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Scanning and Transmission Electron Microscopy Observations on the Surface Lining of Aortic Intimal Plaques in Rabbits on a Hypercholesterolic Diet*

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Summary. In rabbits on a hypercholesterolic diet, the surface of the plaques, at scanning electron microscope examination, looks discontinuous even in specimens obtained with the "critical point" device. Discontinuities of the endothelial lining of the plaques are found also in ultrathin sections by transmission electron microscopy: foam cells do then constitute the superficial row. In proliferative lesions, smooth muscle cells are actively surfacing, reaching sometimes the lumen.

In previous works we have demonstrated, by means of scanning and transmission electron microscopy, alterations of the "polysaccharide containing coat" (surface carbohydrates after Cook and Stoddart, 1973) of the aortic endothelial cells during the early phases of atherogenesis in cholesterol fed rabbits (Weber *et al.*, 1973, 1973 in press; 1972a, 1973a, b, 1974 in press). Unfortunately information on endothelial lesions during atherogenesis is still highly incomplete and contradictory especially when one consideres that the role of smooth muscle cell hyperplasia in the histogenesis of the plaque is unanimously recognized and that importance is placed on its potential regulation by the endothelial cells (Geer and Haust, 1972; Jellinek, 1973; Björkerud, 1973).

By means of scanning electron microscopy we have repeatedly observed that, during experimental cholesterol atherogenesis in Guinea pigs and rabbits, the surface of the plaques (fatty streaks), from their first appearance may look uneven, cribriform and discontinuous (Weber *et al.*, 1972b; and Tosi, 1971, 1973). In order to ascertain the basis of this surface discontinuity shown by SEM examination, we have studied, by means of transmission electron microscopy, aortic blocks of rabbits on a hypercholesterolic diet. Ultrathin sections have been obtained from different peripheral and central portions of plaques as well as from interposed aortic areas macroscopically devoid of lesions.

Materials and Methods

10 rabbits fed a standard diet with 1% added cholesterol dissolved in ether were killed by bleeding after 15–35 days. 4 rabbits on normocholesterolic diets were used as controls. Small portions of aorta, fixed for two hours in 2.5% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2, coated with platinum gold (or copper-gold) alloy after vacuum dehydration, have been

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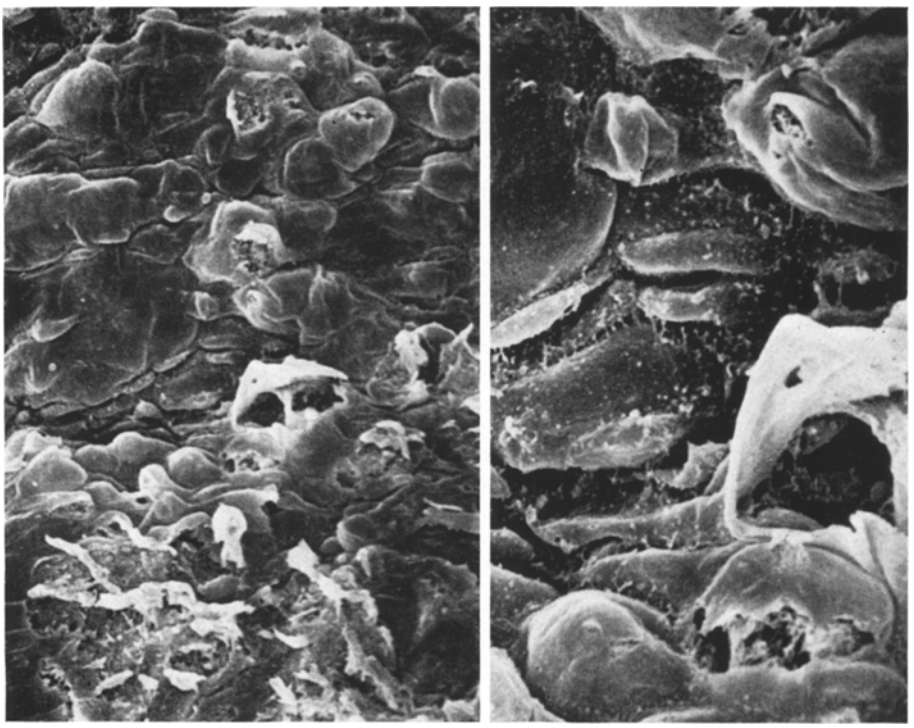

