A COMPARATIVE STUDY OF THE ACTION OF A LIVING CULTURE OF BACILLUS PRODIGIOSUS (SERRATIA MARCESCENS) AND ITS PRODUCTS ON THE GROWTH OF A BROWN-PEARCE TUMOR IN RABBITS

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From sources in the literature we know that certain bacterial toxins (filtrates and lysates of bacterial cultures) and their polysaccharide-containing fractions have the power to depress the growth of malignant tumors in animals, or even of causing their complete absorption. It has been shown, in particular, that a bacterial fraction (Shear's "polysaccharide"), isolated from a culture of Bacillus prodigiosus, causes lasting hemorrhagic changes and necrosis in muscle sarcomas and other tumors [7, 11, 13, 14, 16-19]. Physicochemical methods have demonstrated that such fractions are not only polysaccharide in nature, but consist of a lipo-proteino-polysaccharide complex or of bacterial endotoxin, possessing well-defined antigenic properties [5, 6, 8, 10, 12, 15].

The problem of the antitumor activity of a living culture of B. prodigiosus has received comparatively little study. In isolated researches [4], and also in our own investigations [1, 2, 3], it has been shown that a living culture of this microorganism sharply depresses the growth of sarcomas of rats and, in the majority of cases, leads to the absorption of Brown-Pearce carcinoma in rabbits. Certain authors [9], however, consider that a bacterial infection produced by administration of B. prodigiosum, has no therapeutic influence on the growth of tumors in mice. According to their findings, it is not the living culture but its toxins or bacterial "polysaccharides" which possesses antitumor activity.

In this connection it was of interest to make a comparative study of the effect of living and killed cultures of B. prodigiosus, and also of its "polysaccharide" fraction, on the development of Brown-Pearce carcinoma in rabbits.

METHOD

Experiments were carried out on sexually mature male chinchilla rabbits. The animals, which were divided into five equal groups (with five rabbits in each group), were inoculated intratesticularly with Brown-Pearce carcinoma in a dose of 0.5 ml of a 20% tumor suspension. The "polysaccharide" fraction was obtained from a culture of B. prodigiosus by the method of Perrault and Shear and of Savage [13, 18]. It contained 32.9% of protein as nitrogen and 59.3% of reducing substances, calculated as glucose. The preparation for testing possessed high toxicity towards laboratory animals. After the rabbits had been immunized with this fraction, their blood showed the presence of specific antibacterial agglutinins (against B. prodigiosus), evidence of the antigenic properties of this preparation. The bacterial endoxin used was also a culture of B. prodigiosus, which was killed by heating to a temperature of 60° for $1\frac{1}{2}$ hours. An emulsion of a living culture of this microoganism was prepared in sterile physiological saline 15-20 minutes before the beginning of its injection.

Treatment with the bacterial preparations began on the tenth day after inoculation of the tumor. The rabbits of the first group were given intravenous injections of living B. prodigiosus cells in a dose of 800 million bacterial cells per kg body weight. The rabbits of the second group were treated in the same way, but with a killed culture. The animals of the third and fourth groups received corresponding injections of "polysaccharide" fraction in a dose of 0.3-0.03 mg/kg body weight, also intravenously. Altogether, each animal received seven injections at intervals of four days. The rabbits of the fifth group acted as controls.

In the second series of experiments, 20 rabbits were used, and these animals were inoculated with Brown-Pearce tumor into the right testicle, in a dose of 0.75 ml of a 20% suspension. Six days later, the testicle together with the tumor nodule was removed. On the third day after operation, ten rabbits (experimental group) were injected intravenously with a living culture of B. prodigiosus in a dose of 900 million bacterial cells per kg body weight. The remaining injections were given intraperitoneally in a dose of 2×10^9 bacterial cells per kg body weight. Seven injections were given altogether, five at intervals of three days, and the last two at intervals of ten days. Control rabbits received injections of physiological saline along similar lines.

The efficacy of the antitumor action of the bacterial preparations was assessed by two indices: 1) The

TABLE 1. Study of the Action of Living and Killed Cultures of B. prodigiosus and of Their Fractions on a Brown-Pearce Tumor

Group of rabbits	Preparations injected	No. of rabbits used in experiment	Outcome of the tumor: number of rabbits dying: up to 4th day	up to the 80th day	total	sacrificed after 3 months	length of survival (in days)
First	Living microorganisms . .	5	—	1	1	4	91
Second	Killed microorganisms . .	5	3	1	4	1	59
Third	Fraction (maximum dose)	5	2	1	3	—	41
Fourth	Fraction (minimum dose)	5	3	1	4	1	55
Fifth	Control	5	5	—	5	—	32

Note: In assessing the results of the experiment we ignored two rabbits (of the third group) which died after the first injection of the "polysaccharide" fraction.

