

THE CAPILLARY SUPPLY IN NORMAL AND HYPERTROPHIED HEARTS OF RABBITS*

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PLATES 2 AND 3

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It is common experience to find at the autopsy table hearts which have failed during life, but which show no myocardial abnormality except hypertrophy. It is generally believed that hypertrophy enables the heart to perform its work more advantageously in the presence of certain alterations in physiological conditions. This belief, however, is not supported by sufficient factual evidence and there is no conclusive proof that an hypertrophied heart is actually a better performer than a heart of normal size. Indeed, the question may be raised whether hypertrophy might ultimately become a handicap to the heart and actually contribute to failure.

If, during the process of hypertrophy, the mass of muscle substance should increase without a corresponding increase in the total number of capillaries, the concentration of capillaries per unit of tissue would be decreased. If changes in blood flow could not compensate for this, the heart might suffer from an impairment in the exchange of metabolic substances. It has been shown by Goldenberg (1), Tangl (2), and others (3, 4) that hypertrophy is associated with an increase in the diameter of the individual muscle fibers. Since the capillaries of the heart, for the most part, accompany the fibers in a parallel course, it is obvious that such an increase in the cross sectional diameter of the fibers would push the capillaries farther apart, and, as a result, increase the distance between the wall of a given capillary and the periphery of its region of supply.

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The present study was undertaken in order to determine whether hypertrophy of the rabbit heart is accompanied by such a decrease in the concentration of capillaries or whether the concentration is maintained at a normal level by the birth of new capillaries at sufficient rate to keep pace with the increase in muscle mass. An answer was sought from an analysis of the following material, which was obtained from a series of hypertrophied hearts controlled by a similar series of normal hearts: (a) measurements of the diameter of the muscle fibers; (b) counts of the number of capillaries per square millimeter in the transverse plane of the fibers; and (c) determination of the ratio of the number of capillaries in the tissue to the number of fibers. If it is assumed that in hypertrophy there is no change in the number of fibers, a multiplication of capillaries would be indicated by an increase in this ratio.

Methods

Choice of Animals.—The rabbits of both the experimental and control groups were selected from a variety of breeds, and not more than three were taken from any one litter. There was a fairly even distribution of the sexes in each group.

Operative Procedures.—Cardiac enlargement has been produced experimentally by the creation of various artificial defects in the circulatory system. The most notable are: (a) aortic insufficiency (5); (b) arteriovenous fistula (6); (c) removal of buffer nerves (7, 8); and (d) stenosis of the pulmonary artery (9, 10). Each of these was tried in the present study and each usually produced hypertrophy, but the first was employed in the majority of instances because it necessitated no complicated operative procedure and a satisfactory hypertrophy resulted. Intravenous sodium pentobarbital anesthesia was used, and sterile technique was employed in all operations.

An insufficiency of the aortic valve was produced by the rupture of one of its leaflets. This was accomplished by the introduction of a sound with a bulbous tip through the left carotid artery. (The puncturing of two leaflets almost invariably resulted in the death of the animal within 4 to 48 hours.) The rabbits were killed after 2 to 5½ months had elapsed. In several instances it was noted that false valves had formed in the aortic wall just above the ring of the valve. Arteriovenous fistulae were produced by the creation of a lateral anastomosis between the left carotid artery and jugular vein. The method used for removal of buffers was essentially the same as that of Kremer, Wright, and Scarff (8), except that in most instances the operation was done on both sides at once.

The procedure which was followed in order to produce stenosis of the pulmonary artery in these experiments represents a modification of previously described methods. Reid (9), and Holman and Beck (10) applied occluding bands to the

pulmonary artery in dogs. In their experiments a certain amount of right ventricular hypertrophy occurred, but a permanent stenotic orifice could not be made small enough to insure a markedly increased resistance to the flow of blood.

In the present study a small aluminum band was placed around the pulmonary artery of rabbits when they were about 6 weeks old and weighed from 300 to 500 gm. The band, when used on these very young rabbits, caused a constriction which, although not small in comparison to the size of the vessel at that time, was a relatively narrow stenosis when the rabbits had become full grown. The band, which was of sheet aluminum 0.3 mm. thick, was at first made with an inside diameter of 2 mm., but it was found that the rabbits died after reaching a weight of about 800 gm. With bands which had a diameter of 3 mm. the rabbits grew to maturity without apparent impairment in health. The operative approach was by an incision through the midline of the sternum in the region between the first and third ribs. Artificial respiration was provided by a small tube inserted through an incision in the trachea. Closure of the sternal and tracheal incisions was made with silk. The rabbits were killed 7 to 10 months later.

