

# EFFECT OF VARIOUS METHODS OF ADMINISTRATION OF ANTITUMOR SERUM ON MITOTIC ACTIVITY AND DNA CONTENT IN TUMOR CELLS

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The mitotic activity and DNA content in cell nuclei of an Ehrlich's subcutaneous adenocarcinoma in line A mice are reduced by injection of antitumor serum. This effect is more marked if the serum is injected repeatedly (in the same total volume as when given as a single dose).

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It has been shown that the mitotic activity of tumors is depressed by antitumor sera [2-5], and also that by repeated injections of such sera the mitotic activity of malignant cells can be maintained at a low level throughout the period of injection [6].

In the present investigation the mitotic activity of cells of a subcutaneous Ehrlich's adenocarcinoma was studied in mice receiving the same total dose of antitumor serum but under different conditions. The optical activity (extinction) of interkinetic nuclei, reflecting their DNA content, was also determined.

## EXPERIMENTAL METHOD

To obtain the sera used in the experiments on mice, rabbits were immunized intravenously with extract of ascites cells of an Ehrlich's adenocarcinoma. These sera reacted in the complement fixation test at 37° with antigen from the same tumor in a dilution of 1:400 (+++) and in lower dilutions with spleen and liver antigens.

Experiments were carried out on 42 male line A mice which were divided into 4 groups on the 7th day after subcutaneous inoculation of an Ehrlich's adenocarcinoma in the dorsal region, when the tumor had become palpable. The mice of groups 1, 2, and 3 received injections of the serum obtained by immunization, subcutaneously in the dorsal region as follows: group 1, 1 injection of 2 ml; group 2, 10 daily injections, each of 0.2 ml; group 3, 4 injections, each of 0.5 ml, at intervals of 3 days. The mice of group 4 received no serum (control).

All the experimental animals were sacrificed on the 17th day after inoculation of Ehrlich's adenocarcinoma. The tumors were fixed in Carnoy's fluid and stained by Feulgen's method. The criterion of mitotic activity was the mitotic index (MI) expressed in promille. The DNA content in the interkinetic nuclei of the tumor cells was estimated by determining their optical density (extinction) in the MF-2 microphotometer with cytophotometric attachment [1]\*. Statistical analysis of the numerical results was carried out by the Fisher-Student method (Table 1).

## EXPERIMENTAL RESULTS

The ten injections of antitumor serum given to the animals of group 2 inhibited subsequent growth of the tumor in half these animals, so that it was virtually impossible to count mitoses. Inhibition of mitotic activity in the tumors of the remaining 5 mice of this group (MI 8.014%) by comparison with the control (MI 12.75%) was close to significance ( $P = 0.04$ ).

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TABLE 1. Mitotic Index (in %) and DNA Content in Subcutaneous Ehrlich's Adenocarcinoma after Injection of Antitumor Sera

Mouse No.	Group 1		Group 2		Group 3		Group 4	
	scheme of administration of antitumor sera						control	
	1 ml x 2		0.2 ml x 10		0.5 ml x 4			
	MI	DNA	MI	DNA	MI	DNA	MI	DNA
1	6.522	0.2254	6.083	0.2515	3.277	0.2544	7.435	0.4056
2	6.876	0.2552	6.571	0.2304	4.063	0.2357	7.582	0.3565
3	9.620	0.2889	8.657	0.2984	5.426	0.2283	9.646	0.4043
4	10.337	0.2207	9.279	0.2368	5.820	0.2012	9.681	0.3324
5	10.440	0.2107	9.484	0.2616	6.282	0.1580	11.626	0.3188
6	10.577	0.1982	— <sup>1</sup>	—	7.225	0.2563	14.814	0.2963
7	11.498	0.2398	—	—	7.357	0.2111	16.198	0.3502
8	11.799	0.2412	—	—	7.363	0.2495	16.531	0.3660
9	11.935	0.2470	—	—	8.211	0.3002	21.223	0.3316
10	12.407	0.2625	—	—	9.324	0.2476	Mice Nos. 10, 11, and 12 were destroyed.	
Mean	10.201	0.23896	8.014	0.2563	6.435	0.23423	12.748	0.3513

<sup>1</sup>Here and in the other columns a minus sign (—) means that the tumor did not develop.

Four injections of serum into the mice of group 3 caused significant inhibition of mitotic activity in the tumors (MI 6.435%) compared with the control indices ( $P = 0.002$ ) and in group 1 ( $P = 0.005$ ), when a single injection of serum the day before sacrifice caused only a slight decrease in mitotic activity (MI 10.2%).

After injection of the antitumor serum inhibition of mitotic activity of the tumor cells was accompanied by a decrease in DNA content in their interkinetic nuclei, the optical density of which was significantly lower than in the control ( $P < 0.001$ ). This was also observed in tumors of the animals of group 1, which showed only an ill-defined tendency for the level of their mitotic activity to fall by comparison with the control.

The results show that inhibition of mitotic activity of malignant cells produced by antitumor sera depends to some extent on the mode of administration of the sera (dose, frequency of injections, and so on) and they also indicate that the decrease in DNA content in the interkinetic nuclei of the tumor cells runs parallel to the decrease in number of mitoses in the tumor.

#### LITERATURE CITED

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