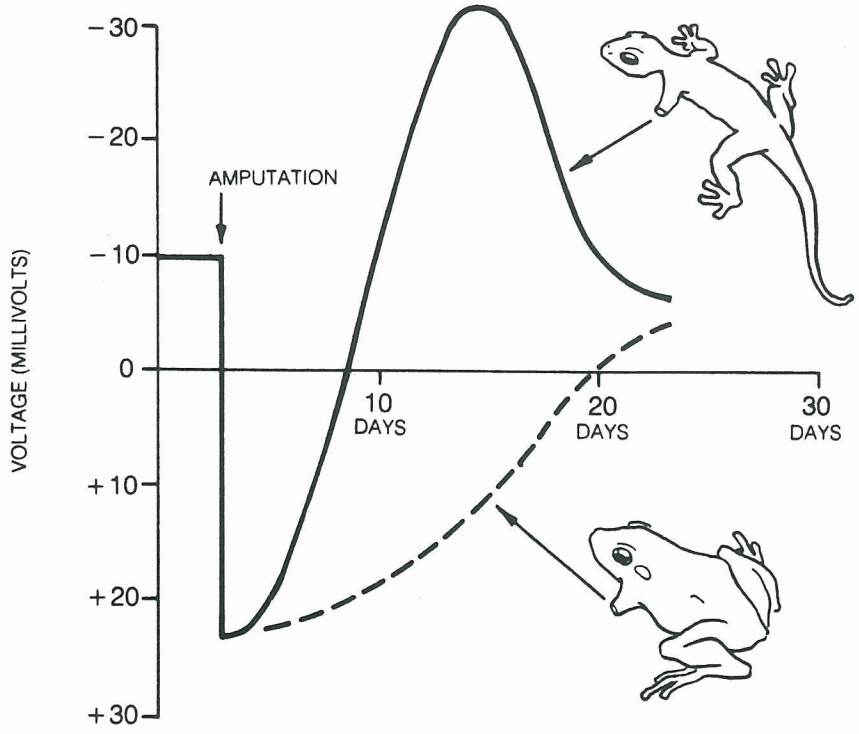
I made measurements daily, expecting to see the salamander voltages climb above those of the frogs as the blastemas formed. It didn't work that way. The force of the current flowing from the salamanders' amputation sites rapidly dropped, while that from the frogs' stumps stayed at the original level. By the third day the salamanders showed no current at all, and their blastemas hadn't even begun to appear.

The experiment seemed a failure. I almost quit right there, but something made me keep on measuring. I guess I thought it would be good practice.

Then, between the sixth and tenth days an exciting trend emerged. The salamander potentials changed their sign again, exceeding their normal voltage and reaching a peak of more than 30 millivolts negative just when the blastemas were emerging. The frogs were still plugging away with slowly declining positive voltages. As the salamander limbs regenerated and the frog stumps healed over with skin and scar tissue, both groups of limbs gradually returned (from opposite directions) to the original baseline of 10 millivolts negative.

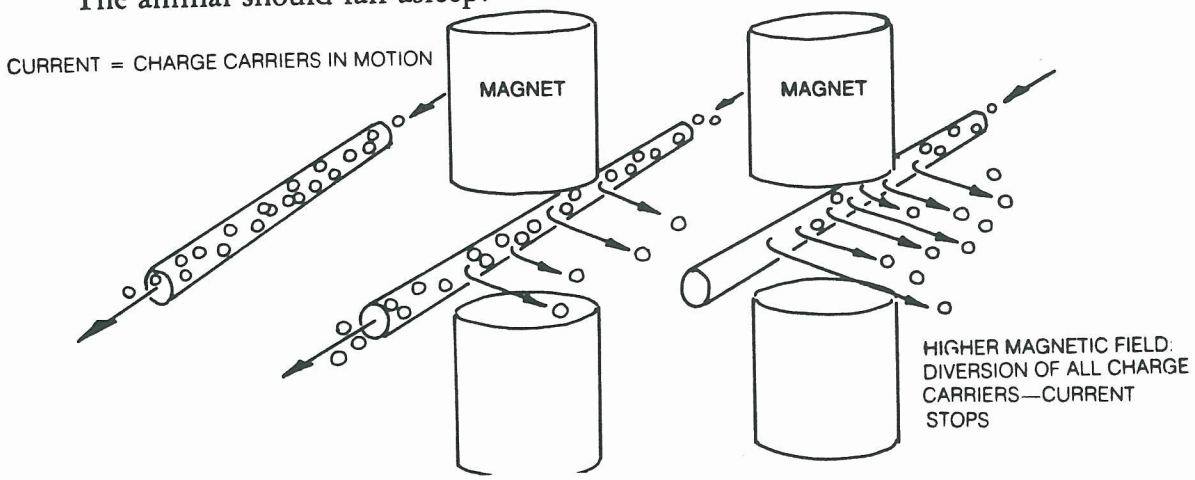


THE CURRENT OF INJURY: SALAMANDER VERSUS FROG

Here was confirmation better than my wildest dreams! Already, in my first experiment, I had the best payoff research can give—the excitement of seeing something no one else had ever seen before. I knew now that

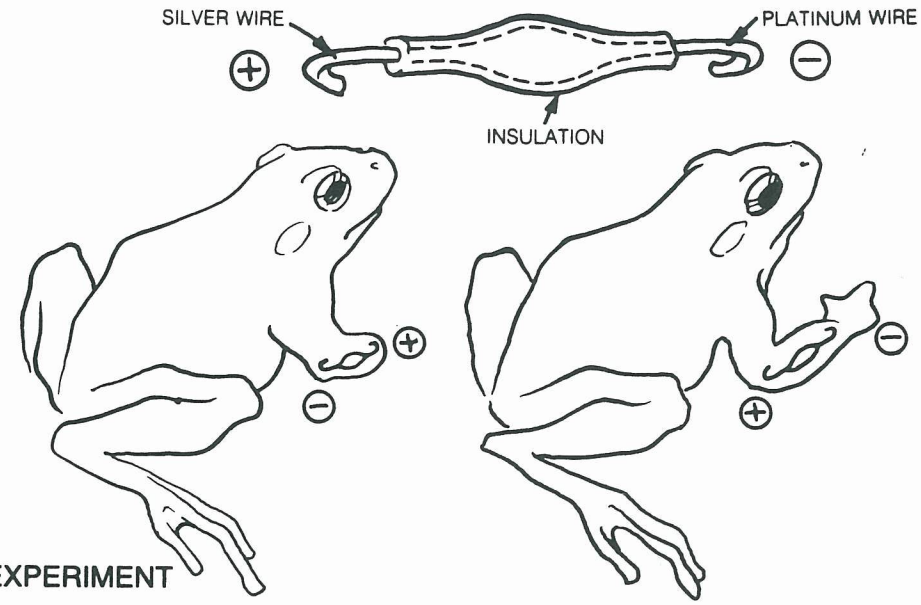
slow waves. Moreover, we could exert the same control from outside by putting current of each type into the head. This was exciting. It opened up vast new possibilities for a better understanding of the brain. It was still on the edge of respectability, too, since it was a logical consequence of the work done by Gerard and his co-workers. The next experiment was harder to believe, however.

I figured the brain currents must be semiconducting, like those in the peripheral nerves. I thought of looking for a Hall voltage from the head but reasoned the brain's complexity would make any results questionable. Then I thought of using the effect backward, so to speak, measuring a magnetic field's action on the brain rather than on the production of the Hall voltage. Since the Hall voltage was produced by diverting some of the charge carriers from the original current direction, a strong enough magnetic field should divert all of them. If so, such a field perpendicular to the brain's midline current should have the same effect as canceling out that normal current with one applied from the outside. The animal should fall asleep.



THE HALL EFFECT—A TEST FOR SEMICONDUCTING CURRENTS

We tranquilized a salamander lightly, placed it on a plastic shelf between the poles of a strong electromagnet, and attached electrodes to measure the EEG. As we gradually increased the magnetic field strength, we saw no change—until delta waves appeared at 2,000 gauss. At 3,000 gauss, the entire EEG was composed of simple delta waves, and the animal was motionless and unresponsive to all stimuli. Moreover, as we decreased the strength of the magnetic field, normal EEG patterns returned suddenly, and the salamander regained consciousness *within seconds*. This was in sharp contrast to other forms of anesthesia.



