# A SEROLOGICAL INVESTIGATION OF HETEROLOGOUS IMMUNITY TO MALIGNANT TUMOURS OF THE MOUSE AND RAT.[1]

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## CONTENTS.

## Introduction.

The experimental investigation of resistance to cancer may be pursued along two lines—homologous and heterologous immunity reactions. A previous paper "On Sarcoma Immunity in Mice" (1915 [39]) dealt entirely with the phenomena of homologous resistance. The present communication is concerned with the phenomena of heterologous resistance as exemplified in the reactions of the sera of animals immunised with transplanted new growths from alien species. Although the properties of such immune sera may be described generally as cytotoxic, the methods employed to study them have mainly been developed along bacteriological lines. The serum reactions examined are—hæmolysis, hæmagglutination, complement fixation, precipitins, and cytolysis *sensu stricto*.

Anatomical and histological investigations have revealed only unimportant differences between the tumours of man and animals, whereas biological studies have proved the existence of fundamental distinctions involving the necessary conditions of growth of the neoplasms of each species. A combined application of the serum reactions mentioned may be expected to afford still more insight into the interrelation of host and tumour. The methods are particularly applicable to the study of tumours from one species growing temporarily in a host belonging to a different species as, *e.g.*, mouse and rat tumours growing in chick embryos (J. B. Murphy, 1914 [1]) and

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mouse tumours growing in baby rats—P. Ehrlich (1906 [2]) and W. E. Bullock (1915 [3]). Experiments were also performed to see if serological differences obtain between the different tumours of the same species. A large amount of transplanted rat sarcoma being available in the laboratory, rabbits were immunised with it, and the serum compared with the rabbit anti-mouse immune sera.

In addition, a series of experiments was carried out to provide a basis for comparison with homologous cancer immunity. These were —(*a*) Immunising rabbits with watery extract of mouse tumour instead of intact tumour cells. For homologous resistance intact cells are necessary. (*b*) Using mouse embryo as antigen for heterologous immunity, mouse embryo being a convenient antigen for homologous resistance. (*c*) Studying the reactions of the sera of baby rats in which mouse tumour had grown for a time and been absorbed.

## I. IMMUNISING METHODS.

In the first instance, the immunising material was obtained by transplanting into mice and also into baby rats the mouse tumours, carcinoma **63** and sarcoma **37 p** used for the earlier work on homologous resistance. Later, the observations were repeated with other strains, carcinoma **199** and sarcoma **37 s**. A useful comparison was obtained by immunising with rat sarcoma **R 16**. The animals immunised were young rabbits varying in weight between 1000 and 1500 grms. The tumours, aged from twelve days to three weeks according to the rate of growth, were removed aseptically, freed from macroscopically necrotic and hæmorrhagic areas, and without any addition minced to a fine emulsion with sharp scissors. The elaborate methods of preparation adopted by Forssmann, Morgenroth, and others with the object of obtaining organ or tumour parenchymata free from blood and connective tissue are largely illusory. Complete freedom from blood cannot be achieved in the case of tumours even by washing out the vessels with salt solution, and the connective-tissue stroma is so delicate and so intimately associated with the parenchyma that only the largest trabeculæ can be separated from the tumour cells by the methods employed.

The injections were made intraperitoneally and repeated at intervals of seven days in ascending doses. Four injections in all were given, the doses being 1, 2, 4, and 5 c.c. Before each injection the rabbits were weighed and their temperatures taken. If loss of weight or rise in temperature had occurred the injection was postponed till recovery had taken place. In some cases the injections were not borne well, temporary malaise being produced: some of the animals died in the course of the immunisation. Consequently it´is rather difficult to immunise very highly, but with the disposition described a relatively high degree of immunity could be obtained. Eight days after the

last injection the animals were bled from the carotid under an anæsthetic.

The peritoneal lymph glands were always found to be much enlarged, to the size of a hen's egg, and filled with sterile, completely degenerated caseous material.


## II. HÆMOLYTIC REACTION.

The hæmolytic action of the sera of men and animals bearing tumours has been studied by many authors.

Thus Kelling (1906 [4]) studied the hæmolytic action of human cancer sera on the erythrocytes of various animals, and Maragliano (1892 [5]) the action on human erythrocytes. The gastric juice from cases of stomach cancer was examined for hæmolysins by Grafe and Roehmer (1908 [6]), while tumour extract was studied in the same way by Kullman (1904 [7]). Fischel (1908 [8]) summarises the results obtained by different investigators as follows. The serum of cancer patients shows increased hæmolytic power in about 50 per cent. of cases both on the erythrocytes of the same and different species. The reaction is not specific for cancer since it is also found in other diseases, *e.g.*, tuberculosis, pernicious anæmia, etc.

The researches of Forssmann and Morgenroth bear more directly on the present investigations. In 1911, Forssmann ([9]) discovered that a hæmolysin for sheep erythrocytes could be obtained by immunising rabbits with emulsions of organs of the guinea-pig. Even when the hæmolytic power on sheep's corpuscles had attained a high degree, no hæmolysin for guinea-pig corpuscles was obtained. Two years later, Morgenroth (1913 [10], 1915 [10a]) obtained a hæmolytic amboceptor for goat corpuscles by immunising rabbits against mouse kidney. In the same way he obtained the hæmolysis of goat erythrocytes with the serum of rabbits inoculated with transplantable mouse carcinoma. In the later paper Morgenroth and Bieling (1915 [10a]) published a fuller account of their researches. Rabbits immunised with mouse kidney and with one strain of mouse carcinoma gave sera hæmolytic for goat erythrocytes, but not for those of the ox and the mouse. The hæmolytic amboceptor of the kidney antiserum was fixed by both kidney and tumour emulsions, but more strongly by the kidney. The amboceptor of the tumour antiserum was usually fixed more energetically by tumour emulsion than by kidney emulsion. The authors concluded that the antigenic substances in kidney and tumour were in part common to both, in part distinct.

In the present researches these results of Forssmann and Morgenroth have been confirmed and extended by experiments with the sera of rabbits immunised against mouse and rat tumours. In addition to goat corpuscles, those of the mouse and rat have also been employed with interesting results.


### *Methods.*

The immune sera were inactivated by heating for half an hour at 56° C. The complement used in the experiments was 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum, the absolute quantity of fresh serum in each tube being thus 0·05 c.c. For comparison, rabbit, rat, mouse, and goat complements were also employed. The erythrocyte suspensions used were from the mouse, rat, goat, rabbit, and guinea-pig, the principal experiments being per-

formed with mouse corpuscles.   To obtain the mouse corpuscles a number of
mice were decapitated with scissors in deep ether narcosis, the blood collected
and defibrinated by stirring with a sterile wire brush.   The defibrinated
blood was centrifuged and washed with normal saline three times, and a
5 per cent. suspension in normal saline made from the corpuscles deposited.
Of this suspension 0·5 c.c. was taken for each tube.   The test tubes were kept
in an incubator at 37° C. for two hours, and then placed in the ice-chest till the
following morning.   After this interval the result was noted, the degrees of
hæmolysis being distinguished as follows: complete, nearly complete, marked,
slight, trace, and none.

### *Experimental Observations.*

The investigations of Forssmann and Morgenroth already referred
to reveal a departure from the specific character of immunity reactions
till then unsuspected in the hæmolysins.   Thus in Forssmann's experi-
ments the serum of the rabbit immunised with organ-emulsions of the
guinea-pig while strongly hæmolytic for sheep's corpuscles was without
action on the corpuscles of the guinea-pig.   Morgenroth stated that
his immune sera, strongly hæmolytic for goat corpuscles, were without
action on the erythrocytes of the mouse, the animal from which the
organ and tumour emulsions used by him as antigens were obtained.
The experiments now to be described show that this discrepancy is
only apparent, since the immune sera obtained from rabbits injected
with mouse-tumour emulsions are hæmolytic for mouse erythrocytes,
as might have been expected from general considerations.   The
hæmolytic immune body for goat erythrocytes will be shown to be
distinct, and, as it were, superadded to the hæmolytic amboceptor
specific to the mouse.

At the same time experiments were carried out to ascertain
whether the fresh sera of the rabbit, rat, and mouse were able to
complement the immune body in the same way as that of the guinea-
pig.   The immune sera were those from rabbits repeatedly injected
with emulsions of mouse carcinoma **63** and mouse sarcoma **37 p.**   It
is to be noted that neither the highest testing dose of inactivated
immune serum, namely, 0·1 c.c., nor 0·05 c.c. of the fresh sera used as
complements, of themselves caused hæmolysis of mouse corpuscles.
Inactivated normal rabbit serum 0·1 c.c., with the addition of 0·05 c.c.
fresh guinea-pig serum as complement was without action on the
corpuscles of the mouse, rat, guinea-pig, and goat.

The experiments (Tables I. and II.) demonstrate clearly that the
sera of rabbits immunised with mouse tumours, carcinoma and sarcoma,
are strongly hæmolytic for mouse corpuscles.   Guinea-pig complement
is seen to be most effective, while those of the mouse and rat are
inert in the dose employed.   The behaviour of rabbit complement is
not the same with the two antisera; while inactive in combination
with the sarcoma serum, it is able to produce marked hæmolysis with
the carcinoma serum.   This difference indicates that the hæmolytic

amboceptors in the carcinoma and sarcoma antisera are not quite identical. The experiment was repeated with two other sera obtained by immunising with sarcoma **37 s** and carcinoma **199**, and a very similar result obtained, the only difference being a slight degree of hæmolysis with the sarcoma antiserum in this case.

TABLE I.—*Hæmolysis of Mouse Blood Corpuscles by Mouse Sarcoma* (**37 p**) *Antiserum, using Various Complements.*

| | | MOUSE BLOOD CORPUSCLES. | | | |
|---|---|---|---|---|---|
| | **37 p** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. | Rat Complement, 0·05 c.c. | Mouse Complement. 0·05 c.c. |
| 1 | 0·1 | Complete. | None. | None. | None. |
| 2 | 0·05 | ,, | ,, | ,, | ,, |
| 3 | 0·025 | ,, | ,, | ,, | ,, |
| 4 | 0·01 | ,, | ,, | ,, | ,, |
| 5 | 0·005 | Nearly complete. | ,, | ,, | ,, |
| 6 | 0·0025 | Slight. | ,, | ,, | ,, |
| 7 | 0·001 | Trace. | ,, | ,, | ,, |

TABLE II.—*Hæmolysis of Mouse Blood Corpuscles by Mouse Carcinoma* (**63**) *Antiserum, using Various Complements.*

| | | MOUSE BLOOD CORPUSCLES. | | | |
|---|---|---|---|---|---|
| | **63** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. | Rat Complement, 0·05 c.c. | Mouse Complement, 0·05 c.c. |
| 1 | 0·1 | Complete. | Marked. | None. | None. |
| 2 | 0·05 | ,, | Slight. | ,, | ,, |
| 3 | 0·025 | Nearly complete. | Nearly complete. | ,, | ,, |
| 4 | 0·01 | Slight. | None. | ,, | ,, |
| 5 | 0·005 | Trace. | ,, | ,, | ,, |
| 6 | 0·0025 | None. | ,, | ,, | ,, |
| 7 | 0·001 | ,, | ,, | ,, | ,, |

In view of the results obtained in the study of blood relationship by the hæmolytic method, it is interesting to study the action of these anti-mouse sera on the red blood cells of the closely related rat. Tables III. and IV. give the results of two of these experiments.

