Chapter 6

THE ROLE OF EPENDYMA IN SPINAL CORD REGROWTH

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IN THE VERTEBATE Central Nervous System (CNS), the epithelial type cells that line the ventricles and the central canal are considered a type of glia and are commonly called ependyma or ependymoglia. They are the cells that remain and retain their epithelial character after the embryonic neuroepithelium produces neurons and astroglia and oligodendroglia. In some adult vertebrates certain of these ependymal cells possess long radial processes, which still reach the pial border, as did all of their neuroepithelial precursors (Schonbach, 1969).

In 1970 a group of scientists at the Boulder Colorado Conference recommended a name change to ventricular cells. That is indeed the "address" for these cells, but it is only accurate in part. The ependymal cells we have studied would more specifically have to be called "ventricular-intermediate-marginal" cells, which defeats the purpose and is very awkward. In addition, ependyma (Gr. meaning "upper garment") is an attractive and distinctive name, so we continue to use it for these most attractive and distinctive cells, even when they are regenerating and embryonic. Some vertebrates may lack other types of glial cells but all have ependymal cells, so it is phylogenetically a very basic type of glue indeed.

Spinal cord regeneration is not a new discovery. It occurs in adult teleosts, urodele amphibians, and in some reptiles. Early in this century it was shown that not only does the spinal cord regenerate when a salamander's tail is cut off, but that it is the regrowing spinal cord that controls or induces the regeneration of the rest of the tail.

In 1955 Kamrin and Singer showed that the spinal cord is necessary also for lizard tail regeneration. A few years later, Simpson (1964) demonstrated that only the ependymal cells of the spinal cord are necessary for lizard tail regeneration. Despite the known trophic action of nerve fibers, it is the ependyma that controls tail regeneration in the lizard and possibly in the salamander, since no amount of nerve fiber transplantation and supplementation can substitute for the spinal cord in this phenomenon of tail regrowth.

The ependyma also plays a role in regrowth of ablated segments of spinal cord, as demonstrated by Stephanelli (1944) for *Triton*. These two

types of spinal cord regeneration — gap replacement and caudal regrowth — represent different problems to the organism, but the ependyma is involved in both solutions. We have studied the ultrastructure of the regenerating spinal cord in the lizard tail *(Anolis carolinensis)* and in the newt tail *(Triturus viridescens)*, so this discussion will deal primarily with caudal replacement of the spinal cord. Recent work in Singer's lab has also included the ependyma of the optic nerve in both embryonic and regenerating animals and embryonic ependyma from other parts of the CNS (Turner and Singer, 1974; Singer et al., 1979).

In caudal regeneration after wound healing and immediately beneath the newly formed epidermis, the ependyma extends from the cut cord as a hollow vesicle of cells continuous as a tube with the old central canal. These cells divide, producing an ever increasing length of ependymal tubing. Sometimes in the lizard they give rise to two vesicles, making a double ependymal tube and hence a bifid tail.

Just as in the old cord ependyma, these newly produced ependymal cells are linked tightly together around the extended central canal by junctional complexes. The external (also called marginal or basal) portions of the cells extend in radial fashion to a surrounding area of collagen and elastic fiber accumulation with a smattering of connective tissue type cells. Just proximal to the new terminal vesicle the elongating ependymal tube is enclosed by a basement lamina, continuous with that of the old cord at the amputation stump.

An interesting aspect of this new ependymal tube is the occurrence of empty-looking intercellular spaces just inside the marginal surface of the basal processes. If one looks at a more proximal section taken from an older tail regenerate, one sees an occasional axon within these spaces. More proximal still will be found more numerous axons and growth cones. In regions close to the amputation plane in older regenerates, these marginal interependymal spaces have become packed and expanded with neurites (Simpson, 1968). At this level the new spinal cord has greatly increased in diameter from the initial outgrowth, but the ependymal cells still span the entire radius of the new cord. Their basal processes have become greatly elongated and at the basement lamina are mushroomed out to form pial endfeet, which completely enclose the increased masses of sprouting axons and dendrites. There are now additional channels forming and side branches or filopodia from the radial ependymal processes, which subdivide the intercellular spaces and the contained fascicles of neurites. In older regenerates in the proximal region of new growth will be found newly formed myelin sheaths; in the newt, new neurons are also produced by this "rejuvenated" ependyma.

Serial section reconstruction has shown that these basal interependymal

spaces are continuous from one section to the next and so form tunnels or channels. It is in these ependymal channels that the regenerating neurites extend. Newly formed neurons in the salamander come to lie in the apical region of these channels, i.e. just lateral to the region of the ependymal nuclei but medial to the large neurite fascicles from the old spinal cord.

