

## **CAN SERUM DEPLETION IN THE LOWER ZONE OF EXTENSIVELY THICKENED PLAQUES IN AORTAS INITIATE CALCIFICATION?**

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The issue of pathological implications and the underlying causes of arterial calcification remain enigmatic. The possibility of vascular calcification exerting both beneficial and/or detrimental effects on atherosclerosis has been suggested. Doherty and Detrano [1] proposed that calcification may stabilize aortic walls weakened by the accumulation of lipids and inflammatory substances. Farb et al. [2] provided clinical observations that advanced calcification can cause the aortic walls to become brittle with subsequent ruptures. Other significant clinical effects of vascular calcification include myocardial infarction [3], failures of bioprosthetic cardiac valves [4], and hypertension resulting from inflexibility of the aortic walls [5].

The prevailing concept of a close association of osteogenesis with vascular calcification [6-9] may not be fully appreciated since bone formation in atheromatous regions is a rare event and that several osteogenesis-related proteins are also present in other soft tissue during organogenesis [10,11]. Bone resorption processes [12] and nano-bacterial infection [13] as an alternative cause of arterial calcification is not consistent with a rabbit model in which aortic calcification occurs in youth at a specific site in which bone resorption and infection is least expected [14,15].

Accumulated data in this laboratory demonstrate a close correlation of calcifying vesicle accumulation with calcification in thoracic aortas from rabbits fed cholesterol supplemental diets and human atherosclerotic subjects [16,17]. Histological examinations of rabbit atherosclerotic thoracic aortas indicate that calcification starts specifically at the lower zone of plaques adjacent to smooth muscle layers (Figure 1) [14,15]. Bone-obligatory marker alkaline phosphatase activity was absent at this provisional calcification site using a specific activity staining procedure [18]. Neither calcification nor osteoblast-associated osteoid was present. Since calcification did not occur until plaques are extensively thickened, blockage in the blood supply to the sites may play a role in the underlying mechanism of aortic calcification.

A rabbit smooth muscle cell culture model was used to determine whether lack of serum in culture media could induce calcification. As a result, serum was found to be a profound source of inhibitors for calcification induced by high Ca x P ion products. A mere serum concentration of 0.04-0.07% from fetal bovine and rabbits was able to inhibit cell-mediated or thermodynamically induced spontaneous calcification by half [19]. Serum depletion in cell culture appears to be capable of inducing membrane translocation as evidenced through a specific apoptosis dye uptake by cells [19]. The translocation leads to exposure of calcifying vesicles and probably Pi-rich source of intracellular metabolites such as ATP and nucleotides for calcification. Furthermore, proteomic analyses of calcifying vesicles revealed several calcification-related proteins including calpactin, calreticulin, integrin, fibrillin, ATPase, and ATP-synthase [19]. Moreover, current data indicate that ATP- or AMP-initiated calcification in culture media by vesicles isolated from rabbit aortas can be inhibited by serum.

Although the role of serum in calcification remains unclear, a recent study demonstrates that dehydration by ethanol increased calcium binding activity of serum by 5-fold. Whether the large calcium binding capacity of serum may contribute to mineralization inhibition would be

interesting to investigate. Altogether, the site-specific calcification independent of osteogenesis is a net complex manifestation of several factors such as serum depletion and exposure of intracellular Pi-yielding calcifying vesicles through membrane translocations.

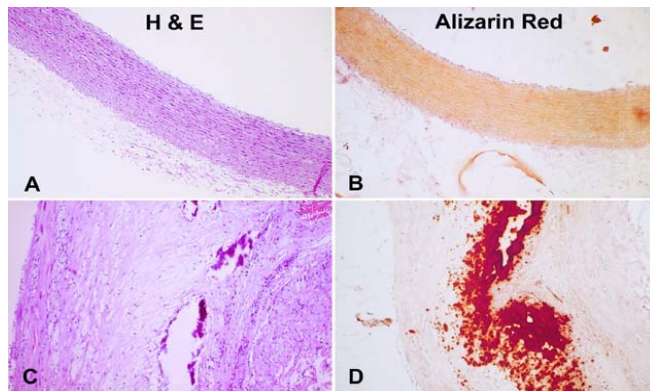


Figure 1. Calcification initiates at the lower zone of plaques. Normal rabbit aortas (panels A and B) show no signs of intimal thickening (A) or alizarin red stains (B) indicative of mineral deposition. Thickened calcified aortas induced by cholesterol supplemental diets are shown in panels C and D.

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