

# A COMPARATIVE STUDY OF THE ANTIGENIC STRUCTURE OF TUMOR TISSUE AND HOMOLOGOUS NORMAL TISSUE.

## REPORT 4. IMMUNOLOGICAL CHARACTERISTICS OF THE ANTIGEN - ANTIBODY COMPLEX

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Experience in the immunology of infectious diseases has shown that if immune sera are mixed with the corresponding transparent extracts or lyzates of bacteria, a specific antigen-antibody complex is formed, and comes down as a precipitate. When antigen reacts with antibody in the precipitation reaction, the bacterial antigens are neutralized or detoxicated by the specific antibodies contained in the immune sera.

On these grounds it is interesting to study the possibility of obtaining a complex of this type between the tissue antigen and tissue antibody, and also to investigate its immunological properties.

The object of the present investigation was to obtain a tissue antigen-antibody complex as a precipitate and to study some of its immunological properties, namely: the immunological properties of the particular complex dissolved in normal serum, the firmness of the bond between the tissue antigens and the attached homologous and heterologous antibodies, the serological activity and specificity of these antibodies, and the biological (cytotoxic) properties of the antibodies dissociated from the complex, for example in tissue culture.

### EXPERIMENTAL METHOD

To study the immunological properties of the antibodies dissociated from the precipitates, sera were obtained from chinchilla rabbits immunized with rat antigens: a saline extract of tissue of a Guerin carcinoma, whole ascites cells from a papillary adenocarcinoma of the ovary, and whole erythrocytes. To obtain precipitates, the sera were mixed with 10% saline extracts from these tumor tissues in the following proportions: 1) 3 volumes of serum against Guerin's carcinoma and 1 volume of extract from tissue of the homologous tumor (first homologous precipitate); 2) 3 volumes of serum against carcinoma of the ovary and 1 volume of extract from the tissue of the homologous tumor (second homologous precipitate); 3) 3 volumes of antierythrocyte serum and 1 volume of extract from the tissue of a Guerin's carcinoma (first heterologous precipitate); 4) 3 volumes of antierythrocyte serum and 1 volume of extract from ovarian carcinoma tissue (second heterologous precipitate). Guinea-pig's serum was added to each mixture as complement.

The controls consisted of the sera of unimmunized rabbits mixed in the same proportions with extracts from these tumor tissues. The mixtures of sera and extracts were poured into graduated tubes (10 ml) and kept in the refrigerator at 4° for 24-48 h. During this period the mixtures separated into two layers: a supernatant fluid and a precipitate. This did not take place in the control tubes. The higher the titer of the immune sera, the greater the volume of the precipitate.

To obtain a better quality of precipitation and to improve its separation from the supernatant fluid, the tubes containing the mixtures were centrifuged for 10 min at 2500 rpm. After centrifugation, the supernatant fluid was carefully aspirated and the residual precipitate was mixed with a small volume of normal rabbit serum. After centrifugation for a second time and removal of the supernatant fluid, the same volume of normal serum as of immune serum was added to the tubes containing the precipitate. The tubes were then thoroughly agitated in order to dissolve the precipitate more completely in the serum.

TABLE 1. Immunological Characteristics (Complement Fixation Reaction) of Immune Sera and Precipitins Dissolved in Normal Serum

| Sera against ascites cells of ovarian carcinoma |                   |       |        |              |                   |       |        |              |                   | Antierthrocyte sera |              |              |                   |       |        |              |                   |       |        |              |  |  |           |  |  |  |
|---|-------------------|-------|--------|--------------|-------------------|-------|--------|--------------|-------------------|---------------------|--------------|--------------|-------------------|-------|--------|--------------|-------------------|-------|--------|--------------|--|--|-----------|--|--|--|
| № 104   |                   |       |        |              |                   |       |        |              |                   | № 132               |              |              |                   | № 197 |        |              |                   | № 181 |        |              |  |  |           |  |  |  |
| Dilution of sera                                | antigens from     |       |        |              |                   |       |        |              |                   |                     | erythrocytes |              |                   |       | kidney |              |                   |       | ovary  |              |  |  | carcinoma |  |  |  |
|   | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary               | kidney       | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes |  |  |           |  |  |  |
| 1 : 20  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 40  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 80  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 160   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 320   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 640   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 1280  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |

