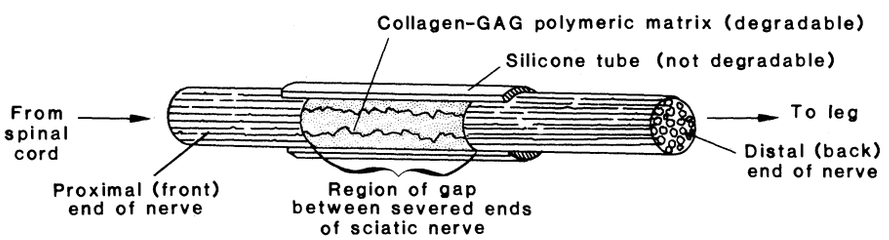
What Makes Nerves Regenerate?

Many experimental strategies revolve around a central issue: What conditions promote regeneration and functional recovery in mammalian nerves?

Damage to nerves often results in a permanent loss of function because the tissue fails to regenerate. This is a particular problem in the mammalian spinal cord and brain. But now it seems that the regenerative capacities of both the peripheral nervous system (PNS) and the central nervous system (CNS) are considerable, if the appropriate conditions are provided.

Research groups headed by Ioannis Yannas at the Massachusetts Institute of Technology (MIT) and Richard Sidman at the Children's Hospital, Harvard Medical School, have worked independently to devise strategies for enhancing the regeneration of peripheral nerves (those outside the spinal cord and brain). Each group has induced nerve fiber outgrowth over a distance of 15 mm or more by using tubes that allow the infiltration of nonneuronal cells whose presence is essential for extensive regeneration.



Cutaway view of severed sciatic nerve regenerating through a synthetic tube used by Yannas and his colleagues (used with permission of I. Yannas).

At the same time, Albert Aguayo and his colleagues of McGill University in Montreal, Quebec, have demonstrated that CNS axons have an extensive ability to regenerate in the presence of peripheral nerve grafts. Aguayo's recent work is with the eye, and it illustrates two important phenomena: damaged CNS fibers can regrow through a PNS graft over a distance greater than normal, and some of the regenerated axons regain functional properties.

Still another approach is to identify the cell types and chemical substances in the peripheral nervous system that support regeneration. This is a direction explored independently by Hans Thoenen of the Max Planck Institute of Psychiatry in Martinsreid, West Germany, Paul Patterson at California Institute of Technology in Pasadena, Randy Pittman of the University of Pennsylvania School of Medicine in Philadelphia, and also by Sidman's group at Harvard. Thoenen's most recent findings indicate that cells from the PNS form a "permissive" substrate for nerve regeneration, but CNS cells form a "non-permissive" substrate. Patterson and Pittman have identified a potential role for protease enzymes and their inhibitors in axon elongation.

Investigators generally agree that severed peripheral nerves, such as the sciatic, which leaves the spinal cord to innervate the leg, will sprout to form the beginnings of new fibers. But some assistance is required to guide the direction of sprouting, if the axons are to make specific functional reconnections with their normal target tissues. One way of directing axonal regrowth is to provide a tube through which cut fibers can grow across a gap. In 1982, Goran Lundborg of Göteborg University in Sweden, Silvio Varon of the University of California at San Diego, and their co-workers described the use of a silicone tube that allowed rat sciatic nerve regeneration across a 10-mm gap (*1*).

Yannas, a polymer chemist at MIT, and Jerry Silver, a neurobiologist at Case Western University in Ohio, induced more extensive regeneration "by restructuring the space inside the guiding tube." Also involved in the project were Dennis Orgill of MIT, Thor Norregaard and Nicholas Zervas of Massachusetts General Hospital, and William Schoene at the Brigham and Women's Hospital in Boston. In a presentation to the American Chemical Society, held in Chicago in September, Yannas reported rat sciatic nerve regeneration across a 15-mm gap using a silicone tube packed with a protein, collagen, and a glycosaminoglycan (GAG) polysaccharide, chondroitin-6-sulfate. These materials are cross-linked to form a porous network that is degradable by enzymes at rates that can be controlled during preparation, although the silicone tube itself is not biodegradable.

Yannas originally developed the polymer in 1975 to promote skin regrowth over an area that had been severely burned. His primary objective with both nerve and skin was the same. He wanted to find a material that was capable of enhancing regeneration without using an autograft and could also be manufactured easily. He believes his most important finding is that almost identical materials stimulate the growth of both nerve and skin, tissues that are very different. The regenerated nerve tissue looks healthy: it is highly vascularized and about 20 percent of the axonal fibers are surrounded by a fatty myelin sheath produced by Schwann cells that have migrated into the tube.

Meanwhile Roger Madison, Ciro Da Silva, and Pieter Dikkes in Sidman's department induced sciatic nerve regeneration over a 20-mm gap through tubes lined with either a collagen matrix or a laminin-containing gel. Their work was described at the Annual Meeting of the Society for Neuroscience*.

Laminin is a glycoprotein associated with the basement membrane (basal lamina) of Schwann cells and is part of the normal substrate for nerve fiber outgrowth during development. Sidman, Earl Henry, and their colleagues at Harvard, in collaboration with Tri-Ho Chin of Allied Corporation, have developed nontoxic nerve guide tubes. The tubes are filled with gels rich in laminin and other extracellular matrix materials. These materials stimulate greater numbers of regenerating axons (many of which become myelinated), faster outgrowth of fibers, and the ability of regenerating axons to span longer gaps (*2*,*3*).

*The Annual Meeting of the Society for Neuroscience was held in Dallas from 20 to 25 October. The Neurobiology of Disease Workshop on Regeneration preceded the meeting.