Injection and Fixation of Hearts.—The method which was used to inject the capillary bed was essentially the same as that devised by Wearn (11). The heart was perfused with oxygenated Locke-Rosenheim solution at a pressure of 50 to 60 mm. of Hg for adult rabbits and 20 to 30 mm. for very young ones. After the heart had begun to beat, a 2 per cent suspension of Berlin blue was injected through the rubber tubing above the aortic cannula by means of a needle and syringe. When the heart appeared uniformly blue, glacial acetic acid was injected. This brought about immediate cessation of contractions and fixed the tissue.¹ Heart weight was determined after all excess fluid had been wiped from the heart and the cavities dried with cotton swabs. The heart was then placed in 10 per cent formalin for fixation. The cavities were first filled with the solution before the heart was immersed so that the natural contour of the ventricles would not become distorted.

Quantitations of capillaries and measurements of fibers could not be made without first controlling the shrinkage which generally occurs during the process of preparation of tissue for microscopic study. Measurements indicated that the tissue shrank about 2 per cent during the first few days in formalin and in a following 2 to 3 months an additional 1 per cent was added. It was considered that the shrinkage from fixation which did not exceed 3 per cent was small enough to be disregarded.

After the hearts had remained in formalin for 3 to 10 days, the auricles were removed and the capacities of the ventricles were determined with mercury. The two ventricles were next separated by the method of Herrmann (12) and each

¹ Registration of contractions on a smoked drum showed that the heart always stopped in complete relaxation. The ventricles, however, were not in a state comparable to physiological diastole when the cavities are distended with blood.

was weighed. Formalin fixation produced considerable weight loss and for this reason ventricular weights were corrected to their original weights before fixation.

Preparation of Sections.—At first attempts were made to prepare sections by the ordinary paraffin and celloidin methods, but it was discovered that the shrinkage during these processes was of such magnitude and subject to such variation that it could not be disregarded. A given section sometimes decreased to 60 per cent of its original area. Such reductions in area could have been corrected mathematically in each separate instance, had it not been apparent that in many sections, even when external dimensions changed very little, there was an internal shrinkage characterized by an increased separation of fibers from each other, or a massing of fibers into artificial bundles which left wide crevasses between. Measurements of external dimensions could not be used to correct for this type of shrinkage. The following method, which was ultimately adopted, produced no shrinkage: Four blocks of tissue, two from the midportion of the right ventricle opposite the septum, and two from the corresponding portion of the left ventricle, were removed from the formalin-hardened heart. These were cut in such manner that the large bundles of fibers which encircle the ventricles in the transverse plane were in cross section. They were washed in running water for 24 hours, placed in 10 per cent gelatin at 37°C. for 24 hours, followed by 20 per cent gelatin for a similar period, and then imbedded in the latter. After the gelatin had solidified, the blocks were hardened in 10 per cent formalin. Frozen sections, about 15 micra thick, were cut, stained in eosin and mounted from water without blotting. The edges of the gelatin were gently touched with absorbent paper, which drew the water from beneath the section and flattened it on the slide. Arabic sugar was used as a mounting medium. Capillary counts were then made immediately, since it was found that in such a preparation the Berlin blue tended to fade out of the capillaries.

Counts and Measurements.—All counts and measurements were done with an oil immersion objective and ruled micrometer ocular square, covering an area of the tissue which varied with the microscope from 3000 to 3500 square micra. In view of the fact that it was desired to determine the number of capillaries and fibers in pure muscle tissue, fields were selected for counting which did not contain connective tissue, arteries, or veins. Crevasses caused by any tears in the section were likewise avoided. Counts were made in areas in which the capillaries and fibers were cut in perfect cross section and in which the capillaries were completely injected, as shown by their even spacing and regular distribution. Except for the restrictions mentioned, no deliberate selection of fields was made. Another system of counting which was tried consisted of choosing adjoining fields in continuity, regardless of their content, in a straight line across the section. Deductions were made for the areas occupied by bare spaces, connective tissue, etc. This method gave results which compared favorably with those of the first method, but it was discarded as it was time-consuming and introduced an additional error in the estimation of blank spaces in a field. The chief criticism of the first method, which was adopted as routine, is that one may be prejudiced and un-

