The nerve response after autotransplantation of the rabbit ear

Neurohistologic investigations on 16 autotransplants of the rabbit ear ranging from 3 weeks to 18 months after replantation are described. This study investigated degeneration and regeneration of the adrenergic nerves of the vascular system and the afferent endings in hairy skin. Histochemical techniques, including hematoxylin-eosin staining by cholinesterase and glyoxylic acid fluorescence, and impregnation techniques with silver and osmium–zinc iodide were used. Central nerve reinnervation was started as early as 3 weeks after autotransplantation and usually was completed after 8 weeks. Spotty small areas of incomplete reinnervation could be found in the skin adnexa (hairs) even after 18 months. After transplantation the regenerating nerves were not attached to the vascular wall and were not incorporated in the Schwann cells of the distal parts of the transected and repaired vessels. The newly formed adrenergic nerve plexus was less dense than the normal one. Areas of patchy reinnervation and denervation were observed in peripheral vessels after 18 months. The defects in the restoration of the adrenergic vascular plexus similar to insufficient afferent reinnervation of skin and vessels may be responsible for the cold intolerance seen clinically and experimentally. (J HAND SURG 9A:121-4, 1984.)

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The observation that cold intolerance persists after replantation of fingers and extremities was the motivation for this neurohistologic investigation of nerve regeneration after rabbit ear autotransplantation. According to Gelberman et al.[1] restoration of an adequate digital blood flow in transplants is essential for the return of digital sensibility and the avoidance of cold intolerance. It has been our observation that blood flow is dependent not only on an efficient readaptation and new formation of vessels but also on the reinnervation of the preexisting vascular system by the efferent and afferent constituents of the nervous system. We therefore decided to study the degeneration and regeneration of the nerve supply to the vascular system in the skin of rabbit ears from 3 weeks to 18 months after autotransplantation.

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Received for publication Sept. 25, 1981; accepted in revised form Jan. 17, 1983.

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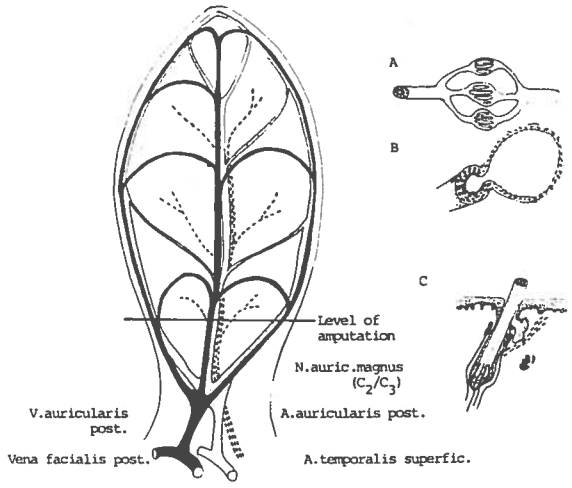

Fig. 1. Vascular and nerve supply of the rabbit's ear: *A*, Capillary system between arterioles and veins; *B*, AV anastomoses: *C*, innervation of the hairs.

The vascular and nerve supply of the rabbit ear

The rabbit's ear is supplied by a large central artery from which project arcade-like branches at different levels. These supply blood to the skin, muscles, and cartilage of the ear. The branches terminate in arte-

