The Response of Mouse Neurons to Mouse Sarcomas 37 and 180.* (25130)

JEAN E. SMALL (Introduced by Jack Davies)
Dept. of Biology, Brown University, Providence, R. I.†

Mouse sarcomas 37 and 180 stimulate differentiation of sympathetic and sensory ganglia in young chick embryos(1,2,3). In these ganglia the growth rate of individual cells is accelerated and the final cell population is greater than that in controls; the motor system is unaffected. This response is mediated by a protein produced by tumor cells(4). An analogous response occurs under *in vitro* conditions, the pattern of fiber outgrowth from chick ganglia being characteristicalliy altered in presence of explants(5) or extracts(4) of the tumors. More recently proteins with identical effects but of much higher potency have been found in snake venoms(6,7) and mouse salivary glands(8,9). The reaction of chick neurons to this substance seemed interesting enough to warrant exposing the developing neurons of other vertebrates to these tumors. In our experiments the responses of the mouse nervous system during fetal and early postnatal stages were studied.

Materials and methods. Spinal ganglia from BUB mice ranging in age from 11th prenatal to 10th postnatal day were grown in plasma clot cultures. In the experimental series pieces of sarcoma 37 or 180 were placed close to the ganglia. In some experiments sympathetic ganglia or pieces of spinal cord were tested, but sensory ganglia were most often used. Parallel studies of chick material served as a standard of comparison. Observations and measurements were made on cultures at intervals after about 12 hours incubation. Fixed material was studied after staining by the silver-reduction method of Cajal-DeCastro or Holmes.

Results. In control cultures the pattern of outgrowth from mouse ganglia is very similar to that from chick ganglia. Neurites first appear after about 12 hours incubation and are accompanied by a migrating population of spindle cells originating from the neural crest and abundantly present in all ganglia. The chief characteristic of fiber outgrowth in these cultures is the tortuous course taken by individual neurites in their generally radial route from the ganglion.

Several differences in the character of outgrowth were noted among ganglia from different age groups. Ganglia from 11- to 13-day fetuses are in the very early stages of differentiation and produce few neurites after explantation. Ganglia isolated between 14th day and term produce an abundance of fibers; ganglia from postnatal animals tend to be retarded (up to 24 hours) in initiation of outgrowth and in general produce fewer neurites (Figs. 1, 2).

In experimental cultures, neurites begin to appear after 9 hours incubation, *i.e.*, at least 3 hours earlier than in controls. The number of neurites far exceeds that in control cultures, and the path of each neurite is a relatively straight radial one. Usually the closely spaced fibers advance in unison, their tips appearing to lie on an invisible boundary of circular or elliptical shape. This phenomenon has been described in chick ganglia(5), and has been termed a "halo" effect. In ganglia of most age groups, migration of spindle cells is completely suppressed for the first 24 hours of incubation. This suppression is gradually overcome, as is the strict regulation of neurite advance. As was true in the chick, sympathetic ganglia respond similarly while neurons of spinal cord are unaffected.

The most interesting age group among experimental cultures is that of the ganglia isolated between 11th and 13th prenatal day. The modestly differentiated cells of these ganglia respond vigorously to the tumor substance and produce large numbers of fibers, whereas in control cultures they produce almost none. On the other hand, the spindle cell element of

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† Present address: Washington Univ. School of Med., St. Louis, Mo.

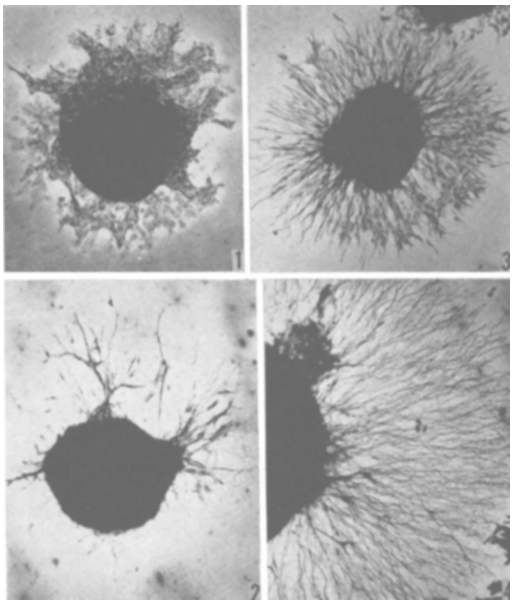

FIG. 1-4. Mouse spinal ganglia in culture. Holmes' silver method. ×35.

FIG. 1. From 11-day fetus; incubated 36 hr; a few neurites at upper right.

FIG. 2. From newborn; incubated 36 hr; few neurites and spindle cells.

FIG. 3. From 12-day fetus; incubated 20 hr with tumor (upper right); many neurites and spindle cells.

