Experimental Irradiated Nerve Heterografts*

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ver the years innumerable methods for repairing large defects in peripheral nerves have been tried with only minimal success. Huber40 reviewed the literature in 1895 and described the following methods: a) nerve stretching (Schüller); b) heterografts; c) tubular structures; d) nerve flaps; e) cross grafting from an adjacent nerve; f) resection grafting of bones (Löbker).

The number of methods has increased considerably since then as various attempts have been made to solve this problem.

Results of end-to-end suture were reviewed by Highet and Holmes8 who found that when the joints were placed in acute flexion, traction injuries occurred and poor results were obtained. The ability to use nerve grafts would furnish an ideal solution to the problem of the large nerve defect. There is no doubt that if heterografts were successful they would be the operation of choice.28

The history of heterografts began in 1880 when Gluck7 implanted a fresh heterograft using a 3.5 cm. section of the sciatic nerve of a rabbit to bridge a 3 cm. defect in the sciatic nerve of a hen. He reported return of function in 11 days which according to modern knowledge is not possible. His experiment, however, is of historical interest. Johnson14 repeated the work in 1882 and found that the heterograft united with the proximal and distal nerve but that there was no sign of innervation after 23 to 34 days. Assaky2 in 1886 reported 4 transplants with 1 successful 3 cm. sciatic graft from a turkey to a rabbit with return of function in 35 days. Huber10 in 1895 reported 5 satisfactory transplants of sciatic nerve of a cat to the ulnar nerve of the dog with return of function within 120 days. He also reported 14 transplants in humans, 3 successful and 7 improved. One of the successful cases occurred within 16 days. Merzbacher20 in 1905 transplanted nerves from various animal species to other species and reported that they did not degenerate but that necrosis of the graft occurred.

Attempts were made also to prepare heterografts to make them more successful. Duroux6 in 1911 placed a cat’s nerve which had been kept on ice for 24 hours into a defect in a dog’s sciatic nerve, but the result compared unfavorably with even a fresh heterograft. Ingebrigtsen12 in 1915 reported that heterografts are unsuitable for bridging nerve defects. In 1916 he reviewed the cases published to date13 and concluded that only 1 heterograft could be considered as successful. Nageotte21 in 1917 conceived the idea of using a heterograft that was fixed in alcohol and kept in 50 per cent alcohol until 24 hours before use when it was transferred to Ringer’s solution. Although he claimed functional recovery, no details were given in his reports. Huber17 in 1919 reported that grafts of this type were innervated in the rabbit. Nageotte21 in 1917, using alcohol-fixed calf heterografts in the sciatic nerve of the dog, observed return of electrical excitability in 188 days. Sencert25 in 1918 performed 15 heterografts of alcohol-fixed calf nerves to humans but he did not follow the cases long enough.

Policard and Leriche27 in 1922 published the results of a 2-year follow-up on a transplant of the sciatic nerve of a calf to a human. The nerve elements had grown into the implant from below, but the upper end was blocked with fibrous tissue. Vargas Salcado29 in 1925 took up the work of Nageotte in Chile and reported 5 successful cases of alcohol-fixed calf nerve transplanted to the human. Sweet26 in 1929 reviewed the literature on nerve regeneration and reported the work of Nageotte and Sencert. He utilized alcohol-fixed fetal calf nerve to bridge 1.5 to 3 cm. gaps in the sciatic nerve of the dog. A significant amount of scar tissue developed at both suture lines hampering the growth of the axons and the percentage of success was very small. Sanders and Young23 in 1942 re-

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ported their work on heterografts using both fresh and alcohol fixed dog and rat nerves transplanted to rabbits. The grafts were attacked by white blood cells which at 25 days had almost destroyed the graft. Weiss and Taylor in 1943 stated that most des- vitalized heterografts behave like foreign bodies. Heterografts subsequently fell into disrepute and little has been added to the literature in the past 2 decades. Seddon stated in 1963, “heterogenous grafts behave as foreign bodies and are completely useless.”

With the advent of cathode irradiation to sterilize homografts and decrease their inflammatory response in the host, it was decided to investigate the value of irradiation in nerve heterografts. The grafts utilized in the following experiments were obtained as follows:

The grafts were removed from various species of animals under unsterile but clean conditions and immediately packaged in heat-sealed polyethylene bags and frozen to \(-12^\circ F\). The grafts were maintained in a frozen state and irradiated with 2,000,000 r.e.p. by a Van de Graaff generator which sterilizes the graft within the bag and alters its rejection by the host. The grafts were then stored in a freezer at \(-12^\circ F\) until used 4 to 8 weeks later.

