A CONTRIBUTION TO THE STUDY OF AMBOCEPTORS AND RECEPTORS.

THIRD COMMUNICATION ON HETEROLOGOUS IMMUNITY TO MALIGNANT MOUSE TUMOURS.

By M. TSURUMI.

(The Lister Institute of Preventive Medicine, London, S.W.)

It must be due to an incidental distribution of receptors that guinea-pig organs and mouse tumours are able to elicit haemolysins for goat erythrocytes, when they are inoculated into rabbit, because antigens are generally found in common only in closely related substances. So, if this relation exists between them, there arises a further question, namely, whether they may not be able to evolve other mutual antibodies; in other words, to what degree they may have antigenic substances in common. I have already noted (1915) that mouse tumour antisera contain complement fixing antibodies, using extracts of mouse and alien tumours as antigens. Michaelis and Fleischmann (1906) reported that rabbit sera immunised against guinea-pig and mouse liver fixed complement in the presence of their homologous antigens, namely, extracts of the livers of guinea-pig and mouse. Later, Fleischmann and Davidsohn (1908) published an extended investigation upon the same subject. So, it is easy to suppose that mouse tumour, guinea-pig kidney and goat erythrocyte antisera contain complement fixing antibodies. Haemagglutination, also, enters into the question, because the sera mentioned above are all haemolytic for goat erythrocytes.

I. COMPLEMENT FIXATION.

The immune sera employed for these experiments are those which were used for a previous work, namely:

1. Mouse carcinoma 199 antiserum,
2. Mouse sarcoma 37 p. antiserum,
3. Guinea-pig kidney antiserum, and
4. Goat erythrocyte antiserum.

All are naturally haemolytic to goat blood cells, and in order to carry out the complement fixation reaction with these sera, it was necessary to eliminate the haemolytic amboceptors from them (goat erythrocytes being used in the haemolytic system), this being done as follows:

In the case of mouse tumour and guinea-pig kidney sera, goat red cells were added in the proportion of 0.5 c.c. goat red cell deposit obtained by washing with saline, to 1.0 c.c. of serum, but in the case of goat erythrocyte serum where this quantity was not sufficient (A.U. = 0.001) it was necessary to absorb twice with 1.0 c.c. of goat blood cells to 1.0 c.c. of the serum. For antigen, watery extracts of mouse carcinoma and guinea-pig kidney, and alcoholic extract of goat blood corpuscles were employed. To prepare alcoholic goat erythrocyte extract I used v. Dungern's method (1912).

The author made up 20 % alcoholic extract from human blood corpuscles in order to test complement fixation with cancer patient serum. As the amount to be used he suggested 0.4 c.c. of this extract after a dilution of 1 in 2 with normal saline. In the present case the same amount of alcoholic goat erythrocyte extract has been employed. Von Dungern's method is, however, not suitable for the preparation of mouse tumour extracts, owing to precipitates being produced in some quantity when dilution with saline takes place. Consequently, watery extracts were employed. As to the method of preparation, healthy tumours were removed from animals which had been bled out, were cut up and washed with normal saline, and 10 % emulsions by weight of the tumour cells were made in physiological saline. These emulsions, after being kept in the cold room for two days, were centrifuged and 0.4 c.c. of the supernatant fluid employed for the reaction—double the quantity having been found not to interfere with complete haemolysis. With guinea-pig kidney extract the same method exactly was applied, and the same quantity employed. In the haemolytic system, 0.5 c.c. of 5 % goat erythrocyte suspension prepared from the deposit was used. The complement dose was 0.5 c.c. of a 1 in 10 dilution of normal fresh guinea-pig serum.

The results were noted after the tubes had been at a temperature of 37° C. for two hours, the degrees of haemolysis being distinguished as follows:

c. = complete	sl. = slight
n.c. = nearly complete	tr. = trace
m. = marked	n. = none

As control, it is necessary to test for complement fixation with normal rabbit serum, using the extracts already mentioned as antigens. For this purpose, sera from several normal rabbits were taken and examined after treating with goat blood cells just as in the cases of mouse tumour and guinea-pig kidney antisera. The results obtained from three of the rabbits are those seen in Table I.

TABLE I.

Complement fixation by normal rabbit serum with mouse carcinoma, guinea-pig kidney and goat erythrocyte extracts.