SMITH'S EXPERIMENT

Smith implanted his wires along the bone remnant, with one end bent over into the marrow cavity. The limbs with the positive silver electrode at the cut showed no growth, and in some cases tissue actually disintegrated. The negative platinum ends, however, started regeneration; the new limbs all stopped growing at about the same distance from the device, suggesting that regeneration might have been complete if the batteries had been able to follow along. In 1974 Smith made a device that could do just that, and achieved full regrowth.

Despite Smith's success, there was no reason to suppose that his method would work in mammals. One researcher had recently noted some regeneration in the hind legs of newborn opossums, but, since marsupials are born very immature and develop in the mother's pouch to a second birth, we suspected that this was merely a case of embryonic regrowth. Most fetal tissues were known to have some regenerative ability while they weren't yet fully differentiated. Richard Goss had shown that the yearly regrowth of deer and elk antlers was true multitissue regeneration, but this feat seemed too specialized to make us confident about restitution in other mammals or other parts of the body.

Many thought all such attempts were doomed, because the process of encephalization had progressed much further in mammals than amphibians. All vertebrates were known to have roughly the same ratio of nerve tissue to other kinds of tissue, but in mammals most of the limited nerve supply went into the ever more complex brain, until, as Singer had shown in a recent study, the proportion of nerve to other tissue in rat legs was 80 percent less than in salamander legs. This was well below the critical mass needed for normal regeneration, and we thought it might be impossible to make up the difference artificially.

Even if we could supply the proper electrical stimulus, we weren't sure there would be any cells able to respond to it. Mammalian red blood cells had no nuclei, so they couldn't dedifferentiate. Based on our work on bone healing in frogs, we suspected that immature red corpuscles in the bone marrow might take over, but perhaps they were programmed to dedifferentiate only for fracture healing. Even if they would respond to an external current, we wondered whether there were enough of them to do the job.

There was also the problem of complexity. Many regeneration researchers believed that mammalian tissues had become so specialized and complicated that they'd simply outgrown the control system. Maybe it couldn't handle enough data to fully describe the parts needed. If so, any blastema we produced would just sit there, not knowing what to make.

A First Step with a Rat Leg

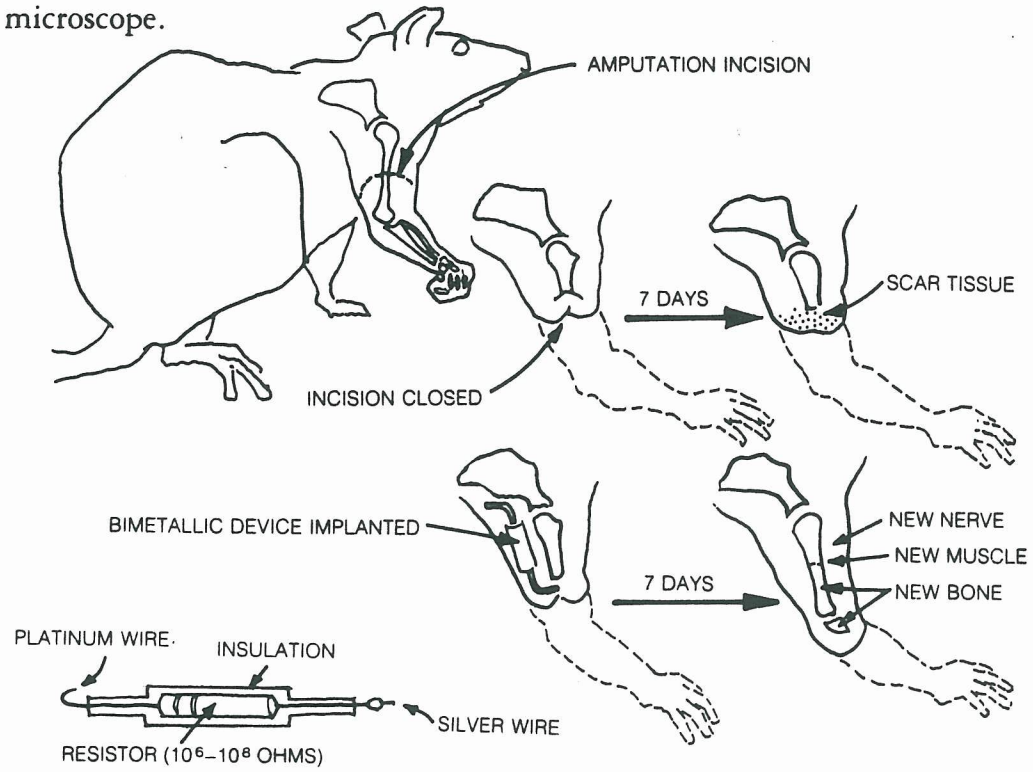
I tested the kind of silver-platinum couplings Smith used and found they delivered several times too much current for ideal dedifferentiation, according to our frog experiments. Joe Spadaro, another of Charlie's grad students, suggested that we put carbon resistors between the two metals, giving us devices of various current levels.

In 1971, Joe and I amputated the right forelegs of thirty-five rats. We made the cuts through the upper foreleg well away from the elbow so that only the bone shaft, which had long ago ceased growing, would remain at the tip. We used all males, to obviate as many hormonal variables as possible. As controls we treated some of the stumps with no device, or one made of a single metal, or one with the silver positive end facing the stump. We did the actual test on twenty-two of the rats, implanting our batteries with the negative platinum electrode at the wound. We tucked the outer electrode into the marrow cavity and sutured the inner one to the skin of the shoulder.

We had an answer fast. After three days the stumps of the controls had begun to heal over or even, in the case of the highest-current couplings, die back a little behind the amputation line. But the experimental legs with our medium-current devices, supplying 1 nanoamp, were doing well. In a week, nearly every one had a well-formed blastema and seemed ready to replace the whole limb.

Since healing is very fast in rats, and because we wanted a uniform sample for our first test, we sacrificed all of the controls and most of the test animals at this time, although we spared a few for a month. We cut

off the entire healing limb, then fixed, stained, and sectioned it for the microscope.



DC STARTS LIMB REGENERATION IN RATS

I shall never forget looking at the first batch of specimens. The rat had regrown a shaft of bone extending from the severed humerus. At the proper length to complete the original bone there was a typical transverse growth plate of cartilage, its complex anatomical structure perfectly regular. Beyond that was a fine-looking epiphysis, the articulated knob at each end of a limb bone. Along the shaft were newly forming muscles, blood vessels, and nerves. At least ten different kinds of cells had differentiated out from the blastema, and we'd succeeded in getting regeneration from a mammal to the same extent as Rose, Polezhaev, Singer, and Smith had done in frogs.

Slides from some of the other animals were even more spectacular. One stump had two cartilaginous deposits that looked like precursors of the two lower arm bones beyond a fully formed elbow joint. All of the regenerates were bent toward the electrode, and in one the lower humerus had formed alongside the old shaft rather than as an extension of it, but otherwise its structure was quite normal.

With one exception, slides from longer than a week were less exciting. They seemed to have gotten *less* organized as time went on. Behind one of these older blastemas, however, at the end of a nearly unformed

ghost of a bone, we found cartilage in a five-fingered shape—this limb had begun to grow a hand.

In general, though, it looked as if the current had to be of a certain duration as well as a certain strength. This was no less disappointing to us than it was to the *Life* photographer who visited the lab at that time and wanted before and after shots with a rat playing the piano at the end, but nonetheless we were very pleased. Since the blastemas always formed around the electrodes and since redifferentiation proceeded into organized tissue, we knew the current had stimulated true regeneration, not some abnormal growth. Mammals still had the means for the orderly reading out of their genetic instructions to replace lost parts. We would simply have to learn more exactly the electrical requirements of the whole process, then make devices to supply the proper current at the proper time in the proper place.

