A CONTRIBUTION TO THE STUDY OF AMBOCEPTORS AND RECEPTORS.

SECOND COMMUNICATION ON HETEROLOGOUS IMMUNITY TO MALIGNANT MOUSE TUMOURS.

By M. TSURUMI.

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

Since Forssman (1911) discovered a heterologous haemolysin obtained by immunising rabbit against guinea-pig organs—kidney, liver, etc.—and having the property of dissolving sheep blood corpuscles but not those of the guinea-pig, many authors have devoted their time to this subject, and to the elucidation of the relationship between antigen and antibody.

Thus, Orudschiew (1913) succeeded in obtaining haemolytic amboceptors by immunising with guinea-pig serum, while Spaet (1914) claimed that the leucocytes of this animal can also evoke the production of the same amboceptors. In 1913 Morgenroth demonstrated two haemolysins obtained by immunising rabbit against mouse kidney and against mouse carcinoma, and noted that it was a difficult task to decide whether the haemolysins were identical or partially different. In a later paper, Morgenroth and Bieling (1915) published a detailed account dealing with this subject. For this purpose they employed the methods of fixation and transgression, and declared that the haemolysins were not identical, but differed from one another to a certain extent.

In a previous paper (1915) I investigated the serological reactions of malignant mouse and rat tumour antisera, *i.e.* haemolysis, haemagglutination, complement fixation, precipitin reaction and cytolysis. My experiments were all made in cases of heterologous immunity and working along bacteriological lines, since in the case of homologous immunity to mouse tumours (*e.g.* immunising mouse against mouse

https://doi.org/10.1017/S002217240000629X Published online by Cambridge University Press

tumours and certain normal tissues—for example mouse embryo-skin) no specific antibody has yet been found.

In the present paper the investigations concern the relationship between mouse tumour and guinea-pig kidney haemolysins and goat-erythrocyte amboceptors respectively, according to the methods of Morgenroth and Bieling.

I. IMMUNISING METHODS.

To immunise with organic cells and obtain heterologous haemolysin many authors have applied the methods, in some cases modified, already used by Forssman. The methods used by Morgenroth and Bieling are very well adapted for the obtaining of exact results. There are, however, in spite of everything, some unavoidable difficulties when immunising with organic and tumour cells, because, apart from the difficulty that exists in obtaining the antigen absolutely free from blood, it is scarcely possible to separate the connective tissue from the parenchyma; an example of this is seen in Bail and Margulies's (1913) use of guinea-pig kidney as antigen.

However, one can probably neglect the influence of connective tissue, because in mouse tumour and guinea-pig kidney the parenchyma is so largely in excess.

Morgenroth and Bieling's methods were therefore adopted and are as follows:

The immunising materials—mouse tumour and guinea-pig kidney—are removed from animals which have been bled out and are cut up under sterile conditions and then washed with normal saline in order to get rid of as much blood as possible. These materials are ground down in a sterile mortar without any addition of sea sand. To the pulp a certain quantity of saline is added and the whole filtered through an ordinary handkerchief under slight pressure. The emulsions thus prepared were injected intraperitoneally into rabbits, twice or three times with intervals of from eight to ten days.

As to the dosage, Forssman had already suggested that a large quantity does not give a proportionately high immunisation, and Morgenroth and Bieling confirming this assertion, used for each injection emulsion corresponding to 1–2 grms. of original material.

Eight days after the last inoculation, the animals were bled from the carotid under narcosis. The immunised sera obtained were inactivated the next day at 56° C. for half-an-hour. To test the

haemolytic reaction 0·5 c.c. of a 5% washed goat erythrocyte suspension was employed. The complement was 1 c.c. of a 1 in 10 dilution of fresh guinea-pig serum, the absolute quantity of complement in each tube thus being 0·1 c.c. The minimal dose which still can cause complete haemolysis under these conditions was named the amboceptor unit.

The degrees of haemolysis were distinguished as follows:

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

The following protocols show the haemolysin antigenic power of guinea-pig kidney and mouse tumour.

Immunisation with guinea-pig kidney.

Rabbit 1.