These spaces or channels have been seen by many investigators in embryonic brain and spinal cord and excused as artifacts. It is certainly true that embryonic and regenerating CNS tissue has more intercellular spaces than does mature brain or spinal cord. It is also true that embryonic tissue is more difficult to fix. In general, developing nervous tissue is more compact than the surrounding mesenchyme. Pysh (1969) and later Caley (1971) have described large intercellular spaces in embryonic brains and a decrease in these spaces with maturation. This change parallels the decrease in CNS glycoaminoglycans (GAG) and the decrease in histochemical staining reactions for these substances.

Krayanek (1977) found an intercellular matrix in embryonic chick tectum prior to neurite invasion and at later stages following denervation. Enzyme digestion eliminated this ground substance, as did routine electron microscopic procedures. These studies suggested a specific intercellular location for concentrations of GAG. Spring and Hope (1978) demonstrated in living epithelia an active change in the size of intercellular spaces with changes in transport. Thus what was once dismissed as artifact must now be examined as very functional aspect of epithelia.

We have been unable to eliminate the ependymal channels by varying the fixation and embedding procedures. Dare we dismiss as artifact the intercellular structure used by neurites in their directed growth just because it is found outside of the cells themselves? We have known since Ross G. Harrison's work that axons do not grow across liquid spaces but require some sort of solid substrate. Letourneau (1975) has reported that axons *in vitro* have favorite substances for substrates and that by controlling the substrate one can control the axonal growth pattern. It has also been shown that an axon lengthens by growth in the region just proximal to the growth cone (Bray and Bunge, 1973) and that the firmest substrate adhesion occurs at the growth cone itself by way of microspikes. Thus, microspikes of the growth cone of the lead axon trip along attracted to whatever signpost lies in its way. Some attachments are withdrawn and some remain as it travels along spinning out the unattached axon in its wake. Subsequent axons use the pioneer or lead axon as a guide and fasciculate in characteristic fashion. Thus the end result is a bundle of axons adhering more closely to each other over longer distances than any of them adhere to the surrounding glial processes.

In the early stages of neurite formation in regenerating as well as

embryonic CNS, blood vessel penetration has yet to occur. The nutritional requirements must be supplied by the surrounding vessels in the mesenchyme where the meninges are developing.

When does the embryonic neuroepithelial tube cease being neuroepithelial and become ependyma? We think it does not change essential character with maturation, and at least in the lizard and newt caudal extremities, the mature ependyma can reexpress the embryonic neuroepithelial developmental role.

There are many differences between the lizard tail regenerates and the salamander tail regenerates, but there is also a striking similarity in the way in which the ependymal tube grows out, pulling its "red carpet" along with it in the form of channels.

If the regenerating ependyma contains within it the genetic information or blueprint for the new spinal cord, then what about the embryonic ependyma in its initial development into a fetal spinal cord? Does it also express this sort of information by production of interependymal spaces or channels prior to neurite invasion? From our electron microscopic examination of *Xenopus* (Figs. 6-1, 6-2), newt (Figs. 6-3, 6-4), and chick embryonic CNS (Figs. 6-5 through 6-8), the answer is "yes." Spaces form between primitive ependymal processes in the right places and at the right times, and also in consecutive sections such that channel formation precedes neurite invasion, and all of this precedes the differentiation of the target tissue.

The feature of preplanning on the part of the ependyma is responsible for the formulation of our blueprint hypothesis. This differs from preexisting hypotheses such as contact guidance and chemotrophic guidance by naming the ependyma and its products as the matrix which forms the scaffolding for future tracts of the CNS involving both contact guidance and chemotrophic features.

Thus we describe pioneering fibers (Weiss, 1941) or lead fibers (Lopresti et al., 1974) as having their free choice or pathways restricted by the preexisting ependymal channels. Perhaps the growth cones with their many microspikes are sampling the signposts on the ependymal membranes or within the intercellular substance produced by the ependymal cells as they venture forth from an already predetermined site of origin of the parent neuron cell body. This blueprint is expressed by Nornes and Das (1972) as the interface between the marginal zone and the ependymal cell body or later between the neuronal soma and the regressing but still productive ependyma. Hendrickson and Vaughn (1974) described dendritic interactions with "radial glial processes" as being the guidelines in a radial direction until newly formed synapses of passing structures should reflect the growth process into a new direction.

