# Metabolism of Arterial Tissue. Oxidative Capacity of Intact Arterial Tissue.* (23241)

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In contrast to the extensive literature dealing with chemical changes in circulating blood in atherosclerosis, the role played by the metabolism of arterial tissue in atherogenesis has received little attention. It is known that while arterial tissue is susceptible to either spontaneous or experimental atherosclerosis in certain species, some arteries are more affected by it than others, and moreover in the same artery, such as the aorta, there are sites of predilection for its development. Investigation of the metabolism of arterial tissue in various species and in various segments of the aorta may be helpful in understanding at least partly, the reason for the site of predilection of this pathologic process. The study of metabolic patterns of the 3 aortic segments (ascending and arch, referred to hereafter as arch, descending thoracic, and abdominal) in man, rabbit and dog was therefore undertaken.

Our investigation deals with the oxidative capacity of normal aortic tissue, namely succinic oxidase and cytochrome oxidase systems, in tissue slices, as determined by oxidative response to succinate and p-phenylenediamine. Study of these 2 enzymatic systems appeared of particular interest since it was shown in various tissues that succinic dehydrogenase, cytochrome oxidase(1) and cytochrome c (2), among others(3), are affected by the level of thyroid function, which in turn plays a role in rendering certain species susceptible to atherosclerosis(4).

*Material and methods.* The tissues used were aorta of man, rabbit and dog. Liver and inferior vena cava (infra-renal segment) were also used for comparison. Animal specimens were secured from male chinchilla rabbits and from male and female mongrel dogs. Animals were sacrificed either by exsanguination or air injection. Human specimens were secured from autopsies within a few to 18 hours after death, during which time they were kept in refrigerators. Preliminary experiments with animal tissues, simulating conditions before autopsy, were performed to ascertain the validity of this study after this lapse of time. Tissues kept at room temperature for 3 to 4 hours, then kept at +4°C for about 16 hours, and kept again at room temperature for 2 to 3 hours, showed the same oxidative capacity as that determined in tissues shortly after removal from the animal, except for endogenous oxygen uptake which became negligible or very small. The used segments of human aortic tissue were free of atherosclerosis. After removal from the host, the tissues were immediately placed in ice-cooled containers with tight-fitting tops to avoid evaporation. The blood vessels were carefully freed of adherent fat and connective tissue, rinsed quickly in ice-cold saline solution and blotted with filter paper. The study of aortic tissue was performed on its 3 segments, *i.e.* arch, descending thoracic and abdominal. Slices of liver and aorta were prepared free-hand by razor blade, while the tissue was placed between 2 frosted glass plates, the bottom one lying on crushed ice. Thin blood vessels, such as rabbit's aorta and inferior vena cava, were opened along the longitudinal axis and cut transversally to provide strips of tissue. The slices or strips were collected in ice-cooled chambers, blotted, weighed and transferred to ice-cooled manometer flasks containing Krebs-Ringer phosphate solution, pH 7.4(5) of final phosphate concentration 0.023 M, in the main chamber and the substrate in the side arm. The thickness of slices was about 0.3-0.5 mm for liver and about 0.5-0.7 mm for aorta. Preliminary experiments with aortic tissue slices of various thicknesses have shown that up to 1 mm the thickness of the slice was not the limiting factor in its oxygen uptake. Oxygen consumption was determined by the direct method of Warburg(6), at

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TABLE I. Oxidative Capacity of Aorta, Inferior Vena Cava and Liver of Man, Rabbit and Dog. Substrate: succinate.

| Species | | Aorta | | | Vena cava | Liver |
|---|---|---|---|---|---|---|
| | | Arch | Thoracic | Abdominal | | |
| Man | $Qo_2$† | .61 ( .23-1.65)* | .73 ( .27-1.62) | .57 ( .17-1.02) | 1.62 (.85-2.32) | 15.00 ( 4.37-25.63) |
| | Determinations | 18 | 20 | 19 | 12 | 15 |
| Rabbit | $Qo_2$ | 2.19 (1.81-2.47) | 2.21 (1.54-2.71) | 2.10 (1.34-2.64) | | 19.28 (13.23-35.18) |
| | Determinations | 11 | 20 | 15 | | 15 |
| Dog | $Qo_2$ | 2.84 (2.00-3.44) | 2.59 (1.92-3.58) | 1.87 (1.06-3.13) | .56 (.39- .84) | 36.76 (20.84-44.91) |
| | Determinations | 20 | 19 | 15 | 10 | 16 |

* Figures in parentheses represent range of variation.
† $Qo_2$ = Oxygen consumption in $mm^3$/mg initial dry wt/hr, corrected for oxygen uptake of tissue in absence of added substrate.

