Capacity of the Terminal Vascular Bed during Normal Growth, in Cardiomegaly, and in Cardiac Atrophy

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ABSTRACT

Vascular capacity representing the terminal vascular bed was determined by albumin 131I in the rabbit myocardium during normal and pathological growth of the heart. A considerable increase in the vascular capacity in the first postnatal weeks indicated growth of the terminal vascular bed during this period. Highest values were reached in animals approximately 6 weeks old. From this time, vascular capacity gradually decreased in relation to the increase in heart and body weight. The growth rate of the terminal vascular bed was very rapid during the first postnatal weeks and later became slower; no growth was detected in adult and old animals. Growth of the terminal vascular bed during pathological increase in heart weight followed the same trend. In young animals pathological growth of the heart was accompanied by an increase in the capacity of the terminal vascular bed; in adult animals the total capacity remained unchanged. A decrease in heart weight in animals kept on a protein-free diet was characterized by a relatively small vascular capacity.

ADDITIONAL KEY WORDS development of vascular capacity experimental aortic stenosis protein-free diet coronary blood volume cardiac atrophy rabbit

Capillary density is one of the most important factors determining tissue oxygen tension. An increase of 70% in the intercapillary distance results in a decrease of oxygen tension in the tissue below the critical level if it is not compensated for by an increase in blood flow, a decrease in oxygen consumption, or an increase of oxygen utilization (1, 2). The importance of the capillary density is even more pronounced in the heart muscle, where the possibility of a decrease of oxygen consumption and of an increase in oxygen utilization is limited. The myocardial oxygen extraction ranges from 70 to 75%, which is greater than that of any other organ. It is very constant over a wide range of arterial oxygen saturations and does not change even with exercise (3).

Most information on capillary blood supply in the heart muscle is derived from morphological observations (4-6). Three years ago, Myers and Honig published a method for the estimation of the capillary density according to the capacity of the terminal vascular bed (7). We used this method in the present study.

In previous morphological studies on the growth of capillaries and muscle fibers in
the normal heart muscle, we found that during the first postnatal weeks normal heart growth is accompanied by a rapid increase in the number of capillaries. Later, however, the number remains constant, and the diffusion distance increases with the growth of individual muscle fibers. From these data we formulated the hypothesis that abnormal growth of the heart probably follows the same trend; in very young animals it is characterized by increase in number of capillaries as well as in size and number of muscle fibers, whereas in cardiomegaly produced in adult animals only muscle fibers grow and vascular capacity therefore diminishes (8, 9). Cardiomegaly is defined as an increase in heart weight that could be due to hypertrophy, hyperplasia or to a combination of both.

In the present study, morphological observations on the developmental changes of the capacity of the terminal vascular bed, here referred to as vascular capacity, were obtained by the isotope method. Finally, the vascular capacity of the large hearts produced by experimental aortic stenosis in rabbits of different age and that of the small hearts of rabbits on a protein-free diet was compared. It has been previously reported that protein-free diet leads to a decrease in cardiac weight.

Methods

Animals were anesthetized with sodium pentobarbital (Nembutal 25 mg/kg). The capacity of the terminal vascular bed was determined by a modification of the method of Myers and Honig (7). Albumin 131I (Albumotope, Squibb) was injected intravenously (5 μc/kg). After 90 sec, two blood samples of 1 ml each were withdrawn from the left ventricular cavity. The heart was stopped with saturated KCl solution and frozen in liquid nitrogen. Samples from the apical part of the left ventricle and septum were taken. Blood on the surface and all visible vessels were removed together with a thin layer of muscle tissue. They were weighed, placed in 2N KOH and allowed to digest overnight. The following day they were counted in a well counter with a standard error of less than 2%. Results were calculated as activity per gram of tissue divided by the activity per milliliter of blood and expressed as milliliters of vascular blood per 100 g of tissue. It is assumed here that under the conditions described above, the hematocrit of capillary blood was uniform and that plasma skimming did not enhance the oxygen-carrying capacity of blood reaching the myocardium (7).