When we published our results, it was hard to shroud our excitement in the circumspect scientific jargon needed. We wrote that we'd activated true, though partial, regeneration with a minuscule direct current and that the marrow cells seemed to be the source of the blastema. I thought this claim was sober enough. Joe and I cautioned that other factors had yet to be studied. Most important, we warned that if such a tiny force could so easily switch on growth, it must be very powerful, and we'd best know it thoroughly before using it routinely on humans, lest we give them unwelcome growths—tumors.

I felt that, within the constraints of scientific propriety, we'd uttered a rousing call for a big research push to open up the benefits of regeneration to humans. It must have been a whisper, though, for it caused no more ripples than a feather settling on a frog pond.

Philip Person, a dental surgeon at the Brooklyn VA hospital and a friend whom I'd known for years, asked me to present our results to the New York Academy of Medicine. Before the academy would permit this, however, it insisted that two experts must visit the lab and look at the actual data. One was Marc Singer, who enthusiastically agreed that we'd really started regeneration in the rat. The other man was totally negative, but he wasn't a specialist in regeneration, so the academy permitted me to speak.

Singer was one of the few who showed much enthusiasm when I'd finished reading my paper at the meeting. Most of the audience was unresponsive; there were few comments or criticisms. To these people, electric growth control was still a vitalistic impossibility, and they seemed unwilling to discuss dedifferentiation. The man who'd visited our lab with Singer complained that the amount of new growth was

small. Phil pointed out that it wasn't the quantity but the quality of new tissue that was important, especially in such a short time. Singer, convinced of the paramount importance of the nerves, thought the current might be stimulating them rather than directly causing dedifferentiation, but still thought the experiment was a big step forward. Nevertheless, it wasn't even attempted again until seven years later, when Phil Person himself took on the task; he, and later Steve Smith, confirmed our findings with even better results.

Meanwhile, buried in the literature we found reports that others had *already* observed some regeneration in mammals. In 1934 Hans Selye, the famous researcher into the effects of stress, discovered that a rat's limbs could partially regenerate of their own accord when the animal was two to five days old. Five years later Rudolph F. Nunnemacher of Harvard confirmed Selye's observation. Nunnemacher, however, ascribed the growth to a remnant of the epiphyseal plate. The growth-plate cells, he thought, simply might have kept on growing as normal in the adolescent animal. Selye replied that he'd specifically made sure to amputate the limbs high enough to get all of the epiphyseal plate so he could be certain that any growth was regenerative.

Thus Joe and I found that we'd really just extended the age limit for regeneration in the rat. Indeed, two years later Phil Person showed that even the young adult rats we'd used occasionally exhibited some regrowth, a fact that had puzzled us in a couple of our control animals. So, to be exact, our electrodes had temporarily but drastically boosted the efficiency of the process as it normally waned with age in the rodent. Still, it was the first time that had ever been done in a mammal.

Childhood Powers, Adult Prospects

The amputation of a fingertip—by a car door, lawn mower, electric fan, or whatever—is one of the most common childhood injuries. The standard treatment is to smooth the exposed bone and stitch the skin closed, or, if the digit has been retrieved and was cleanly cut, to try to reattach it by microsurgery. The sad fact is that even the most painstaking surgery gives less than optimal results. The nails are usually deformed or missing, and the fingers are too short and often painful, with a diminished or absent sense of touch.

In the early 1970s at the emergency room of Sheffield Children's Hospital in England, one youngster with such an injury benefited from a clerical mixup. The attending physician dressed the wound, but the cus-

ing the proper current, and the ability of electrically injected silver ions to dedifferentiate fibroblasts now gives us a possible method for producing an adequate blastema. We should now be able to supply the requirements for phase one in humans. Once this is done, the body itself can probably take care of phase two, even though we don't understand the process. Fingertip regrowth in children suggests that our bodies still have the ability to redifferentiate the cells and organize the missing part, as long as the electrical stimulus and the supply of sensitive cells are sufficient.

Microsurgeons have performed wonders in reimplanting cleanly severed portions of arms, legs, and fingers, but these limbs are subject to atrophy and obviously can't be grafted if they're too badly mangled or riddled with disease. As one who has performed too many amputations in his time, I find the prospect of being able to give a patient the real thing instead of a prosthesis tremendously exciting. There's a good chance that we'll eventually treat some nongenetic birth defects or old injuries by cutting off the defective part and inducing a normal one to grow. Perhaps, combined with gene splicing, such techniques could even rectify genetic birth defects.

Since no one has yet achieved full regeneration in rats or any other mammal, these dreams won't come true overnight. They aren't chimerical, however. The remaining problems could probably be solved in a decade or two of concerted basic research. Meanwhile, human capacities for repair of certain tissues are greater than most people realize, and there are already promising ways of enhancing some of them.

Cartilage

Fossils show that even the dinosaurs had arthritis, but unfortunately it outlived them. Many varieties have been described, all of which result in destruction of the hyaline (glassy) cartilage that lines the ends of the bones. The remaining cartilage cells try to heal the defect by proliferating and making more cartilage. They're almost never equal to the task, and scar tissue fills the rest of the hole. The result is pain, for scar tissue is too spongy to bear much weight or keep the bones from grinding against each other.

After our success at getting rat legs to partially regenerate, we studied this problem in 1973. We reasoned that, since cartilage was made by only one kind of cell, getting it to regrow would be easier than working with a whole limb.

With orthopedic resident surgeon Bruce Baker of the Upstate Medical Center, we removed the cartilage layer from one side of the femur at the knee in a series of white rabbits. The operation left a circular hole of bare bone about 4 millimeters across. In the experimental animals we implanted silver-platinum couplings like those used on the rats, drilling the platinum end into the defect and tucking the rest along the bone. Most of the control animals filled in the defect with scar tissue along with some inferior fibrous cartilage. About a tenth of them grew a millimeter or two of good hyaline cartilage at the edge of the hole. But sure enough, the rabbits with the implants showed greatly enhanced repair. When we used an improved battery implant with silver wires at each end, we got even better results. Two of the rabbits healed the damage completely with beautiful hyaline cartilage just like the original material.

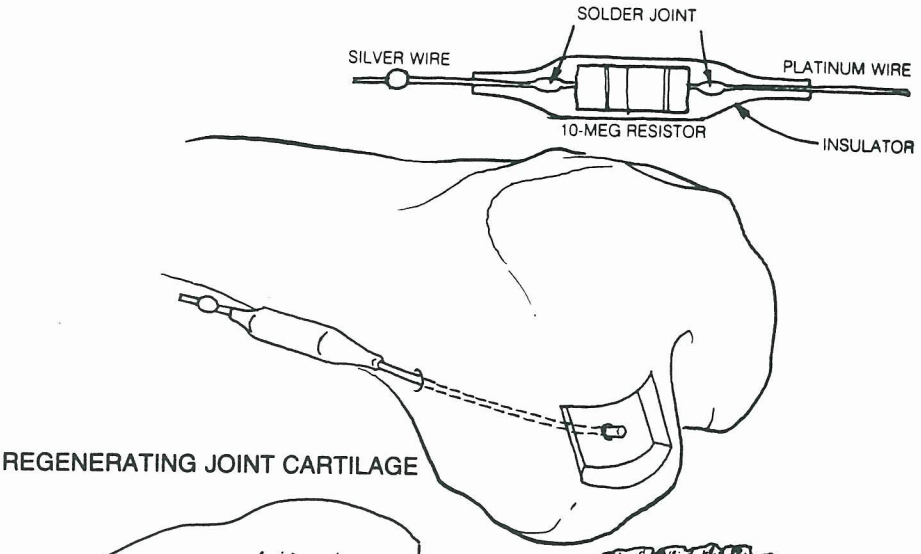
A few years later, when we were testing various electrode metals, we tried a different approach specifically for rheumatoid arthritis, in which runaway inflammation causes phagocytes to attack healthy cartilage cells. Gold salts taken orally sometimes control this disease but often produce toxic side effects. We figured electrical injection of pure gold directly into the joint with no other ions might work better. To find out, Joe Spadaro and I produced rheumatoid arthritis in the knees of both hind legs in forty rabbits, using a standard experimental procedure. Then we treated one knee in each animal with a positive gold electrode stuck right into the space between the two bones for two hours. Joe did the actual treatments. Then we sacrificed the animals gradually over a period of two months, and I examined both arthritic knees, not knowing until later which had been given gold. During the first two weeks about 70 percent of the treated knees were markedly better than the untreated ones. The improvement fell off to about 40 percent thereafter, suggesting that the treatment must be repeated for continued results.