1915. 8th Nov. 5·0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
15th „ 5·0 „ „ „ „
22nd „ 10·0 „ „ „ „
30th „ Bled out.
1st Dec. Amboceptor unit = 0·005.

Rabbit 2.

1915. 8th Nov. 5·0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
15th „ 5·0 „ „ „ „
22nd „ 10·0 „ „ „ „
30th „ Bled out.
1st Dec. Amboceptor unit = 0·002.

Rabbit 3.

1915. 8th Nov. 5·0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
15th „ 5·0 „ „ „ „
22nd „ 10·0 „ „ „ „
30th „ Bled out.
1st Dec. Amboceptor unit = 0·001.

Immunisation with mouse tumour.

For this purpose mouse carcinoma 199 and sarcoma 37 p. which were supplied by the kindness of Dr Murray, the Director of the Imperial Cancer Research Fund, London, were used. The virulence of these tumours is so great that their takes by transplantation are practically 100 per cent., and their proliferative activity so rapid that they reach from 1 grm. to 1·5 grms. two weeks after an inoculation of 0·03 c.c.

Immunisation with mouse carcinoma 199.

Rabbit 4.

1915. 19th Nov. 7·0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
30th „ 6·0 „ „ „ „
10th Dec. 10·0 „ „ „ „
18th „ Bled out.
19th „ Amboceptor unit = 0·005 c.c.

Rabbit 5.

1915. 19th Nov. 5·0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
30th „ 6·0 „ „ „ „
10th Dec. 10·0 „ „ „ „
18th „ Bled out.
19th „ Amboceptor unit = 0·006 c.c.

Rabbit 6.

1915. 19th Nov. 5·0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
30th „ 6·0 „ „ „ „
10th Dec. 10·0 „ „ „ „
15th „ Died.

Immunisation with mouse sarcoma 37 p.

Rabbit 7.

1915. 2nd Dec. 10·0 c.c. of 25 % mouse sarcoma 37 p. emulsion intraperitoneally.
10th „ 7·0 „ „ „ „
20th „ 10·0 „ „ „ „
28th „ Bled out.
29th „ Amboceptor unit = 0·02 c.c.

Rabbit 8.

1915. 2nd Dec. 10·0 c.c. of 25 % mouse sarcoma 37 p. emulsion intraperitoneally.
10th „ 8·0 „ „ „ „
18th „ Bled out.
19th „ Amboceptor unit = 0·025 c.c.

According to Morgenroth and Bieling, mouse tumour—carcinoma—contains more antigenic receptors than mouse kidney, but from the above experiments, although few in number, the antigenic power of mouse tumour, either carcinoma or sarcoma, does not appear to exceed that of guinea-pig kidney.

II. Fixation Experiments.

The guinea-pig kidney and mouse tumour emulsions employed in the following experiments were 10 per cent. by weight, and prepared according to Morgenroth and Bieling; that is to say, the materials were removed from animals, which had been bled out, were cut up, washed with normal saline in order to free them from blood as much as possible, and then centrifuged. Thus the organ and tumour cells were separated from the blood corpuscles, 10 per cent. suspensions of the precipitated cells being made by weight with normal saline. I found 2·0 c.c. of these suspensions an adequate amount for fixation with the serum used.

1. Fixation experiments with mouse carcinoma 199 serum. Rabbit No. 4. A.U. = 0·005 c.c.

For control was used, as in Morgenroth and Bieling's experiments, rabbit kidney emulsion prepared in the way already described.

To 1·0 c.c. (200 A.U.) of the inactivated carcinoma serum was added 2·0 c.c. of 10 % carcinoma, guinea-pig and rabbit kidney emulsions respectively, thus making 3·0 c.c. in all:

1·0 c.c. carcinoma serum (200 A.U.) +2·0 c.c. of 10 % carcinoma emulsion.
1·0 „ „ „ „ +2·0 „ of 10 % guinea-pig kidney emulsion.
1·0 „ „ „ „ +2·0 „ of 10 % rabbit kidney emulsion.

