# EFFECT OF SPECIFIC VACCINATION ON METASTASIS OF THE BROWN-PEARCE RABBIT TUMOR

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Many aspects of the problem of specific antitumor vaccination still await elucidation. There is conclusive evidence of the possibility of prophylactic vaccination against transplanted tumors, effected by resorption of living tumor tissue. It is not clear whether the vaccinatory effect of resorption of living tumor cells applies to tumors already present in the organism. The great majority of researches designed to test this possibility gave negative results [1, 7], from which a number of workers have drawn the conclusion that therapeutic vaccination is not applicable to transplanted, and a fortiori to spontaneously arising tumors [12, 8]. At the present time, however, advances made in the study of immunity to malignant tumors have established the theoretical possibility of therapeutic vaccination, and have delineated the experimental approach towards its achievement [4, 2, 9]. There are only a few reports of the successful application of vaccinotherapy to the treatment of tumors, mostly published during the past few years [10, 11, 5, 6]. From these reports [5] it appears that the therapeutic effect of resorption of the vaccinating tumor is evident only when this takes place at an early stage of development of the original tumor.

The object of the present research was to investigate the therapeutic effect of vaccination on postoperative development of metastatic tumors. We emerged with the assumption [4] that vaccinotherapy might be more effective against metastases after excision of the primary tumor than when it had not been removed. We were unable to trace any literature references to any experimental work of this sort.

## EXPERIMENTAL METHODS

Our experiments were performed on rabbits bearing the readily metastasizing Brown-Pearce tumor. We used 40 chinchilla bucks, of an average weight of 2 kg. Vaccination was effected by introducing virulent tumor tissue under the skin of an ear [3]. The advantage of this procedure was that it assured regular and rapid resorption of the inoculum. Vaccination was effected by a single subcutaneous injection of 0.26-0.27 ml of a 40% suspension of comminuted tumor tissue in physiological saline. The primary tumor was produced by inoculation into the right testicle of rabbits of 0.75 ml of a 20% suspension of fresh tumor tissue.

The experiments were designed to show at what stage of the tumor process vaccinotherapy would be effective. The animals were accordingly divided into 4 groups, of 10 rabbits each. In the first group vaccination was performed 13 days before implantation of the tumor into the testicle. The rabbits of the second group were vaccinated simultaneously with implantation of the tumor. Those of the third group were vaccinated on the 8th day after implantation of the tumor, and one day after its surgical excision. The animals of the fourth group served as controls, and were not vaccinated.

Excision of the testicle containing the implanted tumor was performed on all the experimental and control animals at the same time, on the 7th day after implantation. Observation of the growth of the primary

tumors was continued for 3 months, after which the surviving rabbits were killed. The effectiveness of vaccinotherapy was assessed from the number of successful implants into the testes, and from the incidence and extent of metastases after excision of the primary tumor.

## EXPERIMENTAL RESULTS

Macroscopic observation showed that in the first group of animals the tumor inocula deposited subcutaneously in the pinna at first grew, and then, by the 13-18th day, underwent resorption, in all 10 animals. Similar effects were seen in 8 out of 9 rabbits of the second group, although the process of resorption of the vaccinating inoculum was more prolonged (Fig. 1). In one rabbit (No. 3265) the tumor continued to grow beneath the skin of the ear for a long time, and had not yet undergone resorption when the animal died of the primary tumor. The development of the vaccinating tumor in the third group differed from that in the first and second groups (Fig. 2). As is evident from Fig. 2, the vaccinating tumor either showed no growth at all, or grew only very slowly, in 7 of the rabbits, while in 3 of them (Nos. 1874, 2937, and 3030) its development took a very chronic course, resorption beginning very late, and achieving completion only in one case (rabbit No. 3030), by which time the other two rabbits had already succumbed to the primary malignant process. It thus appears that inoculation under the skin of the ear of an animal at an advanced stage of development of the primary tumor is either not followed by growth of the vaccinating tumor, or is followed by its retarded development.

The effects of vaccination on the course of the primary malignant process are shown in the table and in Fig. 3.

