A NOTE ON THE NON–SPECIFIC ACTION OF SO–CALLED “ANTI–CANCER SERUM”

H. J. PHelps, M.A., D.Phil.¹

(From the Laboratories of the Imperial Cancer Research Fund, London)

The experiments described in this note were undertaken for the purpose of determining whether sera obtained from rats after repeated injections of Jensen rat sarcoma tissue injure specifically Jensen rat sarcoma cells.

OUTLINE OF EXPERIMENTS

Rats were immunized (1) with Jensen rat sarcoma tissue; (2) with emulsions of normal rat spleen; (3) with normal rat blood. Sera from rats of these three series were tested against (a) cultures of Jensen rat sarcoma cells; (b) cultures of normal spleen cells; (c) cultures of mouse carcinoma 63; (d) cultures of cells of normal mouse spleen.

Jensen Rat Sarcoma Tissue as Antigen: Two series of "tumour-resistant" rats were employed. (1) Rats of the Jensen rat sarcoma susceptible strain of the Imperial Cancer Research Fund Laboratories. These rats had been used for routine transplantation of the Jensen rat sarcoma; the implants had grown for a time and had then regressed.
(2) Rats of the Wistar strain in which the Jensen rat sarcoma cells do not flourish readily. Twelve such rats were given routine inoculations with minced Jensen rat sarcoma. Of these, 9 showed transient growth of a tumour; one produced a good tumour of progressive growth which was subsequently used for further transplantation; two rats died, one after a small growth had entirely regressed.

Two series each of six rats were selected for immunization with Jensen rat sarcoma. One series consisted of resistant rats of the J.R.S. susceptible strain and one of Wistar rats which had been given a test inoculation. All these rats were given intraperitoneal injections of 1 c.c. of minced J.R.S.

Normal Spleen as Antigen: A series of 6 normal Wistar rats were similarly injected weekly with doses of 1 c.c. of minced spleen obtained from normal rats of various strains.

Normal Blood as Antigen: Six Balogh rats were injected weekly with 1 c.c. each of citrated whole blood from normal rats, usually the same rats as were used to provide spleen tissue.

RESULTS

The method of cultivating cells in vitro, the method of testing sera and the standards by which the results were computed were all those which have been described by Lumsden (1 and 2).

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After five or six injections of tissue—J.R.S. in one series, spleen in a second series—the immunized animals were bled and the fresh sera tested on twenty-four-hour cultures of rat cells. The sera in every case, from spleen as well as J.R.S.-immunized rats, killed to a greater or less extent the cells which had migrated from the cultures.

The results obtained with blood-injected rats will be dealt with separately.

In order to bring the investigation within the powers of a single worker, the 3 rats of each series showing the greatest titre of cytotoxins were selected for more detailed study. Unfortunately the J.R.S.-immunized rat which initially showed the greatest cytotoxic activity was killed by an accident not directly connected with the investigation.

For test of tissue cultures the rats were bled from the tail on the third or fourth day following an injection.

Tests on Day-old Tissue Cultures of J.R.S.: It has been observed that the lability of such cultures varies considerably from batch to batch; also, the rate and the extent of the production of cytotoxins in any one rat do not follow the same course after each successive injection. In accordance with Lumsden's practice, the titre of cytotoxins in any serum is expressed as the percentage of the total outwandering cells in a tissue culture specimen which are killed in one hour after the application of the serum. The maximum titres observed with the 3 selected rats injected with minced Jensen sarcoma were 80 per cent, 80 per cent, and 75 per cent. The three selected "spleen-immunized" rats all showed a maximum titre of 100 per cent. It can hardly be claimed on the basis of so few animals that injections of spleen produce sera more toxic to J.R.S. than can be produced by injections of minced J.R.S. tissue, but the fact that 3 out of 6 rats should have shown such a high titre of cytotoxins is remarkable. The possibility that the lability of different cultures of the same batch might show considerable fluctuations was tested in a single experiment in which 8 cultures were selected at random from one batch, 4 of them being treated with serum of a rat believed to show a high titre and the other 4 with serum of one believed to have a low titre. The first rat serum gave titre estimates of 95 per cent, 90 per cent, 80 per cent, and 80 per cent, and the second titres of 15 per cent, 20 per cent, 10 per cent, 15 per cent.

Tests with Three-day Cultures of J.R.S.: Of the selected "J.R.S.-immunized" rats, only one appeared to have in its serum cytotoxins capable of killing cells of J.R.S. cultured for three days. The maximum titre shown by this rat was 45 per cent. It is significant that on all occasions this rat showed titres between 15 and 45 per cent, whereas the other two rats never at any time killed three-day J.R.S. cultures.

The behaviour of the spleen-injected rats was similar with respect to the three-day J.R.S. cultures. One of the rats showed in different experiments (i.e. following different injections) titres of 75 per cent, 50 per cent, and 70 per cent; another showed titres of 15 per cent, 1 per cent, and 0 per cent, while the third was without effect at any time. Thus, on the basis of these 6 selected animals, it would seem that the "spleen-immunized" rats show a rather higher titre of cytotoxins than the "J.R.S.-immunized" rats.
**Experiments with Tissue Cultures of Rat Spleen:** Tissue cultures of spleen are far less constant in their growth and reactions than tissue cultures of J.R.S. The outwandering cells obtained from spleen explants are of three types. First, there are many small lymphocytes which are very labile to cytotoxic sera, but since they have in any case only a short life in culture, cells of this type have been discounted as far as possible in estimating the effects of cytotoxic sera on the cultures. Secondly, there are the splenic macrophages, on which so far as possible the tests have been computed, and thirdly, particularly in older cultures of spleen, there appear some relatively large cells which are very stable to cytotoxic sera.

