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Nerve Fibre Regeneration after Traumatic Lesions of the CNS; Progress and Problems

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Nerve fibre regeneration after traumatic lesions of the CNS; progress and problems

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[Abstract not supplied]

INTRODUCTION

Four distinct phases can be distinguished in the regenerative response of a lesioned CNS axon: sprouting of the proximal axon stump, elongation, target recognition, and formation of appropriate synapses. These processes can be observed in such a way only in lower vertebrates, in particular, in the optic system. In these species and systems, both, the guidance mechanisms leading regenerating fibres to their former target areas, and the mechanisms responsible for specific synapse formation are retained throughout life. As during development, *guidance* crucially depends on the presence of favourable substrate molecules, and on chemotropic signals (Dodd & Jessel 1989; Tessier-Lavigne *et al.* 1988; Harris 1989). The cell biological mechanisms responsible for *target recognition* including the arrest of long-distance growth, the initiation of side branch formation and terminal arborization, and the selection of specific post-synaptic partners (cell type; soma, proximal or distal dendrite, spines, axons) remain unknown up to now. Whereas the formation of the retino-topic maps on the optic tectum of amphibia and fish can occur in the absence of all electrical activity of the system, fine tuning and possibly a substantial amount of the detailed wiring go through phases of 'sorting out' and involve activity-dependent mechanisms (Harris 1980; Hartlieb & Stuermer 1989; Constantine-Paton *et al.* 1990). As such mechanisms closely resemble those that operate during plastic modifications in the mammalian brain (Constantine-Paton *et al.*, 1990), the possibility exists that lesioned axons, if they can reach their original target areas, will be able to form appropriate functional synapses. A first, impressive demonstration of these processes has been given recently by Carter *et al.* and Keirsteadt *et al.* who showed that rat retinal axons guided into the superior colliculus by a sciatic nerve bridge, form typical retino-tectal synapses and are able to transmit light impulses from the retina to appropriate post-synaptic tectal partners. This selectivity, however, is not absolute, as shown by the formation of mossy fibre-like terminals in the cerebellum by such retinal fibres (see Aguayo, this symposium).

Our own work has focused on the myelin-associated neurite growth inhibitors, the inactivation of which allowed long-distance regeneration of corticospinal tract axons in the rat spinal cord (Savio & Schwab 1990; Schnell & Schwab 1990; Schwab 1990). In the course of these and related experiments in other parts of the CNS we realized that initiation and maintenance of sprouting and the local situation at the lesion site also require increased attention. Here, we will briefly summarize our findings on long-distance axon regeneration, and then describe and discuss some results and open questions concerning the special conditions at the lesion sites.

NEUTRALIZATION OF MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS ALLOWS LONG-DISTANCE REGENERATION OF CNS AXONS IN THE RAT

By studying the interaction of growing nerve fibres with the various components and cell types of the CNS we found that oligodendrocytes and CNS myelin (the spirally wrapped membrane of oligodendrocytes around axons) exert an inhibitory effect on growing and regenerating neurites (Schwab & Caroni 1988). In culture, the contact of filopodia of neurite growth cones with oligodendrocytes or myelin rapidly leads to a long-lasting or permanent arrest of growth (Bandtlow *et al.* 1990). If neurons are cultured on frozen sections of the CNS or spinal cord, nerve cells exclusively adhered and grew neurites on grey matter (Savio & Schwab 1989). Isolated purified CNS myelin adsorbed to tissue culture dishes as a substrate did not allow neurite growth. Extensive biochemical studies of CNS myelin proteins showed that two components with masses of 35000 and 250000 Da are potent inhibitors of neurite growth (Caroni & Schwab 1988*a*). They were called neurite growth inhibitors NI-35 and NI-250. These two components seem to be closely related. Antibodies were raised, and two monoclonal antibodies (IN-1 and IN-2) could be selected each of which neutralized the inhibitory activity of both these inhibitors in a variety of *in vitro* tests (Caroni & Schwab 1988*b*). In the direct interaction of growing nerve fibres with cultured oligodendrocytes, growth cone collapse did not occur and neurite growth continued in the presence of the antibody IN-1 (Bandtlow *et al.* 1990). Likewise neurons were able to grow on a CNS myelin substrate, and hundreds of axons of cultured sensory neurons grew into co-cultured rat optic nerve explants for several millimetres (axons were absent in explants injected with a control antibody beyond an

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initial sprouting zone of about 1 mm) (Caroni & Schwab 1988*b*).

