Some Histochemical Observations on the Human Aortic Wall in Atherosclerosis

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The presence of various phosphatases in the human aortic wall has been studied biochemically by a number of workers. In a few instances, histochemical studies have been made. In this study, enzymes dephosphorylating ATP, AMP, DPN, TPN, glucose-1 and glucose-6-phosphate were studied and comparisons between normal and atherosclerotic areas of the same and different aortae were made.

Methods

Eight human aortae were obtained within 6 hours post mortem. Taft et al. have shown that the activities of alkaline and acid phosphatases, ATPase, 5-nucleotidase, and glucose-6-phosphatase did not decrease in the rat liver up to 6 hours post mortem. There are many other supporting reports in the literature. The specimens were fixed 3 hours in 85% ethanol, dehydrated in the cold and infiltrated with 56°C paraffin for 12 hours. Sections were cut at 7 μ and incubated with the various substrates, using the standard Gomori phosphatase technic. In all cases, the optimum pH was found to be between 9.0 and 9.3.

Results

The normal young aortae were found to give strong and uniform reaction over most of their length for ATPase (fig. 1) and 5-nucleotidase. However, in an aorta which contained atherosclerotic lesions, the cells in these regions showed a substantial decrease in the 2 reactions (figs. 2 and 3), especially in the intima. In 1 aorta in which atheromas had already developed, there were circumscribed areas appearing not to differ histologically from the normal, which were in fact not atheromatous but showed a considerable decrease in the reactions for 5-nucleotidase and ATPase, a decrease which was comparable with that found in the atherosclerotic areas. Is it possible that there were preatherosclerotic areas? The decrease of ATPase in such regions, and certainly in the atherosclerotic areas, may be of special interest from a biochemical point of view. Apart from these regions, we were not able to detect any histochemical difference in aortae from young or old individuals.

A study of the enzymes which released inorganic phosphate from TPN and DPN showed a distribution interesting and different from that obtained when ATP and AMP were used as substrates. In a number of aortae which appeared reasonably free from atheroma under histologic examination, there was practically no breakdown of TPN (fig. 4). On the other hand, DPN breakdown activity appeared to be intense and evenly distributed through the entire intima and media (fig. 5). In atherosclerotic aortae, there was a considerable increase in TPN dephosphorylation (fig. 6), not only in the "normal" parts of the wall but in the atherosclerotic areas as well. There was no significant difference in DPN dephosphorylation reaction from that found in "normal" aortae. With glucose-1, glucose-6-phosphates and β-glycerophosphate, no reaction was observable over a range of pH’s in either the intima or media of "normal" or atherosclerotic aortae. A good reaction, however, was obtained in each case in adventitia, a usual reaction with many phosphate esters in medium and large blood vessels of all ages.

Discussion

Kirk has shown by biochemical studies that the mean adeny1-pyrophosphatase activity of the atherosclerotic aorta is decreased with that of normal aorta. However, he has not been able to show any decrease of 5-nucleotidase activity. The technic of homogenizing the

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HISTOCHEMICAL OBSERVATIONS ON AORTA

Figure 1

Figure 2
Heat stable ATPase activity in atheromatous area. Great decrease of activity in area. This is partly due to smaller number of cells, but activity in cells is greatly decreased also; pH of substrate 9.0. Incubation time, 16 hours, X 135.

Figure 3
5-Nucleotidase activity in aorta of 58-year-old male. Note great decrease in atheromatous area and in individual smooth muscle cells (arrow); pH 9.0 (found to be the optimum for this tissue in the human). Incubation time, 8 hours, X 135.

Figure 4
Portion of aorta of 47-year-old human male, showing no dephosphorylation of TPN. There were no obvious atheromas in this aorta; pH of substrate mixture 9.2. Incubation time, 16 hours, X 135.

whole thoracic aorta may have obscured the small localized decreases which we observe by histochemical technics.

Carr et al. have investigated the ATPase activity in the aorta of various animals. It is interesting that the activity was highest in the rat, then dog, guinea pig, rabbit and lowest in the aorta of the chicken, in that order. He has also shown a difference of activity in various vessels of the dog. However, again he used homogenates of whole vessels (aorta, carotid, brachial). The highest activity is present in the aorta. We have shown a gradient of ATPase and 5-nucleotidase activity for the cat aorta. Is there a correlation between the ATPase activity of these aortae and their susceptibility to experimentally produced atheroma?

Lupton et al. reported an increase of 5-nucleotidase activity with age which we were not able to see in our histochemical preparations. Kirk, in his studies, reported that he found a significant change in 5-nucleotidase activity with age.
When incubating with the glycolytic intermediates, glucose-1 and glucose-6-phosphate, the results demonstrate the degree of specificity for the reactions obtained with other substrates; they also help to demonstrate that they were not due to some change in the general dephosphorylating activity of the aortic wall.

It has been shown that ATP and TPNH (reduced TPN) are required for the critical reactions of cholesterol and long-chain fatty acid synthesis. The significance of the decrease of the phosphatase activity for ATP and AMP on the one hand, with an increase for TPN breakdown on the other, cannot be interpreted at present, but at least those results indicate some fundamental change in the metabolism of the aortic wall with atheroma and the possibility that these changes precede the development of this condition.

Summary

A decrease in the ATPase (heat stable) and 5-nucleotidase of atherosclerotic regions in human aortae has been demonstrated. An increase in the breakdown of TPN, as measured by phosphate release, between the normal or atherosclerotic aorta. No reactivity for glucose-1 or glucose-6-phosphate or β-glycerophosphate was found in the intima or media. The possible implications in the development of atheroma are discussed.

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