Obviously, these were only preliminary experiments. However, since an estimated 31 million Americans suffer from arthritis, for which there is no cure yet, I think both avenues should be thoroughly explored as soon as possible.

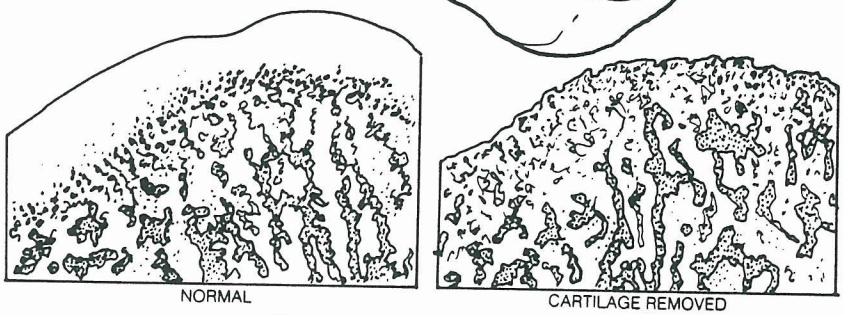
Skull Bones

Lev Polezhaev has spent his career investigating what might be called the Polezhaev principle—the greater the damage, the better the regrowth. He has found he can often enhance repair by adding homoge-

nates, minces, and extracts of the damaged organs, even though this doesn't augment the current of injury as his needling procedure did.



REGENERATING JOINT CARTILAGE



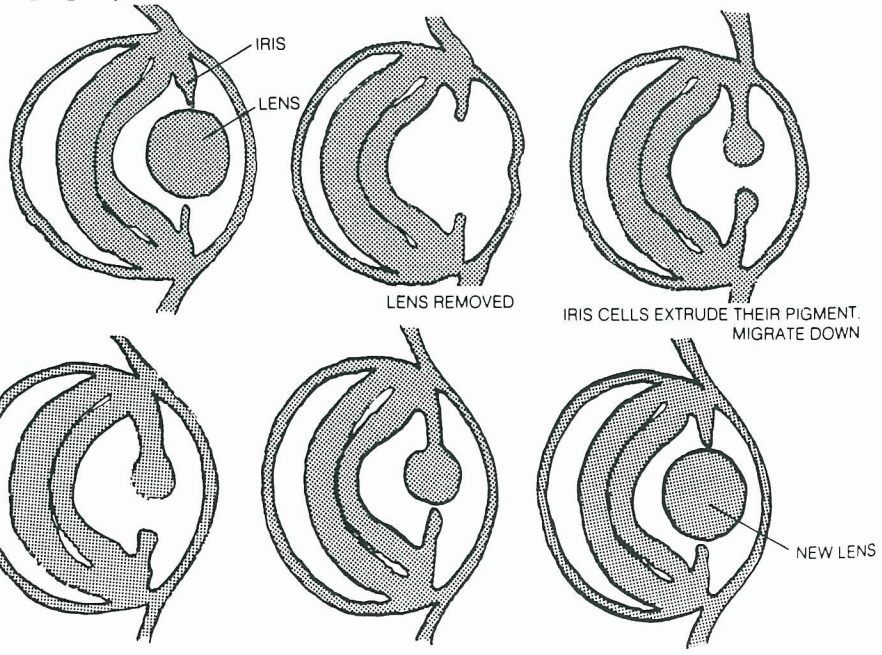
REGENERATING JOINT CARTILAGE—RESULTS

Eventually Polezhaev developed a way to induce regeneration of holes in the skull, which normally heal over with scar tissue. As long as the dura mater, the tough membrane between skull and brain, is intact, a paste of blood and fresh (living) powdered bone will induce the bone cells at the edges to grow and bridge the gap. Microscopic studies have

shown that the few live cells remaining in the paste don't survive and the bone particles themselves soon dissolve. Instead, some substance from the disintegrated bone stimulates repair. Since its first successful trial on humans by several Russian surgeons in the mid-1960s, this method has gradually come into increasing use in the Soviet Union.

Eyes

There is at present no indication whatsoever that humans could ever regenerate any part of their eyes, but the ability of newts (salamanders of the genus *Triturus*) to do so makes this a tantalizing research ideal for the far future. If the lens in a newt's eye is destroyed, the colored cells of the top half of the iris extrude their pigment granules, then transform by direct metaplasia into lens cells. They soon start synthesizing the clear fibers of which the lens is made, and the whole job is finished in about forty days. In case the iris is gone, too, a newt can create a new one from cells of the pigmented retina, and those cells can also transform into the neural retina layer in front of them. If the optic nerve gets damaged, the neural retina in turn can regenerate the nerve tract backward and reconnect it properly with the brain.



SALAMANDER EYE-LENS REGENERATION

No one knows why newts are so much more adept at this than all other creatures; their eyes have no obvious structural or biochemical peculiarities. Steve Smith gave us an important fact to work with when he found two proteins in the lens that seem to prevent the iris cells from changing into new lens cells as long as the old lens is in place. Since the neural retina must be intact for most of the transformations to happen, it may provide a constant electrical stimulus that goes into effect only when the inhibitory proteins are removed by injury.

No blastema is formed; instead the cells change costume right onstage. Furthermore, certain ingenious experiments have shown that a wound isn't really necessary, only the interruption of the inhibitory mechanism. Therefore, the stimulus from the neural retina probably isn't the familiar injury current of limb regrowth. However, despite a voluminous research literature on newt eye regeneration, no one has yet studied its electrical aspects. This may be why we're still so far from understanding the natural process, let alone trying to adapt it to human eyes.

Muscle

Every muscle fiber is a long tube filled with rows of cells (myocytes) laid end to end with no membranes between them—in effect, one multinucleated cell, called a syncytium. These nuclei direct the manufacture of contractile proteins, which are lined up side by side and visible, when stained, as dark bands across the array of myocytes. Each muscle fiber is surrounded by a sheath, and groups of them are bound together in bundles by thicker sheaths. At the edge of each bundle are long, cylindrical cells with huge nuclei and very little cytoplasm, called myoblasts or spindle cells. Also along the edges, between the spindle cells, clusters of tiny satellite cells can be seen at high magnifications.

After a crushing injury or loss of blood from a deep cut, muscle in the damaged area degenerates. The myocyte nuclei shrivel up and the cells die. Soon phagocytes enter to eat the old fibers and cell remnants. Only the empty sheaths and a few spindle and satellite cells are left.

Now these remaining cells turn into new myocytes, fill up the empty tubes, and begin secreting new contractile proteins. Although the early part of this process proceeds without nerves, it can run to completion only if motor nerve fibers reestablish contact with the terminals, called end plates, that remain at specific distances along each fiber sheath. If these end-plate areas are cut out, the nerve endings will enter, sniff

brain, just as all our other amphibian blood donors had. We looked it up just to make sure. The standard works on regeneration all agreed that no animal's heart could repair major wounds. Unlike skeletal muscle, the cardiac variety had no satellite cells to serve as precursors for mature heart-muscle cells. In any case, the textbooks stated, the animal would die long before such repair could occur.

Next week Sharon put our three intended sacrifices in a bowl of water and with a straight face asked me if they looked healthy enough to use. I told her they looked fine. "Good!" she exclaimed. "These are the same three we used last week." Score one for the open mind!

Flabbergasted, I helped anesthetize and dissect this trio of miracles. Their hearts were perfectly normal, with no evidence of ever having been damaged in any way.