TABLE III.—*Hæmolysis of Rat Blood Corpuscles by Mouse Sarcoma* (**37 p**) *Antiserum, using Guinea-pig and Rabbit Complement.*

| RAT BLOOD CORPUSCLES. | | | |
|---|---|---|---|
| | **37 p** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. |
| 1 . . | 0·1 | Nearly complete. | None. |
| 2 . . | 0·05 | Marked. | ,, |
| 3 . . | 0·025 | Slight. | ,, |
| 4 . . | 0·01 | None. | ,, |
| 5 . . | 0·005 | ,, | ,, |
| 6 . . | 0·0025 | ,, | ,, |
| 7 . . | 0·001 | ,, | ,, |

TABLE IV.—*Hæmolysis of Rat Blood Corpuscles by Mouse Carcinoma* (63) *Antiserum, using Guinea-pig and Rabbit Complement.*

| RAT BLOOD CORPUSCLES. | | | |
|---|---|---|---|
| | **63** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. |
| 1 . . | 0·1 | Slight. | None. |
| 2 . . | 0·05 | Trace. | ,, |
| 3 . . | 0·025 | None. | ,, |
| 4 . . | 0·01 | ,, | ,, |
| 5 . . | 0·005 | ,, | ,, |
| 6 . . | 0·0025 | ,, | ,, |
| 7 . . | 0·001 | ,, | ,, |

The experiments demonstrate a much feebler action of the sarcoma serum on rat corpuscles than on those of the mouse, and in the case of the carcinoma serum the action is still less evident. The same result was obtained in the experiment with two other antisera, against sarcoma **37 s** and carcinoma **199**. As in the experiments with mouse corpuscles, guinea-pig complement is much more active than rabbit complement, the dose of the latter used being quite incapable of producing hæmolysis. The sera are thus seen to be specific to a

considerable degree, doses which still produce marked hæmolysis of mouse corpuscles being without action on the corpuscles of the rat.

When these antisera are tested for hæmolytic activity against goat blood corpuscles a very striking contrast is shown. The result of such an experiment is seen in Tables V. and VI.

TABLE V.—*Hæmolysis of Goat Blood Corpuscles by Mouse Sarcoma* (**37 p**) *Antiserum.*

| | GOAT BLOOD CORPUSCLES. | |
|---|---|---|
| | **37 p** Antiserum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . | 0·1 | Complete. |
| 2 . . . | 0·05 | ,, |
| 3 . . . | 0·025 | ,, |
| 4 . . . | 0·01 | ,, |
| 5 . . . | 0·005 | Nearly complete. |
| 6 . . . | 0·0025 | Marked. |
| 7 . . . | 0·001 | Slight. |

TABLE VI.—*Hæmolytic Reaction of Goat Blood Corpuscles by Mouse Carcinoma* (**63 p**) *Antiserum.*

| | GOAT BLOOD CORPUSCLES. | |
|---|---|---|
| | **63** Antiserum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . | 0·1 | Complete. |
| 2 . . . | 0·05 | ,, |
| 3 . . . | 0·025 | ,, |
| 4 . . . | 0·01 | Marked. |
| 5 . . . | 0·005 | ,, |
| 6 . . . | 0·0025 | Trace. |
| 7 . . . | 0·001 | None. |

Hæmolysis is obtained with both sera (sarcoma and carcinoma) to the same degree as in the experiments with mouse erythrocytes. As

in those experiments (cf. Tables I. and II.), the **37 p** antiserum is rather more potent than the serum against carcinoma **63**. Taken in conjunction with the experiments on rat erythrocytes the experiments with goat corpuscles are sufficiently remarkable, for they seem to belie the evidence of specificity in the experiments with the blood cells of the mouse and rat. Experiments were therefore undertaken in the first place with erythrocytes of other species, namely, rabbit and guinea-pig. It is unnecessary to give tables of these experiments as they failed to give a positive result, although the first tube of sarcoma antiserum (0·1 c.c.) caused slight hæmolysis of guinea-pig corpuscles. The antibody, therefore, which hæmolyses goat corpuscles is not active on red blood corpuscles generally, and the conclusion cannot be drawn, from the experiments so far described, that the lysis of mouse corpuscles by these antisera is not due to a specific hæmolysin.

Before passing to experiments designed to elucidate the relation of the agents in these sera responsible for hæmolysis of mouse and goat corpuscles respectively, it is instructive to test the effect on mouse and rat erythrocytes of a hæmolysin for goat corpuscles obtained in the ordinary way by immunising rabbits with goat's blood (Table VII.).

TABLE VII.—*The Behaviour of Ordinary Hæmolytic Amboceptors on Mouse and Rat Blood Corpuscles.*

| | Ordinary Amboceptors. | Guinea-pig Complement. | On Mouse Erythrocytes. | On Rat Erythrocytes. |
|---|---|---|---|---|
| 1 . . . | 0·01 | 0·05 c.c. | None. | None. |
| 2 . . . | 0·005 | ,, ,, | ,, | ,, |
| 3 . . . | 0·0025 | ,, ,, | ,, | ,, |
| 4 . . . | 0·001 | ,, ,, | ,, | ,, |
| 5 . . . | 0·0005 | ,, ,, | ,, | ,, |
| 6 . . . | 0·00025 | ,, ,, | ,, | ,, |
| 7 . . . | 0·0001 | ,, ,, | ,, | ,, |

*Note.*—0·0005 c.c. of this hæmolysin causes a complete hæmolysis of 5 per cent. goat blood corpuscles suspension (0·5 c.c.).

The experiment shows that the goat hæmolysin is without action on mouse or rat corpuscles even in a concentration seventy times the minimal hæmolytic dose for goat corpuscles. Hence the action of the hæmolysin produced by mouse tumour as antigen is not due to identity of the lysinogens for mouse and goat erythrocytes, and the antibodies in the tumour antisera which dissolve mouse and goat corpuscles respectively cannot be the same. To test the validity of

TABLE VIII. (*a*).—*Hæmolysis of Mouse Blood Cells by the Supernatant Fluid of Mouse Sarcoma* (37 p) *Serum, after Treatment with* 10 *per cent. Goat Blood Corpuscles.*

| | Mouse Sarcoma (37 p) Serum. | 10 per cent. Goat Red Cells. | 1 c.c. Supernatant Fluid = Original Serum. | | Guinea-pig Complement. | 5 per cent. Mouse Red Cells. | | | Control (without Treatment). |
|---|---|---|---|---|---|---|---|---|---|
| | | c.c. | | c.c. | c.c. | c.c. | | | |
| 1 | 0·1 | 1·0 | Two hours in an incubator at 37° C., then centrifuged. | = 0·05 | 0·05 | 0·5 | Two hours in an incubator at 37° C. | Complete. | Complete. |
| 2 | 0·05 | ,, | | = 0·025 | ,, | ,, | | ,, | ,, |
| 3 | 0·025 | ,, | | = 0·01 | ,, | ,, | | ,, | ,, |
| 4 | 0·01 | ,, | | = 0·005 | ,, | ,, | | Marked. | Marked. |
| 5 | 0·005 | ,, | | = 0·0025 | ,, | ,, | | Slight. | Slight. |
| 6 | 0·0025 | ,, | | = 0·001 | ,, | ,, | | Trace. | Trace. |
| 7 | 0·001 | ,, | | = – | ,, | ,, | | None. | None. |

TABLE VIII. (*b*).—*Hæmolysis of Mouse Blood Corpuscles by the Supernatant Fluid of Mouse Carcinoma* (63) *Serum, after Treatment with* 10 *per cent. Goat Blood Corpuscles.*

| | Mouse Carcinoma (63) Serum. | 10 per cent. Goat Red Cells. | 1 c.c. Supernatant Fluid = Original Serum. | | Guinea-pig Complement. | 5 per cent. Mouse Red Cells. | | | Control (without Treatment). |
|---|---|---|---|---|---|---|---|---|---|
| | | c.c. | | | c.c. | c.c. | | | |
| 1 | 0·1 | 1·0 | Two hours in an incubator at 37° C., then centrifuged. | = 0·05 | 0·05 | 0·5 | Two hours in an incubator at 37° C. | Complete. | Complete. |
| 2 | 0·05 | ,, | | = 0·025 | ,, | ,, | | ,, | ,, |
| 3 | 0·025 | ,, | | = 0·01 | ,, | ,, | | Slight. | Slight. |
| 4 | 0·01 | ,, | | = 0·005 | ,, | ,, | | Trace. | Trace. |
| 5 | 0·005 | ,, | | = 0·0025 | ,, | ,, | | None. | None. |
| 6 | 0·0025 | ,, | | = 0·001 | ,, | ,, | | ,, | ,, |
| 7 | 0·001 | ,, | | – | ,, | ,, | | ,, | ,, |

this conclusion the following experiment was performed. Goat corpuscles were sensitised for two hours in the incubator at 37° C., with mouse-tumour hæmolysin in the proportions: 1 c.c. of 10 per cent. suspension of washed goat corpuscles to 0·1 c.c. of immune serum. The tubes were then centrifuged and the hæmolytic reaction carried out with the supernatant fluid on mouse erythrocytes. If the

hæmolysis of mouse and goat corpuscles by the mouse-tumour antisera were due to the same antibody, it would all be fixed by the goat corpuscles, and the supernatant fluid would be devoid of hæmolytic action on mouse corpuscles (Table VIII. (*a*) and (*b*)).

The experiment clearly demonstrates that the supernatant fluid contains the hæmolysin for mouse corpuscles in undiminished strength, as compared with the untreated serum. At the same time the sensitised goat corpuscles were again suspended in fresh normal saline, and, with the addition of guinea-pig complement, incubated for two hours in the ordinary way.

TABLE IX.—*Hæmolysis of the Sediment, i.e., Goat Blood Corpuscles sensitised with Mouse Sarcoma (37 p) Serum for two hours.*

| | Mouse Sarcoma (37 p) Serum. | 10 per cent. Goat Red Cells. | Half Portion of Sediment= Original Serum. | | Guinea-pig Complement. | | | Control (without Treatment). |
|---|---|---|---|---|---|---|---|---|
| | | c.c. | | c.c. | c.c. | | | |
| 1 . . | 0·1 | 1·0 | | =0·05 | 0·05 | | Complete. | Complete. |
| 2 . . | 0·05 | ,, | | =0·025 | ,, | | ,, | ,, |
| 3 . . | 0·025 | ,, | Two hours in an incubator at 37° C., then centrifuged. | =0·01 | ,, | Two hours in an incubator at 37° C. | ,, | ,, |
| 4 . . | 0·01 | ,, | | =0·005 | ,, | | Slight. | Slight. |
| 5 . . | 0·005 | ,, | | =0·0025 | ,, | | Trace. | Trace. |
| 6 . . | 0·0025 | ,, | | =0·001 | ,, | | None. | None. |
| 7 . . | 0·001 | ,, | | — | ,, | | — | ,, |

The result is shown in Table IX., and it is seen that the hæmolysin has fixed itself to the goat corpuscles, so that hæmolysis is produced to the same degree as in the experiment with the original serum (cf. Tables V. and VI.). The experiments prove that two separate hæmolysins co-exist in the mouse-tumour antisera, one which is fixed specifically by mouse red cells, the other by goat red cells. The experiments further show that these two hæmolytic amboceptors produced simultaneously are present in practically the same strength, which, unless a coincidence, considerably enhances the difficulty of finding a satisfactory explanation for the phenomenon. The problem is simply that of the nature of lysinogens generally. Many authors believe they are of a lipoid nature (Bang and Forssmann, 1909, 1910 [11]) or a combination of lipoid and protein (Landsteiner, 1909 [12]). The most obvious conclusion seems to be that mouse tissues (and mouse tumours) contain lysinogens capable of giving rise in the rabbit to hæmolytic amboceptors for mouse and goat blood corpuscles simultaneously.

