Studies on the Cardiomegaly of the Spontaneously Hypertensive Rat

By Barbara B. Farmer, Robert A. Harris, Walter W. Jolly, and William J. Vail

ABSTRACT
Changes in size, morphology, and certain relevant biochemical components of hearts from spontaneously hypertensive and normal Wistar rats were studied comparatively. Groups of rats were killed at stages which represented developing and stable cardiac hypertrophy and approaching cardiac failure. Mitochondrial content was determined by comparing cytochrome oxidase activity per milligram of heart homogenate protein with cytochrome oxidase activity per milligram of heart mitochondrial protein. Based on the relationship of heart weight and body weight found in normotensive rats (hearts weight [mg] = 1.85 x body weight [g] + 287), left ventricular dimensions, and micrographs of left ventricular muscle fibers, hypertrophy of the hearts from the spontaneously hypertensive rats was conclusively demonstrated. Approximately 25% of the total heart protein was mitochondrial in both control and hypertensive rats; the percent increased during the period of rapid growth but subsequently decreased with age. The amount of heart mitochondrial protein was greater for spontaneously hypertensive rats relative to body weight but was not greater relative to heart weight. The maximum increase in heart mitochondria relative to body weight was observed in 6-month-old spontaneously hypertensive rats, whereas the maximum increase in heart weight occurred at 12 months of age. A decrease in heart weight occurred in 17-month-old spontaneously hypertensive rats. No preferential retention of mitochondria was apparent during atrophy induced by starvation in either control or hypertensive rats.

KEY WORDS
cytochrome oxidase
deoxyribonucleic acid
cardiac hypertrophy
heart mitochondria
hypertension

Hypertension, accompanied by an enlargement of the heart, can occasionally be related to cardiovascular, neurogenic, endocrine, or renal lesions. However, in 90% of the cases, the etiology of the hypertension is unknown. In his search for the animal model that would most nearly approximate essential hypertension in man, Okamoto (1) has developed, through inbreeding, a strain of Wistar rats known as spontaneously hypertensive rats. Without exception these rats develop hypertension with age; this hypertension is accompanied by cardiomegaly in stages paralleling its development in man. Advantageously, in this animal model the entire life history of the disease is compressed within a period of less than 2 years.

A number of investigators (2-12) have studied in some detail the biochemical changes which occur in induced cardiomegaly. However, the molecular mechanisms and signals responsible for cardiac enlargement in response to an increase in work load remain undefined (13). There is good reason to suspect that the energy-conserving organelles (mitochondria) of the heart are fundamentally involved in the compensatory mechanism which enables the heart to adapt to a greater work load (14); for example, an acute overload of the heart triggers an increase in mitochondrial formation out of proportion to the biogenesis of other cellular components (15). Meerson and Pomointsky (14) have proposed that an increase in the work load of the heart effects a decrease in the steady-state phosphate potential of the cardiac muscle cells which, in turn, activates a compensatory mechanism for cardiac hypertrophy. The purpose of the present study was to investigate the mitochondria of the heart of the spontaneously hypertensive rat at various stages of developing hypertrophy and approaching cardiac failure.

Methods

ANIMALS
Spontaneously hypertensive rats of the Okamoto-Wistar strain were obtained from the colony of rats maintained by the Specialized Center for Research in Hypertension at Indiana University School of Medicine.
Normotensive Wistar rats were obtained from a local supplier. Only male rats were used. Systolic blood pressure was determined using a tail cuff and a mercury manometer.

PREPARATION OF HOMOGENATES AND MITOCHONDRIA

Heart mitochondria were prepared by a modification of the method of Chao and Davis (16). Rats were either lightly anesthetized with ether or killed by decapitation after cervical dislocation. Their hearts were quickly removed and placed in an ice-cold solution of 0.25M sucrose containing 10 mM ethylenediaminetetraacetate (EDTA), pH 7.5. The ventricles were trimmed of atria and great vessels, weighed, and measured for length. The heart was sectioned transversely midway between the aortic root and the apex of the left ventricle. The thickness of the left ventricular wall, the diameter of the left ventricular cavity, and the width of the heart was measured.

