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Vascular Lesions Following Perfusion with Bleomycin

Electron-Microscopic Observations

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Summary. During intra-arterial perfusion therapy of oral squamous cell carcinomas with bleomycin, circumscribed necrotic areas in the perfused region are observed in a few cases. These are usually located in the acral regions. As the cause, a lesion of the blood vessels with formation of microthrombosis could be demonstrated.

Electron-microscopic observations of the blood vessels in five bleomycin-perfused tumor areas demonstrated lesions of the endothelium characterized by swelling of the cells, formation of intracytoplasmatic vacuoles and villous projections into the lumen of the vessel. In arterioles, separation of the endothelium from the underlying tissue, swollen smooth muscle cells and destruction of elastic lamellae were found. These were the pacemakers for the formation of thrombosis. A negative influence of the vascular lesions on the cytostatic effect on the tumor is likely. Vascular lesions also constitute one of the initial factors for the development of the bleomycin-induced lung lesion (bleomycin lung).

Key words: Vascular lesion – Bleomycin – Cytostatic therapy – Intraarterial perfusion.

Zusammenfassung. Eine bisher wenig beobachtete Nebenwirkung der BLM-Perfusionstherapie von oralen Plattenepithelcarcinomen sind umschriebene Nekrosezonen an den Akren. Als Ursache ließ sich ein Gefäßschaden mit Thrombenbildung in den Arteriolen nachweisen. Die elektronenmikroskopischen Beobachtungen an 5 mit BLM perfundierten Tumorarealen und Gefäßen wiesen einen Endothelschaden mit Schwellung und vakuoliger Degeneration der Endothelzellen nach. An den Arteriolen kam es zur Ablösung von Endothelien, Schwellung der glatten Muskelfasern und Zerstörung der elastischen Lamellen. Dies bildet den Boden für eine Mikrothrombosierung. Ein negativer Einfluß des Gefäßschadens auf die Wirkung der Cytostatikatherapie wird diskutiert. Gefäßschäden im Bereich der Lungenstrombahn werden als eine Teilursache der multifaktoriell bedingten Bleomycinlunge angesehen.

Introduction

Bleomycin has proven to be a potent antineoplastic agent in the treatment of squamous cell carcinoma (Mathé, 1970; Takeda et al., 1970). In oral squamous cell carcinomas intraarterial application of the drug further enhances the therapeutic effect (Huntington et al., 1973; Bitter, 1974; Burkhardt and Höltje, 1975; Höltje et al., 1977). Recently Höltje and Burkhardt (1976) described a side-effect of this treatment hitherto unreported. They observed five cases with circumscribed necrotic areas in the perfused area. These were located at the concha, the cheek, the forehead, and the tip of the nose. In one case, necrosis of the perfused half of the tongue was observed (Burkhardt and Höltje, 1975). Histologic examination demonstrated thrombotic occlusions of the smaller arterioles in the respective areas. Further observations in the perfused tumor areas of 20 patients (biopsies and autopsies) showed that more or less severe vascular lesions were present in all cases.

These consisted of circumscribed reactive fibroelastosis of the lamina intima and thrombotic occlusions of various stages, some recanalized. The lamina media, too, was thickened and showed destruction of the elastic lamellae. This was mostly found in cases with additional radiation.

To reach a more thorough understanding and definition of this bleomycin-induced vascular lesion an electron-microscopic study was undertaken.

Material and Methods

In five patients with squamous cell carcinoma of the oral cavity the tumor area was surgically removed following a intra-arterial perfusion with bleomycin. The clinical data and drug doses are presented in Table 1.

The tissue was processed for electron microscopy according to the method specified by Luft (1971a, b). After dehydration in a graded series of alcohol, embedding was done in Epon 812. In semithin sections stained by toluidine blue appropriate areas were selected. Ultrathin sections were contrasted with alcoholic uranylacetate. Analysis was done with the Zeiss Elektronenmikroskop Em 9 S-2.

