Virchows Archiv A Pathol Anat (1989) 415:367–375

Virchows Archiv A
Pathological Anatomy and Histopathology

Intimal thickenings of jugular veins after application of a stimulus known to be sclerogenic in arteries

D. Kling, V. Lindner, J. Fingerle, and E. Betz
Institute of Physiology I, University of Tübingen, Gmelinstrasse 5, D-7400 Tübingen, Federal Republic of Germany

Summary. The present study examined the intimal reactions of rabbit jugular veins to a stimulus known to elicit arteriosclerotic alteration in the artery wall. Repeated transmural electrical stimulation was applied to external jugular veins of both normo- and hypercholesterolaemic rabbits. Endothelial permeability, as well as changes in intimal architecture, were investigated by electron microscopy. Initially, the veins responded to electrical stimulation with an increased transendothelial transport of horseradish peroxidase (40000 daltons). After application of the stimulation program for 4 weeks, intimal fibrous thickening (33%), cellular fibrous proliferation (50%), and organized mural thrombi were observed. The fibrous thickening was characterized by an abundance of connective tissue matrix and paucity of subendothelial cells. The cellular fibrous proliferate predominantly consisted of myocytes with few interspersed monocytes/macrophages and granulocytes. It resembled intimal plaques induced in carotid arteries by the same method. However, the venous thickenings showed limited size and a more pronounced fibrous response when compared with the arteriosclerotic lesions. The morphological similarities between the observed venous intimal thickenings and the different types of phlebosclerotic manifestations described in the literature, especially intimal proliferations in vein grafts, render the model of electrical stimulation suitable for the elucidation of underlying pathogenic mechanisms.

Key words: Venous vessel wall – Endothelial permeability – Intimal fibrous thickening – Cellular fibrous proliferation – Phlebosclerosis

Offprint requests to: Dorothee Kling

Introduction

Venous intimal thickening is a well-known phenomenon. However, it has not been as intensively investigated as arteriosclerotic lesions. The term phlebosclerosis comprises various types of lesions on the venous side of circulation, including the diffuse intimal thickening that can occur with advancing age, or macroscopically visible intimal plaques at sites under mechanical stress (Geiringer 1949; Lev and Saphir 1959; Thurner and May 1967). A very common finding is intimal proliferations in autologous vein segments grafted into arterial circulation (Breyer et al. 1976; Brody et al. 1972; Jones et al. 1973; Kern et al. 1972; Marti et al. 1971; Spray and Roberts 1977; Szilagyi et al. 1973; Vlodaver and Edwards 1971; Vlodaver and Edwards 1972). These thickenings frequently represent a significant cause of late failure of aorta-coronary bypass grafts.

The pathogenesis of the heterogeneous phlebosclerotic manifestations is as unclear as the question of whether similarities exist between the development of these lesions and the morphogenesis of arteriosclerotic intimal proliferation. Experimental denudation of the endothelium of vein walls elicited intimal thickening comparable to that produced in arteries by the same method, however, it was restricted in size and extent (Manderson and Campbell 1986). Transmural electrical stimulation of rabbit arteries induced fibromuscular or lipid-containing plaques depending on the plasma cholesterol level (Betz and Schlote 1979). As already described (Kling et al. 1987a), in this model the arterial endothelium was maintained as a continuous covering of the vessel wall, but after the first stimulation period it displayed an increased perme-

ability to macromolecules and subtle structural changes. These alterations of the endothelium were followed by the invasion of leukocytes from the blood stream into the artery wall and subsequent migration of myocytes from the media into the intima.

In the present study the technique of electrical stimulation was applied to vein walls in order to examine whether veins respond to this stimulus in a similar way to arteries or whether there exist intrinsically determined differences in response.

Materials and methods

In male New Zealand rabbits (2.5–3.7 kg body wt.; $n=16$) external jugular veins were subjected to repeated transmural electrical stimulation, a technique already applied to carotid arteries (Betz and Schlote 1979). Four animals fed with a diet of commercial stock pellets (Altromin; Lage, FRG) were randomly selected to examine the permeability of the endothelium of jugular veins after short stimulation periods; the others were used to investigate the structural changes of the venous wall at electron microscopic level occuring after exposure to a stimulation program of 4 weeks ($n=12$). One half of these animals from the long-term studies was maintained on the standard diet, the other half received a cholesterol-enriched (1%) chow as an additional sclerogenic stimulus.

To investigate the endothelial permeability of the external jugular vein, a procedure similar to that described for carotid arteries was employed (Betz et al. 1985; Kling et al. 1987b). Briefly, under general anesthesia the veins were carefully freed from the perivascular tissue, and a teflon cuff containing two graphite-coated gold electrodes (5 mm × 1 mm) was attached to the adventitia of the right jugular vein so that the electrodes were diametrically opposed. However, the vessel segment was not totally sheathed by the cuff. Direct current impulses (0.1 mA, 50 ms/imp., 10 Hz) were applied to the vessel wall of the anesthetized animal for 30 min, and immediately after the stimulation period horseradish peroxidase (Boehringer Mannheim, FRG; 40000 daltons; 10000 units/kg body wt.) was injected into an ear vein. Six minutes later, fixation of the vessels with cacodylate-buffered glutaraldehyde (1.25%) was started. For this purpose, the chest and the abdominal cavity of the rabbits were cut open, and a cannula was inserted into the left ventricle. Via this cannula 500 ml of the fixative were infused into the ventricle at a pressure of 80–100 mmHg. The pulmonary arteries and the aorta caudal to the diaphragm were clamped so that the buffered glutaraldehyde streamed through the brachiocephalic arterial and venous system for 30–45 min. To ensure the outflow of excess fluid from the vascular bed, the jugular veins were cut at least 2 cm caudal of the cuff-bearing region. The outflow from the veins was regulated so that they didn't collapse. In each animal the contralateral vessel served as an unstimulated control. For ultracytochemical localization of the peroxidase the diaminobenzidine-technique was employed in a modified form (Betz et al. 1985). To avoid masking of the reaction products of the peroxidase, staining of the ultrathin sections was omitted.

