

## THE RESPIRATION AND CYTOCHROME OXIDASE ACTIVITY OF RAT AORTA IN EXPERIMENTAL HYPERTENSION\*

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The search for explanations of changes in arteries with age and various pathologic conditions has led to the accumulation, during the past 15 years, of an appreciable amount of knowledge concerning the metabolism of arterial tissue. Information relevant to such metabolic changes associated with hypertension, however, is quite limited, although structural changes associated with the disease have long been recognized, both in experimental animals and in man (1). Villasante, Jiménez-Díaz, and Nuño reported that the respiration of arterial tissue (unidentified) was not changed in rats with experimental hypertension (2). In the present study the oxygen uptake and cytochrome oxidase activity of aortas of normotensive and hypertensive rats were compared. The results indicate that both values are higher in aortas of hypertensive rats.

### Methods

*Animals.*—Male Sprague-Dawley rats, weighing 250 gm. at the start of the experiment, were used for the production of hypertension with desoxycorticosterone and salt. Male Wistar rats, weighing 200 gm. at the start of the experiment, were used for the production of renal hypertension. Hypertensive animals gained weight at a rate comparable to the control animals. The weights of the animals at the time of sacrifice are given in the legends of the figures.

*Diet.*—All animals were fed Purina laboratory chow *ad libitum*.

*Induction of Hypertension.*—Two methods were used:—

(a) Hypertension was induced by the subcutaneous implantation of a single 25 mg. pellet of desoxycorticosterone acetate<sup>1</sup> (DCA). The implantation site was massaged once a week. The drinking water, which was provided *ad libitum*, contained NaCl 140 m.eq./l. and KCl 35 m.eq./l. Control animals for this study received neither DCA nor salt. (b) Hypertension was also induced by the method of Wilson and Byrom (3). The right kidney was removed, and 2 weeks later, the left renal artery was constricted by the application of a silver clip 0.23 mm. in internal diameter. Control animals for this study had their right kidneys removed.

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*Measurement of Blood Pressure.*—Systolic blood pressure was measured in the foot of the unheated, unanesthetized animal by the method of Kersten *et al.* (4) with a "photoelectric tensometer." The blood pressures of the rats at the time of sacrifice are given in the figures.

*Preparation of Aorta.*—Rats were stunned and decapitated. The complete aorta was excised, and placed in ice-cold saline, when cytochrome oxidase activities only were to be measured, and in ice-cold oxygenated incubation medium when respiration was to be measured. Adherent connective tissue and most of the adventitia were removed. Care was taken to use corresponding portions from each aorta for comparative purposes. This is a matter of importance since it has been demonstrated that thoracic and abdominal portions of rat aorta show differences in oxygen uptake (5, 6) and it is possible that the variation may be continuous from the arch to the bifurcation.

*Cytochrome Oxidase Activity.*—Homogenates of aorta were prepared in glass distilled water using an all glass homogenizer. Cytochrome oxidase activities were measured manometrically at 37°C. by the method of Schneider and Potter as described in reference 7. Warburg flasks of 5 ml. capacity were used. From 6 to 15 mg. of tissue (fresh weight) were used in each flask. Activities of aorta from a control and a hypertensive animal were measured simultaneously. Three samples did not show activity, probably for technical reasons, so that equal numbers of control and hypertensive animals do not appear in all the figures. Cytochrome oxidase activity is expressed as the microliters of oxygen consumed per milligram tissue nitrogen or alkali-soluble nitrogen per hour,  $Q_{O_2}$  (N) or  $Q_{O_2}$  (ASN).

