Careful examination of a number of preparations led to this interpretation of the fluorescent surface markings as rents. In some cases the entire outer surface, as well as the exposed edges, appears somewhat fluorescent with only the exposed under layer, the "holes," being dark. These rents in the hyphal surface are more frequently seen on older and larger hyphae and may arise during the increase in the diameter of the filament. Most tip regions remain free of the fluorescent patterns; however, it is not unusual to find lengths of a single hypha with a fissured area intercalated between two nonfluorescent areas, and with no obvious differences in diameter. The torn appearance is marked in mycelia which have been placed in water for the induction of zoosporangia and in mycelia which have been washed and stored at 4°C. It should be emphasized that in all older cultures where the hyphae show an adsorption of nonspecific labeled serum, the rhizoids never fluoresce, again a demonstration of a difference in surface properties between the two structures.

It thus appears that there are changes in surface properties of hyphae with age, apparently due to exposure of underlying constituents, and that at least some of the hyphal components differ from those present on the rhizoidal surface. Possibly these dissimilarities are a reflection of functional differences between the two structures, and they might be expected if the supposition is true that rhizoids effect nutrient uptake for the organism. The observations herein not only raise the question of the nature of the unique hyphal component or components but the more intriguing possibility that different parts of a wall with a continuous substructure can synthesize different superficial compounds.

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Regeneration in Spinal Neurons: Proteosynthesis Following Nerve Growth Factor Administration

Abstract. *Incorporation of H^3-leucine into dorsal root ganglion cells in rats was markedly increased over that of controls following section of sciatic and femoral nerves. Crush lesion of dorsal roots did not increase the H^3-leucine uptake of these cells except in animals which had received nerve growth factor after the operation.*

Regeneration of nerve cells involves a high rate of nucleoprotein production in the nerve cell related to increased functional demands (*1*), and increased uptake of S^{35}-methionine (*2*) and C^{14}-orotic acid (*3*) has been found during regeneration. Section of motor nerves close to the spinal cord has also resulted in early increase in incorporation of S^{35}-methionine into motor horn cells (*4*), which suggests a close relation between the early intrinsic changes in the neuron and the regenerative process.

Section of the peripheral process of the sensory neuron is followed by active regeneration, but this is not true after interruption of the central process (*5*). A comparable inequality in chromatolytic response of the dorsal root ganglion cells has also been observed (*6*). In our study we sought a basis for this differential regenerative response between the peripheral and central processes of sensory neurons through measurement of protein synthesis.

Marked enhancement of the regenerative response of sensory neurons results from application of nerve growth factor (NGF)(*7*) both in the living chick embryo and in tissue culture, but this result was confined largely to the medio-dorsal cell group of the dorsal root ganglion (*8*). Although this effect of NGF is most pronounced in developing neurons, a regenerative enhancement has been observed in the dorsal funicular axons of kittens 6 to 8 months old (*9*). Augmented growth of sympathetic neurons in the chick embryo has been closely correlated with protein synthesis (*10*). Such initial proliferative growth in the embryo can be contrasted with regenerative growth in the adult by comparing relative rates of protein synthesis. In the second part of this study we examined the effect of NGF on protein uptake by dorsal root ganglion cells 6 days after crush of their central or peripheral processes.

Rats, 1 month old and weighing approximately 100 g each, were used in all experiments, and each experimental procedure and its control employed animals from the same litter. One group of four animals sustained unilateral high section of the sciatic and femoral nerves, the unoperated side serving as control. For comparison, section of dorsal roots L2 to S1 close to the spinal cord was performed on one side in a separate group of four animals.

The second procedure employed unilateral crush lesions (using watchmakers' forceps) of either the central or peripheral process of dorsal root ganglion cells in groups of four animals each. Location and extent of the lesions were similar to those in the first procedure, the normal side serving as control. To examine the effect of NGF, one group submitting to each procedure received this substance for 6 days, while a similar group remained untreated. Each animal received 1 ml per day of a buffered solution of NGF in saline that contained 2.3×10^5 biological units of activity per milliliter. Six days after the operative procedure, H^3-leucine (specific activity, 576 mc/mmole) was injected intraperitoneally at a dosage of 6 mc/100 g of animal weight. Three hours after injection, the dorsal root ganglia of L4 and L5 were removed and fixed in a solution containing 6 percent formol and 0.5 percent trichloroacetic acid. Quantitative evaluation of the autoradiograms was carried out by visual grain counting. In each ganglion the number of grains in 10 large cells at the level of the nucleus was counted and was calculated per unit surface area of the cell (*4*).

