The Presence of Ectopic Xanthine Oxidase in Atherosclerotic Plaques and Myocardial Tissues[1] (37627)

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Plasmalogens, a naturally occurring group of aldehydogenic phospholipids, are found abundantly in human heart and brain tissues. Their phospholipid character makes them an important constituent of many biological membrane systems. Ferrans, Hack, and Borowitz (1) demonstrated plasmalogens in the sarcoplasm, sarcosomes, and intercalate discs of normal human cardiac muscle. Oster and Hope-Ross (2) examined histochemically cardiac muscle from a case of fatal myocardial infarction and found that plasmalogen had disappeared from the infarcted area less than 2 hr after the onset of pain. In this case, there was no necrosis or other significant tissue changes in the affected heart muscle. Similarly, Oster (3) demonstrated the absence of plasmalogen in the aorta of a 22-year-old drowning victim suffering from extensive atherosclerotic changes. Other investigators (4, 5) also demonstrated that plasmalogen depletion in the aortic wall corresponded to an increase in atherosclerosis and that aortic plasmalogen concentrations decreased with age.

It is of significance that certain metabolically active organs—*e.g.*, the liver and mucous membranes of the small intestine—are normally devoid of plasmalogens. Oster and Mulinos (6) ascribed this absence to the activity of the enzyme, xanthine oxidase (xanthine: oxygen oxidoreductase, EC1.2.3.2) which abounds in those tissues where plasmalogen is normally absent. These authors demonstrated that when plasmalogen was split into a fatty aldehyde and lysoplasmalogen by the action of dilute HCl, the resulting aldehydes could be oxidized by purified bovine milk xanthine oxidase preparations. This finding provides a reasonable biochemical explanation for enzyme-mediated plasmalogen depletion. Prior studies by Morgan (7) and Ramboer (8) report the absence of xanthine oxidase in normal human cardiac tissues. The present preliminary investigation endeavors to explain the plasmalogen depletion phenomenon by examining diseased human aortic and myocardial tissues for ectopic xanthine oxidase. No report of the enzyme's presence or absence in diseased tissue has been found in the literature.

Methods. Tissue Sample Selection and Preparation. Unfixed aortic and myocardial tissues of a 54-year-old male and a 74-year-old male were examined (Cases 1 and 2). Their deaths were due to the complications of an abdominal aortic aneurysm and to a myocardial infarction, respectively. In 3 additional cases, the examination for ectopic xanthine oxidase was confined only to the aorta. Case 3 was a 61-year-old male who died from the complications of a bleeding peptic ulcer. He had extensive atherosclerotic calcification of the aortic arch demonstrated by x-ray during life. Case 4 was a 75-year-old male who died from pneumonitis. Calcifications of aorta were examined. Case 5 was a 37-year-old male who died suddenly as a result of a trauma. There was no appreciable amount of atherosclerosis or arteriosclerosis in the aorta.

The entire aortic wall was dissected into little tissue squares (1.0 cm^2) visibly containing yellowish atherosclerotic plaques and similar squares of apparently normal, less involved pinkish aortic tissue. The decision for

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the myocardial sampling was more difficult because of the lack of a clearly visible demarcation between normal and pathological tissue. Sections near the anterior coronary artery branch were compared with apparently more normal-looking tissue closer to the apex of the heart. Grossly, the latter area showed no evidence of scarring. An average of 8 enzyme assays were performed on homogenates of 2–4 tissue samples from each person.

Estimation of Xanthine Oxidase Activity. Xanthine oxidase activity was measured by the method of Haining and Legan (9). Aortic and myocardial tissue samples were homogenized in 10 vol of cold 0.05 *M* phosphate buffer (pH 7.4) with EDTA. The homogenate was then centrifuged for 30 min at 4° and 48,000*g*. The supernatant liquid containing the enzyme was subsequently passed through a Sephadex column (K 9/30) containing G-100 gel which had been preirrigated for 1 hr with 0.1 *M* phosphate buffer (pH 7.4) without EDTA (8). When present, the enzyme would appear in the eluate.

For assay, the reaction mixture consisted of 2.7 ml of 2-amino-4-hydroxypteridine (AHP) in 0.2 *M* phosphate buffer (pH 7.4) as the substrate and 0.3 ml of the enzyme solution. Appropriate fluorometric blanks were prepared by incubating the buffer substrate and the enzyme solutions in separate test tubes and then combining them after the incubation period and just prior to reading. These blanks and the reaction mixtures were incubated for 1 hr at 37°. Following incubation, 3 ml of impurity-free 40% trichloracetic acid were added to 1 ml portions of both blanks and reaction mixtures. The resulting turbid mixtures were then centrifuged for 10 min at 10,000*g* to remove precipitated protein. The supernatant fluorescence was measured in a Beckmann Ratio Fluorometer using a number 5 uranium bar, a Schott UG-11 primary filter, and a second Wratten 2A filter. A quinine sulfate solution (1.6 *M* in 0.1 *N* sulphuric acid) served as the 100% fluorescence standard.

The unit of enzyme activity was established by the Haining and Legan method, so that each fluorometer scale division measured is equivalent to 1.26×10^{-4} μmoles of AHP oxidized to the fluorescent product, isoxanthopterin (9). The unit of enzyme activity is expressed as the number of moles of AHP oxidized per g of tissue per hr.

