Chapter 24

NEURAL-EPIDERMAL JUXTAPOSITION AND ITS EFFECT ON LIMB REGENERATION*

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AMONG THE VERTEBRATES, regeneration of an entire extremity occurs naturally only in tailed amphibians (1). Control over the regenerative process appears to be intimately related to the peripheral nerve supply in the amputation stump. Nerves reportedly must be present at a critical minimum volume (2) and must regrow from the cut end to make contact with overlying wound epithelium (3). This neural-epidermal "junction" (NEJ) has been postulated to take part in an information transfer system that controls organization of the regeneration blastema (4). Evidence gathered over the last twenty years strongly suggests that the modality of transfer involves alteration of the bioelectric field at the distal end of the amputation stump (5-8).

This investigation reports the bioelectrical and epimorphic consequences of repositioning the cut sciatic nerve in the rat to approximate the neural-epidermal relationship in the amphibian limb regenerate.

Animals consisted of young adult male rats (Sprague-Dawley strain) 2½-3 months old at the time of amputation. All surgical procedures were performed under chloral hydrate anesthesia.† No antibiotics were administered. Thirty-six animals in the control group were subjected to unilateral hind-limb amputation through the distal one-third of the femur proximal to the epiphyseal plate. The sciatic nerve was left *in situ* and allowed to retract. With the skin folded over, dermis near the distal end of the stump was removed by gentle scraping with a sharp scalpel. Subsequently, a 2-3 mm diameter hole was made in the remaining epidermis. Skin over the thigh and stump was closed with 4-0 silk sutures, allowing the small hole to persist at the end of the stump.

The forty-four animals of the experimental group received identical amputations except the sciatic nerve was dissected free and its terminal branches fed through the hole in epidermis over the cut femur and sutured to skin with 6-0 polypropylene.

Animals were killed at intervals up to two months following surgery.

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† Each animal received a simple I.P. injection of 3½% (w : v) aqueous chloral hydrate at a dosage of 1 ml per 100 g body weight.

During the first twenty-one days, skin potentials at the apex of the stump were measured using a consistent point on the skin overlying the lumbar vertebrae as reference. Electrical measurements were made with Ag-AgCl electrodes and a Keithley Model 602 electrometer.

At recovery, rats were killed by etherization, hind-limbs x-rayed and fixed in buffered 10% formalin. Tissue was processed routinely for histology and sections stained with either H&E or by Bodian's protagarol method for nerve fibers.

Growth was assessed on a scale of 0 to +5 using a grading system developed for measuring partial limb regeneration in rats (7).

The histogram in Figure 24-1A summarizes the results. Amputation healing in the control group was dominated by proliferation of dense fibrous connective tissue at the end of the stump. Moderate amounts of chondrogenesis were evident at the distal margin of the cut femur by two weeks. The cartilage was replaced, first by spongy then compact bone (Fig. 24-2).

Six out of eight animals in the experimental group at two weeks post-amputation revealed a 0.6-1.0 cm long soft tissue mass (confirmed radiologically) projecting from the cut end of the femur (Fig. 24-3). An extensive capillary network pervaded what was identified as a mesenchymous collection of cells having little extracellular fiber matrix. This blastemal-like cell mass was only recognized in the experimental group.

Beyond two weeks, experimental amputations showed signs of active osteogenesis and myogenesis with epimorphic reconstruction of lost structure. The maximal response at six weeks consisted of a bony outgrowth from the cut femur ending in a cartilagenous structure organized as an epiphyseal plate. This was capped by smooth articular-like cartilage. The femur was met at its distal end by a small ossicle separated by a potential synovial space. Laterally, a second ossicle formed (Fig. 24-5A & B).

In muscle, myogenic changes found muscle fiber basement membranes enclosing clusters of mononuclear myoblastic cells. Also present were chains of myotubes (Fig. 24-6). New muscle fibers tended to project distally.

An important consideration was the nature of the neural-epidermal interaction. Two weeks after amputation, individual nerve fibers had grown out from the cut nerve and ramified throughout the wound epithelium. Careful examination of Bodian stained sections showed small bulbous expansions along the fibers and at their termini (Fig. 24-7). The fate of this neural-epidermal contact is presently unknown, but animals maintained for eight weeks exhibited some moderate amounts of degeneration in the terminal portions of the nerve trunks.