These "radial glial processes" are external ependymal processes

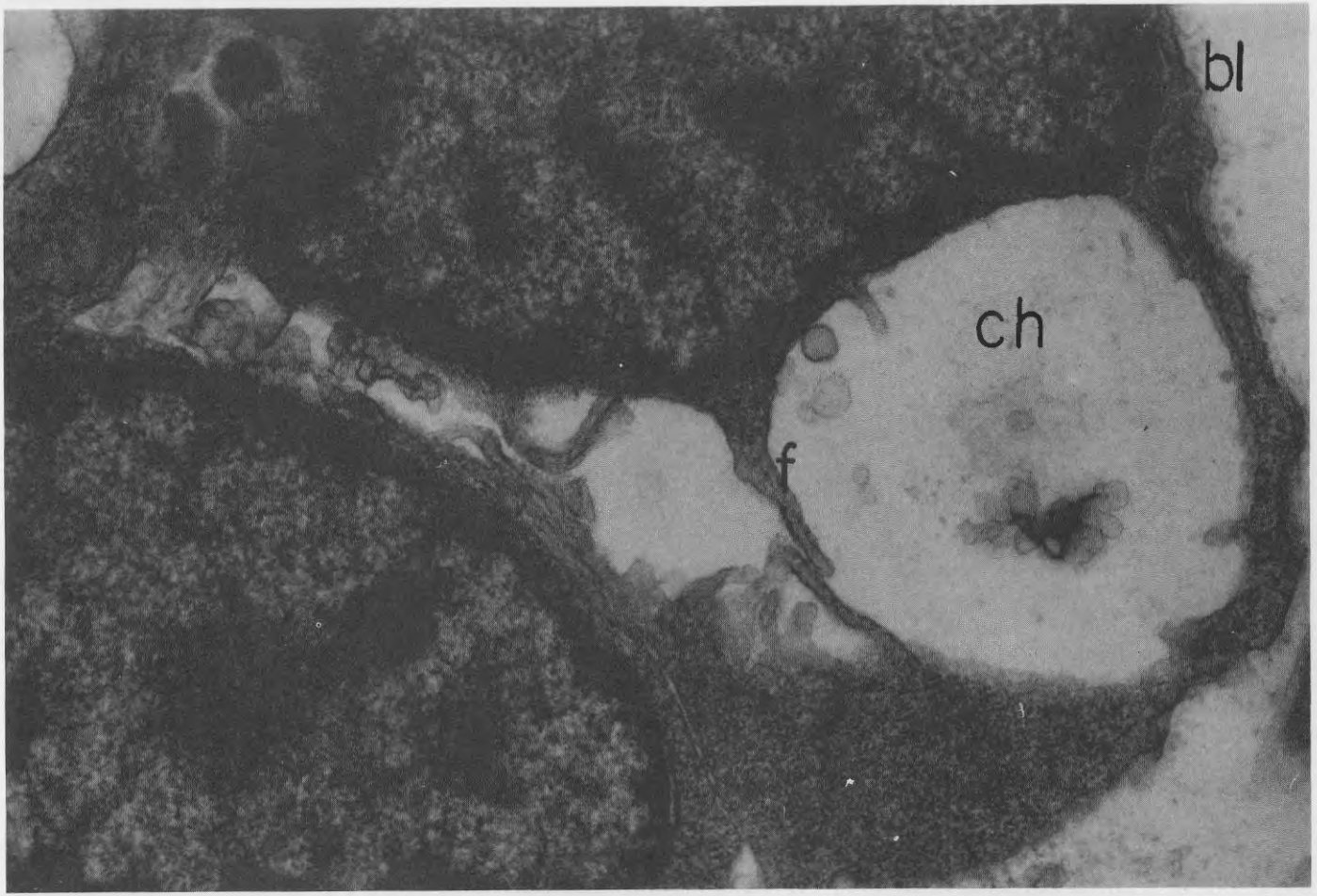

Figure 6-1. Lateral ependymal channels in a cross section of the distal tail of a *Xenopus* tadpole. The basement lamina (bl) is not well formed at this level. Notice the ependymal filopodia (f), which extend into the channels (ch) from the adjacent cells. × 19,000.