The Five-Alarm Blastema

Abruptly I changed my research plans. I asked Sharon to test a series of newts by cutting away large sections of their hearts and sewing up their chests, then killing some of the survivors every day and slicing, mounting, and staining the hearts for study under the microscope. Over 90 percent lived through the first operation, and several weeks later we had hundreds of slides ready for my examination and diagnosis. Unfortunately they all looked the same! Even those from *the day after* that horrendous mutilation showed only normal tissues with no sign of injury.

By now we knew we had come upon a first-class mystery and had better jettison our preconceptions. We reasoned that we could tell when regeneration was finished by finding out when the blood began flowing again. Under the microscope we could easily see blood cells streaming through capillaries in the transparent tail fins of lightly anesthetized newts. The motion stopped when we cut the heart, and restarted about *four hours* later. We sectioned a new series of hearts, this time covering the first six hours at intervals gradually increasing from fifteen minutes to one hour.

While waiting for the specimens, we rummaged more thoroughly through the literature for other reports on heart regeneration. There was evidence for very limited repair—but no true regeneration—of small heart wounds in a few animals. The process seemed limited to the very young. Even then, the results were of poor quality, combining a lot of scar tissue with only a little proliferation of nearby heart cells, but the

mitotic component could be enhanced by various experimental aids.

In 1971, John O. and Jean C. Oberpriller, anatomists at the University of North Dakota School of Medicine in Grand Forks, reported that small wounds in salamander hearts healed this way but required two months. A year after that, the English edition of a book by Lev Polezhaev summarized several decades of Russian research, mainly on the hearts of frogs and lizards. Pavel Rumyantsev, now at the Cytology Institute of the USSR Academy of Sciences in Leningrad, had found in 1954 that newborn mammals (rats and kittens) could repair tiny puncture wounds, and recently he has proved the same capacity in the atria, or receiving chambers, of *adult* rat hearts. We even found a German report of 1914 claiming that human babies had sometimes regenerated small areas of their hearts damaged by diphtheria.

The Russians claimed some progress in extending this marginal native healing. In the late 1950s, N. P. Sinitsyn had repaired large holes (up to 16 square centimeters) in the hearts of dogs by covering the wounds with patches made of muscle sheath, canvas, suede, or other materials. Scar tissue still covered the outside, but the patch guided a thin layer of new muscle fibers forming along its inner surface. Using dogs whose wounds had already closed with scar tissue, Polezhaev then found he could induce heart muscle to fill in part of the gap by cutting away the scar and irritating the edges of the remaining cardiac muscle. Other Soviet researchers enhanced the muscle cell proliferation a little more with vitamins B_1, B_6, and B_{12}, various drugs, extra RNA and DNA, and heart tissue extracts or minces.

Despite such goads, heart regrowth was limited to very small injuries or the border zone around larger ones, and it always took several weeks. No one had even imagined that half a heart could restore its other half, much less in a matter of hours. I could hardly wait until the next batch of slides was ready.

They showed us an unprecedented type of regeneration. Where the missing part of the heart had been, a blastema formed in about two and a half hours. We saw no evidence of dedifferentiation or mitosis in the remaining heart-muscle cells, and indeed it would have been impossible for the processes we'd already studied to make a blastema in such a short time. Instead, the mass of primitive cells arose dramatically from the blood.

As soon as the salamander heart is cut open, blood pools around the wound and clots quickly, usually in about one minute, sealing the hole like wet plaster. Almost immediately, the nearest red blood cells crack open like eggs. Their nuclei, surrounded by a thin coating of cytoplasm, glide by some means yet unknown directly to the raw, frayed edge of the

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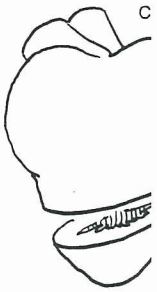

SUPERREGENERA

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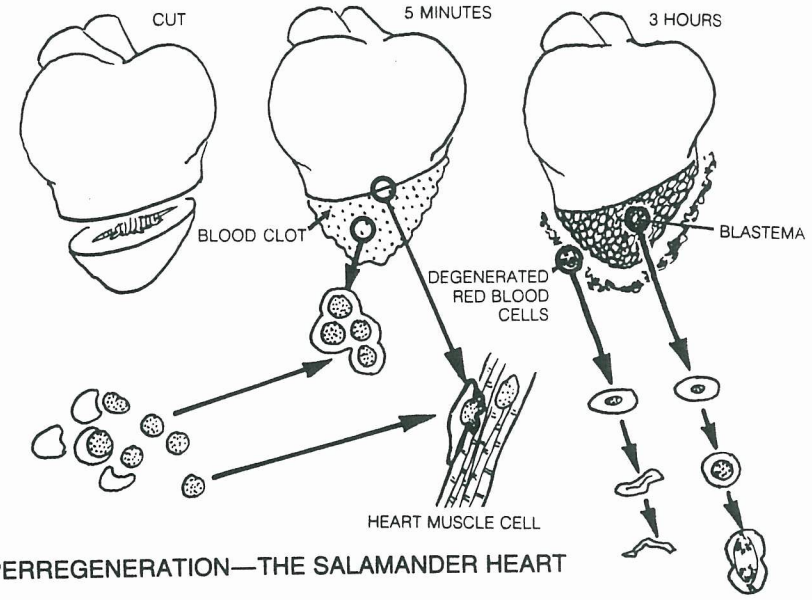
heart muscle and insinuate themselves into the tangle of dying and injured cells. To a biologist this sight is bizarre, uncanny. It's as though the engine of a passing car could walk up to a stranded truck, climb under the hood, and drive it away.



SUPERREGENERATION—THE SALAMANDER HEART

Farther away from the wound surface, the red cells also spill out their nuclei, but these cell yolks clump together, fusing their remaining cytoplasm to form a syncytium. Still farther away from the center of action, the red cells undergo the more leisurely dedifferentiation we observed in our frog fractures and DC culture studies. They turn into primitive ameboid cells that move toward the area of damage and attach themselves by pseudopods to the injured muscle fibers. In all of biology there's no precedent for these virtuosic cellular metamorphoses. In fact, they're so strange that most researchers have simply refused to believe in their existence or try the experiment for themselves.

All these changes are well under way within fifteen minutes. Soon afterward the extruded nuclei, the interconnected syncytial nuclei, and the ameboid cells are all dividing as fast as they can, building up the blastema. It's fully formed within three hours after the injury. By then its cells have already started to redifferentiate into new heart-muscle cells, synthesizing their orderly arrays of contractile fibers and connecting up with the intact tissue. If the clot contained more blood cells than were needed, the extras outside of the area now degenerate, apparently so as not to get in the way of the repair work.

Meanwhile, the newt has survived by absorbing dissolved oxygen from

the water through its skin. Now, at about the four-hour mark, there are enough new muscle cells to withstand contraction, and the heart begins pumping again, slowly. After five or six hours, most of the blastema cells have redifferentiated into muscle, which is still somewhat "lacy" or delicate compared with the established tissue. After about eight or ten hours, however, the heart is virtually normal in appearance and structure, and after a day it's indistinguishable from an uninjured one.

Why did we see this colossal regeneration, while the Oberprillers found only a tiny, slow healing response in the salamander heart? Apparently this was another manifestation of the Polezhaev principle. We made a big wound; they made a small one. Only massive damage unleashed the full power of the cells.

Is this fantastic cellular power forever restricted to salamanders, or does it reside latent in us, ready at the appropriate impetus to repair damaged hearts without problem-filled (and frightfully expensive) transplants of donated or artificial pumps? We don't know, but we've found no other regenerative process that's forever off limits to mammals. At this point we can only speculate on how such a treatment might be accomplished, but at least the idea isn't wholly fantasy.

The first job is to identify human target cells able to dedifferentiate into primitive totipotent cells. Bone marrow cells or immature erythrocytes, the nearest equivalents to amphibian nucleated red blood cells, are one obvious candidate population, especially since they seem to be the crucial cells in rat limb regeneration and the inner part of fracture healing. Fibroblasts despecialized by electrically injected silver ions might be used. Another possibility is lymphocytes, one class of infection-fighting white blood cells. In our lab we've demonstrated that they, too, can dedifferentiate in response to appropriate electrical stimuli.