Fig. 1. Aortic intimal surface. Rabbit on a hypercholesterolic diet. The surface of the plaques appears discontinuous (specimen prepared with "critical point" device). On the right a magnification. SEM ×300, ×1000

observed with a scanning electron microscope JSM 2 at 25 Kv. Some specimens were subjected to the critical point technique (fixed tissue was dehydrated through alcohol, Freon 113, Freon 13 and dried in Freon at the critical point in the Bomar unit; the dried tissue was mounted on a copper holder, coated with evaporated gold-platinum and observed under the JSM 2). Adjacent aortic portions were prepared with the Concanavalin A method, highly specific for surface carbohydrates (after Bernhard and Avrameas, 1972) for TEM examination. The tissue was fixed in buffered glutaraldehyde, washed in buffer, postfixed in osmium tetroxide and embedded in araldite. Micrographs of ultrathin sections have been obtained with Philips EM300 and Siemens Elmiskop 1A, operated at 60 and 75 Kv respectively.

Results

Observations confirming previous work (recently reviewed by Geer and Haust, 1972, and Jellinek, 1973; Weber *et al.*, 1972b, 1973) will not be considered here. It may only be noted, that the surface of the plaques looks discontinuous, in our SEM observations, even in specimens prepared with the "critical point" device (Fig. 1). Our attention has been chiefly focused: 1) on the endothelial lining and its surface carbohydrates and 2) on the mobilization of the subendothelial smooth muscle cells.

1. (a) In the aortic areas interposed among the plaques and macroscopically devoid of lesions, the Concanavalin A reactivity of the endothelial lining, increased

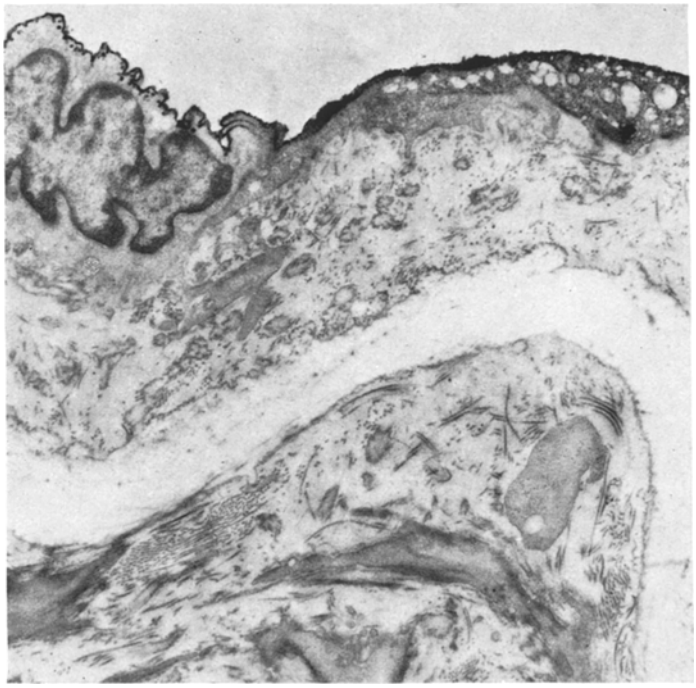

Fig. 2. Idem. A necrotic endothelial cell is still attached to the aortic wall in an area macroscopically devoid of lesions. The Concanavalin A reactivity is evident on a live, adjacent endothelial cell. TEM ×9800

at the 15th day, was afterwards still present, even if reduced in comparison with the rabbits on normocholesterolic diets. Plasmalemmal vesicles and vacuoles are evident in the endothelial cells, the nuclei of which are usually still well preserved.