Table 1

	Case 1	Case 2	Case 3	Case 4	Case 5
Age/Sex	55 M	66 M	87 F	55 F	55 M
Clinical size of tumour	$T_3N_{2b}M_o$	$T_2N_{1b}M_o$	$T_3N_oM_o$	$T_2N_{1b}M_o$	$T_3N_oM_o$
Tumour location	Anterior floor of mouth	Right cheek	Right sulcus glosso-alveolaris	Left cheek	Right cheek
Entry of perfusion catheter	A. thyreoidea dextra et sinistra	A. thyroidea dextra	A. temporalis dextra	A. temporalis sinistra	A. thyreoidea dextra
Bleomycin dose	225 mg	310 mg	420 mg	465 mg	510 mg
Radiation, other antineoplastic agents	1800 mg MTX	500 mg MTX radiation			

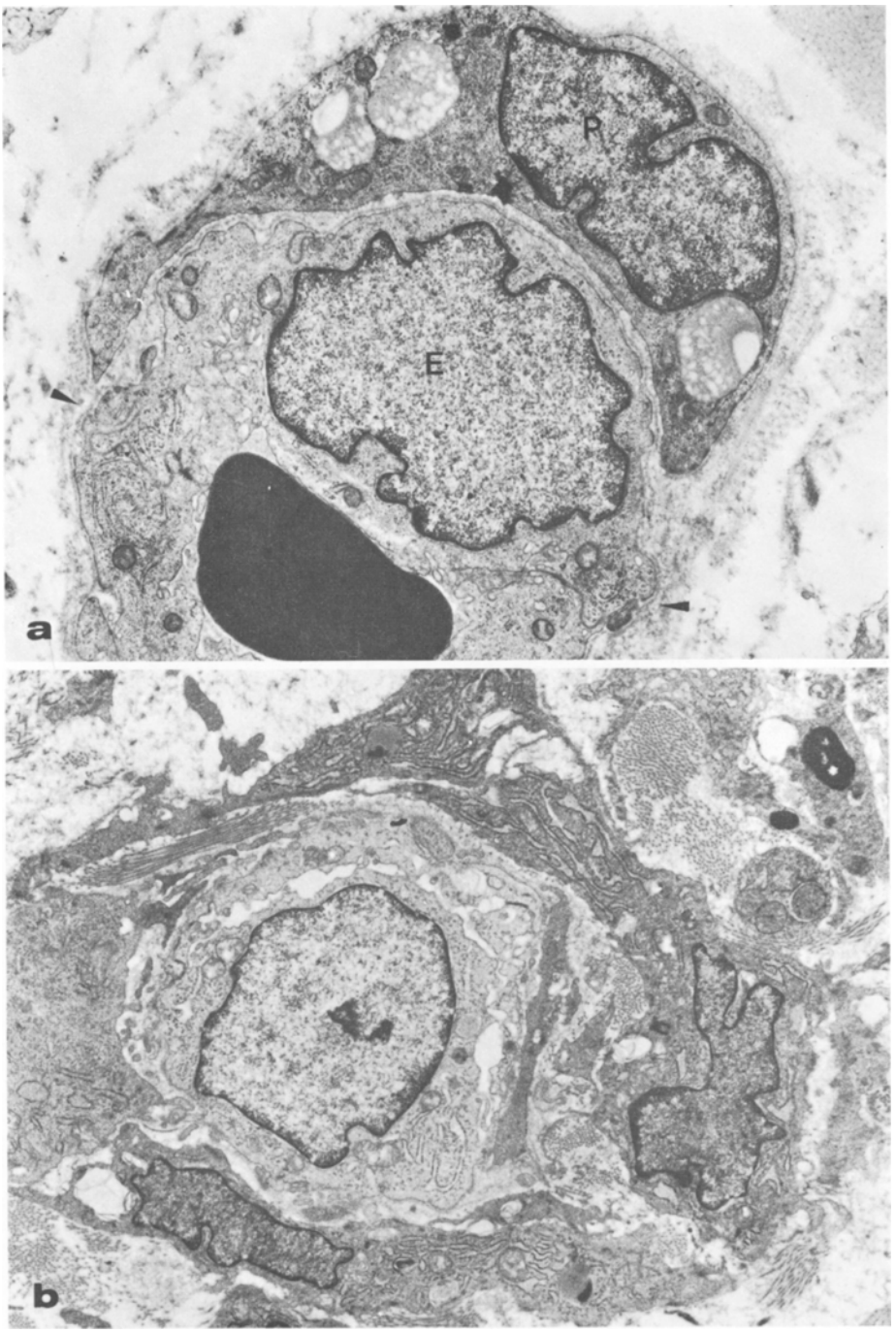

Fig. 1 a and b. Endothelial swelling of capillaries during bleomycin perfusion. **a** Swollen and activated endothelial cell (*E*) and vacuolated pericyte (*P*) with erythrocyte plugging. Note: intact cellular junction (*arrows*). Case 4; ×9000. **b** Subtotally occluded capillary, *L* Lumen. Case 3; ×5900