In order to induce lesions in long-term experiments as in the permeability studies, electrodes (held in position by a cuff) were attached to the adventitia of the right jugular vein of anesthetized rabbits. They were connected s.c. with leads to a small plastic socket fixed in the skull. This stimulation device allowed the application of DC-impulses (0.1 mA, 15 ms/imp., 10 Hz) to the conscious, unrestrained rabbit. The "stimulation schedule" comprised two periods of 30 and 15 min per day and lasted for 4 weeks. The contralateral vein was furnished with a cuff without electrodes, providing an intra-individual control. Unsheathed control segments were obtained from rabbits used for pilot experiments.

At the end of the stimulation schedule, the animals were again anesthetized and the vessels were either fixed in the manner described in the section above or carefully removed after ligaturing the cuff-bearing segment and immersion-fixed with gradual substitution of the intraluminal blood by the fixative. Afterward, the excised and fixed vessel rings were subdivided into various regions (that is anodal, cathodal parts and areas outside of the cuff) and submitted to the usual procedures for transmission electron microscopy (post-fixation in 1% cacodylate-buffered osmium tetroxide, en bloc staining with a 2% alcoholic solution of uranyl acetate, dehydration using ascending concentrations of ethanol, embedding in araldite). Thin sections were mounted on copper mesh grids and stained with lead citrate, prior to examination under a Zeiss EM 10.

Results

Normal jugular vein

In normal external jugular veins an attenuated single layer of endothelial cells overlies a very thin basal lamina which is followed by a narrow space consisting of ground substance and collagen fibrils (Fig. 1). The underlying discontinuous, internal elastic lamina forms an indistinct boundary between the intima and the media. The media usually consists of 2–4 layers of smooth muscle cells organized in a helical fashion. A relatively acellular space containing bundles of collagen fibrils and only scanty elastic fibers adjoins these smooth muscle cell layers. Sporadically, smooth muscle cells or monocytes/macrophages are interspersed within this collagenous connective tissue. This space blends into the adventitia where fibrocytes are arranged between elastic fibers and thick masses of collagenous fibrils. Occasionally capillaries of the vasa vasorum are present.

Responses of the vein wall to electrical stimulation

The external jugular veins responded to electrical stimulation with an enhanced transendothelial transport and an increased uptake of macromolecules into the venous wall. After 30 min of electrical stimulation and subsequent injection of horseradish peroxidase, a massive accumulation of reaction products of the peroxidase was detected in the subendothelial space next to the anode (Fig. 2a). To some extent, the peroxidase had advanced to the juxtaintimal part of the media. Numerous interendothelial clefts were filled with electron-dense reaction products, and cytoplasmic ve-