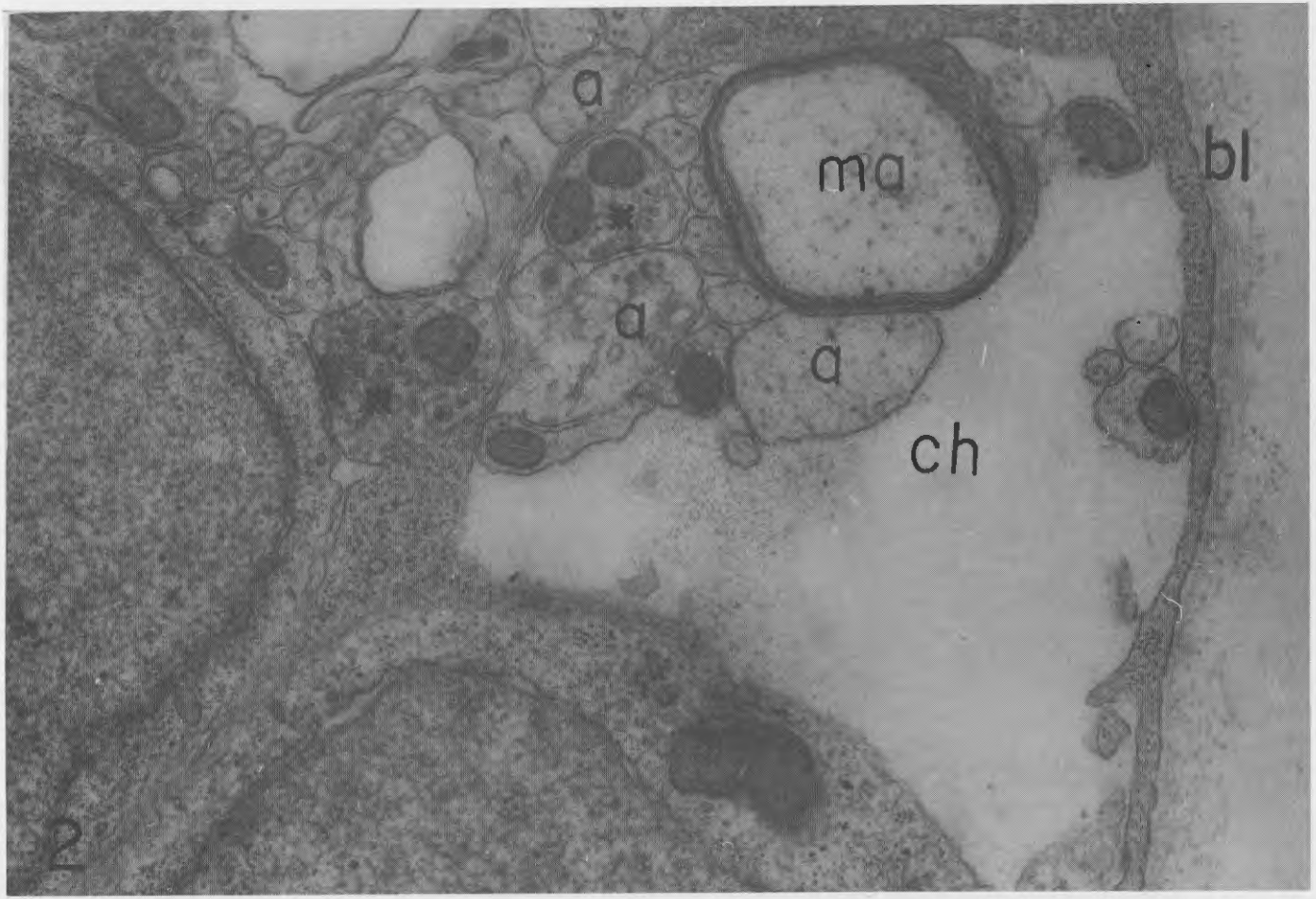

Figure 6-2. A more proximal cross section of a *Xenopus* tadpole tail with axons (a) beginning to fill the ependymal channels (ch). Notice the well-formed basement lamina (bl), the synaptic vescicles (*), and the myelinated axon (ma). ×29,850.