There is another problem which can be approached by means of hæmolytic reactions, namely, the relation which obtains between a tumour and its host when growing temporarily in an animal of alien species, in respect of the extent to which the tissue proteins retain their specific identity under these conditions. As has frequently been pointed out, no difference can be detected histologically between the cells of tumours growing in the maternal soil and in alien soil. Since mouse tumours can be grown in incubating hen eggs and in rats, a variety of dispositions are available for this purpose. Ehrlich (1906 [2]) was the first to point out the possibility of obtaining temporary growth of transplantable mouse tumours in rats. He says: "Impft man dagegen eine Ratte mit einen unserer Sarkome, so sieht man schon in den allerersten Tagen einen schnell wuchernden Tumor sich bilden, der bis ungefähr zum 6. Tage zunimmt. Zu dieser Zeit bietet die Geschwulst ein sehr charakteristisches Aussehen. Der meist lang gestreckte wurstförmige Tumor fübilt sich derb an und besteht aus einem mächtigen Geschwulstmantel, der sackförmig den Rest der nekrotischen Impfmassen umschliesst. Histologisch ist weder im Form und Anordnung der Zellen, noch in dem Reichtum an Mitosen ein irgendwie nennenswerter Unterschied gegenüber einem gleichaltrigen Mäusetumor nachzuweisen. Auch das Carcinom ist, obwohl in beschränktem Masse, fähig, im Rattenorganismus zunächst zu wuchern, und das gleiche gilt von künstlichen Mischungen von Carcinom und Sarkom." A much more uniform and longer continued growth is obtained, as Bullock (1915 [3]) has shown, when mouse tumours are inoculated into new-born rats and success is obtained with carcinomata as well as sarcomata. This method was therefore adopted for the present investigation, and the material for immunisation taken from baby rats in which the same mouse tumours (sarcoma **37 p** and carcinoma **63**) had been inoculated and were still growing rapidly. Bullock [3] showed that although continuous propagation of mouse tumours could be obtained in rats by repeated transference to fresh new-born rats while active growth was still progressing, nevertheless the tumours did not acquire the ability to grow progressively in the rat, nor lose their powers of growth in the original soil of the mouse. The tumours remained mouse tumours in spite of a lengthy sojourn in alien soil.

It was hoped by killing the baby rats at from seven to ten days after inoculation and removing all hæmorrhagic and necrotic areas to obtain the tumour parenchyma relatively free from rat erythrocytes, any considerable admixture of which would introduce an obvious difficulty in interpreting the results.

The experiments (Table X.) show a distinct increase in the hæmolytic action on rat corpuscles, as compared with the sera derived from mouse tumours growing in mice. The sera are still strongly hæmolytic for mouse corpuscles, but not quite equal to those first studied. In view of the necessary admixture of rat tissue, stroma, and

TABLE X.—*Hæmolytic Analysis of Mouse Tumour (Sarcoma* **37 p**) *grown in Mice and in Baby Rats under same Conditions.*

| | Hæmolysis of Mouse Blood Corpuscles. | | | |
|---|---|---|---|---|
| | Mouse Sarcoma (**37 p**) Serum. | Guinea-pig Complement, 0·05 c.c. | **37 p** (from Baby Rat) Serum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . | 0·1 | Complete. | 0·1 | Complete. |
| 2 . . . | 0·05 | ,, | 0·05 | ,, |
| 3 . . . | 0·025 | ,, | 0·025 | ,, |
| 4 . . . | 0·01 | ,, | 0·01 | Nearly complete. |
| 5 . . . | 0·005 | Nearly complete. | 0·005 | Slight. |
| 6 . . . | 0·0025 | Slight. | 0·0025 | Trace. |
| 7 . . . | 0·001 | Trace. | 0·001 | None. |
| | Hæmolysis of Rat Blood Corpuscles. | | | |
| 1 . . . | 0·1 | Nearly complete. | 0·1 | Complete. |
| 2 . . . | 0·05 | Marked | 0·05 | ,, |
| 3 . . . | 0·025 | Trace. | 0·025 | ,, |
| 4 . . . | 0·01 | None. | 0·01 | Marked. |
| 5 . . . | 0·005 | ,, | 0·005 | Slight. |
| 6 . . . | 0·0025 | ,, | 0·0025 | Trace. |
| 7 . . . | 0·001 | ,, | 0·001 | None. |

blood, in the tumours growing in rats, this result is probably as good as could be expected. It does not permit the conclusion that the mouse-tumour parenchyma has acquired specific rat characteristics as a consequence of its temporary dependence on the rat organism for nourishment. The same result was obtained with carcinoma **63** grown in baby rats. The fact that hæmolysis of mouse corpuscles was obtained with the antisera against mouse tumours grown in rats, shows that the hæmolysins in these experiments are not produced in consequence of the presence of blood in the immunising emulsions. No mouse blood or connective tissue is present in the emulsion of mouse tumour grown in rats, these being supplied by the host. The experiments with rat-tumour (**R 16**) antiserum show that rat tissue does not give rise to any very powerful mouse hæmolysin. The mouse hæmolysin in the antiserum against mouse tumours grown in rats

must therefore owe its origin to the mouse parenchyma injected. As a consequence an antigenic rôle must be ascribed to the parenchyma of mouse tumours grown in mice, in the production of the mouse hæmolysins obtained.

While these experiments were in progress, a spontaneous rat sarcoma was obtained which grew progressively in all the normal animals inoculated. The opportunity thus provided was seized to immunise rabbits with this sarcoma **R 16**, for comparison with the anti-mouse sera. The principal experiments only were repeated with this anti-rat serum, and some of these experiments are given in Tables XI., XII., XIII.

TABLE XI.—*Hæmolysis of Rat Blood Corpuscles by Rat Sarcoma* (**R 16**) *Antiserum.*

| RAT BLOOD CORPUSCLES. | | | |
|---|---|---|---|
| | **R 16** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. |
| 1 . . . | 0·1 | Complete. | Complete. |
| 2 . . . | 0·05 | ,, | Nearly complete. |
| 3 . . . | 0·025 | Nearly complete. | ,, |
| 4 . . . | 0·01 | Marked. | Slight. |
| 5 . . . | 0·005 | Slight. | Trace. |
| 6 . . . | 0·0025 | None. | None. |
| 7 . . . | 0·001 | ,, | ,, |

The tables show that the immune serum contains a hæmolysin for rat erythrocytes similar to that for mouse erythrocytes in the anti-mouse tumour sera. The anti-rat serum, however, is not hæmolytic for mouse corpuscles. It is to be noted, however, that the anti-mouse sera were much more potent, hæmolysis being complete even with 0·01 c.c. of antiserum, whereas 0·05 c.c. is the smallest dose of anti-rat serum capable of producing complete hæmolysis of rat red cells. Rabbit complement, again, is able to produce lysis with the anti-rat serum, the corresponding experiment with mouse erythrocytes and antiserum being negative (see Table I.). The action on goat corpuscles also was feebler than that exhibited by the mouse-tumour antisera, in which, as already noted, a goat hæmolysin is present to the same degree as the mouse hæmolysin.

TABLE XII.—*Hæmolysis of Mouse Blood Corpuscles by* **R 16** *Antiserum.*

| MOUSE BLOOD CORPUSCLES. | | | |
|---|---|---|---|
| | **R 16** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. |
| 1 . . . | 0·1 | None. | None. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | ,, | ,, |
| 4 . . . | 0·01 | ,, | ,, |
| 5 . . . | 0·005 | ,, | ,, |
| 6 . . . | 0·0025 | ,, | ,, |
| 7 . . . | 0·001 | ,, | ,, |

TABLE XIII.—*Hæmolysis of Goat Blood Corpuscles by* **R 16** *Antiserum.*

| GOAT BLOOD CORPUSCLES. | | | |
|---|---|---|---|
| | **R 16** Antiserum. | Guinea-pig Complement, 0·05 c.c. | Rabbit Complement, 0·05 c.c. |
| 1 . . . | 0·1 | Marked. | None. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | Slight. | ,, |
| 4 . . . | 0·01 | Trace. | ,, |
| 5 . . . | 0·005 | None. | ,, |
| 6 . . . | 0·0025 | ,, | ,, |
| 7 . . . | 0·001 | ,, | ,, |

The attempt has been made to utilise the resistance of the erythrocytes of cancer patients to hæmolysis, for the differential diagnosis of malignant disease. Braga (1910 [13]) assumed that cancer extracts are more strongly hæmolytic on cancer patients' erythrocytes than on those of normal individuals. Kraus, Plötzl, Ranzi, and H. Ehrlich (1909 [14]) studied the resistance of the erythrocytes of cancer patients and animals to cobra venom. They found increased hæmolysis of the red cells of sarcoma-bearing rats, and decreased hæmolysis on those of carcinoma-bearing mice. They also obtained a similar

result in the human subject. The attempt was made to see whether similar differences in susceptibility to the specific mouse hæmolysin produced by immunisation could be detected between the erythrocytes of mice bearing transplanted tumours and those of normal healthy animals. Tables of these experiments are not given since no such differences could be detected.

*Summary.*

1. By immunising rabbits against mouse tumours, antisera are obtained hæmolytic for mouse erythrocytes.

2. The sera are equally hæmolytic for goat erythrocytes, but the two amboceptors are distinct and can be separated by absorption of one of them by the corresponding erythrocyte suspension, the other amboceptor remaining in undiminished strength.

3. The anti-mouse tumour sera also hæmolyse rat blood corpuscles, but to a much less degree than mouse and goat corpuscles.

4. When mouse tumours inoculated into new-born rats are used to immunise rabbits, antisera are obtained which are nearly as strongly hæmolytic for rat erythrocytes as for mouse erythrocytes.

5. The complement used in all the principal experiments was fresh guinea-pig serum. Rabbit complement was without effect on mouse erythrocytes in combination with the mouse sarcoma (**37 p** and **37 s**) antisera, whereas definite hæmolysis was obtained with it in presence of the mouse carcinoma (**199** and **63**) antisera.

6. The antiserum obtained by immunising rabbits with a rat sarcoma (**R 16**) hæmolysed rat and goat erythrocytes with both guinea-pig and rabbit complement, but was without action on those of the mouse.

## III. HÆMAGGLUTINATION.

Agglutination or clumping of red blood corpuscles by normal serum was described as long ago as 1869 by Creite ([15]). In 1898 Bordet ([16]) showed that hæmagglutinins appeared in the serum of animals immunised against alien red blood cells. The hæmolysin produced at the same time can be eliminated from the experiments in virtue of the fact that although thermostabile (Sachs [17]) like the hæmagglutinin, it requires the presence of fresh serum or complement to produce lysis. The hæmagglutinin acts without the addition of complement, so that if this be omitted from the tubes, no hæmolysis takes place and agglutination can be studied independently. Bordet and Gay (1906 [18]) further showed that ox-serum normally contains a substance which clumps red corpuscles when fresh horse serum is added. To this substance the provisional designation of " colloid" was given by these authors, later changed to " conglutinin " by Bordet and Streng (1909 [19]) after further investigation. There is thus introduced a distinction between hæmagglutination and conglutination,

the former being strictly a product of active immunisation and able to clump erythrocytes without the aid of complement, the latter occurring in the serum of untreated animals of certain species but only able to agglutinate corpuscles which have already been acted on by amboceptor and complement. The present paper deals only with the former, namely, immune hæmagglutinins. It is to be noted that in the first place these were produced by injection of alien blood corpuscles, but Skrobansky (1903 [20]) has reported that the sera of animals immunised with ovary and corpus luteum of alien species agglutinated the homologous erythrocytes. It was therefore decided to test the sera of rabbits immunised against mouse and rat tumours which, as has just been shown, are strongly hæmolytic, for the presence of agglutinins for the red cells of these animals.

