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TO MY PARENTS

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ABSTRACT

Functional coronary intercapillary distances (ICD's) under normoxemia (PaO2 ¬ 150 mmHg) and hypoxemia (PaO2 < 50 mmHg) were obtained using the technique of stop-motion cinematography of the surface of the beating rat heart in situ.

Between the age of 49 days and 200 days, the coronary ICD under normoxemia increased from 15.68 um to 19.37 um while the coronary ICD under hypoxemia increased from 15.60 um to 19.91 um. When these ICD's were converted to capillary densities, the capillary density under normoxemia decreased almost exponentially with left ventricular weight from 4070/mm2 to a plateau of 2670/mm2 while under hypoxemic conditions, it decreased linearly with left ventricular weight from 4110/mm2 to 2520/mm2. Consequently, the capillary reserve varied with left ventricular weight, appearing when the rat reached 49 days of age, reaching a maximum of 740/mm2 (21%) when the rat was 62 days old and disappearing at 200 days of age.

When the heart was denervated by heterotopic isotransplantation there was no significant difference between the normoxemic (18.46 um) and hypoxemic (18.59 um) mean ICD's in one day transplants. In addition, these ICD

values were similar to the mean ICD (18.47 um) predicted from the left ventricular weight under hypoxemia, suggesting that the closing of coronary precapillary sphincters might have been neurally mediated.

In the longer term (7 days) heterotopically isotransplanted hearts, cardiac atrophy had already started; the average loss of left ventricular weight was 8%. There was no significant difference between the normoxemic (17.10 um) and hypoxemic (17.09 um) mean ICD's. In addition, these ICD values were shorter than the mean ICD (17.76 um) predicted from the left ventricular weight under hypoxemia, suggesting that existing capillaries did not obliterate during the process of cardiac atrophy.

When cardiac hypertrophy was induced by subdiaphragmatic aortic constriction, leading to an average increase of left ventricular weight by 63%, no significant difference between the normoxemic (18.89 um) and hypoxemic (19.33 um) mean ICD's was observed. Furthermore, these ICD's were longer than the mean ICD (16.67 um) predicted from the body weight under hypoxemia, suggesting that cardiac hypertrophy caused an increase in ICD and that coronary capillary reserve was exhausted even under normoxemia.

Finally, frequency distributions of ICD's were computed from data obtained under normoxemia and hypoxemia. The skewness and kurtosis values indicated normal distribution.

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Chapter I

INTRODUCTION

The heart is an organ which is continuously working and has a limited anaerobic capacity. Just two to five minutes of anoxia can lead to irreversible damage and therefore oxygen supply to the heart has to be continuously maintained. This supply is almost entirely provided by the coronary circulation. A large amount of research work has been done on the macroscopic level of the coronary circulation; however, the physiology of the coronary microcirculation remains relatively unexplored, especially on the level of the capillaries. The understanding of the physiology of the capillaries is important because it is where most of the exchanges between blood and tissue occur and this is where oxygen is transported to the myocardium by diffusion. The diffusional transport of oxygen was first described mathematically by Erlang at the request of Krogh in 1919. Since Krogh, similar mathematical studies have been made utilizing more sophisticated models (Blum, 1960, Hudson and Cater, 1965, Grunewald and Sowa, 1977). Besides, Kety (1957) and Rakusan (1971b) studied the importance of individual variables in the Krogh-Erlang's equation. They found that one of the most important determinants of tissue

oxygen tension was the diffusion distance, which could be described as the radius of the Krogh's cylinder or half of the distance between adjacent capillaries. The in vivo changes of the coronary intercapillary distances (ICD's) in response to various stimuli is the subject of this thesis.

Research on myocardial microcirculation in vivo is difficult due to many technical problems. First of all, the heart is an organ which is constantly moving and therefore it is difficult to use the standard stationary microscopic system without external restriction of the cardiac movements. Secondly, the chief working part of the heart, the ventricle, is a thick-walled structure which cannot be studied with the conventional transmission light microscopy. Lastly, the heart is connected to the major blood vessels and is covered up partly by the lungs, therefore handling of the heart can easily damage both the lungs and the heart. Due to these problems, early studies on the coronary terminal vascular bed could be made only on hearts post mortem.

Various methods were used to determine the capillary density in the heart. Initially, they included injection of the coronary network with suspensions, staining of the capillary endothelium and the stainng of the trapped red blood cells. In later studies, a "semi in vivo method" was devised which involved the measurement of terminal vascular capacity after quick arrest and freezing of the heart so as

to obtain the tissue closest to its in vivo state. Finally, the most recent method in use has been the stop-motion cinematography of the beating heart in situ. The details of the individual methods mentioned above are outlined in the following paragraphs.

1.1 INJECTION OF CONTRAST MEDIUM

Wearn was among the first who successfully injected the coronary capillaries with India Ink (Wearn, 1928). Others later tried Berlin Blue (Shipley et al., 1937), Chicago Blue-Gelatin (Roberts and Wearn, 1941), Biological Ink (Tomanek, 1970), iodized pigmented gelatin (Hales and Carrington, 1971), barium sulphate followed by X-ray microscopy (Ljunggvist and Unge, 1972) and silicone rubber (Bassingthwaighte et al., 1974). All these injections were done retrogradely through the aorta and it was found that the beating of the heart was important in assisting the entry of the suspension into the capillaries. Injection of non-beating hearts often yielded very poor results (Brown, 1965). Concerning the size of the injected particles, the following problems arise. When the particles are too big, they will get trapped in the precapillary vessels; on the other hand, when they are too small, they will leave the capillaries after the injection when the perfusion stops, which may be due to the intrinsic instability of the capillaries. Therefore, a range of particle sizes which gives optimal results has to be selected for each species.