Since newt-type heart regeneration doesn't occur naturally in mammals, we would probably have to grow a large mass of the target cells in tissue culture. Then, with the patient on a heart-lung machine, the surgeon could cut away scar tissue and otherwise freshen the wound if it wasn't recent enough, then apply enough of these ready-made preblastema cells to fill the defect. They would be held in place by a blood clot, sutured pericardium, or some type of patch. Then, assuming we'd learned the electrical parameters already, electrodes would induce nuclear extrusion, dedifferentiation, consolidation with surrounding muscle, and the final transformation into normal cardiac muscle. The current would probably have to be adjusted throughout the process to get its various steps in synchrony, and vitamins or drugs might be used to enhance mitosis or protein synthesis. Once the scar had been removed,

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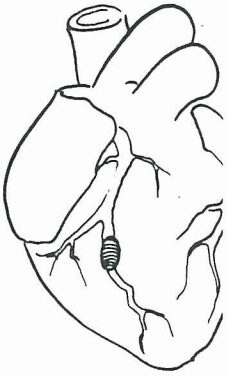

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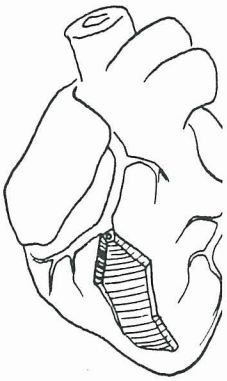

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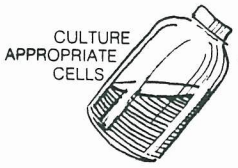

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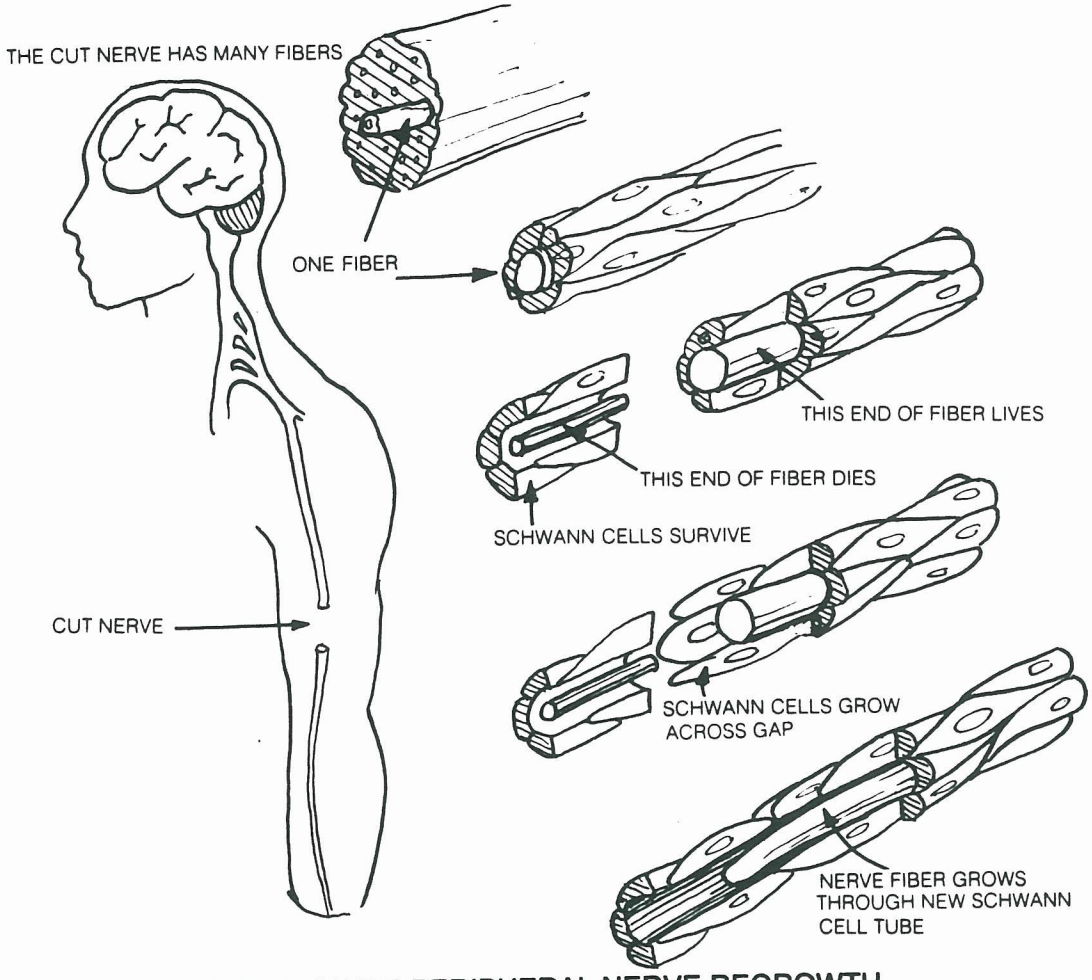
H. Wilson of the Leeds General Infirmary in England, there have been some interesting claims that pulsed electromagnetic fields have speeded recovery of limb function in rats after peripheral nerve damage, but the effect hasn't yet been substantiated for humans. If these findings hold up, we may soon be able to boost nerves past their 1-centimeter limit, even if the action is indirect, and a thorough investigation of the electrical basics could drive nerve regrowth to even greater lengths.



SCHWANN CELLS GUIDE PERIPHERAL NERVE REGROWTH

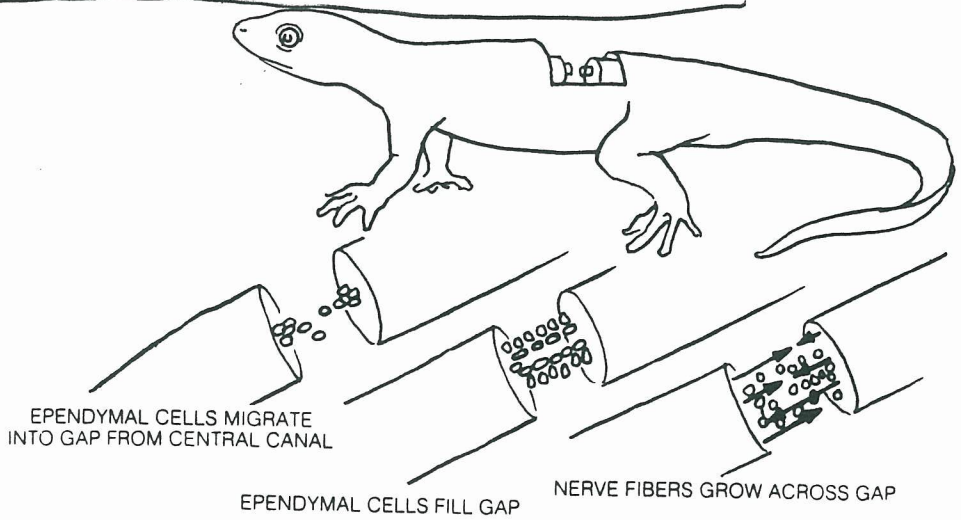
The Spinal Cord

A sad and crucial difference separates peripheral fibers from those in the human spinal cord, for the latter don't reconnect over even a fraction of a centimeter. However, in most injuries relatively few of the neurons themselves are killed. It's important to realize that most of the cord cells below the injury don't die. The reflex arcs remain intact. In fact, reflexes are stronger than normal, because the neurons are now disconnected

from the regulating influence of the brain. For the same reason, the broken bones of paraplegics heal in half the normal time, whereas a bone will heal very slowly or not at all if its *peripheral* nerve supply has been cut. Only the communication between brain and spine is silenced in paraplegia, and that makes all the difference.

Spinal fibers *do* reconnect in some animals, notably goldfish and, as you might expect, salamanders. Their ability seems to decline dramatically with age, however. Jerald Bernstein, a neurophysiologist now at George Washington University Medical School who has studied goldfish spinal regeneration extensively, has found that one-year-old fish heal almost all of the damage. This competence declines to about 70 percent at two years and 50 percent at three. Since salamanders aren't raised in biological supply houses but rather collected from the wild, any group is likely to include young and old individuals, making comparisons difficult. In our lab we found that cord regeneration isn't uniform in salamanders, probably due to age differences.

