

# ONCOLOGY

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

### COMMUNICATION II. INVESTIGATION OF THE ANTIGENIC PROPERTIES OF GUERIN'S CARCINOMA AND THE NORMAL UTERUS OF THE RAT BY THE USE OF THE TISSUE CULTURE METHOD

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In the first communication it was shown, by means of the complement fixation reaction, that rat tissues such as carcinoma of the uterus (Guérin strain) and normal uterus have in their composition common antigens and also antigens which differ from each other. Hence the aim of the present investigation was to study on a culture of rat tumor tissue the action of whole sera of rabbits immunized with antigens from tumor tissue and uterine tissue, and also of sera preliminarily adsorbed on these tissues.

Research in this direction enables first, an idea to be indirectly obtained of the antigenic composition of the tissues investigated, which is very important for the study of the immunology of cancer; and secondly, information to be gained of which of the immune sera possess the highest cytotoxic action in relation to the cancer cells; and thirdly these investigations enable the immediate action of antitumor antibodies of narrow specificity on these cells to be demonstrated.

There are indications in the literature that cancer antisera, adsorbed on the tissues of normal organs, do not lose their cytotoxic properties against cancer cells, whereas the sera obtained by immunization of experimental animals with a suspension of the corresponding normal tissue of homologous origin do not possess this action after adsorption [4, 5, 6]. In other words, this method proves the existence of specific cancer antigens in malignant neoplasms.

In consequence of our own findings and the results obtained by other workers, we decided to investigate whether the tissue culture method could be used to study the antigenic composition of tumor tissue and of homologous normal tissue.

### EXPERIMENTAL METHOD

In explantation experiments we used tissue from carcinoma of the uterus (Guérin strain) in the rat (metastases in internal organs). Fischer's method [3] of tissue cultivation was used. Into each Carrel dish was poured 3 ml of chick plasma, after which the bottom of the dish was rapidly flamed in a spirit lamp and the plasma poured off. Next, into each dish was added 2 drops of penicillin solution (50-100 units) and 2 drops of 20% chick embryonic extract. In isolated cases instead of chick embryonic extract we added an embryonic extract prepared from the tissues of 8- to 10-day-old rat embryos. This medium was inoculated with experimental

cultures. After 5-10 minutes a layer of coagulated plasma, 0.5-0.8 mm thick, was formed at the bottom of the dishes. In this way it is possible to cultivate many pieces of tissue and yet use a comparatively small amount of plasma. Furthermore the bulk of the explantates is to be found on the surface of the coagulated plasma and not in its thickness, and hence is in better contact with the test serum or the other components of the liquid nutrient medium.

Depending on the aim of the experiment, into each Carrel dish was poured 2-3 drops of the test serum on to the tissue fragments, as complement 2-3 drops of guinea pig serum with a complement titer of 0.04-0.12, a balanced saline solution, a 40% solution of glucose with ascorbic acid, 3-4 drops of a 20% solution of chick or rat embryonic extract and 2-3 drops of a 0.5% solution of yeast extract. To give greater sterility, to each dish was added 50-70 units of penicillin. The following immune sera were used in the experiment: 1) serum of a rabbit immunized with antigens from Guérin rat carcinoma tissue; 2) cancer antiserum adsorbed on fresh tumor tissue (for greater sterility); 3) cancer antiserum adsorbed on fresh tissue of a normal rat's uterus; 4) serum of a rabbit immunized with antigens from tissue of a normal rat's uterus; 5) the same serum adsorbed on fresh tumor tissue; 6) the same serum adsorbed on fresh tissue of the particular organ.

As a control the sera of nonimmunized rabbits were used. The titer of the immune sera was given in the first communication. Sera of normal rabbits as a rule did not contain antibodies. We judged the action of the sera tested by the general intensity of growth of the experimental cultures and by the morphological picture of the zones of growth. The intensity of growth of the fragments was estimated by the ratio between the area of the zones of growth to their initial areas. This ratio was designated the growth coefficient. The required area of the fragments was determined by tracing their outline on millimeter squared paper by means of a microscope and a drawing machine (RA-1), and it was calculated in  $\text{mm}^2$ . Perfectly fresh immune sera were used in the experiment, for there are indications in the literature that, during storage of the sera for a long time in the refrigerator, they lose their immunological activity.