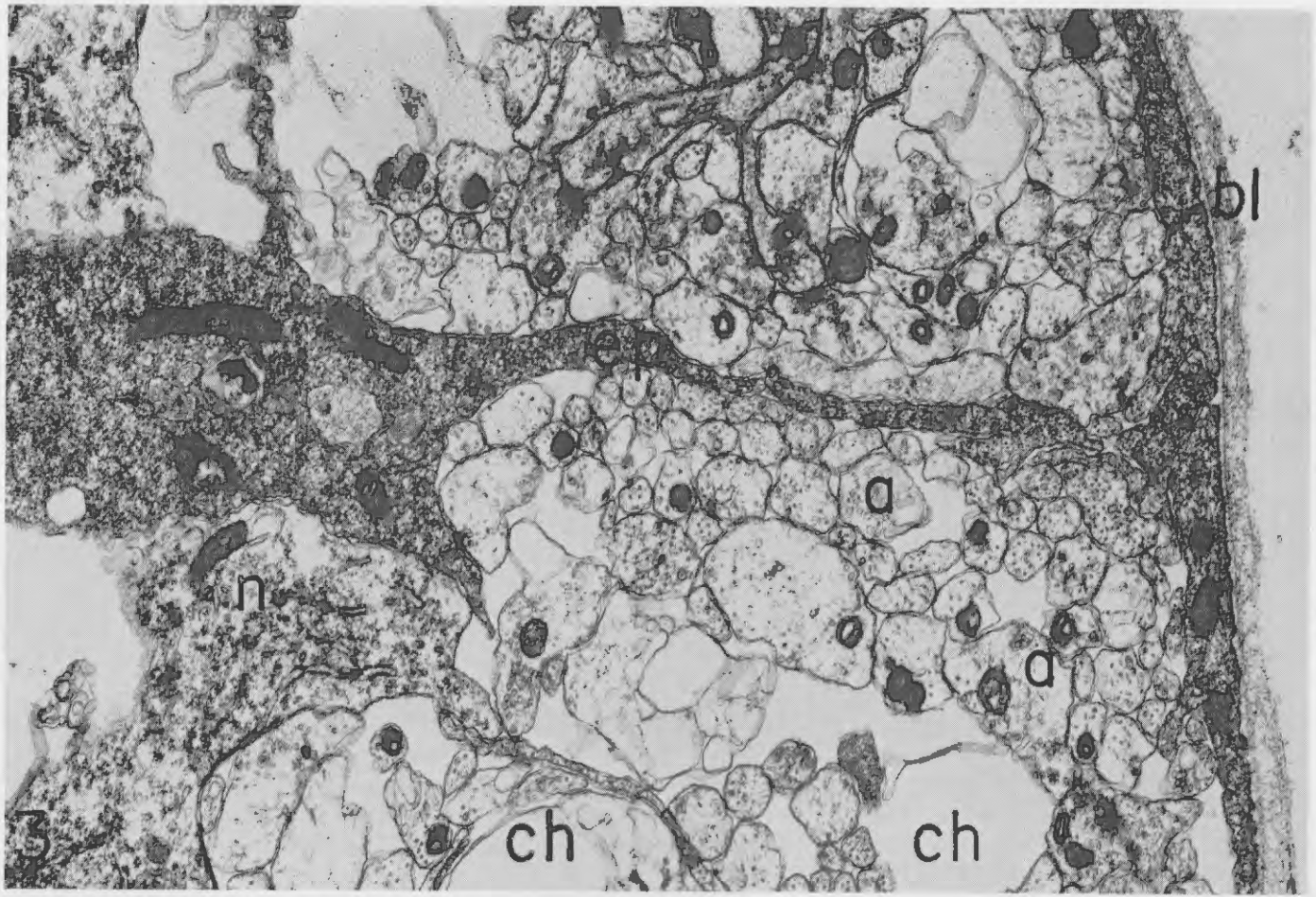

Figure 6-3. Ependymal channels in the lateral region of a *Triturus* larval tail. Notice the external (radial) ependymal process (ep) cytoplasmic density as contrasted to the adjacent neuronal cytoplasm (n). Basement lamina (bl); axon (a); ependymal channel (ch). ×23,000.

Figure 6-4. A lateral neuronal process (np) in an ependymal channel of *Triturus* larval tail. Synaptic connections are numerous (arrows) between this process and the axons that fill the ependymal channels at this level. ×31,000.

