

# ONCOLOGY

## EFFECT OF ANTITUMOR SERUMS ON THE GROWTH OF BROWN-PEARCE RABBIT TUMOR IN TISSUE CULTURE

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The explantation method has been applied to the study of the effect of antitumor serums on the growths of tumor cells since the third decade of the present century. The results of such studies have, however, been very conflicting.

Some authors [8, 9] were unable to detect any differences in the growth in tissue culture of various tumor strains in serum taken from animals bearing tumors which were in process of resorption, or which were growing actively. Other workers [1, 7] arrived at similar results using serum from animals which had been immunized against tumor tissue. On the basis of these observations, these workers either denied the existence of antitumor antibodies in general, or else they admitted their existence, but did not attribute any antitumor properties to them.

Recent papers have shown conclusively [2, 3, 4, 6] that antibodies present in the serums of immunized animals are able to inhibit growth of tumor tissue in vitro. It is not yet known, however, whether this inhibitory effect is due to specific or unspecific antibodies.

The present paper presents the results of a comparative study of the effects of a highly specific adsorbed antitumor goat serum, and of the native serum, on the growth in tissue culture of metastatic nodes of Brown-Pearce rabbit tumor.

### EXPERIMENTAL METHODS

The immune serums were obtained by immunizing goats with 10% aqueous saline extracts of tumor tissue. In order to eliminate unspecific antibodies, and to enhance its specificity, some of the serum so obtained was purified by adsorption on the antigen with formalin-treated kidney and spleen tissues from a normal rabbit. The adsorption procedure was described by us in an earlier publication [5].

The serological examination of our serums was effected by means of the complement-fixation test, at 37°. We used three types of goat serum in our experiments: highly specific immune goat serum, treated by the adsorption procedure, containing basically antitumor antibodies, at a titer of 1:320++; native serum, containing both antitumor antibodies (1:640++) and antibodies to normal organs (1:200++); and, as a control, normal goat serum.

Material from metastatic nodes of Brown-Pearce rabbit tumor was grown in tissue culture in Carrel vessels, diameter 3.5 cm. Fowl plasma was first placed aseptically into the vessels, at a rate of two drops per fragment of tissue. The comminuted fragments of tumor tissue were then transferred to the cups by means of a special needle. Six fragments were placed into each cup. Chick embryo extract was then added. After the plasma had clotted, we added 10 drops of the given serum, and 8 drops of saline solution.

We assessed the action of the various serums on growth of tumor tissue on the basis of the number of

fragments showing growth, and of the intensity of growth of each fragment. The intensity of growth of each fragment was evaluated from the ratio of the area of the growth zone to the initial area. The initial area, and that of the growth zone, were measured by tracing the outlines on millimeter paper, using a microscope and an Ra-1 copying device, and counting the number of square millimeters included in the outlines.

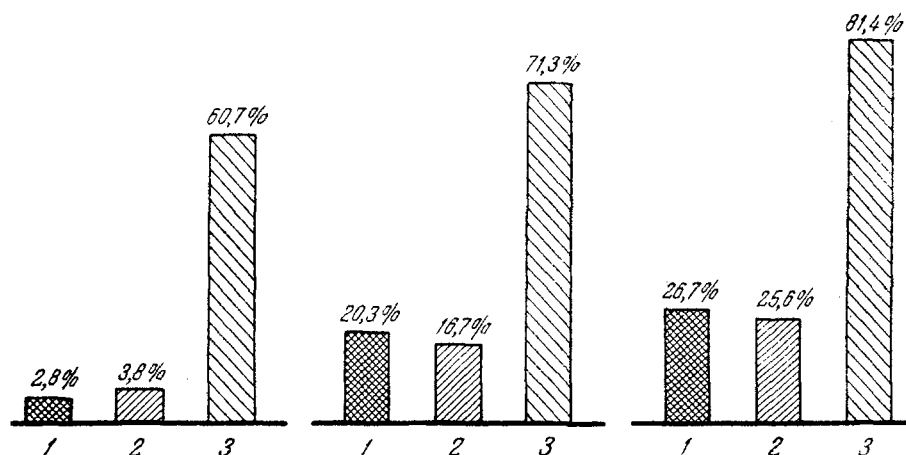


Fig. 1. Growth of tissue cultures of Brown-Pearce rabbit tumor in immune and normal goat serums (area of the growth zone as % of the initial area of the fragment of tissue). On the 5th, 10th, and 15th day of culture: 1) in adsorbed highly specific serum (158 fragments of tissue); 2) in polyspecific serum (174 fragments); 3) in normal goat serum (160 fragments).

#### EXPERIMENTAL RESULTS

Our results are illustrated in Fig. 1, where they are expressed as percentages on the 5th, 10th, and 15th days.

