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KENTUCKY UNIV LEXINGTON DEPT OF ANATOMY
STUDIES OF RAT LIMB PERIPHERAL NERVE REGENERATION. (U)
JAN 80 S D SMITH, W G WINTER

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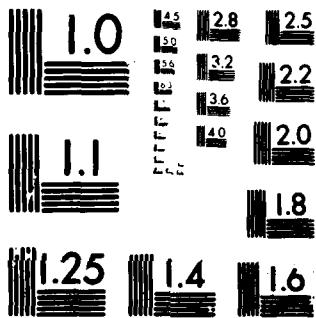
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21. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report outlines the methodology and preliminary results of a study in which adult rat limbs were severed at mid-forearm, then subjected to battery-powered implants with either the positive or negative lead placed at the wound surface. When suitably placed, and if the negative lead were distal, the rats regenerated a remarkable array of tissues in an organized fashion, including cartilage, bone, muscle, and connective tissue. In some cases, structures normal			

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to the limb were formed. These included well-formed joints and bones of the carpal and metacarpal series.

A second set of experiments involved the implantation of silver-platinum electrogenic couples across the severed sciatic nerves of adult rats. If the cathode lay distal to the gap, regeneration of the nerves across the gap was accelerated. This result was confirmed by examination of the nerves by autoradiography, direct gross examination, histological/stereological methods, and measurement of Compound Action potentials.

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ANNUAL REPORT

Contract No. N00014-79-C-0332

Studies of Rat Limb and Peripheral Nerve Regeneration

Task No. NR 207-183

Technical Report No. 1

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Department of Anatomy
University of Kentucky
College of Medicine
Lexington, KY 40536

Date of Report - 1 January 1980

Grant Period: 1 April 1979 - 31 March 1980

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The past year's efforts have proven to be both fruitful and frustrating, in approximately equal proportions. Shortly after the contract was awarded, Dr. Winter left the University of Kentucky to assume duties at the University of Colorado and the V. A. Hospital at Denver. In order to complete the work in as timely a manner as possible, we decided to try to work out a subcontract between Kentucky and Colorado to allow Dr. Winter to continue in the project. The assumption that such a procedure would be simple led us to refrain from initiating the experiments until the arrangements were complete. The final papers were not signed until September, so we received an object lesson of some value.

Needless to say, the experiments called for in the contract are not complete. However, all the implant devices have been fabricated for both experiments, and all those used for the nerve studies have been sent to Dr. Winter. He reports that there was some initial difficulty in keeping the implants in place. Now, however, the implants are attached to the tendon of the piriformis muscle, which prevents them from moving in the distal-proximal axis or the other two axes during the animals' movements and there is no further problem with the nerve implants. The implantations for the nerve study are 80% complete, and will be finished by 1 January, 1980. We anticipate no difficulty in completing the study within the original projected limits. Preliminary results indicate that, as expected, implants with the negative electrode implanted in the distal segment of the severed nerve are most effective in stimulating regeneration. The results with the positive end placed distally are not yet available, but we anticipate no stimulation - even an inhibition, perhaps. We are also as yet uncertain as to whether there is a dose-response

to current level, but previous experience decidedly suggests that an optimal response will be obtained with currents in the 1-10 nA range.

The limb regeneration experiments are also proceeding apace. All implantations should be complete by 1 January, 1980. At present, they are 65% complete. The idea of making the surface electrode follow the limb outgrowth has worked well. The proximal portion of the electrode to be threaded down the limb is simply wound into a loose spring, or series of zig-zags. Either approach works. The spring tension keeps the electrode applied to the wound surface during the animal's movements, yet allows the electrode to advance with the outgrowing regenerate. The results are most encouraging.

Initial indications are that stimulation of the wound surface with a positive potential is ineffective, while stimulation with a negative one is quite effective. It is yet too early to tell whether the regenerates will become morphogenetically well-organized, but the results are encouraging. Considerable outgrowth is being obtained. Perhaps more surprising is the amount of regeneration observed in the controls. It has been known for a long time that if a normally regenerating limb stump (newt) is sutured closed so that skin covers the wound, no regeneration ensues. In defiance of this observation, clinicians and scientists persist in suturing over amputated limbs, thereby eliminating the animals' own efforts. This has obviously been done to prevent infection and to ensure hemostasis - certainly appropriate goals. However, experience with amputated childrens' fingers in others' studies, and with rat limbs in our own, demonstrates that it is not really necessary. Our amputation procedure involves severing the forearm with heavy scissors, then trimming the bone back to the muscle surface. After

such an amputation, the animals lose almost no blood, despite the absence of hemostatic intervention. Neither do they become infected if the animal is allowed to lick his wounds. We have not yet lost a single animal to exsanguination or infection using this seemingly crude technique. In fact, the animals begin to use the limb within a few days of operation. They also seem to be showing quite a bit of outgrowth from the control stumps, albeit not so much as from the negatively stimulated ones.

It is also evident from our preliminary results that the only effective place to stimulate a limb is the ulnar side of the extensor compartment (quadrant IV of the contract proposal). This is not surprising, since that is the position of the Apical Ectodermal Ridge in embryological development, and the AER is known to be influential for the outgrowth and morphology of the developing limb. It appears that we must make the regenerate recapitulate its original ontogeny.

In sum, the experiments are progressing nicely after a long, frustrating delay, and are providing encouraging preliminary data. They should be finished easily by the end of the contract period, and we anticipate exciting and positive results.

Bibliography - None

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