ROGER W. SPERRY

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Nerve Reunion with Sleeves of Frozen-Dried Artery in Rabbits, Cats and Monkeys.*

PAUL WEISS.
From the Department of Zoology, University of Chicago.

Sutureless nerve reunion by arterial cuffs in the rat was described in an earlier paper.[1] Instead of live arteries, frozen-dried ones may be used after rehydration. A more recent analysis[2] revealed the reasons for the superiority of this method. Evidence has now accumulated to show that it is equally satisfactory in larger mammals. This conclusion is based on a total of 132 arterial nerve splices in rabbits (21), cats (30) and monkeys (81).

Material and Methods. The nerves used were mostly the peroneal, tibial, or sciatic. No distinction will be made here between simple reunion of severed stumps and reunion with the intercalation of a nerve graft, mostly of frozen-dried nerve (see following article). For arteries, the aorta or carotid was used in most cases; the former is preferable for its lack of musculature. Freezing, drying, storing, and rehydration were done as previously reported,[3] always with strict aseptic precautions. Elasticity, consistency, and histologic character are not noticeably affected by this treatment. For the reunion of severed nerve stumps, arterial segments of matching caliber and measuring from 6 to 10 diameters in length were fitted over the nerve ends by the following improved technic (Fig. 1):

First, the sleeve is pulled over one nerve end with the instrument illustrated in Fig. 1A-C. A stainless steel tube (a) contains a movable flexible wire (b), one end of which rests against a coil spring (c), while the other end is fastened to a chuck (d) ending in two half-cylinders (e_1 and e_2) of the same diameter as tube a, which act as a clutch. They are manipulated through lever f: depressing the lever (Fig. 1A) produces retraction of the conical chuck and closure of its jaws; on releasing the lever, spring c forces the end piece out and the clutch (e) opens owing to the elastic spread of the jaws (d). When the lever is depressed and the open end plugged by a fitting cone (g), the instrument forms a continuous pointed rod (Fig. 1A). In this condition, it is threaded through the lumen of the arterial segment, and the latter is slipped over the shaft a. The lever is then released, cone g is removed, and the free nerve end is grasped between the jaws of the clutch by depressing the lever again. The sleeve can now easily be slipped back from the shaft onto the nerve. This corresponds to stages a-e of our earlier practice (Weiss,[1] Fig. 1). In a second step, the other nerve stump is introduced into the open end of the sleeve. To facilitate this, the latter is widened and held open by a "spreader" (Fig. 1D, E), consisting of 2 curved metal strips (h) attached to the ends of a steel spring bow (i). Instruments of several sizes were available to fit different nerves.†

It is advantageous to leave a small blood-filled gap between the nerve ends in the sleeve. When grafts are used, their length can be adjusted so as to avoid either slack or exces-

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1 Weiss, P., *Arch. Surgery*, 1943, **46**, 525.

2 Weiss, P., and Taylor, A. C., *Arch. Surgery*, 1943, **47**, 419.

3 Weiss, P., and Taylor, A. C., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 326.

† The instruments were designed jointly with Dr. A. C. Taylor who also did the actual constructing.