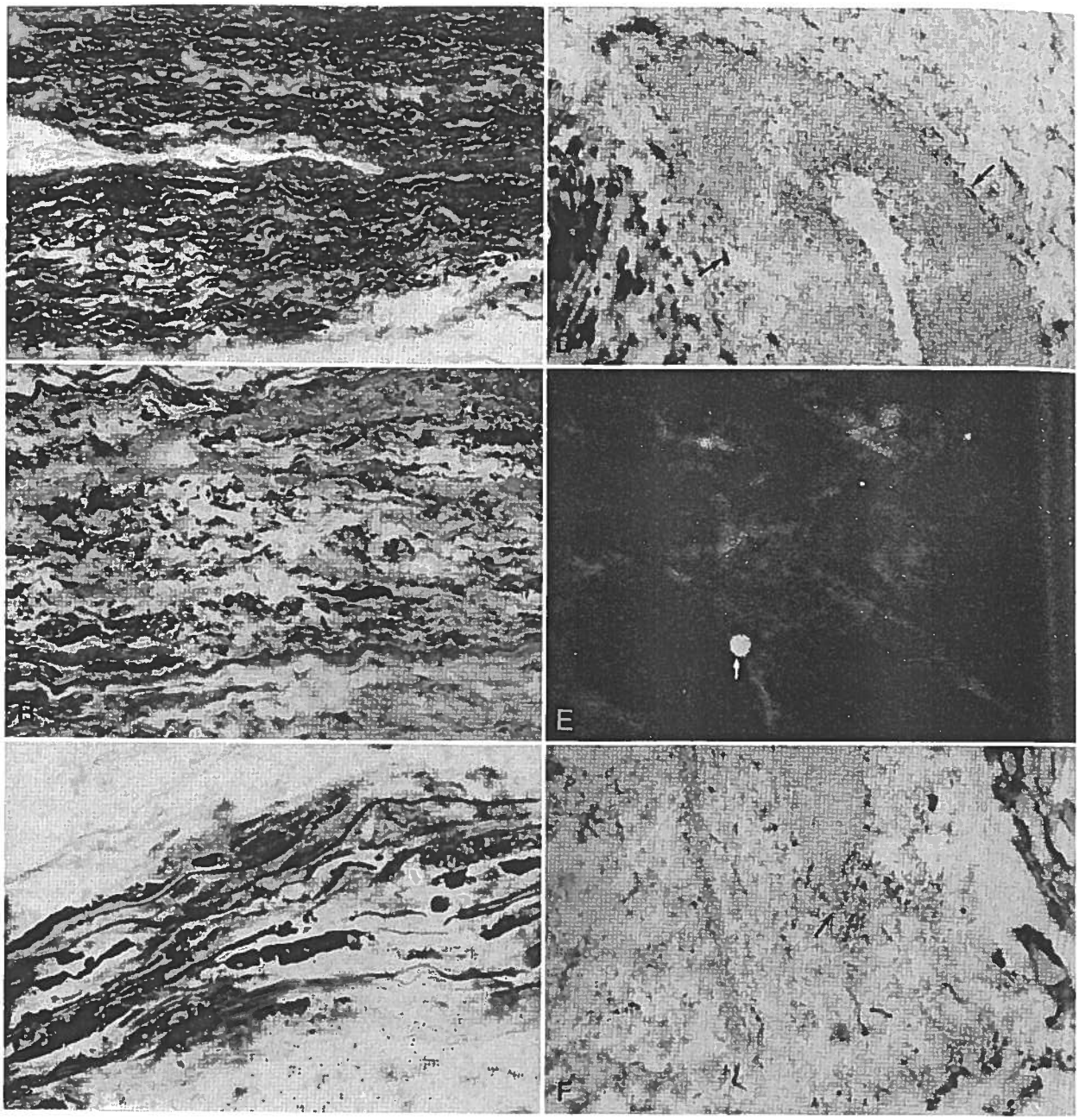

Fig. 2. Rabbit ear 3 weeks after autotransplantation. **A** and **B**, Basal area: axon (**A**) and myelin degeneration in the central nerves. **C**, Middle area: incomplete axon and myelin degeneration in the central nerve. (**A**, Cauna silver impregnation; **B**, Sudan black; **C**, Cauna silver impregnation; **A-C**, Original magnification ×200.) **D**, Basal area: degeneration of the adventitial nerve plexus of the central artery with remnants of cholinesterase-positive fibers *(arrow)*. (Specific cholinesterase method. Original magnification ×125.) **E**, Aggregation of noradrenergic fluorescence material in the course of degeneration of the adventitial plexus of the central artery *(arrow)*. (Original magnification ×300.) **F**, Upper lateral area: incomplete degeneration of the adventitial plexus of a small artery *(arrow)*. (Specific cholinesterase method. Original magnification ×125.)

