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Electron Microscopic Studies of the Innervation of the Human Spleen

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Summary. The innervation of four normal human spleens was investigated by electron microscopy. Unmyelinated nerve fibers accompanied the arterial vascular system up to the arterioles of the red pulp. Neither myelinated nerve fibers nor ganglion cells were seen in the splenic hilum or in the splenic tissue itself. The nerve fibers terminated against the smooth muscle cells of the blood vessels in a manner that is typical of the autonomic nervous system. The terminal axons contained small and large granular vesicles and thus were adrenergic nerve fibers. In contrast to the results of previous studies using silver impregnation methods innervation of the red or white pulp could not be demonstrated. The findings on human spleens agree with those on mammalian spleens obtained by other authors using ultrastructural and fluorescence histochemical methods.

Key words: Human spleen – Splenic innervation – Electron microscopy.

Introduction

The innervation of the human spleen had been investigated only by light microscopy (Harting, 1952) and chiefly with silver staining methods. Since the spleen contains many argyrophilic fibers, such studies are fraught with error. Studies using modern methods have resulted in fundamental changes in views on the autonomic nervous system and especially on the innervation of the viscera. The spleen, however, has been investigated with such methods in only a few animal species. For example, canine (Dahlström and Zetterström, 1965; Zetterström et al., 1973), feline (Fillenz, 1970; Kirpekar et al., 1972), and murine (Reilly et al., 1976) spleens have been studied by fluorescence microscopy. There have also been electron microscopic investigations of spleens from horses and dogs (Zwillenberg

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and Zwillenberg, 1963), cats (Tanzer and Thoenen, 1967; Geffen and Ostberg, 1969; Fillenz, 1970), and rabbits (Olah et al., 1975). Since the human spleen had not yet been analyzed with similar techniques, we investigated by electron microscopy the innervation of the normal human spleen.

Material and Methods

We studied 4 human spleens with no significant pathological changes, which had been removed for accidental splenic rupture and technical reasons during extensive epigastric operations. The age of the patients at the time of splenectomy was 7, 37, 49 and 73 years. Pieces 1–2 mm^3 of red and white pulp, and also specimens containing trabeculae and vessels, and connective tissue from the splenic hilum were examined. The tissues were fixed in 5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.3) at 4° C for 2 h and then in 1% OsO_4 solution (Rhodin buffer). The specimens were gradually dehydrated in acetone and embedded in Araldite®. Embedded material was cut with a glass knife on a Reichert Mikrotom OMU3. Semithin sections were stained with azure II methylene blue. Ultrathin sections were mounted on grids, contrasted with lead citrate and uranyl acetate, and examined with a Siemens Elmiskop 101 at 80 kV.

Results

The detection of nerve fibers in the trabeculae proved to be relatively easy, because they could be recognized in semithin sections by light microscopy. The detection of nerve fibers accompanying the finer branches of the vasculature, however, was extremely difficult, because they could not be identified by light microscopy and were very rare. Thus, a considerable number of splenic blood vessels had to be systematically examined by electron microscopy.

A. Nerve Fibers

I. Splenic Hilum

Relatively large nerve trunks were seen in periarterial adipose tissue, whereas those of the trabeculae were located in the surrounding connective tissue. They consisted of groups of Schwann cells with unmyelinated axons (Fig. 1). A few of the axons contained granular vesicles with a diameter of 1000 Å. All of the axons contained neurofilaments and microtubules. The nerve trunks were separated from the surrounding adipose tissue by perineurial cells. Neither myelinated nerve fibers nor ganglion cells were detected.

II. Trabecular Arteries

The larger trabeculae often revealed bundles of nerve fibers close to the arteries. These bundles of nerve fibers were separated from the collagenous fibers of the trabeculae by a thin perineurial layer, only 1–2 cells thick (Fig. 2). Ultrastructurally, the bundles consisted of closely packed Schwann cells with cytoplasmic processes, enclosed by a basement membrane that separated them from the sparse endoneurial connective tissue. The Schwann cells were associated with variable numbers of