In addition to the above, Ludwig (1971) listed other possible factors causing incomplete filling of the coronary capillary bed such as (a) mechanical alterations during removal of the heart, (b) presence of air microemboli or large size particles in the suspension, (c) systolic cardiac arrest, whereby the contraction of the myocardium could be so strong as to prevent the local penetration of the suspension, (d) endothelial edema, (e) incomplete closure of the aortic valves due to excess pressure, causing the dilation of the left ventricle which, if severe, could compress the coronary arteries to prevent perfusion, (f) possible loss of the suspension through the thebesian veins and the arterioluminal vessels. The question of whether all capillaries can possibly be injected therefore remains unanswered. Krogh early in 1919 reported that he had never observed a capillary containing India Ink without erythrocytes. Willner and Groom in 1977 showed that with Microfil, even with filling pressure of 450 cm H20, only 85% filling was obtained. Lastly, even if all the capillaries were filled, it is not certain if all of them are fully distended; consequently, their diameter may not be the same as the diameter in vivo. This is important because their incomplete expansion may result in false high capillary densities. In view of the above, the injection method is not commonly used at present.

1.2 STAINING OF THE TRAPPED RED BLOOD CELLS

This is a relatively unreliable method because after the heart stops beating, the blood has a tendency to leave the capillaries. This phenomenon was named by Nichol et al. (1951) as "critical closing". Furthermore, even if all the blood should remain in the capillaries, the red blood cells in the capillaries would be separated by a column of plasma, therefore it would be highly unlikely that all the perfused capillaries in a cross section would contain red blood cells. Reynolds et al. (1958) used this method and reported rather low capillary densities. Consequently, this method is rarely in use today. However, a new method for counting the perfused capillaries using trapped erythrocytes as marker has been devised (Honig et al., 1980). It involved the use of thick frozen tissue blocks with capillaries cut in cross section, and tracking for erythrocytes up to 50 um from the surface of the block so that all perfused capillaries would contain erythrocytes provided that they were not apart more than 50 um. This method was used to study capillary recruitement in skeletal muscle and could be applied to the study of the coronary microcirculation as well.

1.3 STAINING OF THE CAPILLARY ENDOTHELIAL LINING

The Periodic-Acid-Schiff's method (PAS) (Hort and Hort, 1958) and the alkaline phosphatase staining method

(Gomori, 1939) have been the common choice. The PAS method, in particular, has been most often used in studying coronary capillaries. This approach is superior to the injection method in a sense that all capillaries may be revealed (Willner et al., 1977). On the other hand, the staining method has all the disadvantages inherent to histological methods such as shrinkage and distortion of the tissue geometry during processing. Also, both the PAS and alkaline phosphatase stains cannot be used in young animals in the early stage of development because capillaries in this stage will not take up enough stain to give an adequate contrast for quantitative evaluation. Nevertheless, the staining method remains the method of choice in postmortem studies. Besides counting the number of capillaries, measurement of the average alkaline phosphatase enzyme activity has also been used as an indicator of capillary density (Dowell, 1975). This is a method which stands between the staining and the indirect method as will be discussed below.

1.4 CAPACITY OF THE TERMINAL VASCULAR BED

Besides the direct observation of the capillary network there was an indirect approach which measured the capacity of the terminal vascular bed using tracers. If the tracer was trapped only inside the capillaries and if the diameter of the capillary was known then the average capillary density could be calculated. The oldest and still

in use tracer is the radioactive I^{131}-Albumin (Myers and Honig, 1964). Other tracers included Cr^{51}-erythrocyte (Myers and Honig, 1964), Fe^{59}-Siderophilin (Weiss and Winbury, 1974), and Cr^{51}-Albumin (Duran et al., 1977). The indirect method can give only the average estimate of the capillary density, but it has the advantage of being less time and work consuming and it does not have problems inherent to histological techniques. It was often used for comparison studies so that the systemic errors such as incomplete trapping, etc. would be minimized. Finally, this method is believed to be one step closer to the in vivo studies than other methods mentioned before because the heart is quickly arrested and frozen so that the state of the myocardium should reflect the condition in vivo.

1.5 IN VIVO STUDIES

With the advancement of light microscopy and new techniques of illumination, the study of the coronary microcirculation in vivo has been made possible. This approach included transillumination of the atrium (Tillich et al., 1971) and epiillumination of the ventricle (Martini and Honig, 1969, Steinhausen et al., 1978). In the transillumination method, the tip of a light conducting lucite rod was placed inside the atrium; light was then transmitted from an external source through the lucite rod and was reflected at the 45 degree cut tip of the rod

through the atrium to the microscope objective. Since the magnitude of the atrial movement was relatively small, it could be further minimized by the transilluminating rod which thus allowed the use of high magnification, and red blood cells inside the capillaries could be observed and the velocity of their movements measured. This system, however, could not be applied to the vigorously moving ventricles without causing severe mechanical trauma. Nevertheless, the light transmitting lucite rod had been tried on the ventricle in a form of needle inserted into the beating ventricle (Tillmanns et al., 1974). However, this approach was by no means ideal because the #20 G needle would surely cause mechanical trauma in its immediate vicinity. Furthermore, it was the area which was affected most, namely, the myocardium on top of the needle that was studied. The degree of damage and experimental artifact imposed on the heart was therefore unpredictable. Since the ventricles are the main working parts of the heart, better methods have to be developed for the study of the ventricular coronary microcirculation. The first approach was developed by Martini and Honig in 1969 which was basically the method used in this project. It involved stop-motion cinematography of the ventricular surface of the in situ beating heart epiilluminated with a strong source of stroboscopic light. When the focal point of the objective lay between the extremes of the excursions of the heart

movement, some of the exposures would have the coronary capillaries in focus. With this method, studies of various aspects of the physiology of the coronary microcirculation has been made and it has been shown that tissue pCO2 and pH had relatively very limited effects on the coronary capillary density (Bourdeau-Martini and Honig, 1973) whereas tissue oxygen tension had a strong influence on the control of the density of the coronary capillaries (Bourdeau-Martini and Honig, 1974). The second approach of the epiillumination method was developed by Steinhausen et al. in 1978 and was similar to the first. However, the movement of the ventricle was restricted by inserting into the ventricular wall a pitchfork type restrainer consisting of five steel needles. The ventricular movement was thus reduced and higher magnification could be employed. Steinhausen et al. used this method to study the influence of arterial oxygen tension on coronary capillary densities and unlike the previous authors, they could not observe any capillary recruitement in hypoxemia. The investigation of this controversy is one of the objectives of this thesis.