TABLE 2. Results of Counts of the Metastases in the Organs of Rabbits after the Action of a Living Culture of B. prodigiosus and its Products on a Brown-Pearce Tumor

Group of rabbits	Preparations injected	No. of rabbits used in the experiment	Outcome of the tumor: number of rabbits: dying	surviving	metastases: present	absent	number of metastases: total	per rabbit	number of organs affected: total	per rabbit
First	Living microorganisms.	5	1	4	4	1	46	11,5	17	4,3
Second	Killed micro. . .	5	4	1	5	—	672	134,4	42	8,4
Third	Fraction (maximum dose) . .	5	3	—	3	—	1 517	505,6	29	9,6
Fourth	Fraction (minimum dose) . . .	5	4	1	5	—	675	135,0	31	6,2
Fifth	Control	5	5	—	5	—	2 394	478,8	60	12

Note: In assessing the results of the experiment we ignored two rabbits (of the third group) which died after the first injection of the subtoxic dose of the "polysaccharide" fraction.

TABLE 3. Effect of a Living Culture of B. prodigiosum on the Survival of Rabbits Inoculated with Brown-Pearce Carcinoma after Removal of the Primary Tumor

Group of rabbits	No. of rabbits used in the expt.	Outcome of the tumor: number of rabbits dying: up to the 35th day	up to the 80th day	total	sacrificed after three months	survival rate, %	mean length of survival (days)
Experimental	10	1	2	3	7	70	80,6
Control	10	7	1	8	2	20	46,8

TABLE 4. The Effect of a Living Culture of B. prodigiosus on Metastases of a Brown-Pearce Carcinoma after Surgical Removal of the Primary Tumor

Group of rabbits	No. of rabbits used in the expt.	Outcome of the tumor: number of rabbits: dying	number of rabbits: sacrificed after 3 mo	metastases: present	metastases: absent	number of metastases: total	number of metastases: per rabbit	number of organs affected: total	number of organs affected: per rabbit
Experimental	10	3	7	5	5	413	82,6	25	5
Control	10	8	2	10	0	6 644	644,4	103	10,3

Fig. 1. Brown-Pearce carcinoma of the testicle in a rabbit. Hemorrhage and destructive changes in the tumor tissue after intravenous injections of the "polysaccharide" fraction of the microorganism (obtained by Shear's method). Magnification: ocular 6 ×, objective 8 ×, coefficient 1.4.

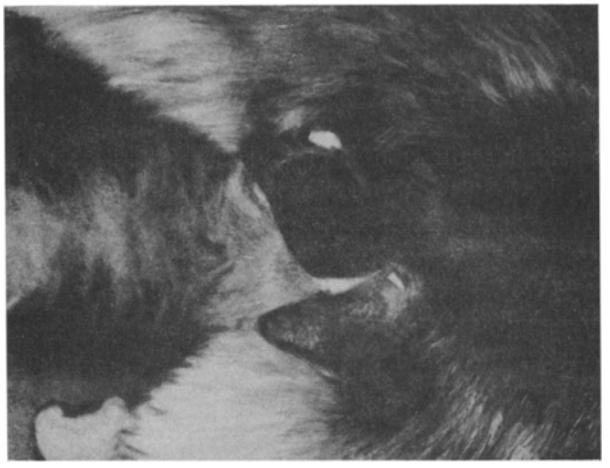

Fig. 2. Brown-Pearce carcinoma of the right testicle in a rabbit. Hemorrhagic necrosis of the tumor after intravenous injections of the "polysaccharide" fraction of the microorganism (obtained by Shear's method).

survival rate of the animals (the rabbits were kept under observation for three months, and were then sacrificed); 2) the degree of metastasization of the tumor.

RESULTS

The results of the experiments are shown in Tables 1, 2, 3, and 4.

It can be seen from Table 1 that the course of development of the tumor differed in each group of animals. All the control rabbits were dead on the 35th-40th day after inoculation of the tumor. In the group in which a living culture was used, no deaths were observed among the animals during this time. In the second group, only one rabbit survived until the day of sacrifice, and of the remainder, three died on the 40th and one on the 80th day from inoculation of the tumor.

After the injection of a subtoxic (third group) or tolerated (fourth group) dose of "polysaccharide" fraction, we observed no therapeutic action. The majority of animals, as in the second and control groups, died at earlier stages of development of the tumor.