Cardiomegaly was produced in rabbits as the result of experimental aortic stenosis by a method that has been described previously (10). Animals were anesthetized with sodium pentobarbital, 25 mg/kg and artificially ventilated. An aluminum clamp with adjustable diameter was placed around the ascending aorta approximately 2 mm above the origin of the coronary arteries. After measuring the external diameter of the aorta, its lumen was reduced to no more than 60% of its original external diameter. A decrease in the heart weight was produced by maintaining animals on a protein-free diet which was purchased through General Biochemicals, Chagrin Falls, Ohio. Ingredients: starch 70%; vegetable oil 10%; salt mix 4%; non-nutritive fiber 15%; cod liver oil (vitamins A and D) 1%.

Experiments were performed on 48 normal rabbits, which were divided into six groups according to their body weight and age as indicated in Table 1. Rabbits in the youngest group were 3 to 4 weeks old and those in the oldest group were over 2 years of age.

Animals with experimental cardiomegaly were divided into two groups according to age at which experimental cardiomegaly was produced. Aortic stenosis was performed in 7 animals, 3 to 4 weeks old, and in 8 adult animals. The last group consisted of adult animals maintained on a protein-free diet for 65 days (7 animals, Table 2).

Results

Normal Growth of the Heart Muscle.—Results are summarized in Table 1 and Figure 1.
There was a considerable increase in vascular capacity in the first postnatal weeks ($P < 0.01$), indicating growth of the terminal vascular bed during this period. The highest values were reached in animals approximately 6 weeks old. After this, vascular capacity gradually decreased in relation to the increase of body weight. This relation is linear and can be described by the equation defined by the method of least squares (11):

$$Y = -0.976 X + 10.764,$$

where $Y$ is the mean value of the capacity of the terminal vascular bed in milliliters of blood per 100 g of tissue and $X$ the body weight in grams.

In all age groups except the youngest and oldest, the vascular capacity was significantly higher in the apex than in the septum (Table 1).

### Influence of Cardiomegaly (Table 2, Figs. 1 and 2).

In the animals in which experimental aortic stenosis was produced, death occurred after an average interval of 4 weeks in the young and after 6 weeks in the adult. In both groups, the ratio of the combined weight of left ventricle and septum to body weight was at least 18% higher than in the control animals. The incidence of death and unsuccessful operations was considerably higher (about 50%) in the young animals. The average increase in left ventricular weight was 31% in young animals and 37% in adults. There was no change in the vascular capacity in young animals, but in adults it was significantly lower.

### Effect of Protein-Free Diet (Table 2, Figs. 1 and 2).

In animals maintained on the protein-free diet, heart weight decreased. During 65 days on the diet, the average body weight decreased from 2,214 g to 1,533 g, and the relative heart weight of the left ventricle and septum was 18% lower than in the control rabbits of similar body weight. This difference was statistically significant. There was a similar decrease in vascular capacity, but this was not statistically significant because of the wide scatter of data.

### Discussion

The assumption is made here that the vascular capacity in any part of the heart muscle truly represents the numbers of small vessels open at the time of death. Since cardiac arrest was the result of KCl, which causes marked dilatation of the heart, measurements as carried out here include the capacity of larger vessels both proximal and distal to the capillaries.

In a previously reported study on the regional differences in the canine heart muscle the highest vascular capacity was in the apex...
### TABLE 2