The most important advantage of the in vivo cinematography of the subepicardium is that coronary microcirculation can then be studied in its functional state. Unfortunately, only the first layer of the subepicardial capillary network can be studied and the phase of the cardiac cycle from which the data are obtained is not

known. Also, the microcirculation at the subepicardium may possibly be different from that in other parts of the ventricle. Since biomicroscopy still cannot be applied to other ventricular layers, the in vivo cinematography of the subepicardium remains the best way to study the coronary microcirculation in its functional state.

As mentioned before, a modification of the first epiillumination method was utilized in this project to study the morphology of the coronary microcirculation in vivo. With this method, the basic architecture of the coronary microcirculatory network up to the level of capillaries could be revealed. From the frames of the film where the subepicardial capillary network is in focus, the perpendicular distance between adjacent capillaries, could be measured and the mean and frequency distribution of the intercapillary distances (ICD's) calculated.

1.6 OBJECTIVES OF STUDY

The objectives of this project are: (1) to reexamine the relationship between coronary capillary density and growth of the heart, (2) to study the density of coronary capillaries in the short term heterotopically isotransplanted heart which is used as a model of empty beating denervated heart, (3) to study the density of coronary capillaries in the long term heterotopically isotransplanted heart which is used as a model of cardiac

atrophy induced by reduction of load, (4) to study the density of coronary capillaries in the pressure-overload hypertrophied hearts, (5) to investigate the existence of capillary recruitement in all the above under hypoxemic conditions. These five objectives are described in more detail in the following paragraphs.

Henquell et al. (1976) studied the relationship between coronary intercapillary distance and growth in rat and reported that throughout the age 40 and 400 days, functional ICD's under normoxemia and hypoxemia both decreased linearly with growth maintaining a constant ICD difference of 2 um between them. Consequently, the capillary reserve decreased from 1200/mm2 to 400/mm2. In our case, we decided to reexamine the relationship between functional coronary capillary density and age under normoxemia and hypoxemia so as to establish control data for later comparison with results from the experimental groups.

The second objective was the study of the capillary density in the short term heterotopically isotransplanted heart which was basically a denervated empty beating heart. In skeletal muscle, reports on the importance of neural innervation on capillary density had been controversial. Hudlicka and Renkin (1968) found an increase in the capillary diffusion capacity (permeability surface area of Rb^{86}) in the denervated dog gracilis muscle whereas Honig et al. (1978), by direct counting, observed no change in

capillary density in the denervated rat gracilis muscle. No similar studies had been done in the heart probably due to the technical difficulties involved in the process of denervation. Feldstein et al. (1978) observed shorter spacing between capillary and vein than the normal intercapillary distances in the rat heart; and since tissue oxygen tension was lowest around the venous end, they further suggested that diffusion distance was influenced by local tissue oxygen tension. Therefore, the importance of neural versus local control of the coronary capillary density was so far uncertain. This problem may be investigated by determining the degree of capillary recruitement under hypoxemia in these short term transplanted hearts and comparing it to that in the normals.

The third objective was the study of the capillary density in the long term transplanted heart when it had already started to atrophy. Very little work had been reported so far on the capillary density changes in atrophied hearts probably due to the difficulties in producing an adequate model of experimental cardiac atrophy. Roberts, et al. (1941) observed an increase in capillary density associated with cardiac atrophy in pathologic human hearts. However, Rakusan et al. (1967) reported a decrease in terminal vascular capacity in atrophied rabbit hearts induced by protein deprivation. Although the degree of cardiac atrophy in both studies are similar, it is difficult

to compare the data from these two groups because of the differences in both the species and methods used. In our case, cardiac atrophy resulted from load reduction, which might be considered a more suitable model of atrophy because the supply of nutrients to the heart was not altered. We studied the functional capillary density in these atrophied hearts in order to find out if any obliteration of preexisting capillaries was associated with the process of atrophy.

The fourth objective was the study of the capillary density in adult pressure overload hypertrophied hearts. Changes in capillary density associated with increased cardiac mass has interested researchers for many years. Shipley et al. in 1937 first reported a decrease of capillary density in adult hypertrophied rabbit hearts. Roberts and Wearn later in 1941 observed a similar decrease in capillary density in adult hypertrophied human hearts and Rakusan (1971a) reported a similar decrease in capillary capacity in aortic-constricted rat hearts. All reports therefore suggested that capillaries did not multiply in adult hearts; the increase in myocyte diameter merely moved capillaries further apart, resulting in lower capillary densities.

Rakusan et al. in 1965 studied the relationship between the early postnatal development and the growth and proliferation of muscle fibres and capillaries of the rat

heart. He separated the period of development of the rat into three stages and showed that coronary capillaries stopped multiplying in the last stage which was the 7th week after birth while the growth of the myocytes continued. This relationship between the growth of muscle fibres and capillaries in the later part of the life span might explain the decrease in capillary density associated with normal postnatal growth and adult cardiac hypertrophy. - Direct confirmation was provided by Rakusan and Poupa in 1966 by comparing young induced cardiac hypertrophy to adult induced cardiac hypertrophy. It was found that cardiomegaly arising early in life was much greater than that arising late in life and yet the ICD was not prolonged. Anversa et al. in 1979 in their extensive light and electron microscopy studies also demonstrated the increase in intercapillary distance in adult hypertrophied rat hearts. All these data, however, were acquired from hearts postmortem so that they reflected the total anatomical capillary content of the heart and how the functional capillary density changed in the hypertrophied heart was unknown.