In vivo experiments to test the functional significance of these neurite growth inhibitors for the lacking regeneration of lesioned CNS axons were a focus of our recent work. In one experimental paradigm, newborn rats were treated with high doses of X-rays directed at the spinal cord. As had been shown before, this procedure selectively eliminates the developing oligodendrocytes and results in virtually myelin-free spinal cords (Gilmore 1963). Frozen sections of such spinal cords represent a good substrate for neurons on grey matter as well as on the fibre tract areas (Savio & Schwab 1989). Complete bilateral transection of the thoracic spinal cord sparing only the ventral and ventrolateral funiculi of young (2–4 week old) myelin-free rats was performed (Savio & Schwab 1990). The corticospinal tract (CST), which runs in the dorsal funiculus in the rat, was subsequently labelled with the highly sensitive anterograde tracer wheat germ agglutinin-horseradish peroxidase (WGA-HRP). Two to four weeks after transection regenerated CST fibres could be followed for long distances caudal to the lesion: most rats showed regenerating CST fibres 4–8 mm caudal to the lesion, some even more than 10 mm (Savio & Schwab 1990). In normally myelinated controls regenerative CST sprouts did not elongate for more than 1 mm distal to the lesion. These exciting and encouraging results were not because of complex side effects of the irradiation as shown by subsequent experiments with antibodies.

The NI-35/250 neutralizing monoclonal antibody IN-1 (Caroni & Schwab 1988*b*) was applied *in vivo* to 2–6 week old rats by implanting an antibody-secreting hybridoma tumour into the brains in contact with the lateral ventricle (Schnell & Schwab 1990). A few days later the thoracic spinal cord including both CSTs was transected as described above. Tracing of the CST after survival times of 2–5 weeks showed a similar result as that observed in the X-irradiated rats: a number of CST fibres elongated caudal of the lesion over distances of 4–10 mm. In rats with control antibodies or in normal, untreated rats, the regeneration distances remained below 1 mm in the majority of the cases (Schnell & Schwab 1990).

In spite of the clear-cut results of both these experiments, it was also clear from these studies that the large traumatic lesions represented an obstacle for the regenerating fibres. In fact, in all these rats showing successful regeneration the number of regenerating CST fibres was small, a few percent of the original CST fibres. Attempts to investigate and improve this condition are described below.

Enhancement of axon elongation was also recently found for cholinergic axons of septal origin (Cadelli & Schwab, unpublished data). A large suction lesion was placed in the fimbria-fornix, interrupting the cholinergic input of the hippocampus from the basal forebrain nuclei. The cavity was bridged by a 1×4 mm strip of nitrocellulose covered with human amnion extracellular matrix (Davis *et al.* 1987) and soaked in nerve growth factor (NGF). Fine bundles of acetylcholinesterase (AchE)-positive axons crossed the bridge and entered the hippocampus. In the control animals these fibres grew for about 1 mm in the caudal and lateral directions. They branched extensively and ended predominantly in the hilus of the dentate gyrus and around the pyramidal and granule cells. Treatment with the neutralizing antibody IN-1 mainly changed the distance of regeneration to 2–4 mm (in 2–4 weeks) in both, the caudal and lateral direction. The smaller distances grown by these fibres in comparison to the CST fibres in the spinal cord could also be influenced by the induction of terminal arborizations by the extensively denervated target region. However, the limited penetration of the antibodies also could play an important role. A major effort of our lab is therefore directed at replacing these antibodies by either small antibody fragments or by other agents interfering with the action of neurite growth inhibitors.