*Methods.*

The immune sera were inactivated by heating in the incubator at 56° C. for half an hour. The 5 per cent. suspension of red corpuscles was prepared in the same way as for the hæmolysin experiments. To each of the test tubes containing falling doses of inactivated immune serum made up to 1 c.c. with normal saline, 0·5 c.c. of 5 per cent. erythrocyte suspension was added, and the tubes placed in the incubator at 37° C. for two hours. At the end of this interval the results were read macroscopically. Landsteiner and Reich (1905 [21]) contend that the reaction must be carried out at room temperature, as a higher temperature disturbs the phenomenon, but in the present series of experiments no difference could be observed between the results under both conditions of temperature. In reading the results macroscopically the main points to observe are the speed at which the corpuscles come down after shaking and their ability to pass through filter paper. It is no advantage to keep the tubes overnight in the ice-chest as in the case of hæmolysin experiments, as spontaneous agglutination is apt to occur even in the control tubes with normal saline alone. The results are recorded as follows :—

| | | | | |
|---|---|---|---|---|
| + + + | . | . | . | . very distinct. |
| + + | . | . | . | . distinct. |
| + | . | . | . | . positive. |
| ± | . | . | . | . suspicious. |
| − | . | . | . | . negative. |

*Experimental Observations.*

Progressive dilutions 1 to 20, 1 to 40, 1 to 80, etc., of each immune serum, were prepared with normal saline and 1 c.c. of each dilution placed in a test tube to which 0·5 c.c. of 5 per cent. suspension of mouse red blood corpuscles was added. For comparison and control similar tubes were prepared with suspensions of rat, guinea-pig, and goat corpuscles. As a general control a tube was prepared with each of the erythrocyte suspensions and inactivated normal rabbit serum diluted 1 : 20. In this dilution rabbit serum does not agglutinate any of the suspensions. The experiments (Tables XIV., XV., XVI.) demonstrate that the immune sera against mouse tumours agglutinate the red corpuscles of the mouse strongly,

TABLE XIV.—*Hæmagglutination by Mouse-Tumour Antiserums on Mouse Blood Corpuscles.*

| | | MOUSE BLOOD CORPUSCLES. | | | |
|---|---|---|---|---|---|
| | Proportion of Diluted Serum. | 5 per cent. Mouse Blood Corpuscles. | **37 p** Serum. | **63** Serum. | **63** (from Baby Rat) Serum. |
| 1 . . | 1 : 20 (1 c.c.) | 0·5 c.c. | + + + | + + + | + + + |
| 2 . . | 1 : 40 ,, | ,, | + + + | + + + | + + + |
| 3 . . | 1 : 80 ,, | ,, | + + | + | + |
| 4 . . | 1 : 160 ,, | ,, | + | ± | ± |
| 5 . . | 1 : 320 ,, | ,, | ± | − | − |
| 6 . . | 1 : 640 ,, | ,, | − | − | − |

TABLE XV.—*Hæmagglutination by Mouse-Tumour Antiserums on Rat Blood Corpuscles.*

| | | RAT BLOOD CORPUSCLES. | | | |
|---|---|---|---|---|---|
| | Proportion of Diluted Serum. | 5 per cent. Rat Blood Corpuscles. | **37 p** Serum. | **63** Serum. | **63** (from Baby Rat) Serum. |
| 1 . . | 1 : 20 (1 c.c.) | 0·5 c.c. | ± | ± | + + + |
| 2 . . | 1 : 40 ,, | ,, | − | − | + + + |
| 3 . . | 1 : 80 ,, | ,, | − | − | + + |
| 4 . . | 1 : 160 ,, | ,, | − | − | + |
| 5 . . | 1 : 320 ,, | ,, | − | − | − |
| 6 . . | 1 : 640 ,, | ,, | − | − | − |

TABLE XVI.—*Hæmagglutination by Mouse-Tumour Antiserums on Guinea-pig Blood Corpuscles.*

| | | GUINEA-PIG BLOOD CORPUSCLES. | | | |
|---|---|---|---|---|---|
| | Proportion of Diluted Serum. | 5 per cent. Guinea-pig Blood Corpuscles. | **37 p** Serum. | **63** Serum. | **63** (from Baby Rat) Serum. |
| 1 . . | 1 : 20 (1 c.c.) | 0·5 c.c. | − | − | − |
| 2 . . | 1 : 40 ,, | ,, | − | − | − |
| 3 . . | 1 : 80 ,, | ,, | − | − | − |
| 4 . . | 1 : 160 ,, | ,, | − | − | − |
| 5 . . | 1 : 320 ,, | ,, | − | − | − |
| 6 . . | 1 : 640 ,, | ,, | − | − | − |

no hæmagglutination taking place with any of the other suspensions, not even of the rat. The antiserum prepared from mouse carcinoma **63 p** in baby rats as antigen shows instructive differences. This serum agglutinates rat corpuscles strongly as well as mouse corpuscles. This result is similar to that obtained with the same serum in the hæmolytic experiments, and the same considerations apply to the interpretation of the result. The experiments were also carried out with mouse sarcoma (**37 p**) antiserum with the same result, so that it is unnecessary to give tables of them. The rat sarcoma (**R 16**) antiserum showed a corresponding behaviour, agglutinating rat erythrocytes distinctly while only feebly agglutinative for mouse corpuscles. It was without action on the red cell suspensions of the guinea-pig and goat.

The observations of Landsteiner (1908 [22]) are not strictly comparable with the results described. He found that watery extracts of mouse and rat tumours agglutinate rabbit erythrocytes, and tried to prove a relation between hæmagglutinating power and the malignancy of a tumour. The immune hæmagglutinins contained in the sera now under investigation are in all probability quite different from those present in watery extracts of tumours.

### *Summary.*

The immune sera obtained by injecting mouse and rat tumours into rabbits, in addition to being hæmolytic, as already shown, contain specific agglutinins for the red blood corpuscles of the corresponding species. Whereas a specific hæmolysin for goat corpuscles is obtained by immunising with mouse and rat tumours, the sera do not agglutinate goat corpuscles in the lowest dilution studied. When mouse tumours grown in rats are used as antigen, the agglutinative power for rat erythrocytes is greatly increased, a result in all probability due to the admixture of rat tissue in the antigenic tumour emulsions.

### IV. COMPLEMENT FIXATION.

It is unnecessary to review the large number of papers by many authors dealing with complement fixation in cancer, since they mostly deal with the subject from a totally different standpoint from that adopted in this paper. They have concerned themselves with the search for specific antibodies for cancer in the sera of men and animals bearing tumours, using tumour extracts and other substances as antigens. That subject is not yet definitely settled, for the several authors are not yet in agreement, some authors claiming to have demonstrated the presence of specific antibodies by this method, while others as emphatically deny their existence.

The problem now under consideration is that of the presence of

complement fixing antibodies in the sera of animals immunised against tumours of alien origin. Cognate studies have been carried out by Michaelis and Fleischmann (1906 [23]), who immunised rabbits against guinea-pig and mouse-liver cells free from blood. The immune sera obtained fixed complement in the presence of the homologous antigens, namely, extracts of the livers of mouse and guinea-pig. The reaction was much feebler when extracts of other organs, *e.g.*, kidney, spleen, and red blood corpuscles, were employed. In addition, they were able to separate the complement fixing antibody from the hæmolytic amboceptors present in the sera, and also to prove its independence of any protein precipitating substance in them. Later, Fleischmann and Davidsohn (1908 [24]) investigated complement fixation in immune sera also prepared against guinea-pig and mouse-liver cells. According to them, the reaction was positive with the homologous antigens, and yet was negative with normal serum, and also with liver extracts of other animals, as, for instance, man, ox, pig, rat, etc.

There is therefore every reason to expect that the mouse- and rat-tumour antisera will contain complement fixing substances, and these sera have therefore been studied in this direction with a considerable variety of antigens.

## *Methods.*

The immune sera were inactivated at 56° C. for half an hour. In addition to the homologous antigens, prepared from the tumours used for immunising, eleven organ extracts were also used for comparison. The extracts were prepared from healthy tumour or normal tissue, which, without any addition, was cut up with scissors to a fine uniform emulsion. To 1 c.c. of the emulsion, 5 c.c. of normal saline containing 0·5 per cent. carbolic acid was added, and the mixture placed in the ice-chest for two days. The fluid was then filtered off, and 0·2 c.c. of the filtrate used as antigen in each tube. It was found by experiment that twice this quantity alone did not interfere with complete hæmolysis. The antigens, prepared as above, were found to remain active for three weeks to a month when kept in the ice-chest. Goat erythrocytes and a homologous hæmolytic serum formed the hæmolytic system. The titre of the goat hæmolysin was 0·0005 c.c., which hæmolysed 0·5 c.c. of 5 per cent. suspension of red cells completely. To prepare the goat corpuscle suspension, blood was drawn by venepuncture from a healthy male goat, defibrinated, and washed three times with normal saline as already described for the mouse erythrocyte suspension used in the hæmolysin experiments. Fresh guinea-pig serum was used as complement in the majority of the experiments, but in some cases rabbit serum was also employed. The dose of complement was 0·5 c.c. of a 1 in 10 dilution, giving an absolute dose of 0·05 c.c. According to the degree of hæmolysis, the results are recorded as complete, nearly complete, marked, slight, trace, and none.

## *Experimental Observations.*

An initial difficulty was caused by the presence in the mouse- and rat-tumour immune sera of a hæmolytic amboceptor for goat

corpuscles. It was found that consistent results could not be
obtained with the untreated inactivated immune sera by using either
goat or mouse erythrocytes in the hæmolytic system. The difficulty
was overcome in the following manner, suggested by the hæmolytic
experiments demonstrating the independence of the two hæmolysins
for mouse and goat corpuscles respectively (Tables VIII. and IX.).
The serum to be tested was incubated for one hour with washed goat
corpuscles, in the proportion of 1 c.c. of a 10 per cent. suspension of
goat corpuscles to 0·1 c.c. of immune serum. At the end of this
period the tube was centrifuged and the complement fixation reaction
carried out with the supernatant fluid, of which, therefore, 1 c.c.
corresponded to 0·1 c.c. of the original serum.

TABLE XVII.—*Complement Fixation by Mouse Carcinoma* (199) *Antiserum,
with Homologous and Heterologous Antigens.*

|  | **199** Antiserum. | Homologous Extract, 0·2 c.c. | **37 p** Extract, 0·2 c.c. |
|---|---|---|---|
| 1 . . . | 0·1 | None. | None. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | ,, | Trace. |
| 4 . . . | 0·01 | ,, | Slight. |
| 5 . . . | 0·005 | ,, | ,, |
| 6 . . . | 0·0025 | Slight. | Marked. |
| 7 . . . | 0·001 | Marked. | Complete. |

TABLE XVIII.—*Complement Fixation by Mouse Sarcoma* (37 s) *Antiserum,
with Homologous and Heterologous Antigens.*

|  | **37 s** Antiserum. | Homologous Extract, 0·2 c.c. | **199** Extract, 0·2 c.c. |
|---|---|---|---|
| 1 . . . | 0·1 | None. | Trace. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | ,, | ,, |
| 4 . . . | 0·01 | ,, | Slight. |
| 5 . . . | 0·005 | Slight. | Marked. |
| 6 . . . | 0·0025 | Marked. | Complete. |
| 7 . . . | 0·001 | Complete. | ,, |

These experiments (Tables XVII., XVIII.) demonstrate the presence of complement fixing antibody in the mouse-tumour antisera, and show a high degree of specificity, so that the reaction is much more evident with the homologous antigen than with the extract of a different mouse tumour. The delicacy of the reaction is therefore such that it reveals differences between tumours, even from the same animal species, by quantitative differences in the power of aqueous extracts to fix complement in presence of the corresponding antisera.

