SCANNING ELECTRON-MICROSCOPIC STUDY OF THE ARCHITECTONICS OF THE FIBROUS SKELETON OF THE GREAT VESSEL WALLS

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Details are given of a method of processing fragments of human large arteries by proteolytic enzymes (protoryzin, trypsin) in order to reveal the fibrous skeleton of the vessel wall. A scanning electron-microscopic study of preparations obtained by this method reveals the arrangement and spatial relations of the connective-tissue bundles and fibers in the various layers of the vessel wall.

KEY WORDS: scanning electron microscopy; arteries; proteolytic enzymes; connective tissue.

In a morphological study of the structure of the walls of large arteries [3] the writers examined the possibility of detecting fibrous skeleton in the different layers of arteries of elastic type in order to analyze the architectonics of the fibrous structures and their interrelationships.

Some idea of the spatial arrangement and interrelationships of the connective-tissue fibers and bundles in blood vessel walls can be obtained by analysis of serial histological sections. The collagen-elastic skeleton of the vessels can be revealed by treating the native or formalin-fixed vessel wall with proteolytic enzymes. Such a skeleton, obtained by the action of pepsin [7], ficin [8-10], or trypsin [6] on the vessel wall, has been used as material for the plastic repair of blood vessels [5].

The method of scanning electron microscopy (SEM) has been used to study the surfaces of vascular prostheses and grafts and also to analyze the special features of thrombosis in them [2].

The object of this investigation was to determine whether the architectonics of the fibrous skeleton of the various layers of human arterial walls of elastic type can be studied with the scanning electron microscope.

EXPERIMENTAL METHOD

The test objects were 12 fragments of major arteries (the aorta, the common carotid and common iliac arteries) from middle-aged and old persons. The material was obtained during postmortem examinations. Fragments of the vessels without previous fixation were treated with proteolytic enzymes (trypsin or protoryzin).

Protoryzin is an enzyme obtained from a culture of the mold Aspergillus oryzae. It has no collagenase or elastase activity, but possesses chiefly amylolytic and low proteolytic activity [1, 4].

Blood vessels for investigation were prepared as follows. The chosen specimens were first weighed, then placed in tubes with phosphate buffer, pH 5.5. Protoryzin was added to the medium in an amount to give an enzyme/substrate ratio of 1:25. The vessels were incubated for 18 h at 37°C. The samples were then washed with tap water for 30 min. Next, after brief alcoholic fixation and dehydration the samples were sprayed with copper and their surface was examined in the Stereoscan 4S-10 scanning electron microscope.

When trypsin was used the vessels were treated in the same way, but the enzyme/substrate ratio was 1:10 and the pH of the medium was 7.6.

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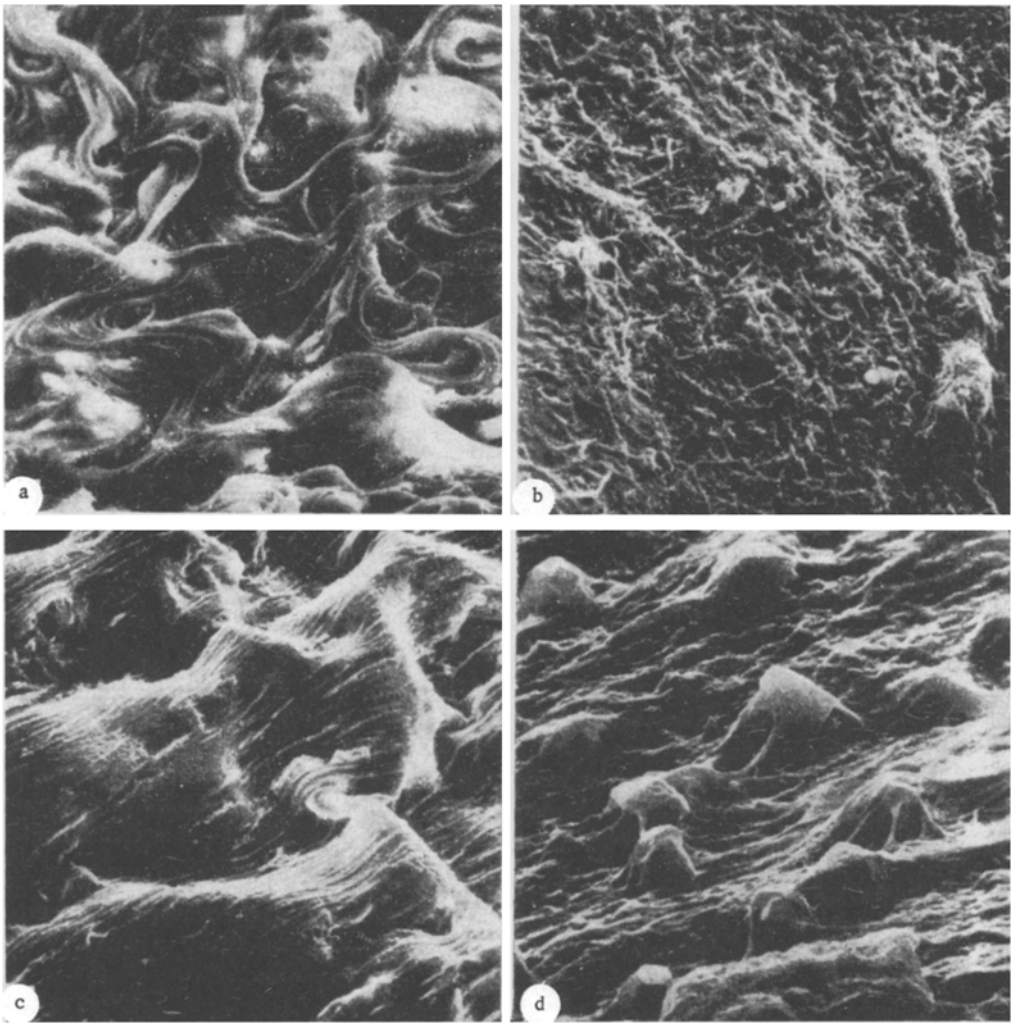