**Pathological Changes**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Age</th>
<th>Body wt (g)</th>
<th>Wt. left ventricle and septum (mg)</th>
<th>Apex (ml blood/100 g)</th>
<th>Septum (ml blood/100 g)</th>
<th>Mean value (ml blood/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult controls</td>
<td>11</td>
<td>1-2 years</td>
<td>3034</td>
<td>3535 (1.20) (8.75)</td>
<td>7.12</td>
<td>7.93</td>
<td></td>
</tr>
<tr>
<td>Adult aortic stenosis</td>
<td>8</td>
<td>1-2 years</td>
<td>2946</td>
<td>4909 (1.67) (6.78)</td>
<td>4.10</td>
<td>5.44</td>
<td></td>
</tr>
<tr>
<td>Difference in %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult controls</td>
<td></td>
<td></td>
<td></td>
<td>-2.9 ±38.9 N.S.</td>
<td>-22.5 ±39.1 P &lt; 0.01</td>
<td>-42.4 ±31.4 P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Young controls</td>
<td>10</td>
<td>7-9 weeks</td>
<td>1303</td>
<td>1993 (1.54) (10.06)</td>
<td>7.83</td>
<td>8.94</td>
<td></td>
</tr>
<tr>
<td>Young aortic stenosis</td>
<td>7</td>
<td>7-9 weeks</td>
<td>1317</td>
<td>2694 (2.02) (10.55)</td>
<td>8.09</td>
<td>9.32</td>
<td></td>
</tr>
<tr>
<td>Difference in %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young controls</td>
<td></td>
<td></td>
<td></td>
<td>+1.1 ±35.2 P &lt; 0.02 N.S.</td>
<td>+4.9 ±31.1 P &lt; 0.01 N.S.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals on protein-free diet</td>
<td>7</td>
<td>1-2 years</td>
<td>1533</td>
<td>2086 (1.27) (8.03)</td>
<td>7.14</td>
<td>7.58</td>
<td></td>
</tr>
<tr>
<td>Difference in %</td>
<td></td>
<td></td>
<td></td>
<td>+17.7 ±4.7 -17.6 -20.2 -8.8 -15.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
<td></td>
<td>N.S. N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average values; standard errors in parenthesis. N.S. = not significant.
and the lowest in the septum (12). For this reason we selected these two parts to represent the vascular capacity in the heart muscle, and the mean values in the heart muscle were calculated from the arithmetic average of these two values. The results reported here confirm that the vascular capacity is always higher in the apex than in the septum. This difference was statistically significant in all age groups except the youngest and the oldest.

Developmental changes in the vascular capacity in the heart muscle of rabbits are in agreement with our previous morphological observation in rats (8, 9). A similar trend in the number of capillaries per square millimeter in the heart muscle of the rat was found by Gautier and co-workers (13). On the other hand, Shipley and co-workers found no difference in the number of capillaries per square millimeter in the heart muscle of the rabbit throughout life (5). Also, Roberts and Wearn (6) reported no developmental changes in the number of capillaries per square millimeter in human hearts. However, their own material demonstrated 4,500 capillaries in hearts of children 3 weeks to 3 months of age and 3,342 capillaries in normal adult hearts.

The question whether the capacity of the terminal vascular bed remains constant during cardiac growth was examined. If this were true, vascular capacity would decrease hyperbolically with cardiac growth: $Y = \frac{k}{X}$

This is an equation describing hyperbole; $Y$ is ml blood/100 g in the left ventricle and septum, $X$ the weight of the left ventricle and septum in milligrams, and $k$ is a constant. Constant $k$ was calculated from the equation by substituting average values for the vascular capacity and heart weight in the group of normal adult rabbits for $Y$ and $X$. Figure 3 represents the results calculated on the basis of this equation as compared to values directly obtained in this study. It may be seen that in adult and old animals as well as in adult animals with cardiomegaly the capacity of the terminal vascular bed in the heart muscle is constant and any further increase in the heart weight results in a decrease in vascular capacity per unit of tissue. This is not the case in younger animals. The difference between values predicted by the equation and those directly measured makes it possible to calculate the percentage of the total capacity of the terminal vascular bed in adult animals, which was already formed at the time of measurement. These values, plotted in Figure 4, demonstrate an increase in vascular capacity in the heart muscle of rabbits, in agreement with several morphological observations on the postnatal development of the fiber-capillary ratio. The growth of the terminal vascu-
Increase of the total capacity of the terminal vascular bed. Broken lines connect animals of the same body weight.

lar bed is very rapid in the first postnatal weeks. The number of 4 to 6 muscle fibers per 1 capillary in the heart muscle at the time of birth rapidly declines toward adult values of 1:1 during the first postnatal weeks (5, 6, 8). Later, this growth becomes slower and finally no further increase can be detected in adult and old animals.