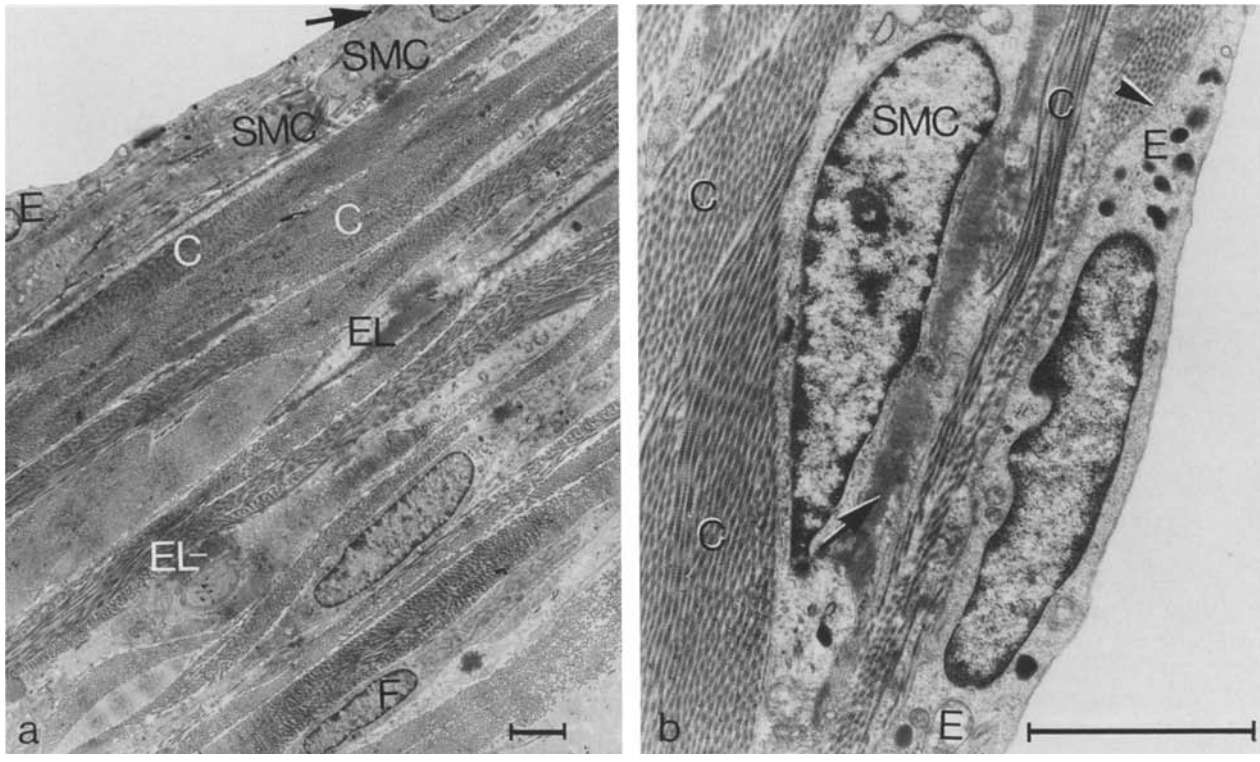

Fig. 1. Normal rabbit jugular vein. (**a**) Low – power view. Fragments of the internal elastic lamina (→) separate the intima from the media where 2 layers of smooth muscle cells and bundles of collagen fibers are present. A distinct external elastic lamina is missing; the media blends into the adventitial layer where fibroelastic tissue dominates. (**b**) High – power view. Between the endothelium and the rudimentary internal elastic lamina (→) a basal lamina (▶) and collagen fibrils embedded in ground substance are discernible. E endothelium; SMC smooth muscle cell; C collagen fibrils; EL elastic fiber; F fibrocyte. Bars indicate 2 μm

sicles displaying positive peroxidase reaction were often localized along these clefts (Fig. 2b). This indicates an intensified intercellular transport possibly combined with parajunctional vesiculation. In the vein wall next to the cathode, a similar pattern in the localization of the peroxidase was observed; however, the amount of the osmiophilic reaction products appeared to be decreased compared to the anodal region. Regarding the areas between the electrodes as well as the unstimulated control vein, no or only slight electron opacity, characteristic of the peroxidase, was detectable in the subendothelial space (Fig. 2c); cytoplasmic vesicles showing peroxidase activity were discernible sporadically within the endothelial cells of these regions. Comparing the structure of the endothelial cells it was notable that those of the stimulated vein wall appeared to be swollen and frequently vacuolated, which was not observed in the controls.

In the following section only the structural alterations observed in the vicinity of the anode in the venous intima are described; the intimal responses found next to the cathode resembled those occuring in the contralateral vessel segments provided with a cuff without electrodes (see below).

After 28 days of electrical stimulation, all jugular veins had developed intimal thickenings in the vicinity of the anodal site. These lesions were not strictly limited to the area of the anode but extended across the borders of the electrode in tangential, caudal and cranial directions. They were heterogenous in their structure, so that 3 types of morphological alterations were distinguished: *cellular fibrous proliferation, fibrous thickening* and *mural thrombus*. All three types occured both in normo- and hypercholesterolaemic rabbits.

Half of the stimulated jugular veins had developed intimal thickening consisting of *cellular fibrous proliferation*; in 3 cases the animals were fed the standard diet, and in the other 3 cases the rabbits were maintained on the cholesterol-enriched chow. Figure 3 shows a low-power view of the cellular fibrous proliferation in the two groups. The thickness of these lesions varied between 16 and 65 μm (note the shrinkage due to fixation and embedding procedure). There was no significant dif-

