Proceedings of the National Academy of Sciences
Vol. 67, No. 3, pp 1521–1527, November 1970

Effect of Muscle Denervation on Growth of Transplanted Tumor in Mice*

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Communicated by Georg von Békésy, August 13, 1970

Abstract. A temporary retardation of transplanted Round Cell Neuroblastoma growth in the gastrocnemius muscle of mice was observed in 20% of the animals after denervation of the muscle. The tumor cells in these denervated animals showed structural alterations and deterioration in function. These altered cells when mixed with associated denervated muscle tissue, to which was added fresh tumor, produced a higher percentage of retardation upon subsequent transplants in innervated muscle.

The importance of adequate innervation and the "trophic" influence on growth and regeneration has been well documented.[1,6,7,12,15,22,23] However, the effects of the nervous system upon neoplasms is still not understood. It has been assumed by some that nervous system changes are only secondary to the presence of a neoplasm.[2,3] Others[17,18,20,21] have suggested, however, that the nervous system may play an integral part in tumor development.

Cheatle reported a clinical investigation in which skin tumors were found to develop and spread mainly in innervated and not in denervated areas.[4,5] Kavetsky in his reviews[17,18] suggested that the course of tumor development can be distinctly modified depending on the state of the nervous system in the host. Forman,[8] in his studies of sarcoma 180 transplants to spinal cord-sectioned mice, found no significant difference between tumor growth of the surgically treated mice from that of controls.

Since the available evidence is inclusive as to whether a definite association exists between the nervous system and tumor development, this study of the effect of denervation on transplanted tumors was instigated.

The present study describes the effects of denervation of, and transplants to, the gastrocnemius muscle in mice. The working hypothesis is that denervation may alter transplanted tumor development.

Materials and Methods. A/J mice, male and female, 6 weeks (±2 days) of age, and Round Cell Neuroblastoma (C1300) obtained from the Jackson Laboratory, Bar Harbor, Me.[11] were used in this investigation. Methoxyflurane (Metofane, Pitman Moore, Division of Dow Chemical Co., Indianapolis) was used to induce general anesthesia. All muscles and tumors utilized for transplantation were obtained immediately after the animals were killed. Donor tissue was kept in cold physiological saline solution at 15°C during the procedures.

Peripheral denervation was performed by section of the left sciatic nerve. Approximately 2 mm of the sciatic nerve was removed at the trochanteric level. Tumor was transplanted 1-day later.

Fresh tumor cells were homogenized manually for 5 min in 10 ml of physiological saline solution. 0.2 ml of the homogenate was injected, via a sterile half-inch 26-gauge needle, bilaterally into the innervated (right) and denervated (left) gastrocnemius muscles. The animals were killed 5–7 days after the tumor cells were transplanted. Seven groups of animals (9–24 per group, total 83) were used in different phases of this first experiment.

When killed the animals were observed for either a tumor "take" or "retarded take." The term "retarded take" merely implies that no visible tumor growth is evident at the time observed. However, as discussed later in the histology section, viable tumor cells were detected in all cases. Scoring was made by direct visual inspection of the exposed gastrocnemius muscle. A "take" was scored if, superficially, the muscle showed discoloration, indicating that tumor tissue of a different hue was located at that site. If no evidence of the transplant was observed superficially the muscle fibers were separated and inspected further for discoloration or a discrete mass. A "retarded take" was scored when no change in muscle appearance or a discrete mass was noted either on the surface or deeper in the separated fibers.

Both the "take" and the "retarded take" muscles were used for additional experiments to determine whether denervation produced alteration in the host tissue which would change its suitability as a host tissue for tumor growth. The muscles were individually homogenized with an equal weight of fresh tumor cells and injected into innervated normal muscles. The following three successive passes of homogenized tumor and muscle transplants were performed.

I. "Retarded take" *denervated* muscle, mixed with fresh tumor, was transplanted into normal innervated gastrocnemius muscle (29 animals in three groups).

II. "Retarded take" muscle resulting from (I) was mixed with fresh tumor and transplanted into normal innervated gastrocnemius muscle (36 animals in three groups).