*Oxygen Consumption.*—30 to 50 mg. portions (fresh weight) of thoracic aorta were split lengthwise and placed in Warburg flasks of 5 ml. capacity, containing 0.75 ml. of medium. The medium was Krebs medium II A (8) or Krebs-Ringer-phosphate (7), containing 160 mg. glucose per 100 ml. The medium employed in each experiment is indicated in the legends of the figures. Oxygen consumption was measured by the direct method of Warburg (7) at 37°C. with 100 per cent oxygen as the gas phase. Carbon dioxide was absorbed by filter paper saturated with 0.05 ml. 2 N NaOH in the center well of the flask. After a 10 minute period of equilibration and flushing with oxygen, manometer readings were made at intervals (usually 10 to 20 minutes depending on the rate of respiration) until the end of the experiment. Oxygen uptake was linear for 3 hours, and the experiments were continued for 2 to 3 hours. Samples from a control and a hypertensive animal were measured simultaneously. In a few cases material from two animals was pooled. This is indicated in the figures. After incubation, the tissue was removed and analyzed for nitrogen and/or desoxypentose nucleic acid content (DNA). Oxygen consumption is expressed as microliters of oxygen consumed per milligram tissue nitrogen or DNA,  $Q_{O_2}^{O_2}$  (N) or  $Q_{O_2}^{O_2}$  (DNA).

*Non-Elastin, Non-Collagen Nitrogen.*—The method of Lilienthal *et al.* was used (9). Homogenates of aorta were treated with 0.05 N NaOH (final concentration) at room temperature for 18 hours. The insoluble elastin and collagen were separated from the soluble proteins by centrifugation, and washed once with alkali. The washing was combined with the first supernatant. The nitrogen contents of supernatant and residue were measured by Kjeldahl digestion followed by nesslerization. The mean of 17 values (representing separate determinations on aortas of 17 normotensive animals) obtained in this manner for collagen plus elastin nitrogen was 71.2 per cent of the total nitrogen. This is in good agreement with the value, 73.3 per cent of the dry fat-free weight, reported by Neuman and Logan by their procedure in which collagen and elastin are determined separately (10).

*Desoxypentose nucleic Acid (DNA).*—Tissue samples were homogenized and washed with 5 per cent trichloroacetic acid (TCA) at 0°C. to remove acid-soluble substances. The nucleic acids were extracted from the residue with 5 per cent TCA at 90°C. for 20 minutes. DNA was measured in aliquots of this extract by the indole reaction as modified by Keck (11).

## RESULTS

The interpretation of comparative data relating to the respiration and respiratory enzyme activity of aorta in normal and hypertensive animals is hampered by the fact that the tissue consists largely of extracellular materials such as elastic and collagen fibers and membranes, which do not contribute to the respiration. Furthermore, the proportion of these elements to cells may change during a prolonged period of hypertension. A change in the proportion of intracellular to extracellular material might be expected to alter the over-all metabolism of the tissue even if the activity of the cells were unchanged.

The total nitrogen content is a sufficient reference basis for studies of respiratory activity in many tissues, as it is proportional to the amount of metabolically active tissue in the sample. However, the total nitrogen content of aorta includes a large amount of extracellular material; therefore additional reference bases were used for calculation of the results. Some of the results were calculated on the basis of the non-elastic, non-collagen nitrogen content of the samples, which was considered to give a better measure of the "active" portion of the tissue.

The desoxyribonucleic acid (DNA) content of the samples was used as another reference basis. It has been shown that the quantity of DNA per cell is constant in all somatic cells of an animal and that the value of the constant is characteristic for each species (12-14). The DNA content of a tissue sample is, therefore, proportional to the number of cells except in special cases such as polyploidy. The use of DNA as a reference basis might make it possible to determine whether the changes in metabolic activity of aorta in hypertension are associated with increased activity of cells or to an increase in the number of cells per unit weight of aorta.