The mixtures were placed for one hour at 37° C. and afterwards centrifuged. The slightly opaque supernatant fluids were then tested haemolytically, using 0·1 c.c. guinea-pig complement and 0·5 c.c. of 5 % goat erythrocyte suspension. After placing for one hour and a half at 37° C., the tubes were kept in the cold room and the result observed the following morning.

The following table shows the results:

TABLE I.

Mouse carcinoma serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Mouse carcinoma	Guinea-pig kidney	Rabbit kidney
1.	1·2 c.c.	80 A.U.	c.	c.	c.
2.	0·6 „	40 „	n.c.	„	„
3.	0·3 „	20 „	n.c.—m.	n.c.	„
4.	0·15 „	10 „	m.	m.	„
5.	0·075 „	5 „	sl.	sl.	„
6.	0·03 „	2 „	sl.—tr.	tr.	„
7.	0·015 „	1 „	tr.—n.	tr.—n.	n.c.
8.	0·01 „	⅔ „	n	n.	m

Mouse carcinoma is thus seen to fix very strongly, while guinea-pig kidney was rather feebler, and by calculating according to Morgenroth and Bieling's method, it is found that mouse carcinoma fixed from at least $\frac{39}{40}$ to $\frac{79}{80}$ of the amboceptors, namely, from 195 to 197 A.U., while guinea-pig kidney deviated $\frac{19}{20}$ to $\frac{39}{40}$; namely, from 190 to 195 A.U. The attempt to fix with rabbit kidney, as Morgenroth has already noted, does not succeed, so that 1 amboceptor unit after absorption still caused practically complete haemolysis. Thus it is clear that rabbit kidney does not fix haemolytic amboceptors, in the same way as, according to Forssman, it does not elicit the antibodies.

2. Fixation experiments with guinea-pig kidney serum. Rabbit No. 3. A.U. = 0·001 c.c.

To tubes containing 0·2 c.c. (200 A.U.) of the serum and 0·8 c.c. saline were added 2·0 c.c. of guinea-pig, mouse carcinoma and rabbit kidney emulsions respectively, the total amount thus being 3 c.c.

0·2 c.c. guinea-pig kidney serum (200 A.U.) + 0·8 c.c. saline + 2·0 c.c. guinea-pig kidney emulsion.

0·2 c.c. guinea-pig kidney serum (200 A.U.) + 0·8 c.c. saline + 2·0 c.c. mouse carcinoma emulsion.

0·2 c.c. guinea-pig kidney serum (200 A.U.) + 0·8 c.c. saline + 2·0 c.c. rabbit kidney emulsion.

After treating the mixtures as before, the supernatant fluids were obtained and tested for haemolytic reaction.

TABLE II.

Guinea-pig kidney serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with		
			Guinea-pig kidney	Mouse carcinoma	Rabbit kidney
1.	1·2 c.c.	80 A.U.	c.	c.	c.
2.	0·6 ,,	40 ,,	n.c.	,,	,,
3.	0·3 ,,	20 ,,	m.	,,	,,
4.	0·15 ,,	10 ,,	sl.	,,	,,
5.	0·075 ,,	5 ,,	tr.	n.c.	,,
6.	0·03 ,,	2 ,,	n.	m.	,,
7.	0·015 ,,	1 ,,	,,	tr.	n.c.
8.	0·01 ,,	$\frac{2}{3}$,,	,,	n.	m.

The fixing power of the guinea-pig kidney emulsion for the amboceptors of the homologous serum lies between $\frac{79}{80}$ and $\frac{39}{40}$, whilst that of the carcinoma emulsion lies between $\frac{9}{10}$ and $\frac{4}{5}$; in other words, although the carcinoma antigens were capable of fixing haemolytic antibodies, these were most strongly fixed by homologous antigens. Rabbit kidney again showed no power to absorb the haemolysin, so

that one amboceptor unit, after treatment, still caused nearly complete haemolysis. However, the behaviour of goat haemolysin, with mouse carcinoma and guinea-pig kidney emulsion, is quite different.

