EFFECT OF ANTITUMOR IMMUNE SERUM ON THE LEVEL OF SULFHYDRYL GROUPS IN TUMOR TISSUE

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Tissue sulfhydryl groups play an important role in metabolic processes and especially in oxidation-reduction reactions in an organism. Since the combining of an antibody with a tissue antigen disrupts the normal course of metabolic processes in tissue [1 et al.], it is natural to assume that a change in the level of the sulfhydryl groups in these tissues can, to a certain extent, be an index of the metabolic disorders which occur in them under the effect of cytotoxic sera.

In the available literature we were able to find a work [6] in which it was shown that when antibodies (γ-globulins of immune serum) combine with antigen, the content of free sulfhydryl groups, determined by amperometric titration, increases. The important role of sulfur-containing compounds in tumor metabolism and carcinogenesis has been demonstrated in a number of works [2, 3, 5]. One of us [4] established that disruption of metabolism in tissues of transplanted tumors by alkylating antitumor substances lowers the level of sulfhydryl groups in them. In the present work we studied the change in the level of SH-groups in tumors of rats injected with antitumor serum.

EXPERIMENTAL

The antitumor serum was obtained from rabbits which were immunized with a 10% salt extract of Guerin carcinoma injected intravenously every other day for a total of 4 injections in amounts 0.5, 1, 1.5, and 2 ml each time. The activity of the serum was determined in the complement fixation test seven days after the last injection of antigen. In the case of a sufficiently high titer (complete inhibition of hemolysis at a dilution of 1:200 and higher) the rabbits were exsanguinated. The experiments were carried out on 84 rats into whom we transplanted a 20% suspension of Guerin carcinoma.

At various periods after transplantation, depending on the nature of the experiment, we injected once or repeatedly the antitumor serum. At the time of the investigation the rats were killed and 100 mg of tumor tissue was extracted and freed from necrotic areas and was homogenized in a physiological salt solution in the cold, using a Potter homogenizer for this purpose. Then the homogenate was centrifuged for 5 min at 1500 rpm. In the supernatant thus obtained we determined, by the method of amperometric mercurimetric titration in tris-buffer, the total SH-groups which contained the SH-groups of reduced glutathione and water-soluble proteins. In all investigations the conditions of homogenization and obtainment of the supernatant were rigorously identical.

The results of each series of experiments were compared with the data obtained with the control animals. The variations in the level of the SH-groups of the supernatant of the tumor homogenate, in the control investigations of the various series, were due to using the animals at different times after transplantation. As was established earlier by one of us [4], the level of the SH-groups in Guerin carcinoma increases as the tumor grows. The obtained results were expressed in micromoles of sulfhydryl groups per 100 mg of fresh tumor weight and were subjected to variance analysis.

TABLE 1. Change in the Content of Sulfhydryl Groups in Guerin Carcinoma Under the Effect of an Injection of Antitumor Serum (M ± m)

Experimental conditions (serum dose 0.5 ml)	Content of SH-groups (in μmoles per 100 mg of tumor tissue)	
	experimental rats	control rats
Single injection of antitumor serum	0.358 ±0.034	0.236 ±0.037
Single injection of normal serum	0.252 ±0.030	0.200 ±0.050
Twofold injection of antitumor serum	0.270 ±0.087	0.287 ±0.038
Sixfold injection of antitumor serum	0.231 ±0.014	0.232 ±0.014

TABLE 2. Change in the Content of Sulfhydryl Groups in Guerin Carcinoma and Blood Serum of Rats Under the Effect of Different Doses of Antitumor Serum (M ±m)

Experimental conditions	Content of SH-groups (in μmoles per 100 mg)		
	in the tumor	in liver	in blood serum
Control rats	0.357 ±0.013	0.9 ±0.037	41.2 ±2.39
Single injection of antitumor serum (dose 0.5 ml)	0.475 ±0.017	1.24 ±0.056	38.2 ±2.04
Single injection of antigimor serum (dose 0.01 ml)	0.506 ±0.024	1.081 ±0.067	33.4 ±3.5

RESULTS

At first we studied the effect of a single injection of 0.5 ml of antitumor serum on the content of the SH-groups in Guerin carcinoma (Table 1). It was established that 2 h after injecting the rats with the serum the content of the SH-groups in the tumor tissue increases 52% ($P < 0.001$).