**Experiment 1**

This experiment was set up to determine the effect of cathode irradiation on peripheral nerve heterografts. Irradiated and nonirradiated implants in animals were to be compared.

**Method.** Eleven rats of different strains and size were utilized for this study. Previously prepared frozen nonirradiated and irradiated peripheral nerves from dogs were cut into 1 cm. sections to serve as grafts. The rats were anesthetized with ether and a subcutaneous incision made in each thigh. The skin flap was dissected to allow the implant to be placed away from the skin incision. An irradiated heterograft was placed in the right thigh and a nonirradiated heterograft in the left thigh. Three rats were killed after one week, 3 after 2 weeks, 3 after 3 weeks, and 2 after 6 weeks. Gross examination and microscopic study were performed to determine the inflammatory response produced.

**Results.** Examination of 3 nonirradiated implants after 1 week revealed a moderate amount of swelling and inflammation about the heterograft. The microscopic sections were stained with hematoxylin and eosin. There were inflammatory cells about the implant with some areas of axis cylinder destruction.

Gross examination of the irradiated heterograft after 1 week showed very little reaction about 2 of the implants and swelling about the third, but very little inflammation. The microscopic sections revealed severe inflammatory reaction in 2 and a slight reaction in the third.

Two weeks after implantation the nonirradiated nerve revealed considerable swelling in all 3 implants. Microscopically there was a tremendous inflammatory response with invasion and destruction of the graft.

The irradiated nerve after 2 weeks revealed a small amount of swelling about one nerve and some slight scarring about the other 2, with little edema. Histologically there was a marked inflammatory response but less destruction of the nerve as compared to that in the nonirradiated nerve.

After the third week the gross appearance of the nonirradiated heterograft showed adhesions, edema, and a moderate inflammatory response. Microscopic sections revealed tremendous destruction with complete invasion of the graft by inflammatory cells.

The irradiated grafts after 3 weeks showed minimal adhesions, no edema and no inflammation. Microscopically the grafts revealed a severe inflammatory response about 2 implants and a minimal response about the third.

Six weeks after implantation, biopsy of the nonirradiated graft showed the implants to be bound down by adhesions. Microscopic sections revealed inflammatory cells in the graft and invasion of the periphery with a few cylinders remaining (Fig. 1 a)).

The irradiated grafts revealed some edema with adhesions about the graft. Histologically the axis cylinders were still present and one rat showed very little scar tissue (Fig. 1 b)).

**Discussion.** It was apparent from this study that irradiation had a slight depressive action on the inflammatory response produced by a nerve heterograft in a rat. Nonirradiated implants were all severely affected in contrast to some of the irradiated heterografts which were still satisfactory, with open axis cylinders.

Although some decrease in the inflammatory response was produced by this method, the degree of response varied considerably.

**Experiment 2**

The second experiment was set up to study the results of heterografts in a small laboratory animal. The facial nerve of the rat was selected as the peripheral nerve to be used in the study due to its accessibility and the fact that there are 2 main branches which innervate the whisker
muscles. This would allow the upper branch to serve as the graft site and the lower branch as a control.

**Method.** Thirty Sprague-Dawley white rats, with an average weight of approximately 200 grams, were selected for the experiment. They were anesthetized with ether and the regional hair was shaved. An incision was made transverse to the facial nerve. The subcutaneous tissue was retracted exposing the nerve and its two main branches. In 10 rats 0.5 cm. defects were created in both branches; in 10 rats 1.0 cm. defects were created (Fig. 2 (a)). Fifteen irradiated and 15 nonirradiated peripheral dog nerves which had been frozen at 12°F. for over 30 days and which were slightly longer than the defect were sutured into the upper branch of the facial nerve of each rat with 6–0 black silk sling sutures. The lower branch of the nerve was left with a defect to serve as a control (Fig. 2 (b)). The skin was then repaired with Michel clips.

The animals were examined every 3 days for evidence of nerve recovery using external electrical stimulation with 0.9 mamp. delivered cutane-
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Fig. 2 (c). Recovery time of the facial muscle in the rat when a heterograft is used; a comparison between irradiated and nonirradiated heterografts when a 0.5 cm. defect is repaired.

Fig. 2 (d). Recovery time of nerve heterograft over 1.0 cm. defect.

sponse actually preventing regrowth of the axons over even a short distance.

Experiment 3

The third experiment was set up to determine the effectiveness of an irradiated heterograft in a larger animal. Five mongrel dogs were used. Each dog was anesthetized and the sciatic nerve exposed. The peroneal branch was separated from the tibial branch and a 2 cm. section was removed from the peroneal nerve. A 3 cm. irradiated heterograft was used in 4 cases and a nonirradiated heterograft in 1 case. The graft was sutured into place using 0–0 atraumatic silk sutures. The gait of the dogs was observed. The graft site was explored 6 months or more postoperatively. The individual experiments will be presented in detail because of the importance of the experiment.

