Virchows Arch. Abt. A Path. Anat. 351, 1—11 (1970)

Acute Vascular Lesions in Developing Coronary Collaterals

MARCEL BORGERS, JUTTA SCHAPER and WOLFGANG SCHAPER
Department of Electron Microscopy Janssen Pharmaceutica, Research Laboratoria Beerse, Belgium

Received February 2, 1970

Summary. Three weeks after the onset of progressive obliteration of the left circumflex coronary artery a number of small arteriolar preexistent collaterals develop into small arteries. Acute focal lesions, localized in the central parts of the collateral, are described at the light microscopic and at the ultrastructural level.

Partial necrosis of the medial smooth muscle cells, leakage of bloodborne elements through gaps and breaks in the intima, and proliferative activity in neighboring parts of the injured zone are the most characteristic features in the early development.

Zusammenfassung. Drei Wochen nach dem Beginn eines fortschreitenden Verschlusses des R. circumflexus der linken Coronararterie entstehen aus kleinen schon bestehenden Kollateralgefäßen von der Größe von Arteriolen kleine Arterien. Es werden lichtmikroskopische und ultrastrukturelle Studien der akuten fokalen Lesionen im Mittelstück der Kollateralgefäße beschrieben.

Typische Merkmale dieses frühen Entwicklungsstadiums sind: Nekrose zahlreicher glatter Muskelzellen der Media, Eindringen von Blutzellen und Plasma durch Lücken der Intima und Proliferation in der beschädigten Zone benachbarten Gebieten.

Injury to arteries has been induced experimentally by a variety of physical (Still, 1967; Hoff and Gottlob, 1968; Cotran and Remensnyder, 1968; Björkerud, 1969) and chemical (Buck, 1962; Hoff and Gottlob, 1967; Gardner and Matthews, 1969) means.

In their experiments on chronic occlusion of the left circumflex branch in canine hearts (Schaper, 1967; Schaper, Schaper, Xhonneux and Vandesteene, 1969) the development of collaterals as early as 2–3 weeks after implantation of an ameroid constrictor was noted. Recently the morphology of the evolution of coronary anastomoses was studied at the ultrastructural level (Schaper, Borgers and Schaper, 1970). Near the time the occlusion was completed severe alterations were observed in the newly developing collaterals caused by acute hemodynamic mechanical trauma.

In the present study the evolution of the vascular injuries along the longitudinal axis of the collateral is reported, indicating the focal nature of the lesion and the involvement of the area surrounding the damaged zone.

Material and Methods

Adult mongred dogs of either sex were used in these experiments. The development of collaterals was induced by progressive constriction of the left circumflex artery by means of an ameroid constrictor (Litvak, Siderides and Vineberg, 1957; Schaper, 1966).

Three weeks after the implantation of the constrictor, the animals were anesthetized and the hearts were fixed in situ by perfusion of a freshly distilled (Fahimi and Drochmans, 1965) solution of 2% glutaraldehyde in sodium-cacodylate buffer 0.1 M, pH 7.4, for 5 minutes. It

was very easy to identify macroscopically the epicardial collaterals connecting the anterior descending artery with the left circumflex artery. The collaterals were rapidly excised, divided into small segments, numbered from stem to reentry, and further fixed for 2 hours by immersion in 3% glutaraldehyde. After rinsing overnight in Veronal-acetate buffer, containing 0.2 M sucrose, the blocs were postfixed in 1.5% osmium tetroxide for 1 hour, stained with uranium acetate, dehydrated in graded series of ethanols and embedded in Epon.

The ultrathin sections, after post-staining with uranium acetate and lead citrate, were examined in a Hitachi HS 8-1 electron microscope. For light microscopy paraffin sections of glutaraldehyde fixed blocks were stained with Hematoxylin-Eosin and with Weigert's resorcin fuchsin. One micron thick sections of Epon embedded segments served as indicators for exact topographical localization of the lesions. These sections were stained with Paragon-1301 (Spurlock, Skinner and Kattine, 1966). Segments of pre-existent collateral junctions, taken from the same region as described above, of control dogs were processed in the same way, for both light and electron microscopy.

Results

A total of 12 collaterals from 4 experimental dog heart were examined and compared with the normal vessel architecture of control dog hearts.

Although the topographical distribution of the morphologic modifications observed in this early stage of vessel growth is the same for all the collaterals definite variations in intensity of the lesions were noted. This probably depended upon the initial vessel size and upon the number of preexistent collaterals before inducing the growth.

The following observations are restricted to the small collaterals which we presumed to respond in the most severe manner to hemodynamic changes occuring at the time of complete occlusion of one major vessel.

