undergone prolonged passage . Naturally CC-32 can be used for research in the experimental diagnosis and treatment of human uterine cervical carcinoma.

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A SIMPLE METHOD OF TESTING SENSITIVITY OF HUMAN TUMORS TO ANTITUMOR AGENTS IN VIVO

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The result of testing hemotherapeutic preparations for their efficacy on human tumors transplanted into nude mice [4] and into mice with immunodepression [11] is in accordance with clinical observations. It has also been found that the treatment of experimental tumors in animals often does not give the same effect in man [10]. To assess the activity of antitumor preparations, the method of implantation of human tumors beneath the renal capsule (RC) of nude mice is used, but the necessity of creating special conditions for the keeping of such animals limits their usefulness. To conduct a similar investigation on mice with temporary immunodeficiency (ID) it is advantageous to use human tumors, such as the nonpigmented melanoma BRO [6], which proliferates rapidly after implantation into nude mice [8] and mice with ID [1, 2, 9]. It has been shown that BRO cells, embedded in a fibrin clot (FC), grow rapidly and remain viable in normal mice previously subjected to whole-body irradiation in a dose of 5.5 or 6.5 Gy [3].

In the investigation described below the conditions for creating optimal ID for growth of human melanoma BRO and for testing the sensitivity of the tumor to cytostatics on this model were determined.

EXPERIMENTAL METHOD

BRO cells were cultured in medium RPMI with 10% embryonic serum and embedded in FC, as described previously [3]. Female (CDF × C57BL/6)F_1 hybrids aged 2-4 months, obtained from the "Stolbovaya" nursery, were used as recipients Whole-body irradiation of the animals was carried out on a ^{137}Cs source (dose rate 0.087 Gy/sec) 24 h before subcapsular transplantation of the tumor. The operation for implantation of FC beneath RC was carried out as described previously [3] and two mutually perpendicular diameters were measured on the day of transplantation and 8 days thereafter, when the mice were killed by cervical dislocation. The antitumor agents were injected intraperitoneally 3 days after

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TABLE 1. Toxicity of Antitumor Preparations in Intact and Irradiated Mice

Serial No.	Adriamycin			Vincristine			Biocarbazine			5-Fluorouracil			Cyclophosphamide		
	dose of preparation, mg/kg	irradiation, Gy	mean change in body weight, %	dose of preparation, mg/kg	irradiation, Gy	mean change in body weight, %	dose of preparation, mg/kg	irradiation, Gy	mean change in body weight, %	dose of preparation, mg/kg	irradiation, Gy	mean change in body weight, %	irradiation, Gy	dose of preparation, mg/kg	mean change in body weight, %
1	—	—	+0,9	—	—	+6,6	—	—	+1,0	—	—	+0,9	0	—	+9,0
2	—	5,5	+4,8	—	5,5	0	—	4,5	+4,4	80	—	—4,0	150	—	+1,5
3	5	—	+1,1	0,5	—	+9,0	—	5,5	—1,9	80	4,5	—6,4	150	4,5	—1,5
4	5	4,5	+3,4	0,5	5,5	+2,7	200	—	—1,8	80	5,5	—14,8	150	5,5	—3,8
5	5	5,5	0	1,0	—	+8,3	200	4,5	+2,1	120	—	—0,6	200	—	+1,8
6	10	—4,0	1,0	5,5	5,5	+3,6	200	5,5	—2,1	120	4,5	—7,4	200	4,5	0
7	10	4,5	—1,5	1,5	—	+1,7	250	—	+2,0	120	5,5	—15,2	200	5,5	—4,2
8	10	5,5	—11,2 (3/18)	1,5	4,5	—1,5	250	4,5	—2,0			(2/10)			
9				1,5	5,5	—1,9	250	5,5	—6,0						
10															
11				3,0	—5,3	—5,3									
12				3,0	4,5	—4,7									
13				3,0	5,5	—4,5									

Legend. Mean change in body weight was determined 9 days after irradiation and 6 days after injection of preparations. Ratio of number of dying animals to number of animals in group given in parentheses.

irradiation. Each group consisted of 3-5 mice. Each experiment was repeated twice. Histological sections were obtained by standard methods and stained with hematoxylin and eosin. Numerical results were subjected to statistical analysis by the U test.

EXPERIMENTAL RESULTS

When the recipient mice were irradiated in a dose of 3 Gy or less, the BRO tumors in FC were no larger on the 8th day after implantation than on the 6th day, probably indicating rejection of the tumor, which began on account of inadequate ID in the mice. After irradiation in a dose of 4.5 Gy, growth of the tumor did not differ from that after doses of 5.5 or 6.5 Gy.

As was described previously for mice irradiated in doses of 5.5 or 6.5 Gy [3], histological investigation of animals irradiated in a dose of 4.5 Gy showed that on the 8th day the tumor cells in the growing transplant remained viable. The cytoplasm of these cells was weakly basophilic, and the cells were branched and joined together. Lymphoid infiltration around the tumor was inhibited. A large concentration of macrophages and lymphocytes was observed only in parts of the capsule and kidney tissue remote from the tumor.