Maturity may reduce the response of the ependymal cells, which are responsible for the first step. They proliferate outward from the central canal and bridge the gap in a few days. Marc Singer, in a recent study of this process, concluded that the ependymal cells extend "arms" radiating outward, which line up like the spokes of wheels stacked one atop another, forming channels for the regrowing fibers to follow. The nerves then reestablish their continuity within a few weeks.

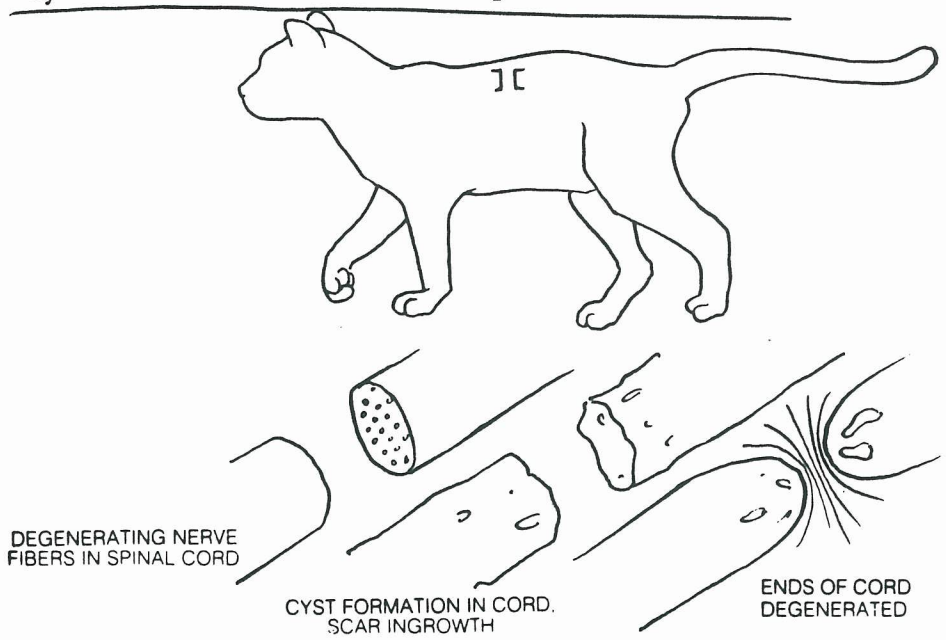


SALAMANDER SPINAL-CORD REGENERATION

Bernstein also found that there's a critical period during which regrowth must be completed or it will fail. After cutting the cords of goldfish, he inserted Teflon spacers to block regeneration. The normal

processes took place, but of course the cells couldn't penetrate the divider. After the cellular activity had died down, Bernstein removed the barriers, but there was no further change. However, when he then cut off each damaged end, producing an even larger gap and reinjuring the cord, the cells started from scratch and healed the defect completely. Thus there's good reason to believe that even long-standing spinal injuries can potentially be regenerated if we can extend the basic capabilities of human cells.

One would expect to see some healing response in mammals, even if it fell short. After all, we only need the elongation and reattachment of fibers, which does take place in peripheral nerves. Instead the opposite happens. The cord cells die a short distance above and below the injury. Cysts form near the ends, and, instead of ependyma, scar tissue fills the gap. Only after this destruction is there an abortive attempt at regrowth. In humans this amounts to only a few millimeters of fiber elongation many months after the injury. By then it's too late; the ependymal cells and nerve fibers can't penetrate the scar.

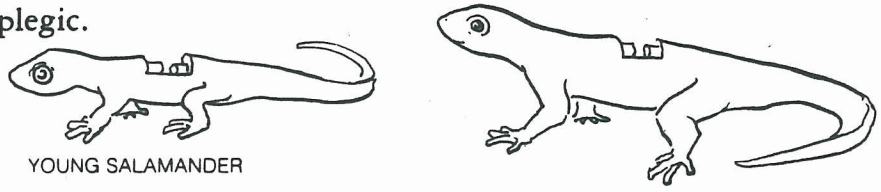


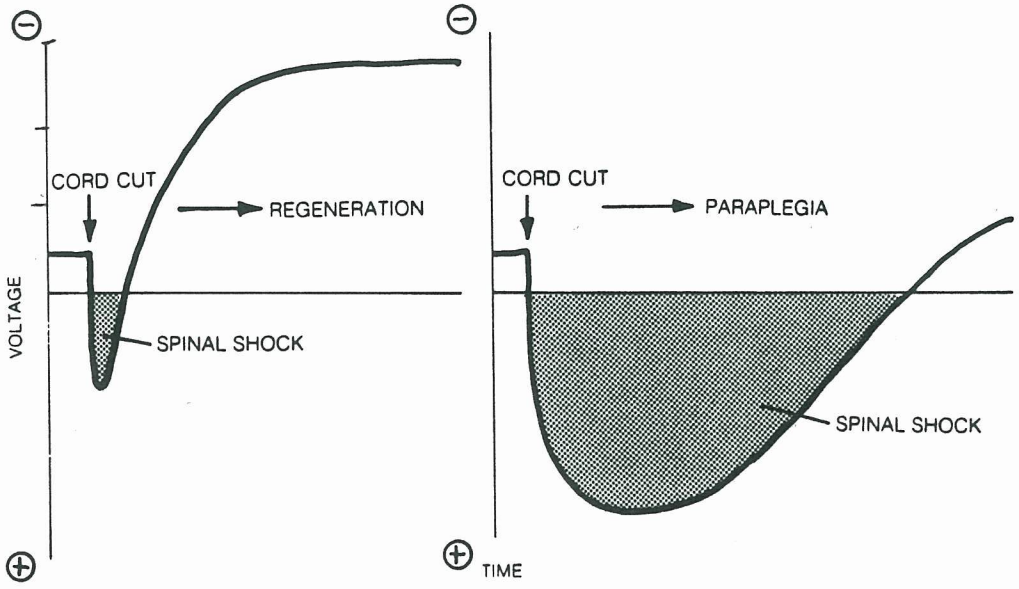
CYSTS AND SCARS PREVENT CORD REGROWTH IN MAMMALS

Why the difference between salamanders and mammals? The reason may lie in the cord's immediate response. In all animals the injury instantly results in spinal shock, during which all neuronal activity is profoundly depressed, especially in the part of the cord still connected to the brain. Even the simplest reflexes disappear. As the shock wears off, the cord *below* the injury becomes hyperactive. Its reflexes become tre-

mendously exaggerated and lead to spastic paralysis of the muscles. The interesting difference is in the duration of shock. In young salamanders and goldfish it lasts only a few minutes, but it may endure for over a week in old ones. In mammals it takes even longer to wear off—as long as six months in humans.

We made some electrical measurements on salamander and frog spines in our lab. The injured area turned out to be strongly positive during spinal shock, even though all direct-current flow ceased in the entire cord and in the peripheral nerves arising from the part below the trauma. Then, as the shock resolved, a steadily increasing negative potential appeared, its size reflecting the amount of outgrowth by ependyma and nerve fibers. We found that we'd only *re*discovered these potentials, however. G. N. Sorokhtin and Y. B. Temper had made the same measurements at the Khabarovsk Medical Institute twenty years before. The patterns of shock and polarity both correlated, not only with the cell activity, but with the end result of regenerative success or failure. A few minutes of shock and a correspondingly short period of positivity led to full repair of the cord. Longer delays produced incomplete regeneration, and, when the shock and positive potential persisted for five to eight days or longer, the salamander became completely paraplegic.

