Two end-piece preparations were selected. The one had been kept for 13 days, the other had been prepared on the day of the experiment. Each preparation of end-piece was tested with a preparation of fresh mid-piece and with five other preparations of mid-piece which had

TABLE VI.

Showing the results obtained by adding new mid-piece to old end-piece and by adding old mid-piece to new end-piece. Date of experiment Oct. 2nd.

0·5 c.c. of 1 in 10 dilutions of various end-piece solutions (date of preparation of end-piece)	+0·5 c.c. of a 1 in 10 dilution of mid-piece (prepared Oct. 2nd)	+0·5 c.c. of a 1 in 10 dilution of mid-piece (prepared Sept. 19th)	+0·5 c.c. saline solution
1. September 19th	■	□	□
2. ,, 21st	■	□	□
3. ,, 28th	■	□	□
4. ,, 29th (A)	■	□	□
5. ,, 29th (B)	■	□	□
6. October 2nd	■	□	□
7. 0·5 c.c. Saline Solution	□	□	

0·5 c.c. of 1 in 10 dilutions of various mid-piece solutions (date of preparation of mid-piece)	+0·5 c.c. of a 1 in 10 dilution of end-piece (prepared Oct. 2nd)	+0·5 c.c. of a 1 in 10 dilution of end-piece prepared Sept. 19th)	+0·5 c.c. saline solution
1. September 19th	□	□	□
2. ,, 21st	□	□	□
3. ,, 28th	□	□	□
4. ,, 29th (A)	■	■	□
5. ,, 29th (B)	■	■	□
6. October 2nd	■	■	□
7. 0·5 c.c. Saline Solution	□	□	

In this experiment the quantities of mid-piece and end-piece shown in the table were mixed together. To every tube was then added 0·5 c.c. of saline solution, 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 400 dilution of haemolytic serum (rabbit v. sheep). The results were read after incubation for two hours at 37° C.

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been kept for various periods. The fresh mid-piece and two preparations of mid-piece which were three days old produced complete haemolysis both with the fresh and the old preparation of end-piece. The three older preparations of mid-piece produced haemolysis neither with the fresh nor with the old preparation of end-piece.

It should be added that if the 13-days old mid-piece was incubated for 20 minutes with corpuscles and haemolysin, the subsequent addition of either old or fresh end-piece produced complete haemolysis.

It is obvious that the inhibition of haemolysis which occurs when old mid- and end-piece are simultaneously added to corpuscles and haemolysin depends on some change which takes place in the mid-piece fraction. The end-piece fraction does not appear to undergo change.

It will be noticed that not only the fresh mid-piece but also the two preparations which were two days old produced haemolysis on simultaneous mixture with end-piece, corpuscles and haemolysin. It was found that great differences existed between the various samples of mid-piece in respect to the time which elapsed before they became unable to produce haemolysis when added simultaneously with the end-piece. Some samples underwent the change within a few hours of their preparation, others did not become changed for several days. The change which occurs is probably intimately connected with the changes which occur in the state of division of the globulin particles in suspension in normal saline solution.

In the preparation of the complement fractions the euglobulin of the serum is precipitated and carries down with it the mid-piece fraction of the complement. The euglobulin is then suspended in 0·85 per cent. saline and under favourable conditions the particles of the suspension are so fine that a perfectly translucent fluid is obtained. After an interval of from one to five days, the various samples varied greatly in this respect, the globulin particles tend to aggregate, the fluid becomes opalescent, then turbid, and finally the greater part of the euglobulin forms a deposit.

It is probable that after a certain stage of aggregation of particles has been reached the fluid inhibits haemolysis by acting on the end-piece fraction which becomes entangled in the small aggregations of globulin.

If, however, corpuscles, haemolytic serum and mid-piece be allowed a preliminary period of incubation the globulin is rapidly aggregated or precipitated on the surface of the corpuscles. It is then no longer present in suspension throughout the fluid and is no longer able to

exert an inhibitory influence on the end-piece. Experimental evidence that euglobulin is actually precipitated by a mixture of red cells and haemolysin or by a suitable mixture of serum and homologous antiserum has been published by one of us (Dean 1911).

On the Mechanism of the Inhibitory Action of old Mid-piece Preparations.

The experiments recorded in Table VI show that some change occurs on keeping in the mid-piece preparation which tends to prevent haemolysis, if mid-piece and end-piece are simultaneously added to red cells and haemolytic serum.

The view was also put forward in the last paragraph that the process depends on the gradual aggregation of globulin particles which may be seen to occur in old suspensions of mid-piece in salt solution.