The longest period of survival was thus observed in the animals injected with the living culture. Only one rabbit of this group died up to the 80th day from inoculation of the tumor, and all the rest survived and were in good condition throughout the three months of observation.

At postmortem examination of all the rabbits dying or sacrificed after three months, we found that tumor metastases had developed in nearly all the animals. It can be seen from Table 2 that the largest number of metastases and of organs affected was observed in the fifth (control) and the third groups of rabbits, in which the length of survival was least. In the second and fourth groups of rabbits the degree of metastasization was approximately the same. Nevertheless, the number of metastases in the control was $3\frac{1}{2}$ times greater than in these animals.

In the first group, despite the high virulence of the inoculated tumor, its degree of metastasization was the lowest. In this group of animals, development of the tumor was shown by the appearance of only solitary (in two or three organs) small metastases with signs of degeneration and absorption.

In connection with the results obtained it is of interest to study the morphological picture of a tumor subjected to the action of the bacterial preparations. We were interested in particular with the animals injected with the different doses of the "polysaccharide" fraction of the microorganism. Histological investigation showed that in the majority of such rabbits some degree of hemorrhage and degenerative and necrotic changes developed in the tissue of the testicular tumor (Fig. 1). In individual cases (in three of the eight rabbits after the second intravenous injection of the preparation) an extensive zone of hemorrhagic necrosis of the testicular tumor was formed, which extended to the subcutaneous cellular tissue and the skin of the scrotum (Fig. 2). Despite such a severe reaction on the part of the tumor vessels, however, metastases developed as a rule in the rabbits and led to the death of 80-100% of the animals.

The results of our investigation fully confirm the earlier findings of Shear and others [17-19] that the polysaccharide-containing fraction of B. prodigiosus does, in fact, cause hemorrhagic changes in the tumors of experimental animals. At the same time, it follows from our results that the intravenous injection of a living culture of B. prodigiosus into rabbits with a Brown-Pearce carcinoma, inoculated intratesticularly, is more effective in its antitumor action than the injection of a heat-killed culture or of its "polysaccharide" fraction. A therapeutic effect was also found from the action of a living culture of this microorganism on the tumor in the second series of experiments.

It may be seen from Table 3 that the majority of animals in the control group (80%) died before the time of sacrifice: seven on the 30th-35th day and one on the 80th day after inocualtion of the tumor. In the experimental group at this time only three rabbits had died, and the remaining seven survived throughout the three months of observation in a good condition.

A longer period of survival was thus observed in the animals treated with the microorganisms.

The results of postmortem examination (Table 4) show that the control and experimental rabbits differed significantly from each other by the degree of metastasization of the tumor.

In the control group, carcinoma nodules were found not only in the rabbits which died, but also in two rabbits sacrificed after 3 months. In one of these, for instance (No. 2261), an enormous tumor of the left eye and one of the wall of the stomach (dimensions 1.5 × 1.5 cm) were found, together with a tumor of the adrenal gland and multiple metastases in the lungs, the large and small intestine and the kidneys. In the other rabbit (No. 2157), the tumor had developed less intensively than in the remaining control animals, although metastases in the kidneys were also observed in this animal. It should be mentioned that in these two rabbits the majority of the metastases were in a state of regression, although among them were some recent, growing tumor nodules.

In five of the seven surviving rabbits in the experimental group no metastases were present in the organs, but in two we observed residual signs of a tumor in the form of scars on the surface of the parenchymatous organs or of pigmented spots (for example, on the serous membrane of the large intestine and the parietal peritoneum). In the experimental rabbits which died, a relatively high degree of metastasization was observed. Nevertheless, considerably fewer metastases were found in these animals than in the dying control animals.

On the basis of the experiments described above, it may be concluded that the intravenous and intraperitoneal injection of a living culture of B. prodigiosus into rabbits with Brown-Pearce carcinoma, inoculated intratesticular-

ly, leads to absorption of the primary tumor and to suppression of metastasization. The results of the experiments fully confirmed our previous findings [1] that a living culture of B. prodigiosus possesses an antitumor action.

SUMMARY

A polysaccharide containing fraction was isolated from B. prodigiosus; when injected intravenously this fraction provoked hemorrhagic changes in a Brown-Pearce tumor inoculated intratesticularly in the rabbit. However, the living culture of this microorganism (administered by the same method) possessed a more effective-suppressive effect on the growth of Brown-Pearce tumors than the culture killed by heating, or its "polysaccharide" fraction.

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