Results of these experiments are summarized in Table 1. Each value expresses the average grain count determined in 80 cells. A significant increase (32 ± 5 percent; $P < .001$) in the number of grains per unit area

Table 1. Comparison of grain counts in normal and regenerating neurons. Values given in number of grains per μm^2 in dorsal root ganglion cells obtained from histautoradiographic studies with H^3-leucine. Unilateral section, or interruption by crushing, of the sciatic and femoral nerve [in experiments I (a), II (a) and (b)] or dorsal roots [in experiments I (b), II (c) and (d)] was performed on groups of four animals each. In experiment II, two groups of animals were treated with NGF; two groups served as controls. Grains were counted in about 20 nerve cells on the control and experimental sides in each animal.

Experiment	Control side (grains per μm^2)	Experimental side (grains per μm^2)	Change in control (%)
I (a) Peripheral nerve section	2.31 ± 0.26	3.06 ± 0.09	+32 ± 5.6*
(b) Dorsal root section	2.82 ± .08	2.76 ± .09	−2 ± 3†
II (a) Peripheral nerve crush, untreated animals	2.92 ± .09	3.00 ± .1	+2.5 ± 2†
(b) Peripheral nerve crush, treated animals	2.56 ± .1	2.69 ± .1	+4.5 ± 1.8†
(c) Dorsal root crush, untreated animals	2.8 ± .1	2.8 ± .1	0
(d) Dorsal root crush, treated animals	2.1 ± .1	2.4 ± .1	+14 ± 4*

* $P < .001$. † Not significant.

of dorsal root ganglion cells was observed after section of sciatic and femoral nerves. No corresponding increase was found after section of dorsal roots. These observations show a marked difference in the rate of incorporation of H^3-leucine into cells of the dorsal root ganglion, depending on whether operative section interrupted their peripheral processes or their dorsal roots.

Crushing sciatic and femoral nerves (Table 1, exp. II) did not increase the incorporation into the dorsal root ganglion cells either in animals treated with NGF or in the control group. This was surprising in view of the effect of nerve section, but the consistency of grain counts in each case suggests that a significant difference exists in the regenerative response of the cell after cutting or crushing its peripheral process.

A crush lesion of dorsal roots did not result in increased incorporation, in agreement with the result from root section. However, administration of NGF following a comparable lesion produced a significant increase (14 ± 4 percent; $P < .001$) of H^3-leucine incorporation into dorsal root ganglion cells.

The increase in incorporation of H^3-leucine into dorsal root ganglion cells which is observed after section of sciatic and femoral nerves is in agreement with chromatolytic changes (*6*). Neither increased incorporation nor chromatolysis has been observed after dorsal root section in untreated animals. Comparable measurements of the rate of regeneration in central and peripheral processes of dorsal root ganglion cells showed a positive response to operative section only in the latter case (*5*).

Operative section of a neural process is characterized by protoplasmic outflow. Its significance to cell metabolism may depend on its absolute magnitude and the residual intact cellular processes (*11*). A marked increase in protein synthesis would be expected after section of the long single process of motor cells, and this has been observed (*4*). On the other hand, dorsal root ganglion cells, which have two processes, showed a significant increase in uptake of H^3-leucine only after section of their long, large-diameter, peripheral processes; section of the central process only deprived the cell of a small fraction of its total protoplasm.

Protoplasmic outflow from peripheral nerve after operative section would be expected to exceed that resulting from a crush lesion. Increased H^3-leucine incorporation was observed after section of sciatic and femoral nerves, but not when the same nerves were crushed, which suggests that enhanced regenerative proteosynthesis was related to rate of protoplasmic depletion of the cell. A greater degree of chromatolysis has also been observed after cell processes were cut than after the same processes were crushed (*6*).

Large variations in the effect of NGF on neurons of differing types have been shown by Levi-Montalcini (*8*). Correlative with this is the fact that the site of action of this material is unknown. There are many reasons to believe that the nerve processes contained in sciatic and femoral nerve differ significantly from dorsal root fibers in their relation to the cell body in the dorsal root ganglion. It is not surprising, therefore, that crushed sciatic and femoral nerves respond differently from crushed dorsal root fibers to the influence of NGF.

Evidence for enhanced regenerative growth of afferent neurons in the dorsal funiculus has been observed in kittens following administration of NGF (*9*). If this were due to increased proteosynthesis of the dorsal root ganglion cell an increase in incorporation would be expected. Our experiments show a significant increase in uptake of H^3-leucine in NGF-treated rats after crushing of the dorsal roots. No such increase was observed either in animals with peripheral nerve lesion or in the untreated control group. Thus, one factor contributing to failure of central regeneration in untreated animals may be lack of adequate stimulation of proteosynthesis in the dorsal root ganglion cell following interruption of its central process. Our observations demonstrate the effect of NGF in enhancing protein synthesis in adult sensory nerve cells while the central process of this cell was responding to a preceding crush lesion.

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