| Normal sera, containing dissolved precipitates |                   |       |        |              |                   |       |        |              |                   | Normal rabbit's serum       |              |              |                   |       |        |              |                   |       |        |              |  |  |           |  |  |  |
|--|-------------------|-------|--------|--------------|-------------------|-------|--------|--------------|-------------------|-----------------------------|--------------|--------------|-------------------|-------|--------|--------------|-------------------|-------|--------|--------------|--|--|-----------|--|--|--|
| homologous (medium No. 2)                      |                   |       |        |              |                   |       |        |              |                   | heterologous (medium No. 4) |              |              |                   | № 167 |        |              |                   | № 167 |        |              |  |  |           |  |  |  |
| Dilution of sera                               | antigens from     |       |        |              |                   |       |        |              |                   |                             | erythrocytes |              |                   |       | kidney |              |                   |       | ovary  |              |  |  | carcinoma |  |  |  |
|  | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary                       | kidney       | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes | ovarian carcinoma | ovary | kidney | erythrocytes |  |  |           |  |  |  |
| 1 : 20   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 40   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 80   | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 160  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 320  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 640  | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |
| 1 : 1280                                       | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++                        | ++++         | ++++         | ++++              | ++++  | ++++   | ++++         | ++++              | ++++  | ++++   | ++++         |  |  |           |  |  |  |

Legend: ++++ greatest fixation of immune sera with antigens; +++ comparatively high fixation; ++ slight fixation; + very weak fixation; h) absence of positive reaction or complete hemolysis of erythrocytes

We conventionally called the first homologous precipitate dissolved in normal rabbit's serum medium No. 1, the dissolved second homologous precipitate medium No. 2, the dissolved first heterologous precipitate medium No. 3, and the dissolved second heterologous precipitate medium No. 4. These media were subsequently tested in the complement fixation reaction and in tissue culture. Media Nos. 2 and 4 were used as test sera for the complement fixation reaction. The antigens were saline extracts of tissues from a carcinoma of the ovary, from normal ovary and kidney, and normal erythrocytes. Untreated media and media heated to 56° for 30 min were used in the experiment.

All four media were tested in tissue culture, the controls for which were 10% saline extracts of the above tumor tissues, the sera of normal rabbits and of rabbits immunized with erythrocytes, ascites cells of carcinoma of the ovary, or extract of the tissue of a Guérin's carcinoma. Tissue culture was carried out by the method described elsewhere [1]. The relative proportions of test substances and the remaining components of the fluid medium in the tissue cultures were 1:1 and 1:1.5. Absolutely fresh sera and extracts were used in the experiments. The action of the test substances on the experimental cultures was assessed by the total number of growing explants, by the intensity of their growth on the 3rd-4th day (the mean coefficient of growth was calculated), and by the morphological picture of the zones of growth.

## EXPERIMENTAL RESULTS

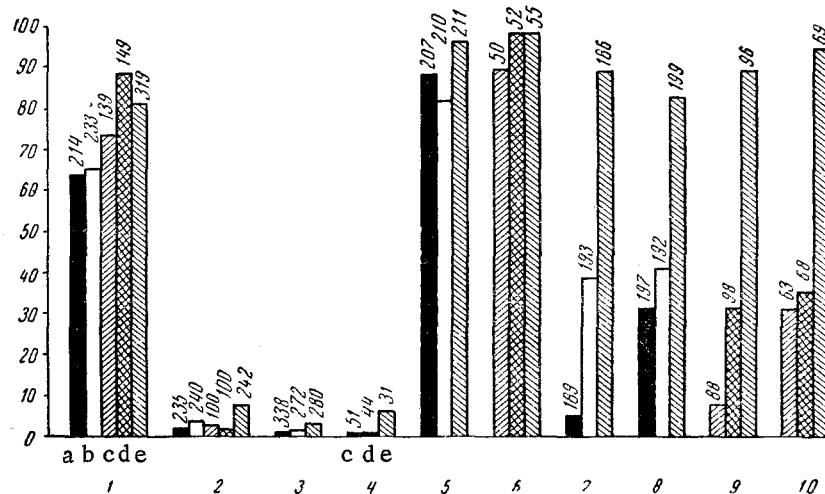
Immunological investigations. In Table 1 we give the immunological characteristics (in the complement fixation reaction) of the sera of rabbits immunized with ascites cells of an ovarian carcinoma and with erythrocytes, and of the precipitates dissolved in normal rabbit sera (media Nos. 2 and 4). It is clear from these facts that the reaction of the anticarcinoma sera with homologous antigens in a dilution of 1:160 was ++ or +, and in much lower titers with antigens from the tissues of the ovary (++ or +++ in a dilution of 1:80) and kidney (++ in dilutions of 1:160 and 1:80) or from the erythrocytes (++ in a dilution of 1:80). The antierythrocyte sera reacted in high titers with homologous antigens (++++ in a dilution of 1:1280) but much less strongly with tissue antigens from ovarian carcinoma and from normal ovary and kidney (++++ in a dilution of 1:160). Positive reactions were also observed when media Nos. 2 and 4 were used. For example, the intensity of the reaction between medium No. 2 and antigens from ovarian carcinoma tissue in dilutions of 1:160 or 1:80 was ++; with antigens from normal ovarian or kidney tissues and with antigens from erythrocytes in a dilution of 1:40 it was ++ or +. A somewhat different picture was observed when the complement fixation reaction was performed with medium No. 4, giving a more intensive reaction with antigens from erythrocytes (+++ in a dilution 1:160) and a much weaker reaction with antigens from ovarian carcinoma tissue (++ in a dilution of 1:80 or +++ in a dilution of 1:40) and from normal ovarian (++ in a dilution of 1:20) and kidney tissue (++ in a dilution of 1:40).