Light Microscopy

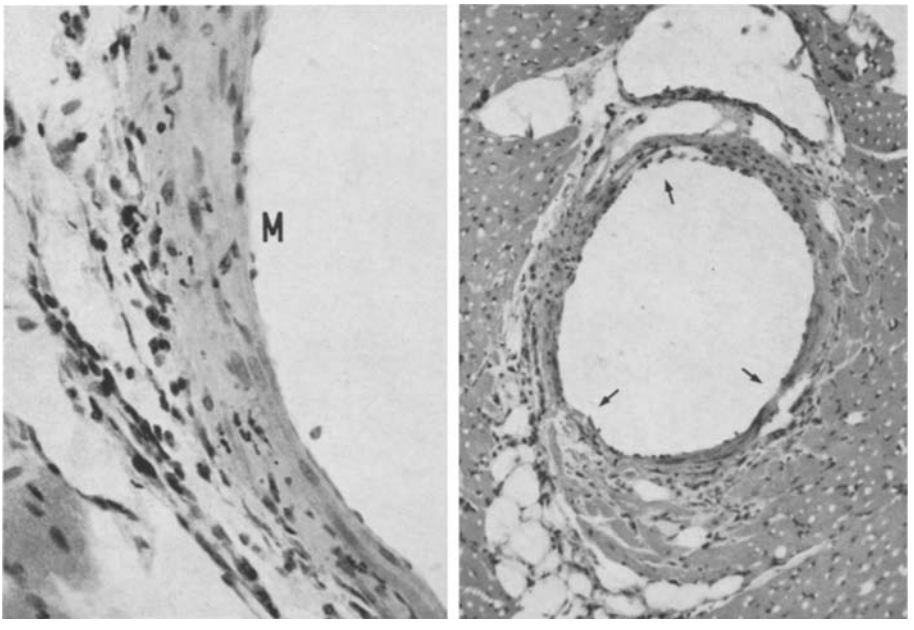
Control Animals. As for the experimental dogs the small collaterals were divided in segments, numbered 1 to 5 from stem to reentry. A gradual decrease in the vessel radius and the medial wall thickness was prominent from segment 1 to 4, ranging from 500 μ and 8 medial muscle layers in segment 1 to 35 μ and 1–2 medial muscle layers in segment 4. The radius and wall thickness increased again in segment 5.

The normal picture of a collateral junction showed continuously arranged endothelial cells with the underlying elastic lamellae, a closely packed thin layer of smooth muscle cells and the joining adventitia. No mitotic activity was observed in any layer.

Experimental Animals. The segments near the stem or reentry had a normal appearance. In Weigert stained sections a continuous elastic layer, closely apposed to the endothelium was observed. No modifications in medial or adventitial architecture were found.

Segment 2 and 3: the modifications found in these parts of the collaterals were rather similar, although more pronounced in 3 than in 2. Both showed definite alterations in a segment involving $^1/_3$ of the vessel in segment 2 to half the vessel in segment 3. Endothelium and elastic lamellae looked well preserved, although intimal diapedesis was frequent. Abnormally large cells filled the media and some of them were found in mitosis (Fig. 1).

A more pronounced mitotic activity was shown in cells occupying the broad adventitia. Leucocytes, probably infiltrating from veins and venules situated in the vicinity of the artery, were abundant in this zone.



1 2

Figs. 1–3 and 5–8 are from experimental animals

Fig. 1. Altered portion of a vessel from segment 2. No signs of damage in the endothelium. The media is filled with abnormally large cells, some of which are heavily vacuolated. Note the mitosis in the media (*M*). Several polymorphonuclear leucocytes are present in the broad adventitia. Hematoxylin-Eosin ($\times$ 525)

Fig. 2. Concentric injury in a collateral at segment 4. Endothelial ruptures are prominent at spots where the vessel wall is very thin (arrows). In the disorganized media, blood-borne cells are abundantly present. Hematoxylin-Eosin ($\times$ 135)

Segment 4: It was generally observed that this segment, which had the smallest radius and the thinnest wall, presented the most striking morphologic alterations. The foci of damage were concentric and occupied the total wall thickness, but were still more pronounced in one large area (Fig. 2). In some foci discontinuity in the endothelial lining was suspected and fragmentation of the elastic lamellae was seen with the Weigert stain. Infiltration of cells belonging to the blood system was the prominent feature in this area. The tunica media looked completely disorganized with few remaining smooth muscle cells and abundant clusters of erythrocytes and leucocytes. In the most altered region one hardly could detect any medial smooth muscle cell among the dominant inflammatory cells. Intimal thickening never occurred. No mitotic ativity was seen in segment 4.

Electron Microscopy

Control Animals. The ultrastructural morphology of a vessel corresponding to segment 4 is illustrated in Fig. 4. The appearance of flat elongated intimal and medial cells is a consequence of the perfusion fixation under a pressure of 100 mm Hg.

Experimental Animals. Segment 1 and 5: no abnormalities in ultrastructure were seen.