This delicacy is strikingly brought out in experiments with the serum against mouse tumours grown in baby rats.

TABLE XIX.—*Complement Fixation by Mouse Sarcoma* (37 p *grown in Baby Rats*) *Antiserum, with Homologous and Heterologous Antigens.*

|  | 37 p (from Baby Rat) Antiserum. | Homologous Extract, 0·2 c.c. | 37 p (from Mouse) Extract, 0·2 c.c. | 199 Extract, 0·2 c.c. |
|---|---|---|---|---|
| 1. . . | 0·1 | None. | Trace. | None. |
| 2. . . | 0·05 | ,, | ,, | Trace. |
| 3. . . | 0·025 | ,, | Slight. | Slight. |
| 4. . . | 0·01 | ,, | ,, | ,, |
| 5. . . | 0·005 | Slight. | Marked. | Marked. |
| 6. . . | 0·0025 | ,, | Nearly complete. | Nearly complete. |
| 7. . . | 0·001 | Marked. | Complete. | Complete. |

As Table XIX. shows, the antiserum from mouse sarcoma **37 p** grown in young rats, in combination with extract of mouse carcinoma **199** as well as of mouse sarcoma **37 p** grown in mice, fixes complement very feebly, whereas with the homologous antigen (extract of **37 p** grown in rats) the reaction is very striking. It is difficult to determine what significance should be attached to this result, which seems to indicate profound biological changes in the mouse sarcoma as a result of sojourn in the rat. The researches of J. B. Murphy (1914[1]) and of W. E. Bullock (1915[3]), however, show that the restrictions to the success of heterologous transplantation cannot be overcome even by a prolonged sojourn by repeated inoculation of a mouse tumour in alien organisms (chick or rat), nor do mouse tumours after such an interlude show any acquired disability of proliferation when returned to the maternal soil.

Normal tissues of the mouse and rat can replace tumour tissue in the production of homologous cancer immunity. It is of interest to ascertain the behaviour of watery extracts of such tissues when employed as antigens for complement fixation with heterologous

mouse-tumour immune sera. For this purpose extracts were prepared of mouse liver and mouse embryo skin, and the reaction carried out with them (Tables XX., XXI.).

TABLE XX.—*Complement Fixation by Mouse Carcinoma* (199) *Antiserum, with Homologous Antigen and Extracts from Normal Mouse Liver and Mouse Embryo Skin.*

|  | 199 Antiserum. | Homologous Extract, 0·2 c.c. | Mouse Embryo Skin Extract, 0·2 c.c. | Mouse Liver Extract, 0·2 c.c. |
|---|---|---|---|---|
| 1 . . . | 0·1 | None. | None. | Trace. |
| 2 . . . | 0·05 | ,, | Trace. | Slight. |
| 3 . . . | 0·025 | ,, | Slight. | Nearly complete. |
| 4 . . . | 0·01 | ,, | Marked. | Complete. |
| 5 . . . | 0·005 | Slight. | Complete. | ,, |
| 6 . . . | 0·0025 | ,, | ,, | ,, |
| 7 . . . | 0·001 | Nearly complete. | ,, | ,, |

TABLE XXI.—*Complement Fixation by Mouse Sarcoma* (37 s) *Antiserum, with Homologous Antigen and Extracts from Normal Mouse Liver and Mouse Embryo Skin.*

|  | 37 s Antiserum. | Homologous Extract, 0·2 c.c. | Mouse Embryo Skin Extract, 0·2 c.c. | Mouse Liver Extract, 0·2 c.c. |
|---|---|---|---|---|
| 1 . . . | 0·1 | None. | Trace. | Slight. |
| 2 . . . | 0·05 | ,, | Slight. | Marked. |
| 3 . . . | 0·025 | ,, | Marked. | Complete. |
| 4 . . . | 0·01 | Slight. | Complete. | ,, |
| 5 . . . | 0·005 | Marked. | ,, | ,, |
| 6 . . . | 0·0025 | Complete. | ,, | ,, |
| 7 . . . | 0·001 | ,, | ,, | ,, |

As before, the reaction with homologous antigen was very energetic, whereas only a feeble effect could be obtained with the normal tissue antigens. It is to be noted, however, that the embryo skin extract fixed complement rather more strongly than that from liver, a result which is of interest because embryo skin elicits a higher degree of homologous resistance than any other normal tissue.

In so far as conclusions can be drawn from complement fixation

as to the chemical constitution of the reacting substances, these experiments indicate chemical differences between normal and tumour tissues, and even between the different tumours of the same animal species. These differences probably involve the protein and lipoid constituents of the tissues, and for the latter there is already evidence of quantitative and qualitative differences in the work of Bullock and Cramer (1913 [25]). These authors investigated the lipoids of transplantable tumours of the mouse and rat. They found qualitative and quantitative differences in the distribution of the lipoid constituents, not only between tumours arising in different tissues, such as carcinomata and sarcomata, but also, though to a lesser degree, between different tumours derived from the same mother tissue, namely, a slowly growing and a rapidly growing mammary carcinoma.

Confirmatory results were obtained when the mouse-tumour antisera were tested with extracts of rat tumours, and, conversely, rat-tumour antisera with mouse-tumour extracts.

TABLE XXII.—*Complement Fixation by Mouse Carcinoma* (199) *Antiserum, with Homologous and Rat Sarcoma* (**R** 16) *Extracts as Antigen.*

| | **199** Antiserum. | Homologous Extract, 0·2 c.c. | **R 16** Extract, 0·2 c.c. |
|---|---|---|---|
| 1 . . . | 0·1 | None. | None. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | ,, | Slight. |
| 4 . . . | 0·01 | ,, | Nearly complete. |
| 5 . . . | 0·005 | ,, | Complete. |
| 6 . . . | 0·0025 | Slight. | ,, |
| 7 . . . | 0·01 | Marked. | ,, |

TABLE XXIII.—*Complement Fixation by Mouse Sarcoma* (37 s) *Antiserum, with Homologous and Rat Sarcoma* (**R** 16) *Extracts as Antigen.*

| | **37 s** Antiserum. | Homologous Extract, 0·2 c.c. | **R 16** Extract, 0·2 c.c. |
|---|---|---|---|
| 1 . . . | 0·1 | None. | None. |
| 2 . . . | 0·05 | ,, | Trace. |
| 3 . . . | 0·025 | ,, | Slight. |
| 4 . . . | 0·01 | ,, | Nearly complete. |
| 5 . . . | 0·005 | Slight. | Complete. |
| 6 . . . | 0·0025 | Marked. | ,, |
| 7 . . . | 0·001 | Nearly complete. | ,, |

TABLE XXIV.—*Complement Fixation by Mouse Sarcoma* (**37 p** *grown in Baby Rats*) *Antiserum, with Homologous and Rat Sarcoma* (**R 16**) *Extracts as Antigen.*

|  | **37 p** (from Baby Rat) Antiserum. | Homologous Extract, 0·2 c.c. | **R 16** Extract, 0·2 c.c. |
|---|---|---|---|
| 1 . . . | 0·1 | None. | None. |
| 2 . . . | 0·05 | ,, | ,, |
| 3 . . . | 0·025 | ,, | ,, |
| 4 . . . | 0·01 | ,, | Slight. |
| 5 . . . | 0·005 | Slight. | Marked. |
| 6 . . . | 0·0025 | ,, | Nearly complete. |
| 7 . . . | 0·001 | Marked. | Complete. |

Tables XXII., XXIII., XXIV. show much feebler complement fixation by rat-tumour extract with mouse carcinoma **199** and sarcoma **37 p** antisera, than with the homologous antigens. The position is reversed when the same antigens react with the rat sarcoma antiserum.

TABLE XXV.—*Complement Fixation by Rat Sarcoma* (**R 16**) *Antiserum, with Homologous* (**199** *and* **37 p**) *Extracts as Antigen.*

|  | **R 16** Antiserum. | Homologous Extract, 0·2 c.c. | **199** Extract, 0·2 c.c. | **37 p** Extract, 0·2 c.c. |
|---|---|---|---|---|
| 1 . . . | 0·1 | None. | Trace. | Slight. |
| 2 . . . | 0·05 | ,, | Slight. | ,, |
| 3 . . . | 0·025 | ,, | ,, | Marked. |
| 4 . . . | 0·01 | ,, | Marked. | ,, |
| 5 . . . | 0·005 | Trace. | Complete. | Complete. |
| 6 . . . | 0·0025 | Slight. | ,, | ,, |
| 7 . . . | 0·001 | Nearly complete. | ,, | ,, |

The rat antigen with rat antiserum gives a strong fixation, the mouse-tumour antigens a very weak fixation (Table XXV.).

It is rather remarkable that the absolute degree of the differences between homologous and strange antigen is much the same whether the experiment refers to two antigens from the same animal or whether they differ specifically as mouse and rat. It was therefore of interest to test the mouse-tumour antisera against tumour extract

from a still more distantly related species, and for this purpose antigens were prepared from two human mammary carcinomata, supplied for the purpose by the courtesy of the Director of the Cancer Research Institute of the Middlesex Hospital, Dr. Lazarus-Barlow. One of the tumours was a relatively soft adeno-carcinoma, the other a scirrhus carcinoma. The extracts were prepared in the manner already described, and the dose was the same as in the other experiments, namely, 0·2 c.c. Three times the testing dose did not deviate complement. In the absence of normal human tissue, from which to prepare an extract, normal human serum from my own arm vein was used as control. The serum was inactivated, diluted 1 in 10, and 0·5 c.c. taken as antigen. Even 0·1 c.c. of the undiluted inactivated human serum did not give the slightest fixation of complement (Tables XXVI., XXVII., XXVIII.).

Human cancer extracts, therefore, give definite evidence of complement fixation with mouse- and rat-tumour antisera, rather more strongly with rat antiserum than with mouse. The indication of the probability of tumours from different species having some constituents at least in common is interesting. Normal human serum, on the contrary, was practically inert. A similar result had already been recorded by Fleischmann and Davidsohn (1908 [24]), who found that the serum of a rabbit immunised against guinea-pig liver exhibited complement fixation in the presence of guinea-pig liver extract, whereas no reaction took place with guinea-pig serum. The combination, normal rabbit serum and guinea-pig liver extract, was also inactive.