Compared with exposure to a dose of 4.5 Gy, mice irradiated in a dose of 3.0 Gy showed disorganization of the tumor tissue on the 8th day. Single tumor cells with basophilic cytoplasm and ill-defined nuclei could be seen among the macrophages, lymphocytes, and other connective-tissue cells; lymphoid infiltration around the tumor was moderate.

Before the beginning of the chemotherapeutic experiments a comparative study was made of the toxicity of several known antitumor agents for intact mice and for animals irradiated in a dose of 4.5 or 5.5 Gy (Table 1). The results showed that irradiation in a dose of 4.5 Gy had virtually no effect on the toxicity of the cytostatics. Meanwhile, after irradiation of the mice in a dose of 5.5 Gy the toxicity of two of the preparations studied, namely adriamycin and 5-fluorouracil, was significantly higher than in intact mice and in animals irradiated in a dose of 4.5 Gy.

The study continued on mice irradiated in a dose of 4.5 Gy. The efficacy of the antitumor preparations was assessed by comparing the size of the tumors on the 8th day after transplantation with their original size. A dose-dependent effect on tumor growth was demonstrated by the use of biocarbazine (dacarbazine (DTIC; Fig. 1) and 5-fluorouracil (Fig. 2). The antitumor efficacy of other cytostatics is shown in Table 2).

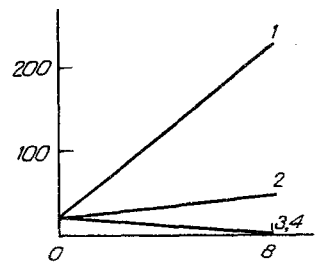

Fig. 1

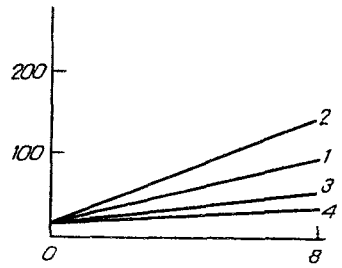

Fig. 2

Fig. 1. Growth of BRO cells beneath RC in intact mice (1) and in mice treated with biocarbazine in doses of 150 mg/kg (2), 200 mg/kg (3), and 250 mg/kg (4). Differences between groups 3 and 4 and control are significant ($p < 0.02$). Four mice were used in each group. Here and in Fig 2: abscissa, time after implantation of tumor (in days); ordinate, relative volume of tumor (in relative units).

Fig. 2. Growth of BRO cells beneath RC in intact mice (1) and mice treated with 5-fluorouracil in doses of 40 mg/kg (2), 80 mg/kg (3), and 120 mg/kg (4). Difference between group 4 and control significant ($p < 0.02$). Five mice were used in each group.

TABLE 2. Effect of Antitumor Preparations on Growth of BRO Tumor Beneath RC

Preparation	Dose, mg/kg	Change in volume of tumor compared with control
Methotrexate	80	1,31
	120	0,52
Vinblastine	1,5	1,43
	3,0	0,87
	4,5	0,71
Cyclophosphamide	150	0,02*
	200	0,00*
	250	0,00*
Adriamycin	5	0,88
	10	0,82
Vincristine	3	0,11*
Platidiam	12	0,31

Legend. *$p < 0.05$: Significant differences between experimental and control groups.

The results show that this model satisfies the three chief criteria essential for in vivo testing of sensitivity of human tumors to antitumor preparations. First, irradiation of the recipient mice in a dose of 4.5 Gy guarantees adequate ID, which permits the BRO cells to remain viable after subcapsular implantation, and to grow rapidly for 8 days. It was shown previously that single irradiation of normal mice allows rapid growth of BRO melanoma cells after their intraperitoneal injection also [2].

Second, a single irradiation of female F_1 hybrids in a dose of 4.5 Gy does not increase the toxicity of the antitumor agents when administered in the doses normally used in chemotherapeutic research. Third, the antitumor effect of known preparations studied on this model depends on the concrete preparation and on its dose.

Moreover, growth of the tumor in FC, which requires only a few tumor cells grown in vitro, rules out the necessity for using nude animals or mice with ID as the source of the tumor.

Cyclophosphamide and biocarbazine were found to be the most effective of the preparations studied When they were used the weight of the tumor was reduced by more than 99% compared with tumors in untreated mice. These observations are in agreement with results obtained in other types of testing of the sensitivity of the BRO tumor to antitumor preparations. In the list of known antitumor preparations (excluding biocarbazine) cytophosphamide is most effective against the BRO tumor after intraperitoneal transplantation [7] and implantation beneath RC of nude mice [4], Biocarbazine also was found to be just as highly active against a BRO tumor growing subcutaneously in nude mice (data not given) Biocarbazine is known as one of a few preparations that are effective in clinical practice against widespread melanoma [5].

To assess the efficacy of antitumor preparations against human tumors implanted into animals, it is logical to select criteria of activity which come close to those used in clinical practice, such as disappearance of the tumor (complete remission) or a sharp decrease in its size (partial remission). By this criterion, cyclophosphamide and biocarbazine can be classed as preparations active against BRO melanoma whereas vincristine and 5-fluorouracil are moderately active preparations.

In the present experiments the various antitumor preparations were injected once only. However, in an experiment lasting 8 days after implantation of the tumor, the efficacy of repeated injections of the same preparation or of a combination of drugs can be evaluated This is yet another advantage of the proposed model for testing.

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