	Inactivated normal rabbit serum	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.	Goat erythrocyte extract 0.4 c.c.
1.	0.1 c.c.	c.	c.	c.
2.	0.05 "	"	"	"
3.	0.025 "	"	"	"
4.	0.01 "	"	"	"

Two further rabbits, however, gave different results, being those shown in Table II.

TABLE II.

Complement fixation by normal rabbit serum with mouse carcinoma, guinea-pig kidney and goat erythrocyte extracts.

	Inactivated normal rabbit serum	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.	Goat erythrocyte extract 0.4 c.c.
1.	0.1 c.c.	m.	m.	c.
2.	0.05 "	n.c.	n.c.	"
3.	0.025 "	c.	c.	"
4.	0.01 "	"	"	"

As the tables show, normal rabbit serum either does not contain complement fixing antibodies or does so to a very small extent.

The reaction carried out with sera immunised against mouse tumour, guinea-pig kidney and goat blood corpuscles however gave the following results:

TABLE III.

Complement fixation by goat erythrocyte antiserum with homologous antigen and guinea-pig kidney and mouse carcinoma extracts.

	Goat erythrocyte antiserum	Homologous extract 0.4 c.c.	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.
1.	0.1 c.c.	n.	n.	n.
2.	0.05 "	"	"	"
3.	0.025 "	"	sl.	"
4.	0.01 "	sl.	m.	sl.
5.	0.005 "	m.	n.c.	m.
6.	0.0025 "	c.	c.	c.
7.	0.001 "	"	"	"

TABLE IV.

Complement fixation by mouse carcinoma antiserum with homologous antigen and guinea-pig kidney and goat erythrocyte extracts.

	Mouse carcinoma antiserum	Homologous extract 0·4 c.c.	Guinea-pig kidney extract 0·4 c.c.	Goat erythrocyte extract 0·4 c.c.
1.	0·1 c.c.	n.	n.	n.
2.	0·05 „	„	tr.	m.
3.	0·025 „	„	m.	c.
4.	0·01 „	„	c.	„
5.	0·005 „	m.	„	„
6.	0·0025 „	c.	„	„
7.	0·001 „	„	„	„

TABLE V.

Complement fixation by guinea-pig kidney antiserum with homologous antigen and mouse carcinoma and goat erythrocyte extracts.

	Guinea-pig kidney antiserum	Homologous extract 0·4 c.c.	Mouse carcinoma extract 0·4 c.c.	Goat erythrocyte extract 0·4 c.c.
1.	0·1 c.c.	n.	tr.	n.
2.	0·05 „	„	sl.	„
3.	0·025 „	„	m.	„
4.	0·01 „	„	n.c.	s.l.
5.	0·005 „	tr.	c.	m.
6.	0·0025 „	m.	„	c.
7.	0·001 „	c.	„	„

Complement fixation tests with these sera gave with all three extracts positive results, although the sera deviated complement most strongly with their homologous antigens. In the case of goat erythrocyte serum this last is least marked, possibly owing to the removal of a certain amount of the more specific complement fixing antibody during the absorption with red cells.

II. HAEMAGGLUTINATION.

The question as to the relationship between haemolysis and haemagglutination does not seem settled yet. Ehrlich and Morgenroth (1913) who deny that haemagglutination is a prelude to haemolysis, as Bordet (1913) and Baumgarten (1913) believe, assert that both antibodies can coexist independently in the same antiserum.

Forssman (1911) has described haemagglutination on goat erythrocytes with guinea-pig kidney antiserum, but he did not give an exact account of his observations. It remained to be seen, therefore, if guinea-pig kidney and mouse tumour antisera contain haemagglutinins, as well as haemolysins, for goat erythrocytes.

The methods employed were as follows:

The immune sera were inactivated by heating in the incubator at 56° C. for half an hour. The 5 % suspension of red corpuscles was prepared in the same way as for the preceding experiments. To each of the test tubes containing diminishing amounts of inactivated immune serum made up to 1 c.c. with normal saline, 0.5 c.c. of 5 % erythrocyte suspension and 0.5 c.c. of normal saline were added. After incubation the tubes were placed at room temperature and the following morning the results were read, the degrees of haemagglutination being distinguished as follows:

+++ = very distinct	± = doubtful
++ = distinct	— = negative
+ = positive	

The normal rabbit sera used in the complement fixation tests served as control.

TABLE VI.

Haemagglutination of goat erythrocytes by normal rabbit sera.