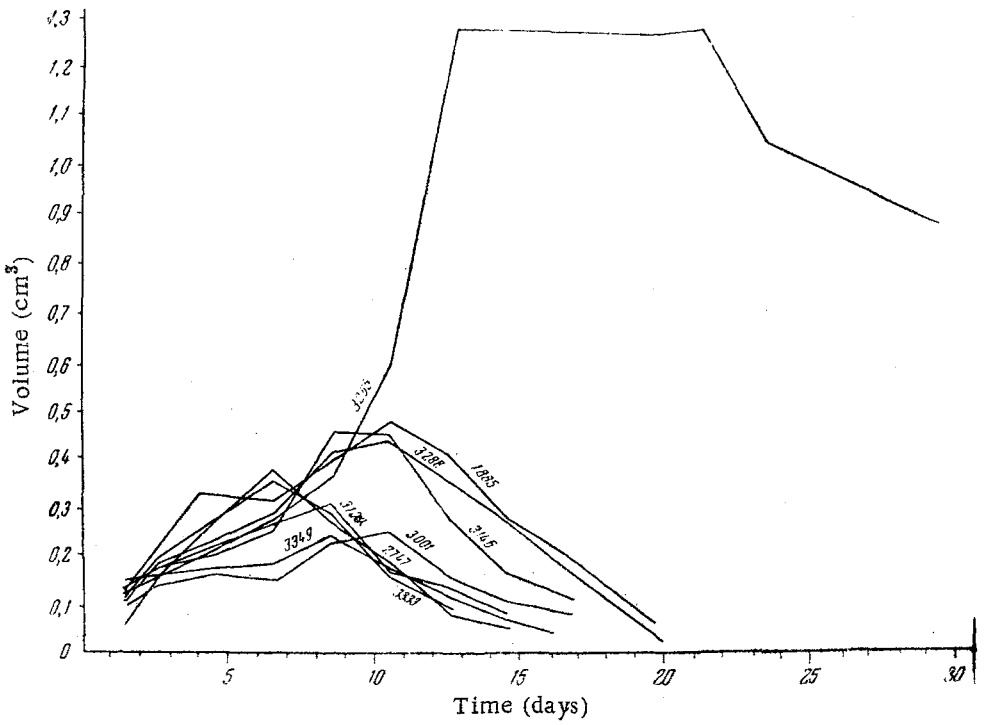


Fig. 1. Growth of vaccinating tumors beneath the skin of the ears of rabbits of the second group of experiments. Curves showing variations in volume of the tumors with time after inoculation.

These data show that, as was to be expected, the animals of the first group differed from those of the other groups; the implants into the testes did not take. Considerable growth of the primary implants took place in all the animals of the second, third, and control groups, as was shown by observations made before excision of the affected testicles, and by histological examination of the excised tissues; the nature and rate of growth of the tumors were the same in these three groups.

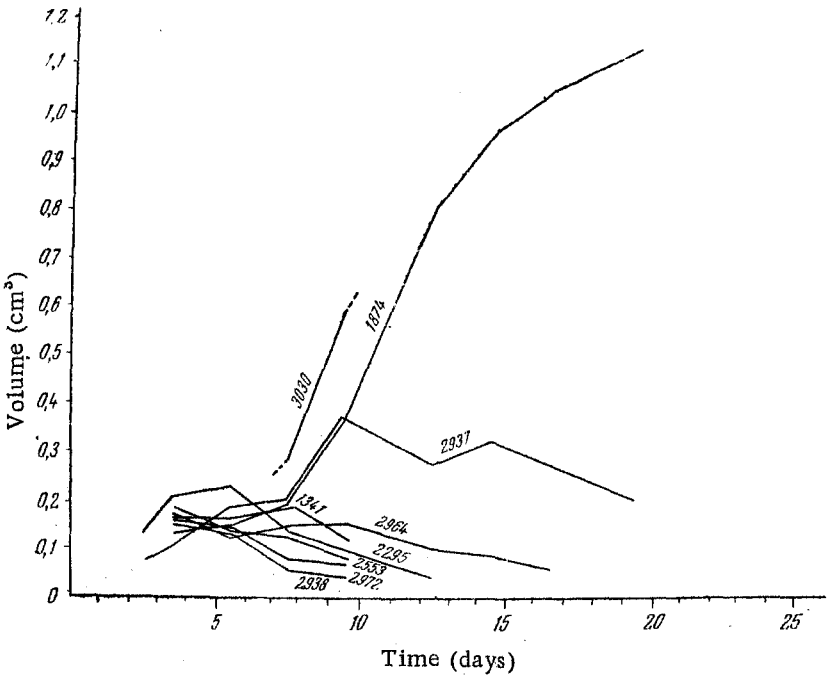


Fig. 2. Development of vaccinating tumors beneath the skin of the ears of rabbits of the third group of experiments. Curves showing variations in volume of the tumors with time after inoculation. Measurement of the volume of the tumor of rabbit No. 3030 was discontinued, for technical reasons.

The evolution of the cancerous process after excision of the testicles proceeded differently in the various groups of animals. The animals of the first group showed no signs of tumor development over a period of 3 months of observation. Anatomopathological examination showed the absence of metastases in 7 of the animals of this group (70%). Solitary, very small metastases were found in some of the organs of the remaining 3 rabbits of this group; histological examination showed that these metastases were in process of encapsulation and resorption.

