PRELIMINARY COMMUNICATION ON THE FIXING OF COMPLEMENT IN HORSE-SICKNESS AND EAST COAST FEVER.

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During my travels in South Africa I visited the Bacteriological Institute at Onderstepoort, near Pretoria, in order to become acquainted with the work carried on there concerning the manufacture of serum and vaccines, as well as the investigations into stock diseases. Whilst at the Laboratory, Dr. Theiler asked me to undertake experiments to see whether the fixing of complement could be made use of in connection with horse-sickness and East Coast fever. These experiments were carried out in the division for sero-diagnosis (Assistant, Dr. H. Sieber). Although these experiments are not yet completed, and on account of my departure had to be abandoned, I consider it advisable to make a preliminary communication. Very likely I shall not have the opportunity of continuing them again in Dar-es-Salam until a later date.

Indication of the value of the method for tracing immunity of cattle against East Coast fever and of horses against horse-sickness.

A suitable method for this purpose would be of enormous practical importance. In addition to this, for the purpose of immunizing equines against horse-sickness it would be of great assistance if one could ascertain first the degree of immunity of such animals and secondly the valuation of the titre of a protective serum in vitro, which so far has not yet been possible. In East Coast fever we do not know of differences in immunity as they exist in horse-sickness, and accordingly the simple proof of the existence of such immunity would be sufficient for the purposes of the practice. Cattle which have recovered from East Coast fever are, as Theiler and myself have proved, protected against future infection and relapses, and therefore no longer capable of propagating the disease. Accordingly such animals, for transport in infected areas, and in areas exposed to infection, would be very valuable, provided that the immunity can be tested in some practical way.

Unfortunately in this short time which was at my disposal, I was not in the position to solve this question completely, but I believe I have brought the proof that it will be possible to do so with the help of the complement fixing method.

Manufacture of Antigen and Serum.

In order to obtain the antigen for horse-sickness I made in the first instance alcoholic extracts from the heart, spleen, liver, and kidneys of an animal killed in extremis, and suffering from horse-sickness. Unfortunately, however, I could not make use of these because already in the quantity of 0·5 c.c. they proved to have a slight haemolytic action. Thereupon I obtained extract with water in the following way: the organs were minced and ground, after mixing with sterile sand, and under gradual addition of an equal quantity of physiological sodium chloride solution. This mixture, after the addition of phenol (0·5 per cent.) was placed on two occasions for twenty-four hours each in the shaking apparatus. The pulp, which was stored in the ice chest (Frigo apparatus) was filtered through a filter paper and the necessary quantity

for the experiments obtained was inactivated for one hour at 55° C.–62° C., and filtered for a second time. Of these extracts those made from the spleen proved to be the most suitable ; they represented a clear pinkish-brown liquid. The test with the serum of an animal immunized some time previously gave the following results :—

TABLE 1.

Serum.	Antigen.	Complement. (3 : 100)	Haemolysin. (1 : 1000)	Sheep Blood Corpuscles. (5 : 100)	Na. Cl.	Result.
1·0	0·5	1·0	1·0	1·0	0·5 —	Complete deviation.
1·0	0·4	1·0	1·0	1·0	0·6 —	
1·0	0·3	1·0	1·0	1·0	0·7 —	
1·0	0·2	1·0	1·0	1·0	0·8 —	
1·0	0·2	1·0	1·0	1·0	0·9 +	Traces of haemolysis.
1·0	0·05	1·0	1·0	1·0	1·0 ++	Partial haemolysis.
—	1·0	1·0	1·0	1·0	1·0 +++	Complete haemolysis.
1·0	—	1·0	1·0	1·0	2·0 ++	
—	—	1·0	1·0	1·0	3·0 +++	
1·0	—	—	—	1·0	4·0 —	
—	1·0	—	—	1·0	4·0 —	
—	—	—	—	1·0	5·0 —	

