the female sitting within 15 cm of the male with her spinal column directly facing the male's ventrum. Back proximity is thought to be a female proceptive behavior in rhesus monkeys [C. G. Cochran, *Behav. Neurol. Biol.* **27**, 342 (1979); K. Wallen and R. W. Goy, *Horm. Behav.* **9**, 228 (1977)].

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Nerve Growth Factor Attenuates Neurotoxic Effects of Taxol on Spinal Cord–Ganglion Explants from Fetal Mice

Abstract. *Most neurons in organotypic cultures of dorsal root ganglia from 13-day-old fetal mice require high concentrations of nerve growth factor for survival during the first week after explantation. These nerve growth factor–enhanced sensory neurons mature and innervate the dorsal regions of attached spinal cord tissue even after the removal of exogenous growth factor after 4 days. In cultures exposed for 4 days to nerve growth factor and taxol (a plant alkaloid that promotes the assembly of microtubules) and returned to medium without growth factor, > 95 percent of the ganglionic neurons degenerated and the spinal cord tissues were reduced almost to monolayers. In contrast, when the recovery medium was supplemented with nerve growth factor, the ganglionic neurons and dorsal (but not ventral) cord tissue survived remarkably well. Dorsal cord neurons do not normally require an input from dorsal root ganglia for long-term maintenance in vitro, but during and after taxol exposure they become dependent for survival and recovery on the presence of neurite projections from nerve growth factor–enhanced dorsal root ganglia.*

Taxol, an antitumor drug (*1*) isolated from the plant *Taxus brevifolia*, promotes the assembly of microtubules and binds to them in cell-free systems; such microtubules resist depolymerization by cold or calcium (*2, 3*). In fibroblasts and other cells exposed to taxol, abnormal bundles of microtubules form throughout the cytoplasm (*4, 5*). Taxol exposure also leads to the formation of unusual numbers and arrays of microtubules in neurons and supporting cells in explants of spinal cord tissue with attached dorsal root ganglia (DRG's) (*6*). The primary effects of taxol appear to be on the tubulin-microtubule system; no other specific mode of action has been reported.

This study demonstrates that nerve growth factor (NGF), a hormone required for the maintenance of sympathetic and DRG neurons (*7, 8*), markedly attenuates the cytotoxic effects of taxol on DRG neurons explanted with spinal cord tissue from fetal mice. Our data suggest that taxol prolongs the critical developmental period during which fetal DRG neurons are dependent on NGF for survival and maturation. Furthermore, exposure of these cultures to taxol (*9–11*) in the presence of NGF permits survival and recovery of many dorsal cord neurons with a large DRG input under culture conditions resulting in extensive degeneration of ventral cord neurons (*12*). Dorsal cord neurons do not normally require a DRG input for long-term maintenance in vitro (*10, 11, 13*), but during and after taxol exposure they become remarkably dependent on factors provided by NGF-enhanced DRG neurites. Some target tissues in the central nervous system have been shown to degenerate after deafferentation during critical periods of embryonic development in vivo (*14–16*), but this, to the best of our knowledge, is the first demonstration of in vitro conditions under which neurons in central nervous system explants become dependent on their afferent inputs (*12*).

Spinal cord tissue with attached DRG's was removed from 13-day-old fetal mice and cross-sectioned. The sections were placed on collagen-coated cover slips (*9, 17*) and incubated in Maximow depression-slide chambers at 35°C in a medium containing human placental serum and chick embryo extract (*17, 18*). The medium was changed twice weekly. Nerve growth factor (300 U/ml) was added to the medium to ensure optimal survival and growth of a large fraction of the DRG neurons (>1000 per ganglion). This led to enhanced innervation of dorsal regions of the attached spinal cord tissue (*9–11*), even when NGF was withdrawn after 4 days (*9, 18*). In the absence of exogenous NGF, > 90 percent of these DRG neurons degenerated. These results agree with the recent finding that exposure of rats and guinea pigs in utero to antibodies to NGF results in the selective destruction of up to 85 percent of DRG neurons and the destruction of sympathetic neurons (*19*). In contrast, injection of antibodies to NGF into postnatal rats leads to destruction only of sympathetic neurons (*7*).