Electrically, control amputees registered a strong positive potential that maximized shortly after amputation (Fig. 24-1B). Over three weeks, this

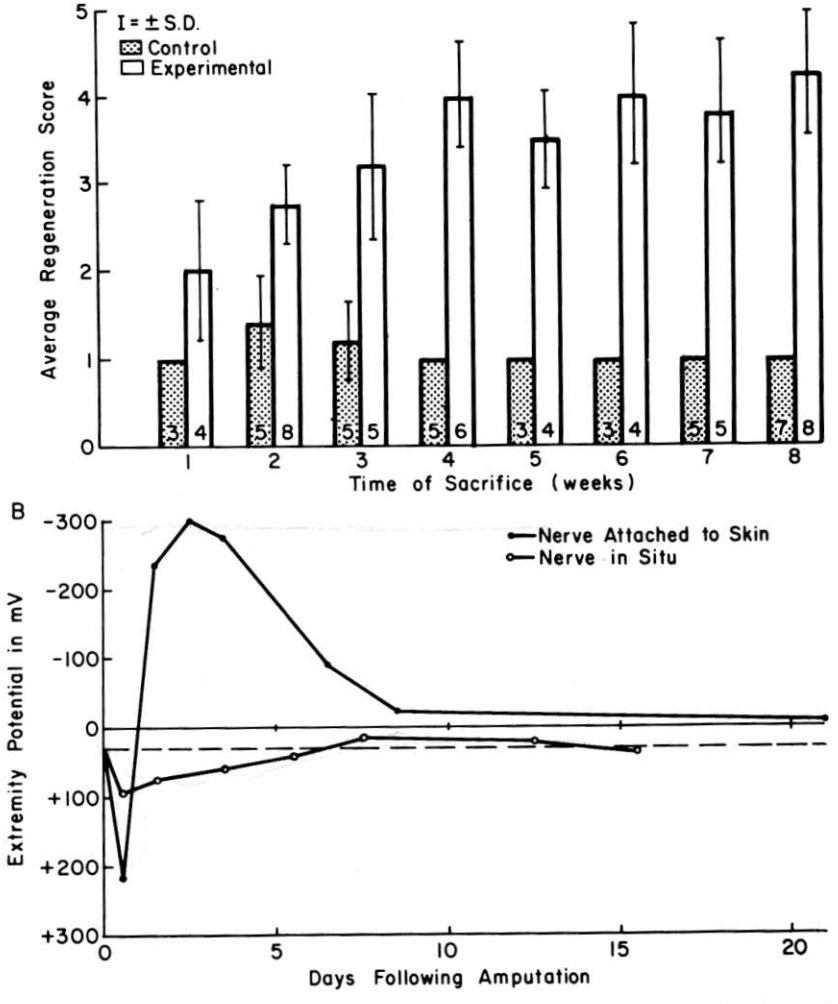

Figure 24-1. Graphical representation of the regenerative response (A). Growth scale ranges from 1 to +5: 1 indicates typical healing following simple amputation; +2 refers to growth exceeding fibrous accumulation, but not recognized as blastema; +3 denotes the presence of a blastemal-like mass; +4 means blastema with differentiation of specific tissue types such as cartilage, bone or muscle; +5 acknowledges the appearance of an organized multi-tissue structure with anatomical features appropriate to the region. The plot in B shows that electrical potentials recorded at the distal end of the amputation stump using silver silver-chloride electrodes of the type used to determine electrical potentials of bone and periosteum (12). The dashed line represents potential readings at the knee without amputation. The points plotted in both experimental and control groups are mean values from five animals per group, followed over a twenty-one day postamputation period. In the control group (nerve *in situ*) there is an initial position deflection after amputation at time 0 that gradually returns to baseline by the tenth day after surgery. The experimental group (nerve attached to skin) presents a strong positive potential shortly after amputation. This potential reverses one day following amputation and maintains a strong negative potential for seven days. At twenty-one days, the potential approaches zero but still lies in the negative range.