For the manufacture of an East Coast fever antigen the so-called " Koch's bodies " and the parasites resulting therefrom come into consideration. The former are particularly numerous in the infarcts of the kidneys as well as in most of the lymphatic glands and spleen of a sick animal. Accordingly, I obtained (after the presence of numerous bodies had been demonstrated in these organs), the extracts in the way described above. For the purpose of obtaining an extract of *Theileria parva* in the corpuscles, I took the blood, which contained enormous numbers of parasites, from the jugular vein of a sick beast, and defibrinated it. The blood corpuscles were washed in physiological water three times and, after removing the latter, were dissolved in distilled water, whereupon the solution with the corresponding quantity of sodium chloride was brought again under izotonic conditions. The parasites freed in this way were centrifugalized twice for a period of ten minutes (3000 revolutions), removing after the first centrifugalizing the super-natent liquid, and replacing it with physiological water. Microscopical examination proved that the parasites were contained in the deposit, together with the stromata of the red corpuscles. This deposit, after drying in the exsiccator, was ground up for two hours in an agate mortar, under the gradual addition of physiological sodium chloride solution (one-twentieth of the original quantity of blood), and then an addition of phenol (0·5 per cent.) was made. The extract obtained in this way was also kept in the Frigo.

The extracts made from the infarcts in the kidney and from the parasites themselves proved to be the most effective. Unfortunately the small quantity obtained did not permit the undertaking of a large number of experiments. The extract of the spleen, on account of its dark brownish-red colour, could not be used in large quantities without interfering with the valuation of the results. Accordingly I had to use the extract of lymphatic glands. The titration with serum of an animal imported from England (No. 928), and suffering from acute East Coast fever, showed complete deviation in the quantity of 0·1 c.c. with 1 c.c. serum.

The serum alone in the quantity of 1 c.c. showed only a slight deviation; 0·6 c.c. of the extract had no haemolytic action and no deviating qualities.

For the purpose of obtaining serum the blood was drawn aseptically from the jugular vein. It was kept for one hour at 37° C., and for twenty-four hours in the ice-box. The serum was collected by means of a pipette. It was then inactivated, an addition of phenol was made, and was placed on the ice. Before each experiment the serum was tested on its haemolytic action.

Technique.

The haemolysine of a rabbit in the form of a serum, injected with blood corpuscles of a sheep, was only used when it proved to be effective in a dilution of at least 0·0005 c.c. in 1 c.c. of physiological sodium chloride. For the experiments the double minimum effective quantity was used. The dilution was accordingly at least 1 : 1000. The minimum effective quantity of complement, that is, 3 : 100 for 1 : 1000 haemolysines, was used in the simple dose in order to prevent a deviation by the superfluous complement. Thereupon the mixture of serum, antigen, and complement was made, and after it had remained for half an hour at 37° C. the haemolysines and the sheep blood corpuscles were added. The tubes were then kept another hour at 37° C. after having been well shaken before placing them into the incubator as well as after removing them. They were then placed in the ice-box, kept from fifteen to eighteen hours, when the results were noted.

Experiments.

(1) *Horse-sickness.*—In the way indicated as above, I tested the sera of two normal horses, 5150 and " H ", of an immune horse 3600, and of an animal suffering from the acute disease " G ", and further, of two horses, Nos. 4429 and 4225, hyperimmunized three months previously, and from which in the meantime 40 litres of blood had been drawn. The results of the experiments are given hereunder :—

Table 2.

Serum. 1·0	Antigen.	Complement. 1·0	Haemolysin. 1·0	Blood Corpuscles. 1·0	Na. Cl. 1·0	Result.
Normal horse of 5150	0·5	1·0	1·0	1·0	2·0	+++
" " "	—	1·0	1·0	1·0	1·5	+++
" " H	—	1·0	1·0	1·0	1·5	+++
" " "	—	1·0	1·0	1·0	1·5	+++
Immune horse 3600	—	1·0	1·0	1·0	2·0	++
" " "	—	1·0	1·0	1·0	1·5	+
Sick horse G	—	1·0	1·0	1·0	2·0	++
" " "	—	1·0	1·0	1·0	1·5	+
Hyperimmunized horses—4429	—	1·0	1·0	1·0	2·0	++
" " "	—	1·0	1·0	1·0	1·5	+
" " 4225	—	1·0	1·0	1·0	2·0	++
" " "	—	1·0	1·0	1·0	1·5	—
Control	—	1·0	1·0	1·0	3·0	+++
	—	—	—	1·0	5·0	—

The titration of serum 4225 as follows :—

TABLE 3.