#### EXPERIMENTAL RESULTS

During explantation of a Guérin rat carcinoma in normal rabbit serum, of the 50 fragments in the first experiment growth was found in 31 cultures, or 62%. All the fragments grew according to an epithelial or a mixed type (Fig. 1). In the second experiment on the 5th day of explantation, growth was observed in 48 fragments of 65, or 73.8%. No destructive changes were observed in the zone of growth in either the first or the second experiment. The zone of growth of the experimental cultures consisted of migrating cells, of different shapes and sizes, in which large, lightly stained nuclei could clearly be seen. We also saw cells of a similar form in case of cultivation of other tumor tissues [1].

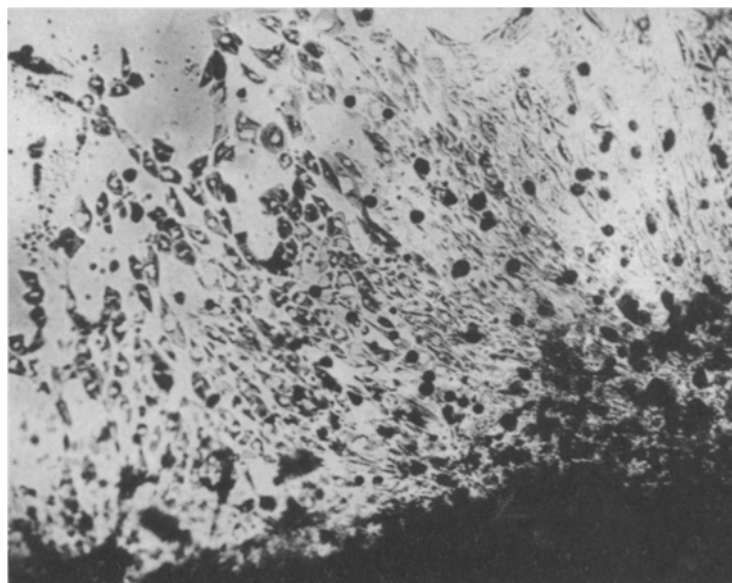


Fig. 1. Zone of growth of a 5-day old culture of a Guérin rat carcinoma explanted in normal rabbit serum. Dilution of serum 1:4. Magnification 126. Living culture.

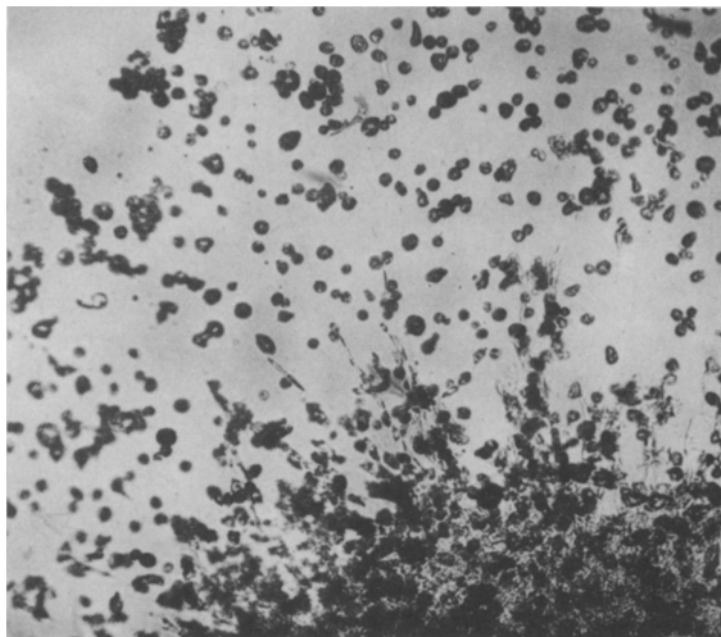


Fig. 2. Zone of growth of a 5-day old culture of tissue from a Guérin rat carcinoma explanted in the serum of a rabbit immune to the particular tumor. Dilution of serum 1:4. Magnification 126. Living culture.