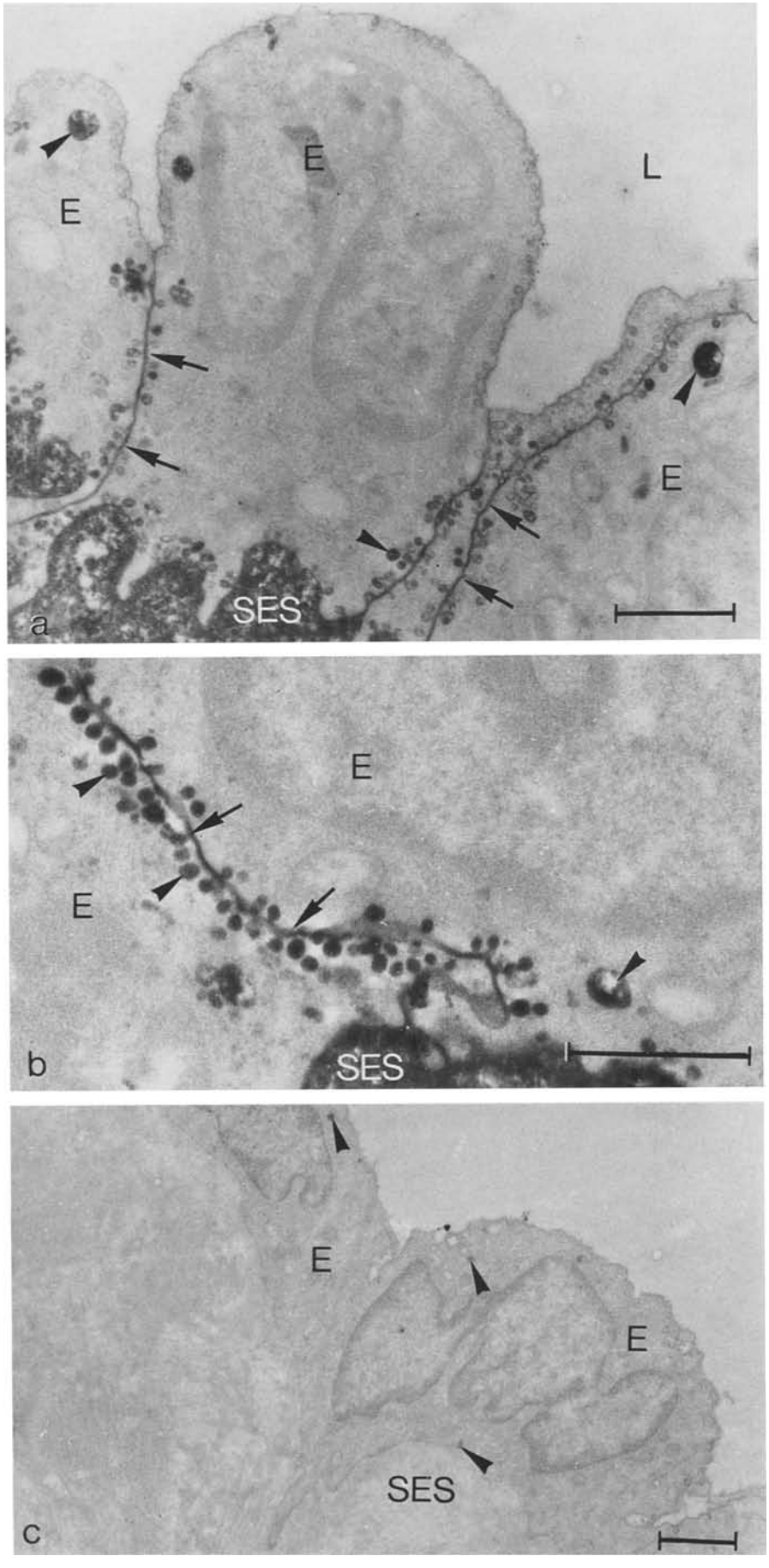

Fig. 2. Deposition of reaction products of horseradish peroxidase in the intima of the external jugular vein. Injection of the peroxidase 6 min prior to perfusion fixation. (**a**) Part of the stimulated vein segment next to the anode after application of DC impulses for 30 min. (**b**) Higher magnification of an interendothelial gap out of the stimulated area displaying peroxidase activity as the vesicles located in the vicinity. (**c**) Part of the unstimulated control vein with very few vesicles exhibiting positive peroxidase reaction. E endothelium; L lumen; SES subendothelial space; → interendothelial gap; ▸ vesicles with peroxidase activity. Bars indicate 1 μm