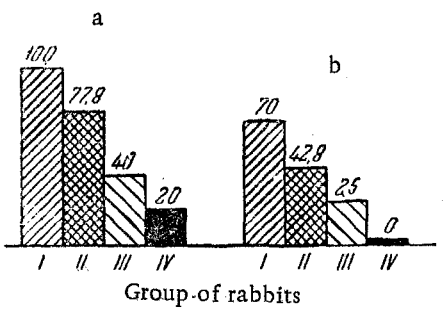


Fig. 3. Mortality and morbidity data for the various groups of experimental animals, over a 3 month period of observation: a) % survival rate; b) percentage of survivors not showing metastases.

In contrast to the animals of the first group, the animals of the second, third, and control groups showed certain changes, which were initially the same for all these groups, in the vicinity of the excised tumor-bearing testicle. These consisted in the appearance in the scrotum and in the spermatic cord of small, dense nodules. The subsequent course of the process varied, however, for the different groups. In the second group these nodules had disappeared by the 15-16th day in 7 rabbits (77.8%), by which time resorption of the vaccinating tumor was almost completed, and these animals subsequently gave no evidence of the continued existence of the neoplastic process. Metastases were not found at autopsy in 3 rabbits (42.8%). Metastases were seen in 4 animals, but they were solitary and small, were found in only 1 or 2 organs, and were in process of encapsulation and resorption. In this group 2 rabbits died owing to the metastases (Nos. 3265 and 3349). Widespread metastases were found in 8 of 10 rabbits of group III, causing the death of 6 animals. In two of the rabbits the metastases began to regress after $1\frac{1}{2}$ -2 months of progressive growth, as shown by observations made on the live animals. The neoplastic process took a benign course in the remaining two rabbits, from the time of implantation. In the four surviving animals

Effect of Vaccination on Development of the Primary Tumor

| Group of rabbits | No. of rabbits | Take of tumor implant in the testicle | | Development of metastases over a 3 month period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | rabbits died | | | | rabbits survived | | | | |
| | | did not take | took | number | mean survival time | average No. of metastases | average No. of organs metastasized | number | rabbits without metastases | rabbits with metastases | average No. of metastases | average No. of organs metastasized |
| First: vaccinated before implantation of tumor in a testicle | 10 | 10 | — | — | — | — | — | 10 | 7 | 3 | 4 | 2 |
| Second: vaccinated simultaneously with implantation of tumor | 9 | — | 9 | 2 | 33.5 | 263 | 10 | 7 | 3 | 4 | 18.2 | 1.5 |
| Third: vaccinated after implantation of tumor in a testicle | 10 | — | 10 | 6 | 38 | 715.6 | 9.5 | 4 | 1 | 3 | 24.3 | 4.7 |
| Fourth: control | 10 | — | 10 | 8 | 35.7 | 675.5 | 10 | 2 | — | 2 | 43.5 | 5 |

(40%) anatomopathological examination did not reveal any metastases in one, and those in the other three were in process of resorption. Metastases were found in all the animals of the control group, of which 8 died during the period of observation, and 2 survived (20%). Metastases were also present in these two rabbits, although most of them had undergone resorption.

The following conclusion may be drawn from our results. Vaccination of rabbits by means of a single implantation of Brown-Pearce tumor tissue under the skin of an ear, performed simultaneously with implantation of the same tumor into a testicle, prevents formation of metastases developing after subsequent excision of the testicular implant, in most of the animals. Vaccination performed at this stage is, however, less effective than is prophylactic vaccination. Vaccination performed on the 8th day after implantation into the testicle, and one day after its excision, has little effect, and metastases develop in most of the animals.

A comparison of the outcome of the primary neoplastic process with the course of development of the vaccinating tumor in the same rabbit shows that a therapeutic effect is found in those cases in which the vaccinating tumor has had time to grow actively, and to begin to regress, in the early stages of the metastatic process. Where this is not the case, the primary neoplastic process may cause the death of the animal before regression of the vaccinating tumor is completed, and it may, moreover, interfere with the development of the vaccinating tumor, so adversely affecting its immunizing effect.

We believe that our experiments show the possibility of applying vaccinotherapy to the treatment of metastases developing after excision of a primary tumor, and that they indicate some of the conditions necessary for the achievement of this effect. Further researches in this direction are essential.

## SUMMARY

Rabbits were vaccinated by subcutaneous implantation of living Brown-Pearce tumor cells into a pinna, before, at the same time as, and after implantation of the same tumor into a testicle, which was excised, together with the implant, after 7 days.

Subsequent development of metastases did not occur, or was greatly reduced, in animals vaccinated before or at the same time as implantation into the testicle. Only a negligible effect of vaccination was found in animals vaccinated 8 days after implantation. It is concluded that vaccinotherapy is only effective when the vaccinating tumor has time to regress before the metastatic process has reached an advanced stage.

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