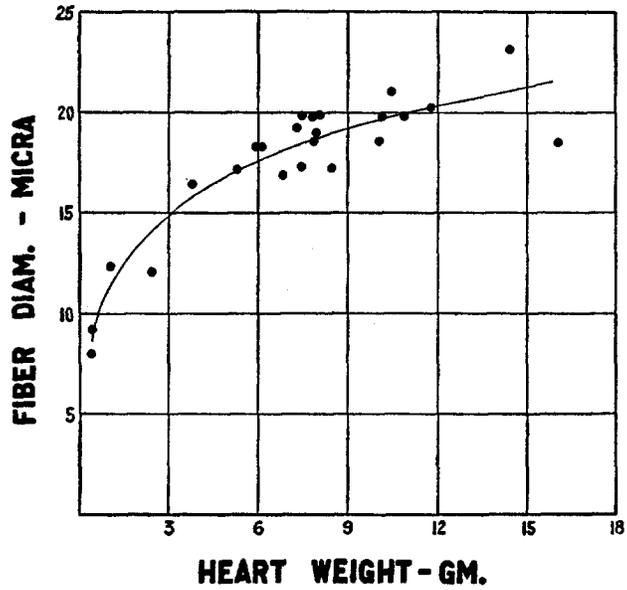
consciously choose fields with a high or low count as his expectation suggests. In all cases, however, the counts were made by at least two observers and often by three. There was almost always one observer who did not know the identity of the heart being counted and yet the counts of all observers agreed very well. The final average was calculated from a total of 120 to 240 fields counted in each ventricle. A fiber count was made simultaneously with the capillary count so that a ratio of the two could be obtained. It is probable that practically all of the capillaries were injected in those regions of the muscle which were selected for counting. In any event, if incomplete injection existed, it was undoubtedly present to an equal extent in both normal and hypertrophied hearts.

Measurements of the diameters of fibers also were made in areas where the fibers were in perfect cross section. Those fibers which were measured were selected in sequence as they touched a single straight line when the slide was moved laterally on the mechanical stage. Most fibers were ovoid in shape and therefore both long and short diameters were measured. From 160 to 240 fibers were measured in each ventricle. The long and short dimensions were first averaged separately and then an average of the two was computed to give the mean fiber diameter. In that the width of the myocardial fibers varies with systole and diastole, these measurements cannot be considered absolute. The number of fields counted to determine the average capillary and fiber count of a given heart, and the number of fibers measured to obtain a representative fiber diameter were found sufficiently large to reduce the probable error of the mean well below the limits dictated by accepted methods of statistics.

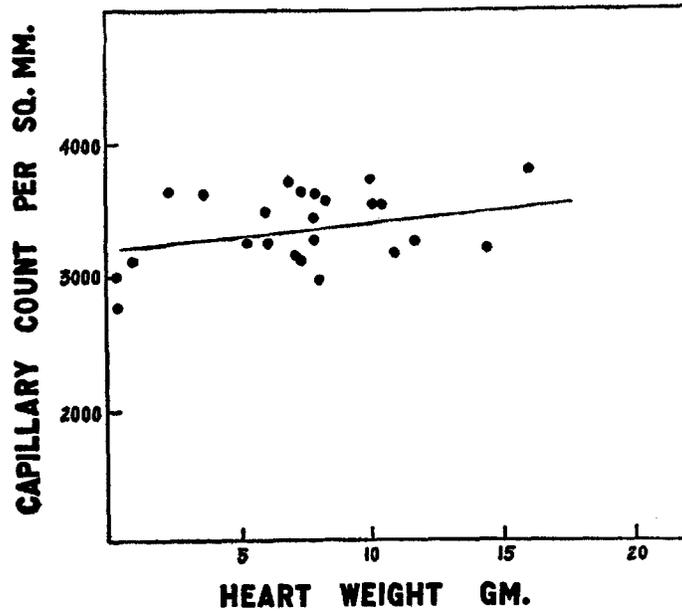
Normal Hearts

A number of hearts from both young and old rabbits at various ages were studied for the purpose of observing the changes in the capillary bed during the normal growth of the heart, and for the comparison of these changes with those occurring in the capillary bed during the process of hypertrophy.

Hearts were obtained from rabbits whose body weights varied from 55 gm. at birth to 5135 gm. in maturity. It was observed that the transverse diameter of the fibers became gradually larger with increase in size and weight of the heart (Text-fig. 1). This mode of growth was first demonstrated by Tangl (2) who found no evidence of multiplication of fibers after birth. The increase in the cross sectional area of an average fiber from birth to maturity was sevenfold and the increase in total fiber length was by estimation nearly sixfold. A sevenfold increase in cross sectional area would reduce the capillary supply to one-seventh of the original concentration if new capillaries were not formed during the growth of the heart. A formation of new capillaries did take place, however, for the capillary count per square millimeter remained essentially the same throughout the period of growth. Indeed, it was a little higher in some of the larger hearts (Text-

