Arteriosclerosis in the Rat: Aortic Enzyme Changes Accompanying Arterial Pathology.* (25926)

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Repeatedly bred rats have been shown to develop arteriosclerosis spontaneously(1-4). This tendency was greatly intensified by unilateral nephrectomy and injection of ACTH (2). This report describes changes in arterial enzyme concentrations which can be correlated with gross pathology.

Methods. Rats were 8- to 9-month-old female breeders from Sprague-Dawley Farms. The majority had 4 to 6 pregnancies, and averaged 330 g in body weight. Animals were used soon after receipt and no treatment was given prior to sacrifice. Animals were sacrificed by decapitation, and their aortas classified as follows: No grossly discernible lesions = *clear;* thin annular streaks in abdominal aorta = *minimal;* annular streaks plus plaques in the arch = *moderate;* plaques throughout the arch, and thoracic and abdominal aorta = *severe.* The entire aorta was removed and the lumen flushed with normal saline. The aorta was weighed after removal of excess moisture and adherent connective tissue, and a 5% homogenate prepared in ice-cold saline with hand operated Tenbroeck tissue grinder. Disintegration of tissue was complete in 3 to 5 minutes. The homogenates were centrifuged at 1000 times gravity for 10 minutes to prepare supernatants for lactic dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) assay. The 6-phosphogluconic dehydrogenase (6-PGDH) was assayed on supernatants obtained by centrifugation at 5000 times gravity for 10 minutes. Enzyme determinations were carried out by modification of Sobel procedure(5). Measurements were made in Beckman DU of rate of change in optical density at 340 mμ at one minute intervals. Assay systems contained TPN or DPNH and are described in legends of Tables I, II and III. Protein content of supernatants was determined by method of Gornall(6).

Results. Enzymatic activities (Tables I, II and III) were calculated from kinetic data obtained during periods when reaction rates were linear with time. Assays were terminated in 5 to 15 minutes. In control experiments, DPNH and TPNH were stable in solutions of buffered enzyme equivalent in concentration to those used in assays. In the absence of substrate, optical density at 340 mμ remained constant for periods exceeding the time required for assay.

Enzymatic activities are expressed as μ-moles of substrate reacting/unit time. Data in first column of Tables I, II and III are based on actual enzymatic activity contained in supernatant protein. Data in second column are calculated assuming that no activity was lost during centrifugation.† Lehninger (9) has pointed out that the aorta is relatively acellular and that extracellular connective tissue contributes little or no metabolic activity. We believe that soluble protein of aorta provides much more significant basis for calculation of enzymatic activity than wet weight. This method also tends to make measurements independent of mucopolysaccharide accumulation, collagen and fibrin deposition and calcification characteristic of pathologic aortas in these animals(4).

Part of TPNH, appearing during assay of G-6-PDH was due to presence of 6-PGDH in the extracts. Some of the 6-phosphogluconate formed in G-6-PDH reaction underwent further reaction with excess TPN to produce additional TPNH. Under these conditions ac-

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† No significant differences in LDH, G-6-PDH and 6-PGDH could be detected when whole homogenates were compared to centrifuged preparations.

TABLE I. Changes in "Hexose Monophosphate Shunt"* Activity in Extracts of Aortas of Arteriosclerotic Female Sprague-Dawley Rats.

Gross pathology and No. animals	Enzyme activity: μmoles TPNH produced/g soluble protein/min.	Enzyme activity: μmoles TPNH produced/g wet tissue/hr	P (t test)
None (6)	42.9 ± 4.0†	133.8 ± 12.5	
Minimal "	38.6 ± 1.8	124.8 ± 5.8	N.S.‡
Moderate "	25.5 ± 3.1	91.8 ± 11.2	<.01
Severe "	32.0 ± 3.1	107.4 ± 10.4	<.05

Assay system: 0.1 M Tris buffer, pH 7.4; 1×10^{-2} M $MgCl_2$; 1.2×10^{-4} M TPN; 3.4×10^{-4} M glucose-6-phosphate; supernatant from 5% homogenate (.025 ml). Total vol 1 ml.

* The observed kinetics are due to combined action of G-6-PDH and 6-PGDH.

† Means ± stand. errors.

‡ Not significant.

tivity units in Table I express maximum capacity of the 2 linked enzyme systems to produce TPNH *via* "hexose monophosphate shunt." Data of Table III indicate that TPNH production by 6-PGDH was approximately 15 to 20% of the total. Data in Table I show that TPNH production by these 2 enzymes is significantly less in extracts obtained from aortas with *moderate* and *severe* pathology. LDH activity was also significantly decreased in these groups (Table II). No significant differences were observed in 6-PGDH activity of normal and arteriosclerotic aortas (Table III). Additional data would be required to establish a lack of correlation between level of 6-PGDH and presence of gross arteriosclerosis.

Discussion. Increasing gross arterial pathology in repeatedly bred female Sprague-Dawley rats can be correlated with decreases in concentration of LDH extracted from homogenates of arterial tissue. Kirk and coworkers reported differences in mean values for LDH activity in normal and arteriosclerotic human aortas. They observed that pathologic aortas were frequently low in LDH activity (7).

