THE DELIBERATE CULTIVATION OF ANTITUMOR PROPERTIES IN BACTERIA*

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(Received June 30, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Data published in the literature in recent years have shown beyond doubt the fact that certain microorganisms exist in nature, the products of whose vital activity have a selective action on the malignant tumors of animals and man [2, 3].

The search for such microorganisms is made much more difficult, however, by the lack of any simple and speedy method of selection of producers of antitumor substances [4].

In the present work we have attempted, by taking advantage of the powers of adaptation of microorganisms, to seek another means of developing antitumor properties in bacteria whose initial, naturally occurring strains do not possess such properties [1, 5, 6].

EXPERIMENTAL METHOD

We used strains of proteolytic bacteria – Proteus vulgaris (isolated from soil) and Bacillus pyocyaneus (isolated from pus). A pure culture was transferred by means of a sterile loop from a solid nutrient medium (Endo's) to a flask containing 200 ml of sterile physiological saline. As a source of nitrogen for the proteolytic bacteria, to the flask was added 0.7-1.0 g of freshly minced tissue from a Crocker's mouse sarcoma, washed twice with sterile physiological saline. The flask was incubated at 37°. After 48 hours the culture was used to seed another flask, perpared the previous day by the same method. From each culture 90 such subcultures were taken. By means of a Seitz filter (disk SF) filtrates of 7-day cultures were obtained and these were used in the experiment.

The experimental animals were white mice. Using a needle with a stilet, small fragments of a Crocker's sarcoma were implanted beneath the skin of the dorsal region of the animals. Each strain of microorganisms was tested on 110 animals.

Sterile filtrate of one of the cultures, in a volume of 0.5 ml, was injected into the animals of the first experimental group on the day of transplantation of the tumor, and into the animals of the second group 5 days after transplantation of the tumor. Filtrate was injected three times into the animals of the third group: on the 1st day after transplantation (1 ml), on the 5th day (0.5 ml) and on the 10th day (0.3 ml). The animals of three control groups were injected with sterile filtrate of a 7-day infusion of tumor tissue in sterile physiological saline; the animals of the first group – on the day of transplantation of the tumor (0.5 ml), of the second group – 5 days after transplantation of the tumor (0.5 ml) and those of the third group – three times: on the 1st day (1 ml), on the 5th day (0.5 ml) and on the 10th day (0.3 ml). The fourth control group of animals were injected with a sterile 7-day filtrate of the original strain of one of the cultures (on the 1st, 5th and 10th days, in doses of 1.0, 0.5 and 0.3 ml respectively); finally, a fifth group was injected with sterile physiological saline. The times and doses were the same as for the fourth group.

* The authors express their deep gratitude to Prof. A. A. Sinitskii, Corresponding Member AMN SSSR M. M. Maevskii and Honored Scientist Prof. P. N. Kashkin, who acted as scientific consultants in the performance of this research.

TABLE 1

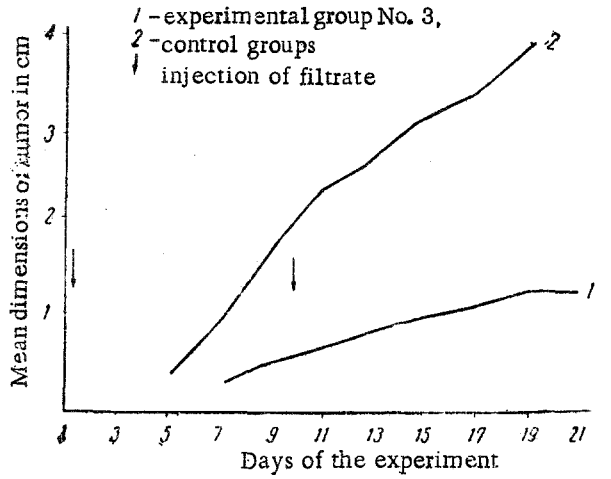
The Effect of Filtrate of a Test Strain of Proteus vulgaris on the Development of a Crocker's Sarcoma in Mice

Group of mice		Number of mice in group		Tumors completely absorbed	
experiment	control	experiment	control	experiment	control
First	First	20	10	5	—
Second	Second	20	10	2	—
Third	Third, fourth, and fifth	20	30	11	—
	Total	60	50	18	

TABLE 2

The Effect of Filtrate of a Test Strain of Bacillus pyocyaneus on the Development of a Crocker's Sarcoma in Mice

Group of mice		Number of mice in group		Tumors completely absorbed	
experiment	control	experiment	control	experiment	control
First	First	20	10	6	—
Second	Second	20	10	3	—
Third	Third, fourth and fifth	20	30	15	
	Total	60	50	24	—



The effect of filtrate of the test strain of Proteus vulgaris on the development and growth of a Crocker's sarcoma in mice. 1) experimental group No. 3, 2) control groups, ↓– injection of filtrate.