To exclude the nonspecific action of the proteins of the immune serum on the content of SH-groups in the tumor, we investigated the effect of normal serum injected in the same dose and under the same experimental conditions as the antitumor serum (see Table 1). Here we also noted a certain increase in the content of the SH-groups in the tumor tissue, but it was almost half that observed with the injection of the antitumor serum. These data indicate that the increase in the content of SH-groups can also be associated with the specific action of antitumor cytotoxins on tumor tissue.

To confirm the specificity of action of the antitumor serum on the level of SH-groups in the tumors, the serum was injected into the rats not only in a dose of 0.5 ml but also in a dose of 0.01 ml. Two hours after the injection of the serum in this dose we investigated the content of sulfhydryl groups in the tumor tissue, liver and blood serum. The level of the SH-groups in the blood serum was expressed in micromoles per 100ml of serum. In this case it was established (Table 2) that small doses of the antitumor serum (0.01 ml) increase the content of sulfhydryl groups somewhat more pronouncedly than do the large doses (0.5 m). The opposite picture was observed in the liver: large doses of antitumor serum led to an increase of the content of SH-groups in the liver tissue to a greater extent than did small doses. The difference in the content of SH-groups in the tumor and liver of the control and experimental animals was statistically significant ($P < 0.001$).

It seems to us that the data obtained confirm the specificity of action of the antitumor sera on the content of SH-groups in tumor tissue, since a high positive effect can be obtained from a small dose. At the same time, the changes in the content of the SH-groups which we observed in the liver upon injecting the antitumor serum can, in all probability, be explained by the fact that the liver renders harmless and inactivates the heterogeneous proteins injected into the organism. The changes in the level of the SH-groups in the blood serum of rats with transplanted tumors under the effect of various doses of antitumor serum were statistically insignificant. It was further established that neither the twofold nor the sixfold intravenous injection of antitumor serum in a dose of 0.5 ml, which caused 12-47% inhibition of tumor growth, substantially changed the level of the SH-groups in the tumor tissue (see Table 1).

TABLE 3. Effect of Antitumor Serum on Sulfhydryl Groups of Guerin Carcinoma in an in vitro Experiment

No. of experiment	Antitumor serum			Normal serum	
	serum titers	level of SH-groups (in μmoles per 100 ml of centrifugate)		level of SH-groups (in μmoles per 100 ml of centrifugate)	
		before incubation	after incubation	before incubation	after incubation
1	1:200	56.2	47.5	62.5	61.2
2	1:400	63.7	55.0	51.2	50.0
3	1:320	57.6	45.0	50.0	50.0
4	1:400	60.0	55.0	52.5	48.0
5	1:320	62.5	55.0	38.7	37.5
6	1:400	50.0	48.7	50.0	50.0
7	1:320	52.5	37.5	–	–
8	1:200	52.5	45.0	–	–
9	1:200	56.2	50.0	–	–
10	1:640	50.0	47.5	–	–
11	1:200	50.0	32.5	–	–
	M ± m	55.5 ±1.7	47.1 ±2.16	50.8 ±3.1	48.9 ±3.3

Finally, we set up experiments to elicit the effect of antitumor serum on Guerin carcinoma in vitro. The tumor-tissue homogenate (100 mg) was incubated for 2 h in a thermostat at 37° with antitumor and normal serum. The sulfhydryl groups were determined in the supernatant by the amperometric titration method. The results were expressed in micromoles per 100 ml of supernatant.

As a result of these investigations (Table 3) it was established that in this experimental setup the antitumor serum lowers the content of SH-groups in tumor tissue by 15% on the average ($P < 0.001$). Under the given experimental conditions, the normal serum had practically no effect on the level of SH-groups in the tumor homogenate. We did not note a direct relation between the titer of the antitumor cytotoxins and the degree of lowering the level of SH-groups. We assume that a single injection of antitumor serum, especially in small doses (0.01 ml), causes a marked stimulation of metabolic processes, which is evidenced in the rise of the level of the SH-groups in the tumor tissue. Upon increase in the concentration of antitumor antibodies in the tumor tissue (an increase in the quantity of antitumor serum injected into the rats with transplanted Guerin carcinoma) or in the in vitro experiments, the increase in the level of the SH-groups in the tumor tissue disappears and a decrease in their level is observed: This, it seems to us, is due to disruption of metabolic processes caused by the effect of the antitumor serum.

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