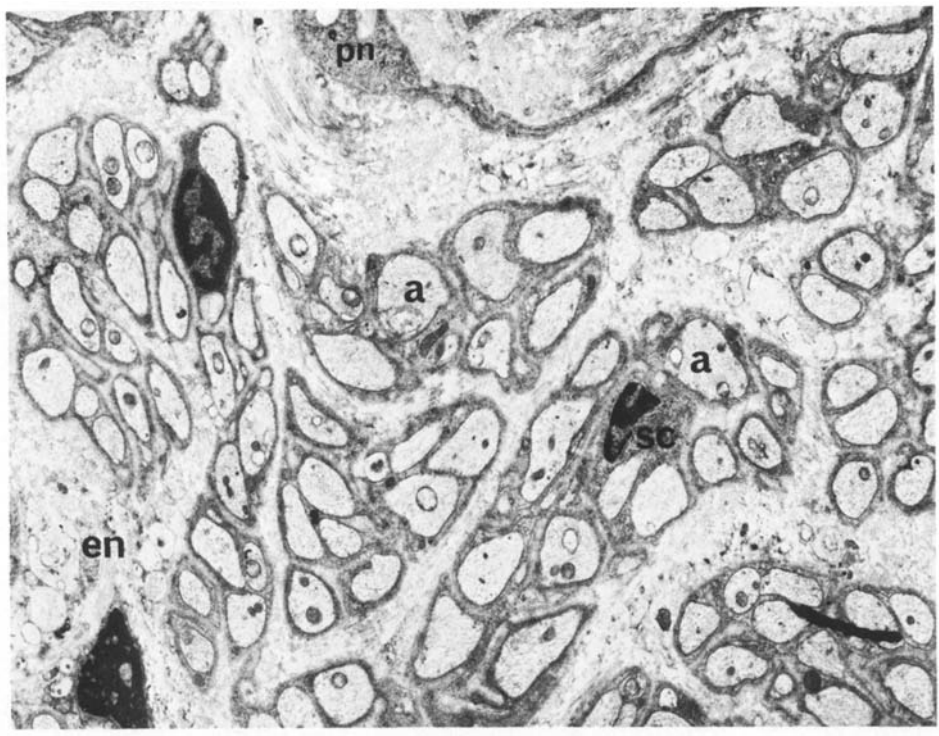

Fig. 1. Portion of a nerve fibers bundle in the splenic hilum showing several Schwann cells (*sc*), with multiple axons (*a*), and endoneurial connective tissue (*en*) between the Schwann cells. *pn*: a perineurial cell process. ×6000

