ELECTRON-CYTOCHEMICAL INVESTIGATION OF DISTRIBUTION OF ACID POLYSACCHARIDES IN THE ENDOCARDIUM OF THE LEFT ATRIAL AURICLE IN RHEUMATIC HEART DISEASE

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Electron-microscopic investigations of the localization of acid polysaccharides on submicroscopic structures of the endomyocardium of the left atrial auricle were carried out on eight patients with rheumatic carditis, tissues being incubated in a solution of colloidal iron after osmium fixation. Extracellular acid polysaccharides are closely connected with the surfaces of collagen and elastic fibers, of smooth-muscle cells of the endocardium, of muscle fibers and capillaries of the subendocardial zone of the myocardium, and also of connective-tissue cells, including cells of the rheumatic granuloma. In ultrathin sections through strongly metachromatic zones of the subendocardium, protein-polysaccharide complexes are revealed as reticulo-fibrillary structures. Intracellular collections of acid polysaccharides are found in cytoplasmic vesicles and in the hyaloplasm of cells of the rheumatic granuloma in its early stages of development.

Hale's reaction is widely used nowadays for the detection of acid polysaccharides (APS) and mucin-containing glycoproteins at the ultrastructural level [1, 6, 11, 14, 15]. The method can be used either in the classical form, when after treatment with potassium ferrocyanide, crystals of Prussian blue 350-850 A in diameter are formed [5], or in the incomplete form, i.e., without counterstaining with ferrocyanide. In the latter case, electron-dense particles, 30-90 A in diameter, are found at the points of location of APS and of mucin-containing glycoproteins [4, 13, 16]. Consequently, the incomplete form gives better resolution and is therefore preferable.

An organ-specific feature of the connective tissue of the heart is its increased content of APS, mainly of chondroitin sulfates, which in the human adult are concentrated in the endocardium and in the "chondroid" tissue of the valves and the fibrous ring. Under pathological conditions and, in particular, in rheumatic carditis, proliferation of connective-tissue cells in the subendocardial layer is accompanied by appreciable accumulation of histochemically detectable APS [2].

Determination of the submicroscopic localization of APS in the endomyocardium of atrial auricles obtained during mitral commissurotomy was the object of the present investigation.

EXPERIMENTAL METHOD

Pieces of tissue from the left atrial auricle from eight patients with a mitral valve defect were fixed immediately after removal in osmium tetroxide [10]. After washing with phosphate buffer, the pieces were incubated in Hale's reagent [12] for 3 and 18 h before and for 6 h after treatment with testicular hyaluroni-

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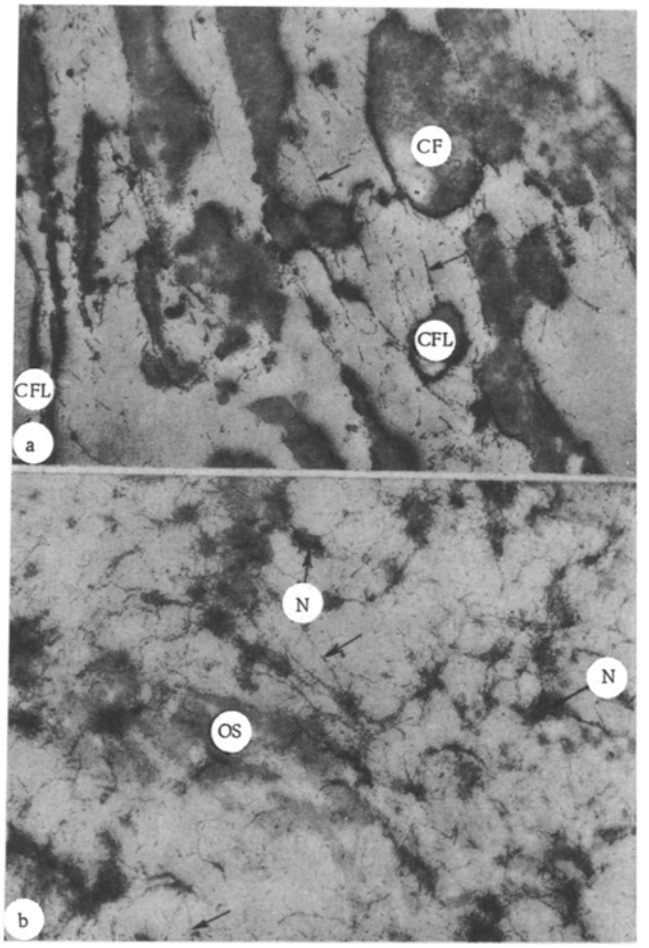

