Peripheral Nerve Grafts: Experimental Studies in the Dog and Chimpanzee to Define Homograft Limitations

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NERVE homografting started experimentally in 1870 with the work of Philipeaux and Vulpian and clinically in 1878 with that of Albert. From the time of these early studies nearly 90 years ago to the present day, nerve homografts have usually failed because they provoked such a severe host reaction. Consequently, after World War II, Spurling, et al., and Seddon and Holmes concluded that homografts were not helpful as a means to bridge an irreducible gap in nerve repair.

However, the problems of homografts did not apply to autografts, for the latter met with nearly immediate success in the early 1900's. It became apparent with the general advances in medicine that the difference between homologous and autogenous tissue lies in the rejection phenomenon. To avoid graft rejection, physicians turned solely to autografts. However, in many cases autografts were not available, because the length of the lesion and the diameter of the nerve involved were so large that the patient was unable to spare a suitable graft without considerable neurological loss.

Due to the lack of suitable autogenous material in the typical patient who needs a nerve graft, experimental studies have again turned to homografts. Sanders and Young proved clearly that the immune reaction must be prevented if this type of graft was to be successful. Wallerian degeneration, irradiation, immunity-suppressive drugs, and a combination of degeneration and irradiation have been partially effective in reducing the host reaction. But the degree of success with these nerve grafts has been openly questioned.

The present study was designed to define the limits of success that can be anticipated with treated homografts. We chose not to study immunosuppressive drugs because the medication alters the immune reaction and endangers the host. Instead, we concentrated solely on reducing the antigenicity of the graft by irradiation and Wallerian degeneration.

**Materials and Methods**

The initial studies were carried out in beagle dogs. Before any operative manipulation, the animals were screened for any prior injury. All animals were anesthetized with intravenous pentobarbital 30 mg/kg, and endotracheal tubes were passed to assure a patent airway. The extremities were shaved and prepared with surgical soap and an antiseptic for exposure of four nerves in each animal. The two radial and peroneal nerves were used because the loss of function of these nerves caused minimal problems to the animal. The radial nerves were exposed laterally at the middle to lower third of the humerus and the peroneals in the popliteal space. The four nerves in each animal were treated differently. In six of the dogs, a 2-cm nerve segment was removed and replaced with 1) a control, measured gap, 2) an autograft, 3) a predegenerated homograft, or 4) an irradiated homograft; each replacement (1 through 4) was 2 cm in length. The remaining six dogs had 4 cm segments removed and the gap replaced with a 4 cm control gap, a 4 cm autograft, etc., as in the first group. With each different animal, one type of repair was rotated and alternated to a different nerve and/or side. All nerve anastomoses were done by the Silastic cuffing procedure developed in our laboratory. A two-layer closure covered each treated nerve. The
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animal was not splinted or braced in the post-operative period. No pre- or postoperative antibiotics were given. Monthly clinical and bioelectrical function studies were performed.

Six months after the grafts had been placed in the animals, direct objective studies of function were performed on the nerves under general anesthesia. Measurements of threshold stimuli of the nerve, conduction times (across the graft), and chronaxie were completed, then the grafts were removed. Threshold stimuli, although crude measurements, are informative and were performed with a micro-needle (32 gauge) placed in 10 different positions within the nerve, with a stimulus of 1 msec delivered at 1/sec, and with the millivolts varied until there was a definite contraction of the distal muscles. The results in volts found on 10 trials were averaged and recorded as the threshold stimuli. Conduction times were measured by using two times the threshold stimulus applied directly to both the proximal and distal nerve segments and with oscilloscope recording in the distal musculature (electromyo-graphic techniques). The difference between the proximal and distal stimulation time in eliciting the electromyo-graphic response over a given nerve graft segment expressed in meters per second gave the conduction time. Determination of chronaxie was carried out on the distal musculature proven to be supplied by the treated nerve and was determined from the duration of a stimulus required for a muscle threshold response to occur from twice the rheobase current. The biopsy segment extended 3 cm distal and proximal to the graft itself, well onto normal nerve. The specimens were pinned on paraffin blocks and fixed in buffered formalin. After fixation, the tissue was embedded in paraffin and sectioned. Staining with hematoxylin and eosin, Bodian techniques (neural elements), Masson techniques (mesenchymal elements), and luxol fast blue (myelin), were all utilized. The stained slides were morphologically compared.

The control gap was guaranteed by placing two epineural stitches in the separated cut nerve ends and tying them exactly 2 or 4 cm apart, as the experiment dictated. The autografts were taken fresh at the time of the initial operative procedure from one limb and placed in another. The predegenerated grafts were from separate beagle donor dogs whose radial and peroneal nerves had been cut under sterile operative conditions 3 weeks before being removed as free grafts. Once they were removed under sterile conditions, they were stored in air-tight sterile tubes in the frozen state in a metabolic balanced (Ringer's) solution. The irradiated homografts were taken from donor beagles under sterile conditions, placed in a metabolically balanced solution, sealed air-tight, slowly frozen, and stored. In the frozen state, the grafts were irradiated to a calculated 2 million rads from the cobalt source of the National Bureau of Standards, Washington, D.C. They remained frozen until used, at which time they were brought to room temperature slowly and sutured in place in the animals.

Simultaneously with the dog studies, we carried out more definitive studies in chimpanzees, whose neural tissue reaction is more analogous to that of man. Previous studies in two chimpanzees had demonstrated that gaps of 2 and 3 cm were not spanned spontaneously by regenerating nerves, indicating that such gaps would have to be grafted successfully if function were to result. With this information, nerve grafts were placed in male and female chimpanzees 3 to 7 years of age and 18 to 32 kg in weight. Preoperative handling of the chimpanzees required 40 mg of phencyclidine hydrochloride delivered intramuscularly via a syringe pistol. Anesthesia was introduced slowly with intravenous pentobarbital 16-20 mg/kg, and endotracheal tubes were placed for assisted respiration. The four extremities were shaved and prepared in the same manner as in the dogs for exposure of four nerves (two radial and two peroneal) in each animal. In the six chimpanzees, each nerve segment received a homograft that was both predegenerated and irradiated. A total of 24 homografts were anastomosed in place; there were four grafts of each of the six different lengths (2 through 7 cm inclusive). These animals also were not splinted or braced in the postoperative period; however, their follow-up period extended for 10 months after which the same clinical and bioelectrical evaluation was carried out.

It was recognized that both in dogs and chimpanzees it might have been desirable to