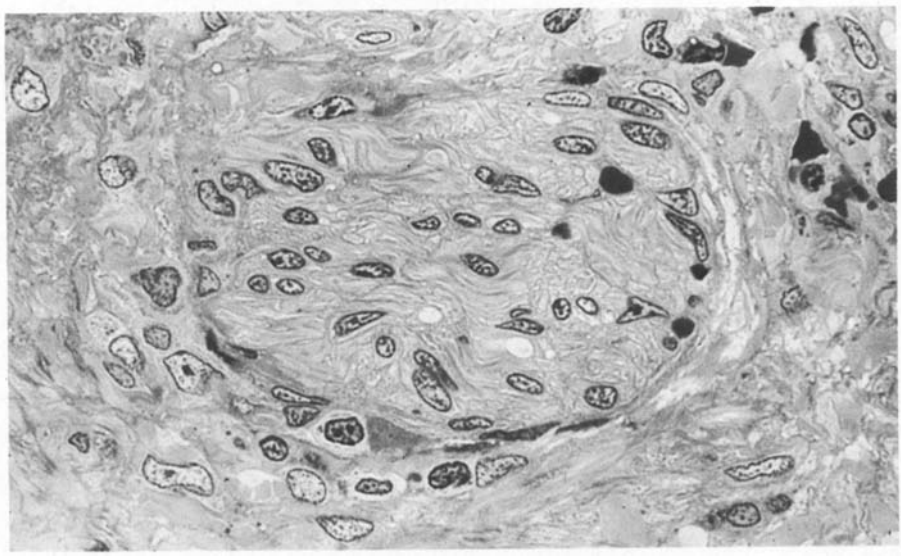

Fig. 2. Bundle of nerve fibers in a trabecula. Semithin section. ×880

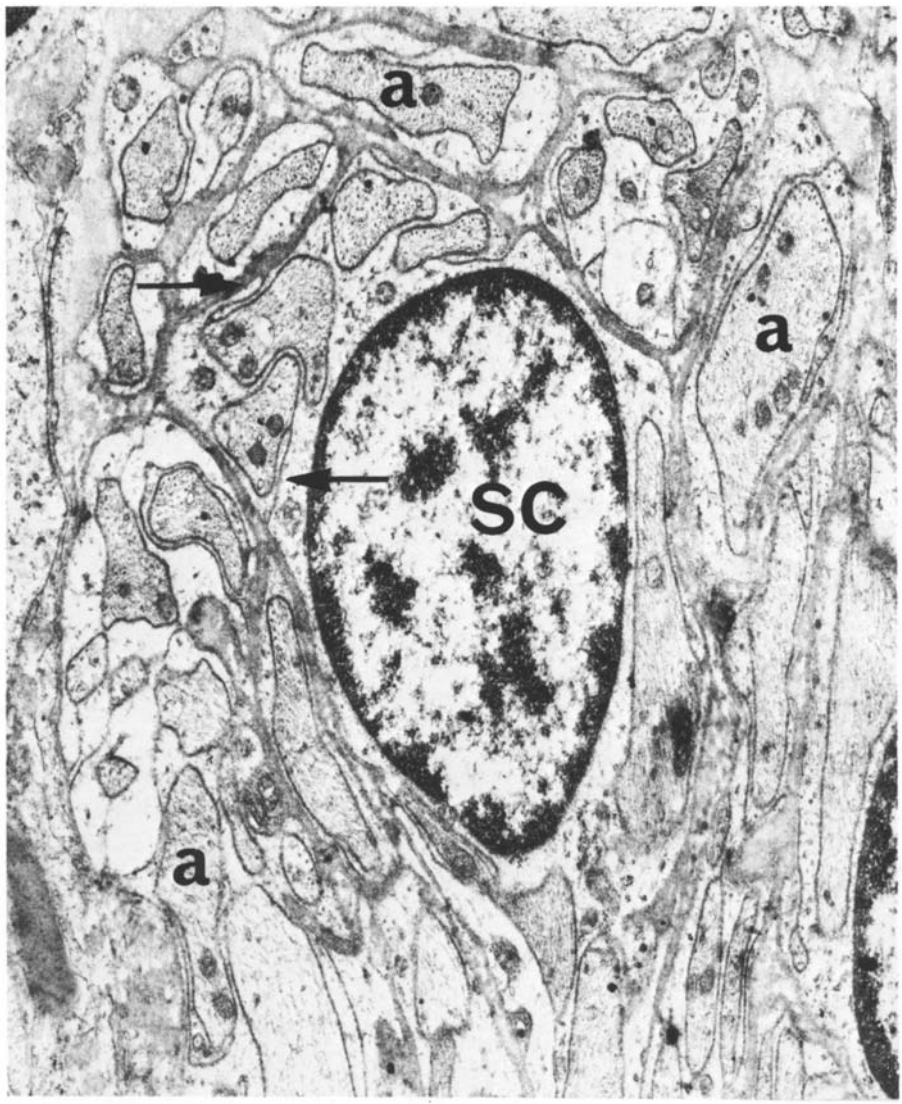

Fig. 3. Portion of a bundle of nerve fibers in a trabecula. Schwann cell (*sc*) with numerous unmyelinated axons (*a*), and mesaxons (arrows). ×16,800

unmyelinated axons with typical mesaxons (Fig. 3). The diameter of the axons ranged from about 0.15 to 1.5 μm. Their axoplasm contained numerous neurofilaments and microtubules, a few round mitochondria, and small electron-dense lysosomes. There were no myelinated nerve fibers or ganglion cells.

III. Central Arteries

In contrast to the arterioles (described below), the central arteries had a well-developed adventitia. By electron microscopy we demonstrated some very small bundles of nerve fibers in the connective tissue surrounding a central artery with a