FIG. 4. From newborn; incubated 36 hr with tumor (lower right); many neurites; spindle cells beginning to emerge.

these ganglia has not yet acquired sensitivity to the tumor substance; these cells fail to show any suppression (Fig. 3). Ganglia isolated at later stages show responses identical to those previously reported for the chick. Ability to respond under *in vitro* conditions extends at least through the first postnatal week (Fig. 4); the generally slower growth of ganglia isolated from older animals makes their response difficult to test under conditions of these experiments.

Additional information on the nature of the response is provided by measurements of fiber lengths made on about 200 living and on many more fixed cultures. Calculation of average rate of fiber elongation during the first 36 hours incubation showed no significant difference between control (7-10 μ/hour) and experimental (7-12 μ/hour) values. Measurements made on cultures in which the distance between ganglion and tumor was sufficient to produce initial differential effects at near and remote poles of the ganglion showed no consistent difference in fiber lengths in the 2 regions at 36 hours.

Attempts to alter development of the nervous system *in vivo* included implantation of tumors subcutaneously in mice during early pregnancy, injection of tumor brei into 16-day fetuses, and subcutaneous implantation in newborn animals, which in some instances had partially amputated forelimbs. The first 2 of these experiments suffered from the fact that sarcomas 37 and 180 do not thrive in pregnant animals. This had been reported for 180(10), and was true of 37 in this study. It is, therefore, unlikely that any animal was actually exposed prenatally to the tumor substance. Tumors implanted in postnatal animals grew rapidly to enormous size without effect upon the intact or injured nervous system in the 2 weeks that the animals survived. The detailed similarity of *in vitro* responses of chick and mouse neurons seems to indicate the possibility of an *in vivo* response from the mouse if proper conditions be found.

Discussion. In evaluating responses of mouse ganglia to the tumor substance, age differences in growth potential must be taken into account. It is impossible to determine whether the few neurites produced *in vitro* by ganglia isolated between the 11th and 13th prenatal day are regenerated from cells which had already produced fibers, or represent newly differentiating cells. In either case it is evident that very few cells isolated at these early stages are able to establish neurites in control cultures. Progressive retardation of initial outgrowth in ganglia from older animals may result from increased trauma to cells which have achieved more elaborate peripheral relationships; or it may simply be due to the phenomenon well known in tissue culture of the slower initiation of activity in older tissues.

Regardless of the interpretation given to the above phenomena, it has been amply demonstrated that the tumor substance has a stimulatory effect upon explanted mouse ganglia from very early stages throughout most if not all of their developmental course. The altered growth pattern described here is exactly simi-

lar, but for minor differences in timing, to that already reported for the chick. There is no explanation for the changed character of neurite growth under these conditions. There can be no doubt that the tumor substance is effective in eliciting fiber formation from cells which would be unable to produce neurites independently; similarly it can cause this process to occur precociously in cells which are themselves able to establish neurites. The substance is, moreover, responsible for the comparatively straight course followed by neurites in experimental cultures. The rate of axon elongation, however, is not increased.

Selectivity of cell type responsive to this substance is one of its more intriguing aspects. In both chick and mouse the motor system is totally refractory, the sensory and sympathetic systems highly responsive. In the chick a further subdivision is possible since the developing sensory ganglia apparently contain 2 morphological and functional groups of neurons. The small, mediodorsal cells are stimulated by the tumor substance; the large, ventrolateral cells are not detectably affected. Since in mouse ganglia corresponding groups of cells are not distinguishable, the comparison cannot be extended to this level.

Further evidence of specificity of responsiveness to the tumor substance is gained by considering the behavior of the spindle cells. The characteristic reaction of these cells in cultures containing pieces of tumor is a failure to migrate for at least 24 hours. After the period of suppression these cells move out from the ganglion in their usual fashion and number. The only exception to this behavior yet reported is the lack of suppression in cultures of ganglia from very young mouse fetuses. Thus, at a stage when the neural elements of sensory ganglia are already responsive to the tumor substance, spindle cell elements are apparently still undifferentiated in this respect. That the inhibition of movement is not a general response extending to fibroblasts is indicated by lack of any effect on chick heart fibroblasts(5) and on mesenchymal cells from mouse limb buds. Sheath cells migrating from segments of pre-degenerated peripheral nerves explanted from young mice are similarly unaffected. Thus, it would seem from limited experiments that suppression by the tumor substance is restricted to a specific class of cells during a particular period of development. That suppression is overcome after 24 hours is probably due to a decrease in amount of tumor substance present; there is a concurrent lessening of effect upon neurites. It is possible that tumor cells *in vitro* lose their ability to produce effective amounts of the critical substance.

Summary. 1) Dramatic responses to a tumor-produced substance occur in cells from mouse as well as chick ganglia. The knowledge that these responses are not unique to the chick adds to the interest which this phenomenon already had for the neuro-embryologist. 2) After conclusion of these experiments, the responsiveness of mouse and rat ganglia to the much more potent snake venom and salivary gland factors was reported(8,9).

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