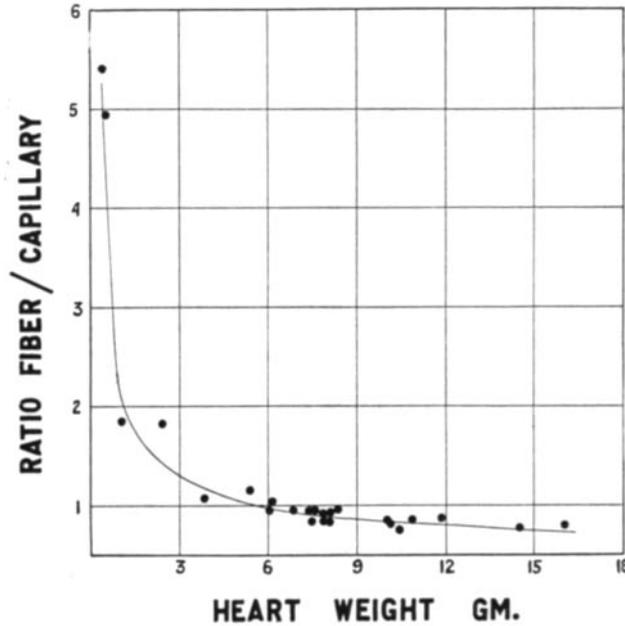


TEXT-FIG. 1. The influence of growth on fiber diameter (left ventricle).

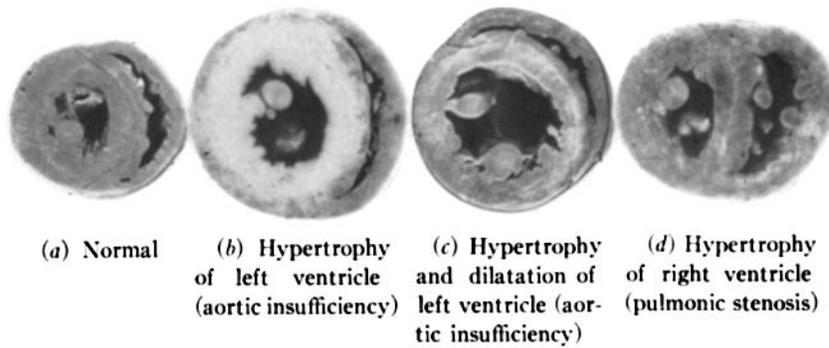


TEXT-FIG. 2. The influence of growth on the capillary count (left ventricle).

fig. 2). At birth there were about five small fibers to each capillary (Fig. 1), but as the fibers enlarged the capillaries multiplied until a ratio of one capillary to each muscle fiber was reached. In the larger adult hearts there were actually



TEXT-FIG. 3. The influence of growth on the number of fibers per capillary (left ventricle).



TEXT-FIG. 4. Cross sections of normal and hypertrophied hearts showing the effect of hypertrophy on the thickness of the walls of the ventricles and on the size of the chambers. Natural size.

a few more capillaries than fibers (Text-fig. 3). The capillary count in the right ventricle averaged slightly lower than that of the left, the fibers of the former were slightly smaller than those of the latter, and the number of fibers in proportion to capillaries was somewhat higher. The lower counts in the right ventricle may have been associated with a tendency for the tissue of this chamber to be less compact than that of the left.²

Hypertrophied Hearts

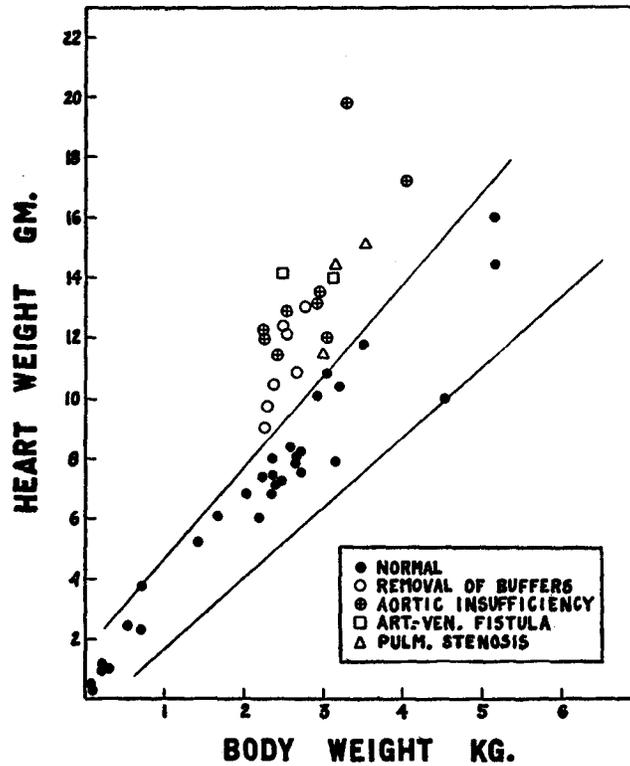
The criterion for hypertrophy was increase in heart weight as compared to body weight. The presence of the Berlin blue in the injected specimens, it was found, caused an increase in weight of 20 to 30 per cent. For this reason only injected hearts were used to establish a series of controls. It will be seen from Text-fig. 5 that all of the hypertrophied hearts fell well above the normal range. The hearts of a few of the rabbits in which the buffer nerves were removed were not included in the study, for their weights were not definitely increased.