The hearts were then finely minced and suspended in ice-cold sucrose-EDTA medium. Each heart was homogenized separately on ice using a Brinkman Instruments Polytron for 10 seconds at low speed and then for 4 seconds at full speed. A sample of the homogenate was removed and used for determinations of protein content by the Biuret method (17) and cytochrome oxidase and adenosine triphosphatase (ATPase) activities.

The remainder of the homogenate was centrifuged at 1,000 g for 10 minutes. The supernatant fluid obtained was centrifuged at 12,000 g for 10 minutes. For 2- and 4-month-old rats, supernatant fluid from four hearts had to be combined to obtain adequate quantities of homogenate and each mitochondrial preparation. The homogenate was kept on ice for 1 hour to remove contaminating actomyosin. The suspensions were then centrifuged at 12,000 g for 10 minutes. For 6- and 12-month-old rats, two hearts had to be combined for each mitochondrial preparation. The resulting mitochondrial pellets were suspended in 20 ml of 0.6M KCl and 5 mM imidazole, pH 7.4, and allowed to stand on ice for 1 hour to remove contaminating actomyosin. The suspensions were then centrifuged at 10,000 g for 10 minutes. The supernatant fluid was discarded and each pellet was suspended in a small volume of sucrose-EDTA medium. The pellets were "cut" at each step with a glass rod in favor of the mitochondria.

DETERMINATION OF THE MITOCHONDRIAL CONTENT OF THE HEARTS

The mitochondrial content of the heart was determined by measuring the activity of the mitochondrial enzyme, cytochrome oxidase, in both the total heart homogenate and each mitochondrial preparation. The proportion of the heart protein that was mitochondrial could then be calculated by the following equations:

\[
\text{Mitochondrial protein (mg)} = \frac{\text{Cytochrome oxidase of heart (units)}}{\text{Cytochrome oxidase of mitochondria, (units/mg protein)}} \times 100 \times \text{total mg of mitochondrial protein} / \text{total mg of heart protein.} \tag{1}
\]

Cytochrome oxidase activity was measured by the method of Griffiths and Wharton (18) except that oxygen consumption was measured polarographically rather than manometrically. Prior to assay, both the mitochondrial preparations and the homogenates were treated for 15 minutes on ice with sodium deoxycholate (1 mg/mg protein), pH 8.0; this procedure fostered maximum activation. The incubation medium for determining cytochrome oxidase had a total volume of 5 ml and contained 20 mM potassium phosphate, pH 7.2, 0.1 mM EDTA, 0.05 mM cytochrome C, 20 mM sodium ascorbate, 6 μg of rotenone, and either 2 mg of homogenate protein or 0.4 mg of mitochondrial protein. Linearity with time and protein concentration was established. Oxygen uptake was measured at 30°C using a Beckman oxygen analyzer with a Clark type of oxygen electrode. This method of determining cytochrome oxidase activity gave reproducible results and consistently low standard errors of the mean. All determinations were conducted in duplicate. In addition, at the end of each series of assays, those done first were rechecked; the results had not changed because of storage on ice. Hearts from both control and hypertensive rats were always homogenized, activated, and assayed on the same day.

The reaction mixture for determining the rate of state-3 respiration with intact mitochondria contained 0.25M sucrose, 5 mM Tris-Cl, pH 7.4, 10 mM potassium phosphate, pH 7.4, 5 mM pyruvate, 5 mM malate, 0.4 mM adenosine diphosphate (ADP), and 1 mg mitochondrial protein/ml. Oxygen uptake was measured at 30°C using a Beckman oxygen analyzer with a Clark oxygen electrode.

DETERMINATION OF HEART DNA

Deoxyribonucleic acid (DNA) was extracted from the 1,000-g pellets of the total heart homogenates by the method of Sasaki et al. (19) and determined by the method of Burton (20). The total DNA content of the hearts was calculated from values derived from standards prepared with Sigma's best grade DNA from calf thymus.

ATPase ACTIVITY

The reaction mixture (1 ml) for determining ATPase activity contained 50 μmoles of Tris-Cl, pH 8.5, 4 μmoles of MgCl₂, 1 mg sodium cholate/mg protein, 2.5 μg of oligomycin (when present), and either 2 mg of total homogenate protein or 1 mg of mitochondrial protein. The reaction mixtures were incubated at 37°C for 3 minutes. The reaction was initiated by adding 10 μmoles of adenosine triphosphate (ATP) and was stopped after 3 minutes by adding 600 μmoles of perchloric acid. The inorganic phosphate (P₄) released was measured by the method described by Lindberg and Ernster (21).