Lumsden’s tests on splenic cultures were carried out mainly with two-day-old cultures of adult spleen. There are obvious difficulties in comparing results obtained from one-day-old cultures of one tissue with those obtained from two-day-old cultures of another. It has been found that it is possible to obtain tolerably good one-day cultures of rat spleen by culturing explants of spleens from young rats taken a few hours after birth. The maximum titres observed for the “J.R.S.-immunized” rats were 40 per cent, 60 per cent, and 80 per cent, using such cultures, while the titres shown by the “spleen-immunized rats” were 75 per cent, 65 per cent, and 80 per cent. Again the cytotoxin content of the J.R.S.-injected rats seems to be slightly lower than that of the spleen-injected rats, and both seem less toxic to one-day spleen cultures than to one-day cultures of J.R.S.

A few experiments were carried out using three-day-old cultures of adult rat spleen. The results of these closely paralleled those obtained with three-day-old cultures of J.R.S.

Of the “J.R.S.-immunized” rats, one showing a maximum titre of 45 per cent with three-day-old J.R.S. cultures showed a maximum titre of 60 per cent with three-day-old splenic cultures. Of the two rats which did not kill three-day J.R.S. cultures at all, one gave a titre of 20 per cent with three-day spleen; the other did not kill three-day splenic cultures at all. Of the “spleen-immunized rats,” one killing 75 per cent of three-day J.R.S. cultures killed 80 per cent of three-day spleen; one killing 15 per cent of J.R.S. killed 2 per cent of spleen, and one killing no three-day J.R.S. cells killed 5 per cent of three-day splenic cultures.

**Results with Tissue Cultures of Mouse Cells:** Anti-sera against rat tissues have also been tested on cultures of carcinoma No. 63 (Bashford’s tumour strain) and on cultures of normal mouse spleen. The notion that malignant cells contain a common antigenic constituent capable of evoking antibodies specifically lethal to tumour cells is derived largely from observations of the effects of anti-sera on cultures of mouse carcinoma 63. The carcinoma cultures, made in normal rat serum, are, as is well known, very unstable; they are destroyed even by the addition of physiological saline. With cultures in such a labile state it is very unsafe to draw definite conclusions. The tests have, nevertheless, been carried out and, as is shown in the next paragraph, the results obtained are similar, so far as mouse tumour 63 is concerned, to those published by Lumsden. The additional information provided by the experiments in which normal mouse-spleen cultures were acted
upon by anti-sera, nullifies the conclusion that anti-Jensen rat sarcoma sera
contain an antibody or cytotoxin specifically noxious to mouse tumour cells.

Sera from both the J.R.S.-injected and spleen-injected rats killed cultures
of both mouse carcinoma 63 and young mouse spleen actively; the mouse
cultures did not show any considerable decrease of lability with age and the
M.63 cultures were more labile to both sera than cultures of mouse spleen.
Sera from the selected “J.R.S.-immunized” rats killed 100 per cent, 100 per
cent and 90 per cent of both one-day and three-day M.63 and sera from two
of these rats killed 80 per cent and 100 per cent, and 80 per cent and 90 per
cent, of one-day and three-day cultures of mouse spleen. Sera from the
“spleen-immunized” rats killed 95–100 per cent of both one-day and three-
day cultures of M.63 and sera from two of these rats killed 100 per cent and
90 per cent, and 90 per cent and 75 per cent, of one- and three-day cultures of
mouse spleen.

Lumsden has made cinematographic records of the action of anti-J.R.S.
sera on cultures of rat tissue (3), showing:

(1) That an anti-J.R.S. serum kills J.R.S. cells which have migrated from
an explant incubated in normal rat serum.
(2) That the same anti-serum has practically no effect on cultured spleen
cells taken from the spleen of a rat immunized with J.R.S.
(3) That the same serum has no effect on peritoneal lining cells taken from
the individual rat which has provided the serum.

The third point has not been investigated since it would hardly be expected
that serum would have an effect on cells taken from the same animal. The
second point has been checked and the results obtained support Lumsden.

Cultures of spleen and peritoneal lining cells were made from an immune
rat. The cultures obtained were not very good but it was possible to show
that the strongest anti-J.R.S. serum available was much less toxic to immune
splenic and peritoneal cells than to corresponding normal cells. Since, how-
ever, it has been shown that normal splenic cells are killed by the serum, the
results obtained with cells from an immune rat are of theoretical interest
only and cannot in any sense be regarded as a justifiable “control.”

Rats Injected with Normal Whole Blood: Citrated whole blood proved a
far less effective antigen than J.R.S. or spleen. When the sera of such rats
were tested on one-day cultures of J.R.S. no titre higher than approximately
5 per cent was observed. Somewhat higher titres were obtained with one-
day cultures of rat spleen, two rats giving titres of 25 per cent and one a titre
of 20 per cent. One “blood-immunized” rat showed a titre of 80 per cent
with a one-day culture of M.63 and 50 per cent with a three-day culture of
the same material. The corresponding figures for another rat were 50 per cent
and 20 per cent.

Summary and Conclusions

The observations recorded in this note show that anti-Jensen rat sarcoma
sera kill both sarcoma cells and normal spleen cells; and that anti-spleen
sera kill both normal spleen and sarcoma cells.
The conclusions drawn from the facts elicited are: (1) that "anti-cancer sera" have no specific action on malignant cells; (2) that the antigen in cancer cells which gives rise to the antibodies studied is not peculiar to malignant cells; (3) that the antibodies are probably iso-antibodies formed in response to injections of foreign, though homologous cells; (4) that the anti-sera, from their nature, probably cannot be therapeutically useful.

References