DIRECT LOCAL LESION RESPONSES AND PROBLEMS AT THE LESION SITE

Whereas developing neurons often die rapidly following axotomy, adult neurons survive for long timespans at least if the lesion is not very close to the cell body (Merline & Kalil 1990; Aguayo, this Symposium). A spontaneous sprouting reaction of the proximal axon stumps has been observed already by Ramon y Cajal (1828). These sprouts, however, seem to be unable to elongate over more than 0.5–1 mm within differentiated CNS tissue, in particular, in white matter. Often, a phase of retraction and die back over 1–2 segments in the spinal cord then follows (Ramon y Cajal 1928). In analogy to the situation for peripheral neurons, neurotrophic factors may be required also in the CNS to maintain the sprouts and initiate or maintain the transcription of growth-associated genes in the cell bodies of the lesioned neurons. Fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF) have been shown to exert such a maintenance function for axotomized rat retinal ganglion cells (Sievers *et al.* 1987; Thanos *et al.* 1989), and several neurotrophic factors present in the CNS (NGF, BDNF, NT-3, ciliary neurotrophic factor; Leibrock *et al.* 1989; Hohn *et al.* 1990; Stöckli *et al.* 1989) have recently been described and cloned and have now to be investigated for their possible effects on regenerating CNS tracts. Multiple effects including promotion of survival, enhancement of sprouting and fibre growth, and chemotropic effects can be expected.

An important obstacle for sprouting and regenerating axons seems to result from the local reactions of the lesioned CNS tissue. Astrocyte reactions can be observed rapidly after a lesion, although the nature and extent of the changes occurring in astrocytes are still largely unknown (Reier 1986; Topp *et al.* 1989). In addition, the substrate nature of this scar tissue, i.e. its permissiveness or non-permissiveness for neurite growth, is far from being clear (Reier 1979, 1986; Smith *et al.* 1986; Fawcett *et al.* 1989). Many attempts have been done to bridge spinal cord lesions with implants of embryonic tissue or bridges containing

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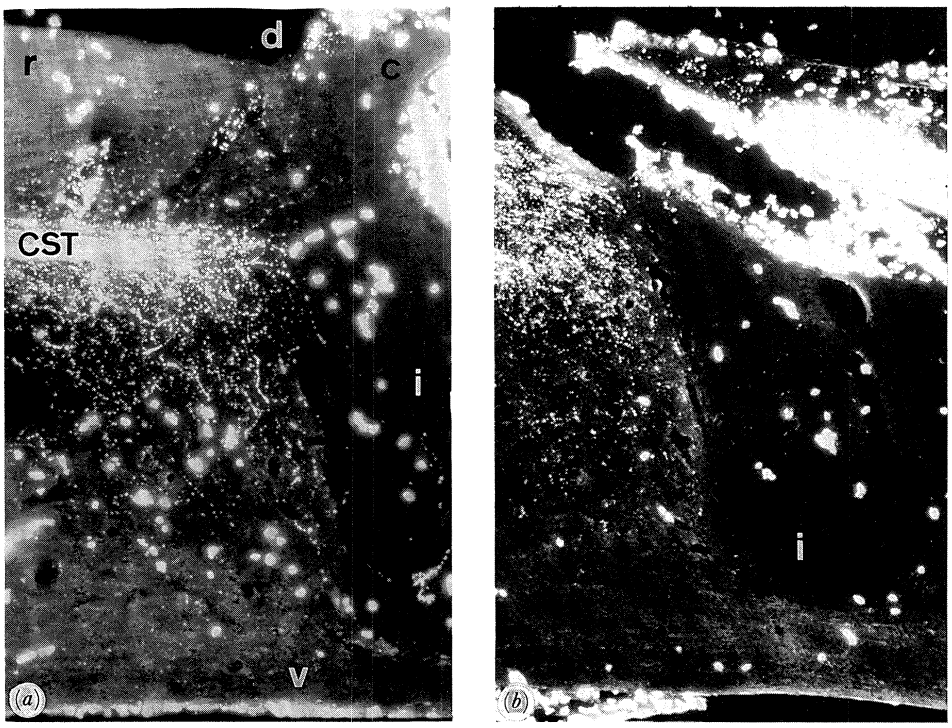

