TWO INSTANCES OF HUMAN SERA SHOWING ABNORMAL ANTI-COMPLEMENTARY POWER.

By E. L. KENNAWAY, M.D., D.Sc., and Capt. A. M. WRIGHT, N.Z.M.C.

(*From the Bland-Sutton Institute of Pathology, Middlesex Hospital, and V.D. Sect., No. 3 New Zealand General Hospital.*)

In the great majority of cases human sera which have been heated at 55–57° C. for thirty minutes, as is the usual practice preparatory to the Wassermann test, exhibit only very slight anti-complementary properties. That is to say, complete haemolysis of the test corpuscles (0·5 c.c. 3 per cent. suspension of ox or sheep red cells sensitised with five doses of immune body from the rabbit) usually occurs when these are added to a mixture of the amount of heated serum commonly employed (0·025 to 0·05 c.c. in 0·5 c.c. saline) along with $1\frac{1}{2}$ to 2 doses of guinea-pig's complement previously incubated for $1\frac{1}{2}$ hours at 37° C., the dose of complement being the amount which causes complete lysis when incubated with saline in the absence of the human serum. Scattered references to unusually anti-complementary sera occur in the literature, thus Thomsen and Bjarnhjedinsson recorded that the sera of lepers possessed very considerable anti-complementary action and this has been confirmed by Mathis and Beaujean among others. But excluding special treatment of sera, *e.g.* prolonged keeping as found by Browning and Mackenzie and confirmed by Zinsser and Johnson, or heating at higher temperatures, pronounced anti-complementary action is very rare.

In this paper we wish to draw attention to two instances of sera showing considerable anti-complementary power; in neither of these could any certain explanation of this abnormality be found either in the condition of the patient or in the mode of treatment of the serum. These are the only cases of the kind which we have observed in the performance of many thousands of Wassermann tests. The practical importance of this anti-complementary action is obvious, since such sera would inevitably be regarded as giving a positive Wassermann reaction if no control test of the serum alone were made.

The sera were inactivated as usual by heating for half-an-hour at 56° C. The serum control tubes contained 0·5 c.c. saline, 0·05 c.c. patient's serum, and amounts of complement as shown in the tables. The antigen employed in the Wassermann tests was an alcoholic extract of human heart plus cholesterol. The phenomenon was found to be independent of the species of red corpuscles employed, since the examination of Case I was carried out with those of the sheep, and that of Case II with those of the ox. The sera

https://doi.org/10.1017/S0022172400007488 Published online by Cambridge University Press

were preserved after the first examination by freezing at from $-10°$ to $-20°$ C. All the results quoted below are based upon repeated examinations of which single examples only are given in the tables.

Case I. Five samples of this serum (A, B, C, D and E) were examined.

The patient contracted trench fever in June, 1918; on August 3rd of that year he received a gun-shot wound in the right forearm which caused considerable injury to the flexor muscles and tendons; the wound was dirty when the patient came under treatment; the damaged portions were then excised. A week later severe haemorrhage from the wound occurred and 1000 c.c. of blood of Group II was given by transfusion.

On November 14th excision of the scar and secondary suture were carried out; sample A was taken before the anaesthesia, and sample B twelve hours later; the temperature was at this time within normal limits. After the operation the arm became oedematous and showed lymphangitis, and five days later the patient had a febrile attack, the temperature rising to 105° F. Sample C was obtained on November 29th, when the temperature had been normal for a week. Sample D was taken on December 19th, and E at the beginning of March, 1919. The state of the serum was thus observed over a period of four months. The patient presented no clinical evidence of syphilis.

The estimations of the full anti-complementary power of the serum are shown in Table I, and the results of the Wassermann tests carried out in the ordinary way in Table II.

Table I. Case I. *Anti-complementary Action of Serum.*

Date when sample taken	Complement, No. of M.H.D. ...	1·5	3	4·5	6	7·5
16. xi. 18	Sample A ... lysis	none	trace	marked	very marked	almost complete
	Sample B ... „	none	trace	distinct	very marked	almost complete
29. xi. 18	Sample C ... „	none	faint trace	trace	very marked	just complete
	Complement, No. of M.H.D. ...	1·3	2·6	4	5·2	—
19. xii. 18	Sample D ... lysis	none	trace	just complete	complete	—
	Complement, No. of M.H.D. ...	2	—	—	—	—
1. iii. 19	Sample E ... lysis	complete	—	—	—	—
	Samples A, B and C mixed and kept frozen since Nov. 1918; re-examined 3. iii. 19	lysis complete	—	—	—	—

(1) Table I shows that samples A, B and C (Nov. 16th and 29th) showed almost identical anti-complementary action, lysis being in each case not quite complete in the presence of 7·5 M.H.D. of complement; the anaesthetic administered between the withdrawal of samples A and B had therefore no influence upon the condition. All other sera tested with the same batch of complement showed, as is almost invariably the case, complete or almost complete lysis with 1·5 or 2 M.H.D.; the inhibitory action of the serum in question must therefore have been six or seven times greater than normal.

Sample D, taken three weeks after sample C, showed just complete lysis with 4 M.H.D.; the anti-complementary power had therefore undergone diminution, though it was still distinctly abnormal. In sample E, taken two months after D, the normal condition was found to be re-established, lysis being complete with 2 M.H.D. The observations show, therefore, the decline and disappearance of the inhibitory character of the serum in the course of four months. These results obtained by one of us were practically duplicated by the other working independently at another laboratory. One other point may be mentioned: after the experiments carried out in November, 1918, the residue of the heated samples A, B and C were mixed and kept frozen at − 10° to − 20° C. until the following March, and then tested in the same way as before; the inhibition of lysis was found to have disappeared completely (Table I). This property must therefore be due to some quite unstable factor, since freezing is very effectual in preserving many of the properties of serum.