TABLE XXVI.—*Complement Fixation by Mouse Carcinoma* (199) *Antiserum, with Homologous Extract, Human Cancer Extracts, and Normal Human Serum.*

|  | **199** Antiserum. | Homologous Extract, 0·2 c.c. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer (A) Extract, 0·2 c.c. | Human Cancer (S) Extract, 0·2 c.c. |
|---|---|---|---|---|---|
| 1 . . . | 0·1 | None. | Marked. | None. | None. |
| 2 . . . | 0·05 | ,, | Complete. | ,, | Trace. |
| 3 . . . | 0·025 | ,, | ,, | Trace. | Slight. |
| 4 . . . | 0·01 | ,, | ,, | Slight. | Marked. |
| 5 . . . | 0·005 | Slight. | ,, | Marked. | Complete. |
| 6 . . . | 0·0025 | Marked. | ,, | Complete. | ,, |
| 7 . . . | 0·001 | Nearly complete. | ,, | ,, | ,, |

TABLE XXVII.—*Complement Fixation by Mouse Sarcoma* (37 s) *Antiserum, with Homologous Extract, Human Cancer Extracts, and Normal Human Serum.*

|  | 37 s Antiserum. | Homologous Extract, 0·2 c.c. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer (A) Extract, 0·2 c.c. | Human Cancer (S) Extract. |
|---|---|---|---|---|---|
| 1 . . . | 0·1 | None. | Complete. | None. | None. |
| 2 . . . | 0·05 | ,, | ,, | Trace. | Slight. |
| 3 . . . | 0·025 | ,, | ,, | Slight. | Marked. |
| 4 . . . | 0·01 | Slight. | ,, | Marked. | Complete. |
| 5 . . . | 0·005 | Marked. | ,, | Complete. | ,, |
| 6 . . . | 0·0025 | Complete. | ,, | ,, | ,, |
| 7 . . . | 0·001 | ,, | ,, | ,, | ,, |

TABLE XXVIII.—*Complement Fixation by Rat Sarcoma* (R 16) *Antiserum, with Homologous Extract, Human Cancer Extracts, and Normal Human Serum.*

|  | R 16 Antiserum. | Homologous Extract, 0·2 c.c. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer (A) Extract, 0·2 c.c. |
|---|---|---|---|---|
| 1 . . . | 0·1 | None. | Marked. | None. |
| 2 . . . | 0·05 | ,, | Complete. | ,, |
| 3 . . . | 0·025 | ,, | ,, | ,, |
| 4 . . . | 0·01 | ,, | ,, | ,, |
| 5 . . . | 0·005 | Trace. | ,, | Slight. |
| 6 . . . | 0·0025 | Slight. | ,, | Marked. |
| 7 . . . | 0·001 | Nearly complete. | ,, | Complete. |

Complement fixation is said to have been obtained with the serum of cancer patients in some cases, using lecithin as antigen in the same way as has been found possible in the Wassermann reaction for syphilis. An experiment was performed to see whether the rabbit immune sera now under consideration would fix complement in the presence of lecithin emulsions. The testing dose employed was 0·5 c. c. of a 0·1 per cent. watery suspension, 1 c.c. of the suspension producing no inhibition (Table XXIX.).

TABLE XXIX.—*Complement Fixation by Mouse Carcinoma* **(63)** *Antiserum, with Lecithin as Antigen.*

| | **(63)** Serum. | Lecithin, 0·1 per cent., 0·5 c.c. | **(37 p)** Serum. | Lecithin, 0·1 per cent., 0·5 c.c. | **(63** in Baby Rat) Serum. | Lecithin, 0·1 per cent., 0·5 c.c. |
|---|---|---|---|---|---|---|
| 1 . . . | 0·1 | Slight. | 0·1 | Complete. | 0·1 | Complete. |
| 2 . . . | 0·05 | Complete. | 0·05 | ,, | 0·05 | ,, |
| 3 . . . | 0·025 | ,, | 0·025 | ,, | 0·025 | ,, |
| 4 . . . | 0·01 | ,, | 0·01 | ,, | 0·01 | ,, |
| 5 . . . | 0·005 | ,, | 0·005 | ,, | 0·005 | ,, |
| 6 . . . | 0·0025 | ,, | 0·0025 | ,, | 0·0025 | ,, |
| 7 . . . | 0·001 | ,, | 0·001 | ,, | 0·001 | ,, |

With two of the antisera no fixation took place, and with the third serum tested only the faintest inhibition was obtained. As is well known, lecithin undergoes changes with age, and in this case the lecithin used was not above suspicion.

*Summary.*

1. Complement fixing antibodies are present in the immune sera obtained by immunising rabbits with mouse and rat tumours.

2. In every case the reaction was most strongly positive when the homologous antigen was used ; it was feeble with other antigens even when these were prepared from similar tumours from the same species.

3. Antigens prepared from normal tissues of the mouse and rat reacted only feebly with the immune sera prepared against the tumours of the same animals.

4. Tumour extracts from alien species gave distinct complement fixation with the mouse- and rat-tumour antisera. In particular the rat-tumour antiserum fixed complement strongly with extract of human cancer. Normal human serum was practically inactive.

5. When lecithin emulsion was used as antigen the reaction was negative (in one case feebly positive).

## V. PRECIPITIN REACTION.

The majority of the numerous references to the precipitin reaction in cancer concern themselves with attempts to utilise the method for serum diagnostic purposes in man and animals bearing tumours. It is unnecessary to refer to these studies in detail, as they have little con-

nection with the present investigation, which is restricted to the investigation of the precipitating properties of heterologous immune cancer sera. Even in this restricted domain much work has been done, and some authors claim a diagnostic value for the method, while others have come to the conclusion that the reaction is not specific.

Engel (1903 [26]) immunised rabbits by intraperitoneal injection of inactivated serum from two cancer patients. The immune serum thus obtained was precipitated by the sera of the cancer patients, but only feebly by the sera of other patients (anæmia, epilepsy, nephritis). Maragliano (1904 [27]) immunised rabbits with the gastric juice of patients with carcinoma of the stomach. The immune serum gave the reaction with the gastric juice of other cases of stomach cancer. Later, Serafini and Diez (1908 [28]) continued these observations on a number of cases, obtaining positive results in cases of stomach cancer, and negative results with the gastric juice from other patients with stomach diseases. Kullmann (1904 [29]) immunised the animals with tumour extract. The antiserum obtained, however, showed an equally strong precipitin reaction with normal as with cancer serum. Ranzi (1908 [30]) repeated these experiments on a large scale, using extracts of carcinoma and sarcoma. The antiserum obtained was not specific, as there was no difference in the reaction when homologous antigen and other organ extracts or normal human serum were employed. Romkes (1903 [31]), however, claimed good results with the method.

The purpose of the present investigation was to determine whether the immune sera against mouse and rat tumours were precipitated in a specific manner by their homologous antigens, a subject which apparently had not been previously studied.

### Methods.

The immune sera used for the precipitin reaction were not inactivated, control experiments having shown that inactivation made no difference to the reaction. The extracts employed as precipitinogen were the same as those which served for complement fixation. The concentrations and doses used will be described later. The chemical reaction of the extracts was acid from the presence of 0·5 per cent. carbolic acid in the saline used to make the extracts. The duration of incubation has a great influence on the results of the reaction, and in these experiments the result was read after a uniform interval of twenty-four hours, while the examination of the tubes after a few hours showed no change.

The successive dilutions of the immune sera were prepared in the following manner. The serum was diluted 1 : 10 in normal saline, and 1 c.c. of this diluted serum placed in the first two tubes. To each of the tubes, except the first, 1 c.c. of normal saline was added. From the second tube, after thorough mixing, 1 c.c. of fluid was withdrawn by a pipette and transferred to the third tube. This in turn was well mixed and the process repeated. Thus a series of tubes was obtained containing serum 1 : 10, 1 : 20, 1 : 40, 1 : 80. Lastly, to each tube 1 c.c. of tumour extract was added, the final concentration of antiserum in each tube being 1 : 20, 1 : 40, 1 : 80, etc.

### Experimental Observations.

In the first instance, 1 c.c. of the undiluted extract (1 : 6) was used as the testing dose. No reaction took place even with homologous

antiserum. From a consideration of the peculiarities of precipitin re-
actions generally, this failure might be due to unsuitable conditions as
to concentration, reaction, or temperature. Before proceeding to altera-
tions in reaction and temperature, alterations in concentration of the
extracts were tried in order to find the optimum concentration. For
this purpose falling doses of extract were added to 0·1 c.c. of anti-
serum (Table XXX.).

TABLE XXX.—*The Optimum Concentration of Mouse-Tumour
Extracts for Precipitin Reaction.*

|  | **199** Extract. | Ten times diluted Homologous Serum, 1 c.c. |
|---|---|---|
| 1 . . . . | 5 x in 1 c.c. | − |
| 2 . . . . | 10 x ,, ,, | − |
| 3 . . . . | 20 x ,, ,, | + + |
| 4 . . . . | 40 x ,, ,, | + + + |
| 5 . . . . | 80 x ,, ,, | + + + |
|  | **37 s** Extract. |  |
| 1 . . . . | 5 x in 1 c.c. | − |
| 2 . . . . | 10 x ,, ,, | − |
| 3 . . . . | 20 x ,, ,, | ± |
| 4 . . . . | 40 x ,, ,, | + + |
| 5 . . . . | 80 x ,, ,, | + + |
|  | **37 p** (from Baby Rat) Extract. |  |
| 1 . . . . | 5 x in 1 c.c. | − |
| 2 . . . . | 10 x ,, ,, | ± |
| 3 . . . . | 20 x ,, ,, | + + |
| 4 . . . . | 40 x ,, ,, | + + + |
| 5 . . . . | 80 x ,, ,, | + |

The tables demonstrate that with dilutions higher than tenfold, pre-
cipitation sets in with the homologous antiserum, and the optimum
concentration lies between the forty-fold and eighty-fold dilution of
each extract. In these experiments, also, the tubes were maintained
at 37° C. for twenty-four hours before the results were noted. Kelling
(1907 [32]) also found that high dilutions of precipitinogen (1 : 1000 or
1 : 2000) gave better results. It was of interest to know whether the

percentage protein content of the watery extracts showed constant differences between the several extracts. For this purpose Esbach's albuminometer was used, the extracts being diluted twenty times with normal saline containing 0·5 per cent. carbolic acid (Table XXXI.).

TABLE XXXI.—*Protein Contents in Various Tumours and Organ Extracts.*

| 1. Mouse carcinoma (63) extract | . | . | . | . | Over 1 per cent. |
|---|---|---|---|---|---|
| 2. ,, (199) ,, | . | . | . | . | About 1 ,, |
| 3. Mouse sarcoma (37 p) ,, | . | . | . | . | Just 1 ,, |
| 4. ,, (37 s) ,, | . | . | . | . | ,, 0·5 ,, |
| 5. ,, (37 p) (from baby rat) extract | . | . | . | ,, 0·5 ,, |
| 6. Rat sarcoma (R 16) extract. | . | . | . | . | About 1 ,, |
| 7. Human cancer (adeno-carcinoma) extract (A) | . | . | ,, 1·2 ,, |
| 8. ,, (scirrhus) extract (S) | . | . | . | Just 1 ,, |
| 9. Mouse embryo skin extract | . | . | . | . | About 0·5 ,, |
| 10. Mouse liver extract. | . | . | . | . | . | ,, 0·8 ,, |
| 11. Rat liver extract . | . | . | . | . | Just 0·5 ,, |

NOTE.—Extracts were diluted twenty times with saline.

The protein content varied from 0·5 per cent. to 1 per cent. No special difference could be found between the extracts from normal tissues and tumours or between the tumours themselves. The protein content in the testing dose which was suitable comes out at about 1 : 2000, which agrees with Kelling's results. In spite of these precautions the precipitin reaction with tumour immune sera did not give very conclusive results.

TABLE XXXII.—*Precipitin Reaction by Mouse Sarcoma* (37 s) *Antiserum, with Homologous and Heterologous Extracts.*

| | 37 s Antiserum. | Absolute Dilution. | Homologous Extract, 1 c.c. |
|---|---|---|---|
| 1 . . . | 10 x in 1 c.c. | 20 x | + + + |
| 2 . . . | 20 x ,, ,, | 40 x | + |
| 3 . . . | 40 x ,, ,, | 80 x | ± |
| 4 . . . | 80 x ,, ,, | 160 x | − |
| | | | **199 Extract, 1 c.c.** |
| 1 . . . | 10 x in 1 c.c. | 20 x | − |
| 2 . . . | 20 x ,, ,, | 40 x | − |
| 3 . . . | 40 x ,, ,, | 80 x | − |
| 4 . . . | 80 x ,, ,, | 160 x | − |
| | | | **R 16 Extract, 1 c.c.** |
| 1 . . . | 10 x in 1 c.c. | 20 x | − |
| 2 . . . | 20 x ,, ,, | 40 x | − |
| 3 . . . | 40 x ,, ,, | 80 x | − |
| 4 . . . | 80 x ,, ,, | 160 x | − |

As Tables XXXII., XXXIII., XXXIV. show, the reaction although not very strong was definitely specific, the homologous extract in every case reacting more strongly than the others. This specificity is still more marked when human cancer extracts react with the mouse-tumour antisera, no precipitation taking place (Tables XXXV., XXXVI.).