Figure 1. Sagittal sections of a 40-day-old rat spinal cord, lesioned and implanted with embryonic day 14 spinal cord and an antibody IN-1 source at day 17. The CST is labelled by anterograde transport of WGA-HRP, visualized under dark field illumination. The CST approaches the lesion and exhibits massive sprouting, partly because of factors released by the implant. Most CST fibres are directed ventrad. Few fibres crossed the ventral bridge of remaining tissue and regenerated up to 6.3 mm caudal to the lesion (not shown). Only very few fibres grew into the embryonic spinal cord implant (i). Magn. × 60. (c, caudal; d, dorsal; r, rostral; v, ventral.)

extracellular matrix material, astrocytes, or Schwann cells (Schreyer & Jones 1987; Bregman *et al.* 1989; Kuhlengel *et al.* 1990; Schnell & Schwab, unpublished data). Unfortunately, these efforts were almost totally unsuccessful up to now with regard to their function as a bridge for regenerating fibres in adult rats (in contrast to newborn rats where embryonic spinal cord implants are used by regenerating fibres as bridges across the lesion area).

In our own experiments two conclusions could be reached up to now: confirming the results of Bregman *et al.* (1989) in young rats, embryonic day 14 rat spinal cord implants have pronounced survival and sprouting effect on lesioned corticospinal axons (figure 1). These effects are not only seen with direct implants, but were also present if the embryonic tissue was sealed into nucleopore filter 'raviolis' and placed on top of a spinal cord lesion. Most probably, trophic factor(s) released from embryonic neurons or astrocytes account for this effect. Second, even with well-integrated embryonic spinal cord transplants or bridges of nitrocellulose filters coated with laminin or human amnion extracellular matrix material only very rare CST axons could be seen to cross the interface and grow into the transplant or onto the bridge (figure 1). This is true for the same bridges which allowed abundant growth of cholinergic septal fibres over the bridge into the hippocampus (see above), and the same transplants that serve as bridges in newborn and young post-natal rats (Bregman *et al.* 1989). The majority of the sprouting CST fibres proximal to the lesion were directed ventrad, and fibres with an initial straight caudal direction seemed to be diverted by the tissue at the lesion site (figure 1). This tissue stains brightly with the astrocyte marker glial fibrillary acidic protein (GFAP). In the future, the timecourse, the electron microscopic structure, and the biochemical changes occurring at spinal cord lesion sites, in particular in the astrocytes, have to be carefully investigated. Additional repulsing or inhibitory proteins, but also mechanical barrier effects can be expected.

Additional destructive effects at lesion sites include effects of free radicals, cytotoxic effects through the release of glutamate and other excitotoxic amino acids, effects of local ischemia, and the multiple and very poorly understood effect of immigrating macrophages.

CONCLUSION

Although a multitude of difficult problems remain to be solved, our understanding of CNS and spinal cord lesions has greatly advanced in the recent past. Stimulated by neurotrophic factors, regeneration of a variety of fibre tracts over long distances may become possible by neutralization of the powerful myelin-associated neurite growth inhibitors. How far specific pathfinding can be reactivated is still unknown, but the results obtained in the spinal cord and hippocampus, and the persistence of many cell adhesion molecules in the adult CNS are encouraging. Once in the target area, synapse formation, possibly followed by sorting out of correct synaptic connections, can occur in the adult, both in lower vertebrates, and by retinal fibres

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guided into the optic tectum through peripheral nerve bridges.