The immunological reactions thus showed that normal serum, in which homologous precipitates were dissolved, reacted more intensively with homologous antigens and much less so with antigens from normal tissues and erythrocytes; normal sera in which heterologous precipitates were dissolved (complex of tumor antigens and antierythrocyte antibodies), on the other hand, reacted more intensively with antigens from erythrocytes and much less so with antigens from normal and tumor tissues. These results demonstrate the presence of both antigenic similarities and antigenic differences between the tissues of the ovarian carcinoma, the ovary, the kidney, and the erythrocytes.

Biological investigations. In order to study the biological properties of media Nos. 1, 2, 3, and 4, we cultivated Guérin's carcinoma, normal uterus, ovarian carcinoma, and the ovary and kidney of young rats from 1 to 5 days old. The kidney was investigated for comparison as a normal tissue nonhomologous with the tumors.

The results of cultivation are summarized in the figure in absolute units and in percent. Sera of normal rabbits slightly inhibited growth of the experimental cultures of tissues of ovarian carcinoma, the ovary, and the kidney (growth was observed in 75.5, 91.9, and 83.7% of cases, respectively). Experimental cultures of tissues from Guérin's carcinoma and the uterus grew slightly less intensively on these sera (growth took place in 66.3 and 68% of cases, respectively). The antierythrocyte sera had a very marked inhibiting action on growth of all the experimental cultures. The intensity of growth was expressed numerically as follows: Guérin's carcinoma in 1.7% of cases, normal uterus in 3.7% of cases, carcinoma of the ovary in 3% of cases, ovary in 2% of cases, and kidney in 7.9% of cases.

The antitumor sera acted in a similar fashion on growth of the experimental cultures. In some experiments this growth was totally suppressed. The opposite pattern was observed when explants were cultivated in saline extracts of tumor tissues. For instance, during cultivation of Guérin's carcinoma, uterus, and kidney on tissue extract of a homologous tumor, growth was found in 90.4, 85.8, and 99% of cases, respectively. Similar results were obtained during cultivation of ovarian carcinoma, ovary, and kidney in extract of ovarian carcinoma tissue.



Number of three- or four-day growing tissue cultures on experimental media (in %). 1) normal serum; 2) antierythrocyte serum; 3) serum against Guerin's carcinoma; 4) serum against ovarian carcinoma tissue; 5) saline extract from Guerin's carcinoma; 6) saline extract from ovarian carcinoma tissue; 7) medium No. 1; 8) medium No. 3; 9) medium No. 2; 10) medium No. 4. a) Guerin's carcinoma; b) uterus; c) ovarian carcinoma; d) ovary; e) kidney. The number of experimental cultures is given above the columns in absolute numbers.

A different picture was observed during tissue culture in media Nos. 1, 2, 3, and 4, for the sera of rabbits, containing dissolved homologous precipitates, possessed some degree of specificity. For example, in medium No. 1, Guerin's carcinoma grew in only 4.2% of 189 explants, and only 38.9% of 193 uterine explants. This medium did not inhibit growth of kidney explants: growth was found in 90.3% of 186 cultures. Medium No. 2 had a similar action on the experimental cultures. Normal rabbit's sera containing dissolved heterologous precipitates had a slightly weaker inhibiting action on growth of the experimental cultures, but in this case too, the weakest growth was shown by explants of tumor tissues, and slightly more intensive growth by explants of homologous normal tissues, while hardly any inhibiting action was observed on the growth of the kidney explants (see figure).