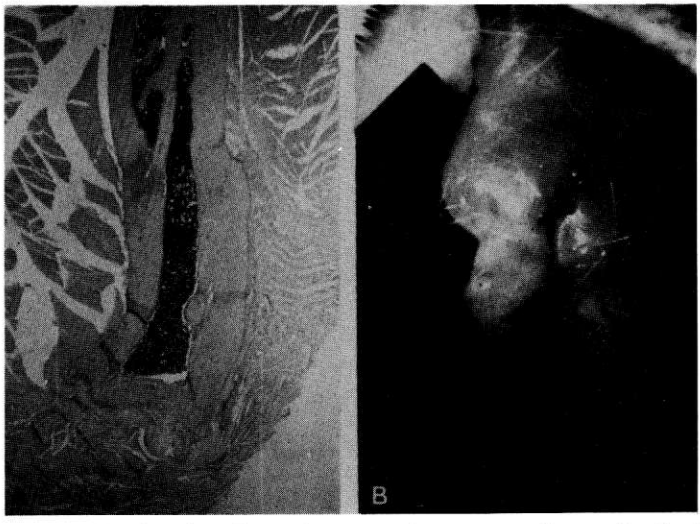

Figure 24-2. Longitudinal section through amputation stump of control animal, seven days postoperative, showing minimal growth response classified as type VI. ×10.

Figure 24-3. Gross appearance of experimental animal amputation stump at seven days postoperative showing apparent new growth beyond original resection line. ×5.

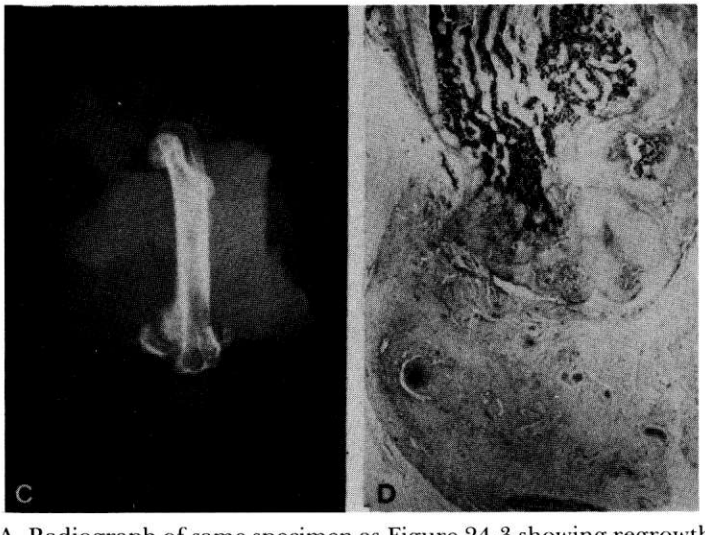

Figure 24-4A. Radiograph of same specimen as Figure 24-3 showing regrowth of femoral condyles at angles to original shaft and new soft tissue growth beyond original resection line.

Figure 24-4B. Photographic enlargement. ×5. D. Longitudinal section through experimental amputation stump at three weeks postoperative showing apparent blastema formation.

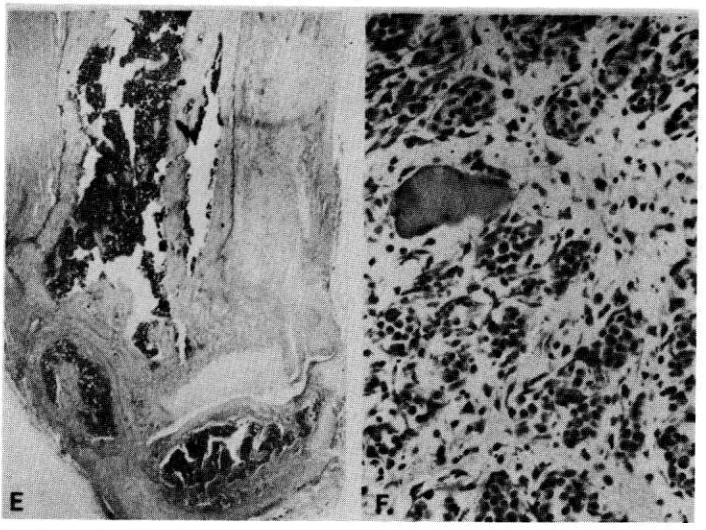

Figure 24-5A. Longitudinal section through experimental specimen at six weeks post-amputation, showing new growth of femoral condyle and tibial ossicle classified as type 4.