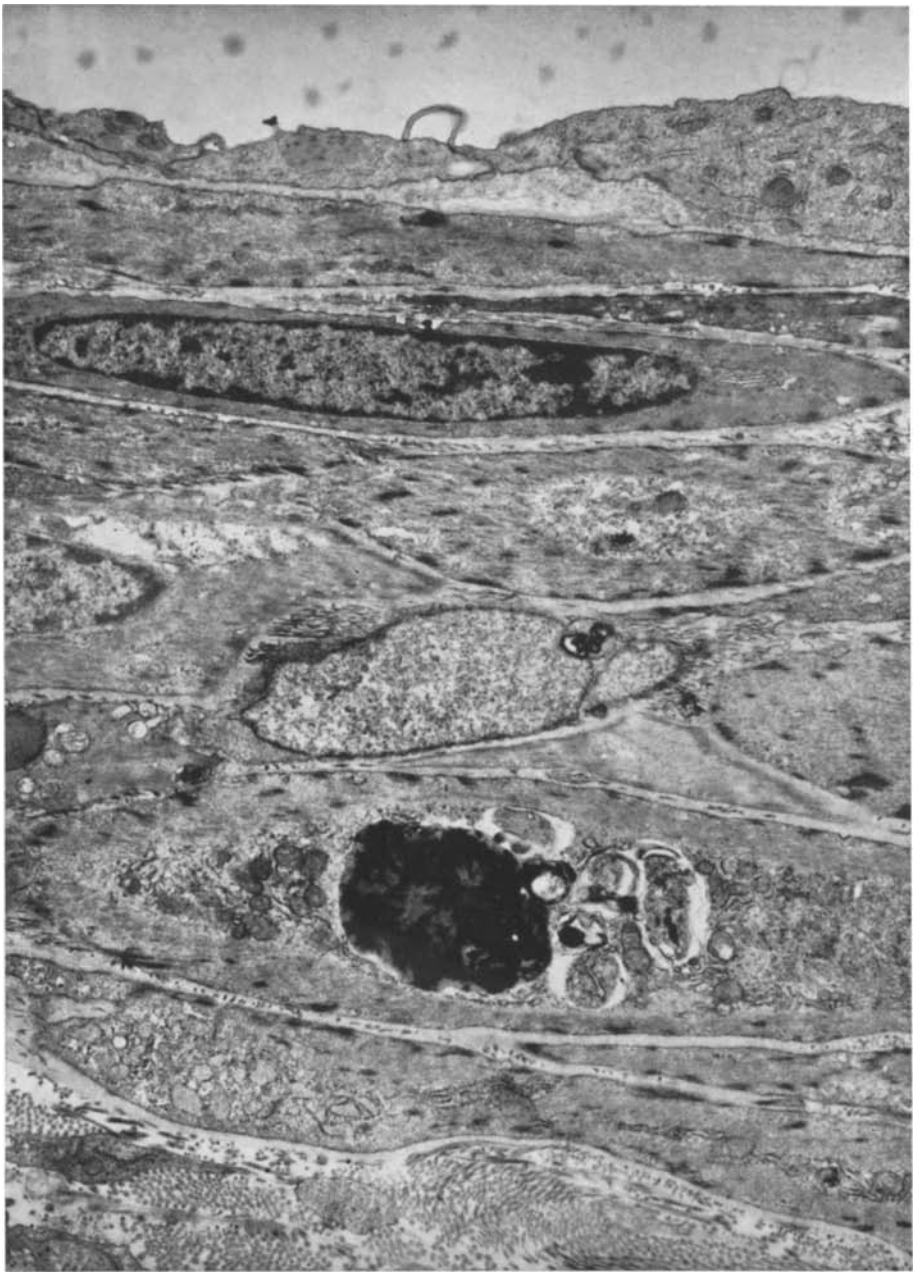


Fig. 3. Segment 2. Medial smooth muscle cells showing various degrees of alteration, such as displacement of myofibrils, swelling of cytoplasmic organelles, pyknosis and formation of autophagic vacuoles ($\times$ 7,700)

Segment 2 and 3: the endothelial cells and the elastic lamellae seemed intact. Only a few leucocytes were found localized between endothelial cells or interposed between endothelial and elastic layer. Focal morphologic differences in the media