3. Fixation experiments with goat haemolysin. A.U. = 0·0005 c.c.

The mixture this time consisted of 0·1 c.c. (200 A.U.) of the goat haemolysin, 0·9 c.c. saline and 2·0 c.c. of goat erythrocytes (10 % by weight), guinea-pig and rabbit kidney suspensions respectively. After treating the mixtures as before, the haemolytic power of the supernatant fluids was tested.

TABLE III.

Goat red cell serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with			
			Goat erythrocytes	Mouse carcinoma	Guinea-pig kidney	Rabbit kidney
1.	0·6 c.c.	40 A.U.	c.	c.	c.	c.
2.	0·3 ,,	20 ,,	,,	,,	,,	,,
3.	0·15 ,,	10 ,,	m.	,,	,,	,,
4.	0·075 ,,	5 ,,	sl.	,,	,,	,,
5.	0·03 ,,	2 ,,	n.	,,	,,	,,
6.	0·015 ,,	1 ,,	,,	n.c.	,,	,,
7.	0·0075 ,,	½ ,,	,,	m.	m.	n.c.
8.	0·0004 ,,	¼ ,,	,,	tr.	tr.	sl.

As the table demonstrates, goat blood cells fix the amboceptors well, while not only rabbit kidney, but carcinoma and guinea-pig kidney do not absorb them practically at all. This result is very curious, because both mouse carcinoma and guinea-pig kidney contain antigen for the production of amboceptors haemolytic to goat red cells. To explain this fact, the following statement is probably admissible: goat erythrocytes contain many groups of receptors able to produce amboceptors haemolytic to goat blood cells, while mouse carcinoma and guinea-pig kidney contain some of these groups only.

If, therefore, haemolytic serum produced by immunisation with goat blood cells is absorbed with mouse carcinoma or guinea-pig kidney emulsions, those amboceptor groups corresponding to their own receptors are fixed, while there still remain the other groups of antibodies to cause haemolysis. It is certainly due to a want of any receptors in common that rabbit kidney did not fix goat haemolysin amboceptors.

Another question approached in this paper is concerned with the fixing power of goat blood corpuscles for the haemolytic amboceptors of mouse carcinoma and guinea-pig kidney serum. Although Morgenroth and Bieling observed that goat erythrocytes fix the haemolytic

antibodies of mouse carcinoma serum more strongly than mouse kidney emulsion, they did not publish an exact account.

4. Fixation experiments with goat blood cells.

To 1·0 c.c. (200 A.U.) of mouse carcinoma serum and 0·2 c.c. (200 A.U.) of guinea-pig kidney, plus 0·8 c.c. saline, 2·0 c.c. of 10 % goat erythrocytes were added, while the same amount of mouse carcinoma and guinea-pig kidney emulsion was added, each to its corresponding serum as control.

1·0 c.c. mouse carcinoma serum (200 A.U.) + 2·0 c.c. of 10 % goat blood cells.
1·0 „ „ „ „ + 2·0 „ of 10 % mouse carcinoma emulsion.
0·2 „ guinea-pig kidney serum (200 A.U.) + 0·8 c.c. saline + 2·0 c.c. of 10 % goat blood cells.
0·2 c.c. guinea-pig kidney serum (200 A.U.) + 0·8 c.c. saline + 2·0 c.c. of 10 % guinea-pig kidney emulsion.

The mixtures were treated as before and the haemolytic reaction of the supernatant fluids tested.

TABLE IV. A.

Mouse carcinoma serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Goat erythrocytes	Haemolysis after absorption with: Mouse carcinoma (control)
1.	1·2 c.c.	80 A.U.	m.	c.
2.	0·6 „	40 „	sl.	n.c.
3.	0·3 „	20 „	tr.	n.c.—m.
4.	0·15 „	10 „	„	m.
5.	0·075 „	5 „	n.	sl.
6.	0·03 „	2 „	„	sl.—tr.
7.	0·015 „	1 „	„	tr.—n.
8.	0·01 „	⅔ „	„	n.

TABLE IV. B.