TABLE 3

The Effect of Filtrate of a Deliberately Cultivated Strain of B. pyocyaneus on the Success of Transplantation of a Crocker's Sarcoma in Mice

Material used for preparation of the tumor suspension	Number of mice		
	altogether in experiment	with tumor	without tumor
Sterile filtrate of deliberately cultivated strain of B. pyocyaneus	10	—	10
Sterile filtrate of original strain of B. pyocyaneus (control)	10	10	—
Sterile physiological saline (control)	10	10	—

TABLE 4

Loss of Antitumor Activity of a Filtrate of the Test Strain of B. pyocyaneus after Contact for 8 Hours with Cells of a Crocker's Sarcoma in Mice

Material used for preparation of the tumor suspension	Number of mice		
	altogether in experiment	with tumor	without tumor
Sterile filtrate of test strain of B. pyocyaneus after contact with tumor for 8 hours	10	9	1
Sterile filtrate of test strain of 7-day culture of B. pyocyaneus (control)	10	0	10
Sterile filtrate of test strain of B. pyocyaneus after contact with cardiac muscle for 8 hours (control)	10	7	3
Sterile physiological saline (control)	10	10	—

EXPERIMENTAL RESULTS

In the experiments using filtrates of the test strain of Proteus vulgaris, 36 mice of the experimental groups survived for 50 days from the beginning of the experiment, and in 18 of these animals the tumors were completely absorbed (Table 1). In none of the control groups was absorption of the tumors observed. The best results were obtained in the third group, after three injections of filtrate. Of 20 mice in this group, 16 were still alive after 50 days, and only 5 of these had tumors of small dimensions. Differences were also noted in the times of appearance and in the dimensions of the tumors in the animals of the experimental and control groups. In the mice of the control groups the tumors as a rule, could be palpated as small granules at the site of implantation on the 5th-7th day after inoculation of the sarcoma, and they reached their largest size on the 20th-25th day. In the experimental groups receiving three injections of filtrate, the developing tumors reached an appreciable size only at the end of the 2nd week, i.e., the time of manifestation of the tumor was delayed approximately 2-3 times. These tumors were also much smaller than those observed in the animals of the control groups (see figure).

Experiments using filtrate of the test animals of Bacillus pyocyaneus gave analogous results (Table 2).

In order to obtain data to confirm the selectivity of action of the culture filtrates on tumor cells in mice in vivo, 2 experiments were carried out.

The first experiment was conducted on 10 white mice into which a Crocker's sarcoma was implanted subcutaneously in the dorsal region in the form of a tumor suspension in sterile filtrate of a 7-day culture of the test strain of B. pyocyaneus. As controls we used mice into which was implanted a tumor suspension in sterile physiological saline and in sterile filtrate of the original strain of the 7-day culture. The results of the experiments were read on the 10th day.

It is apparent from Table 3 that no tumor development was observed in the experimental group of mice, whereas in the controls, the original strain did not produce a fall in the percentage of successful transplantation of the tumor. This indicates the presence of a direct action of the filtrate of test strain of B. pyocyaneus on the sarcoma cells, depriving them of their power of transplantation.

In the second experiment, minced Crocker sarcoma tissue was placed in sterile filtrate of the test strain of B. pyocyaneus, and left for 8 hours at a temperature of +4°, with uniform mixing. The filtrate was then freed from tumor particles by contrifugation, and in it was prepared a suspension of fresh tumor tissue of a sarcoma destined for transplantation into the animals of the experimental group. The control animals were injected with a tumor suspension prepared as indicated in Table 4.

On the 15th day of observation it was found that the filtrate of the test culture produced practically no fall in the percentage of successful transplantation after preliminary contact with tumor tissue. Evidently during the time of contact, a selective adsorption by the tumor cells of the active antitumor principle contained in the culture filtrate took place.

SUMMARY

Experiments were performed with the cultures of Proteus vulgaris and Bacillus pyocyaneus, the initial strains of which did not posses natural antitumor properties.

These bacteria were cultured for a long period in a medium in which Crocker's sarcoma cells formed the source of nutritive substances. This resulted in the appearance of certain substances among the metabolites of the bacteria able to selectively depress the development and the growth of Crocker's sarcoma in mice.

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