Henquell et al. in 1977 measured the functional and minimum coronary intercapillary distances in the hypertensive rats with hypertrophied hearts using in vivo cinematography and reported that capillary reserves in these hypertrophied hearts were exhausted even in normoxemia. In their case, they produced cardiac hypertrophy by salt

loading and unilateral nephrectomy. The other commonly used method for producing cardiac hypertrophy is aortic constriction. Both methods induce cardiac hypertrophy by pressure overloading; however, the onset of cardiac hypertrophy in the case of aortic constriction is more sudden. As part of this project, functional and minimum intercapillary distances in the hypertrophied rat heart induced by aortic constriction were measured using the technique of in vivo stop-motion cinematography.

The last objective was concerned with the existence of capillary recruitement under hypoxemia. Bourdeau-Martini et al. (1974) showed that in normoxemia, only half of the total number of subepicardial capillaries were perfused and hypoxemia could cause the recruitement of the remaining half to decrease the ICD from 17 um to 11 um. However, Steinhausen et al. (1978), using their method, could not observe any capillary recruitement in hypoxemia. According to Steinhausen et al., the capillary recruitement observed by Bourdeau-Martini et al. might have been due to a better optical penetration in the case of hypoxemia. Since Bourdeau-Martini et al. used low magnification and their microscope had a theoretical depth of focus of 20.72 um which was similar to the average ICD, it was possible that more than one layer of capillaries might have been focused. On the other hand, in Steinhausen et al.'s experiments, capillary recruitement could have been impeded by the

mechanical trauma induced by the pitchfork type device which restrained the movement of the heart. It was therefore uncertain whether coronary capillary recruitement did exist and if so how significant it was. The reason why Bourdeau-Martini et al. did not use higher power objectives was that high power objectives had such short working distances (2.2 mm for 22X) that they could not be used on a freely beating heart in situ.

To resolve this controversy, it would be necessary to have an in situ heart which is stationary enough to allow the use of Bourdeau-Martini et al.'s system with a higher power objectives but without the restraining device. As part of this project, in vivo cinematography was applied to the heterotopically isotransplanted hearts which were anastomosed to the two major abdominal vessels where the beating transplants were not influenced by the respiratory movements of the lungs. Since the left ventricle of the transplanted heart was practically empty beating, its small excursions would allow the use of a high power objective with a small depth of focus. Data could then be obtained using high and low power objectives on the same heart under the same conditions. These data would be used to answer the question of whether low power objectives give higher capillary density measurements under hypoxia.

Chapter II

METHOD

2.1 PRODUCTION OF CARDIAC HYPERTROPHY AND ATROPHY

2.1.1 Cardiac hypertrophy

Cardiac hypertrophy was induced by subdiaphragmatic aortic constriction. The method was similar to that used by Beznak et al. in 1969 except that surgical silks instead of silver rings were used to constrict the abdominal aorta in our experiments. Male Sprague-Dawley rats between 300 and 350 gms were anesthetized with pentobarbital sodium (Nembutal) i.p., 5.4 mg/100 g body weight. Mid-line laparotomy was performed and the abdominal aorta close to the diaphragm carefully dissected. A blunt #23 G needle (diameter 0.8 mm) was placed next to the dissected aorta and a piece of 4-0 silk was used to ligate the aorta to the needle. The needle was then removed and the ligature stayed in place. Since the heart then had to pump against a greater afterload, cardiac hypertrophy developed in about one week's time.

2.1.2 Cardiac atrophy

Cardiac atrophy was induced by heterotopic isotransplantation of a donor's heart onto the abdominal aorta and inferior vena cava of another inbred recipient.

The surgical procedure was similar to that used by Ono and Lindsey in 1969. Male inbred Buffalo rats, 70th generation, body weight 300 - 400 g were anesthetized with pentobarbital sodium (Nembutal) i.p., 5.4 mg/100 g body weight. The donor rat was heparinized by injecting 0.5 ml of 1% heparin in saline into the inferior vena cava and five minutes were allowed for equilibration. The thorax of the donor rat was then quickly open through mid-line incision, the superior and inferior vena cavae draining into the heart were ligated, and the ascending aorta and the main pulmonary artery were transected. The remaining pulmonary veins and left atrium were ligated as a whole and the heart was then removed. The excised heart was perfused with normal saline to wash out the residual blood in the coronary circulation and then preserved in ice cold normal saline ready for transplantation.

Mid-line laparotomy was performed on the recipient rat and the abdominal aorta and inferior vena cava were dissected just below the kidneys. The ascending aorta of the donor's heart was then anastomosed to the recipient abdominal aorta and the pulmonary artery of the donor's heart anastomosed to the recipient inferior vena cava. This way, arterial blood went from the recipient abdominal aorta to the ascending aorta of the transplanted heart and retrogradely to its coronary circulation and finally came out of the coronary sinus in the right atrium. The blood

from the right atrium then flowed into the right ventricle and was pumped through the transplant's pulmonary artery to return to the recipient's inferior vena cava. Assuming that the aortic valve of the transplant was competent, the left ventricle of the transplanted heart was then practically empty beating performing a minimum work load represented by the occasional ejection of outflow from thebesian veins and arterioluminal vessels into the aorta. Consequently, the left ventricle started to atrophy after about one week.

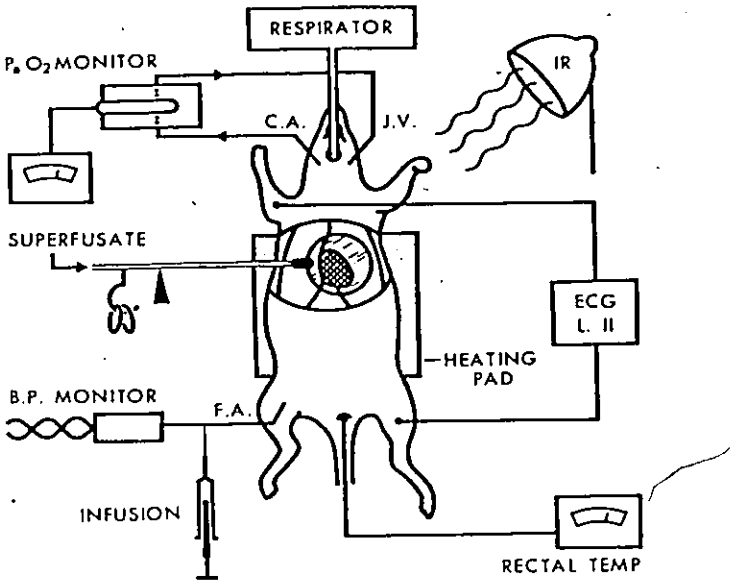
2.2 EXPERIMENTAL PROTOCOL

2.2.1 Normal and hypertrophied hearts

Male Sprague-Dawley rats between 300 and 400 grams of body weight were anesthetized with intraperitoneal injection of pentobarbital sodium (Nembutal) 5.4 mg/100 g body weight. The trachea, femoral artery, carotid artery and external jugular vein were catheterized and the animal was then tied to the stage carrier.