TABLE XXXIII.—*Precipitin Reaction by Mouse Carcinoma* (199) *Antiserum, with Homologous and Heterologous Extracts.*

|  | 199 Antiserum. | Absolute Dilution. | Homologous Extract, 1 c.c. |
|---|---|---|---|
| 1 . . . | 10 x in 1 c.c. | 20 x | + + + |
| 2 . . . | 20 x ,, ,, | 40 x | + + |
| 3 . . . | 40 x ,, ,, | 80 x | + |
| 4 . . . | 80 x ,, ,, | 160 x | ± |
|  |  |  | 37 p Extract, 1 c.c. |
| 1 . . . | 10 x in 1 c.c. | 20 x | + + |
| 2 . . . | 20 x ,, ,, | 40 x | + |
| 3 . . . | 40 x ,, ,, | 80 x | — |
| 4 . . . | 80 x ,, ,, | 160 x | — |
|  |  |  | R 16 Extract, 1 c.c. |
| 1 . . . | 10 x in 1 c.c. | 20 x | + + |
| 2 . . . | 20 x ,, ,, | 40 x | — |
| 3 . . . | 40 x ,, ,, | 80 x | — |
| 4 . . . | 80 x ,, ,, | 160 x | — |

The rat-sarcoma antiserum shows much the same state of affairs, reacting strongly with the rat-tumour extract, but not at all with those from mouse tumours. Human cancer extracts behave in an anomalous manner, giving as strong a reaction as the homologous rat-tumour extract (Table XXXVII.).

*Summary.*

The precipitin reaction with mouse- and rat-tumour antisera while quite definite was not very strong. Although quite specific in the majority of cases, there were notable exceptions.

TABLE XXXIV.—*Precipitin Reaction by Mouse Sarcoma (37 **p** grown in Baby Rats) Antiserum, with Homologous and Heterologous Extracts.*

|  | 37 p (from Baby Rat) Antiserum. | Absolute Dilution. | Homologous Extract, 1 c.c. |
|---|---|---|---|
| 1 . . . | 10 x in 1 c.c. | 20 x | + + + |
| 2 . . . | 20 x ,,   ,, | 40 x | + + |
| 3 . . . | 40 x ,,   ,, | 80 x | ± |
| 4 . . . | 80 x ,,   ,, | 160 x | — |
|  |  |  | R 16 Extract, 1 c.c. |
| 1 . . . | 10 x in 1 c.c. | 20 x | + + |
| 2 . . . | 20 x ,,   ,, | 40 x | + |
| 3 . . . | 40 x ,,   ,, | 80 x | — |
| 4 . . . | 80 x ,,   ,, | 160 x | — |
|  |  |  | 199 Extract, 1 c.c. |
| 1 . . . | 10 x in 1 c.c. | 20 x | — |
| 2 . . . | 20 x ,,   ,, | 40 x | — |
| 3 . . . | 40 x ,,   ,, | 80 x | — |
| 4 . . . | 80 x ,,   ,, | 160 x | — |
|  |  |  | 37 p (from Mouse) Extract, 1 c.c. |
| 1 . . . | 10 x in 1 c.c. | 20 x | — |
| 2 . . . | 20 x ,,   ,, | 40 x | — |
| 3 . . . | 40 x ,,   ,, | 80 x | — |
| 4 . . . | 80 x ,,   ,, | 160 x | — |

TABLE XXXV.—*Precipitin Reaction by Mouse Carcinoma (199) Antiserum, with Homologous and Human Cancer Extracts.*

|  | 199 Antiserum. | Absolute Dilution. | Homologous Extract, 1 c.c. | Human Cancer (A) Extract, 1 c.c. | Human Cancer (S) Extract, 1 c.c. |
|---|---|---|---|---|---|
| 1 . . . | 10 x | 20 x | + + + | — | — |
| 2 . . . | 20 x | 40 x | + + | — | — |
| 3 . . . | 40 x | 80 x | + | — | — |
| 4 . . . | 80 x | 160 x | ± | — | — |

TABLE XXXVI.—*Precipitin Reaction by Mouse Sarcoma* (**37 s**) *Antiserum,*
*with Homologous and Human Cancer Extracts.*

| | 37 s Antiserum. | Absolute Dilution. | Homologous Extract, 1 c.c. | Human Cancer (A) Extract, 1 c.c. | Human Cancer (S) Extract, 1 c.c. |
|---|---|---|---|---|---|
| 1 . . . | 10 x | 20 x | + + + | – | – |
| 2 . . . | 20 x | 40 x | + | – | – |
| 3 . . . | 40 x | 80 x | ± | – | – |
| 4 . . . | 80 x | 160 x | – | – | – |

TABLE XXXVII.—*Precipitin Reaction by Rat Sarcoma* (**R 16**) *Antiserum,*
*with Homologous and Heterologous Extracts.*

| | R 16 Antiserum. | Absolute Dilution. | Homologous Extract, 1·0 c.c. | Human Cancer (A) Extract, 1·0 c.c. | 199 Extract, 1 0 c.c. | 37 p Extract, 1·0 c.c. |
|---|---|---|---|---|---|---|
| 1 . | 10 x | 20 x | + + | + + | – | – |
| 2 . | 20 x | 40 x | + | + | – | – |
| 3 . | 40 x | 80 x | + | ± | – | – |
| 4 . | 80 x | 160 x | – | – | – | – |

## VI. CYTOLYSIS.

Used in a wide sense, cytolysis may be held to include hæmolysis, and one would expect that the antisera to mouse tumours which have been shown to be strongly hæmolytic for mouse corpuscles, would also produce lysis of mouse-tumour cells. In view of the hæmolytic strength of these antisera, it was anticipated that the demonstration of cytolysins for tumour-cell emulsions would present no difficulty.

The discovery and application of bacteriolytic antisera to the treatment of infectious diseases has induced many workers to attempt an analogous solution of the problem of a rational cancer therapy. Investigations in this direction start from the discovery by v. Dungern (1899 [33]) of an epitheliolysin for ciliated epithelium in the serum of rabbits immunised with the tracheal epithelium of the ox, and by Metchnikoff (1900 [35]) of a spermotoxin obtained by injecting guinea-pigs with emulsions of rabbit testicle. A. v. Wassermann (1912 [33]) mentions, in the introduction to his paper on cancer chemotherapy, unsuccessful experiments carried out in collaboration with Borchardt in v. Bergmann's clinic with the serum and milk of goats immunised against human mammary cancer. No effect was produced on the course of the disease.

Lambert and Hanes (1911 [36]) found that mouse and rat sarcomata, which grow well *in vitro* in the normal plasma of alien species, do not grow at all in the plasma of animals of such species after they had been immunised against these tumours of the mouse or rat. They ascribed this failure to presence of cytotoxins in the plasma of the immunised animals.

The present attempt was carried out by applying the ordinary methods used for bacteriolysis. No success was obtained, but it may be as well to describe the experiments for the benefit of other workers. The immune sera against mouse carcinoma **199** and mouse sarcoma **37 p** were inactivated by heating at 56° C. for·half an hour. To 1 c.c. of each serum 0·5 c.c. of the respective tumour emulsions, very finely divided, was added. Complement, 0·5 c.c. of fresh guinea-pig serum, was added, and the whole placed in the incubator at 37° C. for three hours. At the end of this interval the serum was removed and the tumour emulsion inoculated into a dozen mice. The following controls were inoculated at the same time :

1. Untreated emulsion.

2. Emulsion incubated for three hours at 37° C. with inactivated immune serum only.

3. Emulsion incubated for three hours at 37° C. with complement only.

No differences could be observed in the number or rate of growth of the tumours obtained.

## VII. OTHER EXPERIMENTS.

I. *Mouse-tumour extract can replace intact tumour cells as antigen for heterologous immunity.* — Haaland (1910 [37]) showed that intact living normal or tumour cells were indispensable to induce homologous resistance to transplanted mouse carcinomata. It was immaterial in what way the cells were killed, whether by mechanical disintegration, by heat, or by exposure to radium in the ice-chest, the power to produce resistance was annihilated along with the power of growth.

The case of heterologous resistance is quite different. A rabbit was injected intraperitoneally with 5 c.c. of a watery extract from 1 c.c. of an emulsion of mouse sarcoma **37 p**. After an appropriate interval the rabbit was bled and the serum examined for the presence of hæmolysins for mouse corpuscles (Table XXXVIII.).

The experiment shows clearly that the watery extract is sufficient to give rise to the specific hæmolysin, and the same result was obtained by using disintegrated tumour cells. The serum also gave a positive result when tested for hæmagglutination and complement fixation. Whatever may be the cell constituent of mouse tumours which acts as lysinogen, it is soluble in water or at any rate passes into the watery extract, as Takaki (1908 [38]) showed for the lysinogen of red blood corpuscles. Bulloch (1913 [37]) demonstrated that extraction with ether

for six hours robbed erythrocytes of their power to produce hæmolysins, but a similar experiment has unfortunately not yet been performed with mouse and rat tumours. The experiments, therefore, do not decide the nature of the lysinogen concerned.

TABLE XXXVIII.—*Hæmolytic Reaction by Rabbit Serum which was obtained by immunising against Watery Extract of Mouse Sarcoma* (**37 p**).

| MOUSE BLOOD CORPUSCLES. | | |
|---|---|---|
| | Serum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . . | 0·1 | Complete. |
| 2 . . . . | 0·05 | Nearly complete. |
| 3 . . . . | 0·025 | Slight. |
| 4 . . . . | 0·01 | Trace. |
| 5 . . . . | 0·005 | None. |

II. *Mouse embryo emulsion as antigen for heterologous immunity.*— The great activity of mouse embryo emulsions in eliciting homologous resistance naturally raises the question of its suitability as antigen for heterologous immunity. The complement fixation experiments with mouse embryo skin extract as antigen showed great apparent differences between mouse tumour and mouse embryo skin, and it was therefore decided to obtain an antiserum to mouse embryo. A rabbit was immunised intraperitoneally with three ascending doses of mouse embryo, 1 c.c., 3 c.c., and 4 c.c., at intervals of one week. Ten days after the last injection the serum was collected and tested for the presence of hæmolysins (Table XXXIX.).

TABLE XXXIX.—*Hæmolytic Reaction by Rabbit Serum obtained by immunising against Mouse Embryo Skin.*

| MOUSE BLOOD CORPUSCLES. | | |
|---|---|---|
| | Serum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . . | 0·1 | Complete. |
| 2 . . . . | 0·05 | Nearly complete. |
| 3 . . . . | 0·025 | Slight. |
| 4 . . . . | 0·01 | Trace. |
| 5 . . . . | 0·005 | None. |

The hæmolytic action is quite evident, as was to be expected. The serum of mice, on the other hand, inoculated three times at intervals of one week with 0·05 c.c. of mouse embryo skin does not produce hæmolysis of mouse erythrocytes. Similar hæmolytic and complement fixation experiments with the serum of mice in which transplanted tumours had grown and been absorbed were equally negative. In this respect, also, the sharp distinction between homologous and hetero-logous immunity is very evident.