Graft from Calf to Dog 3–1 (Nonirradiated). A brown and white mongrel Collie dog was operated on July 9, 1963, and a 1.5 cm. section of the peroneal nerve was removed and replaced with a 3 cm. nonirradiated calf nerve graft. At examination 4 months postoperatively the dog still had a foot drop and limp. One year after grafting the dog still had a foot drop. On March 4, 1965, the graft site was explored and the old scar incised. The grafted area appeared thin and atrophic. Stimulating the nerve with 4 volts produced no evidence of nerve conduction. There appeared to be hard fibrous tissue about the proximal suture line and the graft felt fibrotic. Microscopic sections of the graft including the proximal suture line revealed a large neuroma with swirling of the axons at the distal suture line and replacement of the graft by fibrous tissue (Fig. 3 a).

This nonirradiated graft failed in a manner comparable to that reported by others experimenting with heterografts.
Fig. 3 (a). Microscopic sections of nonirradiated calf heterograft (3-1) prepared with Glees' silver stain.
Upper left. Large neuroma evident at distal suture line: \( \times \)3.
Upper right. Swirling of axons in the neuroma is evident and boundary between scar and axons indicated by arrow: \( \times \)40.
Lower left. Axons are seen in the neuroma (arrow): \( \times \)120.
Lower right. Section of distal scar indicating the collagenous tissue with no evidence of axons: \( \times \)290.

Graft from Calf to Dog 3-2 (Irradiated). On July 16, 1963, a 2 cm. section of the peroneal nerve of a mongrel dog was removed and replaced with a 3 cm. irradiated calf nerve sutured in place with interrupted 6-0 mersilene sutures. At 4 months after the procedure the dog was observed to have a slight limp but a foot drop was not apparent.

On May 24, 1964, the graft site was explored through the old incision and an electromyogram was performed. There was no evidence of denervation of the muscles supplied by the peroneal nerve. Electrical stimulation of the peroneal nerve and graft produced vigorous dorsiflexion of the foot. The graft was well incorporated with only a minimal amount of scarring. The junctions of the graft at the suture lines were smoothly united. The nerve graft was left in place and the incision repaired. The dog was sent out to the farm after the wound healed.
On February 25, 1965, the dog appeared to be walking normally and again the graft site was re-explored. The nerve graft appeared to be smooth and well incorporated with only slight thickening at the proximal and distal ends. There was only minimal scarring about the graft despite the previous exploration. The graft was removed and microscopic sections were made. Silver stains revealed axons passing through the graft. This could be classified as a successful heterograft of a calf nerve to a dog (Fig. 3 b).

*Fig. 3 (b).* Microscopic section of irradiated calf heterograft (3-2) prepared with Glees' silver stain.  
*Upper left.* Longitudinal section of irradiated graft revealing continuity of specimen: $\times 5$.  
*Upper right.* Axons take a dark silver stain and have a beaded appearance within the graft: $\times 40$.  
*Lower left.* Section of graft with axons running through graft: $\times 120$.  
*Lower right.* Axons passing through graft at higher magnification: $\times 240$.

Graft from Human to Dog 3-4 (Irradiated). On August 6, 1963, a mongrel dog was operated on and a 1.5 cm. section was removed from the peroneal nerve and replaced by a 3.5 cm. irradiated human nerve graft which was sutured into place with 7-0 black silk. The dog was examined on November 18, 1963, and only a slight limp was apparent and no evidence of a foot drop. On May 7, 1964, the peroneal nerve graft was explored and a minimal amount of scar tissue was seen about the graft. Stimulation of the nerve
proximal to the graft and of the graft itself produced vigorous dorsiflexion of the foot. The incision was repaired and the graft was left in place. On March 4, 1965, the graft site was re-explored and a minimal amount of scar tissue was found. Stimulation of the graft produced good dorsiflexion of the foot. The graft was excised and was well incorporated into the proximal and distal ends of the nerve. Microscopic sections revealed axons traversing the graft into the distal end (Fig. 3 c). This can be classified as a successful irradiated human heterograft to a dog.