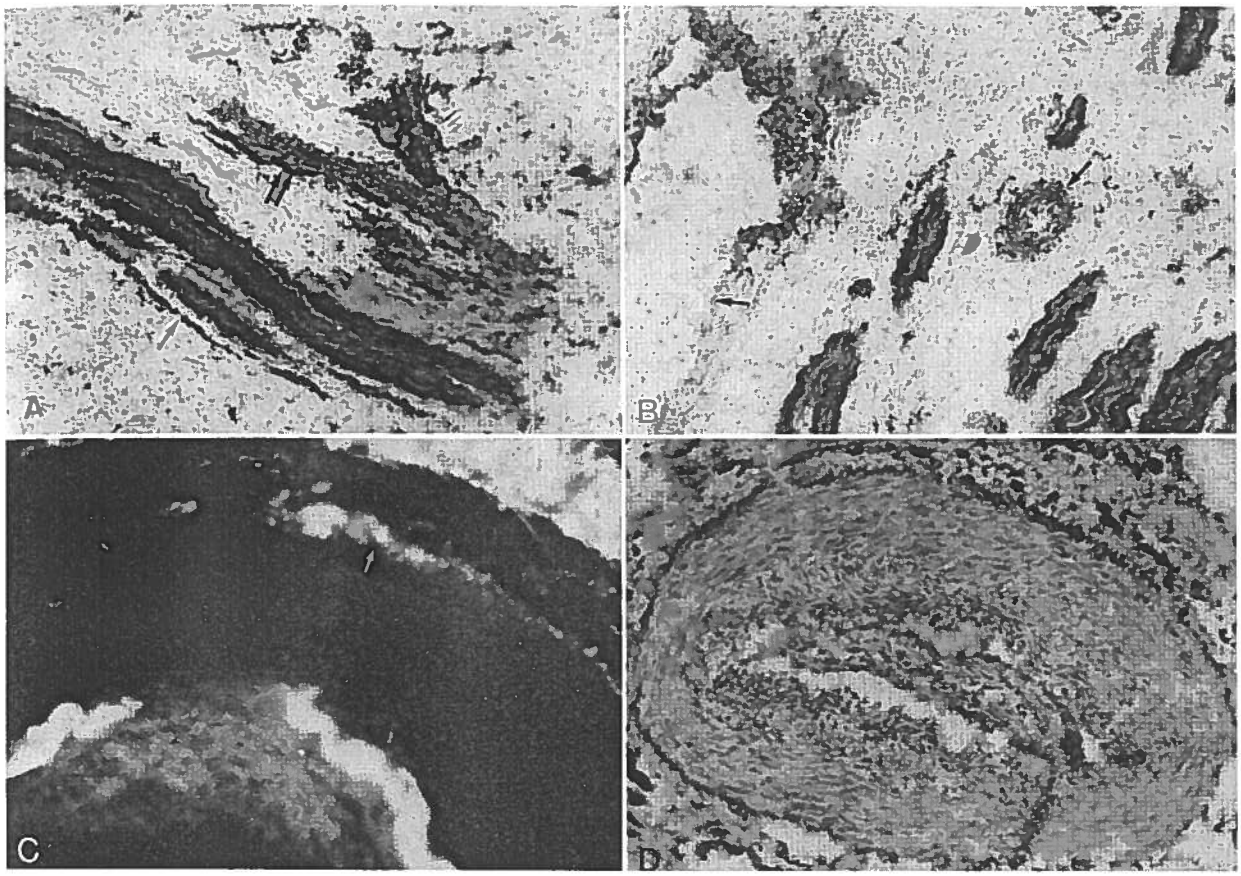

Fig. 3. Eight weeks after transplantation. **A,** Regeneration of the central nerve *(one arrow),* incomplete regeneration of the vascular plexus in a small artery *(two arrows),* and arterial segment of an AV anastomosis *(three arrows).* **B,** Regenerated central nerve and complete lack of adventitial nerve plexus in a small artery and vein *(arrow)* in the upper peripheral part of the ear. (**A** and **B,** Specific cholinesterase method. Original magnification ×125.) **C,** Restitution of the noradrenergic vascular plexus in the central artery in the basal parts of the ear with noradrenergic nerves in the media *(arrow).* (Glyoxylic fluorescent method. Original magnification ×300.) **D,** Restitution of the adventitial nerve plexus in the central artery with typical separation of parts of the plexus by connective tissue. Endothelial valve similar to that in arteries of the sclera in other species. (Specific cholinesterase method. Original magnification ×200.)