III. "Retarded take" muscle resulting from (II) was mixed with fresh tumor and transplanted into innervated gastrocnemius muscle (24 animals in three groups).

The following control experiments were performed at the same time.

(*a*) From the "take" group, *denervated* muscle was homogenized with fresh round cell tumor and injected into normal innervated gastrocnemius muscle (10 animals).

(*b*) A sham operation was performed in which skin incision, muscle fiber separation, and exposure of the left sciatic nerve was done without the sciatic nerve being cut. Homogenized round cell tumor was injected into both left and right gastrocnemius muscle 1 day after surgery (10 animals).

(*c*) Tumor cells were homogenized with normal gastrocnemius muscle and injected into innervated gastrocnemius muscle (20 animals).

(*d*) Homogenized tumor without any muscle tissue was injected into normal gastrocnemius muscle (40 animals).

In order to determine time-course data on the "retarded take" tumor development, an additional 30 animals were studied for 19 days after transplant as follows. Homogenized tumor was injected 1 day after section of the sciatic nerve. 5 days after the transplant, the animals were anesthetized and the muscles were examined. "Retarded takes" were evident in 7 of the 30 animals (Table 1, *c*). One of the seven animals was killed at this time for histological examination of tumor development, and the remaining six animals were killed at 3-day intervals for similar histological examinations.

The samples of muscle and tumor from both the experimental (including the "take" and "retarded take" in the successive passes) and control groups were fixed in 10% buffered formalin, sectioned (5 μm) and stained with Harris alum hematoxylin and eosin for the histological studies.

Results (Table 1). The original group of 83 animals, in which the transplants were made to denervated muscles consistently produced 20% fewer "takes"

TABLE 1. *Percentage of "takes" on tumor transplant.*

	Transplant of	Transplant into	% "take"*
a.	Tumor cells	Innervated right leg	100 ± 0 (83)
b.	Tumor cells	Denervated left leg, same animals	80 ± 6 (83)
c.	Tumor cells	Denervated left leg only	77 ± 0 (30)
d.	"Retarded take" from (*b*)	Innervated muscle	63 ± 14 (29)
e.	"Retarded take" from (*d*)	Innervated muscle	60 ± 10 (36)
f.	"Retarded take" from (*e*)	Innervated muscle	40 ± 10 (24)
g.	Controls (described in *Methods*)	Innervated muscle	99 ± 1 (80)

* Mean ± SD, number of animals in parentheses.

(line *b*) than in the innervated gastrocnemius muscle (line *a*). The tumor cells in the denervated "retarded take" showed morphological alterations (see *Histology*) and were used for successive transplants to normal innervated muscles. The tumor cell alterations and percentage of "retarded takes" increased in the three successive passes.

Using "retarded take" *denervated* muscle from the original group of 83 animals mixed with fresh tumor and passed to normal innervated gastrocnemius muscle of 29 animals (*Methods*, Expt. I), we observed a 63% "take" (line *d*).

On the second pass (see *Methods*, II) a 60% "take" occurred (line *e*), and on the third, 40% (line *f*) (see *Methods*, III). In the control experiments, which included sham operations, a 99% "take" occurred.

Histology. Distinct histological differences were observed in the "take" and "retarded take" both in the muscles and tumors. Sections from the "take" group show characteristically dense localizations of tumor cells at the periphery of the muscle with invasion and disruption of the muscle. The tumor was diffusely cellular and characterized by large round cells and oval nuclei with many mitotic figures (Fig. 1).

The general features conformed to the usual morphology of neuroblastoma. However, while many pyknotic nuclei are reported to be present in the subcutaneous transfers in A/J mice,[11] this feature was not evident in these "takes" of tumor transplant to muscle in this study. They were observed, however, in the successive transplant experiments.

The "retarded take" group differed in that the density of tumor cells at the periphery of the muscle was low. There appeared to be little or no invasion or disruption of the muscle. There were alterations in the "round cell" appearance of the tumor, with reduced nuclear size and change in shape. Mitotic figures were less frequent (Fig. 2).