The skin covering the thorax was incised along the mid-line, detached, and retracted to the side. A small perforation was made in the diaphragm just behind the xiphoid process and artificial ventilation was initiated with a tidal volume of 3 mls, respiratory rate 50/min. Two long hemostats were used to clamp the two internal mammary arteries to the sternum by clamping the anterior wall of the thorax. The thoracic wall was then lifted slightly and was

slit open between the two hemostats with a scalpel. Ligatures were placed at the first and sixth intercostal spaces to ligate the internal mammary arteries to the halves of the sternum. The two hemostats were then released and bilateral cuts were made on the second and fifth intercostal spaces. The detached rib cages were then clamped and cut off, allowing a complete thoracotomy with minimal bleeding. In case of bleeding, blood from a donor rat was transfused through the jugular venous catheter. The final layout is displayed in Figure 1.



Abbreviations:
C.A. - Carotid Artery
F.A. - Femoral Artery
J.V. - Jugular Vein

Figure 1: Surgical preparation of the animal

The beating heart was covered with a piece of saline-soaked gauze and the carrier with the animal was then placed on a tray where the animal could be positioned so that the left ventricle would face upward to the objective of the microscope. The tray was quickly transferred to the stage of the microscope and artificial ventilation resumed with 30% O2 - 70% N2 gas mixture and the water recirculating warming under pad connected to warm up the animal. The femoral arterial cannula was connected to a Statham P23Dd strain gauge transducer for blood pressure monitoring. Furthermore, the animal was continuously infused (0.1 ml/min) through the femoral artery with 5% Albumin-Ringer's solution (Abbott) containing 20 mg% of heparin to prevent blood clotting inside the cannula and to replace fluid loss during the experiment. The oncotic pressure of the infusate was maintained with albumin instead of dextran because dextran had been found to have deleterious effects on the rat hemodynamics (Flaim et al., 1978).

The blood pressure was continuously monitored throughout the experiment to ensure that the mean arterial blood pressure was not lower than 100 mm Hg. If necessary, blood or packed red blood cells were given through the venous catheter. The rectal temperature of the rat was also continously monitored with a thermistor probe connected to the telethermometer (Yellow Spring Instrument). ECG needle electrodes were placed subcutaneously and lead II

electrocardiogram of the animal was occasionally recorded to monitor the electrical activity of the heart.

The gauze covering the heart was then removed and a coverslip held by a light weight balanced lever system was placed on the surface of the heart. A warm superfusate (37°C) with the following composition was constantly dripping between the heart and the coverslip to keep the cardiac surface moist and warm.

NaCl	128.6 mM	
KCl	3.6 mM	
MgSO4	1.2 mM	
C6H12O6	5.5 mM	
NaHCO3	10.0 mM	
CaCl2	1.8 mM	
HEPES	20.0 mM	--------BUFFERING
NaOH	6.3 mM	--------COMPONENTS

(pH=7.4)

HEPES in combination with HCO_3^- had been shown to be an excellent buffer system (Chagnon and Corbeil, 1973) and was convenient to use in this setting. Since the coverslip was well balanced, it adhered to the cardiac surface by surface tension without exerting mechanical trauma on the heart and eliminated the excess reflections from the cardiac surface and thus made epiilluminating microscopy easier.

The superfusate was not bubbled; therefore the oxygen tension of the superfusate should be atmospheric. However, the diffusion of oxygen from the superfusate to the heart was probably minimal because of the following reasons. First of all, the preparation was not a usual type of superfusion because the superfusate between the coverslip and the cardiac surface was flowing very sluggishly due to surface tension. Furthermore, the amount of oxygen dissolved in this thin layer of superfusate was also small. Consequently, after the initial warming period, the oxygen tension of the superfusate between the coverslip and the heart was probably similar to that in the subepicardium. The animal and the coverslip system were then carefully manipulated so that the heart was immediately below the .....

objective. In most cases, the rectal temperature of the animal up to this stage was lower than 37°C and an infrared lamp was used to warm up the animal to 37°C. Meanwhile, the catheters in the common carotid artery and external jugular vein were connected to the oxygen electrode bypass chamber for the continuous monitoring of arterial oxygen tension.

The animal was then heparinized with 10 mg heparin sodium in 1 ml saline, the clamp on the carotid artery released and the extracorporeal bypass perfused. The arterial oxygen tension could be read directly from the Radiometer panel and was maintained around 150 mm Hg by adding oxygen to the breathing mixture if necessary. Arterial oxygen tension of 150 mm Hg was chosen because Bourdeau-Martini et al. in 1974 reported that the coronary ICD was longest around this arterial oxygen tension. Finally, arterial blood pressure, electrocardiogram and arterial oxygen tension were also recorded on the Grass polygraph.

Cinematography was then performed on different areas of the left ventricular subepicardium of the beating heart until approximately half roll of film (50 ft.) was utilized. Four samples of 100 microlitres each of blood were then withdrawn for pH, pCO2 and hematocrit determination. pH and pCO2 were determined by Radiometer BMS 2 electrode system interfaced with the Radiometer PHM 71 acid base analyzer. Hematocrit was determined by the microcapillary centrifuge

International Equipment). All of these three measurements were determined within three hours after the experiment to ensure that no significant metabolic or physical changes had occurred.