Observations on hearts in which hypertrophy was manifested primarily in the left ventricle are recorded in Table I (see also Text-figs. 6 and 7 and Fig. 3). It will be noted that the fibers were of larger diameter in the hypertrophied group than in the control, and the capillary count was reduced.³ The reduction in capillary count agreed fairly well with an expected figure which was calculated on the assumption that no new capillaries were formed, and that the pre-existing capillaries were pushed apart by fibers which increased in area from 361 to 493 square micra. (Fiber area was calculated by squaring fiber diameter.) This observation, along with the fact that the ratio of capillaries to fibers did not change, allows a fair conclusion that multiplication of capillaries did not occur.

The outcome was somewhat different when hypertrophy developed gradually in young growing rabbits as a result of pulmonic stenosis. Although this group was too small for proper statistical analysis, the

² The capillary counts of normal rabbit hearts in this study were somewhat lower than those reported by Wearn. This was undoubtedly due largely to the elimination of shrinkage in the present study and to the fact that we made no attempt to select areas in which the count was at a maximum.

³ The ratio of the long diameter of the fibers to the short diameter was almost identical with that of the normal hearts. In other words, the fibers increased in size equally in both dimensions.



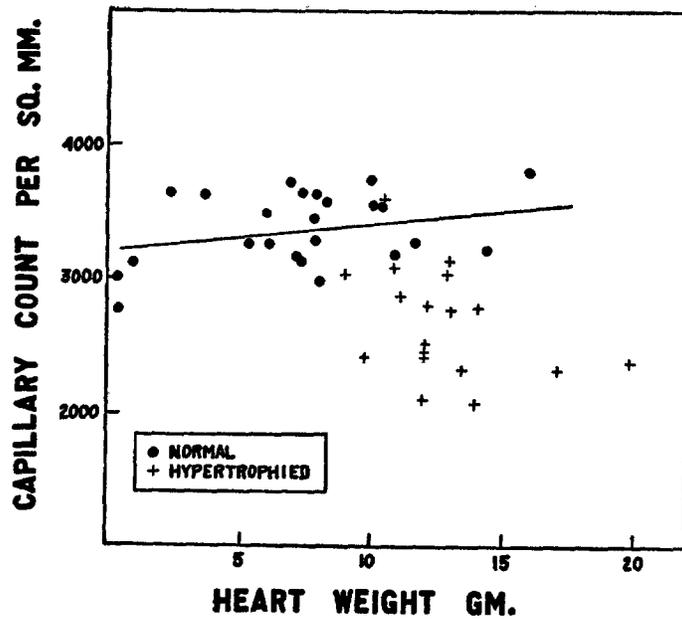
TEXT-FIG. 5. Heart weight in relation to body weight. The hypertrophied hearts fall well above the normal controls.

TABLE I

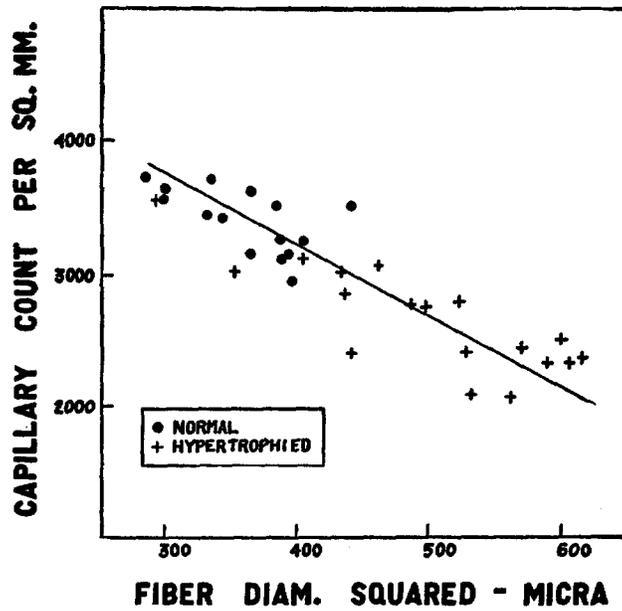
The Effect of Hypertrophy (Left Ventricle)