Sections made of "takes" from the successive passes showed greater alterations in tumor-cell morphology. Many of the tumor cells were decreased in size and now showed frequent pyknotic nuclei (Figs. 3 and 4).

"Retarded take" sections from the successive passes, however, showed only a scattering of residual tumor cells, and some of the sections were almost indistinguishable from those of normal muscle (Figs. 5 and 6).

Sections of the muscle and tumor taken 5, 8, 11, and 19 days from the group of 30 animals studied 19 days after transplant (which were regarded as "retarded takes" at 5 days) showed increased tumor development beginning at day 8 and

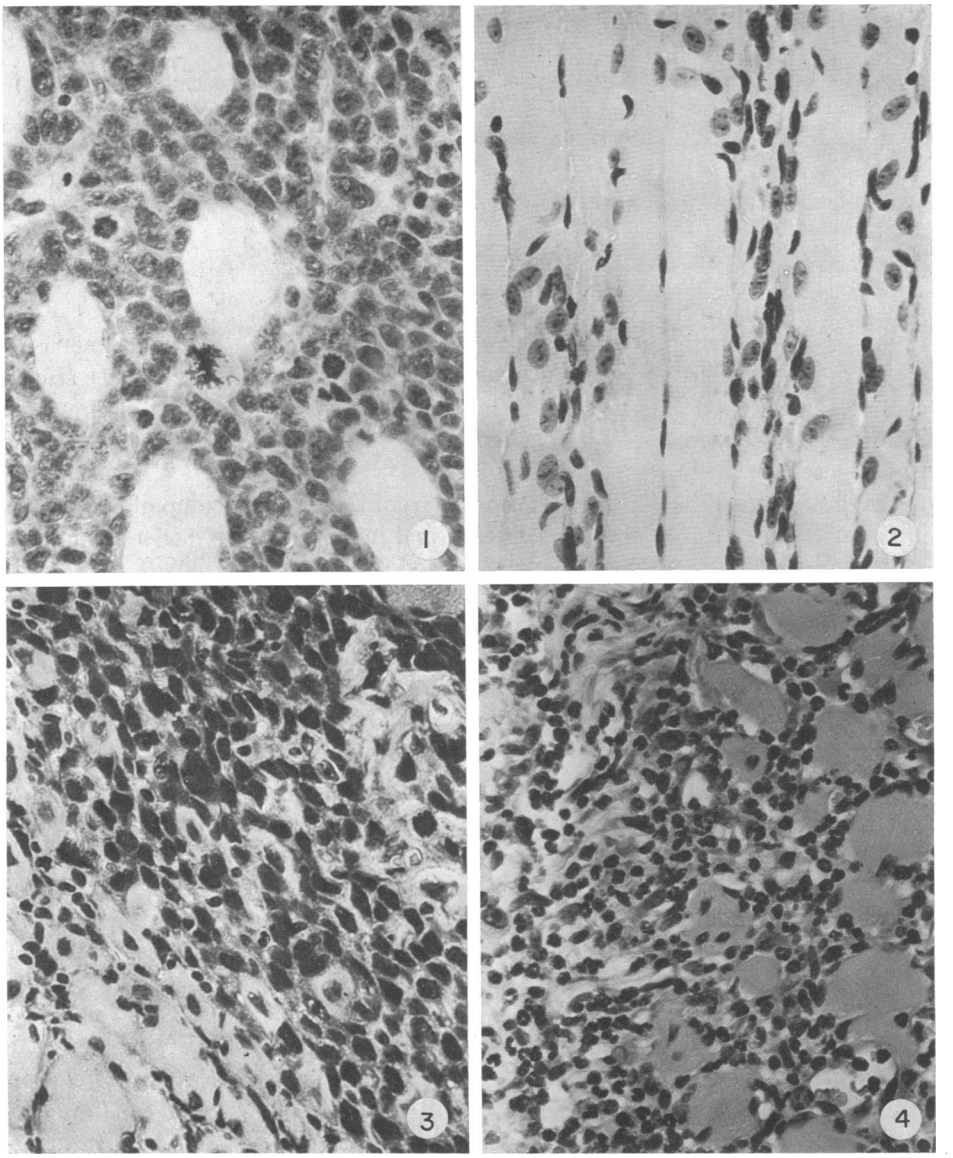

FIG. 1. Section of denervated muscle, scored as "take," showing large round tumor cells with frequent mitotic activity. H & E, ×315. Sections for Figs. 1–8 were taken from site of tumor implantations.