Serum.	Antigen.	Complement.	Haemolysin.	Blood Corpuscles.	Na. Cl.	Result.
0·75	0·5	1·0	1·0	1·0	1·75	—
0·5	0·5	1·0	1·0	1·0	2·0	+
0·25	0·5	1·0	1·0	1·0	2·25	++
0·1	0·5	1·0	1·0	1·0	2·4	++

East Coast fever. (Note : 0·3 c.c. of extract was used.)

The serums of six animals, immune to redwater (Nos. 1053, 1081–1085), of six animals immune to East Coast fever (Nos. 1047, 895, 615, 829, 883, and 836), and of two animals suffering from an acute attack of the disease (Nos. 905 and 928) were tested.

The result was as follows :

TABLE 4.

Serum. 1·0	Extract.	Complement.	Haemolysin.	Blood Corpuscles.	Na. Cl.	Result.
Redwater immune cattle—1053	0·3	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
1081	—	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
1082	—	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
1083	—	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
1084	—	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
1085	—	1·0	1·0	1·0	2·0	+++
,,	—	1·0	1·0	1·0	1·7	+++
East Coast Fever, immune cattle—						
1047	—	1·0	1·0	1·0	2·0	++
,, ...	—	1·0	1·0	1·0	1·7	++
895	—	1·0	1·0	1·0	2·0	+++
,, ...	—	1·0	1·0	1·0	1·7	++
829	—	1·0	1·0	1·0	2·0	+
,, ...	—	1·0	1·0	1·0	1·7	(+)
883	—	1·0	1·0	1·0	2·0	++
,, ...	—	1·0	1·0	1·0	1·7	+
836	—	1·0	1·0	1·0	2·0	++
,, ...	—	1·0	1·0	1·0	1·7	+
615	—	1·0	1·0	1·0	2·0	+++
,, ...	—	1·0	1·0	1·0	1·7	++
Sick cattle—905	—	1·0	1·0	1·0	2·0	+
,, ...	—	1·0	1·0	1·0	1·7	—
918	—	1·0	1·0	1·0	2·0	+
,, ...	—	1·0	1·0	1·0	1·7	—
Control	—	1·0	1·0	1·0	3·0	+++
,, ...	—	—	—	1·0	5·0	—

The further titration of serum 928 gave a total deviation in the dose of 0·25 c.c. with 0·3 c.c. antigen; that of serum 905 is shown in the following table:—

TABLE 5.

Serum. 905	Extract.	Complement.	Haemolysin.	Blood Corpuscles.	Na. Cl.	Result.
0·8	0·3	1·0	1·0	1·0	2·2	+
,,	—	1·0	1·0	1·0	1·9	—
0·6	—	1·0	1·0	1·0	2·4	++
,,	—	1·0	1·0	1·0	2·1	—
0·4	—	1·0	1·0	1·0	2·6	+++
,,	—	1·0	1·0	1·0	2·3	—
0·2	—	1·0	1·0	1·0	2·8	+++
,,	—	1·0	1·0	1·0	2·6	—
0·1	—	1·0	1·0	1·0	2·9	+++
,,	—	1·0	1·0	1·0	2·6	—
0·05	—	1·0	1·0	1·0	3·0	+++
,,	—	1·0	1·0	1·0	2·7	+
0·01	—	1·0	1·0	1·0	3·0	+++
,,	—	1·0	1·0	1·0	2·7	++
—	—	1·0	1·0	1·0	3·0	+++
—	—	—	—	1·0	5·0	—

The foregoing experiments can be summarized as follows:—

(1) Extract from the spleen of an animal which died of horse-sickness, in the quantity of 0·5 c.c. and extract from the lymphatic glands of a beast suffering from East Coast fever, and containing numerous plasma bodies, in the quantity of 0·3 c.c. as well as extracts of parasites from the blood proved to be effective as antigenes.

(2) The serum of normal horses and cattle, and of cattle immune to red-water, in the dose of 1 c.c., does not cause deviation when pure or mixed with antigen.

(3) The serum of animals suffering from horse-sickness or of East Coast fever, or which were immune against these diseases, with few exceptions, gave a distinct deviation, in the dose of 1 c.c.

(4) The deviation with the sera enumerated under (3) was obtained in every instance after the addition of antigen, and was stronger than that caused by the serum alone.