ELECTRON-MICROSCOPIC INVESTIGATION OF ALLOGRAFTS AND XENOGRAFTS OF THE AORTIC VALVES AFTER PROLONGED FUNCTIONING IN THE HUMAN HEART

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An electron-microscopic investigation was made of allografts and xenografts of the aortic valves after various methods of chemical sterilization and γ-ray irradiation. Changes in the ultrastructural components were demonstrated in the cells of the valve grafts after chemical methods of sterilization, but the cells were intact after γ-ray irradiation. The dynamics of development of specific degeneration of the connective tissue of the valves during prolonged functioning of the grafts in the human heart was revealed. Degeneration of the collagen was shown not to be connected with its enzymic destruction, but to be the result of passive mechanical injury.

KEY WORDS: aortic valves; allografts and xenografts; sterilization; degeneration of collagen.

Investigation of transplanted valves at the ultramicroscopic level can shed light on the character, intensity, and extent of their injuries, so that the best type of sterilization of the valves can be chosen and their fate in the body studied.

There are a few reports in the literature of ultramicroscopic changes in grafted valves after sterilization with γ rays and also after clinical use [3, 5, 7]. The reports of these investigations contain no information on the mechanisms of development of the specific degenerative processes in the valves after transplantation and a long period of functioning in the human heart.

This paper describes an attempt to obtain such information.

EXPERIMENTAL METHOD

Recent human and porcine aortic valves were investigated unsterilized (control) and also after sterilization: 1) in a solution of β-propiolactone, 2) in a 4% solution of formaldehyde at pH 5.6, 3) by Carpentier's conditions method, and 4) by γ-ray irradiation in a dose of up to 2.5 Mrad for 11 min after preliminary freezing to $-79°C$.

In addition, 10 allografts and xenografts of valves sterilized by the methods described above and implanted in the mitral and tricuspid positions in patients with rheumatic heart disease were investigated. The periods of implantation ranged from 12 to 52 months.

Fragments of the transplanted valves were fixed in 2.5% glutaraldehyde and in 1% OsO_4. The tissue was embedded in Epon 812. The sections were stained with lead citrate by Reynolds' method.

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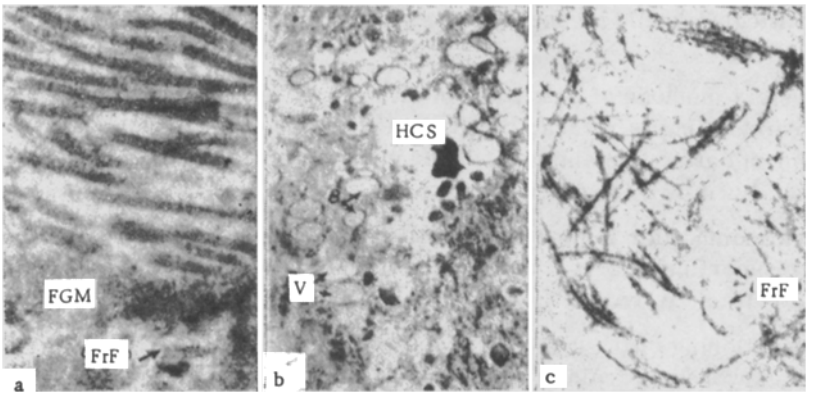

Fig. 1. Ultrastructure of transplanted valves after sterilization and a long period of functioning in the human heart: a) among unchanged collagen fibrils finely granular material and fragments of destroyed collagen fibrils can be seen at well-marked regular intervals (time of implantation of the valve 21 months; 25,000 ×); b) numerous vacuoles present in the ground substance, forming honeycomb-like structures (period of implantation of the valve 52 months, 20,000 ×); c) irregular orientation of collagen fibrils and their fragmentation (period of implantation of the valve 25 months, 15,000 ×). FrF) Fragmentation of fibrils; FGM) finely granular material; V) vacuoles; HCS) honeycomb-like structures.

EXPERIMENTAL RESULTS

After sterilization of the valves with β-propiolactone, formaldehyde, and the conditioning method, tiny areas of loosening of the collagen bundles were found between bundles of intact collagen fibrils with a period of 640 Å; they were most marked and extensive after sterilization with β-propiolactone. Fibroblasts with a well preserved cytoplasmic membrane and an elongated nucleus were observed. The cytoplasmic matrix was translucent and most of the mitochondria were damaged or destroyed. After sterilization of the valves by γ-ray irradiation, the bundles of collagen fibrils were unchanged. In the fibroblasts after γ-ray irradiation, unlike those treated by chemical methods of sterilization, a well-developed granular endoplasmic reticulum and intact mitochondria were seen.