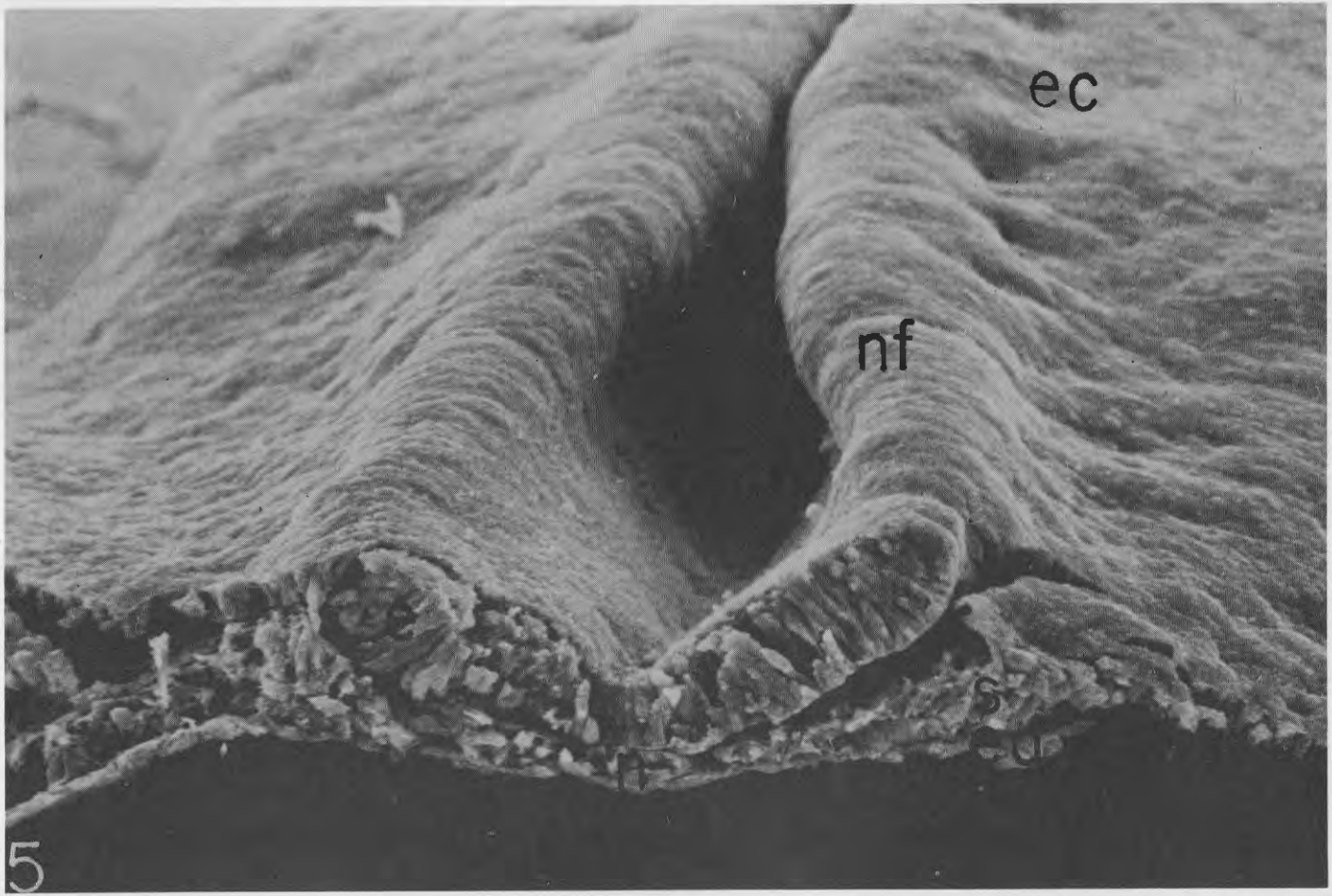

Figure 6-5. A scanning electron micrograph of the ependymal cells (e), which compose the neural folds (nf) of an early chick embryo (stage 8, Hamburger-Hamilton, 1951). The folds are closing in the region of the hindbrain. Notochord (n); somite (s); ectoderm (ec); and endoderm (ed). × 575.