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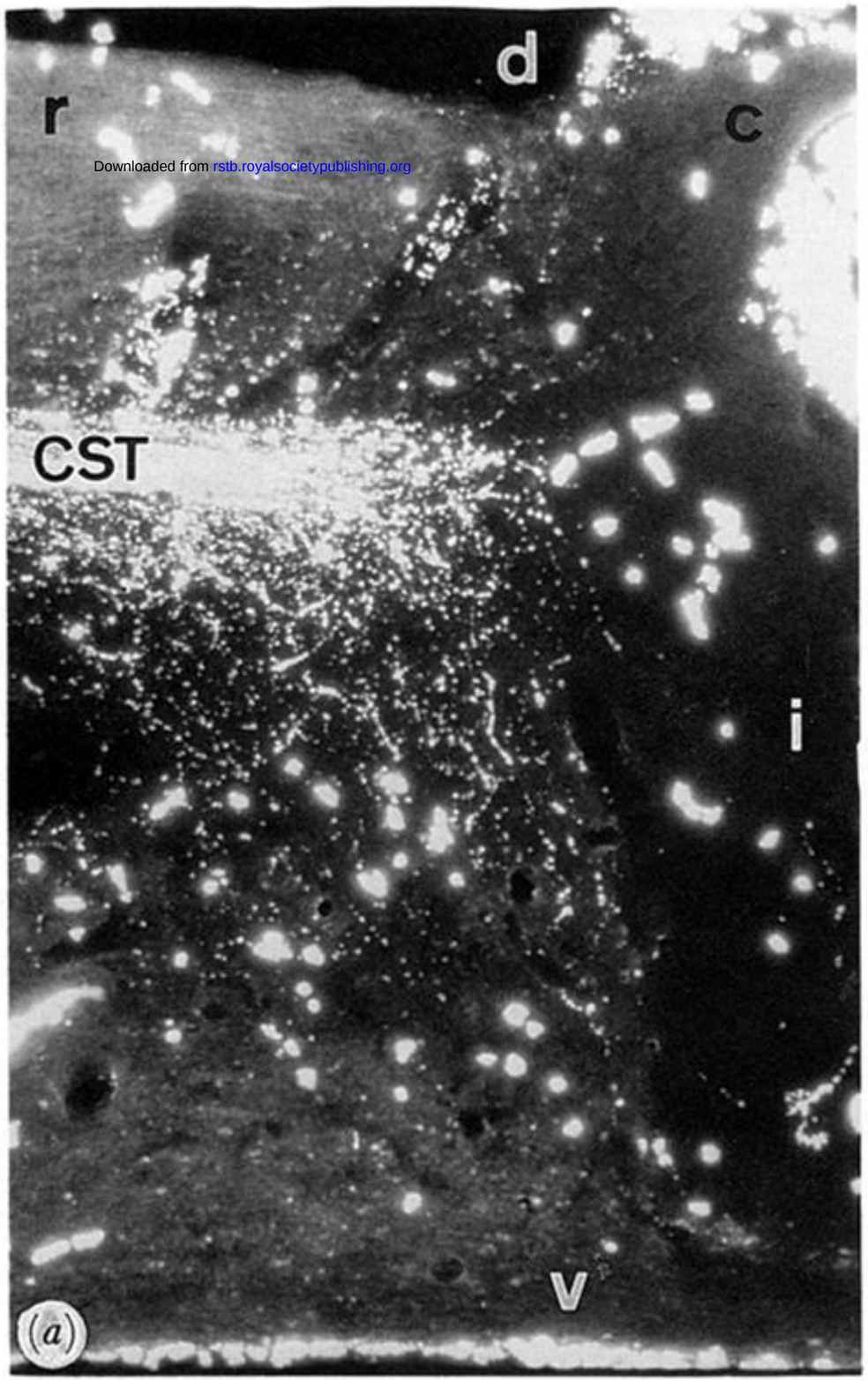