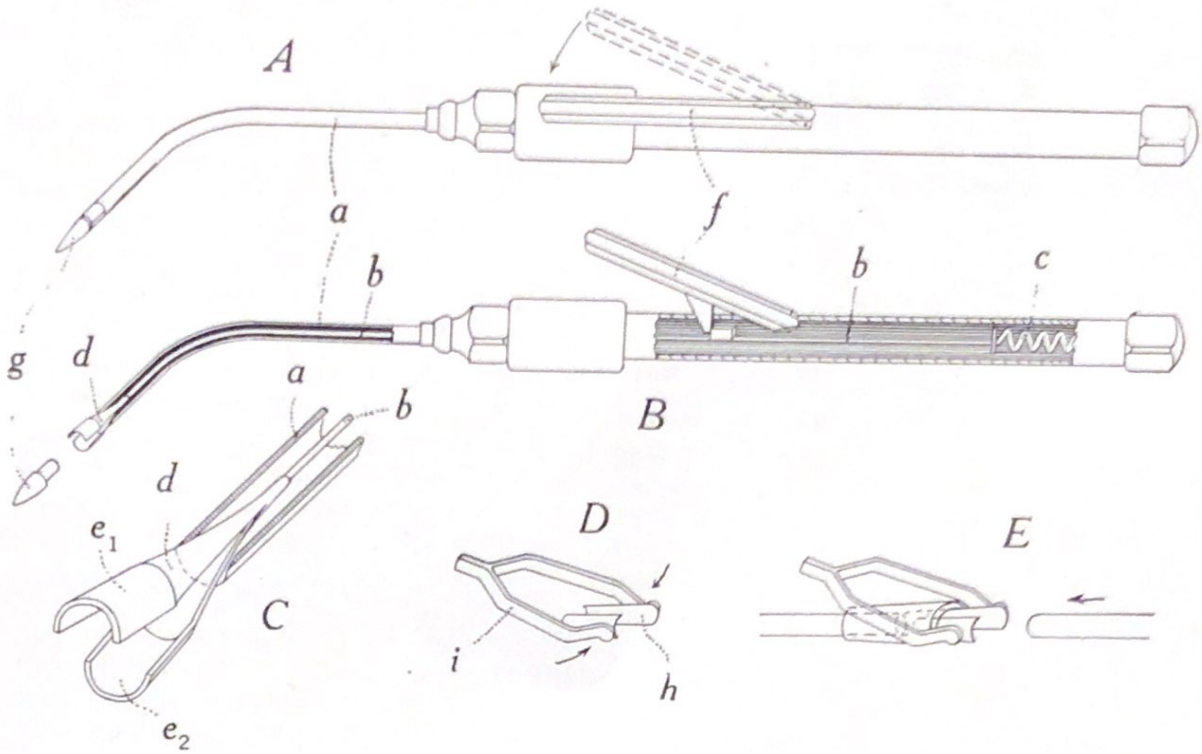

FIG. 1.
Splicing instrument. Explanation in text.

sive stretch, and no further attachment between sleeve and nerve is necessary. The same applies to nerve ends approximated under moderate stretch. Higher degrees of stretch require special provisions (see below). No sutures have been used in any of the cases dealt with in this report: the elastic sleeve, sealed to the nerve by clotting blood, was the only link. No casts or other means of restraining active and passive use of the operated limbs were employed. This placed a severe test upon the arterial unions.

The animals (rabbits, cats, spider monkeys, and rhesus monkeys) have been under observation for up to 10 months. Histological studies thus far completed include 4 rabbit, 8 cat, and 25 monkey splices, in addition to 30 monkey biopsies.

Results. Only in one rabbit and one monkey nerve has the arterial link failed to hold. In all other cases, nerve regeneration has occurred much in the same way as described for the rat under similar conditions. Criteria of success were the rate and completeness of functional recovery and the histological appearance of the union. From the functional standpoint, the results were optimal; histologically, there was a greater proportion of cases with some fiber entanglement than was seen in the rat—a difference readily accounted for by the greater variability in the operative handling of larger nerves. An average case is illustrated in Fig. 2 showing a sleeve-spliced rabbit sciatic nerve 2½ months after the operation; it represents the average in that the line of junction is more evident than in optimal cases, yet less than in the worst cases.

Nerves properly operated upon regenerate in the orderly fashion peculiar to arterial sleeve splicing:[1,2] the great mass of the regenerating fibers pass straight, unbranched, unobstructed across the gap into the distal stump, and there is neither fibrosis nor neuroma formation. This orderly regeneration pattern makes it possible for the majority of fibers of a given fascicle to remain together and, therefore, to reinnervate a relatively localized muscle group, instead of becoming dispersed over the whole denervated periphery at random, as commonly happens after ordinary sutures. As an experimental test of this fact, the following example may be cited:

The right tibial nerve of a spider monkey was severed in the proximal half of the thigh,