(2) The results of the Wassermann tests carried out in the usual manner with these sera are given in Table II; a comparison of these with the data given in Table I shows that the amounts of complement fixed in the presence of antigen (Table II) are in each of the five tests practically identical with

Table II. Case I. *Wassermann Tests.*

					Serum control
Complement, No. of M.H.D.		1·5	3	4·5	1·5
Sample A ...	lysis	none	trace	distinct	faint trace
Sample B ...	„	none	trace	distinct	faint trace
Negative control serum	„	almost complete	complete	—	complete
Antigen control ...	„	distinct	complete	—	—
Complement, No. of M.H.D.		1·5	3	4·5	1·5
Sample C ...	lysis	none	faint trace	trace	none
Negative control serum	„	complete	—	—	complete
Antigen control ...	„	almost complete	complete	—	—
Complement, No. of M.H.D.		1·5	3	4·5	1·5
Sample D ...	lysis	faint trace	marked	complete	faint trace
Negative control serum	„	complete	—	—	complete
Antigen control ...	„	complete	—	—	—
Complement, No. of M.H.D.		2	4	—	2
Sample E ...	lysis	complete	—	—	complete
Negative control serum	„	just complete	complete	—	complete
Antigen control ...	„	complete	—	—	—

those inhibited by the sera alone (Table I). For instance, sample C gave a trace of lysis with 4·5 M.H.D. of complement both in the presence and in the absence of antigen. The Wassermann reaction was, therefore, negative; as was mentioned above, the patient showed no clinical evidence of syphilis. The results with the negative control serum included in Table II show that sera such as samples A, B, C and D would inevitably be regarded as giving a positive Wassermann reaction if no control observations were made with

the serum alone; whereas the employment of proper serum controls, such as are recorded in the last column of the table, causes the abnormality to be at once detected.

The apparent positive Wassermann reaction diminished *pari passu* with the loss of anti-complementary power, until in the last sample (E) the behaviour of the serum is seen to be practically identical with that of the negative control. Incidentally the results in Table II show that the administration of the anaesthetic (Nov. 16, samples A and B) had no influence upon the strength of the Wassermann reaction. This is of interest in view of statements that an anaesthetic may cause the serum to react positively.

Case II. Female, aged 56. As regards the clinical history of the case, we have been able to learn no more than that the patient showed "mental symptoms." The serum (sample A, Table III) showed somewhat less inhibitory power than did the first samples in Case I, lysis being complete with 5 M.H.D.

Table III.

Case II. (1) *Anti-complementary Action of Serum.*

Date	Complement, No. of M.H.D.	2	3	5
3. iii. 19	Sample A ... lysis	none	distinct	complete
2. iv. 19	Sample B ... ,,	complete	—	—

(2) *Wassermann Test.*

					Serum control
	Complement, No. of M.H.D.	1·5	3	4·5	1·5
3. iii. 19	Sample A ... lysis	none	none	none	faint trace
	Negative control serum ,,	complete	—	—	complete
	Antigen control ,,	complete	—	—	—
	Complement, No. of M.H.D.	2	4	6	2
	Sample B ... lysis	none	none	none	complete
2. iv. 19	Negative control serum ,,	complete	—	—	complete
	Antigen control ,,	complete	—	—	—

of complement. In contrast to Case I, the Wassermann reaction was found to be positive, no lysis occurring in the presence of antigen with 4·5 M.H.D., whereas in the absence of antigen lysis would no doubt have been almost if not quite complete with this amount. All other sera tested on this occasion showed complete or practically complete lysis with 2 M.H.D. The serum was somewhat deeply tinted with haemoglobin, but we have examined hundreds of such sera from partially lysed bloods without encountering any other instances of anti-complementary action.

A month later a second sample (B), free from haemoglobin, was examined. In the meantime the patient had received anti-syphilitic treatment ("914" and calomel), an injection having been given a week before the withdrawal of the blood. The Wassermann reaction was found to be as strongly positive as before (no lysis with 6 M.H.D.), but the abnormal anti-complementary power had disappeared, lysis being complete in the serum control tube with 2 M.H.D.

The second case thus resembles the first in that the anti-complementary property was transient only, disappearing in the course of from one to three months. That this fixation of complement is independent of that which is the basis of the Wassermann reaction is shown by the facts that (1) the Wassermann was in Case I negative and in Case II positive, and (2) in Case II the anti-complementary power disappeared while the Wassermann remained positive. The two cases do not present any common feature which would suggest the cause of the abnormality in question.

SUMMARY.

Two instances are described of sera showing abnormal anti-complementary power. The amount of complement fixed by the serum was in the one case about six times, in the other about four times, greater than is normal. Examination of subsequent specimens from the patients showed that this inhibitory character was transient only. No feature common to the two cases was found to which the abnormality could be attributed. Attention is drawn to the rarity of this condition, but it is of practical importance in that such sera would be regarded as giving a positive Wassermann reaction if their behaviour in the absence of antigen were not observed.

REFERENCES.

BROWNING and MACKENZIE (1908). *Journ. of Path. and Bact.* XIII. 325: see also *Recent Advances in the Diagnosis and Treatment of Syphilis*, London, 1912.

MATHIS and BEAUJEAN (1915). *Bull. Soc. Pathol. exot.* VIII. 252.

THOMSEN and BJARNHJEDINSSON (1910). *Zeitschr. f. Immunitätsforsch.* Orig. VII. 414.

ZINSSER and JOHNSON (1911). *Journ. Exp. Med.* XIII. 31.