37.5°C. with oxygen as gas phase. Amounts of fresh tissue flask were 100-150 mg for rabbit and dog aorta, 150-200 mg for human aorta, 125-150 mg for inferior vena cava, 30-40 mg for rabbit and human liver, and 20-30 mg for dog liver. The aorta of man and dog provided sufficient material for duplicate determinations whereas that of the rabbit, particularly arch and abdominal segments, did not provide enough material, making it necessary to pool tissues of 2 to 3 rabbits. Initial dry weights were determined for each experiment, by drying tissues to constant weight at 106°-108°C. Substrates used were sodium succinate (pH 7.4) of final concentration 0.05 M for determination of the succinic oxidase system and p-phenylenediamine of final concentration 0.03 M, dissolved in phosphate buffer (pH 7.4) under nitrogen, for the cytochrome oxidase system. Their concentration appeared to be optimal since rate of oxygen consumption remained constant when the concentration was doubled, and it increased proportionately with increasing amounts of tissue. Both these substrates are known to penetrate the tissues. In the presence of succinate, oxygen uptake was constant during 30-40 minutes for liver and 40-60 minutes for artery and vein. In the presence of p-phenylenediamine it was constant during 10-20 minutes for liver and 40-60 minutes for artery and vein. The oxygen consumption/hour was computed from the period of linearity. It was related to initial dry weight and was expressed as $Qo_2$, *i.e.* oxygen consumption in cmm/mg initial dry weight/hour, corrected for oxygen uptake of tissue in absence of added substrate.

*Results.* Tables I and II summarize the results obtained with the 3 aortic segments and liver of man, rabbit and dog, and inferior vena cava of man and dog. All ages were included in these groups. They varied from one day to 73 years for man, 5 months to 2½ years for the rabbit and 5 months to 17 years for the dog.

These Tables reveal that in man and rabbit the 3 aortic segments showed no appreciable difference in oxidative response to either succinate or p-phenylenediamine. In contrast, in the dog, while the 2 first aortic segments exhibited no appreciable difference in their oxidative response to both substrates, the abdominal segment showed a markedly lower response to succinate and a moderately lower to p-phenylenediamine. It is noteworthy that in man the oxidative response of the inferior vena cava to both substrates was about 3 times higher than that of the aorta, whereas in the dog it was about 3-5 times lower with succinate and 2 times lower with p-phenylenediamine. The oxidative response of arterial and venous tissues to both succinate and p-phenylenediamine appears to be quite low in the 3 species studied, when compared to that of the liver. The oxidative response of aortic and venous tissues to succinate was approximately 1/24th and 1/9th that of liver in man, 1/13th, 1/20th and 1/65th for the first 2 aortic segments, the ab-

TABLE II. Oxidative Capacity of Aorta, Inferior Vena Cava and Liver of Man, Rabbit and Dog. Substrate: p-phenylenediamine.

| Species | | Aorta | | | Vena cava | Liver |
|---|---|---|---|---|---|---|
| | | Arch | Thoracic | Abdominal | | |
| Man | $Q_{O_2}$† | .67 ( .33-1.31)* | .74 ( .28-1.29) | .71 ( .43-1.26) | 2.20 (1.24-3.01) | 11.26 ( 4.93-17.00) |
| | Determinations | 17 | 20 | 18 | 12 | 14 |
| Rabbit | $Q_{O_2}$ | 2.34 (1.63-3.13) | 2.42 (1.81-3.10) | 2.35 (1.80-3.04) | | 13.07 ( 9.78-20.80) |
| | Determinations | 12 | 18 | 12 | | 15 |
| Dog | $Q_{O_2}$ | 2.32 (1.49-2.95) | 2.53 (1.50-4.58) | 2.09 (1.27-3.42) | 1.38 (1.12-1.57) | 27.56 (15.48-33.45) |
| | Determinations | 20 | 18 | 15 | 10 | 16 |

* Figures in parentheses represent range of variation.
† $Q_{O_2}$ = Oxygen consumption in $mm^3$/mg initial dry wt/hr, corrected for oxygen uptake of tissue in absence of added substrate.

dominal aorta and inferior vena cava respectively in the dog, and 1/9th for the 3 aortic segments of the rabbit. Using p-phenylenediamine as substrate, the oxidative response of aortic and venous tissues was approximately 1/15th and 1/5th that of liver in man, 1/13th and 1/20th respectively in the dog and 1/5th for the aortic tissue in the rabbit.