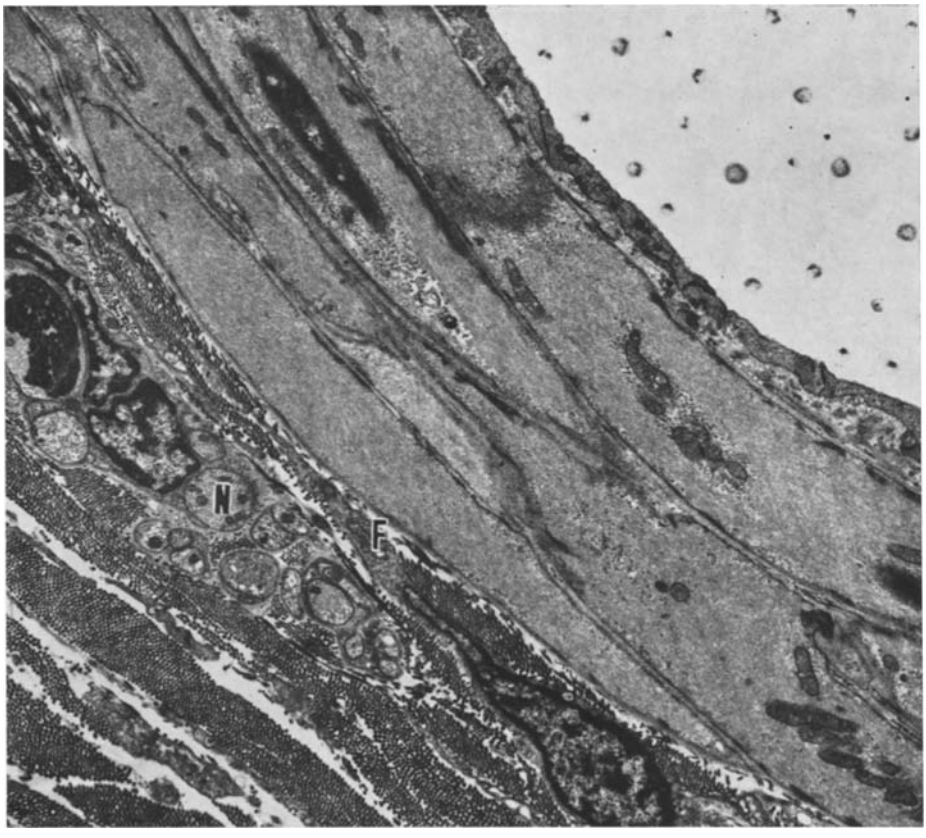


Fig. 4. Control. Electron micrograph of a vessel corresponding to segment 4. The flattened endothelial layer is followed by a small rim of well organized medial smooth muscle cells. A nerve cell (*N*) and a fibroblast (*F*) are embedded in abundant adventitial collagen (× 5,600)

and in the adventitia as found with the light microscope were clearly established revealing completely normal areas and markedly modified ones.

Ultrastructural modifications in medial smooth muscle cells were prominent varying from early changes such as dilatation of endoplasmic reticulum and lateral displacement of myofibrils to more severe alterations such as nucleolar disorganization, nuclear disintegration, cellular swelling, lack of myofibrils, formation of large autophagic vacuoles, fat deposition in the cell cytoplasm, and even complete cell necrosis (Fig. 3). Leucocytes and fibroblasts were found intermingled between the muscle layers. Mitotic activity in muscle cells was not encountered in the media. In the broad adventitia numerous very active fibroblasts, frequently in mitosis and infiltrating leucocytes, most of them neutrophils, were present.

Segment 4: in the best preserved part of this vessel segment the endothelium consisted of continuously arranged but enlarged cells, frequently multinucleated and filled with numerous strands of ergastoplasmic reticulum and a very active Golgi apparatus. Basement membranes and fragments of elastic material were still present. A small rim of smooth muscle cells was prominent in the media, but they were all in a more or less necrotic stage.