*Cytochrome Oxidase Activity.*—In the first experiment hypertension was induced in 12 animals by the administration of DCA and salt. The animals had been hypertensive for 3 months at the time of sacrifice. The 10 control animals received neither DCA nor salt and were normotensive. Cytochrome oxidase activities of homogenates of aortas of these animals were compared, and the results are summarized in Fig. 1. Samples of the same homogenate were used for measurement of cytochrome oxidase activity and nitrogen content. On the basis of total nitrogen the mean activity of aortas of hypertensive rats was 66 microliters of oxygen per mg. nitrogen per hour, while the mean activity of the normotensive group was 40. The probability ( $P$ ) that the difference between the means was the result of chance is less than 0.001. The per cent of alkali-soluble nitrogen (non-collagen, non-elastic nitrogen) was 40.4 in the hypertensive group and 28.9 in the control group ( $P < 0.001$ ). Cytochrome oxidase activities were 165 microliters of oxygen per mg. alkali-soluble nitrogen per hour for the hypertensive group, and 136 for the normotensive group ( $P < 0.05$ ). Values of  $P$  were calculated by applying the  $t$  test.

In order to make certain that the difference between the hypertensive and normotensive groups was not an effect of DCA and/or salt and unrelated to the blood pressure, a comparable study was undertaken in which rats with renal hypertension were employed. Hypertension was induced in 7 rats by removing one kidney and placing a clip on the renal artery of the remaining kidney 2 weeks later. The animals had been hypertensive for 2 to 3 months at the time of sacrifice. The 6 control animals had one kidney removed.

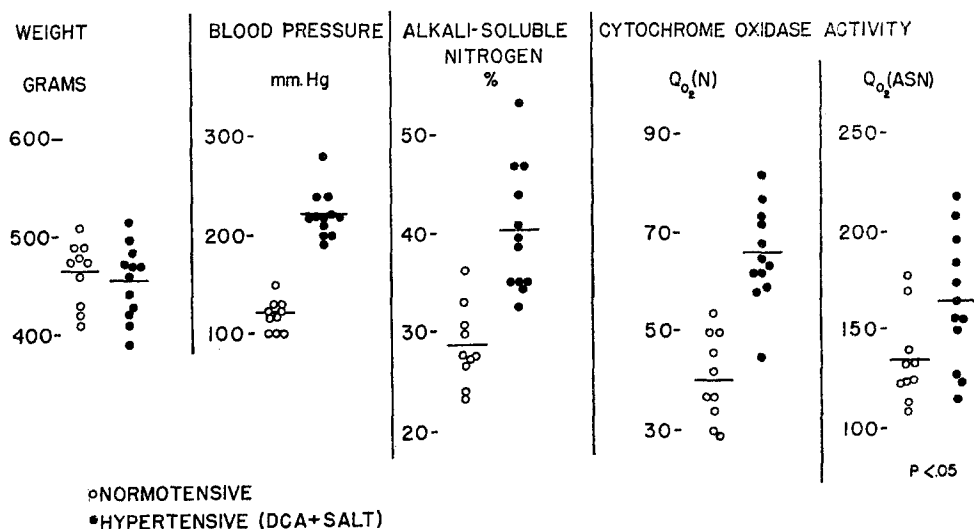


FIG. 1. Comparison of cytochrome oxidase activities of aortas of normotensive and hypertensive rats. Each circle represents one animal; horizontal lines indicate group means. Weights and blood pressures are those measured at the time of sacrifice.

Cytochrome oxidase activity, expressed as  $QO_2$  (N or ASN) = microliters of oxygen consumed per milligram total nitrogen or alkali-soluble nitrogen per hour.

$$\text{Per cent ASN} = \frac{\text{alkali soluble nitrogen} \times 100}{\text{total nitrogen}}$$

The data indicate that aortas of hypertensive rats show higher cytochrome oxidase activities per milligram total nitrogen or alkali-soluble nitrogen than aortas of normotensive rats.

The results are presented in Fig. 2. The mean cytochrome oxidase activity of homogenates of aortas of hypertensive animals was 76 microliters of oxygen per mg. total nitrogen per hour compared to 45 microliters of oxygen per mg. total nitrogen per hour for the normotensive group. The mean values for the per cent of alkali-soluble nitrogen were 35 for the hypertensive group and 27 for the normotensive group ( $P < 0.05$ ). The mean values of cytochrome oxidase activity per milligram alkali-soluble nitrogen were 212 microliters of oxygen per hour for the hypertensive group and 169 for the normotensive group ( $P < 0.05$ ).