Similarly, the possibility was considered that the growth of the terminal vascular bed is realized in only one dimension; namely, that the cross section of the terminal vascular bed remains unchanged and the only growth is in length of terminal vessels. The results showed no clear pattern.

A decrease of the capillary density in cardiomegaly was reported by several investigators (5, 6, 14-16). Our findings are in agreement with these results when it is assumed that the vascular capacity equals the capillary density. The decrease in vascular capacity in our experiments is proportional to the increasing heart weight. A completely different situation, however, exists in animals in which the pathological growth of the heart started in early life, when the organism could still respond with a growth of the terminal vascular bed. The first observation of this kind was made by Shipley and co-workers, who reported high counts of capillaries in three growing rabbits with pulmonic stenosis. These counts were higher than counts anticipated on the basis of the degree of the cardiomegaly alone. Previously, in reported studies, it was found that in anemic rats on an iron-free diet following the weaning period heart weight increased 100% with no changes in the number of capillaries per square millimeter (17). Under such experimental situations anemic hypoxia could have had a direct effect on the circulation. Our studies with experimental aortic stenosis showed that the growth of the terminal vascular bed during the development of cardiomegaly probably follows the same trend as during normal heart growth. In young animals, an increase in muscle mass is accompanied by growth of the terminal vascular bed, and in adult animals only individual muscle fibers grow, and the total capacity of the terminal vascular bed remains unchanged. Therefore, in adult animals with cardiomegaly as a result of decrease in vascular capacity, diffusion distance becomes longer and conditions for the oxygen supply are impaired.

In animals maintained on a protein-free diet, decrease in heart weight was greater than decrease in body weight.

References
8. RAKUŠAN, K., JELÍNEK, J., KORECKÝ, B., SOUT.


or not. The usual effect of the extract is to produce contraction of such isolated organs. The limb shrinks in size (figs. 8, 9), the kidney diminishes greatly in volume (fig. 3), and the spleen contracts enormously (figs. 3, 4), while the heart and the larger blood vessels are enormously distended. Sometimes however an organ, especially a limb, expands (figs. 2, 7).

FIG. 1. Dog, weight 16 kilos. Chloroform, afterwards morphia. Vagi uncut. Effect of injecting extract of 0.2 gramme dog-suprarenal. Delayed inhibition. A, auricle; B, ventricle; C, femoral artery; D, injection of suprarenal (watery extract), the line D also indicates 0 pressure; E, time in 0.5 sec.*

In some experiments where two or more organs (such as the right and left fore-limb, or one fore-limb and one kidney) were under investigation at the same moment one was found to contract and another to expand (figs. 2, 4, 6, 7), the former owing its contraction to the active dimi-

* All the curves except those given in fig. 12 are to be read from left to right. They are photographically reproduced and are for the most part half the actual size.

from George Oliver and E. A. Schafer
The physiological effects of extracts of the suprarenal capsules
Journal of Physiology (London), vol. 18, pp. 230-276, 1895

A record showing the major cardiovascular effects following the intravenous injection of an extract from the adrenal gland. Lines A and B show movements of fine threads hooked into the auricles and ventricles. Femoral artery pressure is recorded with a mercury manometer. The main effects were the marked rise in systemic arterial pressure which the authors showed by other experiments to be due to direct constriction of arterioles by the extract, inhibition of the auricles (after a brief augmentation), and ventricular slowing, sometimes delayed as in this record. Prior injection of atropine or cutting both vagus nerves invariably removed the cardio-inhibitory effects.
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