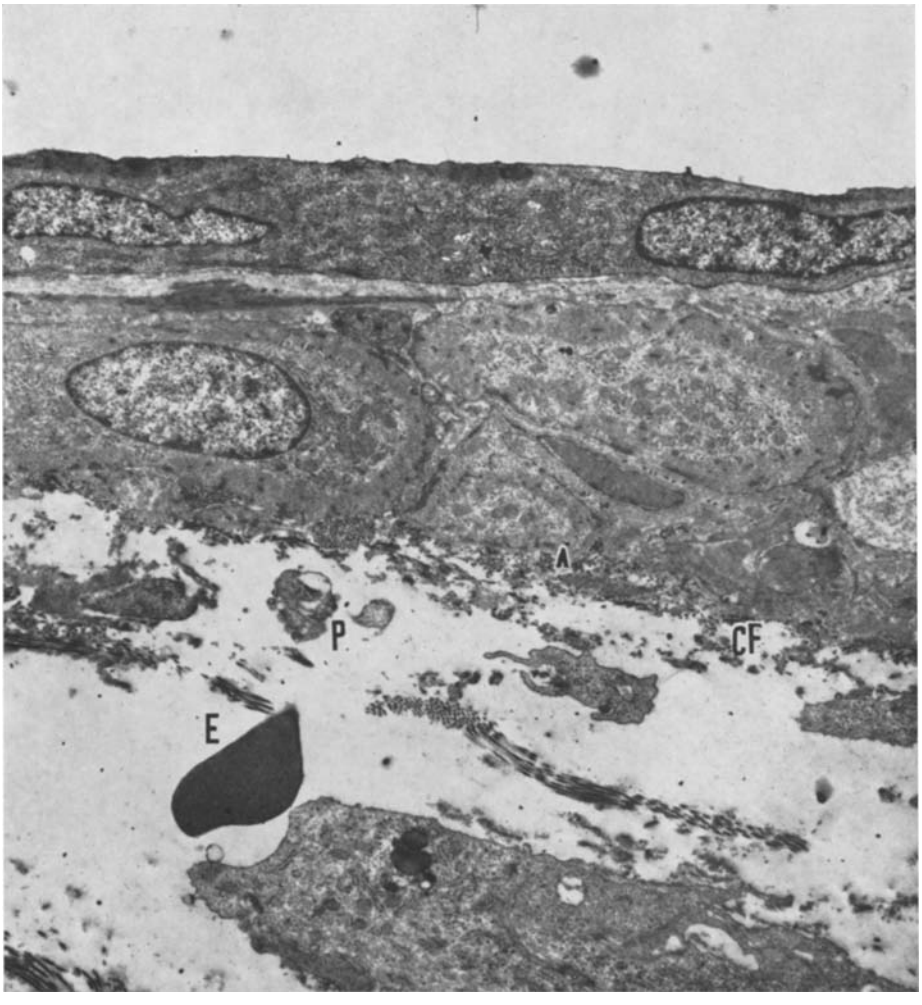


Fig. 5. Segment 4. Less injured area. A multinucleated "giant" endothelial cell rests on a well preserved basement membrane and elastic lamellae. A small rim of modified, but still organized, smooth muscle cells is closely apposed to the intima. Platelets (*P*), erythrocytes (*E*) and some necrotic cell fragments (*CF*) are seen in the media and in the adventitia (× 6,300)

Between these still identifiable smooth muscle cells, fragments of totally necrotic cells, erythrocytes and platelets were seen (Fig. 5). In the heavily damaged area the extremely thin endothelial lining was ruptured at some spots. Platelets and erythrocytes were found frequently situated near these discontinuities. However, no denudation of the endothelium was observed. Near the disruptions, basement membranes and elastic material were absent (Fig. 6).

The cells encountered in the media were mainly cells infiltrating from the blood stream, largely platelets and erythrocytes and various kinds of leucocytes (Figs. 7 and 8). Some heavily vacuolated smooth muscle cells and portions of

