THE Experimental Alteration of Malignancy with An Homologous Mammalian Tumor Material

II. Intracutaneous Inoculation of Preserved Material

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A material obtained from the Brown-Pearce rabbit tumor, when injected intratesticularly, was found to render rabbits more susceptible to transplantation two weeks later with the tumor into the same or opposite testicle, and to enhance greatly every observed phase of local and metastatic tumor growth (1, 2). By this means the homologous tumor material brings about the occurrence of primary tumors, and metastases were produced in animals which would not otherwise have developed them.

It may be recalled, however, that testicular inoculation of the Brown-Pearce tumor is usually followed by a local growth and the development of metastases, while inoculation into the skin or subcutaneous tissues gives fewer primary tumors and, under ordinary conditions, metastases do not occur. It appeared, therefore, that the use of the homologous material in conjunction with intracutaneous inoculation offered a more severe test of the potency of the material and, at the same time, afforded a means of determining whether the homologous material retained its activity when injected by a route other than the testicle.

The present paper is concerned with results obtained when both

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the tumor inoculations and the injection of the homologous material are made in the skin instead of the testicle.

**Material and Methods**

Three experiments were carried out between December 1931 and June 1932 (Table I) on a group of 32 rabbits divided equally between experimental and control series. The animal material differed from that used for intratesticular inoculation in several respects. First, the animals were raised in the laboratory and with two exceptions were paired litter mates from standard bred stocks; paired females were used as well as males, and the animals were two to three and a half months old instead of four to ten months, as in previous experiments.

The homologous material was prepared, as in the previous experiments, from excised masses of rabbit tumor tissue embedded aseptically in paraffin and preserved in the ice box (24° F.) for nineteen to seventy-nine days. When an injection was to be made, the preserved tumor tissue was removed from the paraffin, minced and ground in a mortar without the use of sand. Two parts of normal saline were then added to form the emulsion used. Experimental animals were given an intracutaneous injection of 0.3 to 0.5 c.c. of this emulsion in the skin of the flank, and two weeks later both the control and experimental animals were inoculated intracutaneously with an emulsion of fresh tumor tissue in the same or opposite flank.

Comparisons of results were made as in previous experiments. Measurements of the tumors resulting were made in three dimensions with calipers, twice weekly, during a sixty-day period. At the termination of this period, the surviving animals were killed by the injection of air into an ear vein. At post-mortem examination the incidence and volume of the primary tumor, and the incidence, number, volume and distribution of the metastases were recorded for each ani-
mal, as described in the previous paper (2). Similar statistical procedures in analyzing the results were employed.

**Results**

**Incidence of Primary Tumors:** Of the 16 animals in the control series, 2 (13 per cent) had primary tumors at the termination of the experiments. This compared with 11 primary tumors (69 per cent) in the 16 animals injected with the preserved material (Table II, Fig. 1). This difference is significant ($\chi^2 = 9.76$, $n = 1$, $P = 0.01$).

**Growth and Volume of the Primary Tumor:** The primary tumors in the experimental animals grew at a more rapid rate than those in the control animals (Fig. 2). At twenty-seven days the primary tumor in the experimental animals averaged 2.57 c.c., while that in the control series averaged 0.50 c.c. The curves were not plotted after this date, as the tumor in most of the control animals soon disappeared. At post-mortem examination only 2 of the 16 controls and 11 of 16 experimental animals had primary tumors. These averaged 1.5 and 5.4 c.c. respectively by water displacement (Table II), a probably significant difference (diff. $= 3.88 \pm 1.56$ c.c., $n = 11$, $t = 2.5$, $P = 0.03$). In the two animals in the control series no living tumor remained, only yellow infiltrated scars. All other primary tumors had completely regressed.

**Metastatic Tumor Growths:** In accordance with general experience with this tumor, no control animals developed metastatic growths...
In the experimental series, however, six animals developed metastases (38 per cent). This was a significant difference ($x^2 = 6.959$, $n = 1$, $P = 0.01$). Animals with metastases had from 1 to 30 metastatic foci, with an average of 10 per animal. The total amount of the metastatic tumor per animal with metastases was 20.5 c.c. Thirty-six of 50 possible tumor sites were represented with metastases in one or more of the six animals. The muscles of mastication and the right kidney were involved in four animals; the left kidney, the mandible, subcutaneous tissue of the trunk, the muscles of the trunk, the left perirenal area, the mesentery and the muscles of the scapulae were involved in three animals; the left suprarenal, the interscapular space, the muscles of the tongue, the right perirenal area, the nasal sinuses, the posterior cervical region and the upper metaphysis of the tibia were involved in two animals, a total of 63 metastases in all.

**Mortality from the Tumor:** At post-mortem examination all the control animals were negative except two which had small entirely necrotic primary growths and no metastases. According to the arbitrary method of calculating mortality (outlined in the first paper of this series), this was an actual mortality of 0 per cent and an estimated and total mortality of 3 per cent. Among the experimental animals, 3 died of tumor during the observation period, giving an actual mortality of 18 per cent. Of the survivors, 1 had a primary tumor and ten metastatic foci, 2 had a primary tumor and one metastatic focus, and 5 had a primary tumor and no metastatic foci. This gave a total mortality (actual and estimated) for the series of 35 per cent. This was twelve times the total mortality for the control series and was statistically significant ($x^2 = 5.32$, $n = 1$, $P = 0.022$).
Intracutaneous inoculation of the Brown-Pearce rabbit tumor into the flank has been carried out in a number of other experiments for the purpose of inducing immunity to the tumor. Spontaneous regression of the local tumors in a period of three to eight weeks has always occurred, and no metastases have resulted. The mere fact, therefore, that 11 of 16 rabbits treated with preserved material had primary tumors eight weeks after inoculation and that 6 of the 16 developed metastases in distant parts of the body is a very significant alteration in the expected course of malignant disease. There was no evidence in these or in the experiments already reported that the preserved material will of itself produce tumor growth during a two months observation period. After four to ten days no palpable lesion remains at the site of the injection of the preserved material and the animals show no clinical signs of illness or infectious process. Also a first injection of fresh tumor tissue followed two weeks later by a second inoculation of living tumor does not result in a more malignant course of disease. The phenomenon of enhancement is not, therefore, due to the cumulative effect of repeating the dose of tumor tissue, but must be explained on some other basis. Berkefeld "V" filtrates of the preserved material have the same potency as the unfiltered emulsion. Experiments bearing out these points will be published later.

SUMMARY AND CONCLUSIONS

Experiments were carried out to determine whether an homologous tumor material which enhanced every observed phase of the rabbit tumor following intratesticular inoculation would have similar effects following intracutaneous inoculation into the flank. Groups of rabbits injected in the skin of the flank with the material and inoculated into the same or neighboring areas two weeks later with tumor were compared with control groups inoculated with the tumor alone.

The results for the control animals confirmed previous experience that the rabbit tumor, although malignant following intratesticular inoculation, is a spontaneously regressing, non-metastasizing growth following intracutaneous inoculation into the flank. Nevertheless, in the experimental animals which had been treated with the homologous material a higher incidence of and more rapidly growing and lingering local tumors occurred. Furthermore, distant metastases were discovered in a large proportion of the animals. The preserved material, in enhancing both the primary and metastatic phases of malignancy, is, therefore, not dependent upon intratesticular inoculation, and is able to overcome the natural resistance of the rabbit’s skin to this tumor. Neither are sex and immaturity barriers to the action of this material.

BIBLIOGRAPHY