FIG. 2. Section of denervated muscle, scored as "retarded take," showing decreased density of tumor cells with alterations in cell appearance. H & E, ×315.

FIG. 3. Section of innervated muscle into which "retarded take" muscle plus fresh tumor were transplanted. Muscle scored as "take" showed alterations in cell appearance as compared to Fig. 1. H & E, ×315.

FIG. 4. Section of muscle scored as "take" after a second pass of "retarded take" muscle plus fresh tumor to an innervated muscle. Increased alteration in cell morphology is observed. H & E, ×315.

rapidly increasing at day 11. By day 19 there was a fully developed tumor but the size and shape of tumor cells were still changing.

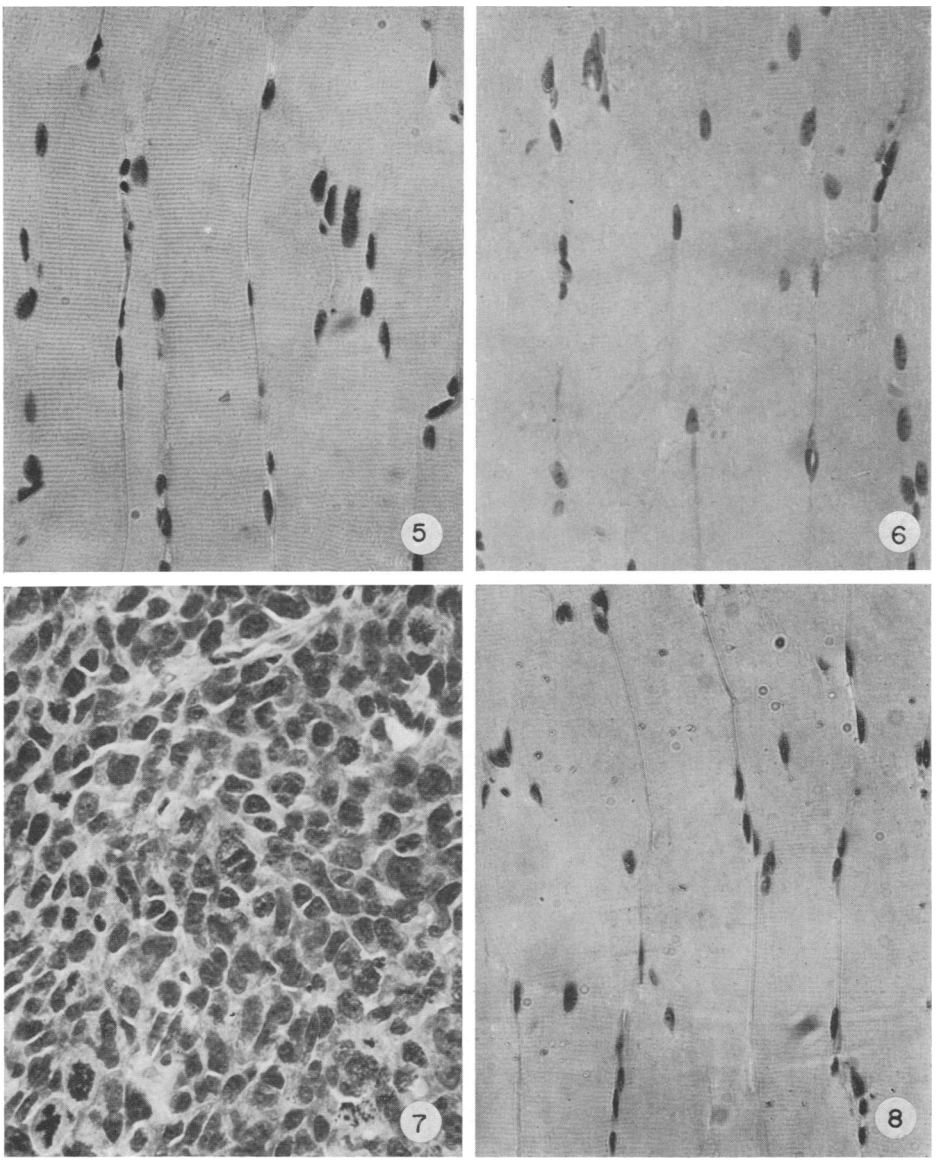

FIG. 5. Section of muscle after transplant identical to Fig. 3 except that muscle was scored as "retarded take." Muscle gives all the appearance of normal muscle with reduced tumor cells (Fig. 8). H & E, ×315.

FIG. 6. Same as Fig. 4, except that muscle was scored as "retarded take." Again a sharp reduction in apparent tumor cells is observed. H & E, ×315.

FIG. 7. Sham-operation control followed by transplant of fresh tumor plus normal muscle tissue. High density of tumor cells, identical in appearance to those in the whole tumor tissue itself. H & E, ×315.

FIG. 8. Sham operation without transplant of tumor. Note similarity in this normal muscle to the "retarded takes" of Figs. 5 and 6. H & E, ×315.

Sections of the sham-operation muscle into which a tumor was transplanted showed normal tumor development at 5 days (Fig. 7). The histologic appear-

ance of sections of the sham-operated muscle without a tumor transplant was identical to that of normal muscle (Fig. 8).

Discussion. Laird[19] has suggested that growth retardation in tumors reflects some retained property of its normal tissue origin. Host denervation with its associated "trophic" effects and attendant structural, chemical, and cell division changes[12–16,24] may influence these retained normal tissue properties.