Investigation of valves implanted in the mitral position after sterilization in formaldehyde and in β-propiolactone revealed areas of loosening of the bundles among bundles of well preserved collagen fibrils. The cross striation in such fibrils was not equally distinct. In some areas an amorphous, finely granular material could be seen close to well preserved collagen fibrils and fragments of others (Fig. 1a). In the interstitial space there were numerous vacuoles from 150 to 400 nm in diameter. Most of these vacuoles were surrounded by a distinct single membrane (150-200 Å), but in some of them it was partly or completely absent. In some areas the vacuoles had joined together to form honeycomb-like structures, especially in valves sterilized with β-propiolactone (Fig. 1b).

Where the cusps of the valves were torn the collagen fibrils were very much thinner, the fibrils were fragmented in some places and appeared to have "melted," and to be converted into finely granular, amorphous material.

During the investigation of valves implanted in the tricuspid position after sterilization in formaldehyde or by Carpentier's method most of the bundles of collagen fibrils were seen to be well preserved. In the cusps of these valves small areas of collagen fibrils with an irregular orientation could be seen. Cross-striation within the periods (about 50 Å) was observed in some of the fibrils. Characteristically some fibrils showed weakened osmiophilia, thinning of their ends, and fragmentation (Fig. 1c). These changes were much more marked in valves sterilized in formaldehyde. In all cases the elastic fibers showed no structural changes.

In all transplanted valves implanted in the mitral and tricuspid positions newly formed cells of the fibroblast type could be seen; their nucleus was elongated, they were surrounded by a distinct double membrane, and chromatin was concentrated at the periphery of the nucleus. A well-developed network of

granular endoplasmic reticulum could be seen in the cytoplasm. The numerous mitochondria had a dense matrix with many cristae.

Chemical and physical methods of sterilization thus do not lead to destruction of the collagen-elastic skeleton of the valve, maintaining its mechanical strength, but the cells of the graft are gradually destroyed. By the end of the first month after implantation, the donor's cells were almost completely absent in the graft, even in valves sterilized by γ-ray irradiation.

Approximately one month after implantation of the valves, newly formed cells of the fibroblast type began to appear in their superficial parts. These were undoubtedly recipient's cells; their ultrastructure pointed to active biosynthetic activity.

After prolonged functioning of the grafted valves, progressive changes were observed in the ground substance. These changes were particularly well marked after implantation in the mitral position: areas of loosening of the bundles of collagen fibers, loss of their complex structure, and thinning of their ends were observed. More severe changes indicating molecular disturbances in the structure of the collagen fibrils took the form of disturbance of the precise period of 640 Å between the striations and the appearance of cross-striations within the periods, as fragmentation of the fibrils and, finally, as cloudy swelling. These changes indicate the gradual destruction of the collagen skeleton of the valve cusps.

An unusual and not completely clear phenomenon, that has not been observed in other types of disorganization of the connective tissue under pathological conditions, is the formation of vacuoles with or without a surrounding membrane in the ground substance. The vacuoles are not cellular in origin for there are no cells in the valve except a few newly formed cells in the superficial zones. The formation of honeycomb-like structures with breakdown of the ground substance can be seen on the electron micrographs. This picture probably corresponds to the formation of the large vacuoles visible in the light microscope [6, 8]. This phenomenon is evidently a specific mechanism of destruction of the ground substance after transplantation of the valves [1, 2]. As a result of the absence of cells in the implanted valve, the enzymic catabolism of collagen cannot take place (no collagenolytic or mucolytic enzymes, proteases, or hydrolases are produced) and a passive mechanical wearing away of the collagen structures therefore takes place. These processes taking place at the fibrillary and molecular levels progress particularly rapidly where the hemodynamic load is greater (in the mitral position), where they lead to thinning of the collagen fibers, fragmentation of the fibers, and even to perforation and rupture of the valve cusps.

LITERATURE CITED

1. A. B. Shekhter and L. P. Stranov, Arkh. Pat., No. 7, 3 (1970).
2. A. B. Shekhter and Z. P. Milovanova, in: Simulation, Methods of Study, and Experimental Treatment of Pathological Processes [in Russian], Moscow (1973), p. 117.
3. J. Binet, C. Planche, and M. Weiss, in: Biology of Tissue in Heart Valve Replacement, London (1972), p. 409.
4. R. Donelly, L. Aparicio, and F. Dexter, Thorax, 28, 91 (1973).
5. J. Gavin, P. Herdson, and B. Barrat-Boyes, Pathology, 4, 175 (1972).
6. F. Herlein, Arch. Klin. Chir., 324, 154 (1969).
7. A. Rose, Thorax, 27, 401 (1972).
8. T. Luterland, in: Biology of Tissue in Heart Valve Replacement, London (1972), p. 887.