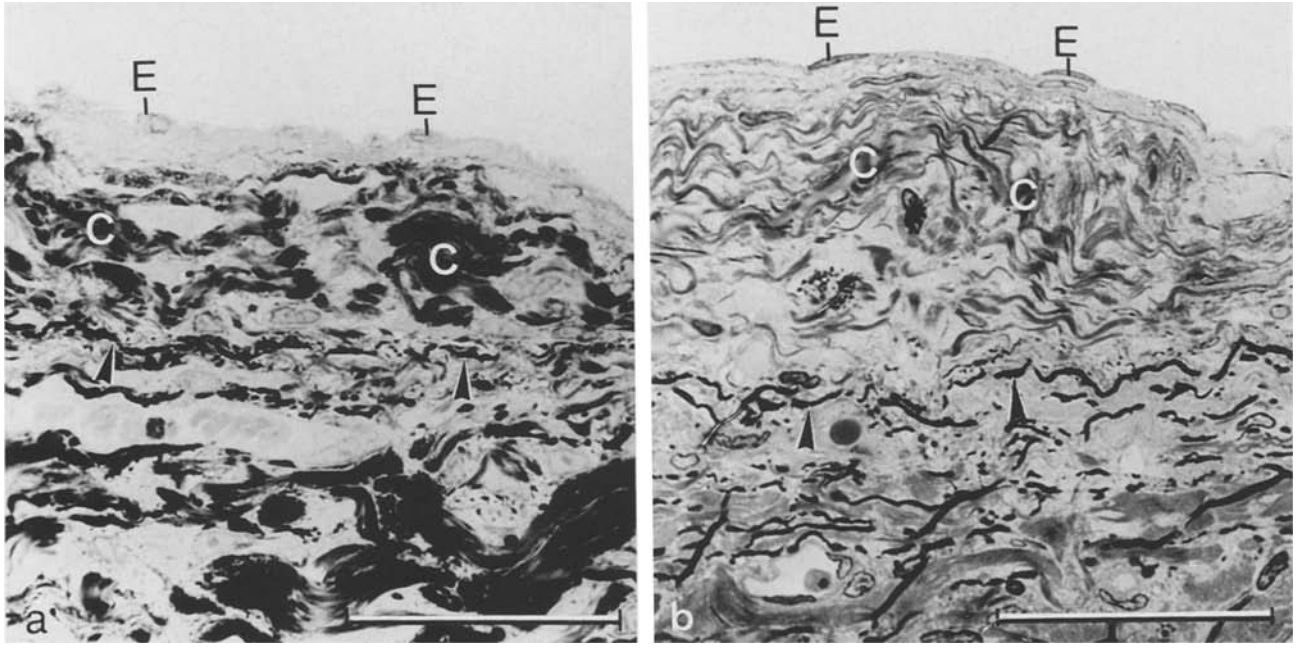

Fig. 3. Light micrographs of cellular fibrous proliferations in jugular veins which were exposed to electrical stimulation for 28 d. The rabbits received either a standard diet (**a**) or a cholesterol-enriched chow (**b**). Note the abundance of extracellular material, especially collagen fibers. E endothelium; C bundles of collagen fibers; ▶ internal elastic lamina. Toluidine blue staining. Bars indicate 50 μm

ference in the number of cell layers between the two experimental groups (standard diet: 4.0 ± 0.5 cell layers; cholesterol-enriched diet: 5.0 ± 0.6 cell layers).

The cellular fibrous proliferate was covered by a continuous endothelial lining. It was composed predominantly of myocytes which were embedded in well developed extracellular material that mainly consisted of bundles of collagen fibrils (Fig. 4a). Some of the smooth muscle cells were oriented with their longitudinal axes parallel to the direction of the blood flow, others were arranged in a more helical fashion like the media myocytes. Most of the intimal smooth muscle cells exhibited the contractile phenotype, whereas only a few of them were in a synthetic state after 28 d of electrical stimulation. Sporadically, granulocytes and mononuclear cells were not only observed within the intimal lesion but also adhering to the endothelium (Fig. 4a, d).

In those cases with additional hypercholesterolaemia, lipid vacuoles were recognized both in intimal myocytes and monocytes/macrophages (Fig. 4a, c). Whereas clusters of nonmembrane-bound, electron-lucent vacuoles were present in macrophages, only a small number of lipid inclusions was observed in some of the smooth muscle cells. Extracellular fatty droplets were also noted in the vein wall.

In its morphology, the cellular fibrous proliferation resembled the atherosclerotic plaque induced by the same method in carotid arteries (Fig. 4b). However, the comparison of these intimal proliferations on the arterial and venous side revealed two important differences: the venous lesions were restricted in size, even after the same stimulation time, and the development of extracellular material, especially of collagen fibrils, was more pronounced in the intimal cushions of the jugular vein than in those of the carotid artery.

Fibrous thickening was present in one third of the stimulated veins and differed from the cellular fibrous intima proliferation by the paucity of cells and the predominance of the fibrous component. The space between the endothelium and the distinct, mostly continuously developed internal elastic lamina was mainly composed of collagen fibrils embedded in ground substance (Fig. 5). Among the fibrils, amorphous material was present, resembling the homogenous component of elastic fibers. The arrangement and direction of the collagen fibrils was often disturbed; they appeared to be disordered and pointing in all directions in the intercellular space, a phenomenon called collagen dysplasia and frequently described in cases of human vascular disease such as diabetic angiopathy and varicose veins (Staubesand and Fischer 1979). Only few intimal cells, namely myocytes and monocytes/macrophages were interpersed in the fibrous thickening (Fig. 5). When the animals were fed with the cholesterol-enriched diet, extracellular lipid deposits were discernible.

