after exposure to a large virus dose and independent of whether the HSC per se are virus infected.

This study provides additional support to the suggestion that the microenvironmental stimulus promoting the HSC renewal can diffuse only over very short distances and certainly not throughout the C portion across the P_1-C-P_2 boundaries. (Otherwise, calculations show that portion C would have gradually disappeared in < 4 days after irradiation[2].) Finally, our results do not support the idea that Friend virus transforms infected HSC into autonomously growing cells. If so, the HSC within the C portion would have continued to grow with their initial t_D of 22 h to reach about 20,000 CFU by day 14. The HSC, however, slowed their renewal to a level of 2400 CFU, the stimulating capacity of a normal local microenvironment, in accordance with the original hypothesis. As a last remark, the interpretations of our results are valid only when the virus is injected after irradiation. Preliminary data (Matioli, unpublished) suggest that spleen microenvironments infected before irradiation may require a much higher radiation dose to reduce their stimulatory efficiency, perhaps because unirradiated microenvironmental sources become hyperactive rapidly after virus infection.

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Skeletin immunoreactivity in peripheral nerves

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Summary. By use of monospecific antibodies against the cow heart intermediate filament protein, skeletin, an antigenic relationship between skeletin and neurofilament protein of peripheral nerves is demonstrated. Crossreactivity is also demonstrated in the filament-containing Schwann cells. The results are consistent with the existence of several subclasses of related intermediate-sized filament proteins.

Neurofilaments occur in most nerve cells and comprise a major structural component of the myelinated axons of most species[3,4]. Neurofilaments seem to be the neural variant of a filament class which is present in all types of eukaryotic cells. Until recently, the identification of these filaments has been restricted to electron microscopic studies in which they have been characterized as smooth-surfaced, fairly rigid filamentous organelles of indeterminable length and with a diameter of 8–10 nm[5,6]. For this reason, the filaments of nonneural tissues have often been called '10 nm filaments' or 'intermediate filaments', as they have an intermediate diameter as compared with actin and myosin filament[5].

The purification of the neurofilament protein[7] and the glial filament protein (GFA)[8], made possible the production of antisera and the application of immunomicroscopic methods to the field of neurofilament research. Such investigations would have been expected to elucidate the immunological relatedness of neurofilaments, glial filaments and intermediate filaments from other cells. However, the results from such immunomicroscopic studies have been confusing in the sense that the question of cross-immunoreactivity has been answered in different ways by different authors. Evidence of both cross-reactivity[9–11] and nonreactivity[12–14] have been presented[15].

Recently, we have reported a method for preparing antibodies to intermediate filament protein (skeletin) from the cow heart conduction system, a cell system of myogenic origin buth with primarily nerve-like properties[16]. We have now adopted the indirect immunofluorescence method in order to demonstrate the immunoreactivity of heart skeletin in peripheral nerves. The results suggest that skeletin filaments and neurofilaments share antigenic determinants.

Experimental. The study was performed in the lateral cutaneous nerve of the thigh (n cutaneus femoris lateralis) of the cow, obtained within 5 h after death. Specimens were frozen in Freon-12 chilled with liquid nitrogen. Longitudinal and cross sections were cut in a cryostat and mounted on glass slides. The mounted sections were fixed in cold absolute acetone and air dried. Incubations were carried out in antiskeletin prepared as previously described[16] and diluted 1:50 in phosphate buffered saline (PBS) (1 h, +37 °C, moist chamber). After repeated washes in PBS, sections were incubated in FITC-conjugated goat antirabbit globulin (GARG) under the same conditions as for antiskeletin, washed in PBS and mounted in PBS-glycerol.

Control sections were treated in a similar way but with absorbed or nonimmune sera or with GARG alone. Sections were viewed in a Leitz Orthoplane Photomicroscope with epifluorescent optics, or in a Leitz Dialux-20 Photomicroscope equipped with interference contrast accessoires.