DETERMINATION OF CYTOCHROMES A, B, AND C

The spectrums of reduced vs. oxidized cytochromes of the mitochondria from 1-year-old spontaneously hypertensive and normotensive rats were measured by the method of Rieske (22). The mitochondria were suspended in 0.1M Tris-Cl, pH 8, to a final protein concentration of 1 mg/ml. Antimycin A (1 μg/mg protein), potassium deoxycholate (0.5 mg/mg protein), and potassium cholate (1 mg/mg protein) were added. The oxidized state of the cytochrome was produced by adding ferricyanide (3 μmoles) and cyanide (6 μmoles). Spectra were recorded with an Amino-Chance double-beam spectrophotometer equipped with a chamber cooled to 0-5°C. From the spectrum obtained after reduction with sodium ascorbate, the concentrations of cytochromes C (+ C₅) were calculated. The concentration of cytochrome B was determined by complete reduction with dithionite.
PREPARATION OF MICROGRAPHS

The hearts used for micrographs were rapidly removed following decapitation of the rats, quartered on dental wax with a razor blade, and fixed in Bouin's solution (75% saturated picric acid, 5% glacial acetic acid, and 9% formaldehyde in water). Sections were stained with hematoxylin and eosin.

STARVATION OF RATS

In the starvation experiments, food was removed from the cages for 6 days prior to killing; water was given freely. Control rats with similar initial body weights were fed ad libitum during the 6 days.

STATISTICAL ANALYSIS OF THE DATA

Regression lines for the ratios of heart weight to body weight and heart mitochondrial protein to body weight in normotensive rats were determined by the least-squares method using a linear regression program. Confidence limits (95%) for this regression line at any value of x are given by \( Y = Y_e + 2SEY_e \). Deviation of the data for the hypertensive rats above these lines was analyzed for statistical significance by a one-tailed Student's t-test. Other results were analyzed for statistical significance by a two-tailed Student's t-test.

Results

EVIDENCE FOR CARDIAC HYPERTROPHY IN SPONTANEOUSLY HYPERTENSIVE RATS

Growth curves and blood pressure data are plotted for normotensive and spontaneously hypertensive rats in Figure 1. As reported by others (23, 24), the spontaneously hypertensive rat is considerably smaller at maturity than the normotensive Wistar rat. This differential in body weight was not found in the original studies by Okamoto and Aoki (25). The blood pressure of spontaneously hypertensive rats was elevated significantly at 2 months of age and rose steadily until it reached 235 ± 11 mm Hg at 12 months of age. The normotensive rats underwent a significant increase in blood pressure only at 17 months of age.

A linear regression program determined by the least-squares method using 43 normotensive rats showed that the relationship of heart weight and body weight could be expressed by the equation:

Heart weight (mg)  
= 1.85 \times \text{body weight (g)} + 287. \ (3)

This result confirms the linear relationship between body weight and heart weight of the rat reported by Tolnai and Beznak (26). The values for the slopes and intercept are similar to those previously reported (26) for another strain of rats. Confidence limits (95%) for the line were determined (Fig. 2A).

In striking contrast, the hearts of the spontaneously hypertensive rats were greatly enlarged relative to the rats' body weights. For example, at 12 months of age, the weight of hearts from normotensive rats averaged 2.13 ± 0.07 mg/g body weight, whereas the weight of hearts from spontaneously hypertensive rats averaged 3.69 ± 0.07 mg/g body weight. Thus, as seen in Figure 2A, the spontaneously hypertensive rats deviated markedly from the linear relationship between body weight and heart weight established for normotensive rats. A significant increase in heart size relative to body weight was noted at all ages (\( P < 0.02 \)); the maximum increase of 42% was noted in 12-month-old rats (\( P < 0.001 \)). The hearts of 17-month-old spontaneously hypertensive rats were significantly larger on a body weight basis than those of normotensive rats (\( P < 0.01 \)) but smaller (\( P < 0.1 \)) than those of 12-month-old spontaneously hypertensive rats (see Discussion). The difference in heart size between normotensive and spontaneously hypertensive rats was confirmed by determination of total heart protein. For example, at 12 months of age, normotensive and spontaneously hypertensive rats had 0.39 ± 0.01 mg heart protein/g body weight.
Deviation with age of the spontaneously hypertensive rats from normotensive rats with respect to the relationship of (A) heart weight to body weight and (B) heart mitochondrial protein to body weight. Age in months at the top of the figure refers only to spontaneously hypertensive rats. A: Solid line represents the relationship of heart weight to body weight in 43 normotensive rats determined by the least-squares method using a linear regression program. The relationship is expressed by the equation heart weight (mg) = 1.85 × body weight (g) + 287. The dotted lines represent the 95% confidence limits for the regression line. The solid circles represent the means of hypertensive rat heart weights at ages 2, 4, 6, 12, and 17 months. Vertical bars represent SE. At all points N = 8. At all ages the weight of the hypertensive rat hearts was significantly greater on a body weight basis (P < 0.02) than that of the normotensive rat hearts.