III. *Hæmolysis of mouse erythrocytes with the serum of rats in which mouse tumour had grown and been absorbed.*—The rapid and complete disintegration of mouse-tumour grafts in rats in which mouse tumours had grown and been absorbed has been ascribed to the development of antibodies to mouse tissue in the rat. The serum of young rats in which mouse sarcoma **37 p** had grown temporarily was taken as soon as absorption was complete and tested for hæmolysis of mouse red blood corpuscles (Table XL.).

TABLE XL.—*Hæmolytic Reaction with Serum of Baby Rats in which Mouse Sarcoma (**37 p**) developed and afterwards was absorbed.*

| | MOUSE BLOOD CORPUSCLES. | |
| --- | --- | --- |
| | Serum. | Guinea-pig Complement, 0·05 c.c. |
| 1 . . . . | 0·1 | Trace. |
| 2 . . . . | 0·05 | ,, |
| 3 . . . . | 0·025 | None. |
| 4 . . . . | 0·01 | ,, |

The reaction was so faint that it cannot properly be described as positive. It is probable that this result is in part due to the immature state of the rats, and in part to the fact that only one injection of tumour was given, both of which conditions are unfavourable to the production of a potent antiserum. The interval after inoculation may also have been too prolonged.

CONCLUSIONS.

The malignant new growths of man and animals, under a great diversity of anatomical forms, are united loosely together by a number of characters common to all. While it is comparatively easy, as well as a descriptive convenience, to distribute them into the main sub-divisions of carcinoma and sarcoma and their subsidiary histological groups, this method of examination by itself breaks down when the attempt is made to find morphological criteria which can be relied on

to separate the similar histological groups of different species and, still more, the similar tumours of the same species. The experimental study of transplantable tumours has elicited the fact that tumours, apparently structurally identical, may possess constant biological characters which differentiate them completely from each other. The criterion of growth, again, usually suffices to separate the tumours belonging to different species from each other, tumours being easily propagable in animals of the parent species, and failing to proliferate for any length of time in alien species. These and other differences have their counterpart in the serum reactions of which an account has been given. Although we are still far from a complete correlation of the results of these distinct methods of investigation, it is of interest to attempt a general survey and to formulate provisional conclusions.

These serum reactions, as was to be expected, are to a certain extent examples of the reactions characteristic for tissues of the species from which the antigens are derived, and show features which may be interpreted as due to blood-relationship. Due allowance must be made for these features before it is permissible to utilise the reactions for an analysis of the characters of the tumours as tumours. In this respect the reactions fall naturally into two groups: in the first, comprising hæmolysis and hæmagglutination, the reactions of mouse tumours *quâ* mouse tissue overshadow all minor differences; in the second group, comprising complement fixation and precipitins, the species characters are overlaid with the effects of other differences of a more individual kind enhancing the difficulties of interpretation but promising fresh insight into the biochemistry of malignant new growths. In the case of the hæmolysins it is found that the mouse-tumour antisera are considerably more hæmolytic for mouse erythrocytes than for those of the nearly related rat. These antisera are practically without action when red blood corpuscles of the rabbit or guinea-pig are substituted for mouse or rat corpuscles in the hæmolytic system. The rat-tumour antiserum, equally inactive on rabbit and guinea-pig corpuscles, is strongly hæmolytic for rat corpuscles, and only faintly hæmolytic for those of the mouse. The phenomena of hæmagglutination show corresponding features. The antisera obtained by immunising with mouse tumours grown in young rats show an intermediate condition. They are nearly equally hæmolytic (and agglutinative) for mouse and rat corpuscles, a condition in all probability due to the unavoidable intimate mixture of mouse parenchyma and rat stroma in the tumour emulsions used as antigens, in this case.

The hæmolysin for goat erythrocytes present in the antisera against mouse and rat tumours is a distinct antibody comparable to and probably identical with the hæmolysins discovered by Forssmann and Morgenroth in animals immunised with organ emulsions of the guinea-pig and mouse. Morgenroth also found this goat hæmolysin in rabbits immunised with mouse tumours; with excessive caution he refrained

from identifying it with the hæmolysins obtained by immunising with mouse kidney. There seems to be no valid reason for doubting their identity. This exception to the general specificity of hæmolysins has led to interesting speculations on the nature of the mother substance of specific hæmolysins, lysinogens, by Forssmann, Landsteiner, and others. Forssmann brought forward the view that the lysinogen would prove to be of a lipoid nature; Landsteiner, that a combination of lipoid and protein corresponded more closely with the facts. It is unnecessary to discuss the matter further, as the present investigations do not permit of definite conclusions on this difficult question.

The several antisera prepared with different mouse tumours agree closely in hæmolytic strength. A difference was noted between the antisera to carcinoma and sarcoma in that the strength of the sarcoma sera (of which two had been prepared with different sarcomata) was slightly greater than that of two carcinoma antisera. As far as possible the doses and intervals of immunisation were the same in all cases. The carcinoma antisera, on the other hand, were hæmolytic when complemented with normal rabbit serum in a dose quite inactive with the sarcoma antisera. The difference, therefore, can hardly be purely quantitative. Apart from this the fresh sera of the mouse, rat, and rabbit were without action in the dose adopted when substituted for fresh guinea-pig serum as complement to these antisera.

The most constant feature of the complement fixation experiments is the regularity and energy with which fixation occurs when the homologous extract is put in contact with an antiserum (Tables XLI., XLII.). The other extracts, whether from similar tumours of the same species or from tumours of other species, give a much weaker reaction. The evidence in this respect implies the possession of substances of importance in the reaction by each tumour strain, peculiar to itself. This is remarkable when it is remembered that the strains of mouse tumour employed have all been propagated for years in a succession of normal mice, and the tumours for immunisation came from one set of animals, those from which the extracts were prepared from another series. Nevertheless, the Bordet-Gengou reaction picks out the homologous extract in every case. There is here an exact counterpart to the phenomena of propagation, such as the persistence in individual tumour strains of characteristic features in the rate and percentage of growth, and also in histological structure.

The extracts prepared from normal tissues turn out to be unexpectedly weak in complement-fixing power, in many cases weaker even than extracts of tumours belonging to other species. This result may be due to differences between tumour and normal tissues in the ease with which the reacting substances pass into the watery extracts, but in any case it points to essential differences between heterologous and homologous immunity, since it has been shown that in the latter case

TABLES XLI. AND XLIII.—*Summary of Complement Fixation Experiments.*

**Table XLI.**

| 199 Antiserum. | 199 Extract, 0·2 c.c. | 37 p Extract, 0·2 c.c. | Mouse Embryo Skin Extract, 0·02 c.c. | Mouse Liver Extract, 0·2 c.c. | R 16 Extract, 0·2 c.c. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer. Extract A, 0·2 c.c. | Human Cancer. Extract S, 0·2 c.c. |
|---|---|---|---|---|---|---|---|---|
| 1 . . . 0·1 | None. | None. | None. | Trace. | None. | Marked. | None. | None. |
| 2 . . . 0·05 | ,, | ,, | Trace. | Slight. | ,, | Complete. | ,, | Trace. |
| 3 . . . 0·025 | ,, | Trace. | Slight. | Nearly complete. | Slight. | ,, | Trace. | Slight. |
| 4 . . . 0·01 | ,, | Slight. | Marked. | Complete. | Nearly complete. | ,, | Slight. | Marked. |
| 5 . . . 0·005 | ,, | ,, | Complete. | ,, | Complete. | ,, | Marked. | Complete. |
| 6 . . . 0·0025 | Slight. | Marked. | ,, | ,, | ,, |  | Complete. | ,, |
| 7 . . . 0·001 | Marked. | Complete. | ,, | ,, | ,, |  | ,, | ,, |

| 37 s Antiserum. | 37 s Extract, 0·2 c.c. | 199 Extract, 0·2 c.c. | Mouse Embryo Skin Extract, 0·2 c.c. | Mouse Liver Extract, 0·2 c.c. | R 16 Extract, 0·2 c.c. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer. Extract A, 0·2 c.c. | Human Cancer. Extract S, 0·2 c.c. |
|---|---|---|---|---|---|---|---|---|
| 1 . . . 0·1 | None. | Trace. | Trace. | Slight. | None. | Complete. | None. | None. |
| 2 . . . 0·05 | ,, | ,, | Slight. | Marked. | Trace. | ,, | Trace. | Slight. |
| 3 . . . 0·025 | ,, | ,, | Marked. | Complete. | Slight. | ,, | Slight. | Marked. |
| 4 . . . 0·01 | Slight. | Slight. | Complete. | ,, | Nearly complete. | ,, | Marked. | Complete. |
| 5 . . . 0·005 | Marked. | Marked. | ,, | ,, | Complete. | ,, | Complete. | ,, |
| 6 . . . 0·0025 | Complete. | Complete. | ,, | ,, | ,, | ,, | ,, | ,, |
| 7 . . . 0·001 | ,, | ,, | ,, | ,, | ,, | ,, | ,, | ,, |

Table XLII.

| | 37 p (in Rat) Antiserum. | 37 p Rat, 0·2 c.c. | 37 p Mouse, 0·2 c.c. | 199, 0·2 c.c. | R 16, 0·2 c.c. |
|---|---|---|---|---|---|
| 1 | 0·1 | None. | Trace. | None. | None. |
| 2 | 0·05 | ,, | ,, | Trace. | ,, |
| 3 | 0·025 | ,, | Slight. | Slight. | ,, |
| 4 | 0·01 | ,, | ,, | ,, | Slight. |
| 5 | 0·005 | Slight. | Marked. | Marked. | Marked. |
| 6 | 0·0025 | ,, | Nearly complete. | Nearly complete. | Nearly complete. |
| 7 | 0·001 | Marked. | Complete. | Complete. | Complete. |

| | R 16 Antiserum. | R 16, 0·2 c.c. | 199, 0·2 c.c. | 37 p. | Normal Human Serum, 0·5 c.c. (diluted ten times). | Human Cancer (A) Extract, 0·2 c.c. |
|---|---|---|---|---|---|---|
| 1 | 0·1 | None. | Trace. | Slight. | Marked. | None. |
| 2 | 0·05 | ,, | Slight. | ,, | Complete. | ,, |
| 3 | 0·025 | ,, | ,, | Marked. | ,, | ,, |
| 4 | 0·01 | ,, | Marked. | ,, | ,, | ,, |
| 5 | 0·005 | Trace | Complete. | Complete. | ,, | Slight. |
| 6 | 0·0025 | Slight. | ,, | ,, | ,, | Marked. |
| 7 | 0·001 | Nearly complete. | ,, | ,, | ,, | Complete. |

normal tissues can elicit the immune or resistant condition as well as do tumour tissues. It is perhaps well to exercise caution in appraising the significance of the experiments with extracts of other tumours whether of the same or other species. There is no clear evidence of a community of antigenic substances in the histological groups, since the sarcoma extracts, for example, do not react more strongly with the sarcoma antisera than do the extracts of carcinomata. There is a further consideration which must be given considerable weight in this respect. As is well known, organ extracts can be replaced by artificial cholesterin-lecithin mixtures as antigen for the Wassermann reaction for syphilis. The physical state of these lipoid emulsions has a great influence on their suitability, so that slight changes in the mode of preparation abolish their complement-fixing properties completely. It is now fairly well established, however, that the cell lipoids of malignant new growths show significant variations in their relative proportions from one tumour strain to another, and may also differ from those present in normal tissues in a similar way. It may therefore well be that similarity in the state of physical

aggregation is largely responsible for the results obtained in complement fixation and precipitin experiments. There is apparently no correspondence with the other known characters of these growths, so that here a new set of independent properties is revealed. It remains for further investigation to determine whether the antigenic substances in the different tumours from the same and other species are to some extent common or closely related, as these experiments seem to show, and should this prove to be the case, the nature of the cell constituents involved.

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