In cultures exposed for several days to 1 to 2 μ*M* taxol (*20*) in the absence of exogenous NGF, most neurons degenerated, whereas in the presence of the NGF (300 U/ml), survival of DRG neu-

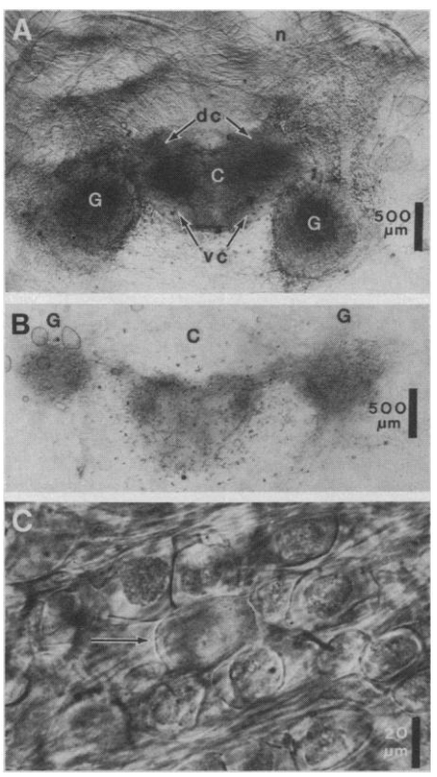

Fig. 1. Photomicrographs of cross sections of living spinal cord (~ 0.5 mm thick) with attached DRG's, explanted from 13-day-old fetal mice. (A) Explant cultured for 5 weeks in medium with NGF (300 U/ml) and exposed to 1 μ*M* taxol for 24 hours during days 4 to 5 in vitro. Neuronal development in DRG (*G*) and cord (*C*) tissues was not altered, although growth of Schwann cells was severely depressed. Dorsal (*d*) and ventral (*v*) regions of the cord contain abundant neurons. Note the profuse outgrowth of neurites (*n*) without Schwann cells. (B and C) Extensive degeneration of cord and DRG's in an explant where NGF was withdrawn from the culture medium for 3 weeks following the exposure to 1 μ*M* taxol during the fourth to eighth days in vitro. Nerve growth factor was present in the medium for 4 days prior to and during the exposure to taxol. The cord and DRG tissues have been reduced to thin layers and neuritic outgrowth is sparse. The number of neurons per DRG has been reduced from more than 1000 [see (A)] to less than 100. A surviving DRG neuron is indicated by the arrow in (C). The damage would have been even more severe had taxol been added at the time of explantation.

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rons was comparable to that in control cultures without taxol. During taxol exposure the DRG's showed some cytoplasmic aberrations and nuclear distortions, neuritic outgrowth was essentially blocked, and Schwann cell development was sharply depressed (*6, 21*). The spinal cord showed severe necrosis of glial cells and dose-dependent patterns of neuronal degeneration. When exposure to taxol and NGF was limited to 1 to 2 days, the explants showed characteristic organotypic features after recovery in NGF-supplemented medium (Fig. 1A), although the neurites, whose outgrowth resumed 1 to 2 days after drug withdrawal (*21*), remained remarkably free of Schwann cells for many weeks thereafter. In cultures exposed for 4 days to taxol and NGF and returned to medium without NGF, < 5 percent of DRG neurons (about 60 per ganglion) survived and spinal cord tissues were reduced almost to monolayers, with very few neurons (*22*). Comparable numbers of DRG neurons survive in control cultures grown without NGF (*9*). Even when the introduction of taxol was delayed until 4 to 8 days after explantation (to permit an initial period of NGF-stimulated DRG growth), similar widespread cytotoxic effects occurred if NGF was omitted after drug withdrawal (Fig. 1, B and C).

On the other hand, when NGF was maintained in the culture medium following a 4-day exposure to taxol, there was not only a > 90 percent survival of DRG neurons but also a remarkable survival of dorsal—but not ventral—spinal cord tissue (Fig. 2). Injection of horseradish peroxidase into the DRG's (*23*) in these taxol-exposed cultures showed that DRG neurites arborized profusely throughout the dorsal target regions of the attached cord and that only a few invaded the ventral cord (Fig. 2B)—as occurs regularly in control explants (*24*). Dorsal cord tissue in explants exposed to taxol for 4 days not only showed abundant neurons after return to high NGF but also characteristic dorsal horn network responses (*9–11*) could be evoked with DRG stimuli in bioelectric tests 2 weeks later.

Exposure of explants with only one attached DRG to taxol and NGF led to severe degeneration in a significant portion of the dorsal cord on the side lacking an attached DRG, even when a detached DRG was located close to the edge of the cord. Neurites from nearby detached DRG's were unable to project into the cord tissue since meningeal barriers had not been removed (*10, 17*). In cultures grown without NGF for the first week in order to reduce greatly the DRG input to the cord (*9*), severe degeneration of dorsal cord tissue followed a 4-day exposure to taxol, even though high concentrations of NGF were present during and after drug exposure. These data rule out the possibility of a direct effect of NGF on the survival of dorsal cord neurons.