B: Solid line represents the relationship of heart mitochondrial protein to body weight in 43 normotensive rats determined by the least-squares method using a linear regression program. The relationship is expressed by the equation heart mitochondrial protein (mg) = 0.075 × body weight (g) + 17.8. The dotted lines represent the 95% confidence limits for the regression line. The solid circles represent the means of mitochondrial protein content for the hypertensive rat hearts at 2, 4, 6, 12, and 17 months. Vertical bars represent SE. At each point eight rats were used, but because of the small size of the hearts from younger rats they were pooled to prepare mitochondria; therefore, N = 2 at 2 and 4 months, N = 4 at 6 and 12 months, and N = 8 at 17 months. At 6 months there was significantly more (P < 0.01) mitochondrial protein in relation to body weight.
Microscopic changes in the form of enlarged muscle fibers were readily apparent in sections of left ventricular tissue from spontaneously hypertensive rats examined by light microscopy (Fig. 4). The diameter of the muscle fibers was approximately 20 μ for 12-month-old normotensive rats compared with approximately 30 μ for 12-month-old spontaneously hypertensive rats.

MITOCHONDRIAL CONTENT OF THE HYPERTROPHIED HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS

The cytochrome oxidase activity of mitochondria prepared from normotensive and hypertensive rats was identical at all ages studied. For example, the activities were $2.23 \pm 0.12 \mu$ atoms oxygen consumed/min mg$^{-1}$ protein and $2.27 \pm 0.12 \mu$ atoms oxygen consumed/min mg$^{-1}$ protein for 12-month-old normotensive and hypertensive rats, respectively ($N = 4$). Likewise, the total ATPase activities and state-3 rates of respiration with pyruvate plus malate were identical for mitochondrial preparations from these rats (data not presented). Spectrums of the cytochromes (reduced vs. oxidized) for mitochondria prepared from normotensive and spontaneously hypertensive rats were superimposable. Therefore, the use of Eqs. 1 and 2 appears justified; the results using this method are given in Table 1. The amount of mitochondrial protein in the heart on a body weight basis was greater for spontaneously hypertensive rats in each age group. However, when mitochondrial protein was expressed as a ratio to either