*Oxygen Uptake.*—Changes in cytochrome oxidase activity of aorta homogenates might be expected to be correlated with changes in the respiration of strips of aorta. The respiration of thoracic aorta from hypertensive and normotensive rats was compared. In Fig. 3 values for oxygen uptake of aortas of 6 animals made hypertensive with DCA and salt are compared with those of six normotensive controls. The mean oxygen consumption of aortas of hyper-

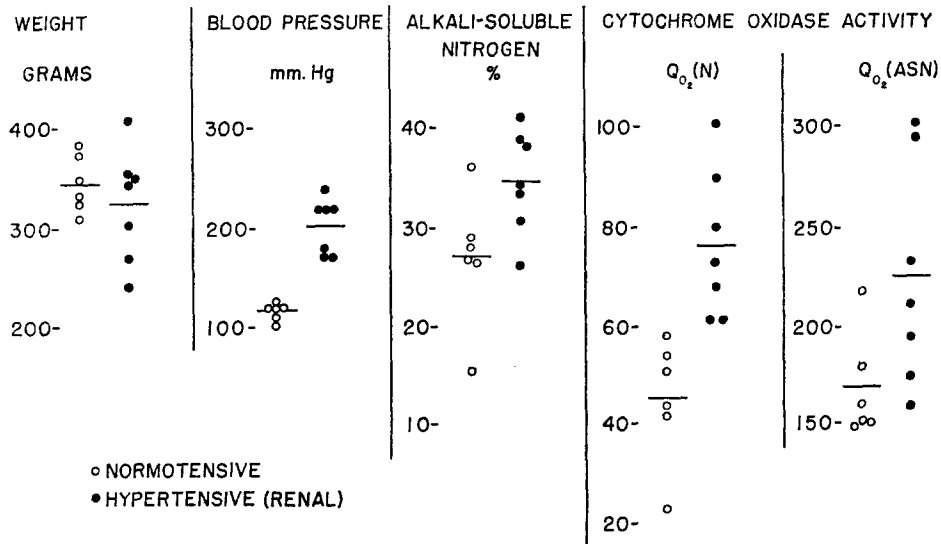


FIG. 2. Comparison of cytochrome oxidase activities of aortas of normotensive and hypertensive rats. Each circle represents one animal; horizontal lines indicate group means. Weights and blood pressures are those measured at the time of sacrifice.

Cytochrome oxidase activity, expressed as  $Q_{O_2}$  (N or ASN) = microliters of oxygen consumed per milligram total nitrogen or alkali-soluble nitrogen per hour.

$$\text{Per cent ASN} = \frac{\text{alkali soluble nitrogen} \times 100}{\text{total nitrogen}}$$

The data indicate that aortas of hypertensive rats show higher cytochrome oxidase activities per milligram total nitrogen or alkali-soluble nitrogen than aortas of normotensive rats.

tensive animals is 10.4 and of controls, 7.4 microliters of oxygen per mg. total nitrogen per hour ( $P < 0.01$ ).

In Fig. 4, values obtained for aortas of 9 renal hypertensive rats and 9 controls are compared. Only six values of oxygen uptake are shown for each group, because samples from two animals were pooled in three cases. The mean oxygen consumption was 8.1 for aortas of the hypertensive group and 5.2 for aortas of the control group ( $P < 0.01$ ).