	Normal hearts (mean of 15 with probable error)	Hypertrophied hearts (mean of 18 with probable error)
Fiber diameter, <i>micra</i>	19.0 ±0.20	22.2 ±0.33
Capillaries per sq. mm.	3420 ±39	2670 ±62 (Expected count—2510)
Capillaries per fiber.....	1.14 ±0.013	1.16 ±0.013

Limits of body weights of normal rabbits used for controls were 2050 to 4530 gm., average 2790 gm. Limits of body weights of rabbits with hypertrophy of the left ventricle were 2245 to 4055 gm., average 2710 gm.



TEXT-FIG. 6. The capillary count as influenced by hypertrophy (left ventricle). Note that the counts in the hypertrophied hearts fall below the range of the normal.



TEXT-FIG. 7. The relation between cross sectional area of fibers and capillary count (left ventricle). Coefficient of correlation = -0.89 ($P.E. \pm 0.025$).

findings were fairly uniform. The size of fibers in the right ventricle was well above normal, but the capillary count per unit area, although somewhat low, was not reduced to the expected figure and the ratio of capillaries to fibers averaged somewhat above the normal (Table II). The only tenable explanation for the failure of a reduction in the capillary count to the level which would be anticipated from the increased size of fibers is that there was a certain degree of multiplication of capillaries in response to the hypertrophy which in these hearts did not occur after the heart had reached a relatively mature state, but developed simultaneously with active growth.

Measurements of ventricular weight and fiber diameter indicated that in all of the experimental hearts except those with pulmonic

TABLE II
*The Effect of Hypertrophy in Growing Rabbits with Pulmonic Stenosis
(Right Ventricle)*

	Normal hearts (mean of 15 with probable error)	Hypertrophied hearts (mean of 3)
Fiber diameter, <i>micra</i>	17.2 ±0.23	23.0
Capillaries per sq. mm.....	3310 ±59	2740
Capillaries per fiber.....	0.99 ±0.19	(Expected count—1850) 1.15

Limits of body weights of rabbits with pulmonic stenosis were 3025 to 3530 gm., average 3220 gm.

stenosis hypertrophy was not confined to the one ventricle, but was shared by the other to a smaller degree. The capillary count was reduced to an extent corresponding to the degree of hypertrophy.

It would be expected that the presence of any appreciable degree of dilatation would cause an elongation and narrowing of the muscle fibers. This might offset the effect of hypertrophy which produces thickened fibers. An analysis of the influence of dilatation as expressed by the ratio of chamber volume to chamber weight, however, disclosed the fact that increases in chamber capacity of the magnitude encountered generally affected the fiber size and capillary count to only a slight degree.

DISCUSSION

It is evident that the process of hypertrophy differed from that of growth in its effect on the capillary bed. It appears that some factor which was responsible for the genesis of capillaries during natural growth was not in evidence during hypertrophy. An indication of the presence of some stimulating factor which influences capillary formation during normal growth may be secured from the findings in the hearts with pulmonic stenosis. In these hearts, in which hypertrophy developed simultaneously with growth, the capillary count was higher than in those in which hypertrophy occurred after the normal growth process had nearly ceased.

In the mature group the period during which the hypertrophy developed was of sufficient length to allow the capillaries to multiply. It is hardly probable that if a longer period had been allowed before injection the results would have been any different. The capillary counts in general were no higher in hearts injected $5\frac{1}{2}$ months after operation than in those after 2 months.

The obvious question to ask oneself at this point is whether or not the decreased capillary supply could embarrass the myocardium because of an impairment in metabolic exchange. An enlarged heart would be expected to consume and discharge greater quantities of metabolic substances than a normal one. An augmented blood flow might serve to supply the increased mass of muscle tissue, and might also compensate for the relatively sparse distribution of capillaries. It cannot be stated at present, however, whether an increase in blood flow really occurs in these hearts, or whether an increase, if present, can actually meet the demands of a larger amount of tissue with relatively few capillaries. The physiological significance of the present findings cannot be estimated without further data bearing on these problems.