### Table 1

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Type of animal</th>
<th>Body wt (g)</th>
<th>Heart wt (mg)</th>
<th>Heart protein (mg)</th>
<th>Heart mitochondrial protein (mg/heart)</th>
<th>Heart mitochondrial protein (mg/g body wt)</th>
<th>Heart mitochondrial protein (mg/g heart wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Normotensive</td>
<td>179 ± 3</td>
<td>577 ± 16</td>
<td>101 ± 2</td>
<td>28 ± 1</td>
<td>0.157 ± 0.004</td>
<td>48 ± 1</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>194 ± 4*</td>
<td>728 ± 23*</td>
<td>123 ± 11</td>
<td>35 ± 1†</td>
<td>0.181 ± 0.004</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>Normotensive</td>
<td>316 ± 5</td>
<td>915 ± 24</td>
<td>144 ± 4</td>
<td>44 ± 1</td>
<td>0.139 ± 0.003</td>
<td>48 ± 1</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>280 ± 7*</td>
<td>926 ± 30</td>
<td>152 ± 9</td>
<td>48 ± 3</td>
<td>0.174 ± 0.007†</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>Normotensive</td>
<td>506 ± 14</td>
<td>1230 ± 60</td>
<td>216 ± 12</td>
<td>62 ± 6</td>
<td>0.120 ± 0.005†</td>
<td>51 ± 3</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>345 ± 9*</td>
<td>1112 ± 24</td>
<td>190 ± 3</td>
<td>59 ± 2</td>
<td>0.171 ± 0.004*†</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>12</td>
<td>Normotensive</td>
<td>618 ± 32</td>
<td>1307 ± 44</td>
<td>239 ± 9</td>
<td>60 ± 3</td>
<td>0.098 ± 0.006</td>
<td>46 ± 2</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>369 ± 10*</td>
<td>1359 ± 29</td>
<td>234 ± 18</td>
<td>52 ± 4</td>
<td>0.142 ± 0.007†</td>
<td>38 ± 2†</td>
</tr>
<tr>
<td>17</td>
<td>Normotensive</td>
<td>622 ± 16</td>
<td>1547 ± 56</td>
<td>281 ± 11</td>
<td>65 ± 3</td>
<td>0.106 ± 0.003†</td>
<td>42 ± 1</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>395 ± 14*</td>
<td>1243 ± 60†</td>
<td>215 ± 9†</td>
<td>48 ± 2*</td>
<td>0.123 ± 0.005†</td>
<td>39 ± 2†</td>
</tr>
</tbody>
</table>

Groups of eight normotensive and eight hypertensive male rats were used at each age. For the smaller rats the hearts were pooled for the preparation of mitochondria; thus, $N = 2$ at 2 and 4 months, $N = 4$ at 6 and 12 months, and $N = 8$ at 17 months for the mitochondrial protein, although all values represent the mean for eight rats of each type at each age.

* $P < 0.001$.
† $P < 0.05$. 

Circulation Research, Vol. 35, July 1974
CARDIOMEGALY IN THE HYPERTENSIVE RAT

heart weight or total heart protein, the ratio was not significantly greater in the hypertensive rats (Table 1). When the ratio of heart mitochondrial protein to body weight of normotensive rats (N = 43) was analyzed on a linear regression program, the relationship could be expressed by the following equation:

Heart mitochondrial protein (mg) = 0.075 \times \text{body weight (g)} + 17.8. \ (4)

The mitochondrial content of hypertensive rat hearts deviated from this relationship. At 6 months of age there was a significant increase in the mitochondrial content of the hypertensive hearts on a body weight basis (N = 4, P < 0.01) (Fig. 2B); at 17 months of age the mitochondrial content had decreased to within normal limits. Therefore, the maximum increase in mitochondria in the 6-month-old hypertensive rats appeared to precede the maximum hypertrophy of their hearts observed at 12 months of age (Fig. 2A and B).

DNA IN HEARTS OF NORMAL CONTROL RATS AND SPONTANEOUSLY HYPERTENSIVE RATS

The total DNA content of both groups of rats increased rapidly up to 6 months of age. Thereafter, the DNA content of the hypertensive rat hearts (1098 \pm 33 \mu g/heart) did not change and the DNA content of the normotensive rat heart increased slightly (from 1206 \pm 29 \mu g/heart to 1450 \pm 50 \mu g/heart, N = 8).

STARVATION-INDUCED ATROPHY OF THE HEARTS OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

Starvation of normotensive rats produced a significant decrease in the wet weight, total protein, and total mitochondrial protein of the heart (Table 2). On the basis of published studies on skeletal muscle (27), we thought that the hearts of spontaneously hypertensive rats would be affected differently by starvation. However, we observed that the hearts of spontaneously hypertensive rats were induced to atrophy under conditions of starvation (Table 2). The decreases in heart weight, total heart protein, and total heart mitochondrial protein content noted for spontaneously hypertensive rats were similar to those noted for normotensive rats. Starvation produced a significant decrease in blood pressure only in the spontaneously hypertensive rats.

Discussion

The stages in the development of cardiac hypertrophy in the spontaneously hypertensive rats paralleled the stages outlined by Fizelova and Fizel (28) in their studies of cardiac hypertrophy induced by aortic insufficiency in rabbits. These stages are (1) developing cardiac hypertrophy (0–12 months), (2) relatively stable cardiac hypertrophy (12 months), (3) relative cardiac atrophy (17 months), and (4) presumably, failure (> 17 months).