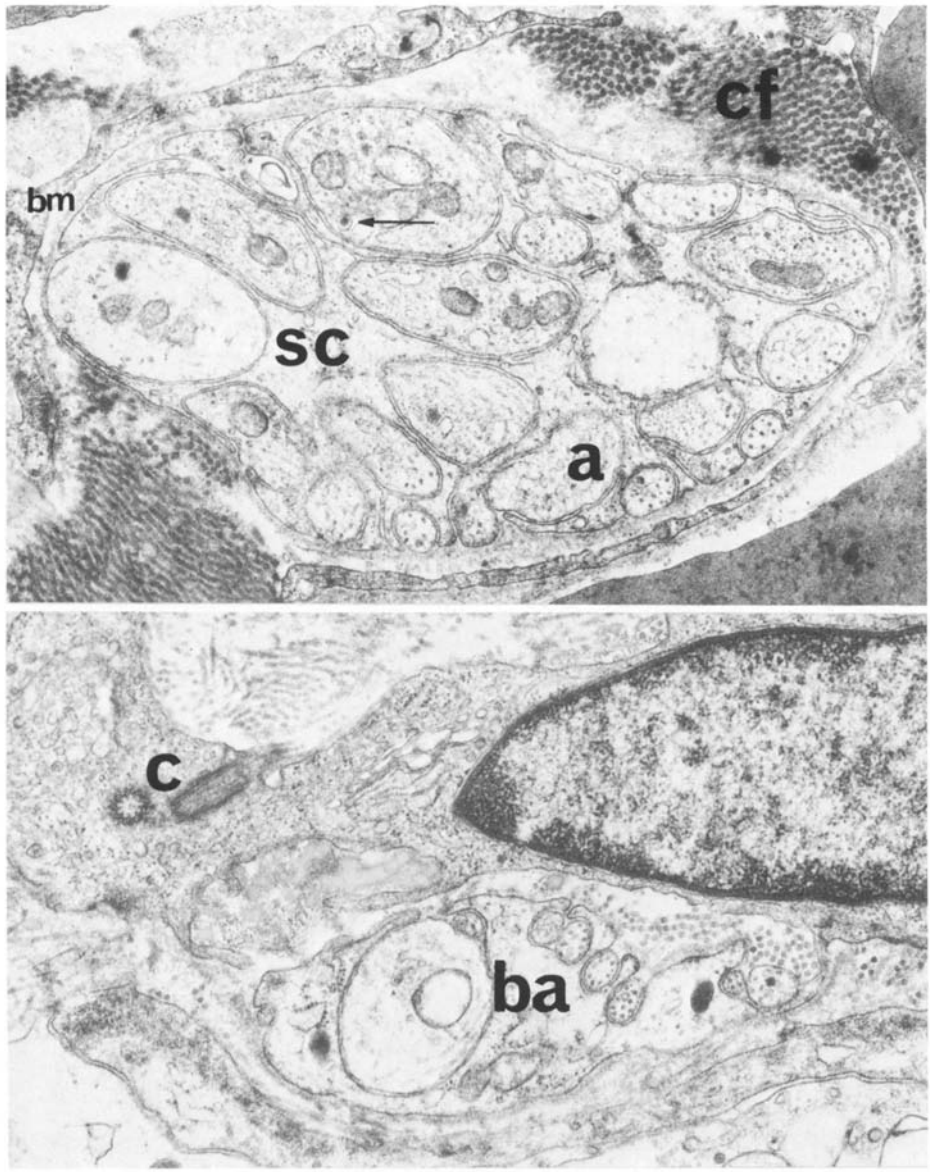

Fig. 4. Small nerve fiber bundle in the adventitia of a central artery. Schwann cell (*sc*) with multiple axons (*a*). Collagenous fibrils (*cf*), the basement membrane (*bm*) and granular vesicles (arrow) are also indicated. × 24,000

Fig. 5. Bundle of very small axons (*ba*) ensheathed by a single Schwann cell in the adventitia of an arteriole in the red pulp. *c* centriole in an adventitial cell. × 24,000

diameter of 120 μm. These bundles consisted of 10–20 axons ensheathed by one to four Schwann cells or their cytoplasmic processes. The fine nerves did not have a perineurium. The cytoplasmic processes of the Schwann cells were surrounded by collagenous fibrils of the adventitia (Fig. 4). The axoplasm sometimes contained granular vesicles about 1000 Å in diameter, as well as microtubules, neurofilaments, and mitochondria.

IV. Arterioles of the Red Pulp

Blood vessels of this caliber had a very thin (1–2 μm) adventitial layer. The amorphous ground substance of the connective tissue contained loosely arranged bundles of collagenous fibrils and some cytoplasmic processes of Schwann cells. The cell processes were very small with each process only partially ensheathing a few (three to six) thin axons (Fig. 5). The axoplasm often contained some relatively large granular vesicles as well as microtubules with a diameter of about 200 Å.

V. Splenic Microvasculature

By electron microscopy we were not able to demonstrate innervation of the more peripheral parts of the blood vessels or innervation of the red or white pulp itself. Nerve fibers or nerve terminals were not found along the sheathed capillaries, the arterial capillaries in the white and red pulp, or the sinus and pulp venules.

VI. Trabecular Veins

Small nerve fibers could be demonstrated in the connective tissue surrounding trabecular veins. The fibers consisted of only a few axons, which were not completely ensheathed by the cytoplasm of Schwann cells, and were found next to the outer smooth muscle cells of the media of these vessels.

B. Nerve Terminals

Nerve terminals were demonstrated in the central arteries and pulp arterioles. The media of the central arteries consisted of several layers of smooth muscle cells. There were no terminals in the media, but terminal axons were seen at the border between the adventitia and the outer layer of medial smooth muscle cells (Fig. 6). In addition to microtubules and mitochondria, these axons contained three different types of vesicles: (1) agranular vesicles with a diameter of 300–600 Å. Most of these were electron-lucent, but some contained sligthtly electron-dense homogeneous material. (2) Granular vesicles, also 300–600 Å in diameter, with a highly electron-dense core that was often eccentric. (3) Granular vesicles with a diameter of 900–1200 Å, also with a highly electron-dense core.