Fig. 1. Subendocardium: a) particles of iron surround collagen fibers (CF) and cover surfaces of isolated collagen fibrils (CFL); they form delicate fibrils in spaces between collagen fibers (arrows). 11,500×. b) Ultrathin section with strongly metachromatic area of subendocardium. Network of fibrils (arrows) and nodules (N) can be seen. OS – clumps of osmiophilic material. 14,700×.

dase (Reanal, Hungary), washed with buffer, dehydrated in alcohols, and mounted in methacrylates or araldite. Areas where, under the optical microscope, a diffuse positive reaction was found in semithin sections stained with potassium ferrocyanide were chosen for cutting ultrathin sections. Sometimes inspection of the unstained section in transmitted light was sufficient to pick out zones of intensive iron fixation. Ultrathin sections were studied in the JEM-7 electron microscope without additional shadowing.

RESULTS

Examination of half-thin sections stained with ferrocyanide under the optical microscope revealed a positive reaction mainly in the subendocardial layer and, less frequently, in the endocardium itself (for terminology, see [8]). On examination in the electron microscope, the reaction product was found in the endocardium, in the form of discrete angular particles of iron, 30-70 A in diameter, on the surface of collagen and elastic fibers, smooth-muscle cells, and fibrocytes. Deposition of iron was found in the subendocardium on the surface of collagen and elastic fibers, undifferentiated and mast cells, cells of the rheumatic granuloma at different stages of maturity, fibroblasts, and subendocardial muscle fibers (Figs. 1 and 2). No particles of iron were seen between membranes of the intercalary disks of the muscle fibers. Subendocardial capillaries were surrounded by a border of electron-dense particles. Isolated collagen fibrils were thickly spattered with iron particles (Fig. 1a). The cross-striation of the fibrils was usually masked.