Meerson et al. (15) have reported a preferential induction of mitochondrial protein that precedes an increase in other muscle cell components in response to an acutely induced work overload of the heart. In our study, when the total milligrams of mitochondrial protein from spontaneously hypertensive rat hearts of each age group were compared with those from the hearts of normotensive rats of the same body weight there was a 43% increase in mitochondrial protein above normal values in the normotensive rats (N = 4, P < 0.01) (Fig. 2B). This peak of mitochondrial protein preceded maximum hypertrophy which occurred at 12 months. By 17 months there was no difference in mitochondrial protein content on this basis between normotensive and hypertensive rats. These results suggest preferential accumulation of mitochondria at 6 months of age. However, this conclusion is tenuous because the slower growth rate of the spontaneously hypertensive rats greatly complicates the interpretation. In addition, a statistically significant increase in the quantity of mitochondrial protein expressed on the basis of total heart protein was not observed when normotensive and hypertensive rats of the same age were compared. Other investigators have not found an
increase in mitochondria when hypertrophy of the heart has been induced slowly. The development of compensatory hypertrophy in response to a work overload is a dynamic process; if mitochondrial induction precedes an increase in myofibrillar protein, it appears difficult to unequivocally separate the two processes.

The dynamic nature of the changes in the heart of the spontaneously hypertensive rat with age has recently been shown in terms of hemodynamics and myocardial function by Pfeffer and Frolich (24). They have found a hyperkinetic state in the young (2-3 months) spontaneously hypertensive rat manifested by an increase in heart rate, stroke and minute work, and peak flow velocity and by a maximum acceleration of flow. Between 4 and 8 months of age, the total peripheral resistance in the spontaneously hypertensive rat increases, and maximum acceleration of flow, peak flow velocity, and stroke work cease to have different values from those measured in control rats. Thus a transition from a hyperkinetic state to a pressure-overloaded state occurs. It was during this transition (6-month-old rats) that we measured the maximum increase in mitochondrial protein with respect to body weight. The reduction in the index of myocardial contractility found by Pfeffer and Frolich (24) in these oldest rats (15-24 months) paralleled our findings of a return to normal amounts of mitochondrial protein in 17-month-old rats and a fall in the ratio of heart weight to body weight from the level at 12 months (see Fig. 2A and B).

Our data showed that the mitochondrial mass varied from 22.5% to 31.9% of total heart protein (38 to 54 mg mitochondrial protein/g wet weight). Rabinowitz et al. (29), on the basis of electron microscopic analysis, have reported that the mitochondrial mass is 20% of the total heart mass. Page et al. (7), using special techniques with electron micrographs, have reported that the mitochondrial content of normal rat hearts is 35%. In contrast, Fizelova and Fizel (30) have reported only 3.8-7.3 mg mitochondrial protein/g wet weight heart in rabbits. This difference is probably not species related but rather due to the fact that, in the preparation of heart mitochondria, a yield of only about 10% is realized. The results of Fizelova and Fizel (30) were based on total yield, whereas our data were based on activity of the mitochondrial enzyme, cytochrome oxidase. In both normotensive and hypertensive rats, the mitochondrial content was greatest on a percent basis during the periods of rapid heart growth. In the hypertensive rat heart growth would represent a response to the greater work load caused by increased peripheral resistance, whereas in the normotensive rat it would represent a response to the greater work load imposed by the rapidly increasing body size. By definition, this heart growth would not be hypertrophy in the normotensive rat, since the heart increased proportionately with body size. In spite of increases in heart weight, the DNA content of the spontaneously hypertensive rat heart did not change after 6 months of age. This finding suggests hypertrophy and atrophy as opposed to hyperplasia and necrosis; however, since it is impossible to differentiate biochemically between interstitial cell DNA and muscle cell DNA, the data are inconclusive.

Although many hypotheses have been proposed regarding the signals for cardiac hypertrophy, the exact mechanism is still unexplained. Rabinowitz and Zak (31) have summarized current notions as follows: (1) increased work demands might lead to local tissue anoxia or depletion of energy stores with accumulation of materials causing genetic activation, (2) increased wall tension or hypoxia might lead to macromolecular breakdown and a similar accumulation of control molecules, (3) stretching of the muscle cells secondary to enhanced preload or afterload might activate growth processes, and (4) humoral or hormonal factors might be involved.