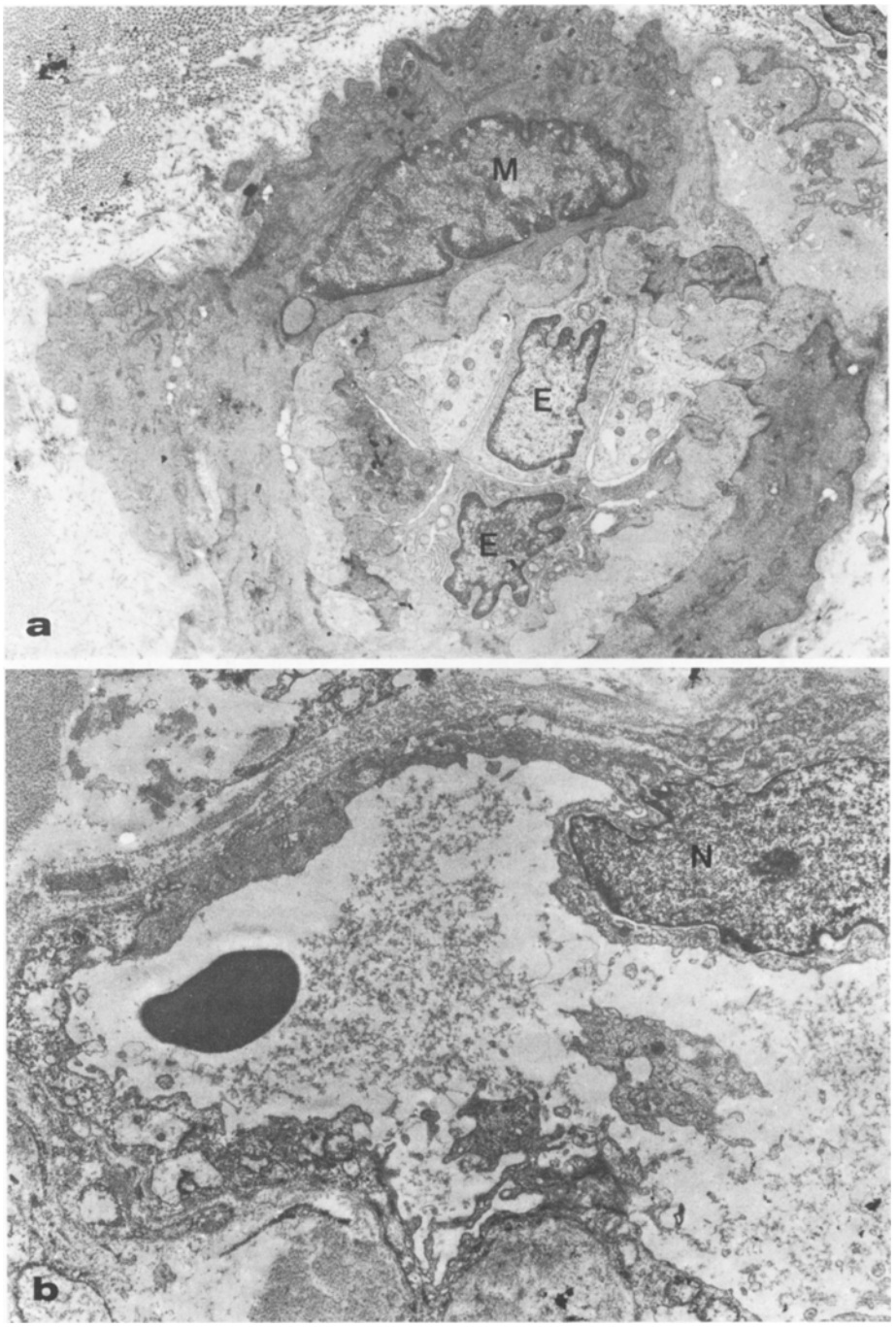

Fig. 2a and b. Endothelial swelling and damage during bleomycin perfusion. **a** Occluded metarteriole, *M* smooth muscle cell, *E* Endothelial cell. Case 3; ×5700. **b** Venular capillary with severe endothelial damage: vacuolization, luminal cytoplasmatic projections and evisceration of endothelial cell. *N* Nucleus of endothelial cell. Case 5; ×6000