The animal was then ventilated with room air with reduced minute volume so that the arterial oxygen tension decreased to 50 mmHg or below. This was considered adequate for the studying of the effect of hypoxemia on coronary ICD.

It would be ideal to have the arterial oxygen tension decreased closer to 0 mmHg. However, this was not feasible because then the blood pressure of the animal would be low and unstable and the animal would probably die before the experiment could be completed. Nevertheless, this degree of hypoxia is believed to be sufficient to open up all precapillary sphincters because functional capillary density measured under this degree of hypoxia was similar to that obtained by other utilizing histological technique as will be discussed in section 4.1. In most cases, the diaphragm in this stage exhibited spontaneous contractions probably as a result of CNS hypoxia. This would periodically pull the big vessels and the heart, which would make focusing extremely difficult and therefore 0.2 ml of curare (Sigma), 3 mg/ml was administered intravenously to paralyze the diaphragm. Cinematography was then resumed on the left ventricular supepicardium during this hypoxemic period. Four samples of 100 microlitres each of blood were again taken for pH, pCO_2 and hematocrit determinations.

Finally, the heart was excised and appendages such as the aorta, atria, etc. were trimmed off. The ventricle was then divided into right ventricular free wall, septum and the left ventricular free wall, the these individual parts were weighed and then dried at 85°C for overnight. The dried individual parts were weighed again for the determination of their water content.

2.2.2 Transplanted hearts

The in vivo cinematography of the transplanted heart was easier to perform than that of the normal or hypertrophied hearts because thoracotomy was not required. After the cannulation of the femoral artery, carotid artery, jugular vein and the trachea, the abdomen was opened along the previous mid-line suture from transplantation and the intestines were retracted aside to expose the transplanted heart anastomosed to the aorta and the inferior vena cava of the recipient. Due to the position of the aorta and the pulmonary artery of the transplant, the left ventricular surface was facing upward and no further manipulation of the transplanted heart was necessary.

The animal was transferred onto the microscope stage, connected to the blood pressure transducer, electrocardiographic leads, rectal thermistor probe, and the oxygen electrode bypass chamber. The electrocardiograms of the transplant and the recipient's heart were monitored with precordial leads. For the recipient's heart, V4 was used while for the transplant, the exploring electrode was placed on the abdominal wall close to the transplant. This way, electrical interferences between the two hearts were minimized. Both ECG's were recorded on the Grass polygraph. In order to maintain the arterial oxygen tension around 150 mm Hg, artificial ventilation was again required. The coverslip held by the lever system was then placed on the

transplanted heart with the warm superfusate dripping between the heart and the coverslip. The coverslip was further stabilized by placing cotton wools beside the transplanted heart.

The resulting preparation was stable with the excursion of the coverslip less than 2 mm. This allowed the use of a higher magnification objective (22X) which had a working distance of 2.1 mm. The transplanted heart was therefore filmed with 11X and 22X objectives under normoxemia and hypoxemia. Since the 22X objective had a theoretical depth of focus of only 5.95 um, data obtained with it should therefore be representative of only one layer of capillaries. At the end of each stage of filming, samples of blood were taken for pH, pCO2 and hematocrit determinations. Finally, the transplanted heart was removed from the abdomen, and the left ventricular free wall, right ventricular free wall and the septum were weighed and then dried for the determination of water content.

2.3 OPTICS AND FILMING

The optical system used was the Leitz Ortholux microscope equipped with the "Ultropak" objectives. The source of stroboscopic light was the "Chadwick-Helmuth 136 Strobex" which was capable of delivering high intensity xenon discharge light with a short duration of 130 microseconds. The light from the "Strobex" entered the

microscope from the side and was reflected by a mirror to the objective. Owing to the design of the mirror, the light came down to the objective as a hollow cylinder and was then concentrated to a point by the circumferential collimator of the objective for the epiillumination of the heart . It was the reflected light from the heart that entered the objective lens and was displayed by the eye piece. The ray diagram of the optical system is shown in Figure 2.

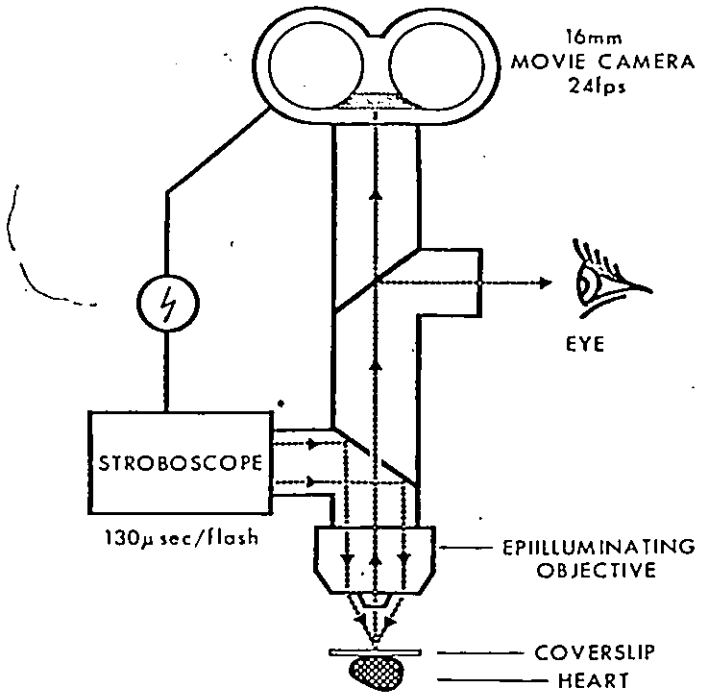


Figure 2: Ray diagram of the optical system

For the periods of filming during the experiment, the "Strobex" was triggered through a photo-electric coupler by the "Bolex H16" motor-driven camera. The film speed was set at 24 frames per second and the room darkened so that the cardiac movement could be optically "frozen" by the "Strobex" during the opening of the shutter due to the short duration (130 usec) of one stroke of light and exposed on the Kodak Ektachrome 7239 (daylight ASA 160) film. Before every experiment, a stage micrometer slide with 50 um X 50 um grids was filmed for calibration purposes. The relatively long working distance (5.8 mm for the 11X objective) of the "Ultropak" objective allowed free movements of the heart during the experiment without touching the objective. However, because of these movements, focusing could only be done in such a way that capillaries could be seen in focus for a brief moment only. Theoretically, when the focal point of the objective lay between the extremes of the cardiac movements, one or two focused frames would be obtained from each cycle of the cardiac movement.