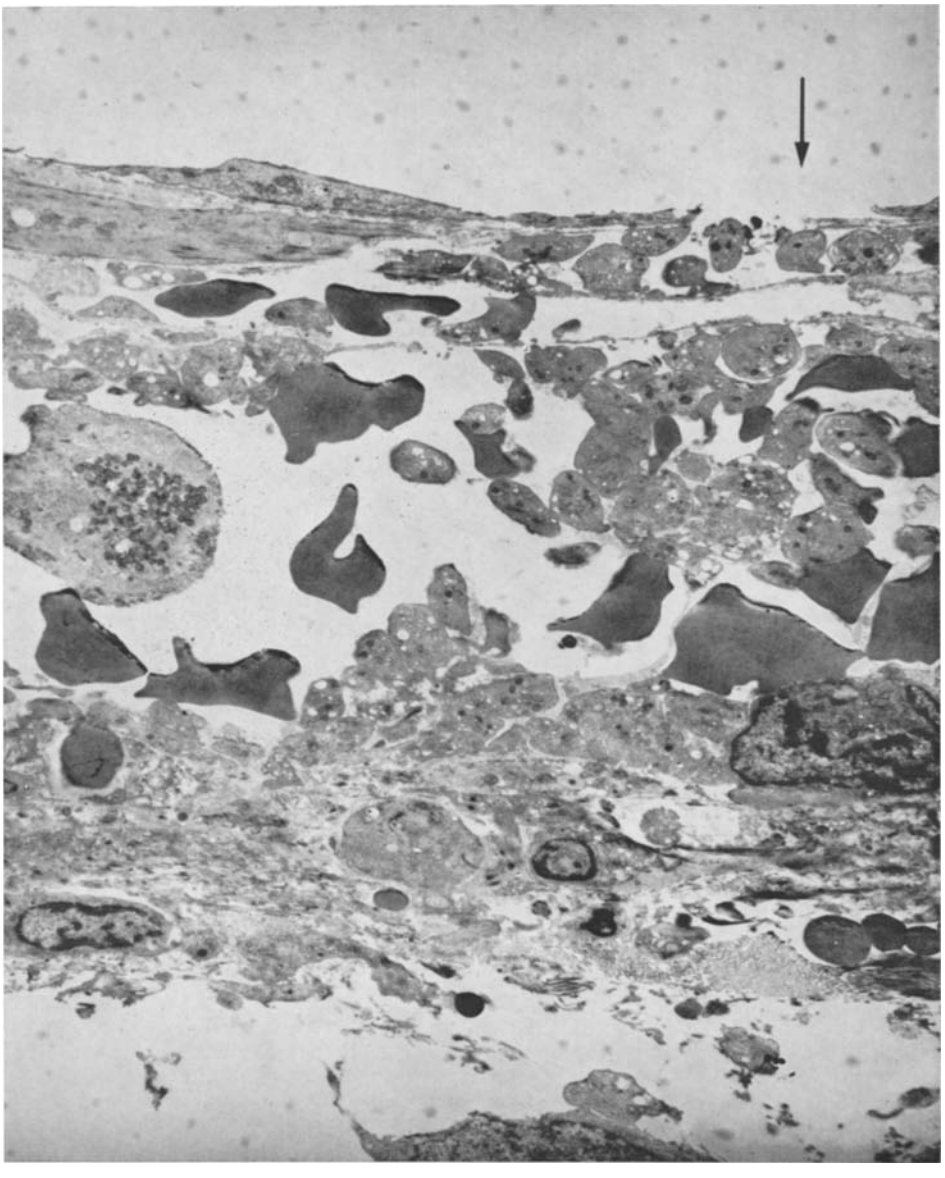
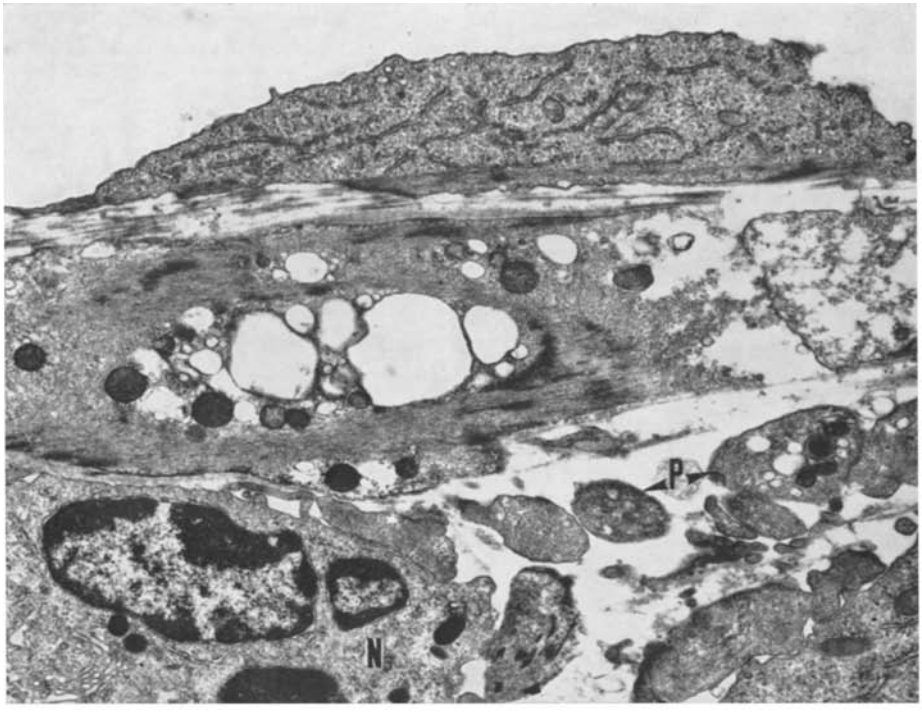
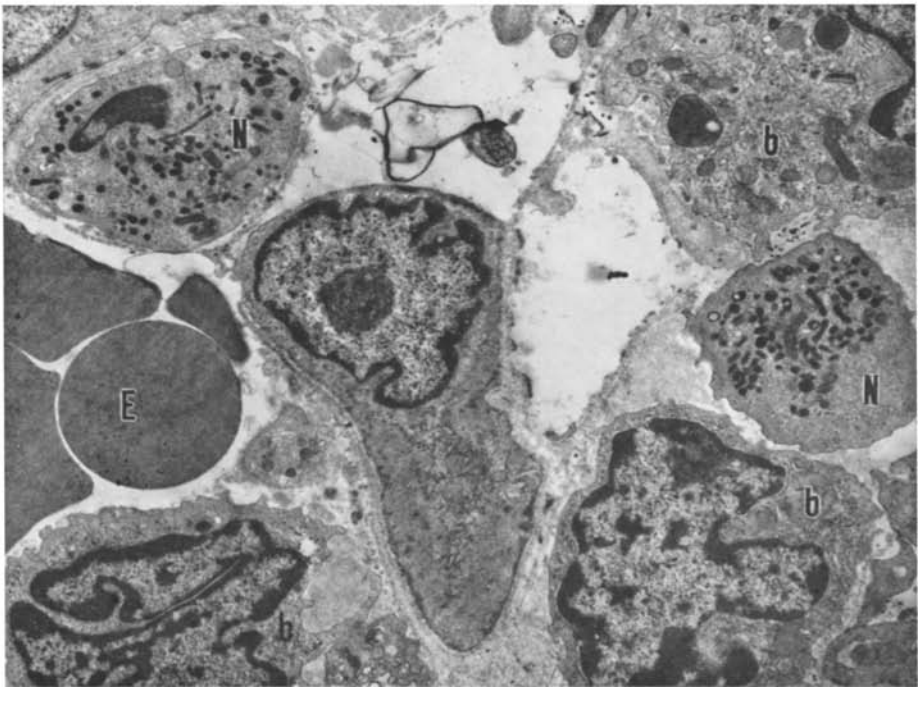


Fig. 6. Segment 4. Markedly damaged zone. Rupture of endothelial lining (arrow). Note the absence of basement membrane and elastic material. Platelets and erythrocytes are situated close to the disruption. In the edematous media only a few pyknotic muscle cells remain between the mass of blood cells. There is no definite boundary between media and adventitia ($\times$ 2,300)

necrotic smooth muscle cell were still present (Fig. 7). One could hardly discern the boundary between the tunica media and the tunica adventia where fibroblasts, collagen and mucopolysaccharide substances were rarely seen (Fig. 6).