Figure 24-5B. Section through experimental specimen at six weeks postoperative, soft tissue with apparent myotube formation. ×100.

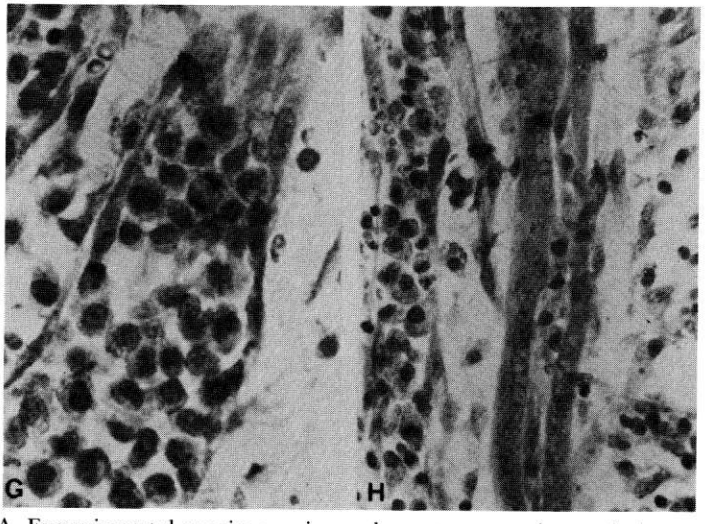

Figure 24-6A. Experimental specimen, six weeks postamputation, soft tissue cross section showing myoblasts and early myotube formation. ×450.

Figure 24-6B. Experimental specimen six weeks postamputation, soft tissue longitudinal section, showing multinucleated myotube formation. ×450.

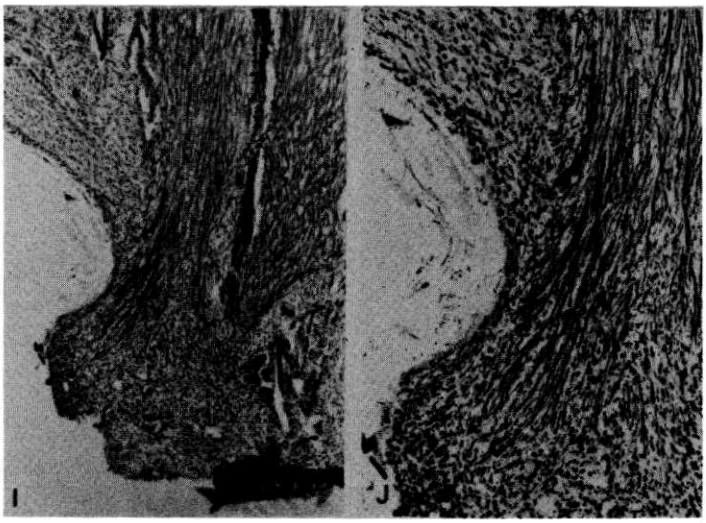

Figure 24-7A. Experimental animal, section through epidermis, sciatic nerve association at fourteen days postamputation. ×10.

Figure 24-7B. ×100.

Figure 24-7C. ×450, showing new nerve fibrils growing at angle to original sciatic nerve, penetrating epidermis and making junction with epidermal cells, similar to those described for neuro-epidermal junctions in the salamander.

potential gradually drifted back towards baseline. Amputations from the experimental group had a biphasic response. After an initial positive deflection, the limb polarity reversed approximately twenty-four hours after surgery. This negative potential persisted for several days before returning to baseline (Fig. 24-1B).

In approximating sciatic nerve with epidermis, the nerve fiber epidermal ingrowth that takes place in the rat amputation stump appears very similar histologically to nerve fibers in the epidermis of the regenerating salamander limb (9). Additionally, the electrical potential in the experimental group follows the polarity reversal found in the salamander (5), the classical regenerator (10), while the potential measured in the control group resembles the pattern established for the frog, a non-regenerator (5).

Discovery of a soft tissue blastemal-like mass with subsequent reorganization to form discrete tissues and structures adds support to the concept that limb regeneration, regardless of vertebrate class, proceeds along the same general pathway. The close similarities between salamander and rat experimental group electrical potentials suggest that, rather than nerve bulk being responsible for generation of the appropriate electrical environment, the NEJ is the primary structure that produces this factor.

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