Our results indicate that exposure of spinal cord tissue and DRG's from fetal mice to taxol greatly enhances the dependence of DRG neurons on NGF and the dependence of dorsal cord neurons on DRG neurite projections; in the absence of sustained increases in these extrinsic influences, taxol produces severe cytotoxic effects on DRG and dorsal cord neurons. Furthermore, in preliminary experiments in which 2- to 3-week-old, NGF-enhanced explants were exposed to taxol for 4 days, a fraction of the DRG neurons showed dependence on NGF for survival during and after drug exposure. Taxol appears therefore to alter DRG's so that even some of the more mature sensory neurons in culture become dependent on exogenous NGF. While NGF does not influence the survival of adult DRG neurons, these mature nerve cells show specific uptake and transport of NGF (*25*). Injecting NGF into neonatal and adult rats leads to increased levels of substance P in DRG's (*26–28*). Furthermore, injecting postnatal rats with antibodies to NGF, while no longer depleting the neuronal populations in DRG's, does decrease the content of substance P (*27*).

Our demonstration that NGF attenuates the neurotoxic effects of taxol on fetal DRG neurons in culture is of interest in relation to evidence that NGF prevents the cytotoxic effects of vinblastine on sympathetic ganglia in rodent neonates in vivo (*29*). Although taxol promotes the assembly of microtubules (*2, 3*) whereas vinblastine inhibits such assembly (*30*), the toxic effects of both drugs on these neurons may be due to similar alterations in the organization of microtubules or of other tubulin-associated systems—alterations that are counteracted by NGF.

Central neurons in deafferented spinal cord explants from rodent fetuses develop quite normally for long periods in control media (*13*), and dorsal horn neurons remain receptive to innervation by cocultured DRG neurons introduced after 2 to 3 weeks in vitro (*10, 11*). Taxol exposure of cord-DRG explants appears, therefore, to alter cord tissue so that dorsal cord neurons become dependent for survival and recovery on factors provided by NGF-enhanced DRG neurites projecting into the dorsal cord. Similarly, taxol exposure of DRG's may lead to enhanced dependence on trophic influences normally provided by the peripheral inputs to these sensory neurons (*14, 16*), for example, NGF (*8, 29*). Further studies are required to determine whether the altered requirements for survival of DRG and dorsal cord neurons after taxol exposure are due to direct effects of the drug on these nerve cells [for example, on microtubules or other tubulin-associated systems (*4–6*)] or to antimitotic actions on proliferating neuroglia and Schwann cells (*6*) that may block possible survival factors produced by these supporting cells (*8, 31*).

Irrespective of the mechanisms underlying taxol-induced alterations in organotypic cord-DRG explants, the experimental paradigm in this study clearly is useful for determining the effects of pos-

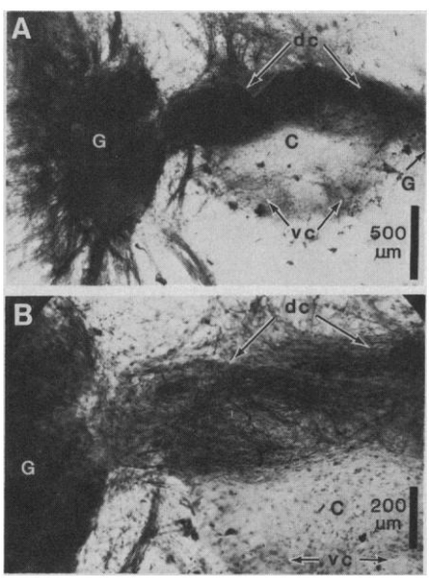

Fig. 2. (A and B) Photomicrographs showing survival of dorsal—but not ventral—spinal cord tissue in an explant where NGF was maintained in the culture medium for 7 weeks after exposure to 1 μ*M* taxol during the fourth to eighth days in vitro. The ventral region of the cord has been reduced to a thin layer, whereas the DRG and dorsal cord tissues have retained characteristic multilayered neuronal populations (the right DRG is out of the field). Horseradish peroxidase (HRP) was injected into the DRG's (*23*) to produce orthograde labeling of DRG neurites projecting into cord tissue (the explant was counterstained with toluidine blue). Note that the HRP-labeled neurites are profusely arborized throughout the dorsal cord and that few DRG neurites have invaded the ventral region, as in control explants (*24*). In (B) the monolayer of residual ventral cord tissue is out of focus. Abundant nerve cells were visible throughout dorsal cord tissue, whereas very few surviving neurons were seen in ventral tissue.

sible trophic factors provided by peripheral inputs on the survival of central nervous system neurons during normal development and in certain types of injury or neurologic disorders.