7



8

Figs. 7 and 8. Same area as Fig. 6. Details of abnormal endothelial cell, vacuolated smooth muscle cells and different kinds of blood cells which occupy the whole vessel wall (*N* neutrophil, *b* basophil; *P* platelet; *E* erythrocyte). (Fig. 7 × 10,600; Fig. 8: × 7,700)

Discussion

The morphologic transformation in the early development of collateral coronary arteries can be summarized as follows:

A variety of alterations, going from slight subcellular modifications to complete cell necrosis, has been observed.

These alterations can be classified topographically. The severity of injuries varies mainly with the vessel radius and wall thickness, the damage being most pronounced at spots where the smallest radius and wall thickness are noted.

Leakage of blood-borne elements through the arterial wall is prominent in the more altered zones.

Characteristic signs of repair, as invasion of leucocytes from the adventitial side and mitotic activity in the surrounding parts of the damaged zone are noted.

The signs of "damage and repair" as earlier stated by some of the authors (Schaper, Schaper, Xhonneux and Vandesteene, 1969) at the light microscopic level, are completely confirmed in this electron microscopic investigation.

The observation that the degenerative process is localized at sites of minimal wall thickness and radius is in agreement with the conception that damage is prokoved by greatly increased tangential wall stress (Schaper, 1967a; Hoff and Gottlob, 1967). Almost identical morphologic lesions have been described by Liebow in his experimentally induced hypertension studies on the pulmonary arteries (Liebow, 1967).

The eccentric nature of the lesions is poorly understood. The localization of the damaged foci is variable. No predilection for the myocardium or towards the pericardium is noted.

The early changes observed in the medial smooth muscle cells of segment 2, characterized by the abundance of ergastoplasmic reticulum, a voluminous Golgi apparatus, and the lateral displacement of myofibrils, recall the modified smooth muscle cells as described by Wissler (Wissler, 1968). They may represent formerly inactive cells changing into a proliferative state (Hoff and Gottlob, 1968).

These proliferative modifications together with the observed mitotic activity suggest a mechanism of repair, to fill the gaps left by the necrotic cells in the heavily damaged area.

Björkerud (Björkerud, 1969) found intense mitotic activity in the endothelium and smooth muscle cells surrounding the injured areas after mechanical trauma of the rabbit aorta.

The most active proliferation in our study is in the adventitia where mitosis of fibroblasts frequently occurred. Mitotic activity of endothelial cells as found in the rabbit aorta following mechanical trauma (Spaet and Lejnieks, 1967) and in experiments related to the present study (Schaper, 1967) has not been found at the ultrastructural level. Nevertheless, giant multinucleated endothelial cells are frequently observed. As suggested by Gottlob and Zinner and by Hoff and Gottlob they could be produced by cells undergoing nuclear, but no cytoplasmic division (Gottlob and Zinner, 1962; Hoff and Gottlob, 1968).

Diapedesis of leucocytes is a common response to various kinds of physically and chemically provoked injuries in blood vessels, mostly veins and venules, as studied following thermal injury (Cotran and Remensnyder, 1968) and after

mechanical irritation (Robertson, Moore and Mersereau, 1959; Stehbens, 1965; Ts'ao and Spaet, 1967).

In the present study the various types of leucocytes and platelets normally present in canine blood are encountered in the vessel wall. The ultrastructural details of these blood cells were demonstrated by Shively, Feldt and Davis (1969).

The assumption that leucocytes enter through gaps between endothelial cells is strongly supported by their subendothelial position at spots where the elastic lamellae seemed wel preserved. Another way of entry is represented by the definite breaks in the endothelial lining. The absence of elastic and mucopolysaccharide material in the intima at the heavily injured spots and the position of platelets and erythrocytes close to the rupture suggest further that this is a free passage way for blood-borne elements.

The possibility should be considered that these breaks are artifacts of the perfusion fixation. This seems unlikely, however, since the pressure at which the fixative is introduced into the circulation never exceeded the normal blood pressure of the dog. The intramural localization of leucocytes in venules and veins near the adventitia and their abundance in the adventitia strengthen the belief that leucocytes penetrate the vessel wall from both sides. The adventitial entry could indicate a supplemental defense reaction for clearance of plasma proteins and necrotic cellular elements. Diapedesis in the aortic wall was observed following double ligation of the rabbit carotid artery and hypertension (Hackensellner, David and Uerlings, 1965; Still, 1967).