The following experiments (Table VII) show that (1) an old mid-piece preparation does not interfere with the combination of corpuscle with haemolytic serum and fresh mid-piece, (2) an old mid-piece has the power of directly inhibiting the action of fresh end-piece solution.

The first part of the table shows that old mid-piece solution does not interfere in the least with the union of fresh mid-piece antibody and red cell. The only result of adding the old mid-piece was to reinforce the action of the fresh mid-piece.

In the experiment recorded in the second part of the table the old mid-piece was allowed to act directly on the fresh end-piece solution. After half an hour the old mid-piece was found to have inhibited the end-piece markedly. It is quite clear that the old mid-piece acts directly on the end-piece.

This inhibitory action of the mid-piece on the end-piece is probably the direct result of the physical state of the euglobulin suspended in salt solution. It is most marked, as has been shown, in preparations which have been allowed to stand for some days, but it is demonstrable even in freshly prepared mid-piece solutions. It was always found, even with freshly prepared fractions, that better results were obtained by the preliminary incubation of red cells, haemolysin, and mid-piece and the subsequent addition of the end-piece, than when the mid-piece and end-piece were added simultaneously to the red cells and haemolysin.

TABLE VII.

Experiment to determine the mechanism of the inhibitory action of old mid-piece solution. Date of experiment Oct. 9th.

0·5 c.c. of various dilutions of a mid-piece solution prepared Oct. 9th	+0·5 c.c. saline solution	+0·5 c.c. of a 1 in 10 dilution of mid-piece solution prepared Oct. 2nd.
1. 1 in 10		
2. 1 in 20		
3. 1 in 40		
4. 1 in 80		
5. 1 in 160		
6. 1 in 320		

Duplicate sets of dilutions of fresh mid-piece were prepared. To each tube of one set was added 0·5 c.c. of saline solution, to each tube of the other set was added 0·5 c.c. of a 1 in 10 dilution of old mid-piece solution. To every tube was then added 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 400 dilution of haemolytic serum (rabbit v. sheep). After incubation for half an hour at 37° C., 0·5 c.c. of a 1 in 10 dilution of fresh (prepared Oct. 9th) end-piece solution was added to each tube. The amount of haemolysis was recorded after incubation for two hours at 37° C.

0·5 c.c. of various dilutions of an end-piece solution prepared Oct. 9th	+0·5 c.c. saline solution	+0·5 c.c. of a 1 in 10 dilution of mid-piece solution prepared Oct. 2nd
1. 1 in 10		
2. 1 in 20		
3. 1 in 40		
4. 1 in 80		
5. 1 in 160		
6. 1 in 320		

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Duplicate sets of dilutions of fresh end-piece were prepared. To one set was added saline solution, to the other set the old mid-piece solution. The tubes were then incubated for half an hour at 37° C. There was also prepared a mixture of equal parts of sheep corpuscles (1 in 20), haemolytic serum (1 in 400), and fresh mid-piece solution (1 in 10). This mixture was incubated for half an hour and 1·5 c.c. of it was added to each tube shown in the table. The results were read after two hours incubation at 37° C.

On the Haemolytic value of the Fractions of Rabbit Complement as compared with the Fractions of Guinea-pig Complement.

A considerable number of experiments have been recorded in which the complement fractions obtained from the sera of different animals have been interchanged.

Liefmann and Cohn (1910) found that the mid-piece derived from sheep serum could be activated by guinea-pig end-piece.

Landsteiner (1911) found that rabbit mid-piece could be activated by guinea-pig end-piece.

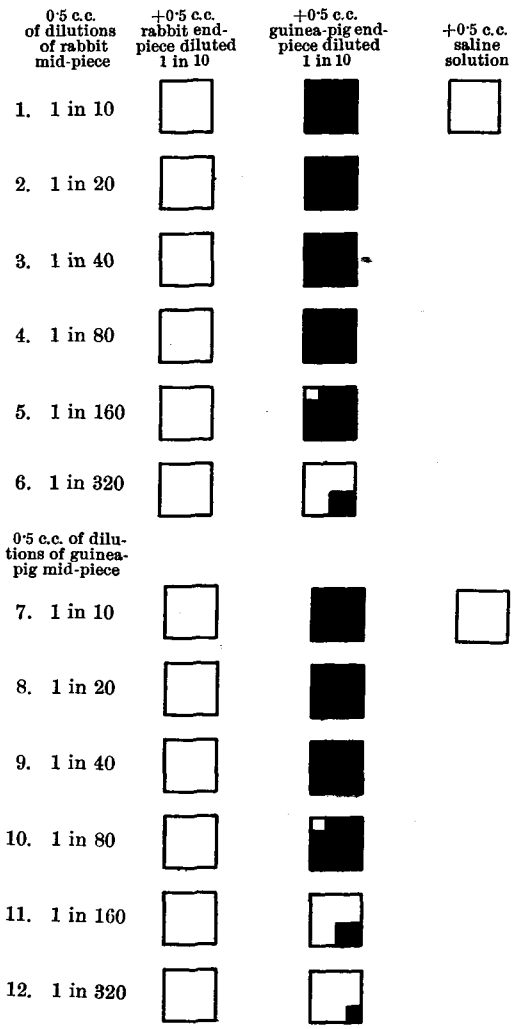
Marks (1911) found that the mid-piece of the ox and of the sheep could be activated by guinea-pig end-piece.