_Graft from a Human to Dog 3-6 (Irradiated)._ On June 4, 1964, a large mongrel sheep dog was operated upon; a 1.5 cm. section of the peroneal nerve was removed and replaced with a 2 cm.
section of human irradiated nerve graft sutured into place with 6-0 silk (Fig. 3 d). In October, 1964, the dog was observed to walk with a limp and a prominent foot drop. In January, 1965, the dog still walked with a limp at times and on January 28, 1965, the peroneal nerve was explored. A minimal amount of scarring was observed about the nerve graft and it was well united at both ends (Fig. 3 e). Stimulation of the nerve produced good dorsiflexion of the foot. The graft was removed for study and appeared larger than the dog peroneal nerve, but it should be noted that the original human graft was larger in diameter than the dog peroneal nerve. Microscopic sections revealed axons traversing the graft. This may be classified as a successful human irradiated peripheral nerve graft.

It appears that irradiation of the nerve heterograft will prevent rejection of the graft in the dog. Severe fibroblastic reaction with replacement of the graft by scar tissue did not occur.

**Graft from Calf to Dog 3-3 (Irradiated).** On July 30, 1963, a 1 cm. section was removed from the peroneal branch of the sciatic nerve of a mongrel dog. A 2 cm. irradiated nerve graft from a calf was sutured into place with 2-0 interrupted silk sutures repairing the defect. The dog was evaluated on November 18, 1963, and was observed to have a slight foot drop. On May 14, 1964, the graft site was explored and the nerve graft had very little scar tissue about it (Fig. 3 f). The graft was well incorporated into the peroneal nerve. Electrical stimulation produced vigorous dorsiflexion of the foot. The wound was repaired and the animal allowed to be active in a kennel. On April 22, 1965, the animal was noted to walk without a limp and there was no evidence of a foot drop. At exploration the peroneal nerve and heterograft could be easily dissected out and were well united. Stimulation of the graft produced vigorous dorsiflexion of the foot. Microscopic sections stained with Glees' silver stain revealed axons within the graft (Fig. 3 g).

This experiment represents the successful transplant of irradiated calf nerve to the peroneal nerve of a dog.
Discussion

Review of related research reports beginning with the early work of Gluck has revealed some successful heterografts, but documentation of results has been poor. Many, but not all, of the early concepts of nerve regeneration have been disproved with the passage of time. Huber wrote in 1895, "I may state that in all cases of division of a peripheral nerve, the regeneration of the peripheral end depends on the outcome of a struggle between the downgrowing axis cylinders and the developing connective tissue between the severed ends." This statement is as true today as it was in 1895, as it is the inflammatory response and connective tissue that destroys the graft. The progress that has been made in tissue transplantation has assisted us to understand statements that were made in the past. Marinesco in 1907 stated that new fibers growing out of the central stump definitely avoid a heterograft
as though it contained an "antineurotropic" substance. It is the rejection of the graft by the host which produces the violent inflammatory response that interferes with the advance of the axons. Kilvington in 1908 believed that nerve fibers could regenerate unassisted over a gap of less than 2 cm. It was our experience in Experiment 2 that the facial nerve of the rat would regenerate over 0.5 cm. but could not span a 1.0 cm. gap. In the rats receiving a nonirradiated graft, regeneration was retarded by the inflammatory response. Many authors have concluded that heterografts do not work because of the inflammatory response produced. Sweet stated in 1929 that the regenerative power of the nerve is so great that good results may often be obtained, not because of any particular method but in spite of it.

Our first experiment in the rat with dog heterografts revealed that irradiation with 2,000,000 r.e.p. associated with freezing will decrease but not prevent the inflammatory response in the host. Destruction of some of the implants occurred with white cell invasion as described in the experiments of Sanders and Young in 1942. The difference between the 2 grafts was more obvious grossly than by histological examination.

The use of irradiation of the heterograft in Experiment 2 in the rat was much more encouraging.

In contrast with the irradiated heterografts, none of the nonirradiated nerve heterografts recovered function. Fifty per cent of the irradiated heterografts were able to span a 1.0 cm. defect whereas none of the 20 nonirradiated control animals showed return of function. Although some inflammatory response was present in the irradiated heterograft it still allowed regeneration to occur.

Experiment 3 was of great interest in view of the excellent documentation of the cases. There was recovery of function in 4 cases with irradiated heterografts and no recovery in the one nonirradiated heterograft. The gap spanned by these grafts was 3 cm. long and from previous work it was apparent that regeneration over this distance is not very certain. The fact that all 4 of our animals recovered function should tend to stimulate interest in heterografts as a method of bridging nerve defects and encourage further study of this problem.

Summary

We have reviewed all available reports on nerve heterografts. We have presented a method of preparing nerve grafts that utilizes cathode irradiation and freezing. We have reported experiments on the use of irradiated heterografts including 5 types of heterografts used successfully in animals. We suggest that irradiation of nerve heterografts may be useful in tissue transplantation in man.

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References

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