Measurements of capillary diameter were not made. Simple inspection revealed no difference in size between the capillaries of the normal and those of the hypertrophied hearts. It should be kept in mind, however, that the postmortem appearance of a capillary is not necessarily an indication of its premortem state. It must also be remembered that even if capillary dilatation were demonstrated, this in itself would not be an indication of actual augmentation in the

volume flow of blood. The size of the other vascular channels, and the pressure within them must also be considered.

Findings in the hypertrophied rabbit heart cannot be applied to the human heart without some reservation. A study of the capillary bed in normal and hypertrophied human hearts is now in progress.

CONCLUSIONS

1. During normal growth of the rabbit heart, muscle fibers enlarge, and the capillaries multiply so that a relatively constant capillary supply per unit of tissue is maintained from the time of birth to maturity.

2. In cardiac hypertrophy the muscle fibers enlarge, but the capillaries do not multiply and, as a result, the capillary supply per unit of tissue is reduced.

3. The decreased concentration of capillaries in the hypertrophied heart would constitute an impediment to the adequate exchange of metabolic substances, but the seriousness of the impediment cannot be estimated without further physiological data.

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EXPLANATION OF PLATES

PLATE 2

FIG. 1. The capillary supply at birth (ventricular septum) $\times 500$. Note the small fibers and compare with Fig. 3a.

FIG. 2. X-ray photographs showing the increase in size of the cardiac silhouette after operative procedures. Top row, before operation—bottom row, after operation. (*a*) removal of buffers; (*b*) aortic insufficiency; (*c*) arteriovenous fistula.

PLATE 3

FIG. 3. The capillary supply of the normal heart (*a*) compared with that of an hypertrophied heart (*b*) (left ventricle). $\times 500$.