*Influence of age.* Values of oxidative responses to succinate and p-phenylenediamine summarized in Tables I and II represent averages of results obtained in groups including all ages. When correlated with age, it was found that in the rabbit (5 months to 2½ years) and in the dog (5 months to 17 years) there was no appreciable change in oxidative responses to both substrates in either aorta, liver or vena cava. In contrast, in man, the oxidative capacity diminishes with age in aortic tissue while showing no appreciable change in vena cava and liver. Tables III and IV summarize the influence of age on oxidative capacity of the 3 aortic segments, inferior vena cava and liver, in man. These Tables reveal that average values of oxidative responses of the aortic tissue to succinate and p-phenylenediamine are markedly lower in the group 21 to 73 years old, as compared to those of the group one day to 17 years old. Table III also reveals that the oxidative response to succinate is lower in the abdominal aortic segment in the younger group, and that this difference between thoracic and abdominal aorta seems to level off in the older group. The oxidative response of aortic tissue to succinate in the older group is about 50% and 30% lower in the thoracic (arch and descending) and abdominal segments respectively as compared to that found in the younger group. Using p-phenylenediamine as substrate, oxidative response of the 3 aortic segments in the older group is about

TABLE III. Comparison of Oxidative Capacity of Aorta, Inferior Vena Cava and Liver of Man One Day to 17 Years and 21 to 73 Years Old. Substrate: succinate.

| Age | | Aorta | | | Vena cava | Liver |
|---|---|---|---|---|---|---|
| | | Arch | Thoracic | Abdominal | | |
| 1 day-17 yr | $Q_{O_2}$† | 1.00 (.49-1.65)* | 1.17 (.57-1.63) | .72 (.38- .93) | 1.54 (.90-1.96) | 16.29 (4.37-25.63) |
| | Determinations | 6 | 8 | 8 | 5 | 6 |
| 21-73 yr | $Q_{O_2}$ | .44 (.23- .75) | .50 (.27- .90) | .53 (.17-1.02) | 1.66 (.85-2.32) | 14.13 (8.58-24.50) |
| | Determinations | 12 | 12 | 11 | 7 | 9 |

* Figures in parentheses represent range of variation.
† $Q_{O_2}$ = Oxygen consumption in $mm^3$/mg initial dry wt/hr, corrected for oxygen uptake of tissue in absence of added substrate.

TABLE IV. Comparison of Oxidative Capacity of Aorta, Inferior Vena Cava and Liver of Man One Day to 17 Years and 21 to 73 Years Old. Substrate: p-phenylenediamine.

| Age | | Aorta | | | Vena cava | Liver |
|---|---|---|---|---|---|---|
| | | Arch | Thoracic | Abdominal | | |
| 1 day-17 yr | $Qo_2$† | .98 (.79-1.31)* | 1.07 (.79-1.29) | 1.08 (.96-1.26) | 2.20 (1.40-3.01) | 12.20 (5.01-17.00) |
| | Determinations | 6 | 8 | 6 | 6 | 7 |
| 21-73 yr | $Qo_2$ | .55 (.33- .79) | .45 (.28- .63) | .59 (.43- .69) | 2.20 (1.24-2.61) | 10.45 (4.93-15.88) |
| | Determinations | 11 | 12 | 12 | 6 | 7 |

* Figures in parentheses represent range of variation.

† $Qo_2$ = Oxygen consumption in $mm^3$/mg initial dry wt/hr, corrected for oxygen uptake of tissue in absence of added substrate.

50% lower.