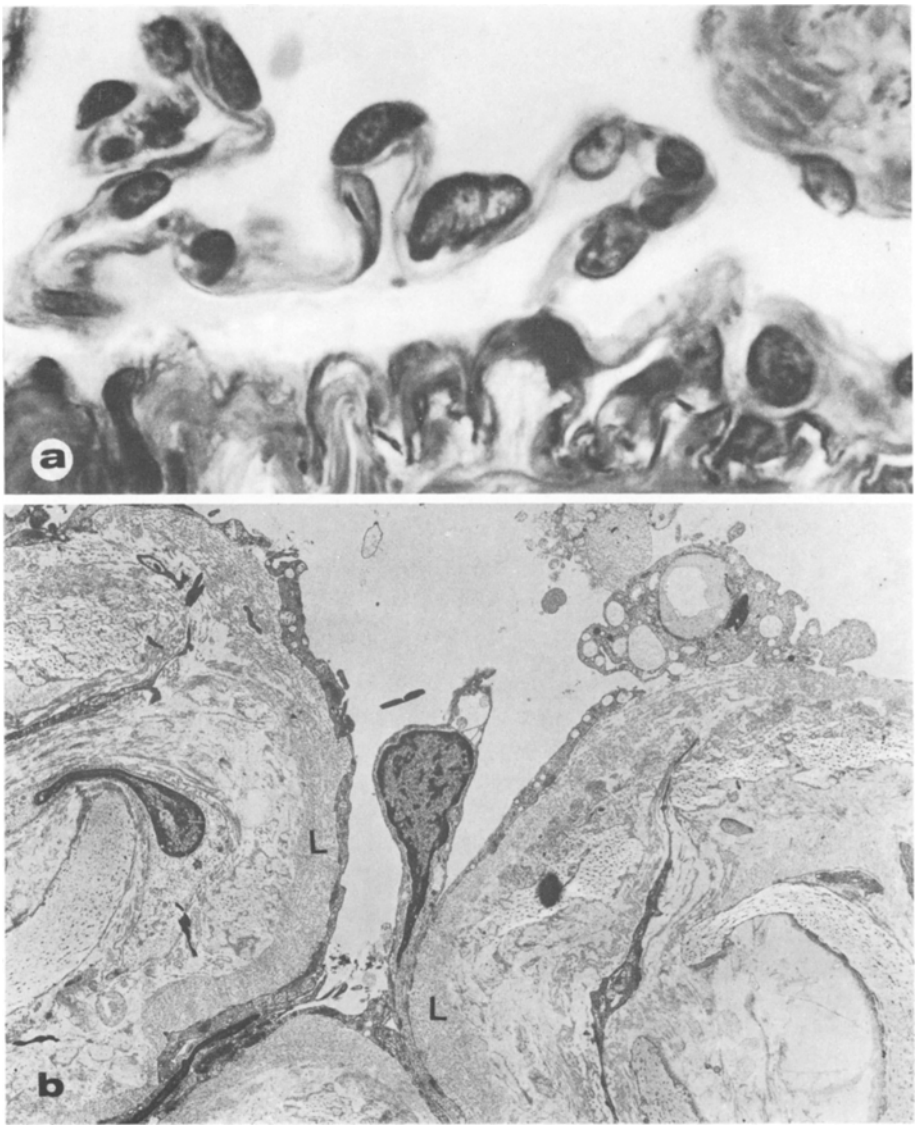

Fig. 3a and b. Lesion of small arterioles during bleomycin perfusion. **a** Detachment of endothelial lining. Case 4; Semithin section, toluidine blue, ×1200. **b** Vacuolisation, detachment and evisceration of endothelial cells, *L* Lamina elastica interna. Case 4; ×3800