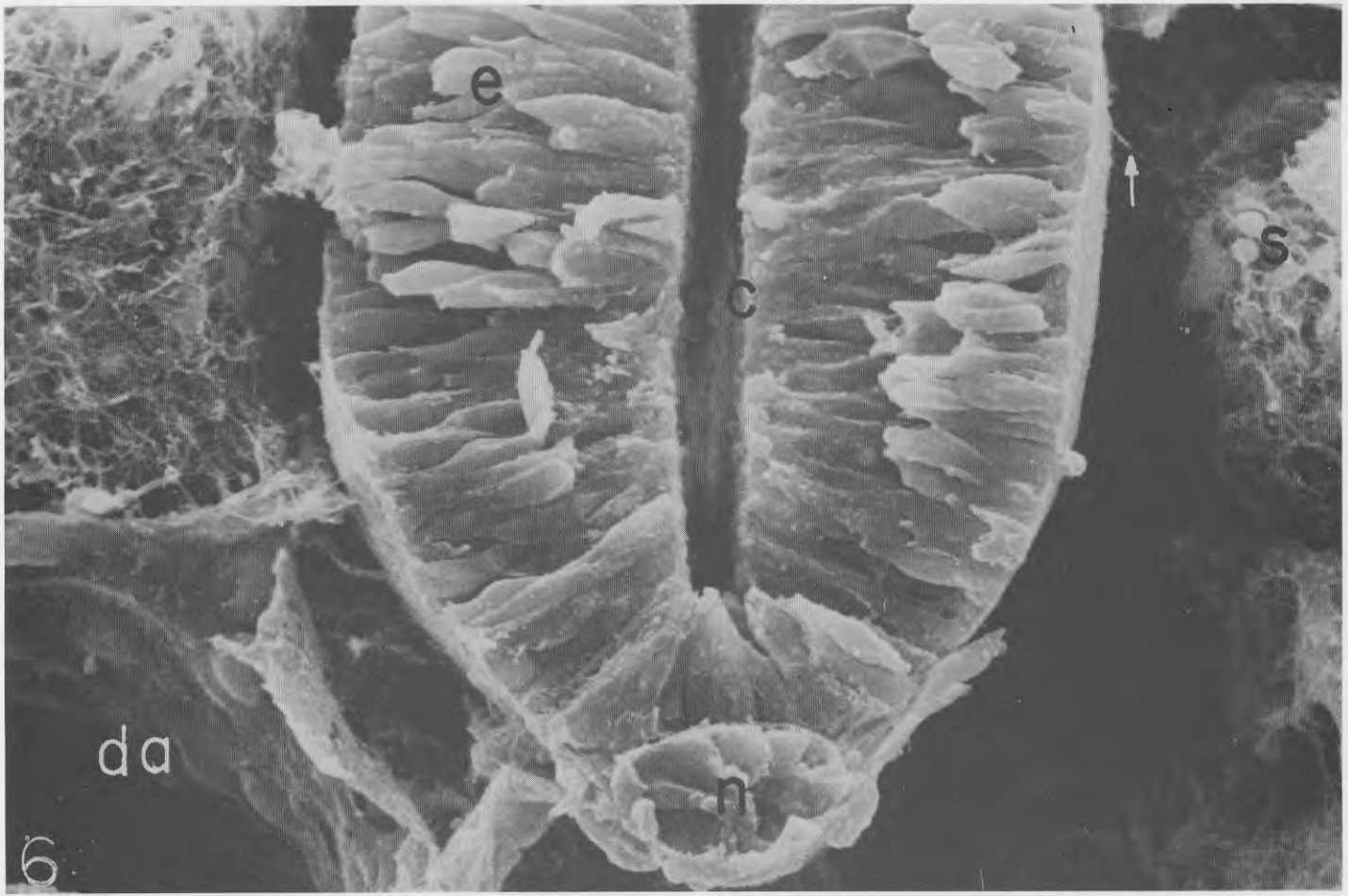

Figure 6-6. A scanning electron micrograph with a cross-sectional view of the thoracic spinal cord of a chick embryo at stage II (Hamburger-Hamilton, 1951). Central canal (cc), notochord (n); somite (s); dorsal aorta (da). Notice the fine filament connecting the somite to the lateral spinal cord at the white arrow. The notochord is more closely attached by many similar fibers. ×1,600.

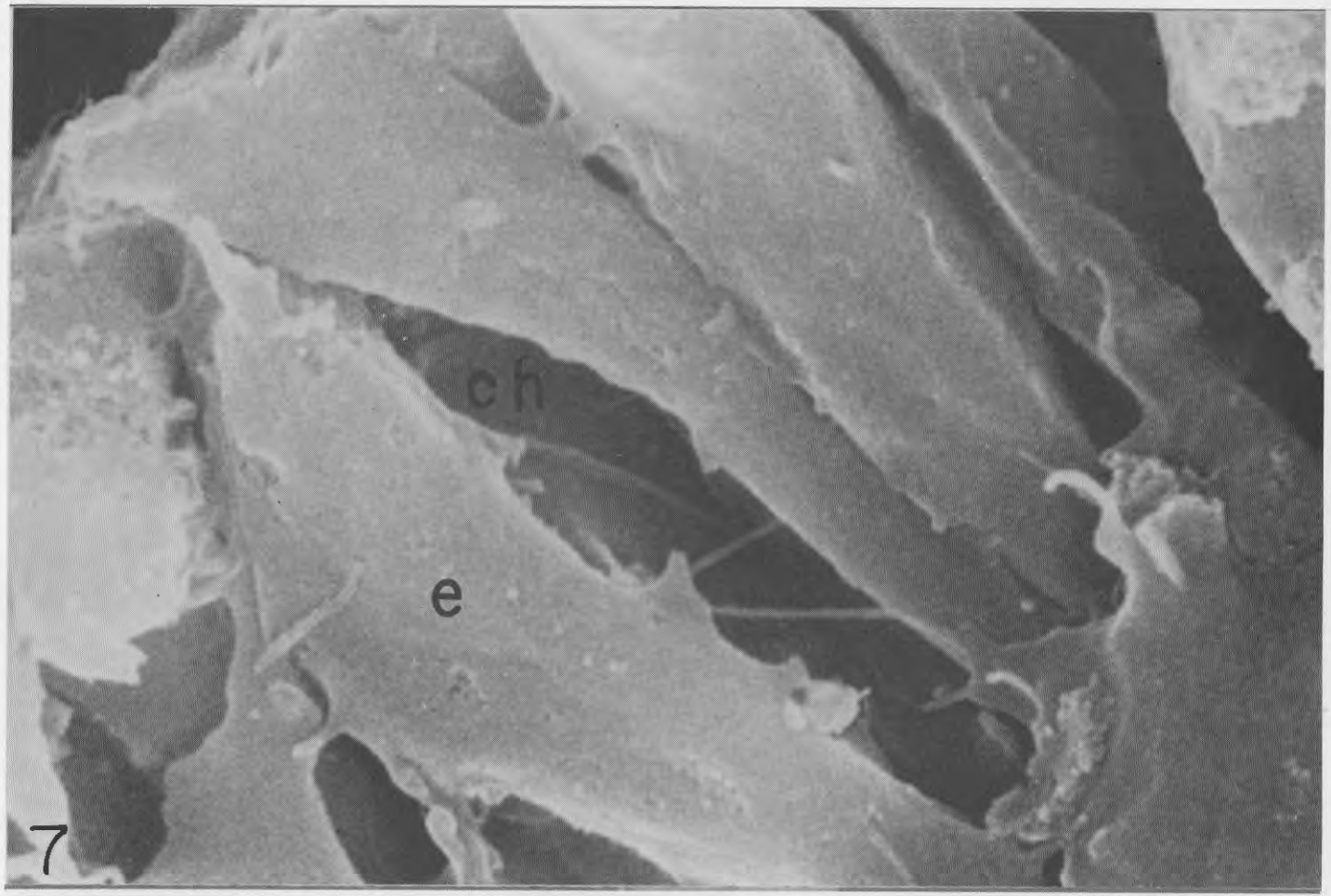

Figure 6-7. An early chick embryo spinal cord ependyma with ependymal channels (ch) crossed by lateral ependymal filopodia (f). ×14,000.