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22. Cell counts were made during microscopic observations of the living cultures with an oil-immersion objective lens (×40) and verified after fixation and staining.
23. Horseradish peroxidase was injected iontophoretically into the DRG's through glass micropipettes (tip diameter, 5 to 10 μm) filled with 20 percent HRP by applying two 100-msec pulses per second for 30 minutes. After 1 hour a modified Hanker-Yates histochemical procedure (*24*) was used prior to fixation (D. R. Friedlander and S. M. Crain, in preparation).
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Histocompatibility and Isoenzyme Differences in Commercially Supplied "BALB/c" Mice

Abstract. *BALB/c mice obtained commercially were found to differ significantly from the standard phenotype of BALB/c strain mice. Isoenzyme tests and H-2 haplotype analyses indicated that the majority of mice from two of the three sources tested appeared mixed, frequently heterozygous, and did not consistently express either the expected H-2 or glucose phosphate isomerase type.*

In experiments conducted by us during the past year, several hundred commercially bred BALB/c mice were obtained from Charles River Breeding Laboratories to supplement BALB/cAu females produced at the University of Wisconsin. These animals were used in long-term experiments whose aim was to produce chimeric mice of BALB/c ↔ CBA/T6 constitution by injecting CBA/T6 cells into BALB/c embryos. In the course of analyzing internal chimerism among the 48 mice that were ultimately born to foster mothers during the period of December 1980 to July 1981, it became apparent that there was a serious discrepancy in the phenotype of the majority of these mice. Normally, the two parental strains are characterized by differences in the isozyme glucose phosphate isomerase (GPI) and H-2 haplotype (BALB/c: *Gpi-1*^{a}, *H-2*^{d}; CBA/T6: *Gpi-1*^{b}, *H-2*^{k}), in addition to external pigmentation and chromosome markers. Electrophoretic analysis of the isozyme samples from the injected animals, derived from embryos of mice obtained from Charles River Breeding Laboratories, showed that the GPI pattern exhibited by the majority of the animals

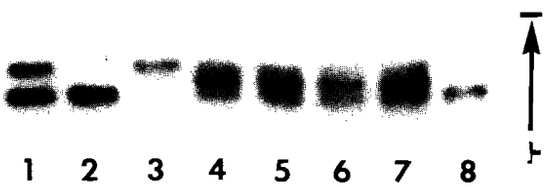

Fig. 1. GPI isozyme patterns of mice from inbred strain controls and of mice received from Charles River. Blood lysates were subjected to electrophoresis in tris-glycine buffer, *p*H 8.5, on cellulose acetate plates (Titan III, Helena Laboratories). (Slot 1) A 1:1 mixture of blood lysates from BALB/cAu and CBA/T6Au; (slot 2) BALB/cAu; (slot 3) CBA/T6Au; (slot 4) BDF1; and (slots 5 to 8) four different BALB/c animals received from Portage.

Table 1. MLC reactions among individual BALB/c mice from different facilities.

Stimulating cell*	Responding cell incorporation of tritiated thymidine†	
	BALB/c-IRC-M	B10.M
BALB/c-IRC-M	6,312 ± 2,053	63,952 ± 7,101
BALB/c-S_1	51,354 ± 5,983	63,091 ± 6,374
BALB/c-S_2	4,412 ± 1,938	35,870 ± 10,790
BALB/c-S_3	108,155 ± 10,792	55,353 ± 5,358
BALB/c-P_1	125,802 ± 25,739	47,909 ± 12,277
BALB/c-P_2	8,286 ± 2,018	70,973 ± 6,436
BALB/c-P_3	5,954 ± 1,046	57,644 ± 6,684
BALB/c-W_1	6,389 ± 1,552	56,479 ± 7,754
BALB/c-W_2	6,250 ± 1,830	59,068 ± 5,679
BALB/c-W_3	8,170 ± 1,136	37,238 ± 6,706
B10.M	123,045 ± 27,737	4,850 ± 1,717

*IRC-M, BALB/c^J colony, Immunobiology Research Center, University of Minnesota; S, C.R. Stoneridge; P, C.R. Portage; W, C.R. Wilmington; B10.M, H-2^f allogeneic control. †Results are given as number of counts per half-minute ± the standard deviation. Average of six replicate cultures of 5×10^5 stimulating spleen cells plus 5×10^5 responding spleen cells. Stimulating cells were exposed to x-rays (2500 roentgen) prior to mixing with responding cells. Tritiated thymidine was added on day 5 for 6 hours before assay.

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