The principal cause of the up and down cardiac excursions turned out to be the respiratory movements of the lungs. Since the period of stationary phase was longest at the end of expiration, capillaries were mostly focused at the end of expiration. With the 11X objective, each focused frame contained about 20 capillaries. One roll of

100 feet film was used for each experiment. For hypertrophied and normal hearts, there were about 20 focused frames from the 100 feet of film. For the transplanted hearts, the yield was better, there were about 35 to 40 focused frames from 100 feet of film.

2.4 OXYGEN ELECTRODE BYPASS SYSTEM

This system was designed and built for continuous monitoring of arterial oxygen tension during the experiment. A Clark type oxygen electrode (Radiometer E5046) was secured in a small electrode chamber which was in turn housed in a water recirculating chamber maintained at 37°C and had a total internal volume of about 1 ml. This small volume allowed fast exchange of blood inside the electrode chamber and it also would not cause severe pooling of blood from the animal when the bypass was opened. The spiral shape of the polyethylene tubing inside the water recirculating chamber allowed the warming of the blood back to its in vivo temperature (37°C).

The system was calibrated by flushing gas-equilibrated solution of 30% glycerol in water into the electrode chamber. Gas mixtures containing either 100% oxygen or 10.3% oxygen in nitrogen were used to bubble the glycerol solution in a water jacketed muscle chamber maintained at 37°C. The glycerol solution was chosen instead of blood because it had been shown that 30% glycerol

in water had very similar oxygen diffusion characteristics as blood (Hulands et al., 1970). Furthermore, this solution had several advantages over blood as it was readily available, would not foam, was not subject to bacterial contamination and it could be reused.

With the administration of heparin 5 mg per 45 minutes to the animal during the experiment, the extracorporeal circulation could be maintained without blood clotting. Since the major blood vessels were all ligated or clamped, heparinization of the animal to this degree did not cause severe bleeding problems. Finally, the rubber segment of the venous side to the bypass allowed administration of drugs, packed red blood cells and withdrawal of blood samples using hypodermic needles.

2.5 DATA PROCESSING AND STATISTICS

Images of the subepicardium obtained in the form of 16 mm Ektachrome microfilm strips were projected with the Zeiss DL2 microfilm projector onto a blank paper for tracing. The calibration grid was first projected, the final magnification adjusted to 600X and then focused frames were projected on the blank paper and the network of capillaries traced by drawing lines along the image of the centre of the capillaries. Every traced frame was coded to allow future retrieval. Capillaries were defined as vessels whose diameter were less than 7um (Sobin and Tremer, 1972).

The area on the tracing suitable for further measurements was selected based on the sharpness of focus and then sets of auxiliary lines approximately perpendicular to the capillaries were drawn randomly on the identified area.

Figure 3 shows a typical tracing of the capillary pattern in the rat subepicardium with the auxiliary lines. The lines were not absolutely parallel since the capillaries were not absolutely parallel either. The space between adjacent random lines was maintained at approximate equal distances. Individual centre to centre distances on the random auxiliary lines intersected by adjacent capillaries were measured and recorded as ICD's. The data were further separated into two groups, those obtained during normoxemia and hypoxemia. Since the total number of ICD measurements involved was in the order of hundreds in each experiment, the data were punched on computer cards and processed in the IBM 360 computer to calculate the mean ICD, standard deviation, standard error and coefficient of variation of the ICD's, skewness (/B1) and kurtosis (B2) of the ICD frequency distribution and finally to plot the histogram of the ICD frequency distribution. This was the basic statistics performed on all raw experimental data. Finally, paired and standard t-tests were used for testing the significance of difference between means.