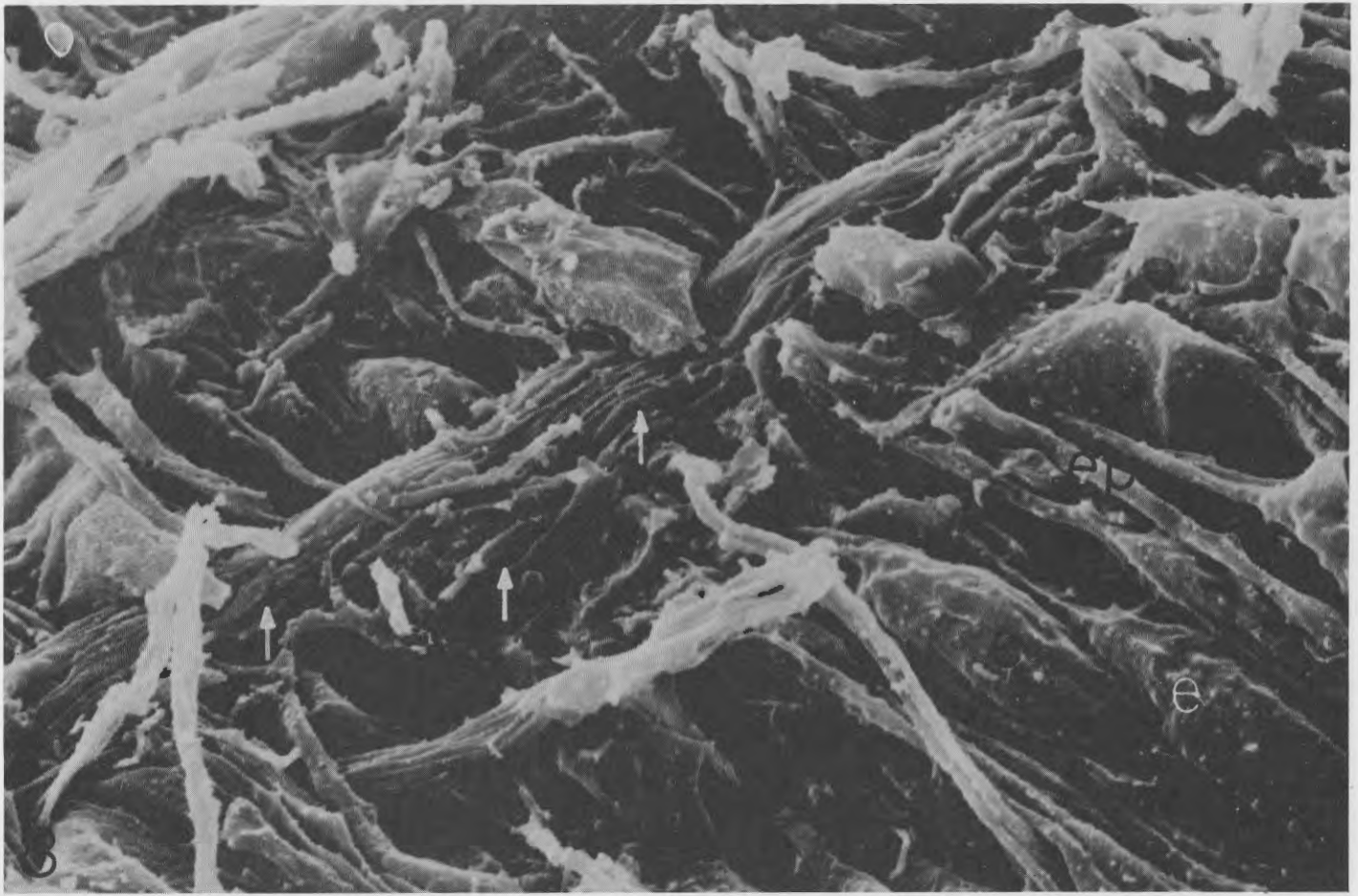

Figure 6-8. A scanning electron micrograph from a stage 17 chick embryo hindbrain. Axon fascicles (white arrows) are seen to cross perpendicular to the external (radial) ependymal process (ep). ×4,600.

(Seymour and Berry, 1975) in the tissues we have studied, and the descending neurites travel perpendicular to the ependymal cell orientation but in conformity to the intercellular channels. As Taylor (1944) pointed out, "thus selection of the pioneer fibers would be in terms of preneural pathways rather than of the terminal tissues themselves."

Kalil and Reh (1979) have recently reported regrowth of pyramidal tract axons in infant hamsters. These axons do not regrow following lesions in adult hamsters, yet in the infant the axons regenerate along a foreign route to reestablish appropriate connections. The factors guiding the axons are unknown, but our work suggests a possible ependymal channel open at the time the neurons are still responsive.

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