Of the 170 fragments of tumor tissue cultivated in cancer antiserum (whole) with a high titer of immune antibodies, not one showed growth. The cancer antiserum thus completely suppressed growth of the tumor in the tissue culture. In the same way no growth was found on the 5th day of the experiment in the culture of tumor tissue explanted in the serum of a rabbit immunized with tissues from a normal rat's uterus. Growth was not apparent in any one of the 150 cultures. Isolated cultures were found in which the zone of growth consisted of individual migrating cells of altered shape (round, small in size and with a coarse, granular protoplasm).

The serum of a rabbit immune to normal rat's uterus thus also completely suppressed the growth of experimental cultures of a Guérin rat carcinoma.

In order to test the action of immune sera directly on the zone of growth of the cultures, fragments of tumor tissue were cultivated for 2-3 days at the beginning of the experiment in normal rabbit serum, after which the sera to be tested were added to the medium. In the first series of experiments the cultures were grown for 3 days on normal rabbit serum, after which cancer antiserum was added to the nutrient medium. In the first few days of explantation growth was observed in 41 of the 60 fragments. On the 5th day of cultivation, i.e., in the course of 2 days of contact between the fragments and the cancer antiserum, changes in the cells and breaking up of the zone of growth began to be observed. The cells became round, vacuolated, reduced in size and grouped together in small agglomerations rather reminiscent of the phenomenon of agglutination; in some places lysis was observed (Fig. 2).

Thus a cancer antiserum with a relatively high titer of immune antibodies caused severe destructive changes in the cells of the tumor.

In the second series of experiments, carried out in the same way, we tested the action of the serum of a rabbit immune to normal rat's uterus on the zone of growth of the experimental cultures. On the 5th day of the experiment growth was observed in 39 fragments of the 60 in culture. In this case modified cells were also observed in the zone of growth, but not to the same degree as after the action of the cancer antiserum on the cells (Fig. 3). The cells in the zone of growth of these experimental cultures were rounder and smaller in size than in the controls. In isolated places, however, groups of cells with a perfectly viable appearance were encountered, as shown by the preservation of their cell structure, membrane and nucleus. No severe destructive changes were observed in the cells.

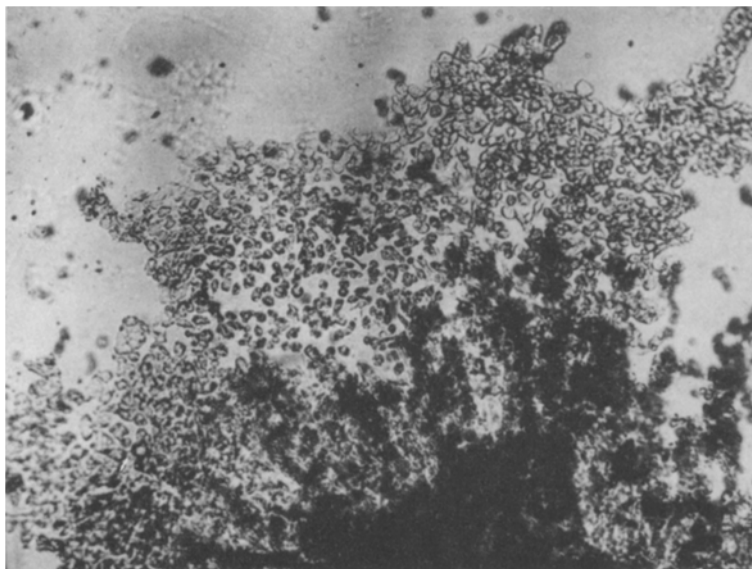


Fig. 3. Zone of growth of a 5-day old culture of Guérin rat carcinoma tissue explanted in the serum of a rabbit immune to normal rat's uterus. Dilution of serum 1:4. Magnification 126. Living culture.

It is clear from the results of these experiments that a cancer antiserum with a high titer of antibodies had a more marked cytotoxic action on the culture of tissue of the corresponding tumor than the serum of a rabbit immunized with tissue of an organ homologous in its origin with the tumor tissue. The results of the experiments described are shown in Table 1.

In the next experiments we tested the action on the tissue cultures of the tumor, of immune sera preliminarily adsorbed on the tumor and on tissue from a rat's uterus. On the 5th day of the experiment, of 100 fragments of tumor tissue cultivated in normal rabbit serum, 75 explantates showed growth, with an average growth coefficient of 1.35 (Table 2). Of 100 fragments of tumor tissue cultivated in cancer antiserum preliminarily adsorbed on tumor tissue, on the 5th day of the experiment growth was observed in 81 cultures, with an average growth coefficient of 1.08. No destructive changes were observed in the cells in the zone of growth of the experimental cultures. Thus the cancer antiserum, after preliminary adsorption on the tumor tissue, no longer contained any antibodies in its composition, for if such had been present, they would have inhibited the growth of the experimental cultures.