In Table 2 we give the mean coefficients of growth of the experimental cultures of rats' tissues on the 3rd-4th day of the experiment. The most intensive growth of the explants was found when they were cultivated on saline extracts of tumor tissues, and the least on anticarcinoma anti-erythrocyte sera. When the tissues were cultivated on media Nos. 1, 2, 3, and 4, the lowest coefficient of growth was found in explants of tumors and homologous normal tissues, and the highest in explants of the kidney.

Although the immune sera in every case caused destructive changes in the cells of the zone of growth of the explants, media Nos. 1, 2, 3, and 4 had a cytotoxic action on the cells of the zones of growth of the tumor tissues and of the homologous normal tissues, but not on the cells of the zone of growth of the kidney explants.

Hence, the immunological and biological investigations showed that the antigen-antibody complex, when dissolved in normal serum, undergoes dissociation and the antibodies display serological activity in the complement fixation reaction. The dissociated antibodies retain their biological (cytotoxic) properties, and these may be demonstrated by the method of tissue culture. Antibodies dissociated from a homologous complex (tumor antigens-antitumor antibodies) react more strongly in the complement fixation reaction with tumor antigen and less strongly with antigens from homologous and nonhomologous normal tissues and erythrocytes. In tissue culture, the antibodies depress the growth of the experimental cultures of tumor tissue more than growth of the cultures of the homologous normal tissue, and have no appreciable depressing action on the growth of kidney tissue cultures. Conversely, the antibodies dissociated from a heterologous complex (tumor antigens-antierythrocyte antibodies) react more intensively in the complement fixation reaction with antigens from erythrocytes, and much less strongly with antigens from tumor tissue and

TABLE 2. Mean Coefficient of Growth of 3-4-Day Experimental Cultures of Rat Tissues Growing on Experimental Media

| Medium tested   | Tissue             |        |                   |       |        |
|---|--------------------|--------|-------------------|-------|--------|
|   | Guerin's carcinoma | uterus | ovarian carcinoma | ovary | kidney |
| Serum of control rabbits  | 1.22               | 1.07   | 1.37              | 2.29  | 2.10   |
| Serum of rabbits immunized with erythrocytes                            | 0.24               | 0.15   | 0                 | 0     | 0.19   |
| Serum of rabbits immunized with tissue of a Guerin's carcinoma          | 0.02               | 0.27   | —                 | —     | 0.11   |
| Serum of rabbits immunized with ascites cells of carcinoma of the ovary | —                  | —      | 0                 | 0     | 0.60   |
| Medium No. 1  | 0.45               | 0.75   | —                 | —     | 1.00   |
| Medium No. 2  | —                  | —      | 0.47              | 0.62  | 2.42   |
| Medium No. 3  | 0.52               | 0.45   | —                 | —     | 0.93   |
| Medium No. 4  | —                  | —      | 0.85              | 1.29  | 2.71   |
| Extract of tissue of a Guerin's carcinoma                               | 1.18               | 1.18   | —                 | —     | 2.55   |
| Extract of ascites cells of ovarian carcinoma                           | —                  | —      | 1.87              | 2.39  | 4.44   |

normal tissues. In tissue culture, these antibodies depress the growth of experimental cultures of tumor tissues more strongly, and depress growth of cultures of the homologous normal tissues less strongly, while they have no such action on the growth of cultures of kidney tissue.

#### SUMMARY

In dissolving the homologous (tumor antigen-antitumor antibody) and heterologous (tumor antigen-antierythrocytic antibody) in the normal rabbit serum there is dissociation into the solution of antibodies which manifest their serological activity in the complement fixation reaction. Dissociated antibodies also retain their biological (cytotoxic) properties manifested in the growth depression of experimental cultures of tumor and homologous normal tissues and cellular destruction in the growth zone. Antibodies dissociated from the homologous precipitate react more intensely in the complement fixation reaction with the antigens from the corresponding tumor tissue and much less with the antigens from the normal tissues and erythrocytes. In the tissue cultures these antibodies manifest some degree of specificity; they depress the culture growth of tumor tissues, and to a much lesser extent the growth of the cultures of homologous normal tissue; they do not affect the growth of the kidney tissue culture of newborn animals. The antibodies dissociated from the heterologous precipitate react more with the antigens from erythrocytes, and much less with the antigens from the tumors and normal tissues. The greatest depressive effect is produced by these antibodies on the growth of experimental cultures of the tumor tissues; the least on the growth of the kidney tissue culture.

#### LITERATURE CITED

1. M. S. Lomakin. Byull. éksper. biol., 6, 85 (1959).

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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