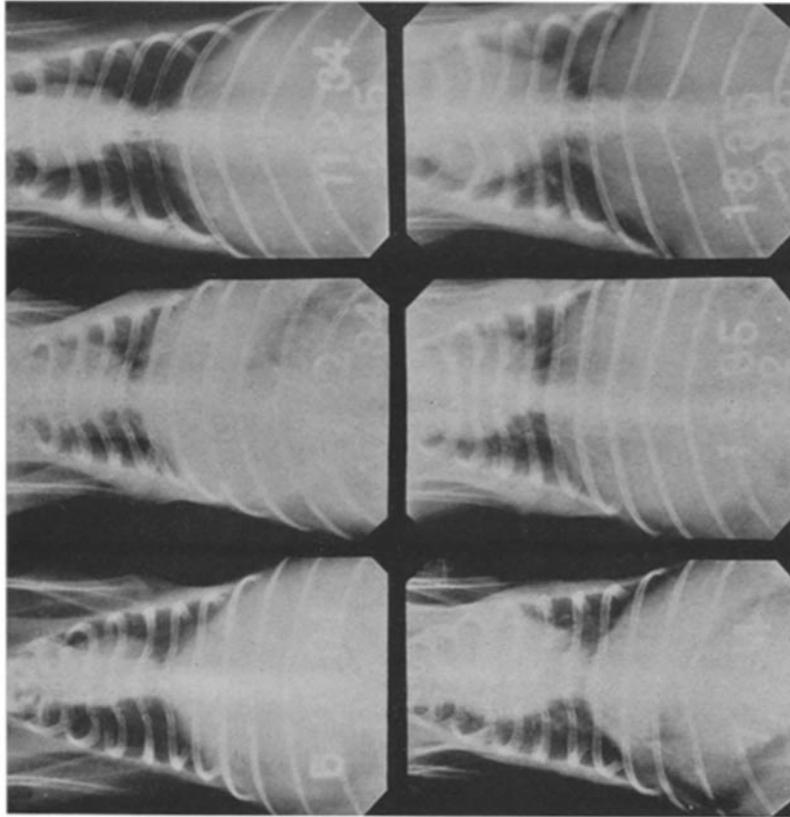


FIG. 2

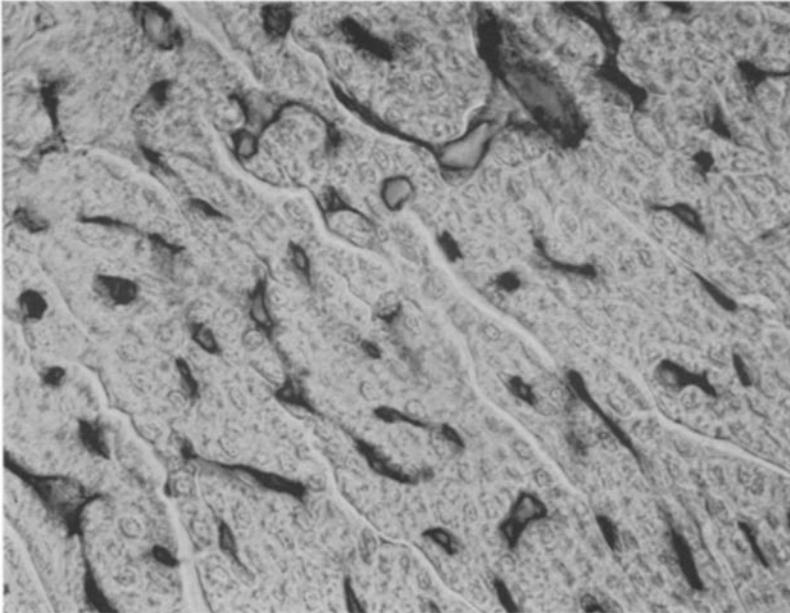
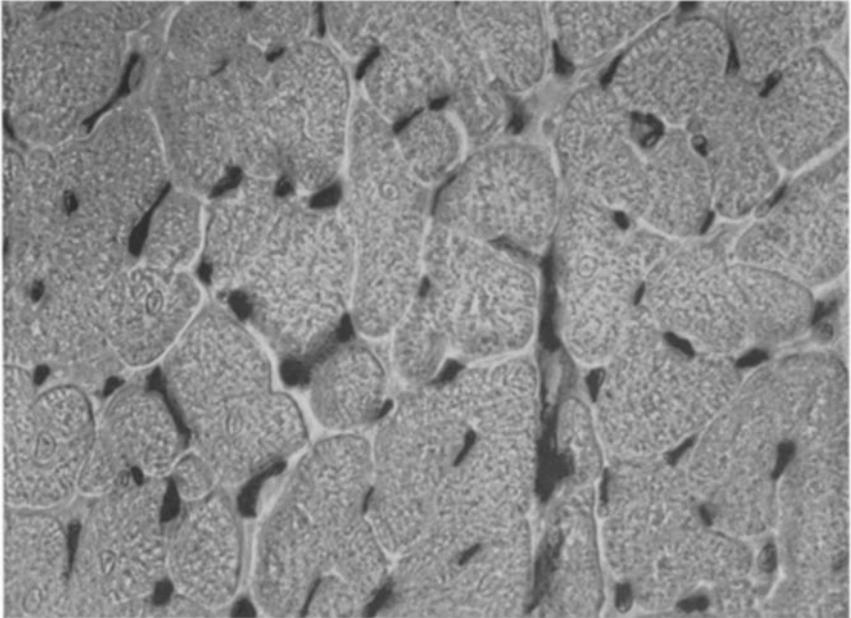
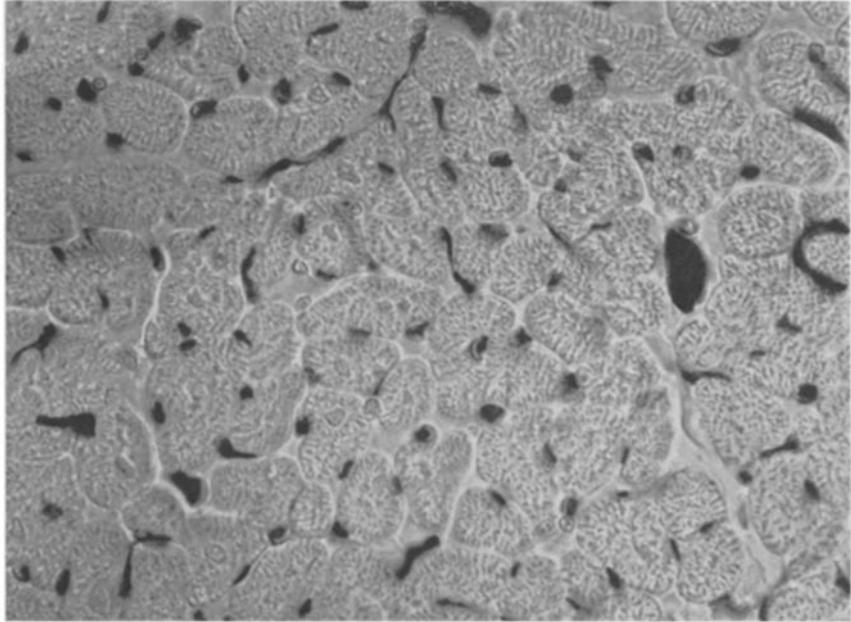


FIG. 1

(Shipley *et al.*: Capillary supply in hypertrophied hearts)



b



a

FIG. 3

(Shiple *et al.*: Capillary supply in hypertrophied hearts)