In 18 cases oxygen consumption was calculated on the basis of DNA content in order to obtain a measure of the oxygen consumption per cell. Values for 3

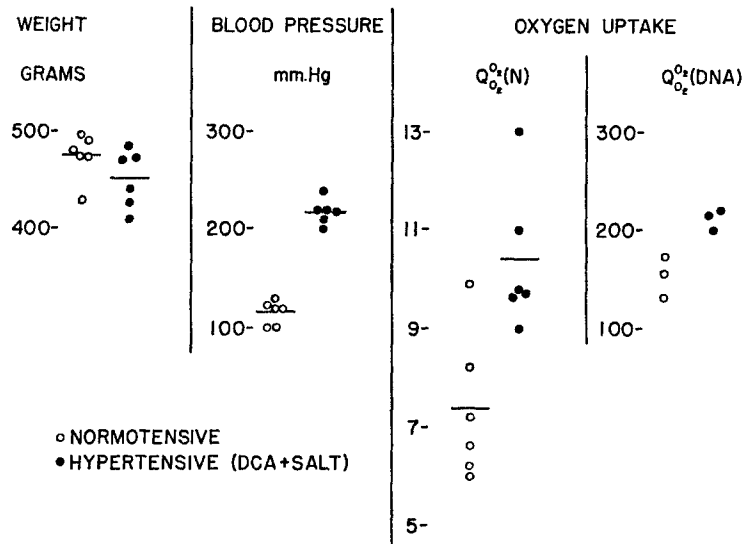


FIG. 3. Comparison of oxygen uptake of aortas of normotensive and hypertensive rats. Each circle represents one animal. Horizontal lines indicate group means. The weights and blood pressures are those measured at the time of sacrifice.

Oxygen uptake, expressed as  $Q_{O_2}^{(N)}$  (N or DNA) = microliters of oxygen consumed per milligram total nitrogen or DNA per hour in an atmosphere of 100 per cent oxygen. The medium employed was Krebs medium II A (8).

The data indicate that aortas of hypertensive rats show higher rates of oxygen consumption than aortas of normotensive rats.

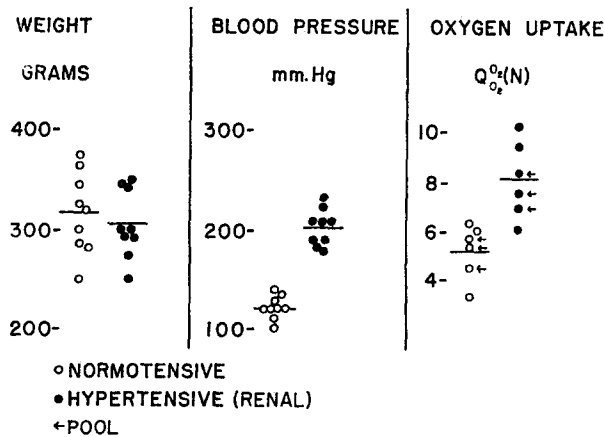


FIG. 4. Comparison of oxygen uptake of aortas of normotensive and hypertensive rats. Each circle represents one animal except for those indicated by arrows, when pools of tissue from two animals were used. Horizontal lines indicate group means. Weights and blood pressures are those measured at the time of sacrifice.

Oxygen uptake, expressed as  $Q_{O_2}^{(N)}$  (N) = microliters of oxygen consumed per milligram nitrogen per hour in an atmosphere of 100 per cent oxygen. The medium was Krebs-Ringer-phosphate (7) containing 1.6 mg. of glucose per ml.

The data indicate that oxygen uptake is higher for aortas of hypertensive rats than for those of normotensive rats.

animals with hypertension produced with DCA and salt and their controls are given in Fig. 3. The number of determinations was small, but the results suggested a difference in distribution between the two groups. In Fig. 5 are presented values for a group of 6 renal hypertensive rats and 6 controls. The mean value for the hypertensive group was 306 microliters of oxygen per mg. DNA per hour and for the control group 210 ( $P < 0.01$ ).