Sidman also notes that the presence of the distal stump at one end of the tube is critical for regeneration. He believes that the "tube isolates the local milieu," thus establishing conditions that encourage fiber outgrowth. The "milieu" consists of all the tissues invading the tube, including nerve fibers, fibroblasts, blood vessels, macrophages, and Schwann cells, as well as any growth-promoting factors they may secrete and any extracellular matrix material they produce. David Shine, Paul Harcourt, and Sidman are now in the process of defining the role of the distal stump and of identifying which cellular components and their products are essential to add (to a blind-ended tube lacking a distal stump) to allow fiber regeneration.

Although significant progress has been made in the field of peripheral nerve regeneration, inducing regrowth of central nervous system axons has been especially difficult. Until recently, most researchers believed that differentiated central nerves were incapable of regenerating after an injury. However, Aguayo, M. Vidal Sanz, and their colleagues at McGill University capitalized on the greater regenerative capability of the peripheral nervous system to induce fiber outgrowth in the central nervous system (*4*).

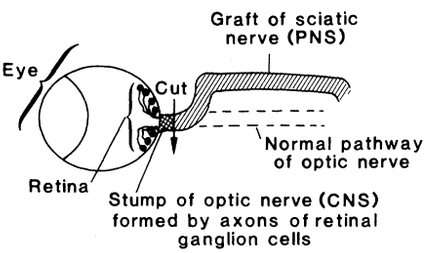
In recent work with Susan Kierstadt and Michael Rasminsky, they removed a section of the optic nerve (a CNS fiber tract that carries visual information from the eye to the brain) and replaced it with a section of sciatic nerve (PNS). New CNS optic nerve fibers grew into the PNS graft. The greatest regenerative response occurred when the CNS lesion and the PNS graft were made very close to the cell bodies of the retinal ganglion neurons, whose axons form the optic nerve.

Aguayo's group reported their latest results at the Society for Neuroscience meeting. They showed that severed axons of adult rat retinal neurons can regenerate through an entire 20- to 30-mm PNS graft, a distance about twice the length of a normal optic nerve. Significantly, they were also able to demonstrate some restoration of physiological function.

By using light to stimulate specific receptive fields in the retina, they produced electrical activity in regenerated axons that had been teased from the PNS grafts. The electrical responses were "indistinguishable from normal responses." Although this does not indicate that sight was restored in the experimental animals, these new findings are especially significant. Additionally, it seems to be important to the growing axons that they find their normal target in the brain, because axons denied access to target tissue eventually lose physiological function.

The mechanism by which peripheral nervous tissue encourages CNS fiber outgrowth is still an open question, but differences in the chemical composition of the substrates for the two tissues appear to be crucial. Support for this view comes from Thoenen and Martin Schwab, also in Martinsried, West Germany. They report that neurons will choose a peripheral nerve substrate rather than a CNS substrate for extending new fibers, possibly because adult central nerve tissue contains certain substances that inhibit growth (*5*).

Schwab and Thoenen used dissociated rat sympathetic or sensory ganglion cells



Side view of the eye and optic nerve showing sciatic nerve graft used by Aguayo and his colleagues.

growing in culture and gave the neurons a choice. They could extend axons along a "bridge" of either optic (CNS) or sciatic (PNS) nerve explants. When possible, axons grew along other axons, forming bundles of fibers. Besides this tendency to "fasciculate," the consistent choice for both sympathetic and sensory axons was to extend along the peripheral nerve explant and to avoid the CNS explant completely.

Specifically, nerve fibers grew along Schwann cell surfaces and the basal lamina. They made the same choice even if the explanted tissues were not living. According to Schwab and Thoenen, this points to "the presence of a nonpermissive substrate in the CNS" that persists even after the cells producing it have died.

The tissue culture conditions were optimized for extensive fiber outgrowth by adding nerve growth factor. Because the growth conditions were so ideal, Schwab and Thoenen cite the failure of axons to grow along the CNS explant as evidence that, "in the differentiated CNS, inhibitory substrate molecules should be considered."

In presentations at the Neurobiology of Disease Workshop on Regeneration and also in a symposium at the Society for Neuroscience meeting, Paul Patterson summarized further evidence that certain materials outside cells, in the extracellular matrix, are essential for fiber outgrowth.

Two components of the extracellular matrix that appear to support neuronal outgrowth are the protein, laminin, and a proteoglycan, heparin sulfate. These materials form a complex in the extracellular matrix, which is associated with Schwann cells in the peripheral nervous system but not in the central nervous system. Patterson explained that, in order to grow, axons must be able to both attach to their substrate and detach from it.

Citing work done in collaboration with Randall Pittman, Patterson offered an intriguing hypothesis by which growing axonal tips control their own behavior, providing there is an appropriate substrate.

Pittman studied the elongation of rat sympathetic ganglion axons growing in culture (*6*) and reported his most recent results at the Society for Neuroscience meeting. Axons appear to modulate their own growth by producing substances that alter their attachment to the substrate. Patterson and Pittman suggest that this modulation is achieved by a balance between certain enzymes and their inhibitors that exist in the local environment of growing neurons. With such a balance, a cycle of attachment and detachment of growing axons could be explained.

Thus, there is increasing evidence for cell-cell interactions and cell-substrate interactions during regeneration of differentiated nerve tissue. The interactions may be positive, or negative, or absent. What was not apparent before is that nerve cells in the mammalian central and peripheral nervous systems can regenerate if they are given the right environment, and that some of their functional characteristics can be preserved. Ultimately, it must be shown that regenerated axons connect in a specific manner with their target tissues, and that normal stimulation of the regenerated nerve produces an appropriate response.

—DEBORAH M. BARNES

References and Notes

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