Guinea-pig kidney serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Goat erythrocytes	Haemolysis after absorption with: Guinea-pig kidney (control)
1.	1·2 c.c.	80 A.U.	n.c.	c.
2.	0·6 „	40 „	m.	n.c.
3.	0·3 „	20 „	sl.	m.
4.	0·15 „	10 „	tr.	sl.
5.	0·075 „	5 „	n.	tr.
6.	0·03 „	2 „	„	n.
7.	0·015 „	1 „	„	„
8.	0·01 „	⅔ „	„	„

As the preceding experiments demonstrate, goat blood cells have a much greater fixing power for both haemolytic amboceptors than their homologous antigens, this being particularly the case with the carcinoma amboceptors.

It has been already stated that rabbit kidney fixes the amboceptors of neither mouse carcinoma, guinea-pig kidney, nor goat erythrocyte antiserum, and the immunising of rabbit with rabbit kidney cells conversely does not elicit the production of haemolysins. Consequently, it is natural to suppose that the organs of other animals, for example rat kidney which, according to Forssman and Morgenroth, does not contain the haemolysin antigen, cannot have any fixing power for the amboceptors of mouse carcinoma, guinea-pig kidney and goat erythrocyte antisera.

5. Fixation experiment with rat kidney emulsion.

The experiment was performed as follows:

A. 0·5 c.c. (100 A.U.) mouse carcinoma serum + 0·5 c.c. saline + 2·0 c.c. rat kidney emulsion.

B. 0·1 c.c. (100 A.U.) guinea-pig kidney serum + 0·9 c.c. saline + 2·0 c.c. rat kidney emulsion.

C. 0·1 c.c. (200 A.U.) goat haemolysin + 0·9 c.c. saline + 2·0 c.c. rat kidney emulsion.

These mixtures were treated as before and the haemolytic reaction of the supernatant fluids tested.

TABLE V. A.

Mouse carcinoma serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Mouse carcinoma	Haemolysis after absorption with: Rat kidney
1.	1·2 c.c.	40 A.U.	c.	c.
2.	0·6 ,,	20 ,,	n.c.	,,
3.	0·3 ,,	10 ,,	,,	,,
4.	0·15 ,,	5 ,,	m.	,,
5.	0·075 ,,	$2\frac{1}{2}$,,	sl.	,,
6.	0·03 ,,	1 ,,	sl.—tr.	,,
7.	0·015 ,,	$\frac{1}{2}$,,	n.	n.c.
8.	0·01 ,,	$\frac{1}{3}$,,	,,	sl.

TABLE V. B.

Guinea-pig kidney serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Guinea-pig kidney	Haemolysis after absorption with: Rat kidney
1.	1·2 c.c.	40 A.U.	c.	c.
2.	0·6 ,,	20 ,,	n.c.	,,
3.	0·3 ,,	10 ,,	m.	,,
4.	0·15 ,,	5 ,,	sl.	,,
5.	0·075 ,,	$2\frac{1}{2}$,,	tr.	,,
6.	0·03 ,,	1 ,,	n.	,,
7.	0·015 ,,	$\frac{1}{2}$,,	,,	n.c.
8	0·01 ,,	$\frac{1}{3}$,,	,,	m.

https://doi.org/10.1017/S002217240000629X Published online by Cambridge University Press

TABLE V. C.

Goat erythrocyte serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Goat erythrocytes	Haemolysis after absorption with: Rat kidney
1.	0·6 ,,	40 A.U.	c.	c.
2.	0·3 ,,	20 ,,	,,	,,
3.	0·15 ,,	10 ,,	m.	,,
4.	0·075 ,,	5 ,,	sl.	,,
5.	0·03 ,,	2 ,,	n.	,,
6.	0·015 ,,	1 ,,	,,	,,
7.	0·0075 ,,	½ ,,	,,	m.
8.	0·004 ,,	¼ ,,	,,	sl.

The tables show that the supposition was right, that is to say, rat kidney emulsion does not fix the amboceptors practically at all; so that one amboceptor unit in all cases still caused after treatment nearly complete haemolysis. So, it is confirmed that, as Morgenroth asserted, the fixation reaction is specific.

The mouse tumour used in the foregoing experiments has throughout been carcinoma.

It still remained to be seen whether mouse sarcoma serum would behave in the same way. For this purpose serum 37 p. was used, and exactly the same method applied as before.