	1 c.c. of serum diluted	5 per cent. goat blood corpuscles	Normal saline	Result
1.	1 : 5	0.5 c.c.	0.5 c.c.	—
2.	1 : 10	„	„	—
3.	1 : 20	„	„	—
4.	1 : 40	„	„	—

Normal rabbit serum is thus seen not to contain haemagglutinin for goat erythrocytes at all.

The following tables show the same reaction carried out with various goat red cells, guinea-pig kidney and mouse tumour antisera.

TABLE VII.

Haemagglutination of homologous erythrocytes by goat erythrocyte antisera.

	1 c.c. of serum diluted	5% goat blood corpuscles	Normal saline	Dilution	Goat erythrocyte serum		
					No. 1 A. U. = 0.001	No. 2 A. U. = 0.001	No. 3 A. U. = 0.0025
1.	1 : 5	0.5 c.c.	0.5 c.c.	1 : 10	+++	+++	+++
2.	1 : 10	„	„	1 : 20	+++	+++	+++
3.	1 : 20	„	„	1 : 40	+++	+++	+++
4.	1 : 40	„	„	1 : 80	+++	+++	++
5.	1 : 80	„	„	1 : 160	+++	±	+
6.	1 : 160	„	„	1 : 320	++	—	—
7.	1 : 320	„	„	1 : 640	+	—	—
8.	1 : 640	„	„	1 : 1280	—	—	—

TABLE VIII.

Haemagglutination of goat blood corpuscles by guinea-pig kidney antisera.

	1 c.c. of serum diluted	5% goat blood corpuscles	Normal saline	Dilution	Guinea-pig kidney serum		
					No. 1 A. U. = 0·001	No. 2 A. U. = 0·005	No. 3 A. U. = 0·005
1.	1 : 5	0·5 c.c.	0·5 c.c.	1 : 10	—	—	—
2.	1 : 10	„	„	1 : 20	—	—	—
3.	1 : 20	„	„	1 : 40	—	—	—
4.	1 : 40	„	„	1 : 80	—	—	—
5.	1 : 80	„	„	1 : 160	—	—	—
6.	1 : 160	„	„	1 : 320	—	—	—
7.	1 : 320	„	„	1 : 640	—	—	—
8.	1 : 640	„	„	1 : 1280	—	—	—

TABLE IX.

Haemagglutination of goat blood corpuscles by mouse tumour antisera.

	1 c.c. of serum diluted	5% goat blood corpuscles	Normal saline	Dilution	Mouse carcinoma serum	Mouse sarcoma serum
					A. U. = 0·005	A. U. = 0·02
1.	1 : 5	0·5 c.c.	0·5 c.c.	1 : 10	—	—
2.	1 : 10	„	„	1 : 20	—	—
3.	1 : 20	„	„	1 : 40	—	—
4.	1 : 40	„	„	1 : 80	—	—
5.	1 : 80	„	„	1 : 160	—	—
6.	1 : 160	„	„	1 : 320	—	—
7.	1 : 320	„	„	1 : 640	—	—
8.	1 : 640	„	„	1 : 1280	—	—

The tables show that goat erythrocyte antiserum certainly contains a haemagglutinin for goat blood corpuscles but that guinea-pig kidney and mouse tumour antisera do not. In other words, the receptors which are able to produce haemagglutinin for goat blood cells are not distributed in guinea-pig kidney and mouse tumours. One must therefore conclude that as the antigens for haemagglutinins and haemolysins for the same blood cells do not always exist side by side, but can be found separated from one another, haemagglutination is not necessarily a prelude to haemolysis.

SUMMARY.

By using watery extracts of mouse tumours and guinea-pig kidney, and alcoholic goat erythrocyte extract, it has been shown that mouse tumour, guinea-pig kidney and goat erythrocyte antisera contain complement fixing antibodies for all three extracts, although the reaction is most evident when the homologous antigens are employed.

Goat erythrocyte antiserum agglutinates goat blood corpuscles strongly while guinea-pig kidney and mouse tumour sera do not contain haemagglutinin at all. Haemagglutination is therefore not to be regarded as a prelude to haemolysis; the antibodies for both processes can exist independently of one another. It has thus been seen that mouse tumour, guinea-pig kidney and goat erythrocytes have in common the property of producing, when inoculated into the rabbit, besides haemolytic amboceptors, complement fixing antibodies, but receptors for goat haemagglutinins cannot be found in either guinea-pig kidney or mouse tumours.

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