activity on the 10th day. No enzyme reactivation could be seen throughout the period investigated. At the same time, both the electron dense sinusoid terminals in the Rolando substance, exerting AP activity under normal conditions, and other primary axon terminals in this area, exhibited signs of ultrastructural desorganization essentially identical with those occurring in the course of degenerative atrophy that ensues after surgical transection or traumatization of the respective peripheral spinal nerve (figures 1–4). No alterations could be seen in the control (saline-treated) group.

Discussion. It has been shown that local colchicine and vinblastine treatment induces blockade of axoplasmic transport mechanisms in peripheral nerves[3–8]. In pioneering studies performed by injecting small amounts of these drugs, nerve truncs may have suffered microinjuries resulting in Wallerian degeneration of peripheral axons[9]. The use of perineural cuffs, however, excludes the possibility of a mechanical injury; therefore, the effects of colchicine and vinblastine observed by us should be ascribed to arrested axoplasmic transport.

Both colchicine and the Vinca alcaloids are known to induce a metaphase mitotic arrest[10]. By virtue of the analogy between mitotic spindle filaments and neurotubuli, the theory was forwarded that fast axoplasmic transport is related to mechanochemical activity of neurotubuli. Our observations indicate that, in addition to arresting axoplasmic transport, mitotic metaphase inhibitors induce histochemical and ultrastructural alterations in the central terminals of primary sensory neurons identical with degenerative atrophy caused by transection, crush or ligature of peripheral axons[11]. Such 'transcellular' or 'transganglionic' alterations were sporadically reported since 1880 (Stiénon, cit. Scharf[12], Grant[13], etc.[14]); however, only recent studies in this laboratory revealed the fine structural and histochemical aspects of this process[15]. Degenerative atrophy, obviously necessitating a new formulation of the doctrine of 'neuronal trophical entity', is characterized by the disappearance of AP activity from the central terminals of sensory neurons in the Rolando substance, and by a series of ultrastructural alterations resembling or identical with, those of a secondary Wallerian degeneration.

The present investigations support the idea that the very reason of degenerative atrophy is the blockade of microtubule-dependent fast transport mechanisms in the peripheral axon. It can be assumed that axoplasmic arrest elicits a signal for the perikaryon, initiating a 'state of emergency' throughout the domain of the primary sensory neuron. Degenerative atrophy of the central terminal appears to be one of the symptoms of this emergency.

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Effect of the pentosanpolysulfate SP 54 on the collagen of embryonic limb buds cultured in vitro

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Summary. After addition of SP 54 to limb buds from 11-day-old mouse embryos in tissue culture, collagen with an altered structure is produced.

Proteoglycans (PG) and glycosaminoglycans (GAG) are bound to collagen by salt-like bindings. These substances influence the aggregation of collagen to filaments and fibrils in vitro, the strongest effects being produced by highly sulphated PG and GAG respectively[1–8]. They also seem to play an important part in the development of some properties of the fibrils in vivo, such as thickness, length, cross-striation pattern, and packing density[9–11]. However, rather contradictory results on the influence of GAG on collagen have been obtained from in vitro experiments. These are probably due to the different properties of the various collagen and GAG preparations used. For studying the influence of GAG on collagen fibril formation with natural procollagen and natural collagen respectively, limb bud cultures from 11-day-old mouse embryos are especially suitable as they produce large amounts of type II collagen[12–14]. We therefore studied in this system the influence of GAG with different degrees of sulfatation on fibrillogenesis.

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Materials and methods. Limb buds of 11-day-old mouse embryos were cultured on organ culture[12, 13, 15] without and with the addition of 1.74 mg glycosaminoglycans (GAG) per ml culture medium. Hyaluronate, chondroitin sulfate, heparin, and SP 54 (a pentosanpolysulfoester, mol.wt 2000 daltons, 3.8 SO_4/disaccharide) were used. After a 6 days' growth period, the cultures were fixed in glutaraldehyde/paraformaldehyde and subsequently in O_sO_4 + ruthenium red (RR), as has been described in detail earlier[16]. Contrasting: uranyl acetate/lead citrate. Pictures: Zeiss EM 10.