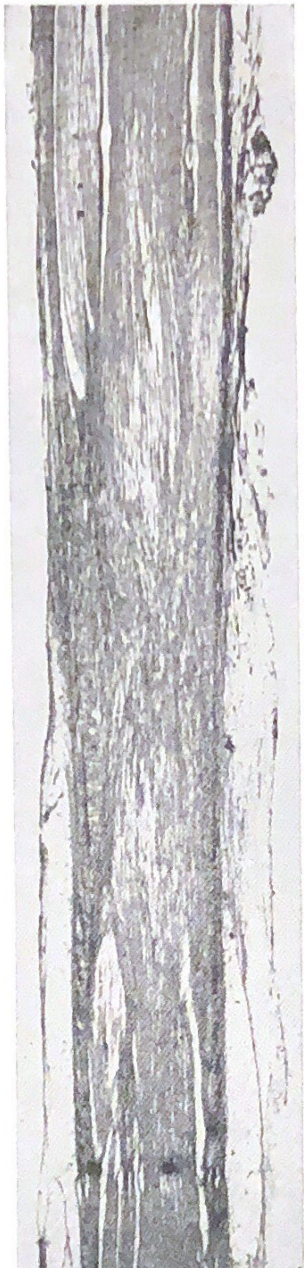

FIG. 2.

Rabbit sciatic nerve 2½ months after reunion with sleeve of frozen-dried artery. Bodian impregnation. × 12. This illustrates an average case considerably below optimum.

and the ends were spliced with a segment of frozen-dried artery from a spider monkey, which had been stored for 2 months, then rehydrated and kept in Ringer's solution in the refrigerator for 2 days before use. (The right peroneal nerve, likewise severed, had received a graft). When examined 6 months after the operation, motor function was completely recovered. The site of the nerve was then exposed and the tibial trunk proximal to the splice was split into 8 constituent fascicles. By stimulating these individually with induction shocks, 6 different and distinct motor effects were obtained, which demonstrates the preservation of fascicular topography in regeneration through the splice. The combined calf muscles of this animal weighed 56 g on the regenerated side and 52 g on the control side.

Branching and commingling of fibers at the junction result from defective operations, *e.g.*, sagging arteries; slack nerves; overhanging epineurium or perineurium. Aside from greater irregularity of the fiber courses, regeneration seemed, however, unimpaired even in these instances.

The frozen-dried arterial sleeves persist as such for many months, perhaps indefinitely. They become partially repopulated by host cells. In contrast to live sleeves, their walls absorb a certain number of regenerating fiber branches. Homoplastic frozen-dried sleeves provoke no marked inflammatory reaction. However, adhesions are not uncommon, their extent depending more on the condition of the wound bed than of the artery. Heteroplastic frozen-dried arteries (macaque in spider monkey) cause heavier adhesions than do homoplastic ones. Keeping rehydrated arteries for a few days refrigerated in Ringer's solution does not seem to harm them. Fresh arteries stored for several weeks in aqueous solution of Merthiolate 1:1000 and then washed, proved less adequate. Other methods of preservation (boiling, alcohol, formaldehyde) are definitely contraindicated, as they transform the artery into a foreign body and deprive it of many properties essential for nerve splicing. Veins tried in the monkey have proved too flabby for the purpose. No artificial substitute has as yet been found that would come up to the standards of the arterial sleeve; they all fall short of one or more of the principal functions of the sleeve, which are:[2] to align the nerve ends; to permit longitudinal stress to act on the "scar"; to prevent dissipation of fibrinolytic agents from the gap and thus insure proper liquidity of the "scar"; and to keep fibrous tissue and other local growth from penetrating. Continued research may yet produce a synthetic sleeve answering these demands.

For end-to-end reunion of stumps under tensions greater than an arterial cuff can hold, means must be devised to reconcile the need for forcible approximation with the cardinal precept for good regeneration, viz., to keep the zone of union free from artificial intervention, particularly sutures. A solution may lie in approximating the stumps by means of a permanent loop of Tantalum or Columbium wire, stitched either through the nerve stumps or the epineuria at considerable distance (more than one inch) from the cuts, and then splicing the free ends with an arterial sleeve just as in unstretched unions. It may be expected that the gradual "give" at the points of attachment will place enough strain on the young tissue connecting the stumps to produce in it the desired longitudinal organization. Results of this operation will be reported on a later occasion. Experiments are also under way to determine the maximum length of a blood filled gap between stumps compatible with optimal nerve regeneration.

Summary. Results obtained with sutureless nerve reunion by means of cuffs of live or frozen-dried artery in 21 rabbit, 30 cat, and 81 monkey nerve splices have confirmed for the larger mammals the conclusion previously reached in the rat, that optimal nerve regeneration may be secured by this method.