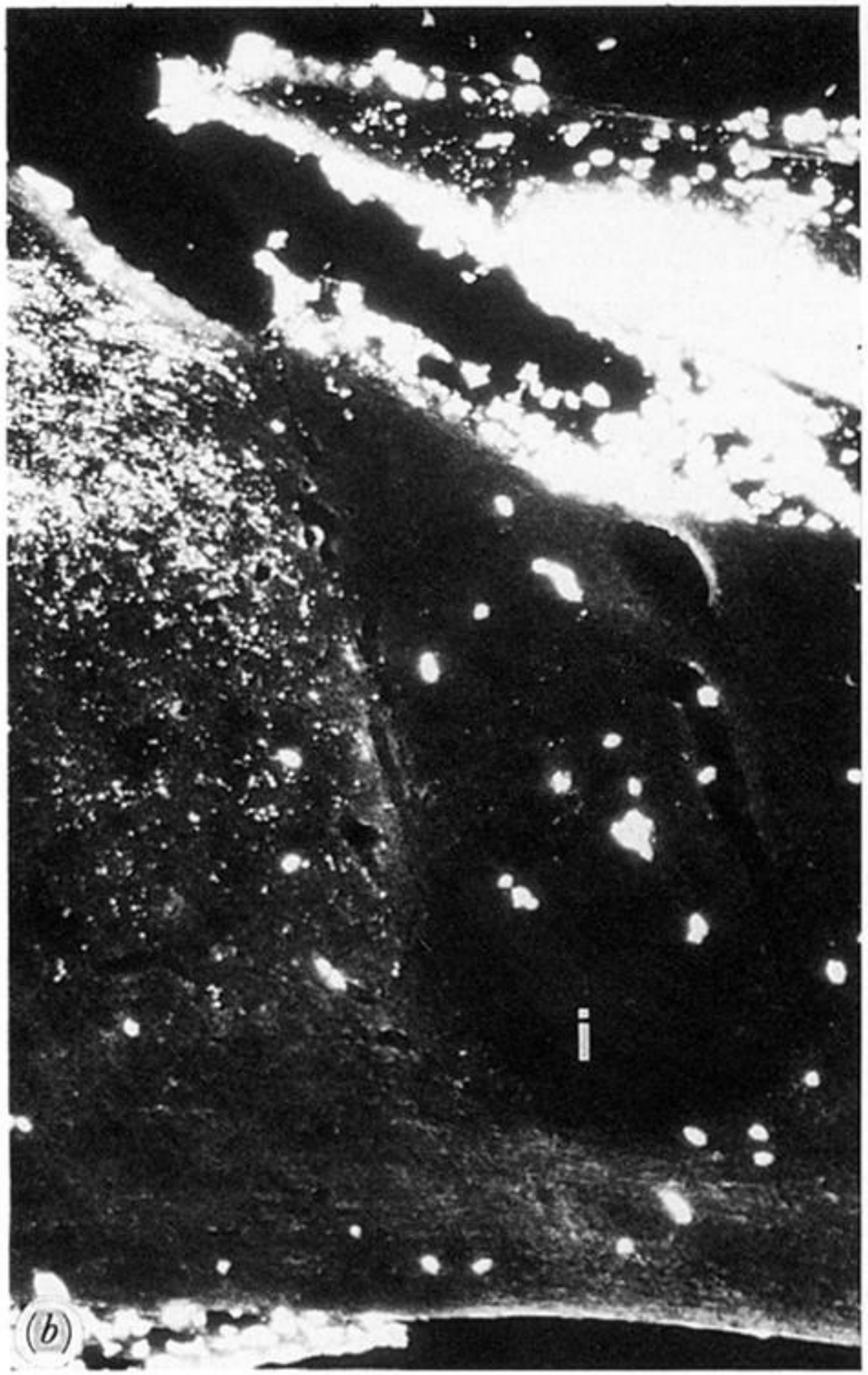

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