The terminal axons were only partially surrounded by cytoplasmic processes of Schwann cells. Schwann cell cytoplasm was absent on the side facing the smooth muscle cells, but the axon was separated from the amorphous ground substance of the connective tissue by a typical basement membrane. The distances between the terminal axons and the muscle cells ranged from 2000–4000 Å. In the pulp arterioles, whose media consisted of only a few layers of circular muscle cells,

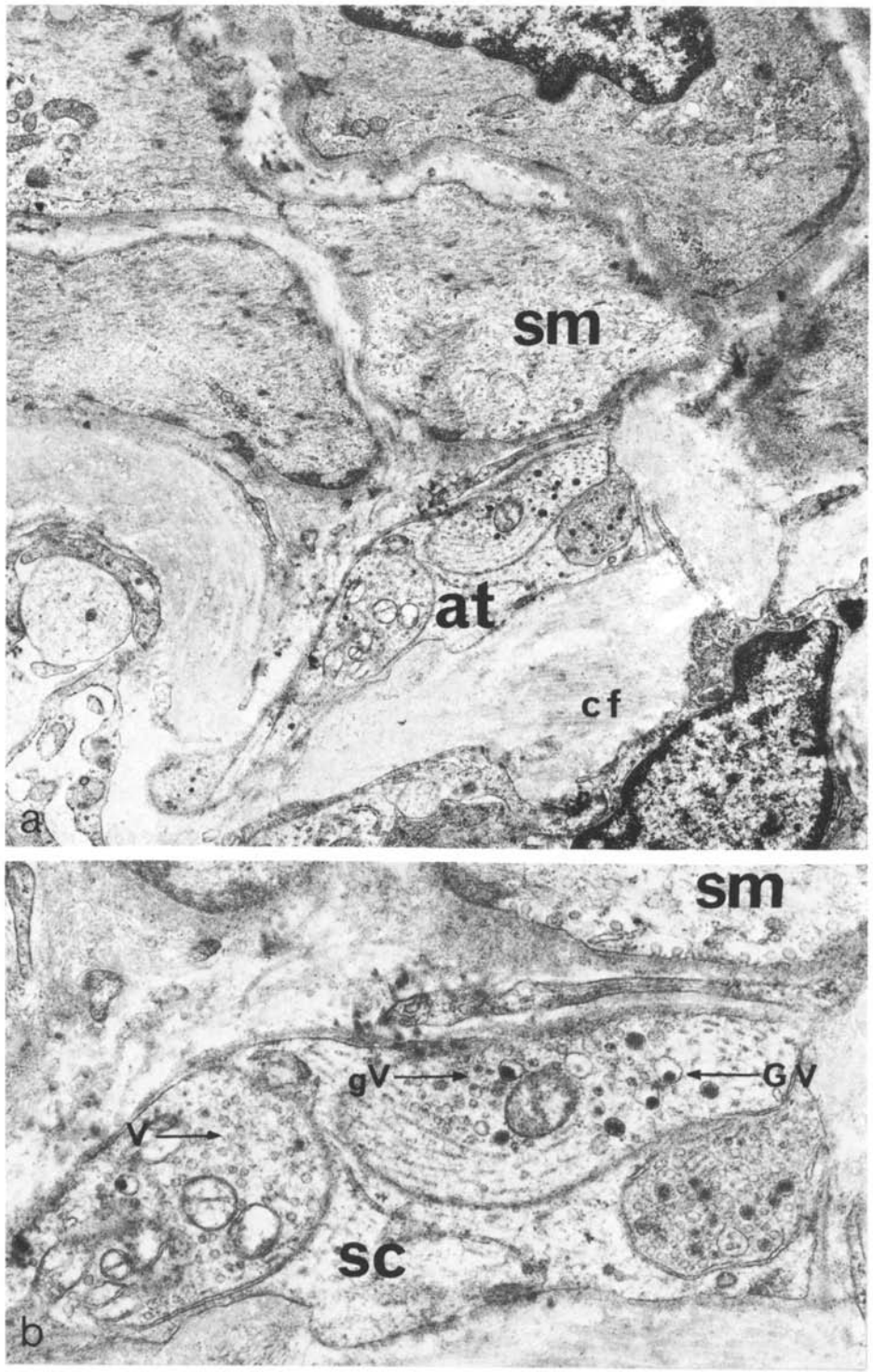

Fig. 6a and b. Adrenergic nerve terminal (*at*) at the border between the adventitia and media of a central artery. Smooth muscle cells (*sm*), and collagenous fibrils in the adventitia (*cf*) are indicated. **b** Detail of **a**. Three nerve terminal profiles only partially surrounded by the cytoplasm of a Schwann cell (*sc*) with three different types of vesicles: granular vesicles with a diameter of about 1000 Å (*GV*), granular vesicles with a diameter of about 400 Å (*gV*), and agranular vesicles (*V*). **a**: ×13,000; **b**: 39,200