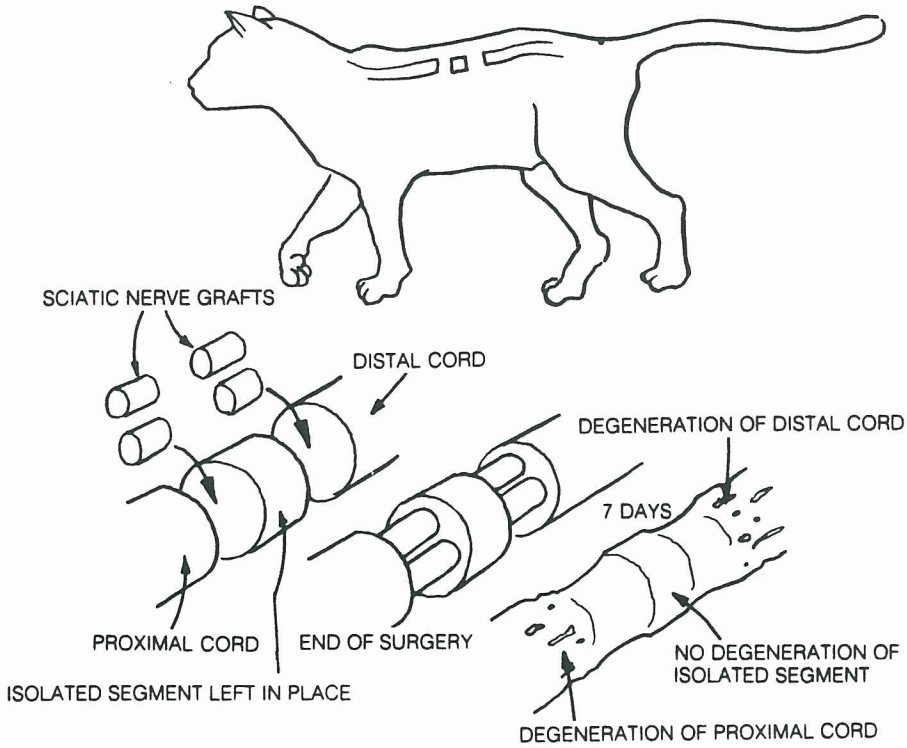




SPINAL SHOCK AND AGE INHIBIT CORD REGROWTH

As far as I know, the only electrical measurements of spinal shock in mammals were preliminary ones done at our laboratory in conjunction with Carl Kao of the VA hospital in Washington, D.C. We tested the severed cord ends in cats for twenty-four hours and found only an increasing positive potential. The situation seemed quite similar to the electrical difference between salamander and frog limbs. As in most instances, positive potentials appeared to inhibit constructive cellular activity while negative ones fostered it.

An experiment Kao did several years ago provided some supporting evidence. Kao made two cuts through the spinal cord in each of several cats, producing a central fragment about 5 millimeters long, separated from each end. He then grafted pieces of sciatic nerve as spacers in the two cuts. Typical degeneration with cysts occurred in each end of the cord but not in the isolated piece. In fact, this part showed some growth of its ependyma and nerve fibers. The small piece was probably isolated from the positive potentials produced in the rest of the cord. Hence it escaped inhibition and grew. It seems the prolonged electrical positivity of spinal shock is the main roadblock in the way of human cord repair.



KAO'S EXPERIMENT

It should be possible to cancel that polarity and replace it with a growth-stimulating negative one, using a properly shaped electrode. Older injuries in which spinal shock has subsided might require a dif-

trical problems in spinal healing may be tackled sooner than in other fields.

The public imagination has been captured by the computerized muscle-stimulation techniques being developed by Jerrold Petrofsky, an engineer at Wright State University in Dayton. The nationally televised sight of his patient Nan Davis and other paraplegics taking tentative steps and pedaling tricycles with their own muscle power was tremendously exciting. But if we can get the body to do the same things by itself, that will be even better. Any amount of regeneration would only make other techniques more effective. Even restoring 10 percent of lost function would be an unimaginable blessing to those who are now helpless. I feel the electrical manipulation of spinal shock must be tested vigorously now, for this is perhaps the one area where the barriers of tragedy are closest to being broken.

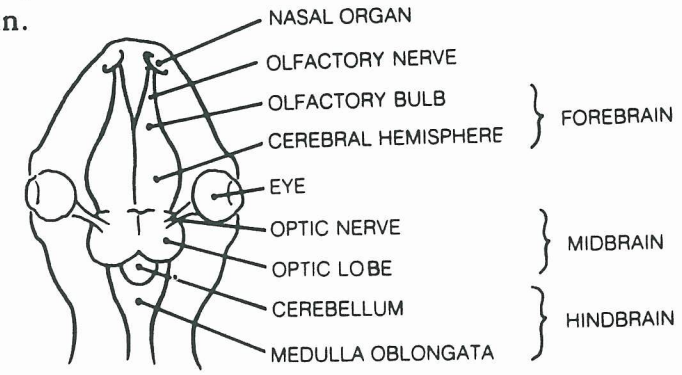
The Brain

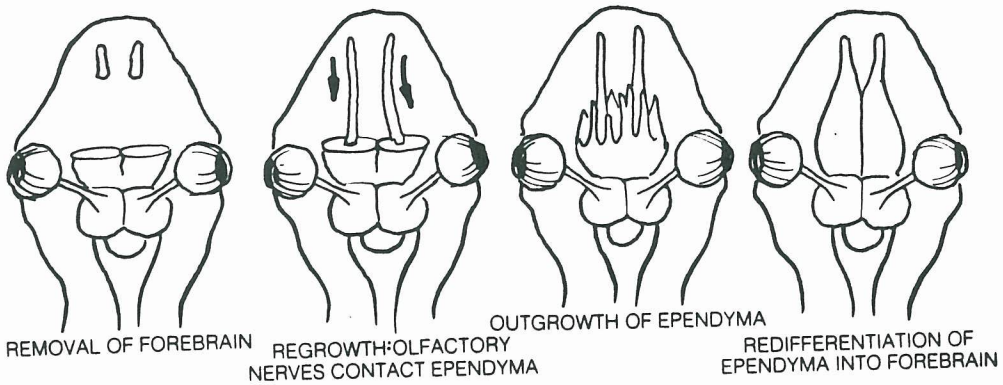
It might seem foolish to expect any regeneration in the most complex of all biological structures, the brain, yet salamanders, some fish, and most frogs in the tadpole stage can replace large parts of it, including the optic lobes and the olfactory lobes, or forebrain, the part from which our prized cerebral hemispheres developed in the course of evolution. Replacement depends on ingrowth of remaining sensory nerves, the olfactory nerves in the case of the forebrain and the optic nerves for the optic lobes. When these nerves grow back into the area where brain has been destroyed, they stimulate the ependymal cells in the brain ventricles, which proliferate outward into the damaged part and then differentiate into new neurons and glial cells. If the animal's nose or eyes are removed so that the injury zone receives no nerve input, no regeneration occurs.

Thus brain regrowth begins much like that of limbs, with the connection of nerve fibers to an epithelial tissue. The ependyma, remember, is embryologically a close relative of the epidermis, and in fact can be considered the central nervous system's "inner skin." Since the electrical environment produced by the neuroepidermal junction is what stimulates cells to dedifferentiate and divide in the salamander limb stump, and since we started limb regeneration in the rat by crudely mimicking this signal, it seems likely that a similar stratagem could induce brain regeneration in animals normally lacking this ability.

A form of shock, called the spreading depression of Leão after its discoverer, neurologist A. A. P. Leão, occurs after brain injuries. Start-

ing at the site of damage, it extends in all directions until the entire cortex becomes electrically positive and all its neurons shut down. Leão studied it only in response to small injuries, when it persists for a few hours. We don't know whether it occurs in the salamander or how long it lasts after major damage to the mammalian brain. Concerted study of Leão's depression combined with experiments in electrically stimulating the ependymal cells could open the way to self-repair of the human brain.





AMPHIBIANS CAN REGENERATE LARGE PARTS OF THE BRAIN

Recovery from stroke and head wounds taught us long ago that the brain has a great deal of plasticity; that is, it can reorganize so that undamaged regions take over tasks formerly done by the lost cells. Supplementation of this ability with even a small amount of regeneration might make recovery nearly complete for many brain-damaged people. For the first time in history, neurologists can hope to progress from describing the brain and cord to mending them. As Geoffrey Raisman of London's Laboratory of Neurobiology recently reminded his colleagues: ". . . no immutable natural laws have been discovered that forever rule out repair of the nervous system."