In previous investigations, Schaper et al. came to the conclusion that not all of the developing collaterals survived (Schaper, Schaper, Xhonneux and Vandesteene, 1969). Our opinion now is that collaterals, so heavily injured as those investigated in this paper, could be those which degenerate completely.

Acknowledgements. The authors are greatly indebted to Mr. R. Xhonneux and Mr. P. Van Even for their cooperation.

References

Björkerud, S.: Reaction of the aortic wall of the rabbit after superficial, longitudinal, mechanical trauma. Virchows Arch. Abt. A Path. Anat. **347**, 197–210 (1969).

Buck, R. C.: Lesions in the rabbit aorta produced by feeding a high cholesterol diet followed by a normal diet. An electron microscopic study. Brit. J. exp. Pathol. **43**, 236–240 (1962)

Cotran, R., Remensnyder, J.: The structural basis of increased vascular permeability after graded thermal injury. Light and electron microscopic studies. Ann. N.Y. Acad. Sci. **150**, 495–509 (1968).

Fahimi, H. D., Drochmans, P.: Essais de standardisation de la fixation au glutaraldehyde. Purification et détermination de la concentration du glutaraldehyde. J. Microsc. **4**, 725–736 (1965).

Gardner, D. L., Matthews, M. A.: Ultrastructure of the small arteries in early experimental rat hypertension. J. Path. Bact. **97**, 51–62 (1969).

Gottlob, R., Zinner, G.: Über die Regeneration geschädigter Endothelien nach hartem und weichem Trauma. Virchows Arch. path. Anat. **336**, 16–32 (1962).

Hackensellner, H. A., David, H., Uerlings, I.: Licht- und elektronenmikroskopische Untersuchungen an doppelt ligierten Arterien (A. carotis des Kaninchen). Acta biol. med. germ. **14**, 34–51 (1965).

Hoff, H. F., Gottlob, R.: A fine structure study of injury to the endothelial cells of the rabbit abdominal aorta by various stimuli. Angiology **18**, 440–451 (1967).
— — Ultrastructural changes of large rabbit blood vessels following mild mechanical trauma. Virchows Arch. Abt. A Path. Anat. **345**, 93–106 (1968).
Liebow, A. A.: Arteriosclerosis of the pulmonary circulation. In: Cowdry's arteriosclerosis, Chapt. 14, p. 248–328, ed. H. T. Blumenthal. Springfield, Illinois: Publ. C. C. Thomas 1967.
Litvak, J., Siderides, L. E., Vineberg, A. M.: The experimental production of coronary artery insufficiency and occlusion. Amer. Heart J. **53**, 505–518 (1957).
Robertson, H. R., Moore, J. R., Merserau, W. A.: Observations on thrombosis and endothelial repair following application of external pressure to a vein. Canad. J. Surg. **3**, 5–16 (1959).
Schaper, J., Borgers, M., Schaper, W.: Coronary collateral vessels. An electron microscopic study (In press).
Schaper, W. K. A.: Der Einfluß physikalischer Faktoren auf das Radialwachstum von Kollateralgefäßen im Koronarkreislauf. Verh. deutsch. Ges. Kreisl.-Forsch. **32**, 282 (1966).
Schaper, W.: The collateral circulation in the canine coronary system. Thesis, University of Louvain, Belgium (1967).
— Tangential wall stress as a molding force in the development of collateral vessels in the canine heart. Experientia (Basel) **23**, 595–596 (1967).
— Schaper, J., Xhonneux, R., Vandesteene, R.: The morphology of intercoronary anastomoses in chronic coronary artery occlusion. Cardiovasc. Res. **3**, 315–323 (1969).
Shively, J. N., Feldt, C., Davis, D.: Fine structure of formed elements in canine blood. Amer. J. Vet. Res. **30**, 883–905 (1969).
Spaet, T., Lejnieks, I.: Mitotic activity of the rabbit blood vessels. Proc. Soc. exp. Biol. (N.Y.) **125**, 1197–1201 (1967).
Spurlock, B. O., Skinner, M. S., Kattine, A. H.: A simple rapid method for staining epoxy-embedded specimens for light microscopy with the polychromatic stain paragon-1301. Amer. J. clin. Path. **46**, 252 (1966).
Stehbens, W.: Reaction of venous endothelium to injury. Lab. Invest. **14**, 449–458 (1965).
Still, W. J.: An early effect of hypertension on the aortic intima of the rat. Amer. J. Path. **51**, 721–734 (1967).
Ts'ao, C., Spaet, T. H.: Ultramicroscopic changes in the rabbit inferior vena cava following partional constrictions. Amer. J. Path. **51**, 789–813 (1967).
Wissler, R. W.: The arterial medial cell, smooth muscle or multifunctional mesenchyme? J. Atheroscler. Res. **8**, 201–213 (1968).

M. Borgers
Janssen Pharmaceutica
Research Laboratoria
Beerse, Belgium