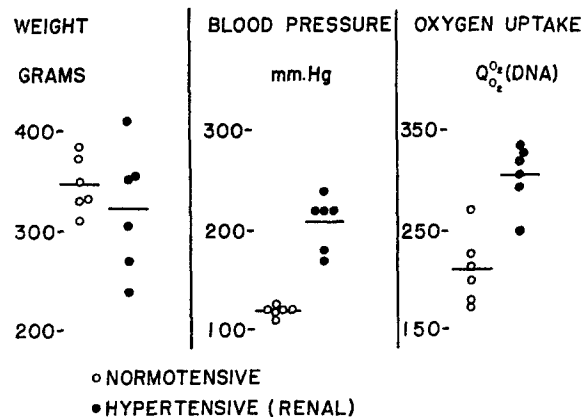


FIG. 5. Comparison of oxygen uptake of aortas of normotensive and hypertensive rats. Each circle represents one animal. Horizontal lines indicate group means. Weights and blood pressures are those measured at the time of sacrifice.

Oxygen uptake, expressed as  $Q_{O_2}^{O_2}$  (DNA) = microliters of oxygen consumed per milligram DNA per hour in an atmosphere of 100 per cent oxygen. The medium was Krebs medium II A (8).

The data indicate that oxygen consumption per milligram DNA is higher for aortas of hypertensive rats than for those of normotensive rats.

#### DISCUSSION

Villasante, Jiménez-Díaz, and Nuño reported that the respiration of arteries was not changed in rats with experimental hypertension (2). The arteries were not identified, but, if aorta was studied, there is a discrepancy between their results and those presented in this report. The lack of agreement cannot be easily explained, but may be accounted for by marked differences in experimental conditions such as the technique used to induce hypertension and the duration of the hypertension.

The present report indicates that aortas of rats which have been hypertensive for 2 to 3 months, show higher rates of oxygen uptake and cytochrome oxidase activity than aortas of normotensive rats. This was shown for both hypertension produced by DCA and salt and that produced by compression of the renal artery. These changes are associated with an increase in the proportion of alkali-soluble nitrogen of the tissue. The increase in the proportion of alkali-

soluble nitrogen is interpreted as an indication of a higher proportion of muscle cells to connective tissue fibers and membranes in aortas of hypertensive animals, a concomitant of medial hypertrophy. As medial hypertrophy is generally considered to be a work hypertrophy, it is probable that the results reported here represent largely the effects of the hypertensive process.

The higher metabolic activity of aortas of hypertensive rats is due not only to a change in the proportion of muscle cells to extracellular elements, but also to a higher activity of the intracellular portion of the tissue. This is suggested by measurements based on the non-elastic, non-collagen nitrogen content, and on the DNA content of the tissue. The difference between cytochrome oxidase activities per unit of alkali-soluble nitrogen in aortas of normotensive and hypertensive rats is of marginal significance, but the results suggest that the activity of the non-elastic, non-collagen portion of the tissue is higher in aortas of hypertensive animals. The higher oxygen consumption per unit of DNA in aortas of hypertensive rats indicates a greater oxygen consumption per cell. This can be explained by an increased activity of individual cells, by an increase in the proportion of more active cell types, or by a combination of both types of changes.

The changes reported here may not be relevant to the etiology of hypertension, but may have a bearing on the development of the vascular complications characteristic of the advanced disease. Prolonged elevation of the blood pressure may be accompanied by adverse changes in all parts of the arterial system (1). In the larger vessels, such as the aorta, the vasa vasorum penetrate into the outer layers of the media only. It is possible that with the thickening of the vessel wall in hypertension and the increase in metabolic activity, the demands of the vessel wall for oxygen and other nutrients may exceed the rate at which they can be transferred to the tissue from the blood. Such a situation might facilitate the development of degenerative changes.

#### SUMMARY

Oxygen consumption and cytochrome oxidase activity of aortas of rats with experimental hypertension were found to be higher than the corresponding values for aortas of normotensive animals. The higher metabolic activity of aortas of hypertensive animals appeared to be due both to an increase in the proportion of muscle cells to connective tissue fibers and to a higher activity of the intracellular portion of the tissue.

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