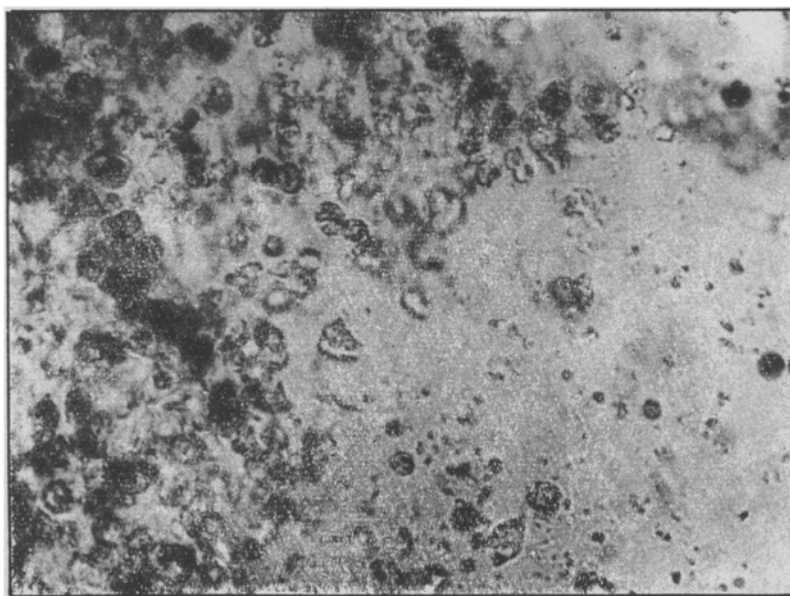


Fig. 2. Growth of tissue from a metastatic node in native immune goat serum (15th day of culture). Magnification 268 x.

As is evident from Fig. 1, the highest percentage growth of the growing fragments was found in nutrient medium containing normal goat serum (81.4%), on the 15th day. The least percentage growth in this time

was found in media containing native (untreated) goat immune serum (25.6%). Growth in treated goat immune serum amounted to 26.7%. The small difference in growth (1.1%) between media containing treated and untreated sera is not statistically significant.

The rate of growth, up to the 15th day, was greatest in medium containing normal goat serum. In a nutrient medium containing "adsorbed" serum the growth coefficient was 0.18, and with untreated immune serum it was 0.15. The small difference between the growth coefficients (0.03) in the two media is similarly not significant.

The results of our experiments (we used 492 fragments of tumor tissue for explantation, in all) thus showed that explants in nutrient media containing treated or untreated immune sera either did not grow at all, or they grew much more slowly than in media containing normal goat serum, and total or partial destruction of the cells of the explant had taken place by the 15th day of culture (Fig. 2).

The greatest growth in tissue culture of Brown-Pearce rabbit tumor was found in media containing normal goat serum. In such media we observed growth in the form of a unicellular layer, in which we could readily distinguish polymorphous cells with large, clear nuclei, situated very close to each other (Fig. 3).

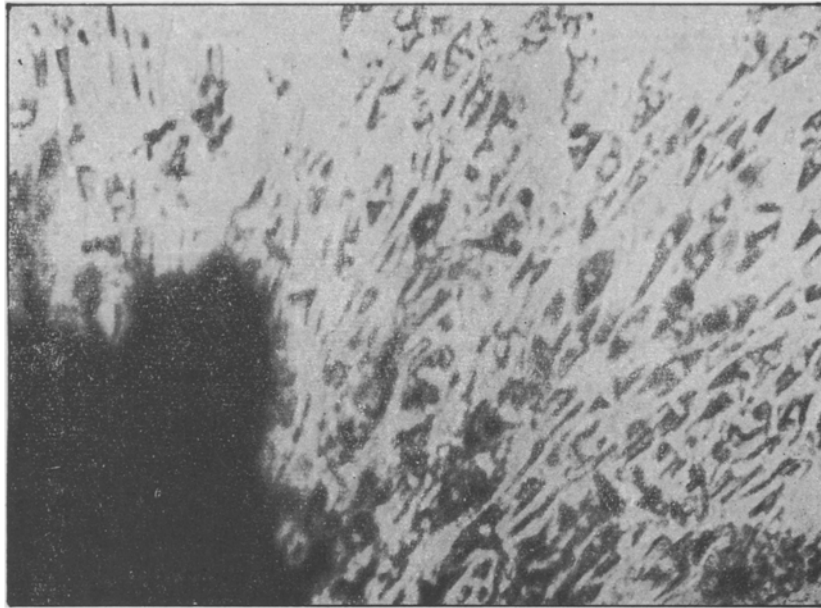


Fig. 3. Growth of tissue from a metastatic node in normal goat serum (12th day of culture). Magnification 268  $\times$ .

We concluded from our findings that sera containing antitumor antibodies powerfully inhibited growth of explants of Brown-Pearce rabbit tumor.

Comparison of the number of explants which showed growth, and of their rate of growth in media containing "adsorbed" and untreated sera showed that they inhibited growth to an equal extent.

Our experimental results, as well as those of other authors [1, 2], justify the conclusion that inhibition of growth of tissues of metastatic nodes of Brown-Pearce rabbit tumor is ascribable to the action of specific antitumor antibodies.

#### SUMMARY

Growth of Brown-Pearce rabbit tumor in tissue culture is powerfully inhibited by serum from goats which had been immunized against this tumor. Removal from the immune serum of unspecific organ antibodies, by adsorption on formalin-treated rabbit tissues, gave a highly specific antitumor serum, which inhibited growth of explants to the same extent as did the untreated serum. It is concluded that the inhibitory effect is due to the presence of specific antitumor antibodies.

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\*Original Russian pagination. See C. B. Translation.