6. Fixation experiment. Amboceptor unit of serum 37 p.=0·02 c.c.

It is regrettable that this time I could not for certain reasons use homologous tumour emulsion; mouse carcinoma emulsion had to be employed instead.

A. 2·0 c.c. (100 A.U.) of the serum + 2·0 c.c. mouse carcinoma emulsion.

B. 2·0 c.c. (100 A.U.) of the serum + 2·0 c.c. guinea-pig kidney emulsion.

Here the mixtures amounted to 4·0 c.c., it being necessary to take 2·0 c.c. of the serum in order to obtain 100 amboceptor units.

After absorption the haemolytic reaction was carried out on the supernatant fluids.

TABLE VI.

Mouse sarcoma serum.

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with: Mouse carcinoma	Haemolysis after absorption with: Guinea-pig kidney
1.	0·8 c.c.	20 A.U.	c.	m.
2.	0·4 ,,	10 ,,	n.c.	,,
3.	0·2 ,,	5 ,,	m.	sl.
4.	0·1 ,,	2½ ,,	m.—sl.	tr.
5.	0·04 ,,	1 ,,	sl.	n.
6.	0·02 ,,	½ ,,	tr.	,,

https://doi.org/10.1017/S002217240000629X Published online by Cambridge University Press

It is seen that guinea-pig kidney fixes the amboceptors of mouse sarcoma serum better than mouse carcinoma. In other words, it seems that the former contains more lysinogen than the latter for the amboceptors of sarcoma 37 p. antiserum.

III. Transgression Experiments.

The cell-sediments produced by centrifugation (either mouse tumour or guinea-pig kidney) were carefully separated from their supernatant fluids and 10 % suspensions prepared each time before the experiment. The only difference was that the quantity of suspension consisted of 2·0 c.c. while Morgenroth and Bieling used 2·5 c.c.

1. Transgression experiment with mouse carcinoma serum.

Here the sediment obtained in fixation experiment 1, whose protocol is produced here, was used.

1·0 c.c. (200 A.U.) mouse carcinoma serum + 2·0 c.c. carcinoma 199 emulsion = 3·0 c.c.
1·0 c.c. (200 A.U.) mouse carcinoma serum + 2·0 c.c. guinea-pig kidney emulsion = 3·0 c.c.

To the sediments produced by centrifugation was added saline to the original volume (3·0 c.c.). With diminishing amounts of these cell suspensions 0·5 c.c. of 5 % goat erythrocytes was mixed; after incubating at 37° C. for one hour, 0·1 c.c. complement was added, and the tubes again placed in the hot room for two hours.

The following table shows the results:

TABLE VII.

	Amount of cell suspension	Haemolysis after transgression with: Mouse carcinoma	Haemolysis after transgression with: Guinea-pig kidney
1.	1·0 c.c.	sl.—m.	n.c.
2.	0·5 ,,	tr.	m.
3.	0·3 ,,	n.	,,
4.	0·2 ,,	,,	sl.
5.	0·1 ,,	,,	tr.
6.	0·05 ,,	,,	n.

Both suspensions, but especially carcinoma emulsion retained a considerable quantity of the fixed amboceptors and gave to the goat blood cells some of them only. In other words, carcinoma cells fixed amboceptors from carcinoma serum more permanently than guinea-pig kidney did.

2. Transgression experiment with guinea-pig kidney serum.

Here the cell sediments obtained in fixation experiment 2 as shown in the following protocol were used:

0·2 c.c. (200 A.U.) guinea-pig kidney serum + 2·0 c.c. guinea-pig kidney emulsion + 0·8 c.c. saline = 3·0 c.c.

0·2 c.c. (200 A.U.) guinea-pig kidney serum + 2·0 c.c. mouse carcinoma 199 emulsion + 0·8 c.c. saline = 3·0 c.c.

To the sediments was added saline to the original volume (3·0 c.c.). The haemolytic reaction after the transgression was tested in the same way as before.

TABLE VIII.