Environmental changes have also been reported as being relevant in neuroblastoma alterations. Goldstein[10] noted differentiation and maturation of neuroblastoma cells taken from young children and grown in tissue culture. Varon[25] suggested that the condition of the host is a prime factor in the morphological and growth changes in neuroblastomas. Inasmuch as a positive "take" depends also in part on the interaction of the tumor inoculum with the host tissue,[9,26] an alteration in the host tissue by denervation as presented here is significant. A decreased percentage of "takes" and changes in tumor cell morphology resulted initially in the tumors transplanted to a denervated muscle environment. The changes in cell morphology and an increased percentage of "retarded takes" in serial passes is still evident when the tumor mixtures (fresh tumor cells plus "retarded take" muscle and its residual tumor cells) are transplanted into normal innervated muscle.

The initial results may be related strictly to denervation effects on growth. In successive passes *to innervated muscle*, this original denervation environment is no longer present but an increased number of "retarded takes" still occurs. A residual carryover from the previous denervation does exist in the presence of some altered tumor cells in the transplant inoculum mixture used for successive passes. By using these altered cells as a component of the tumor cell mixture, the growth retardation effect remained for 5–7 days after transplant. However, when the post-transplant interval was increased to 19 days the animals that were scored as "retarded takes" at 5 days now became positive "takes" even though some alteration in the cells was still present.

Thus the retardation effect on tumor cell growth following denervation is a temporary one. The active tumor cells overcame this initial effect by day 8 after transplant in the present series.

This study suggests that alterations in the nervous system play a distinct role in tumor development by altering the host environment. Further studies are now underway to identify the mechanism of the temporary retardation.

We thank Dr. Nicholas E. Palumbo and Dr. Terence A. Rogers for their continued interest and guidance, and Barry Lino for photographic assistance at the School of Medicine, University of Hawaii.

* This study was supported in part by the National Science Foundation grant GB-5768, the Institute of Neurological Diseases and Blindness, U.S. Public Health Service grant NB-06890-03, and the National Cancer Institute grant CA-10977-04.

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