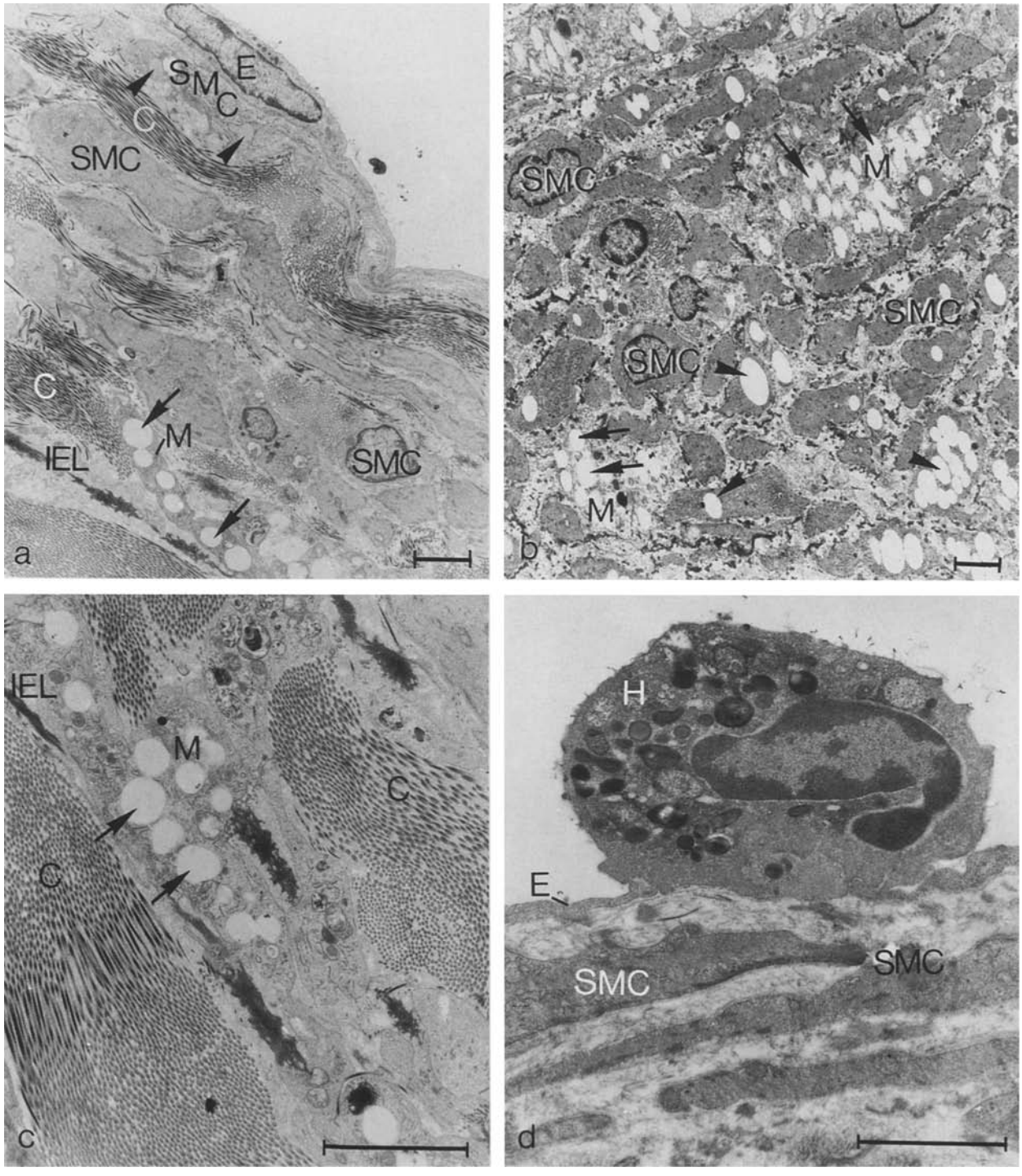

Fig. 4. (**a**) Part of an intimal cellular fibrous thickening of the vein wall induced by electrical stimulation for 28 d and additional hypercholesterolaemia. (**b**) For comparison, cross-section through an intimal proliferate of the carotid artery elicited by the same combined stimulation technique. The 28-day-old plaque consists mainly of myocytes oriented with their longitudinal axes parallel to the blood flow. Few macrophages are interspersed among the smooth muscle cell. Lipid vacuoles are present in both myocytes and macrophages. (**c**) Intimal macrophage containing nonmembrane-bound lipid vacuoles in a venous cellular fibrous proliferate. (**d**) Heterophil granulocyte adhering to the venous endothelium covering a cellular fibrous thickening. E endothelium; SMC smooth muscle cell; M macrophage; ➞ lipid vacuoles in macrophages; ▸ lipid vacuoles in SMC; C collagen fibrils; IEL internal elastic lamina; H heterophil granulocyte. Bars indicate 2 μm

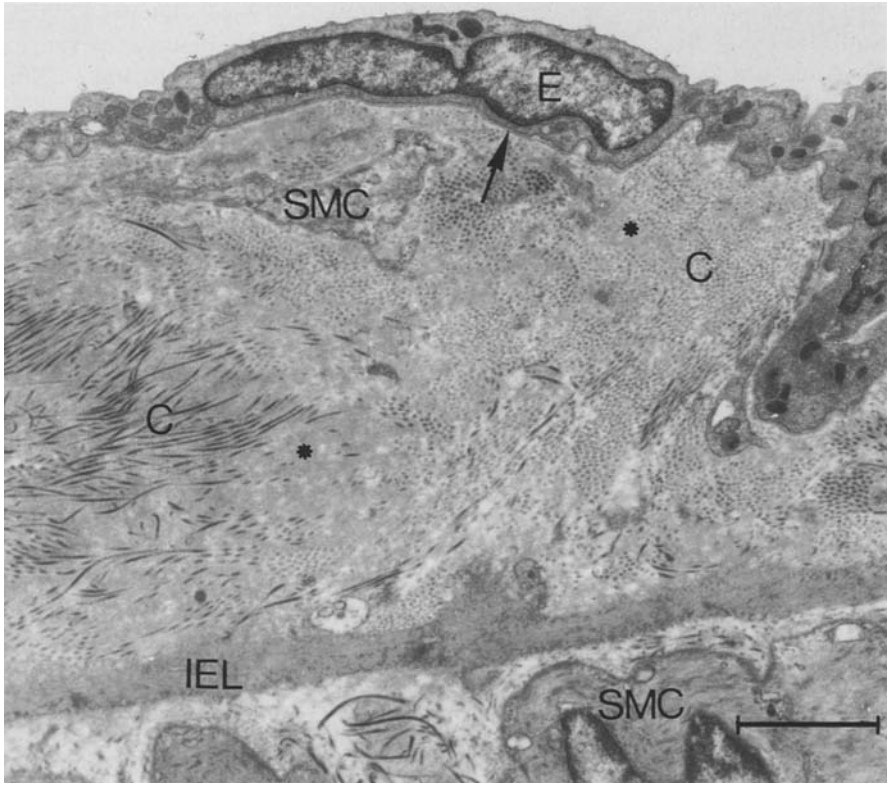

Fig. 5. Intimal fibrous thickening characterized by the abundant existence of intercellular substance and very few interspersed subendothelial cells. E endothelium; C collagen fibrils; * amorphous material; SMC smooth muscle cell; IEL internal elastic lamina; ➙ basal lamina. Bar indicates 2 μm