(b) In smaller plaques and in the peripheral portions of the larger plaques (proliferative lesion, pre-atheroma) the reactivity of the endothelial surface to Concanavalin A was strongly diminished, even to the point of being scarcely demonstrable; sometimes it appeared completely lost, in which case a plasmatic deposit covered the "denuded" endothelial surface. The number of the pinocytotic vesicles was strongly reduced, often they were no longer visible; many vacuoles of large or even of huge dimensions were found in the endothelial cytoplasm. The nuclei were frequently very pale. Other endothelial cells, even in areas macroscopically devoid of lesions (Fig. 2), were degenerating, having completely lost their characteristics, as if they were necrotic (cfr. Still, 1963, and Daoud *et al.*, 1968) and about to detach: in many points, the loss of single endothelial cells had given place to an "endothelial ulcer" (Gutstein and Farrel, 1972; Gutstein and Parl, 1973; Gutstein *et al.*, 1973).

(c) In larger plaques, very few endothelial cells (if any) lined the luminal surface (Fig. 3). The Concanavalin A reactivity was scarcely demonstrable. Where the endothelial lining is lost, the surface appeared almost exclusively constituted

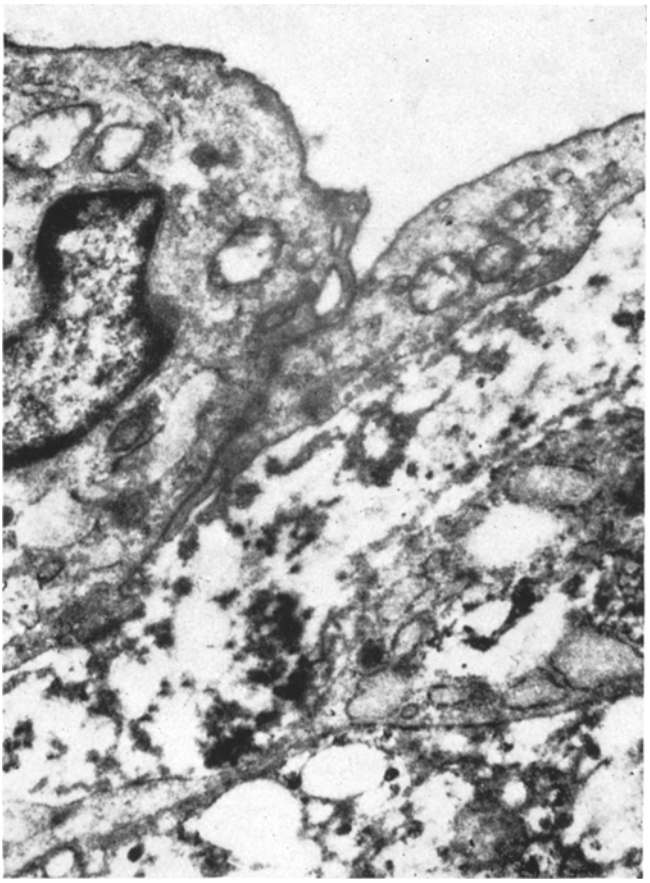

Fig. 3. Idem. In the middle portions of a plaque, in an area where the endothelial lining is present, the Concanavalin A reactivity is still (but scarcely) evident. TEM ×12700

by an irregular row of foam cells facing the lumen (cfr. Still, 1963). These were often partially covered by an acellular layer (chiefly collagen, fibrin and some cellular debris).

2. (a) In the aortic areas, interposed among the plaques, the occasional smooth muscle cells, present in the widened subendothelial space, were usually in a disposition roughly parallel to the endothelial surface and showed the well established characteristics of resting (myointimal) smooth muscle cells.

(b) In smaller plaques and in the peripheral portions of the larger ones, smooth muscle cells differed in certain features from resting ones as has been described in previous works (Geer and Haust, 1972; and Jellinek, 1973). We only wish to underline that, beneath the degenerating (necrotic) endothelial cells, subendothelial smooth muscle cells, surrounded by collagen and practically without lipid inclusions, often appeared with their axis perpendicular to the vessel surface. Finger-like projections similar to pseudopodia, emerged from that portion of their outline facing the endothelium. The finger-like projections of these smooth muscle