TABLE 1

Intensity of Growth of 5-Day Cultures of Guérin Rat Carcinoma Explanted in Sera of Rabbits Immunized with Tumor Tissue or Tissue of Normal Rat's Uterus

Type of rabbit serum	No. of fragments cultivated	No. of fragments showing growth		Morphological picture of the zones of growth	
		in absolute units	in %	epithelial or mixed	destructive
Normal	50	31	62.0	31	0
	65	48	73.8	48	0
Cancer antiserum	170	0	0	0	0
Immune serum to normal rat's uterus	150	0	0	0	0
Normal + cancer antiserum	60	41	68.3	0	41
Normal + immune serum to normal rat's uterus	60	39	65.0	3	Modified form of cells

Of 100 fragments of tumor cultivated in cancer antiserum preliminarily adsorbed in tissue from normal rat's uterus, on the 5th day of the experiment growth was observed in 15 cultures, or 15%. The average growth coefficient of these experimental cultures was 0.15. Of the cultures showing growth, 13 fragments had a zone of growth of epithelial or mixed type, and in two fragments the zone of growth bore traces of destructive changes.

The results of these experiments showed that the normal rat tissue did not adsorb on itself all the immune antibodies present in the cancer antiserum, as was demonstrated by the intensity of growth of the experimental cultures. This was presumably due to the fact that tumor tissue contained antigens which were not present in the tissue of the normal uterus.

TABLE 2

Intensity of Growth of 5-Day Cultures of a Guéren Rat Carcinoma Explanted in Immune Rabbit Sera Preliminarily Adsorbed on Tumor Tissue or Tissue of Normal Rat's Uterus

Type of rabbit serum	No. of fragments cultivated	No. of fragments showing growth			Morphological picture of the zones of growth	
		in absolute units	in %	average growth coeff.	epithelial or mixed	destructive
Normal	100	75	75.0	1.35	75	0
Cancer antiserum adsorbed on tumor tissue	100	81	81.0	1.08	81	0
Cancer antiserum adsorbed on tissue of rat's uterus	100	15	15.0	0.15	13	2
Immune serum to uterus adsorbed on tumor tissue	100	48	48.0	0.83	48	0
Immune serum to uterus adsorbed on tissue of this same organ	100	79	79.0	1.10	79	0

Of 100 fragments of tumor tissue cultivated in rabbit serum (immune to normal rat's uterus) preliminarily adsorbed on tumor tissue, 48 cultures showed growth on the 5th day, with an average growth coefficient of 0.83 (see Table 2). No destructive changes were produced by this serum in the cells of the zone of growth. This serum thus had an inhibitory effect on the growth of the experimental cultures, but this action was weakly expressed and mainly affected the growing fragments. Finally, of 100 fragments of tumor tissue cultivated in rabbit serum (immune to normal rat's uterus), which had been preliminarily adsorbed on tissue from the uterus, on the 5th day of the experiment growth was found in 79 cultures, with an average growth coefficient of 1.11. In other words, this serum had no inhibitory effect whatsoever on the growth of the experimental cultures.

The results of our experiments showed that of the two types of immune sera (cancer antiserum and immune serum to homologous normal tissue), the cancer antiserum possessed the greatest cytotoxic action in tissue culture. The tissue culture method was able to demonstrate the presence of specific cancer antigens in a malignant tumor, which distinguished it in its antigenic properties from the corresponding normal tissues.

These investigations using the method of tissue culture thus confirmed the results which we obtained previously and which were described in our first communication [2].

#### SUMMARY

It is possible to detect the antigenic differences between the normal tissue (uterus) and the homologous in origin tumor tissue (Guéren carcinoma) by the method of tissue culture. Such difference consists in the following: anticancer serum when adsorbed on the tumour tissue does not possess any cytotoxic effect with respect to the cancer cells, while after adsorption of this serum on the normal tissue of the homologous organ, it begins to inhibit the growth of experimental cultures.

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