TABLE II. Changes in Lactic Dehydrogenase Activity in Extracts of Aortas of Arteriosclerotic Female Sprague-Dawley Rats.

Gross pathology and No. animals	Enzyme activity: μmoles DPNH consumed/g soluble protein/min.	Enzyme activity: μmoles DPNH consumed/g wet tissue/hr	P (t test)
None (7)	370 ± 22	1924 ± 114	
Minimal "	358 ± 36	1933 ± 194	N.S.
Moderate "	288 ± 22	1728 ± 132	<.03
Severe (5)	253 ± 16	1416 ± 90	<.005

Assay system: 0.1 M phosphate buffer, pH 7.8; 1.2×10^{-4} M DPNH; 4.0×10^{-3} M Na pyruvate; supernatant from 5% homogenate (.005-.01 ml). Total vol 1 ml.

TPNH production by combined action of G-6-PDH and 6-PGDH is significantly lower in preparations obtained from aortas showing *moderate* and *severe* pathology. Kirk reported similar observations using human arteriosclerotic aortas(8).

In vitro enzymatic reaction rates do not necessarily reflect rate limiting processes *in vivo*. Nevertheless, changes observed in pyridine nucleotide linked enzymes could conceivably affect critical rate limiting processes in the intact animal.

TABLE III. Comparison of 6-Phosphogluconic Dehydrogenase Activity in Extracts of Aortas of Normal and Arteriosclerotic Female Sprague-Dawley Rats.

Gross pathology	No. animals	Enzyme activity: μmoles TPNH produced/g soluble protein/min.	Enzyme activity: μmoles TPNH produced/g wet tissue/hr
None	10	7.81 ± .88	40.6 ± 4.57
Arteriosclerotic*	6	8.16 ± 1.49	45.7 ± 8.34

Assay system: 2.7×10^{-2} M glycylglycine buffer, pH 7.5; 1×10^{-2} M $MgCl_2$; 1.2×10^{-4} M TPN; 4.2×10^{-4} M Na 6-phosphogluconate; supernatant from homogenate (.2 ml). Total vol 1 ml.

* This group includes 3 aortas classified as *minimal*, 2 as *moderate*, and 1 as *severe*.

Fatty acid synthesis in liver requires DPNH and TPNH and probably occurs mostly in the extramitochondrial portion of the cell(10). Cholesterol synthesis also is dependent on a supply of TPNH. Synthesis of reduced pyridine nucleotides by "soluble" cytoplasmic enzymes of aorta, *e.g.*, LDH, G-6-PDH and 6-PGDH may be a rate limiting factor in reductive synthesis of fatty acids and cholesterol(9,10). In addition, Lehninger suggested that decreased LDH activity may be important in processes leading to calcification of the aorta(9).

Enzymatic activities based on tissue wet weight in Tables I, II and III can be directly

compared with data reported by Kirk(7,8). LDH, G-6-PDH and 6-PGDH activities are remarkably similar in human and rat aortas.

Summary. (1) Extracts of arteriosclerotic aortas of female Sprague-Dawley breeder rats were assayed for lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase. (2) Extracts obtained from moderately and severely arteriosclerotic aortas contained significantly less lactic dehydrogenase than extracts of aortas grossly normal or only slightly affected. TPNH production by combined action of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase was significantly depressed in extracts from moderately and severely arteriosclerotic aortas. No significant differences were observed in levels of 6-phosphogluconic dehydrogenase.

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Comparison of Ultracentrifuge and Polyanion Precipitation Methods for Serum β-Lipoproteins.*† (25927)

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Isolation of human serum β-lipoproteins by precipitation of a complex with polyanions has been described by Burstein(1) and confirmed by Oncley(2), Bernfeld(3) and other authors. The polyanion precipitation method yields an insoluble complex which, in the human β-lipoprotein, can be dissociated readily (4). It has the advantage of rapid separation of β-lipoproteins from other serum constituents. This precludes exchange of lipid components between α and β lipoproteins(5) and makes possible kinetic studies of β-lipoprotein, which previously could not be done precisely due to slowness of electrophoretic and ultracentrifugal isolation technics. Completeness of precipitation technic has been checked against electrophoretic methods of β-lipoprotein isolation(6), but this comparison seems insufficient in view of poor quantitation of lipids possible by means of paper electrophoresis(7) and the ease with which mobility of β-lipoproteins can be altered by adsorbed fatty acid anions(8). We therefore studied completeness of precipitation of serum β-lipoproteins by comparison with ultracentrifugal lipoprotein analyses(9). We extended this study to include a number of animal sera, since no adequate evaluation of applicability of the precipitation method to sera of common laboratory animals has been published. Much of published work used dextran sulfate as the polyanion, but this compound is not available in this country. Mepesulfate, the sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside (Hoffmann - LaRoche,) whose use was described parenthetically by Burstein(4), is the only suitable polysulfonated macromolecule available at reasonable cost. We therefore used it and compared its

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