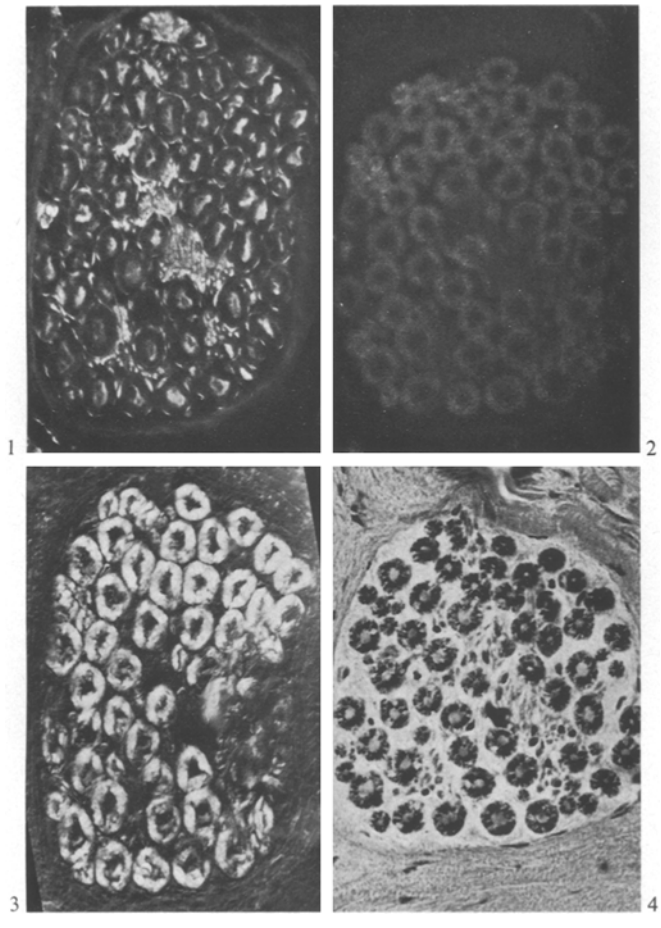
Results. In crosscut nerves a characteristic starry sky appearence was observed, consistent with the staining of myelinated axons. Surrounding myelin was not stained, but a distinct staining did occur in the Schwann cell cytoplasm. Endoneurial and perineurial connective tissue were negatively stained (figure 1), while small vessels showed specific fluorescence of the smooth muscle layer. The findings were confirmed by correlation with interference contrast microscopy of identical areas (figure 3) and sections stained with Gomori trichrome (figure 4). In longitudinal sections, the axons appeared as slender fluorescent ribbons while surrounding areas were dark. Control sections were all dark except for a faint nonspecific staining of myelin sheaths (figure 2).

Discussion. The demonstration of skeletin immunoreactivi-

ty located in the peripheral axons of large myelinated nerves clearly indicates an immunological relatedness of skeletin and neurofilament protein, which is known to be present in the axons in great amounts. The results thus corroborate a previous investigation in which antiskeletin immunoreactivity was specifically present in the neurofilament coils of cultured neuroblastoma cells[15].

A number of investigations have recently been concerned with the problem of the relation between different types of intermediate filaments, e.g. neurofilaments, glial filaments and the intermediate filaments of muscle and connective tissue cells. Opinion is divided as to whether cross-reactivity between various types of filaments exists[17] or not[18]. Antibodies against neurofilaments and glial filaments have been widely used in experimental neurological research. The results of these studies have, however, been controversial and diverging results have indicated the multivalency of the antibodies (compare references 9–11, 14 with references 8, 12, 13). This may be due to the heterogeneous cell populations in the starting material, which contained neuronal, glial, Schwann cells and vascular tissues, all known to contain intermediate-sized filaments as demonstrated by electron microscopy[4,6,9,10,13,14,19–21]. The techniques used for the preparation of the primary antigens have also been criticized on the grounds that they yield quite different proteins, as e.g. tubulin[13,21–23]. By the use of heart Purkinje fibres as a starting material, intermediate filament protein can be produced which is free from these objections. This is possible since these cells can be isolated free from impurities; they are nearly completely filled with intermediate filaments, and contain only extremely sparse amounts of microtubules.

By the use of antibodies to intermediate filament proteins from smooth muscle (skeletin[24] or desmin[25]), epidermis (prekeratin[26]), and fibroblasts (FC-protein[27] or vimentin[26]), 4 subclasses of filaments have been suggested on the basis of their lack of cross immunoreactivity. The intermediate filaments were accordingly divided into epithelial, mesenchymal, muscular and brain filaments[26,28]. The present investigation adds evidence for a greater complexity in intermediate filament protein classification, consistent with recent findings by use of antisera against intermediate filament protein from fibroblasts and smooth muscle[29,30] or, possibly, the coexistence of several subtypes of intermediate filament proteins in the same cell. The results also indicate the possibility of using antiskeletin antisera for further investigations into the mysteries surrounding neurofilaments, e.g. the characteristics of the abnormal filaments formed in certain degenerative and proliferative disorders of the central and peripheral nervous system[20,31,32]. The question of the relationship between skeletin and glial filament protein also merits further study.



Figures 1–4. Serial cryostat cross-sections of peripheral nerve from the cow. ×275. Fig. 1. Section treated with antiskeletin by the indirect immunofluorescence method. Fluorescence is confined to axons and surrounding Schwann cells. Bright areas correspond to tightly packed unmyelinated axons. Note that these areas are dark in figures 2 and 3. Fig. 2. Control section treated with nonimmune serum. A faint nonspecific staining is seen in the myelin sheaths. Fig. 3. Interference contrast micrograph of the same section as in figure 1. Birefringence of myelin sheaths. Fig. 4. Gomori trichrome-stained section. Myelin sheaths are darkly stained. In the areas corresponding to the bright areas of figure 1, several small axons are seen.