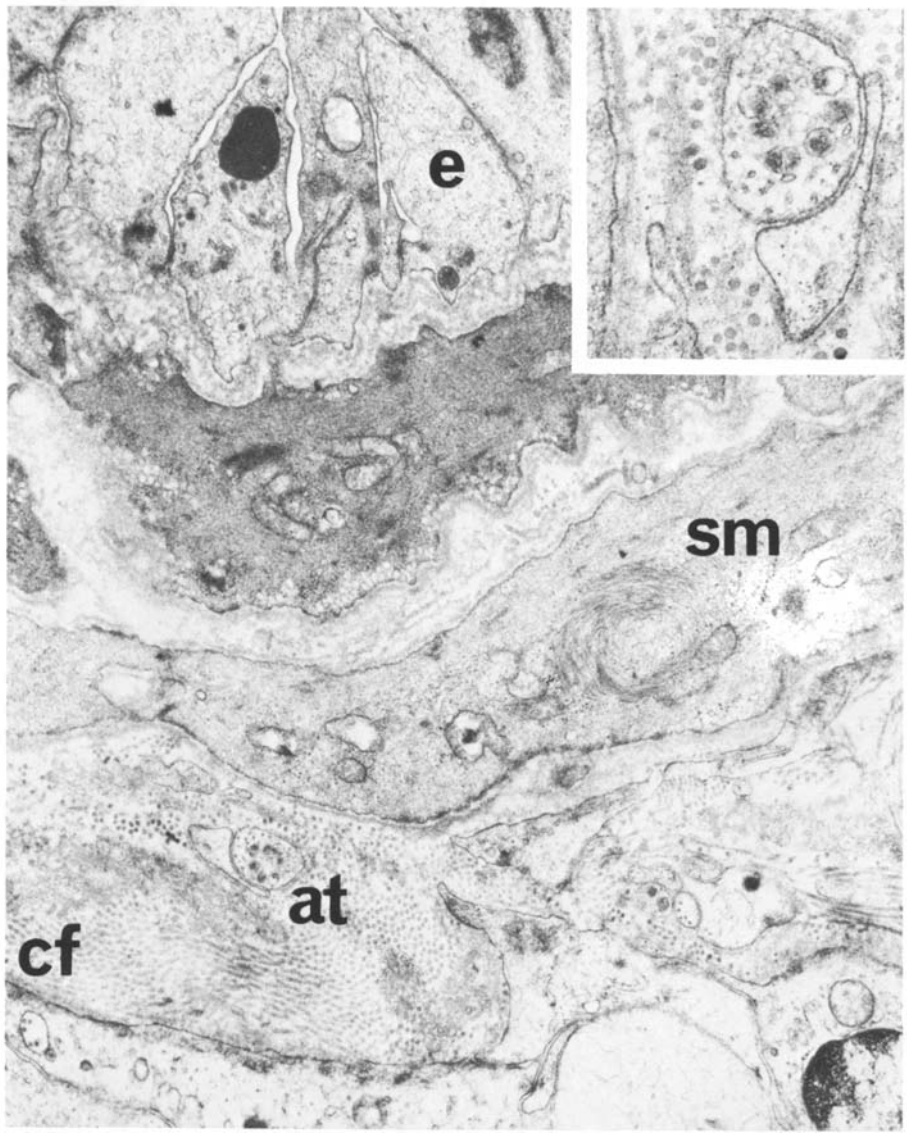

Fig. 7. Terminal axon (*at*) (enlarged in inset) facing a smooth muscle cell of a pulp arteriole. Collagenous fibrils (*cf*) in the surrounding connective tissue and an endothelial cell (*e*) are labelled. ×17,400. Inset: ×45,000

terminal axons were also detected only in the neighborhood of the outer muscle cells (Fig. 7).

