

RESISTANCE OF THE SPECIFIC ANTIGEN OF HUMAN MALIGNANT  
TUMORS TO A HIGH TEMPERATURE

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The improvement of methods of detection of the specific antigens of human malignant tumors has made it possible to study the physicochemical properties of these antigens. The detailed study of the nature and the physicochemical properties of the specific antigens of malignant tumors is of great interest, but the facts at present known about this subject are extremely limited and controversial. This is because of the complexity of the antigenic structure of both normal and malignant tissue, and also because of the great technical difficulties in the way of their immunological differentiation.

Our method [3, 4, 5] of selective absorption has shown [2] that the specific antigen of human carcinoma is resistant to the action of formalin and glycerol, and does not lose its antigenic and immunogenic properties as a result of such treatment; the tissues of malignant tumors retain the ability to specifically absorb the corresponding antibodies from immune sera, and saline extracts of tissues treated with these substances are active in serological tests and cause antibody formation in rabbits.

The tumor antigens are also resistant to the action of low temperatures and to vacuum drying. In our researches we have constantly used antigens prepared from tumor tissues frozen at  $-20^{\circ}$  or dried by the lyophilic method without thereby observing any decrease in their antigenic activity by comparison with untreated tissues.

Information in the literature regarding the action of a high temperature on the antigenic properties of tumors of man and animals is scanty and indicates merely the disappearance of the antigenic properties of the tumors on heating [1], or their thermostability [7-10 and others]. As has been pointed out above, the contradictory nature of the results obtained by different workers may be attributed to differences and imperfections in the methods used.

In the present research our object was to study the resistance of the specific antigens of human malignant tumors to a high temperature.

## EXPERIMENTAL METHOD

The material used for this study was tissue from malignant tumors (metastases in the liver) of patients dying from cancer. As controls we used tissue from organs (liver, kidney, spleen, heart muscle, lung and brain) from the same patients not affected by tumors, and also spleen tissue from healthy persons killed in accidents.

As antigens we used saline extracts of the crude tissues and of tissues heated for 30 minutes at  $100^{\circ}$ . A suspension of finely minced tissue in physiological saline (1:10) was boiled, then ground again in a mortar and centrifuged. The supernatant fluid was used as antigen. Rabbits were immunized with antigens from the untreated and boiled tumor tissues, and also with antigen from the untreated spleen from the healthy individual.

TABLE 1

Comparative Study of Antigens from Various Human Tissues (untreated and boiled) by Means of the Complement Fixation Reaction

Specific serum	Dilution of serum	Antigens from tissues of patient P													
		untreated							boiled						
		malignant tumor	liver	kidney	spleen	heart muscle	lung	brain	malignant tumor	liver	kidney	spleen	heart muscle	lung	brain
No. 211 to untreated tumor tissue of patient P	1:40	++	++	++	++	++	++	++	+++	++	++	++	++	++	++
	1:80	++	++	++	++	++	++	++	+++	++	++	++	++	++	++
	1:160	++	++	++	++	++	++	++	+++	++	++	++	++	++	++
	1:320	++	++	++	++	++	++	++	+++	++	++	++	++	++	++
No. 834 to untreated spleen tissue of a healthy person	1:40	—	—	—	++	++	++	++	—	—	—	—	—	—	—
	1:80	—	—	—	++	++	++	++	—	—	—	—	—	—	—
	1:160	—	—	—	++	++	++	++	—	—	—	—	—	—	—
	1:320	—	—	—	++	++	++	++	—	—	—	—	—	—	—

Conventional signs: +, ++, +++, +, +, + various degrees of positive reaction; — negative reaction.

The method of obtaining antitumor sera and of freeing them from contaminating antibodies, and also the method of performance of the complement fixation and absorption reactions which we used in our experiments are described in our previous communications [3, 4].

# EXPERIMENTAL RESULTS

In Table 1 we give one of the typical experimental records from the comparative study of antigens from untreated tissues and tissues subjected to boiling, taken from patient P (blood group A), who died from carcinoma of the stomach with metastases in the liver. It will be clear from Table 1 that the specific antitumor serum No. 211, obtained against untreated antigen from the tumor in patient P and free from contaminating antibodies, gave a positive complement fixation reaction not only with antigen from the untreated tumor but also with the extract of tumor tissue subjected to boiling. Antigens from the liver, kidney, spleen, heart muscle, lung and brain of the same patient, whether untreated or boiled, gave no reaction. This is evidence of the presence in the tumor tissue, both before and after heating, of a specific antigenic component which is absent from the investigated organs of the same patient. The organ-specific serum against spleen tissue, used as a control, reacted only with untreated antigen from spleen tissue and gave no reaction with spleen antigen heated for 30 minutes at 100°. It follows from the experiments that the antigen which is specific for human spleen is destroyed by boiling the tissue for 30 minutes, as was previously shown by the work of Kuznetsova [6].