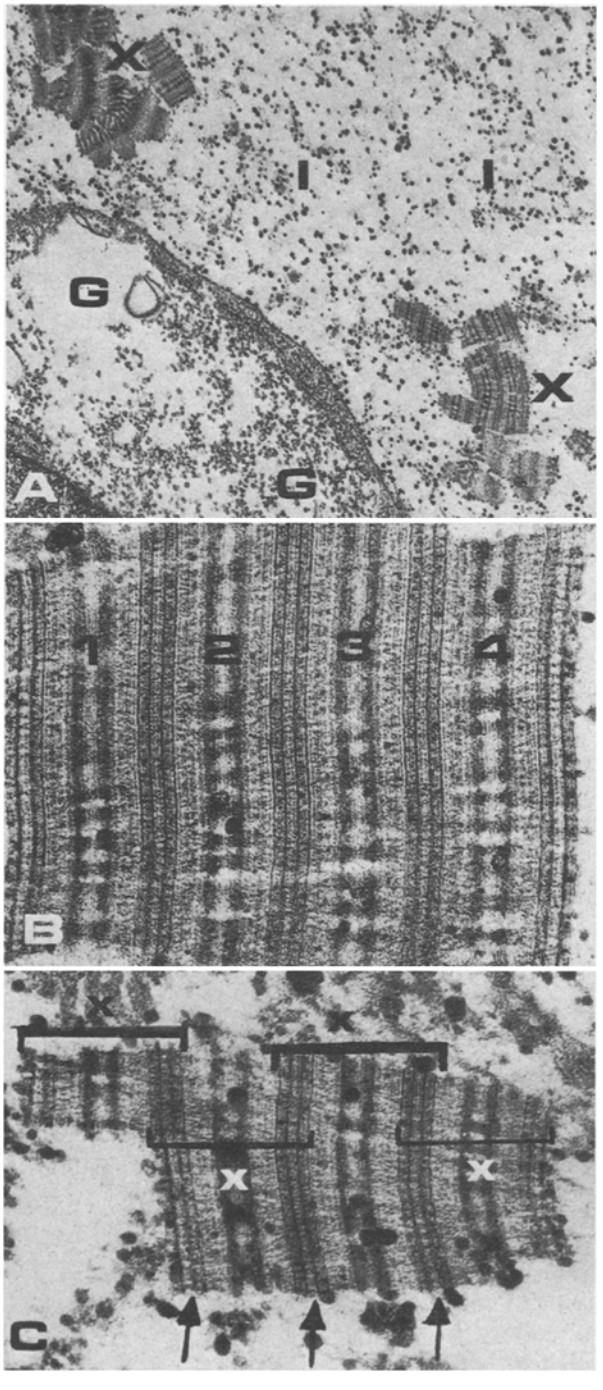
Results. The intercellular substance of the cartilage which develops in vitro contains 2 structures which can be demonstrated electronmicroscopically:

1. There are irregularly running 120–180 Å thick collagen filaments. At the highest magnification they show a continuous cross-striation with alternating 50 Å wide light and dark bands.
2. There are RR-positive granules, approximate size 500 Å, resting either on the filaments at varying distances or in the spaces between the filaments. As reported in detail earlier[16], these are probably chondroitin sulphate containing PG.

After addition of SP 54, the collagen filaments in the cultures almost disappeared (figure, A). Especially in the vicinity of the chondroblasts, there are many structures which are very similar to collagen segments. Their length varies from 2800 Å to 16 000 Å and their width from 0.1 μm to 1.2 μm. In the shortest segments (2800 Å), there is cross-striation with a central band pair whose single bands are 75 Å thick and 200 Å apart. At each side of this central band pair, up to 12 electron-dense bands arranged symmetrically can be seen. The length of the shortest segment corresponds to the length of tropocollagen. The longer segments are formed by end-to-end aggregation of a various number (up to 6) of the shortest segment. The short segments overlap at the ends, so that the resulting pieces become somewhat shorter than a multiplicity of 2800 Å. Thus they are 2800 Å, 5400 Å and so on up to 16,000 Å long. The amount and the structure of the Ruthenium red granules appear unchanged (figure, B, C).

Discussion. SP 54 itself cannot be demonstrated with RR under our experimental conditions. Therefore, one may conclude that SP 54 has no influence on amount or structure of the RR-positive, chondroitin sulphate containing PG, and that their biosynthesis may be unchanged. This is supported by the fact that the ultrastructure of the chondroblasts is not altered by SP 54.

The typical collagen structures of this tissue can no longer be seen under the influence of SP 54. The newly formed structures contain collagen, because they are broken down by collagenase. Therefore, collagen exists in another form of aggregation, namely short and thick segments with a regular cross-striational pattern different from normal collagen. The alteration in the collagen structure is produced by a highly sulphated carbohydrate, apparently by its high negative charge density. Heparin and the other GAG did not show this effect, even when used in higher concentrations. This special effect of SP 54 was also observed in experiments with soluble collagen type II. After precipitation of soluble collagen type II in the presence of SP 54, collagen segments were formed which show a cross-striational pattern similar to that seen in the collagen segments from SP 54 containing cultures (unpublished).



A Part of a cartilage cell with a large glycogen-area (G). In the intercellular space (I) proteoglycan-granules and collagen segments (X). 1:12,500. *B* Collagen structure made of 4 (1–4) overlapping units. 1:65,000. *C* Collagen structure with 3 or 4 overlapping units (X). ↓ = overlapping zones. 1:62,000.

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