With respect to this last hypothesis, low insulin levels or low ratios of insulin to glucagon have been shown to signal protein breakdown for gluconeogenesis (32, 33). Under such conditions, muscles which have been actively used are spared. Indeed, Largis et al. (34) have shown that the hearts of the spontaneously hypertensive rats are apparently deficient in receptors for glucagon. Less sensitivity to glucagon and, thereby, relatively more sensitivity to insulin might contribute to the preferential growth of heart muscle over other muscle in the spontaneously hypertensive rat. We attempted to test this hypothesis in our starvation experiments. Skeletal muscle is known to undergo considerable atrophy on starvation. Likewise, Addis et al. (35) have reported that the hearts of rats starved acutely for 7 days lose 18% of their original protein. This loss is an indispensable response for survival, because muscle protein becomes the major source of carbon for gluconeogenesis during periods of starvation. In the soleus muscle, the loss has been found to be blocked by work overload (27). Thus, it would not have been surprising if the pressure overload in the hearts of the hypertensive rats had blocked starvation-induced atro-
phy. However, this blockage was not found. Instead, equal loss of total heart protein and mitochondrial protein occurred in normotensive and hypertensive rats. During starvation, the ratio of glucagon to insulin should be large, and catabolic events would be expected to predominate over synthetic processes such as protein synthesis. Thus, these results suggest that hypertrophy of the hearts of spontaneously hypertensive rats is probably not a consequence of decreased sensitivity to glucagon. Likewise, the results demonstrate that the signal(s) provided by starvation for atrophy to maintain a source of carbon for gluconeogenesis is not overridden by the signal(s) provided by pressure overload for hypertrophy. These results also indicate that hypertrophy is probably not a consequence of suppressed degradation. Therefore, by elimination, hypertrophy must be a result of an increased rate of synthesis of most, if not all, components of the heart.

The starvation experiments are of interest also, because they implicate the heart as a source of carbon for gluconeogenesis. However, these experiments do not provide any insight into the signals responsible for cardiac hypertrophy. Meerson and Pomoïnitsky (14) have suggested that pressure overload produces a greater energy demand on the heart which, in turn, produces a lower intracellular phosphate potential \((ATP/ADP \times P_i)\). They have also suggested that a decreased phosphate potential is a signal for greater synthesis of mitochondria to meet the demand for greater amounts of ATP. We suggest that other cellular components increase only after the energy crisis has been solved by a proliferation of mitochondria. Some evidence for preferential accumulation of mitochondria in the hypertrophied heart of the spontaneously hypertensive rat was noted in this study. However, during acute starvation of the hypertensive rats, no preferential retention of mitochondria was apparent. If the Meerson model is valid, pressure overload of the hypertensive rat heart should influence cytoplasmic phosphate potentials.

The extent of cardiac hypertrophy decreased rather markedly in the oldest spontaneously hypertensive rats studied. This phenomenon has also been reported to occur with time in rabbits with hypertrophied hearts induced by aortic insufficiency (28, 30) and in rats with hypertrophied hearts induced by aortic stenosis (9). This decrease in the extent of cardiac hypertrophy might indicate approaching cardiac failure in these animals as a result of the inability of the heart to continue to cope with the pressure overload. The spontaneously hypertensive rat in the terminal stages of hypertension might be a useful model for the pressure-overloaded failing heart.

Acknowledgment

We thank Mrs. Jennifer Anderson and Dr. Ronald R. Beck for their assistance with the statistical analysis of the data and Dr. Allan Katzberg for his valuable comments.

References

5. MEERSON FA, POMOÎNITSKY VD: Role of high-energy phosphate compounds in the development of cardiac hypertrophy. J Physiol (Lond) 200:285-294, 1969

Circulation Research, Vol. 35, July 1974
Studies on the Cardiomegaly of the Spontaneously Hypertensive Rat
BARBARA B. FARMER, ROBERT A. HARRIS, WALTER W. JOLLY and WILLIAM J. VAIL

Circ Res. 1974;35:102-110
doi: 10.1161/01.RES.35.1.102

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/35/1/102

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/