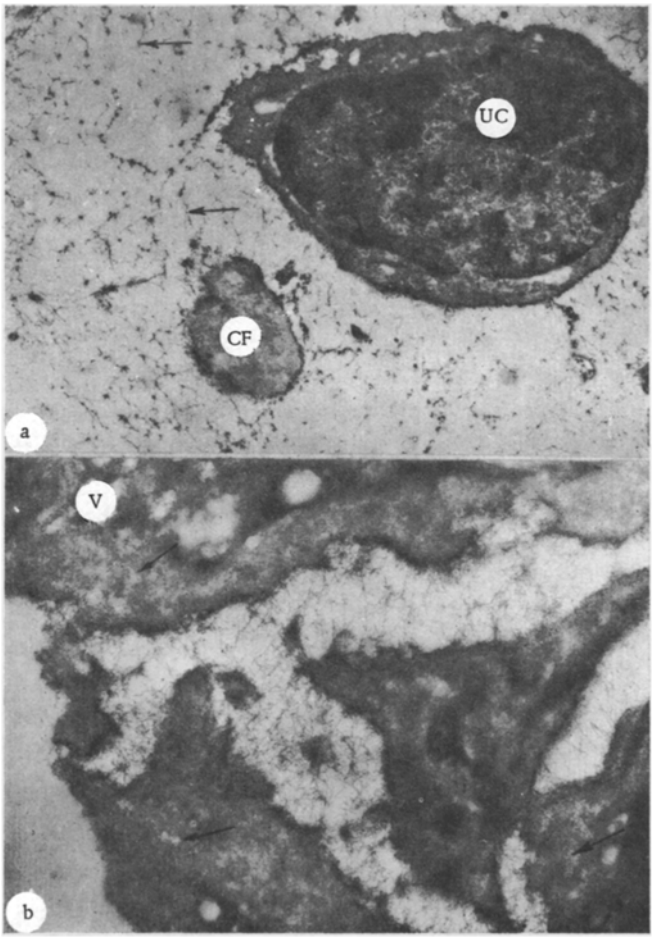

Fig. 2. Deposit of iron particles in connective-tissue cells of subendocardium: a) deposit on surface of undifferentiated connective-tissue cell (UC) and of collagen fiber (CF). Iron particle deposited in regular sides between cells and fibers (arrows). 10,800 ×. b) Deposit on surface of granuloma cells in early stages of development, inside cytoplasmic vesicles (V), and in hyaloplasm (arrows). Fibrils composed of regular lines of iron particles in intercellular substance organized into a delicate network. 14,100×.

On the surface of the smooth-muscle cells of the endocardium and also of capillaries and muscle fibers of the subendocardial zone of the myocardium, iron was deposited uniformly both in the basement membrane and in the osmiophobic layer between the basement and cell membranes.

In the spaces between fibrous and cellular components of the subendocardium, most iron particles were arranged in regular lines, so that under low power they appeared as very thin fibrils (Fig. 2a, b). Most commonly the fibrils formed a delicate network. In some areas, especially where γ-metachromasia was found under the optical microscope after staining with toluidine blue, nodules 500-600 mμ in diameter or more could be seen at the points of intersection of the fibrils (Fig. 1b); these were very clearly visible in ultrathin sections about 2000 A in thickness. This reticular organization was probably associated with the state of increased polymerization of the APS responsible for intensive metachromasia. Clumps of amorphous osmiophilic substance were frequently found in these areas (Fig. 1b). The fibrils described above in some cases appeared to be woven into elastic fibers and collagen fibrils, and into the basement membranes of the smooth-muscle cells, capillaries, and muscle fibers of the subendocardial myocardium, i.e., they corresponded in their appearance and distribution in the tissues to microfibrils [9], such as are observed in large numbers in metachromatic zones of control sections immersed without incubation in colloidal iron, and most probably consisting of protein-polysaccharide complexes.

Lannigan and Zaki [7] found particles of iron inside some cytoplasmic cells of a granuloma in the auricular endomyocardium of patients with rheumatic carditis, but they consider that the meagerness of these vesicles cannot be taken as evidence in favor of secretion of APS by granuloma cells. They never observed structures of this type inside the endothelial cells of capillaries, pericytes, fibroblasts, undifferentiated cells, or mast cells, and only rarely in macrophages. The present findings disagree to some extent with these observations. Intracellular deposits of iron particles in the writers' material were found mainly in immature granuloma cells, and most of the deposit was localized actually in the hyaloplasm, less of it in the vesicles (Fig. 2b). Intracellular particles were never arranged in regular lines, whereas extracellular particles as a rule were formed into fibrils.

No reaction product was present either outside or inside the cells in control sections treated with hyaluronidase. Consequently, colloidal iron revealed APS or, more exactly, chondroitin sulfates A and C and hyaluronic acid.

Frequently, especially in larger blocks incubated for 3 h, the distribution of deposit was irregular. This probably reflected uneven permeation of reagent into the blocks and confirms the view [3] that bound iron can prevent the permeation of further amounts of reagent.

Extracellular APS in the endocardium in rheumatic carditis are thus closely connected with the surfaces of cells and connective-tissue fibers,and they are present in the cytoplasm of cells of the rheumatic granuloma in the early stages of its development.

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