In two cases *mural thrombi* were present, one in the stimulated vein of a rabbit fed the standard diet, the other in the stimulated segment of a hypercholesterolaemic animal. Based on ultrastructural criteria, the cells covering the thrombi were identified as endothelial cells. The cellular composition of the thrombi showed a predominance of granulocytes and mononuclear cells. The cells in close apposition to the internal elastic lamina displayed morphological features of myofibroblasts and smooth muscle cells. At this point in thrombus organization, platelets were rarely seen. Within the body of the thrombus, clefts lined by cells closely resembling endothelium and forming capillary channels were a prominent finding.

To evaluate the real influence of electrical stimulation on the intima reactions of the vein wall, one must examine the changes of the contralateral vessel equipped with similar cuffs but without electrodes. Here the findings were somewhat inconsistent. Some of the sheated vessels showed an apparently normal intima or at the most a slight augmentation of the subendothelial connective tissue material, also observed in regions adjacent to venous valves. In others a cellular reaction could be recognized within the intima; however, the protrusions of these cellular thickenings into the lumen were only small and flat compared with those occuring in the stimulated vessel segments.

Discussion

The present study demonstrates the intimal reaction elicited in vein walls by electrical stimulation and allows a comparison of the venous responses with those induced in artery walls by the same sclerogenic stimulus and with the phlebosclerotic alterations described in literature. These comparisons may help to elucidate the pathogenesis of phlebosclerosis, especially of the severe lesions occuring in autologous vein grafts inserted into the arterial circulation.

One of the earliest responses to electrical stimulation was the increased transendothelial transport and uptake of macromolecules into the vein wall, a finding which also occured in carotid arteries (Kling et al. 1987b). In the latter the increased endothelial permeability was followed by the invasion of leukocytes and subsequent migration of myocytes from the media into the intima and their proliferation (Kling et al. 1987a); after 28 d of stimulation fibromuscular and lipid-containing plaques had developed, depending on the plasma cholesterol level. With the low stimulus intensity

used, mural thrombi were never observed in stimulated arteries. Veins, however, manifested very different types of intimal alterations after the same exposure time to electrical stimulation of identical intensity. Despite the very close resemblance of the initial functional changes of the endothelium of arteries and veins, the latter displayed a broader scope of further reactions, namely cellular fibrous and fibrous intimal thickenings as well as mural thrombi had developed after the stimulation program of 28 d. The dynamics of cellular events beginning with the initial enhancement of endothelial permeability up to the formation of the 3 types of intimal thickenings have not yet been clarified. However, based on the morphological parallels it is very likely that the development of the venous cellular fibrous proliferate is similar to that of the arteriosclerotic plaque induced by the same method. The observation of leukocytes adhering to the endothelium and being interspersed with the intimal cell population suggests that invasion of leukocytes is also involved in the genesis of the cellular fibrous thickening.

Whether fibrous intimal lesions can be derived from the cellular fibrous ones or vice versa, or whether they represent final points of two different lines of development are still unanswered questions. In any case, it is notable that jugular veins developing sclerotic changes after exposure to electrical stimulation display a much more pronounced fibrous reaction when compared with carotid arteries. This fibrous reaction was not only confined to the intima but was also very prominent in the media and adventitia. Funaki et al. (1987), who induced intimal thickenings of jugular veins and carotid arteries in rabbits by investing the vessels with a polyethylene tube found an elevated content of collagen fibers in the intimal lesions of veins compared to those of the arteries in agreement with our findings. Although the mechanisms underlying the increased formation of intercellular substance, especially collagen, are not known, it is obvious that veins display a greater tendency towards fibrous reactions under pathological conditions than arteries. This may be due to the structure of the vein wall with its abundance of intercellular substance.

Another striking difference between venous and arterial intimal lesions occuring after the same period of electrical stimulation is the restricted size of both fibrous and cellular fibrous thickenings on the venous side of the vessel tree; that is their protrusion into the lumen of the vessel is reduced compared with that of the arteriosclerotic plaque. This finding is in accordance with the observations of Manderson and Campbell (1986). One possible explanation may be the smaller percentage of venous smooth muscle cells able to proliferate and to contribute to lesion formation. However, it must be noted that despite the relative paucity of smooth muscle cells, veins grafted into arterial circulation can develop haemodynamically significant intimal thickening which may progress to total occlusion (Breyer et al. 1976; Kern et al. 1972; Vlodaver and Edwards 1971). This indicates that not only the number of venous myocytes present in the media determines the extent of the thickening but that, for example, the supply of smooth muscle cells with growth stimulating or – inhibiting factors (which may differ in arterial and venous circulation), their sensitivity to these factors, their rate of synthesis of extracellular material all influence the degree of lesion formation.

The present study also investigated the behavior of electrically stimulated jugular veins in hypercholesterolaemia. Like the intimal proliferations of the arteries induced by the same method, the intimal thickenings of the veins incorporated lipids in both monocytes/macrophages and smooth muscle cells as well as in the extracellular material. At first sight, the extent of lipid deposition appeared to be reduced in the venous intimal thickenings compared to the arterial ones. This is in agreement with other reports in the literature describing only scanty incorporation of fat in phlebosclerotic lesions (for a review, see Thurner and May 1967). However, an exact quantitative evaluation requires extensive morphometric studies which take into consideration the extent and the cellularity of the respective lesions.