Fränkel, who has made the most extensive observations on this point, succeeded in producing various degrees of haemolysis with the mid-piece fractions of man, ox, sheep, goat, pig, cat and pigeon by the addition of guinea-pig end-piece.

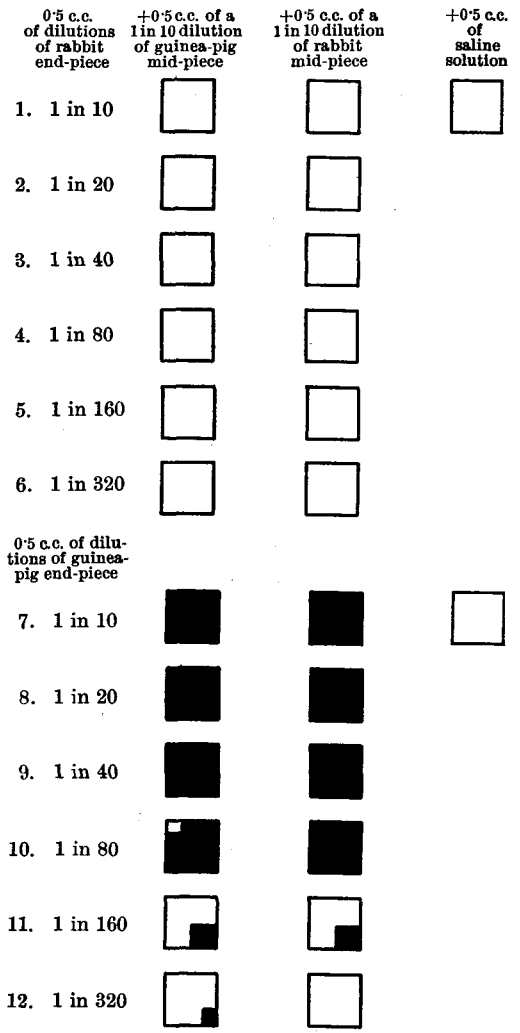
The experiments described hitherto have been concerned with the fractions separated from fresh guinea-pig serum. It was decided to try the action of the complement fractions of the rabbit in both the phagocytosis and the haemolytic experiments. Guinea-pig serum is, as is well known, a very active haemolytic complement, while rabbit serum has comparatively little power to complement inactivated haemolytic serum. Rabbit serum is on the other hand quite an efficient complement for phagocytosis. The following experiment (Table VIII) illustrates the haemolytic value of the fractions obtained from normal rabbit and guinea-pig serum. A portion of each serum was retained in order that the complement value of the whole serum might be estimated. The guinea-pig serum produced complete haemolysis in a dilution of 1 in 40, and three-quarters haemolysis in a dilution of 1 in 80. The rabbit serum even in a dilution of 2 in 5 failed to produce complete haemolysis. The table shows that the end-piece of the rabbit in the strongest dilution (1 in 10) which could be employed, failed to produce even a trace of haemolysis with either rabbit or guinea-pig mid-piece. The rabbit mid-piece, on the other hand, produced excellent lysis when

TABLE VIII.

Haemolytic value of complement fractions of rabbit and guinea-pig.



To the various dilutions of mid-piece were added 0·5 c.c. of sheep corpuscles (1 in 20) and 0·5 c.c. haemolytic serum 1 in 400. After incubation for half an hour the end-piece solution was added. The results were read after a second period of incubation lasting two hours.



A mixture in equal parts of sheep corpuscles (1 in 20), haemolytic serum (1 in 400) and mid-piece (1 in 10), was prepared and incubated for half an hour at 37° C. 1·5 c.c. of the mixture was then added to all tubes shown in the table. The results were read after incubation for two hours at 37° C.