Results

The most marked alteration of the blood vessels is located in the endothelium. The endothelial cells are swollen (Figs. 1, 2a, 4a). The lumina of the capillaries are compressed by swollen endothelial cells and pericytes. Often, subtotal occlusion with plugging of erythrocytes is seen (Fig. 1a, b). Depending on the degree of the damage, the endothelial cells exhibit an activation with increased or-

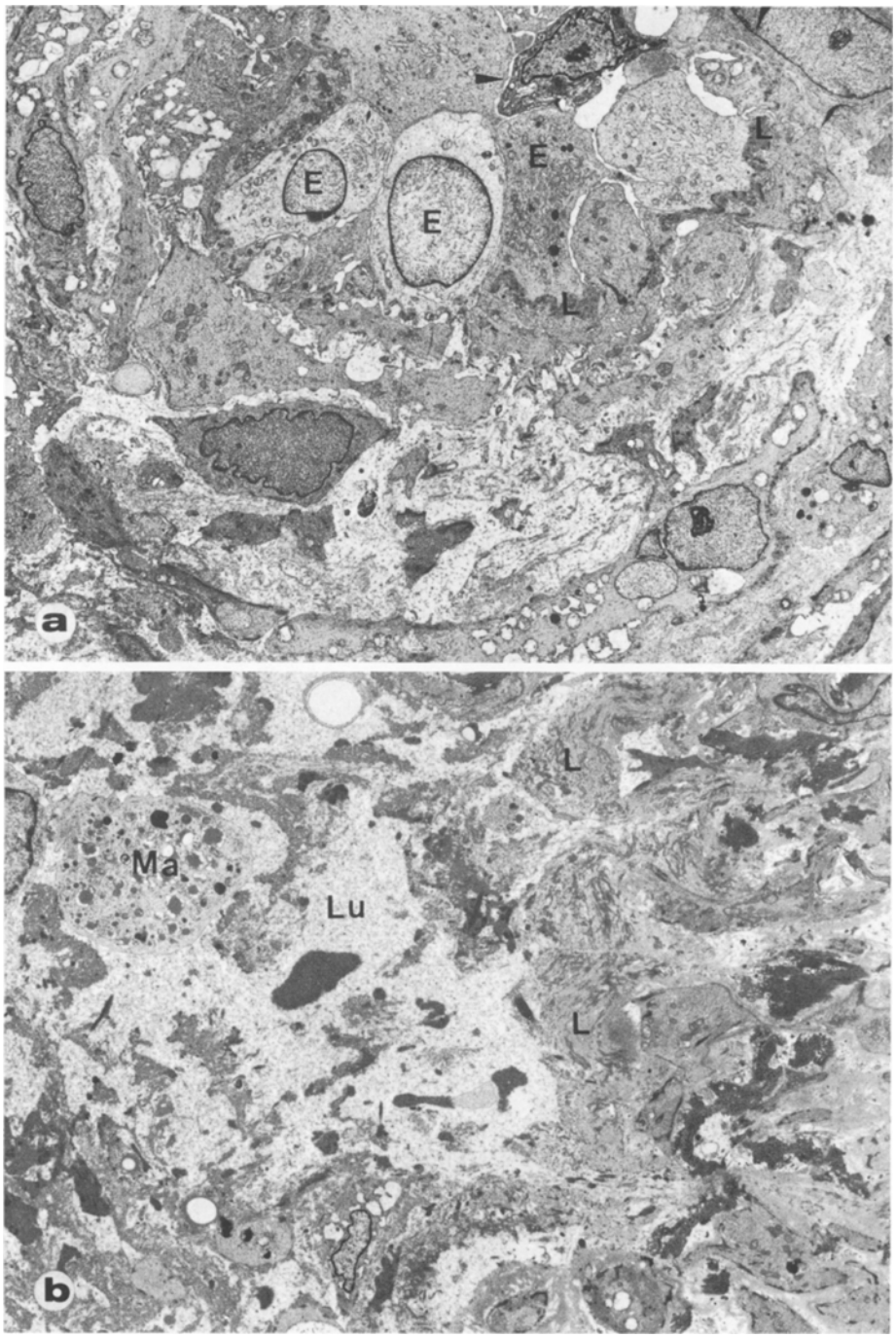

Fig. 4a and b. Occlusion of small arterioles during bleomycin perfusion. **a** Swollen endothelial cells (*E*) with degenerative changes (*arrow*), edematous disintegration of the vascular wall. *L* Lamina elastica interna. Case 4; ×2800. **b** Necrosis and disappearance of endothelial cells with cellular detritus and macrophage (*Ma*) in the former lumen (*Lu*). *L* Lamina elastica interna. Case 4; ×2800

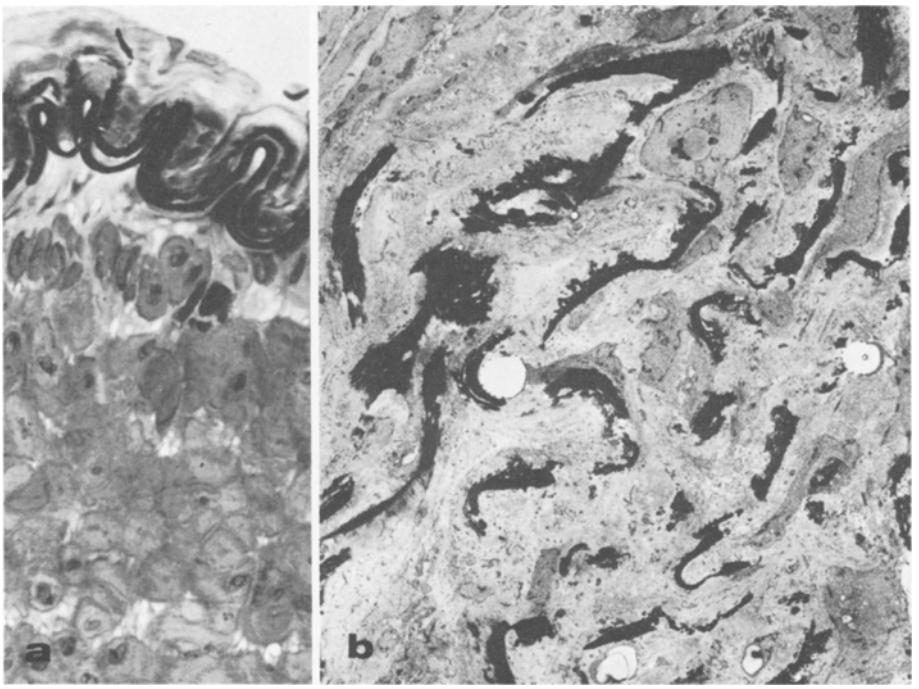

Fig. 5a and b. Changes in deeper layers of vascular wall of arterioles during bleomycin perfusion. **a** Swelling of smooth muscle cells (note palisade arrangement on top). Case 4; semithin section, toluidine blue; ×480. **b** Disintegration of elastic lamellae. Case 4; ×2800

ganelles and nuclear volume (Figs. 1a, b, 2a), or degenerative changes with swollen organelles, formation of intracytoplasmatic vacuoles, irregular luminal projections and detachment of cytoplasmatic particles ("evisceration", Fig. 2b). The intercellular junctions of the endothelial cells are intact, formation of gaps or clefts cannot be observed. The small arterioles show formation of subendothelial blebs and detachment of the endothelium from the internal elastic lamina together with severe degenerative changes and necrosis of the endothelial cells (Fig. 3a, b). In advanced cases the lumina of the arterioles are occluded by swollen and necrotic endothelial cells, show accumulation of cellular debris, invasion of macrophages, and thrombosis (Fig. 4a, b).

The other constituents of the arteriolar wall show edema and swollen smooth muscle cells. These are sometimes arranged in a palisade-like pattern (Fig. 5a). The elastic lamellae are occasionally disrupted (Fig. 5b) and are taken up by macrophages.