Discussion

The innervation of the human spleen had never before been investigated by electron microscopy and spleens of other mammals only rarely. Galindo and Imaeda (1962), for example, mentioned in only one sentence the rare occurrence of unmyelinated

nerve fibers in the murine spleen. Moore et al. (1964) studied rabbit spleens and observed unmyelinated nerve fibers throughout the white and red pulp. Some of the fibers were said to be intimately associated with small blood vessels that resembled sheathed capillaries. Unfortunately, these findings cannot be verified, because they were not illustrated. The occurrence in the red and white pulp of free nerve fibers, i.e., nerve fibers that are not associated with blood vessels, would be a remarkable and unusual observation. Neither we nor other authors (Fillenz, 1970; Reilly et al., 1976) have been able to confirm this finding in human, feline, or murine spleens.

More detailed studies of the innervation of the spleen have been performed in cats (Zwillenberg and Zwillenberg, 1963; Tranzer and Thoenen, 1967; Fillenz, 1970). During investigations of sheathed capillaries, Zwillenberg and Zwillenberg (1963) detected Schwann cells and unmyelinated nerve fibers among the surrounding cells. Some of the axons were said to be closely related to the endothelial cells, which were picked with filaments. So far, we have not found nerve fibers in the poorly developed sheathed capillaries of the human spleen. In rabbit spleens Olah et al. (1975) demonstrated very small unmyelinated nerve fibers more at the periphery, i.e., in association with the terminal capillaries. The axons were surrounded by the cytoplasmic processes of reticulum cells accompanying the vessels and were in contact with the basal cytoplasmic processes of endothelial cells. Fillenz (1970) analyzed feline spleens by means of electron microscopy, fluorescence microscopy, and enzyme histochemistry for adrenergic and cholinergic nerve fibers. Until now, she was the only investigator who had presented ultrastructural findings on terminal axons in the spleen. She distinguished four different types with respect to the spatial relationship with smooth muscle cells:

(1) axons containing vesicles, completely surrounded by Schwann cell cytoplasm, and located more than 300–1000 Å from the muscle cell; (2) axons containing vesicles, only partly surrounded by Schwann cell cytoplasm and located 300–1000 Å from the muscle cell; (3) naked axons in close contact with a muscle cell, the intercellular gap being 200 Å; and (4) partially-surrounded axons in close contact with a cytoplasmic process of a muscle cell.

We found only the first two types of axons in the human spleen. Fillenz mentioned, however, that the latter two types were very rare. Earlier findings (Dahlström and Zetterström, 1965) encouraged Zetterström et al. (1973) to examine the red pulp of the canine spleen for adrenergic nerves by means of electron microscopy. According to their findings, the pulp cords contained numerous terminal axons that showed no relation to smooth muscle cells. The terminal axons were sometimes swollen. They contained small and relatively large granular vesicles. The axons ran freely between the splenic cells, but frequently appeared to be in close contact with erythrocytes. The interpretation of the latter finding is still not clear; Zetterström et al. (1973) mention the possibility that the adrenergic system influences red and white blood cells. It contradicts other observations that sympathetic adrenergic nerves normally end at smooth muscle cells or glandular tissue. Fluorescence microscopic studies of spleens from other species have also provided contradictory findings. The "nerve terminals" found by Zetterström et al. (1973) might be of a completely different nature. These structures might be thrombocytes, which can occur in large numbers in the spleen (Elgjo and Hovig, 1972). Like nerve terminals, thrombocytes reveal granules and vesicles on electron microscopy and they also contain catecholamine (Marcus and Zucker, 1965).

Fluorescence microscopic studies of adrenergic nerve fibers in the spleen were performed by Kirpekar et al. (1972) and Fillenz (1970) on cats, by Reilly et al. (1976) on mice, and by Dahlström and Zetterström (1965) and Zetterström et al. (1973) on dogs. With the previously mentioned exceptions, it can be concluded that the capsule and trabeculae contain a variable number of adrenergic nerves, depending on the number of smooth muscle cells. All of the spleens revealed a predominance of adrenergic nerves at the border between the adventitia and media of arteries and arterioles. The red and white pulp have no adrenergic nerves.