The comparison of the described venous intimal thickenings with phlebosclerotic alterations reported in literature sometimes proved to be difficult since most of them were only described on a light microscopic level. Most intimal thickening (for a review, see Thurner and May 1967) is characterized as scarcely rising above the neighboring intima and being mainly composed of connective tissue material with few subendothelial cells, suggesting a resemblance to the fibrous thickenings in our model. Others, however, for example the intimal cushions often observed in the inferior vena cava and iliac veins (Geiringer 1949; Lev and Saphir 1959) and the intimal proliferations in autologous vein grafts (Brody et al. 1972; Jones et al. 1973; Vlodaver and Edwards 1971) resemble cellular fibrous thickening. Some of the authors (Kern et al. 1972; Vlodaver and Edwards 1971) studying the histological fate of aorta-coronary bypass grafts have stressed the fibrous nature of intimal

thickenings, especially of grafts inserted in arterial circulation only for a short time.

Various factors may be responsible for the different phlebosclerotic changes; elevated intravascular pressure or increased shear stress (Jones et al. 1973; Spray and Roberts 1977; for a review, see Thurner and May 1967; Vlodaver and Edwards 1971). These conditions may lead to ill-defined endothelial damage, assumed to be one factor involved in the development of phlebosclerotic changes. Based on our investigations and the similarities between the lesions studied here and the phlebosclerotic lesions in the literature including those of the vein grafts, it is very likely that increased endothelial permeability, as one sort of functional endothelial injury, is involved in the pathogenesis of phlebosclerosis.

Acknowledgements. The authors thank Dr. H. Heinle for the helpful suggestions during the preparation of the manuscript. Ms. M. Beck is kindly acknowledged for her skilful and valuable technical assistance.

References

Betz E, Schlote W (1979) Responses of vessel walls to chronically applied electrical stimuli. Basic Res Cardiol 74:10–20

Betz E, Hämmerle H, Strohschneider T (1985) Inhibition of smooth muscle cell proliferation and endothelial permeability with Flunarizine in vitro and in experimental atheromas. Res Exp Med 185:325–340

Breyer RH, Spray TL, Kastl DG, Roberts WC (1976) Histologic changes in saphenous vein aorto-coronary bypass grafts. J Thorac Cardiovasc Surg 72:916–924

Brody WR, Angell WW, Kosek JC (1972) Histologic fate of the venous coronary artery bypass in dogs. Am J Pathol 66:111–129

Funaki C, Naito M, Nahayama Y, Hayashi T, Kuzuya M, Asai K, Kuzuya F (1987) Experimental intimal thickening of jugular veins by investment in rabbits. J Jpn Atheroscler Soc 15:1471–1473

Geiringer E (1949) Venous atheroma. Arch Pathol Lab Med 48:410–420

Jones M, Concle DM, Ferrans VJ, Roberts WC, Levine FL, Melvin DB, Stinson EB (1973) Lesions observed in arterial autogenous vein grafts. Supplements to Circulation 47 and 48 (suppl III):198–210

Kern WD, Dermer GB, Lindesmith GG (1972) The intimal proliferation in aortic-coronary saphenous vein grafts. Am Heart J 84:771–777

Kling D, Holzschuh T, Betz E (1987a) Temporal sequence of morphological alterations in artery walls during experimental atherogenesis – occurrence of leukocytes. Res Exp Med 187:237–250

Kling D, Holzschuh T, Strohschneider T, Betz E (1987b) Enhanced endothelial permeability and invasion of leukocytes into the artery wall as initial events in experimental arteriosclerosis. Int Angio 6:21–28

Lev M, Saphir O (1959) Endophlebohypertrophy and phlebosclerosis II. The external and common iliac veins. Am J Pathol 28:401–411

Manderson JA, Campbell GR (1986) Venous response to endothelial denudation. Pathology 18:77–87

Marti M-C, Bouchardy B, Cox JN (1971) Aortocoronary bypass with autogenous saphenous vein grafts: histopathological aspects. Virchows Arch [A] 352:255–266

Spray TL, Roberts WC (1977) Changes in saphenous veins used as aortocoronary bypass grafts. Am Heart J 94:500–516

Staubesand J, Fischer N (1979) Collagen dysplasia and matrix vesicles. Researches with the electron microscope into the problem of the so-called "weakness of the vessel wall". Path Res Pract 165:374–391

Szilagyi DE, Elliott JP, Hageman JH, Smith RF, Dall'Olmo CA (1973) Biological fate of autogenous vein implants as arterial substitutes. Ann Surg 178:232–246

Thurner J, May R (1967) Probleme der Phlebopathologie, mit besonderer Berücksichtigung der Phlebosklerose. Zbl Phlebol 6:404–482

Vlodaver Z, Edwards JE (1971) Pathologic changes in aortic-coronary arterial saphenous vein grafts. Circulation 44:719–728

Vlodaver Z, Edwards JE (1972) The pathologist approaches clinical coronary disease. Hum Pathol 3:3–9

Received March 7, 1989 / Accepted April 26, 1989