TABLE 2

Investigation of Antigens from Tissues of Patient P in an Absorption Experiment

Specific serum	Tissue used for absorption of serum	Dilution of serum	Antigens from tissue of patient P			
			untreated		boiled	
			malignant tumor	spleen	malignant tumor	spleen
No. 211 to untreated tumor from patient P	—	1:40	++++	—	++++	—
		1:80	++++	—	++++	—
		1:160	+++	—	++	—
		1:320	++	—	±	—
	Boiled spleen from patient P	1:40	++++	—	++++	—
		1:80	++++	—	++++	—
		1:160	++	—	+	—
		1:320	±	—	±	—
	Boiled malignant tumor from patient P	1:40	—	—	—	—
		1:80	—	—	—	—
		1:160	—	—	—	—
		1:320	—	—	—	—
No. 834 to untreated spleen of a healthy person	—	1:40	—	++++	—	—
		1:80	—	+++	—	—
		1:160	—	++	—	—
		1:320	—	±	—	—

Conventional signs as in Table 1.

By means of the complement fixation reaction we thus demonstrated the resistance of the specific antigen of human carcinoma to heating to 100° for 30 minutes, in contrast to the behavior of organ-specific antigens, especially of the spleen, which lose their serological activity under these conditions.

The resistance of the tumor antigen to a high temperature was also confirmed by absorption experiments. The results of the experiments on the absorption of the specific antitumor serum No. 211 with tumor and spleen tissues from patient P, treated by boiling, are given in Table 2.

TABLE 3

Experiment of Absorption of Serum Obtained Against Antigen from a Tumor in Patient P and Heated to 100°

Serum	Tissue used for absorption of serum	Dilution of serum	Antigens from tissue of patient P										Antigen from spleen of a healthy person		
			untreated					boiled							
			malignant tumor	spleen	heart muscle	lung	brain	malignant tumor	spleen	heart muscle	lung	brain			
No. 211 to untreated tumor from patient P	Normal formalinized spleen	1:40	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:80	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:160	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:320	++	++	++	++	++	++	++	++	++	++	++	++	++
No. 97 to boiled tumor from patient P.	Normal formalinized spleen	1:40	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:80	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:160	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:320	++	++	++	++	++	++	++	++	++	++	++	++	++
No. 97 to boiled tumor from patient P.	Mixture of boiled tissues from organs of patient P	1:40	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:80	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:160	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:320	++	++	++	++	++	++	++	++	++	++	++	++	++
No. 97 to boiled tumor from patient P	Boiled malignant tumor from patient P	1:40	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:80	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:160	++	++	++	++	++	++	++	++	++	++	++	++	++
		1:320	++	++	++	++	++	++	++	++	++	++	++	++	++

Conventional signs as in Table 1.

It is clear from Table 2 that the antitumor serum reacted only with antigens from untreated and boiled tumor tissue. Absorption of this serum with boiled spleen did not remove the specific antitumor antibodies from the serum. On the other hand, absorption of this serum with boiled tumor tissue completely deprived it of its ability to react with the tumor antigens.

It follows from these experiments that not only extracts but also tumor cells themselves, when heated to 100°, preserve their specific antigenic properties and, in contrast to spleen cells, can absorb specific antibodies from the serum.

We tested the integrity of the antigenic properties of the tumor after boiling by means of immunization experiments. The results of the serological study of one of the sera (No. 97) obtained by immunization of rabbits with a saline extract from the tumor in patient P, heated for 30 minutes to 100°, are shown in Table 3. For comparison in this experiment we also tested serum No. 211, prepared against untreated antigen from the same tumor. It will be seen from the results shown in Table 3 that antitumor serum No. 97, after removal therefrom of nonspecific antibodies (by absorption with normal formalinized spleen), was indistinguishable in its reaction from serum No. 211 and contained antibodies not only to the antigen from the boiled tumor tissue against which it was produced, but also led to fixation of complement in the presence of antigen from the untreated malignant tumor. Absorption of serum No. 97 with the tissues of patient P (tumor tissue and a mixture of organ tissues), heated to 100°, also revealed no serological differences between this serum and serum No. 211.

These experiments thus showed that antigen prepared from malignant tissue, after boiling for 30 minutes, was capable of causing the formation of specific antibodies when used to immunize animals.

Identical results showing the resistance of the specific antigens of malignant tumors to a high temperature were also obtained during the investigation of material from five other cases of cancer.

The results described in this communication concerning the difference in behavior between specific antigens of malignant tissues and the antigens of normal human organs to the action of a high temperature may be used for the purification and the separation of the tumor antigen in the course of its preparation.

#### SUMMARY

With the aid of immunological methods of investigation it was shown that the sensitivity of cancer tissues and healthy human tissues to the action of high temperature was different. Human cancers contain a specific antigenic component, resistant to boiling for the period of 30 minutes, in contradistinction to the organ specific antigens, destroyed in these conditions.

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