Results. The results (Table I) indicate the presence of xanthine oxidase in many of the samples investigated. As shown, the lowest values for enzyme activity (< 4.69) were observed in aortic tissue samples which appeared grossly normal. Significantly ($p < 0.01$) higher activities were found in samples from both atherosclerotic aortas as well as the pericoronary and apical myocardial tissues of both heart specimens. The variations among the high readings can be ascribed to the differences in severity of the sample pathology, since it is known that tissue reactivity in atherosclerosis and myocardial damage is uneven.

In Cases 3–5, where just the aorta was examined, only one (Case 3) showed ectopic xanthine oxidase in an atherosclerotic lesion. Case 4 with a history of liver disease had no detectable ectopic xanthine oxidase in the lesions examined. The atherosclerotic process in this case showed the severest degree of calcification. Case 5, whose aorta was essentially normal, exhibited no detectable ectopic xanthine oxidase.

Discussion. For the first time, to our knowledge, the presence of xanthine oxidase in the atherosclerotic plaque and the pathological myocardium has been demonstrated. Grossly normal aortic tissues exhibit very little or no detectable enzyme activity, and the same has been reported for normal heart muscle (7, 8). It is possible that this ectopic xanthine oxidase may encounter a suitable substrate in the aldehyde moiety of the phospholipid plasmalogen which is a normal constituent of the cell membranes of such tissues. The subsequent alteration of the structural integrity of these membranes by such enzymatic activity may produce an initial lesion which could then serve to increase cell permeability, microthrombus deposition, or both.

One source of the enzyme may be the liver cell, since patients with acute liver disease show increased serum levels of xanthine oxidase (8). In patients with chronic liver disease, the serum level of xanthine oxidase

TABLE I. Xanthine Oxidase Activity in Normal and Pathological Human Aortic and Myocardial Tissue Homogenates.

Male patient no.	Age	Tissue sampling	Samples (n)	Mean g tissue/ml homogenate	Mean moles AHP[a] oxidized/hr	Xanthine oxidase activity[a]
1	54	Normal-appearing aorta (control)	3	0.320	$1.89 \times 10^{-4} \pm 0.04$	4.69 ± 0.10
1		Atherosclerotic aorta	4	0.493	$5.56 \times 10^{-3} \pm 0.08$	89.5 ± 0.36
2	74	Normal-appearing aorta (control)	2	0.289	$<5.6 \times 10^{-5}$[b]	
2		Atherosclerotic plaque (aorta)	3	0.239	$1.01 \times 10^{-3} \pm 0.07$	33.5 ± 2.3
1		Normal-appearing myocardium (control)	2	0.220	[b]	
2		Normal-appearing myocardium (control)	2	0.244	[b]	
1		Pericoronary myocardium of right lateral branch of coronary artery	2	0.301	$2.48 \times 10^{-3} \pm 0.04$	65.3 ± 10.8
2		Area from apex of heart with no visible epicardial scarring	3	0.332	$1.10 \times 10^{-3} \pm 0.06$	26.3 ± 1.4
3	61	Atherosclerotic aorta	3	0.249	$7.02 \times 10^{-3} \pm 0.14$	28.19 ± 2.7
4	75	Atherosclerotic aorta	3	0.314	[b]	
5	37	Normal aorta (control)	3	0.219	[b]	

[a] Moles of 2-amino-4-hydroxypteridine (AHP) oxidized/g tissue/hr. An average of 8 enzyme assays were performed on each tissue sample. Results are expressed as averages $\pm$ SD.

[b] Minimal detectable activity level for method employed.

is occasionally moderately elevated. Moreover, in uncomplicated, obstructive jaundice, the serum xanthine oxidase is, at times, slightly elevated (8). In addition to these findings, Ramboer (8) was able to demonstrate slight xanthine oxidase activity in the sera of 10 out of 25 normal human subjects, although Shamma'a *et al.* (10) detected no xanthine oxidase activity in the sera of 18 healthy subjects. Another potential source of the enzyme, *viz.* bovine milk ingestion, is presently under investigation in this laboratory, since it has been shown that milk antibodies are significantly elevated in the blood of male patients with ischaemic heart disease (11).

The postulated enzyme-induced alteration of the phospholipid composition of the cell membrane may point to ectopic xanthine oxidase as one of the factors inducing serious inflammation or perfusion of the arterial endothelium or the myocardium as described by Haust (12). Roussos (13) has shown that bovine xanthine oxidase activity is stimulated by androsterone and testosterone and inhibited by the estrogens (β-estradiol, 17α-estradiol, estrone, and estriol) and progesterone. This could account for the predominance of atherosclerotic heart disease in men.

Summary. Xanthine oxidase activity of five grossly normal aortic tissues of five male patients was compared with that of atheromas from the same aortas. In addition, pathological myocardial tissues of two of the patients were examined. Significantly elevated enzyme activities were found in most abnormal tissue samples. Little or no activity was detected in the normal-appearing samples. These results suggest that xanthine oxidase may be deposited gradually with time, possibly initiating a pathological reaction which culminates in plaque formation or myocardial cellular damage.

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