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Secretory phenomenon of the adenohypophyseal cells viewed with the scanning electron microscope

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Summary. Exocytosis from rat adenohypophyseal cells, probably somatotrophic, was studied in the scanning electron microscope after freeze fracturation of the glands. This technique permitted the distinguishing of a clearly delimited exoplasm interrupted by the passage of the secretory granules. It was postulated that the exoplasm could be involved in the control of the release of secretory granules in the endocrine cells studied. At the basal cell surface a simultaneous discharge of several secretory granules has been detected.

Exocytosis from different endocrine cells has been studied in the transmission electron microscope (TEM) on sections and on freeze-etched replicas[2-8], so that this phenomenon is now well understood. However, the scanning electron microscope (SEM) offers some new details concerning the discharge of hormonal granules which may further our knowledge about this phenomenon.

Materials and methods. Following perfusion through the abdominal aorta of adult male Wistar rats with Ringer's solution (pH 7.3; 330 mosmoles; +4 °C; 13.6 kPa), the hypophyses were prefixed with a mixture composed of 2% glutaraldehyde and 1% formaldehyde in 0.15 M cacodylate buffer (pH 7.2; 710 mosmoles; 13.6 kPa) for 2 min. The glands were then prepared and stored in the same mixture for 45 min. After postfixation in 1% osmium tetroxide, the hypophyses were washed in a fresh buffer solution, dehydrated in increasing concentrations of alcohol, transferred to liquid nitrogen, carefully fractured according to Tokunaga et al.[9] and critical-point dried with Freon 13. After gold metallization, the specimens were observed in a JEOL JSM-35 scanning electron microscope.

Observations. In freeze-fractured cells, probably somatotrophic cells, the secretory granules are clearly visible and measure about 0.3–0.5 µm (figures 1 and 2, G). They are concentrated in the cell pole facing the blood capillary (figure 1), the endothelial cells of which are very flattened (figures 1 and 2, C). The spherical secretory granules (figure 1, arrow) are seen perforating the thin cytoplasmic rim, the exoplasm (figure 1, E) and partially penetrating into the narrow pericapillary space (figure 1, S). Other granules lie in a cytoplasmic protrusion (figure 1, P) that is in contact with the outer aspect of the capillary endothelium. In some areas, the exoplasm shows delimited interruptions (arrowheads), the borders of which may fuse with those of the secretory granules (asterisk). Some irregularly shaped and partially dissolved granules are seen within the pericapillary space (white arrowhead).

On the basal cell surface (figure 2, BS) the secreting adenohypophyseal cells show smaller and larger apertures in which the expulsion of a single granule (figure 2, arrowhead) or more (figure 2, encircled area) is seen.

Discussion. The secretory phenomenon can be studied under the scanning electron microscope on freeze-fractured specimens only, since sectioning of the organs causes serious artifacts.

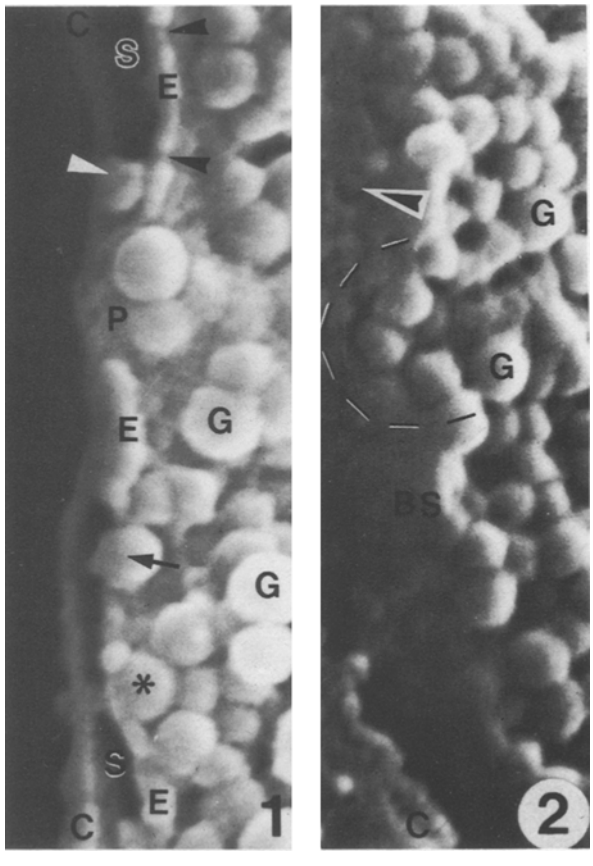

Fig. 1. Profile image of the secretory phenomenon. From the cell body (right) the secretory granules (G) pass a thin exoplasm (E) which shows some interruptions (arrowheads). The arrow points to a hormone granule protruding into the pericapillary space; the white arrowhead shows the remnants of such a granule. An asterisk indicates a hormone granule, the borders of which fuse with the exoplasm. × 22,500.

Fig. 2. Overview of the fractured basal cell surface (BS). An arrowhead shows the protrusion of a single secretory granule. At least 3 secretory granules in the course of exocytosis leave the cell through a large opening (encircled area). × 22,500.