*Discussion.* Oxidative response of arterial tissue slices to succinate and p-phenylenediamine in optimum concentration, may be considered to be a fair index of oxidative capacity of the succinic oxidase (succinic dehydrogenase + cytochrome c + cytochrome oxidase) and cytochrome oxidase (cytochrome c + cytochrome oxidase) systems and affords a quantitative measure of their oxidative capacity, with cofactors available at the concentration existing in the tissues. This method has proven of value in the study of a number of normal and neoplastic tissues(7,8).

Oxidative utilization of succinate indicates the existence of succinic dehydrogenase in the aorta of man, rabbit and dog and vena cava of man and dog. The vena cava of the rabbit was not studied. Our results are in agreement with previous findings of the existence of this enzyme in the thoracic aorta of rat(9) and man(10). The oxidative response to p-phenylenediamine indicates the presence of cytochrome oxidase in the above mentioned vessels.

Our findings reveal that aortic and venous tissues possess a relatively low oxidative capacity for both succinic and cytochrome oxidase systems. Our data further indicate that a difference exists in oxidative capacity of aortic tissue in regard to the above 2 systems, in the 3 species studied, human aorta exhibiting the lowest oxidative response to both substrates. The dog's thoracic aorta exhibits the highest response to succinate, while its abdominal segment and the 3 aortic segments of the rabbit exhibit values of equal magnitude, between those of man and thoracic aorta of the dog. The oxidative response to p-phenylenediamine is of equal magnitude in the aorta of rabbit and dog.

Paralleling these findings, the liver shows the lowest oxidative capacity in man, highest in the dog and intermediate in the rabbit. In contrast, the oxidative response of human vena cava to both substrates is higher than that of the dog.

A similar species difference exists for adenosine triphosphatase, which is higher in the aortic tissue of the dog as compared to that of the rabbit(11). Oxygen uptake of the aorta is also different in several species, in a medium containing simultaneously lactate, glutamate and fumarate(12).

Since the aorta is composed of several layers of different tissues, relationship between enzymatic makeup of aortic tissue and its morphologic components may be raised both with regard to its segments and species. Histologic differences between thoracic and abdominal aorta are known to exist and consist chiefly in greater thickness of the media and more abundant elastic tissue in the thoracic segment. While in the dog, it may be tempting to ascribe differences in oxidative responses of thoracic versus abdominal segment to the above morphologic differences, this does not hold true, however, in rabbit and man. Likewise, the observed differences in oxidative responses of aortic tissue in the 3 species studied do not seem to reflect the known difference in morphologic components. Factors responsible for the above species difference remain to be elucidated.

The lower oxidative capacity of the succinic oxidase system in the abdominal aorta of the dog, as compared to the thoracic, may prove of interest when correlated to its susceptibility to atherosclerosis. Indeed, in the dog on an atherogenic regimen, the abdominal segment is the most susceptible to atherosclerosis, whereas the thoracic aorta is refractory† (13, 14). The decrease in oxidative capacity of human aorta with age may prove equally interesting when correlated to the known greater susceptibility of man to atherosclerosis with advancing age. It is likely that the observed differences between the 3 species studied may be part of a broader difference in metabolic patterns of their arterial tissue. Work now in progress seems to support this view.

*Summary.* The oxidative capacity of succinic oxidase and cytochrome oxidase systems in 3 aortic segments (ascending and arch, descending thoracic, abdominal), inferior vena cava and liver slices of man, rabbit and dog was studied. A species difference was found to exist: a) man exhibited the lowest oxidative values for both systems; b) the dog, the highest values for the succinic oxidase system in the thoracic aorta; c) the rabbit, intermediate values for the succinic oxidase system in all 3 aortic segments; d) oxidative values for the cytochrome oxidase system were of approximately equal magnitude in the aortic tissue of dog and rabbit. A significant decrease in oxidative capacity of aortic tissue was associated with aging in man, while the vena cava and liver remained unaffected. No appreciable change with age was found in rabbit and dog.

The significance of the above findings in relation to atherogenesis was discussed.

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## Metabolism of the Protein Moiety of Rabbit Serum Lipoproteins. (23242)

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Virtually all of the serum lipids exist in combination with proteins(1). On the basis of electrophoretic mobility(2), solubility(3, 4), and density(5) these lipoproteins may be divided into two large groups, the alpha or high density lipoproteins and the beta or low density lipoproteins. Each of these groups may be further subdivided on the basis of density differences(6). In addition to these physical differences, the various lipoproteins differ in their lipid composition(7). Recent

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