Elfvin (1961 a,b) studied splenic nerves by means of electron microscopy. Later, in a number of ultrastructural investigations of bovine spleens, Klein and Thureson-Klein and coworkers examined vesicles in splenic nerves, both in the nerve itself and in isolated fractions. They combined these studies with analysis of noradrenaline content (Klein et al., 1970; Klein and Thureson-Klein, 1971; Thureson-Klein et al., 1973). They reported that large granular vesicles are commonly found throughout the nerve trunk and are distributed to various degrees in terminals and varicosities. Small granular vesicles are chiefly confined to terminals and varicosities, but they can also be found in axon segments immediately adjacent to the terminals. It was also shown that the appearance, i.e., the density and size of the core, depended on the method of fixation. If the material is not fixed immediately after the excision, the vesicles lose their noradrenaline and appear electron-transparent and swollen. When fixation is performed as in our laboratory, namely, with glutaraldehyde, followed by OsO_4, noradrenaline is not lost. Especially when they used cacodylate or collidine buffer, Thureson-Klein et al. (1973) observed that the vesicles had a dense core surrounded by a translucent "halo", which they considered to be an artifact. The density of the vesicle matrix was correlated with the noradrenaline content. The authors presumed that noradrenaline was not the only substance responsible for the electron density. The appearance of the granular vesicles detected in our study (the granular vesicles often had a greater diameter than those found by Klein et al., who reported a diameter of 750 Å, and some of them were electron-transparent) was therefore probably caused by autolysis, since the tissue was not fixed immediately after the excision. The dense core of the vesicles, which was often surrounded by a translucent "halo," was probably attributable to the cacodylate buffer used in our investigation. Tranzer and Thoenen (1967) discussed the importance of the agranular vesicles with a diameter of 450 Å that we also found in nerve terminals. They were able to show in the feline spleen that such vesicles develop from the granular vesicles owing to the loss of noradrenaline during fixation and that they do not constitute a separate type of vesicle comparable to those in cholinergic axon terminals.

So far, modern methods have not been applied in studies of the innervation of the human spleen. Harting (1952) was the only researcher who used silver staining in such investigations. The data given in handbooks on anatomy (Stöhr, Jr., 1957; Tischendorf, 1969) are based almost exclusively on his findings. According to Harting, the bundles of nerve fibers in the trabeculae are found near the arteries and to a greater extent at the periphery in the adventitia of pulp and central arteries. We made similar observations. Technical artifacts were probably responsible for data on the distribution of nerve fibers and their endings in other structures. For example, there have been descriptions of a neural fibrillar network around the

fibrocytes of the trabeculae, of neural reticulation at the media-intima border of the arteries, and of a plexiform distribution of nerve fibers at the edges of Malpighian corpuscles. As Harting mentioned, demonstration of nerve elements in the spleen by means of silver staining is extremely difficult because of the large number of argyrophilic structures. Harting attached particularly great importance to demonstration of innervation of the sinus, which was supposed to consist of a network of neural nucleated "Plasmastränge." Neither in the present study however, nor in a previous one (Heusermann and Stutte, 1975), did we see innervation of the sinus.

At present, most investigators agree that cholinergic nerve fibers do not occur in the spleen. Whereas Stöhr, Jr. (1957) mentioned that the splenic nerves come from the ganglion coeliacum, to which vagal fibers are distributed, Tischendorf (1969) did not say anything about parasympathetic axons. Both authors agreed that the spleen is mainly innervated by sympathetic nerve fibers. In order to solve this problem, mammalian spleens have recently been subjected to fluorescence microscopic analysis for noradrenaline in nerve terminals and to histochemical demonstration of acetyl-cholinesterase, which indicates cholinergic nerves. In feline spleens, Fillenz (1970) found periarterial cholinergic nerves only in the splenic hilum; in the spleen itself she found only adrenergic nerves. These findings agree with those of Tranzer and Thoenen (1976), who demonstrated terminal axons that contained only noradrenaline close to the smooth muscle cells of the splenic capsule in cats. Reilly et al. (1976) showed that cholinergic nerve fibers are also completely absent from murine spleens. All of the nerve terminals we found in the human spleen contained granular vesicles and were thus adrenergic.

Apart from the nerve fibers in the adventitia of central arteries, we did not observe innervation of the white pulp itself. The innervation of other lymphatic organs has been studied by electron microscopy. Pfoch et al. (1971) investigated human thymuses and Pfoch and Unsicker (1972) examined Peyer's patches in Syrian hamsters. Like the human spleens we studied, the parenchyma of the thymus did not contain any nerve fibers. In conclusion, it appears that lymphatic tissue is hardly, if at all, innervated. Thus, it is improbable that the autonomic nervous system influences immune rections via peripheral nerves. This supposition is not contradicted by the finding of Pfoch and Unsicker (1972) that nerve fibers in Peyer's patches are seen in direct contact with lymphocytes. The wall of the small intestine, especially the submucosa, is supplied with abundant nerve fibers. The amount of lymphatic tissue in the small intestine largely depends on the functional status of the immune system. When lymphatic tissue is hyperplastic, lymphocytes probably envelop the nerve fibers in the submucosa and thus simulate innervation of Peyer's patches.

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