	Amount of cell suspension	Haemolysis after transgression with: Guinea-pig kidney	Haemolysis after transgression with: Mouse carcinoma
1.	1·0 c.c.	c.	c.
2.	0·5 ,,	,,	,,
3.	0·3 ,,	,,	,,
4.	0·2 ,,	m.	,,
5.	0·1 ,,	sl.	n.c.
6.	0·05 ,,	tr.	m.

The amount of guinea-pig kidney suspension which caused complete haemolysis is 0·3 c.c., while that of carcinoma suspension is 0·2 c.c. Thus it is seen that the kidney cells have bound the amboceptors of the kidney serum more strongly than the carcinoma cells.

3. Transgression experiment with mouse sarcoma serum.

The sediments used were those obtained in fixation experiment 6, the following being the protocol:

2·0 c.c. (100 A.U.) mouse sarcoma serum 37 p. + 2·0 c.c. mouse carcinoma 199 emulsion = 4·0 c.c.

2·0 c.c. (100 A.U.) mouse sarcoma serum 37 p. + 2·0 c.c. guinea-pig kidney emulsion = 4·0 c.c.

The haemolytic reaction after transgression was carried out as before.

TABLE IX.

	Amount of cell suspension	Haemolysis after transgression with: Mouse carcinoma	Haemolysis after transgression with: Guinea-pig kidney
1.	1·0 c.c.	n.c.	n.c.
2.	0·5 ,,	m.—sl.	m.
3.	0·3 ,,	sl.	sl.
4.	0·2 ,,	tr.	tr.
5.	0·1 ,,	n.	n.
6.	0·05 ,,	n.	n.

Mouse carcinoma cells and kidney cells bound the amboceptors of mouse sarcoma serum 37 p. strongly and practically to the same degree —1·0 c.c. of the suspensions not causing complete haemolysis.

Conclusions.

1. Mouse carcinoma cells fix the haemolytic amboceptors of homologous serum more strongly than guinea-pig kidney cells.

2. Guinea-pig kidney cells fix the amboceptors of guinea-pig kidney antiserum more strongly than mouse carcinoma cells.

3. Goat blood cells are capable of fixing the amboceptors of both sera particularly well.

4. However, while goat blood cells can naturally absorb goat haemolysin, neither carcinoma nor kidney cells have any power of fixing goat haemolysin practically at all.

5. Rabbit and rat kidney cells, as they do not contain the lysinogen, do not absorb haemolytic amboceptors from the three antisera.

6. This fixation reaction is therefore to be regarded as specific.

7. Transgression experiments show that homologous antigens bind their amboceptors more strongly than heterologous; thus in all probability there is no complete identity between the haemolytic amboceptors which are produced by immunising rabbit against mouse tumours—carcinoma and sarcoma—and guinea-pig kidney. It will be remembered that Morgenroth and Bieling could not establish identity between carcinoma and mouse kidney haemolysis.

In conclusion, I desire to express my thanks to Dr Schütze for his advice in the course of this work.

BIBLIOGRAPHY.

Bail and Margulies (1913). Untersuchungen über die Absorption von Schafblut-haemolysinen durch Meerschweinchenorgane. *Zeitschr. f. Immunitätsforsch.* XIX. 185.

Forssman (1911). Die Herstellung hochwertiger spezifischer Schafhaemolysine ohne Verwendung von Schafblut. *Biochem. Zeitschr.* XXXVII. 78.

Morgenroth (1913). Haemolytische Versuche mit gegen Mäusekarzinom immunisierendem Serum. *Berlin. klin. Wochenschr.* 561.

Morgenroth and Bieling (1915). Amboceptoren und Receptoren. *Biochem. Zeitschr.* LXVIII. 561.

Orudschiew (1913). Ueber die Beziehungen der haemolytischen Hammelblut-amboceptoren zu den Receptoren des Meerschweinchens. *Zeitschr. f. Immunitätsforsch.* XVI. 268.

Spaet (1914). Untersuchungen über ein Leucocytenimmunserum. *Ibid.* XXI. 565.

Tsurumi (1916). A serological investigation of heterologous immunity to malignant tumours of the mouse and rat. *Journ. of Pathol. and Bacteriol.* XX. 214.