A Comparative Study of Response of Species to Peripheral-Nerve Injury

I. Severance

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Techniques for repair of peripheral nerves which seem promising in the experimental laboratory often prove disappointing when tried in the human. Some of these clinical failures can be attributed to a lack of appreciation for the basic principles of neural repair and the physiologic and structural needs of regenerating nerves. It is difficult, moreover, to produce in the laboratory animal the type of wounds that exists in most war and many civilian injuries. Infection, scar tissue, and associated injuries frequently complicate the neural wound in the human. These complications are not part of the usual experimental design. Another factor possibly contributing to failure, and one which receives scant attention, is the large number of different species of animals used to evaluate various techniques of peripheral-nerve repair and to study peripheral-nerve regeneration. Rabbits, rats, cats, dogs, rhesus monkeys, and goats have served as experimental animals for studies in neural repair and many more species have been used for basic studies in regeneration. Much useful information has been gained from these studies but, as Woodhall has stated, man cannot be considered as simple a creature histologically as the rabbit, the cat, or even the monkey.

Ramón y Cajal suggested that the species of animal as well as the animal's age influenced the rate of reinnervation of the distal stump following severance but he did not cite evidence to support this impression. Review of the literature has failed to uncover comparative studies of response of various species to peripheral-nerve injury. In particular, the higher primates such as the baboon and the chimpanzee have not been studied in this regard.

The purpose of the studies reported herein is to delineate the reparative response of peripheral nerves to various forms of trauma in the dog, rhesus monkey, baboon and chimpanzee.

This paper will report response to neural severance while another paper will delineate the response to neural crush and to primary suture of nerves in various species.

Method

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed throughout this study. Four groups of adult animals were used. The 1st group was composed of 20 dogs, estimated to be between 1 and 3 years of age; the 2nd of 20 Macaca rhesus monkeys estimated to be 1 to 3 years old; the 3rd of 16 baboons estimated to be 1 to 3 years old; and the 4th of 12 chimpanzees known to be 5 to 8 years of age. The dogs, monkeys and baboons were anesthetized by intravenous pentobarbital. The chimpanzees were anesthetized by a technique utilizing intramuscular Sernylan® and intravenous pentobarbital. Base-line measurements of limbs were made and reflexes were tested whenever possible before each operation.

The peroneal, radial, and ulnar nerves were used for these studies. The peroneal nerves were exposed in the popliteal space, the radial nerves at the level of the middle third of the upper extremity, and the ulnar nerves at the level of the...
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The distal third of the upper extremity. Sharp dissection was used to expose each nerve over a 4–5 cm. length. Thresholds of stimulation were determined by means of a Grass stimulator and a bipolar electrode. The frequency was kept at 5 stimuli per sec. and the duration at 1 msec. The minimal voltage necessary to produce muscular contraction was termed the functional threshold of stimulation. In the intact nerve the threshold ranged from 1 to 2 V. Two epineural sutures of 0–0 silk were placed a measured 3 cm. apart. A 1-cm. segment of nerve was then resected between the markers leaving a proximal and distal stump each with a suture marker 1 cm. from the cut surface. The stumps usually retracted leaving a 2–3 cm. gap between them. Care was taken to excise all traces of connective tissue and neural branches between the stumps so that the only planes of tissue left were the true planes formed by the surrounding muscle. All wounds were closed with 4–0 silk.

The animals were reanesthetized at intervals of 1, 2, 6, 8, 14 and 24 weeks. Nerves were exposed and stimulated to redetermine the threshold of conduction. The dogs, monkeys and baboons were then sacrificed with pentobarbital. The chimpanzees were biopsied in the operating room and were not sacrificed. The proximal and distal nerve stumps as well as any intervening tissue were removed en bloc and pinned on carved paraffin blocks (Figs. 8, 11 and 12). The specimens were kept in 10 per cent buffered formalin for 1–2 weeks and embedded in paraffin. Cross sections of the proximal and distal stumps and multiple longitudinal sections of the proximal stump and central segment were made. Hematoxylin and eosin, Masson's trichrome, Bodian, Morgan's myelin, and Nissl stains were used.

Method of Grading. The gross and histologic characteristics of each specimen were graded on a 0 to 4 basis. The method of grading was as follows:

(i) Gross neuroma. Since all material was fixed and processed by the same method, comparison of the widest portion of a central longitudinal section with the diameter of the normal portion of the nerve 1.5 to 2 cm. proximal to the area of trauma was thought to be a valid estimate for comparative purposes.

0—No enlargement in the area of repair.
1—Less than two times the diameter of the normal nerve as measured 1.5 to 2.0 cm. proximal to the suture marker.
2—Two times diameter of the proximal segment.
3—Three times diameter of the proximal segment.
4—Greater than three times diameter of the proximal segment.

(ii) Connective-tissue spanning and axonal carry through.

0—Less than 0.5 cm. of gap spanned with connective tissue (spanning) and/or with axons (carry through).
1—0.5 to 1 cm. of gap spanned.
2—1 to 1.5 cm. of gap spanned.
3—1.5 to 2 cm. of gap spanned.
4—Complete bridging of the gap.

(iii) Disorganization. Figs. 4 (1), 5 (2), 2 (3), and 9 (4) are representative sections of axonal disorganization.

(iv) Distal collapse. Distal collapse refers to tubular collapse in the distal stump with endoneurial proliferation. Figs. 15 (3), and 16 (4) are representative sections.

(v) Connective-tissue proliferation. This was difficult to gauge in an objective manner and was the most subjective parameter of those graded. The use of multiple macroscopic and microscopic photographs was helpful in this regard.

Results

Table 1 shows the number of nerves and the interval of time they were studied in each species. Tables 2 and 3 contain the characteristics graded and the grades for each specimen studied.

All wounds healed primarily with the exception of the popliteal wounds of Dogs 17S and Z92 which healed by secondary intention. Weakness of limbs in the dogs was evidenced by decreased activity and difficulty in gaining their feet and walking. The monkeys and baboons remained quite active and weakness of the limbs, although present, was not as obvious as in the dogs and chimpanzees. In the chimpanzees there de-

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<thead>
<tr>
<th>Interval (Weeks)</th>
<th>Species</th>
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<tr>
<td></td>
<td>Dog</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<td>6</td>
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<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Total nerves</td>
<td>16</td>
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Table 1
Nerves severed