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Atherosclerotic Plaque: X-ray Diffraction Investigation

Abstract. *Human atherosclerotic plaque material continuously maintained in an aqueous environment has been subjected to examination by x-ray diffraction. The first diffraction pattern from single crystals of human biological apatite was obtained from the plaque material of a freshly excised plaque when it was equilibrated with its aqueous environment. As the plaque material dried, the discrete spots characteristic of single crystal diffraction disappeared, leaving only the powder pattern of apatite.*

The atherosclerotic plaque is a lesion that represents the culminative trauma of atherosclerotic disease. The plaque, which predominantly occurs in man, can be found in the large and medium-sized arteries. Although no explanation completely accounts for all the aspects of atherogenesis, it is generally believed that atherosclerosis results from lipid accumulation in the intimal wall of the artery caused by a local breakdown of the normal process of lipid diffusion (*1*). This initial accumulation of lipid is followed by further deposits of organic cholesterol and other lipids and inorganic material (*2*).

X-ray studies (*3*) established that the major crystalline constituent of all mineralized tissue was the polycrystalline form of the calcium phosphate mineral apatite. Calcified intimal atheroma of the coronary arteries were examined by Carlstrom *et al.* (*4*), who used x-ray powder diffraction techniques. The diffraction patterns indicated a crystalline structure due to the presence of apatite. In all cases, the biological apatite structure consisted of relatively small crystallites, and x-ray diffraction investigation of calcified tissue (*5*) substantiated these findings. The powder patterns were consistent with the patterns obtained from apatite with small crystallites. Posner and Termine (*6*) recognized the presence of polycrystalline hydroxyapatite as well as amorphous calcium phosphate in bone; Perloff and Posner (*7*) prepared the first pure hydroxyapatite suitable for x-ray diffraction studies of single crystals.

Atherosclerotic material was examined by x-ray diffraction to determine to what extent structural order exists in the plaque and to what extent this structure is influenced by its environment. Aortas taken from recently deceased, aged, normal males were placed immediately into physiological saline solution. The moisture equilibrium between the plaque and its surrounding fluid was thus maintained. Plaques from different sections of the aorta were stripped from the arterial wall, and small test specimens were excised from the plaque so as not to include surface material.

Small test samples were placed in a 1-mm glass capillary tube that contained a reservoir of solution at each end. Care was taken not to have the solution in direct contact with the plaque specimen, so that no x-rays would pass through the solution before reaching the sample. The test specimen in the sealed capillary tube was exposed to nickel-filtered copper radiation in a Weissenberg camera for 2 hours. Specimens were examined from 1 to 7 days after autopsy. Tests were run as the plaque samples remained stationary, as the sample went through a 15° oscillation, or as the sample went through a 360° rotation. The capillary tube was then opened at one end and the solution was allowed to evaporate slowly for about a week. The same samples were tested on successive days as the plaque dried so that a change in the diffraction pattern with loss of moisture could be noted.

Five plaque samples from different areas in two aortas taken from recently deceased aged males were examined. Tests were also run, on successive days, on the glass capillary tube containing solution only. Plaque samples in physiological saline were stored in a sealed container at about 5°C for x-ray examination at a future date. Samples stored in this manner were examined as early as 1 day after autopsy and as long as 1 year later with no change in the diffraction pattern.

Since there is some evidence for the presence of hydroxyapatite in calcified tissue (*3*), a control pattern of hydroxyapatite was obtained. This control pattern was obtained by placing powdered calcium tribasic phosphate in a 0.5-mm glass capillary tube for exposure to x-rays for ½ hour to 2 hours.

Initially, as the plaque sample was equilibrated with the solution, two types of patterns were obtained. Continuous rings characteristic of polycrystalline material were obtained, as well as discrete spots characteristic of single crystals. In all cases the discrete spots fell on the continuous rings. Scattering maximums were obtained at scattering angles greater than 160°. As the sample dried the discrete spots disappeared, leaving only the rings. The rings from the plaque sample were compared with those obtained from the hydroxyapatite powder. They were identical.

Discrete diffraction spots falling on powder rings identified as hydroxyapatite were recorded only when the atherosclerotic plaque material was equilibrated with an aqueous environment. These discrete spots disappeared, leaving only rings as the plaque specimen dried. These findings indicate that in vivo plaques contain single crystals of apatite. Although biological apatites in the polycrystalline state have been observed in the human body this is the first observation of single crystals of biological apatite.

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