Fig. 1. Structure of fibrous skeleton in various layers of the wall of large arteries as revealed by SEM (fragments of vessels treated with protoryzin): a) large bundles of connective-tissue fibers in adventitia of common iliac artery (400 ×); b) small-loop connective-tissue network in subendothelial zone of intima of common iliac artery (800 ×); c) surface of fenestrated membrane in media of common iliac artery (800 ×); d) crystals of minerals in media of aorta from man aged 61 years (1600 ×).

Both whole pieces of the vessel wall and separate fragments of them obtained by separation of total preparations into layers were treated with protoryzin or trypsin. In the latter case the surfaces of the fenestrated membranes of the media as well as individual structural details of the fibrous skeleton of the other layers of the vessel could be detected.

EXPERIMENTAL RESULTS

The results indicate that treatment of the wall of large arteries with enzyme preparations enables the architectonics of bundles of connective-tissue fibers to be revealed by the SEM method.

Examination of total preparations clearly revealed large bundles of connective-tissue fibers, in some cases appearing as characteristic bands, on the surface of the adventitia, where they could be followed for a considerable distance. They were oriented longitudinally or inclined at a small angle to the axis of the vessel. Small connective-tissue bundles and single fibers, forming plexuses with small loops, lay in the intervals between them. Because of the great depth of focus of the scanning electron microscope, the mutual arrangement and relationship between the small loops and meshes formed by the thin interwoven connective-tissue bundles in the surface layers of the adventitia are revealed (Fig. 1). On some areas of the adventitial surface spaces or systems of meshes bounded by a small-looped network of connective-tissue bundles of varied caliber can be observed. They spread into the depth of the adventitia. These channels can be tentatively regarded as cor-

responding to the pathways of spread of the nutrient blood vessels and nerve fibers in the vessel wall.

The study of the inner surface of the vessel wall showed a different pattern of distribution of the structures of the fibrous skeleton. After enzyme treatment the surface of the intima became completely deendothelized. Because of removal of the ground substance in the subendothelial layer the bundles of connective-tissue fibers stood out in considerable relief over a large area of the specimen. Connective-tissue bundles and fibers of small diameter, oriented mainly perpendicularly to the axis of the vessel, were predominant in this area. The connective-tissue network of the subendothelial zone appeared to have a fine-mesh construction, characterized by great polymorphism of the small loops and meshes, with no predominant orientation. Large meshes or channels were absent from the connective-tissue stroma of the intima (Fig. 1). The study of specimens of the wall of large arteries stripped into layers and treated with enzyme preparations showed the fenestrated membranes standing out in high relief, with bundles of collagen fibers between them. The surface of the fenestrated membranes was slightly undulating. On some parts of it small parallel irregularities running parallel to the axis of the vessel could be seen (Fig. 1). Openings surrounded by connective-tissue bundles were clearly visible in the fenestrated membranes. The openings in the fenestrated membranes lying in the deeper layers of the media were closed with a fine network of connective-tissue fibers. Bundles of fibers in the intermembranous spaces were oriented tangentially to the surface of the membranes and their caliber varied. In some cases small bundles of fibers could be seen to penetrate from the region of the intermembranous spaces into the fenestrated membranes.

The study of stripped preparations obtained from cadavers of old persons revealed areas with disturbance of the architectonics and the relief of the connective-tissue skeleton of the vessel wall. Zones with an increased content of mineral salts could be detected. In these cases crystals of minerals directly related to elements of the fibrous stroma could be seen (Fig. 1).

It can be concluded from comparing the results of investigation of the fibrous skeleton of the walls of large arteries treated with protoryzin and trypsin that, for the same duration of incubation, protoryzin gives clearer pictures of the relief of the fibrous structures.

This may be because protoryzin, with its higher amylolytic activity than trypsin, destroys the proteoglycans and glycoproteins of the ground substance more deeply than trypsin. Also, trypsin hydrolyzes peptide bonds selectively, mainly those formed by basic amino acids [4].

The SEM method applied to specimens of the vessel wall after preliminary treatment with proteolytic enzymes can thus be used to study the fiber architectonics of the skeleton of blood vessels.

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