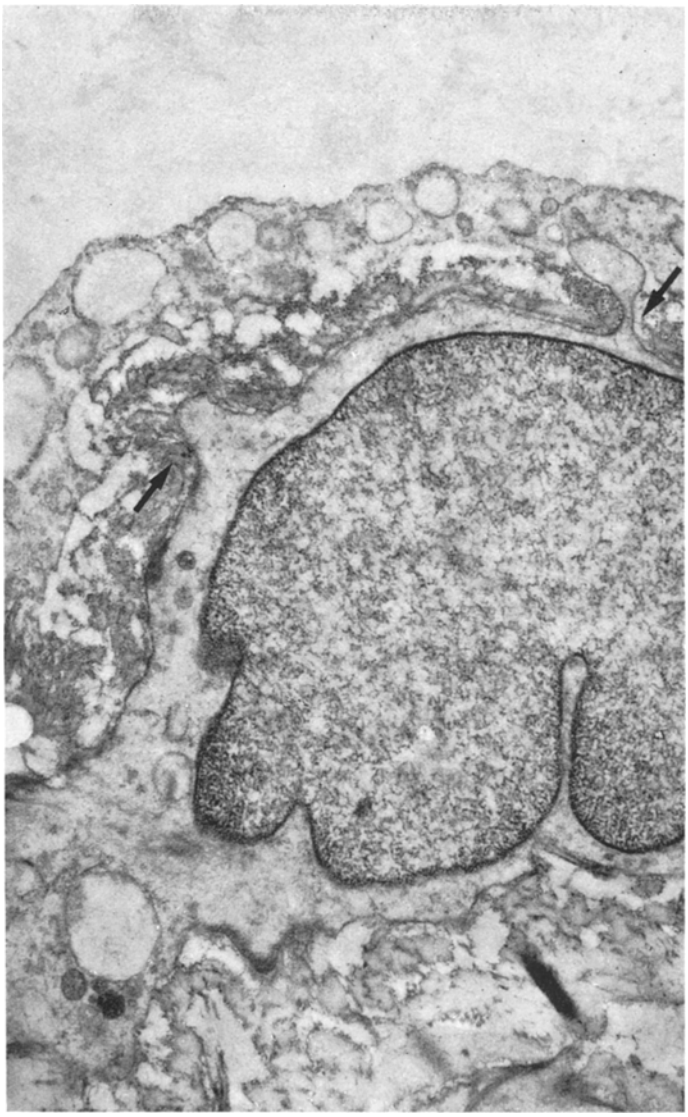

Fig. 4. Idem. In a proliferative lesion, a smooth muscle cell in the subendothelial space, surrounded by collagen, is penetrating, with its finger-like cytoplasmic projections, into (arrows) the overlying degenerating endothelial cell. TEM ×20000

cells sometimes reached the overlying endothelium, and even appeared to actively penetrate its cytoplasma (Fig. 4). Other smooth muscle cells still devoid of or containing only few lipid inclusions and showing evident cytoplasmic myofilaments, had reached an endothelial position, freely projecting into the lumen.

(c) In larger plaques, where the endothelial lining was largely lost, the aforementioned superficial foam cells displayed the characteristics of "myogenic foam

cells" (Geer and Haust, 1972; Jellinek, 1973). Most of them were clearly degenerating.

In synthesis: Our TEM observations on the surface lining of aorta and of aortic plaques in rabbits fed a hypercholesterolic diet have always shown, after an increase at the fifteenth day, a heavy decrease in Concanavalin A reaction at the luminal surface of endothelial cells, both at the periphery of the plaque and in the aortic areas among the plaques macroscopically devoid of lesions. Large discontinuities in the endothelial lining have been found chiefly in the central areas of the plaques. Concomitant with the active "surfacing" of smooth muscle cells, degeneration, necrosis and detachment of single endothelial cells may be observed in the peripheral proliferative areas. Our TEM observations disclose that the surface of many plaques, in rabbits on a hypercholesterolic diet, is practically devoid of any continuous endothelial lining. These findings may partially explain the surface discontinuities we had observed with scanning electron microscopy and may represent a feature of some relevance in explaining the increased aortic permeability (cfr. Dayton and Hashimoto, 1970) of hypercholesterolemic rabbits.

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