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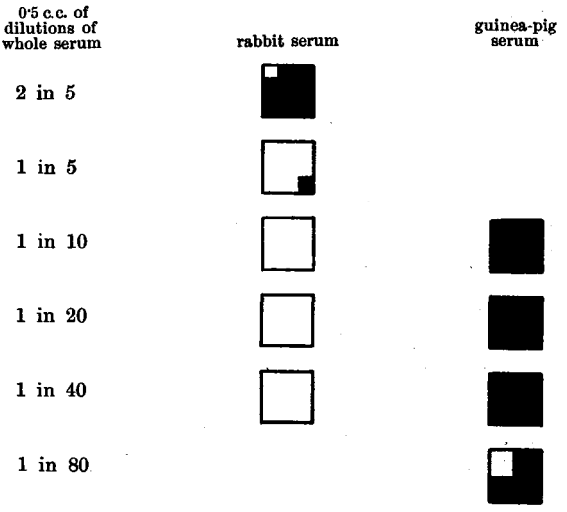
TABLE VIII (*continued*).

To show the haemolytic value of the whole sera from which the complement fractions were prepared.



supplemented by guinea-pig end-piece. The table indeed shows that this particular rabbit mid-piece was haemolytically more active than the guinea-pig mid-piece. Exactly the same result was obtained with another specimen of rabbit serum.

Similar results as regards haemolysis were obtained by splitting human serum and sheep's serum. The end-piece of the sheep and of man was found to have comparatively little haemolytic value, while the mid-piece preparations showed marked haemolytic power when "complemented" with guinea-pig end-piece.

It must be concluded that the well-known haemolytic virtues of guinea-pig serum depend on the lytic activity of its end-piece.

While the end-piece of the rabbit is of little value for haemolysis it acts excellently in phagocytosis (compare phagocytosis experiments in Part I). This result appears to indicate some difference between the phagocytic and haemolytic complement.

General Conclusions.

Phagocytosis System.

(1) Mid-piece as prepared by the CO_2 method is unable by itself to sensitise *B. typhosus* for phagocytosis.

(2) End-piece has a slight sensitising action by itself—an action which is facilitated probably by minute traces of mid-piece remaining after the CO_2 treatment.

(3) With mid-piece and end-piece acting in concert it has only rarely been possible to obtain values approaching those of the original complement.

(4) The addition of mid-piece to immune typhoid serum (tropin) either does not alter the value of the latter or considerably depresses it.

(5) When the mid-piece is "indifferent" or only slightly inhibitory to tropin, the final addition of end-piece gives good complementing.

(6) When the mid-piece is very depressant, the end-piece is no longer able to produce definite complementing.

(7) Heated mid-piece is not inhibitory in conjunction with tropin but its power of acting in the joint system of tropin, mid-piece and end-piece is destroyed.

(8) Mid-piece which has been passed through a Berkefeld filter is not inhibitory but is inactive in the joint system.

(9) End-piece is more resistant to heat than mid-piece and remains longer active on keeping.

(10) Removal of the mid-piece from a fresh immune typhoid serum, greatly increases the tropic action of the latter when used in high concentrations.

Haemolytic System.

(1) Preparations of the mid-piece fraction in 0·85 per cent. salt solution remain active for many weeks if kept at 0° C. Their activity is quickly destroyed by a temperature of 56° C. They are inactivated by passing through a Berkefeld filter.

(2) Preparations of the end-piece fraction in normal saline may be preserved at 0° C. for many months. They are destroyed at 56° C. but somewhat more slowly than the mid-piece fraction. Preparations of end-piece can be filtered through a Berkefeld but not through a gelatin filter.

(3) An end-piece preparation (in conjunction with a suitable dilution of the mid-piece) may be diluted to the same extent as whole serum. The mid-piece fraction (in conjunction with a suitable dilution of the end-piece) can be diluted to a greater extent than the whole serum.

(4) Relative excess of mid-piece inhibits haemolysis. By adopting the method of preliminary incubation of corpuscles, haemolysin and mid-piece (persensitisation) the inhibitory effect is greatly diminished, but not absolutely abolished.

(5) The addition of relatively small quantities of mid-piece to whole complement increases haemolysis; the addition of relatively large quantities of mid-piece inhibits haemolysis. If the persensitisation method be used the inhibitory effect of the large quantities is lessened while the favourable effect of the small quantities is increased.

(6) Preparations of mid-piece vary greatly in respect of the time which elapses before they become unable to produce haemolysis if simultaneously added to corpuscles, haemolysin and end-piece.

(7) This change which occurs in old mid-piece preparations is probably due to an aggregation in the particles of the globulin in suspension. The state of division of the particles of globulin is probably the cause of the inhibitory action of mid-piece preparations.

(8) The mid-piece preparations of the serum of man, the rabbit, and the sheep can be activated with guinea-pig end-piece.

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