riovenous (AV) anastomoses or in smaller arterioles found in the capillary system. Blood flows from these to the veins in the central and peripheral parts of the ear. The major nerve arborizes into smaller branches in the central portion of the ear; these branches are accompanied by arterioles as they spread to the periphery (Fig. 1). The nerves contain myelinated axons of different sizes with some nonmyelinated "beaded" noradrenergic fibers. The skin and its adnexa are innervated by branches of the central nerve. The muscle fibers in the basal parts are supplied by moter fibers forming regular end plates. The central artery is innervated by an adrenergic adventitial nerve plexus as fibers penetrate the muscle coat of the arteries. The density of nerve innervation is highest in the AV anastomoses and arterioles. The nerve supply to the capillaries is variable with neural innervation accompanying the capillaries for different distances without direct connection with the capillary wall. Neurologic influence on the capillaries is possible only by the mechanism of "synapse at a distance," and in such situations we postulate that a hormone such as noradrenalin influences endothelial receptor cells. Venous innervation is less extensive than that of the arteries.

Material and methods

The neurovascular response was studied in 16 rabbit ears after amputation and replantation at 3, 4, and 8 weeks, and at 4, 5, 6, 8, 12, 14, and 18 months. Two normal, unoperated ears were also studied and served as control specimens. Sections were taken from the basal, middle, and upper lateral parts of the ear.

Routine hematoxylin-eosin sections were examined in addition to sections that were stained with Sudan black B in cryocut and paraffin slices. Other techniques used include that of specific cholinesterases developed by Root-Karnowsky. In addition, fluorescence microscopy with the use of the glyoxyl acid method of Lindvall and Bjorkland[2] on native noradrenergic fibers was also used. In addition, osmium–zinc iodide (Maillet-Jabonero) and silver impregnation (Cauna and Jabonero) techniques were also used.

Results

Sixteen autotransplants were investigated from 3 weeks to 18 months after surgery. At 3 weeks wallerian degeneration of the central nerve and of the adventitial nerve plexus in the central vessels was observed. Remnants of cholinesterase-positive particles and granular degeneration of noradrenergic fluorescence particles also were seen. The nerve supply of the small arteries and veins under the skin appeared to be normal (Fig. 2). At 4 weeks wallerian degeneration in the basal parts of the central nerves is complete and the degeneration of the adrenergic nerves in the basal parts of the central vessels is far advanced with only a few fluorescent granules and cholinesterase-positive particles present. The adrenergic plexus of the peripheral arteries and the afferent nerve supply to the skin and its adnexa are reduced. Regeneration of axons into some fascicles was observed in the basal part of the central nerve. Central nerve regeneration to the middle part of the ear was nearly complete after 8 weeks. Regeneration was also observed in the adventitial plexus of the central artery. At 4 months regeneration of the central nerve was advanced but not complete in two animals, and regeneration of the peripheral vessels and vasculature of the skin was incomplete (Fig. 3). At 6 months regeneration of the central nerve and nerves to the central artery, peripheral arteries, and the skin and its adnexa was seen, but differences in completeness were observed in these areas in animals at 18 months. A characteristic feature of new-formed adrenergic adventitial plexus and the central artery is separation of the plexus and the artery muscle by connective tissue.

Discussion

The nerve supply to the vascular system of the rabbit's ear consists mainly of noradrenergic fibers as described by De la Lande,[3] Hume and Waterson,[4] and Grant and Thompson.[5] Unlike the crush experiments on the cervical sympathetic trunk in mice (Romine et al.)[6] where the Schwann cells in the peripheral stump persist and become associated with regenerating axons, the Schwann cells of the adventitial plexus did not associate with regenerating axons after autotransplantation. There appears to be a difference between developing the innervation to peripheral vessels after birth and replantation, but to date it has not been possible to experimentally define the factors responsible for the difference. There is also a lack of understanding of those factors that influence the outgrowth of autonomic nerve fibers; these factors appear to be different from those that influence the outgrowth of the afferent axons. We feel it is important to study the spot-like defects observed in the afferent innervation of skin and its adnexa and a separation of adrenergic innervation and the vessel wall after ear autotransplantation seen in this study. Further studies on the factors that influence the outgrowth of the nerves to the vascular system and skin after replantation will help clarify these questions and we recommend the rabbit's ear as a good model for further research in this area.

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