Repair of Peripheral Nerves by Grafts of Frozen-Dried Nerve.

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Repair of Peripheral Nerves by Grafts of Frozen-Dried Nerve.*

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Orientation, growth rate and grouping of growing nerve fibers are determined by the biophysical and biochemical constitution of the interfaces along which they advance.[1] Their most favorable growth medium is degenerated peripheral nerve.[2] Accordingly, peripheral nerve fragments are the best means of bridging traumatic nerve gaps.[3] In mammals, autografts can be fully, homografts adequately successful;[4] heterografts are largely discredited.[4,5] Nerve grafting in man has met with the difficulties of (1) growth-obstructing fibrosis of the distal suture line, and (2) unavailability of grafts. The former may be obviated by resection of the barrier,[6] or possibly completely avoided if sutureless nerve splicing by arterial sleeves[7] should prove as successful in man as it has been in animals. The supply difficulty is more serious, since commonly no source other than the patient's own intact nerves is available. In order to avoid having to sacrifice a "minor" nerve for the repair of a more vital one, several authors have tried preserved or fixed nerves. Storage in petrolatum[8] leaves the grafts viable, but presumably not indefinitely. Alcohol-fixed grafts[8,9] lead to poor regeneration,[5] formalin-fixed grafts to complete failure.[9]

Basically, these past failures are attributable to the denaturation of the grafts. Therefore, one of us (P. W.) thought of trying a preserva-

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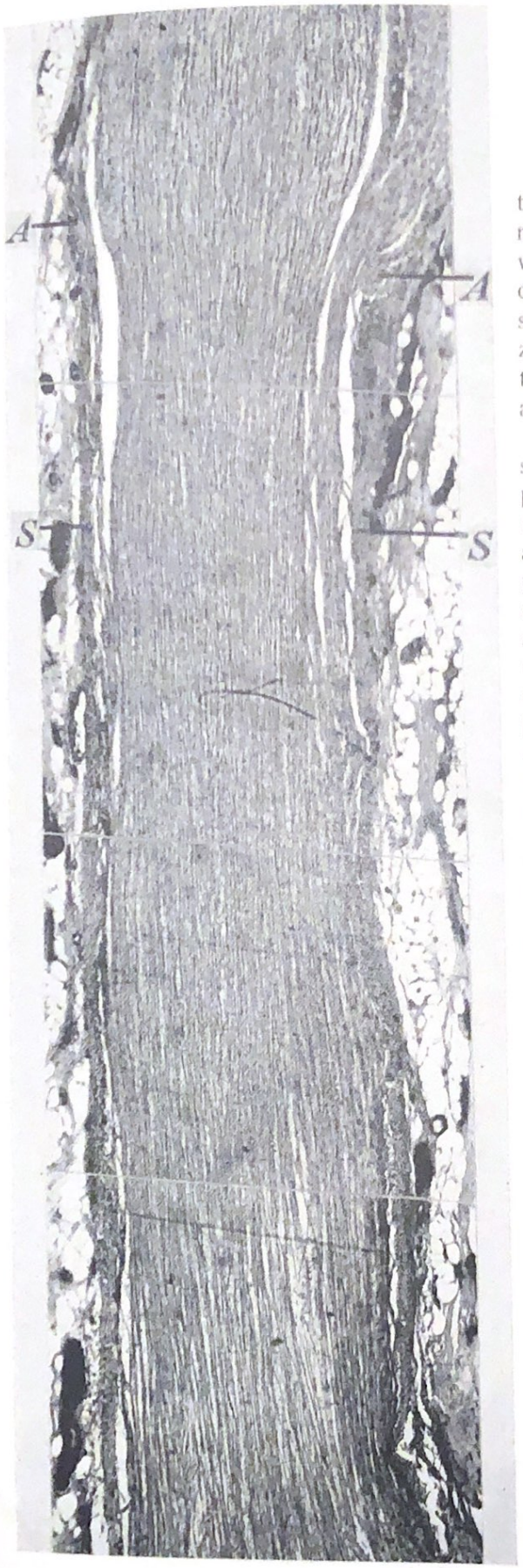

FIG. 1.
Regeneration through frozen-dried-rehydrated nerve graft sleeve-spliced to proximal stump of tibial nerve in rat, 127 days after operation. A, proximal end of arterial splicing sleeve. S-S, original level of fusion of proximal stump and graft.

tive, but non-denaturing treatment: the Altmann-Gersh freezing-drying method[10] used with great success in histology and histochemistry.[11] This method preserves microstructure and major biochemical (*e.g.*, enzyme) constitution without essential alteration. This very feature determines its suitability for the preparation of nerve grafts.

Our procedure is as follows: Nerves dissected aseptically are dropped into Isopentane[12] immersed in liquid nitrogen (−195°C), where they freeze instantaneously, and transferred to high vacuum and mercury pumps for about one week of dehydration over P_2O_5 at −40°C. The dry specimens are stored in sealed sterile containers. Before use, they are rehydrated in vapor at −40°C or in Ringer's solution *in vacuo* at room temperature. They resume their normal appearance and major histological characteristics,[13] including specific staining reaction (silver impregnation and Mallory Triple Azan).

Segments of these "devitalized" nerves of about 1-2 cm length were grafted into gaps of hind limb nerves of 38 rats, 4 cats, and 18 monkeys. These grafts were spliced to the nerve stumps by 2 short arterial sleeves,[7] or a single long sleeve containing the graft. Twenty-nine rat nerves (21 homoplastic, 8 cat-to-rat) have thus far been studied microscopically, and a few oscillographically, from 6 days to 18 weeks after the operation.

Homoplastic grafts heal and promote regeneration much as does live nerve. The regenerating sheath cells and nerve fibers invade them promptly, traveling in straight parallel courses, without appreciable branching or confusion. Fig. 1 shows a typical case, 4 months after grafting: regeneration is so perfect that there is no evidence of the old proximal "suture line" (level marked S-S). The regenerated fibers regain caliber (Fig. 2), impulse conduction is restored, and motility and sensitivity return. Nerves predegenerated

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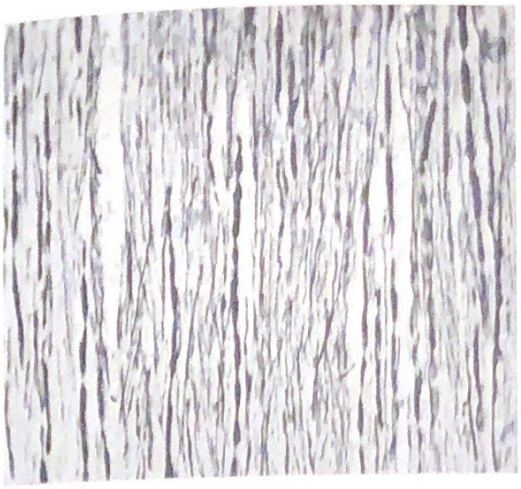

Detail from Fig. 1, showing the regenerated nerve in the zone of the graft. × 230.
FIG. 2.

before treatment do not seem superior to undegenerated ones. Details will be reported later. Sclerotic islands present in a few grafts may tentatively be ascribed to imperfections in the freezing or rehydration process.

Most devitalized heteroplastic grafts behave like foreign bodies. However, masses of nerve fibers use their oriented surface as pathway.

The results indicate that the biophysical and biochemical benefits to regenerative nerve growth inherent in peripheral nerve are essentially preserved in the freezing-drying process. The fitness of devitalized nerve as graft brings the supply problem in nerve grafting nearer its solution. Banks of assorted nerve sizes stored in the dry condition could readily fill a steady demand.