Discussion

The focal, well-demarcated nature of the necroses during bleomycin therapy suggested a vascular pathogenesis. Histologic and electron-microscopic studies confirmed a vascular lesion, which was predominantly located in the endothe-

lium. Hagedorn et al. (1974) described similar light-microscopic findings in a patient with a basal cell carcinoma of the leg treated with intra-arterial bleomycin perfusion. They found defects and vacuolization of the endothelium as well as thrombosis.

The morphologically demonstrable spectrum of vascular reaction to noxae of different kind is apparently limited. Thus the alterations described above conform in many details to the picture of a number of other noxae. Similar alterations could be demonstrated following ischemia and anoxia (Constantinides and Robinson, 1969a; Willms-Kretschmer and Majno, 1969; Armiger and Gavin, 1975; Kjeldsen and Thomsen, 1975), injury (Ashford and Freiman, 1967), perfusion of hypertonic solutions, chemical noxae, vasoactive substances and kinins (Hoff and Gottlob, 1967; Hoff et al., 1967; Burri and Weibel, 1968; Constantinides and Robinson, 1969a–c; Ts'ao, 1970), action of endotoxin and choleratoxin (Hoff et al., 1967; Hashimoto et al., 1974), allergic reaction (Dvorak et al., 1976), and radiation (Andres, 1963; Fajardo and Stewart, 1973; Sholley, 1973).

These consist of endothelial swelling, vacuolization, formation of luminal cytoplasmatic projections and finally necrosis and detachment of the endothelium.

In addition, some of the noxae mentioned above damage the intercellular junctions and lead to the formation of endothelial gaps. This is conspicious in histamine- and cadmium-chloride-induced lesions (Constantinides and Robinson, 1969b; Majno et al., 1969; Gabbiani et al., 1974; Dvorak et al., 1976). This kind of endothelial damage could not be demonstrated in bleomycin-induced lesions.

As part of the action of bleomycin on the tumor tissue seems to be associated with a stabilization of the desmosomal cellular junctions, the described lack of endothelial gaps is in accordance with this interpretation.

The bleomycin-induced vascular lesion is not confined to the capillaries. In the arterioles, alterations are found which are typical of a strong contraction of the vessel such as riding of the endothelial nuclei on the crests of the folds of the gyrated elastic lamellae, empty grooves, teardrop configuration of the endothelium and contraction of the smooth muscle cells (Altschul and Faul-Bochmler, 1963; Phelps and Luft, 1969). But in addition there are evident signs of endothelial lesions ranging from vacuolization to detachment and necrosis. This is the basis for the formation of thrombosis (Ashford and Freiman, 1967; Ts'ao, 1970), which as a consequence leads to infarction of the circulation area, as was observed clinically.

According to our observations, disintegration of the elastic lamellae in the wall of the arteries is promoted by additional radiation.

The vascular lesions during bleomycin therapy affect, without any doubt, the cytostatic effectiveness of the drug. Hagedorn et al. (1974) are of the opinion that the microvascular occlusions enhance the antitumoral action. Our observations, however, seem to indicate that this has a negative influence on tumor destruction. Accordingly, case 3, which was characterized by an advanced obliteration of the blood vessels, showed only minimal therapeutic effect. Bläsig et al. (1971), too, explain the reduced effect of bleomycin in pretreated, especially preradiated cases, with vascular obliteration in these cases.

Although the vascular damage is most extensive in the perfused vessels, there can be no doubt that this side-effect has a significance in other organs, too. Thus, it must be regarded as proven that the most severe complication of bleomycin therapy – the bleomycin-induced lung lesion – is caused partly by it. The increased concentration of bleomycin in lung tissue leads to an endothelial lesion with activation of blood coagulation and formation of microthrombosis (Bedrossian et al., 1973; Burkhardt et al., 1976). In 15 patients treated with bleomycin we could demonstrate microthrombosis in the terminal circulation of the lung at autopsy in six cases (Burkhardt et al., 1976). Luna et al. (1972) as well as Bedrossian et al. (1973) found an endothelial lesion in the terminal vessels of the lung. These findings could be confirmed by experimental results in mice (Adamson and Bowden, 1974; Gebbers et al., 1977).

This study makes it advisable to prevent part of the mentioned side-effects of bleomycin by simultaneous therapy with anticoagulant drugs. This will not alter the primary vascular lesions, but will reduce the formation of thrombosis and its consequences.

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