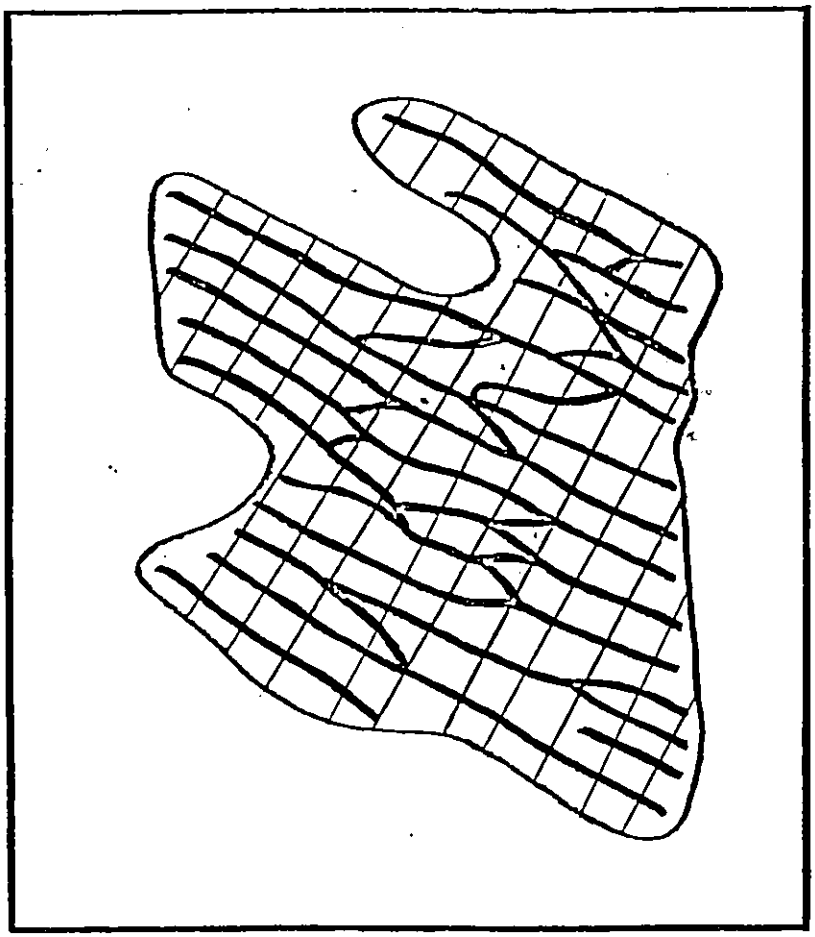
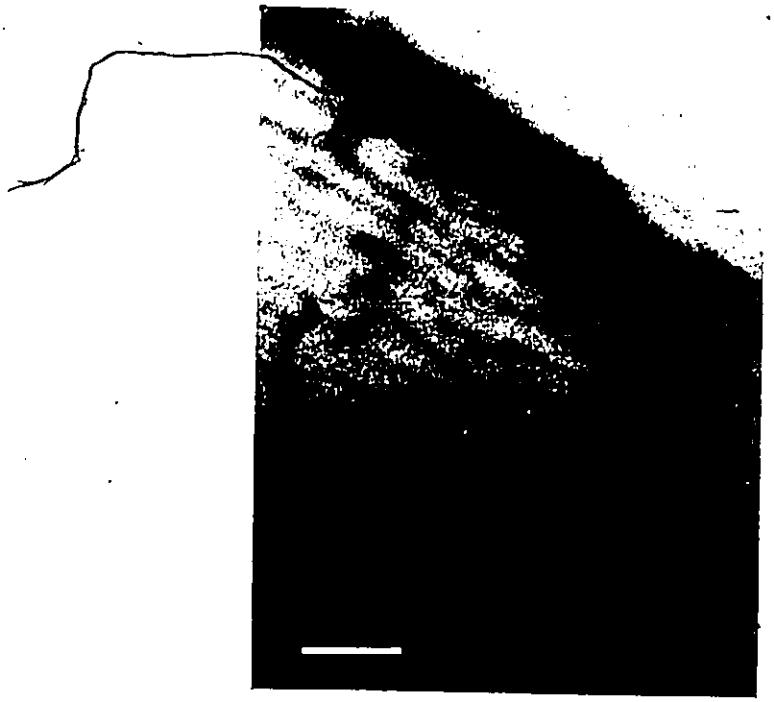


Figure 3: A typical tracing of the capillary pattern in the rat subepicardium

Chapter III

RESULTS

Figure 4 shows the characteristic pattern of the capillary network in the rat subepicardium. The corresponding pencil tracing has been shown in Figure 3. The photograph was developed from a negative made from an Ektachrome film strip. Data presented in this thesis were obtained from such capillary patterns; however, the photograph as presented was not normally developed. Instead, pencil tracings of projected images as shown in Figure 3 were used for measurement. In average, each of such pencil tracing yielded measurement of about 100 ICD's and up to 20 tracings were obtained from each experiment. Cónsequently, the total number of ICD data for each experiment ranged from 100 to 2000 - 3000 and computer was used for all subsequent evaluation of data.



(The horizontal bar at the bottom of the photograph represents 50 um.)

Figure 4: Typical capillary pattern in the rat subepicardium in vivo (270)

3.1 EFFECT OF GROWTH ON CAPILLARY DENSITY

As mentioned in the introduction, Henquell et al. (1976) studied the change of coronary intercapillary distance with growth. They found a relationship between left ventricular weight and coronary intercapillary distance whereby both the functional and minimum ICD decreased linearly with left ventricular weight, maintaining a constant ICD difference of 2 um between them. Coronary capillary density (CD) in this case was again plotted against left ventricular weight (LVW). The established relationship between capillary density and left ventricular weight would also be useful for later comparisons with data obtained from experimental groups because the change in heart weight after subdiaphragmatic aortic constriction or after heterotopic isotransplantation was observed mainly on the left ventricle.

In our experiments, capillary densities was calculated from ICD's using the following formula,

$$CD = 1000000/ICD^2$$

where CD was in capillaries/mm2 and ICD in um, assuming a square model which implies that the cross section of the cylinder supplied by a single capillary was a square with sides equal to half of the intercapillary distance.

The reason why capillary density was used in addition to ICD was two fold. First of all, some authors defined ICD as the distance between the centres of adjacent capillaries while others defined ICD as only the distance between the walls of adjacent capillaries. To avoid confusion, all ICD's measured (centre to centre) were converted to capillary densities. The second reason was that most of the earlier workers presented their findings in capillary densities only because they actually counted the number of capillaries per section. Therefore, presenting our data as capillary densities would make them comparable with reported findings and perhaps would also make them easier to visualize.

The data obtained under normoxemia and hypoxemia are shown in Table 1.

TABLE 1

RELATIONSHIP BETWEEN ICD AND LEFT VENTRICULAR WEIGHT IN NORMAL HEARTS

LVW (g)	NORMOXEMIA ICD (um)	NORMOXEMIA CD (#/mm2)	HYPOXEMIA ICD (um)	HYPOXEMIA CD (#/mm2)
0.263			15.40	4217
0.311	15.68	4067	15.60	4109
0.315	16.49	3678		
0.351	18.22	3012	17.04	3444
0.385	18.98	2776	17.35	3322
0.401	18.69	2863		
0.417			16.63	3616
0.422	20.12	2470		
0.460	19.93	2518		
0.522	20.15	2463	18.69	2863
0.526	20.56	2366	18.51	2919
0.536	19.64	2592	17.90	3121
0.542			18.61	2887
0.580	20.02	2495		
0.619	19.82	2546	19.87	2533
0.645	19.37	2665	19.91	2523

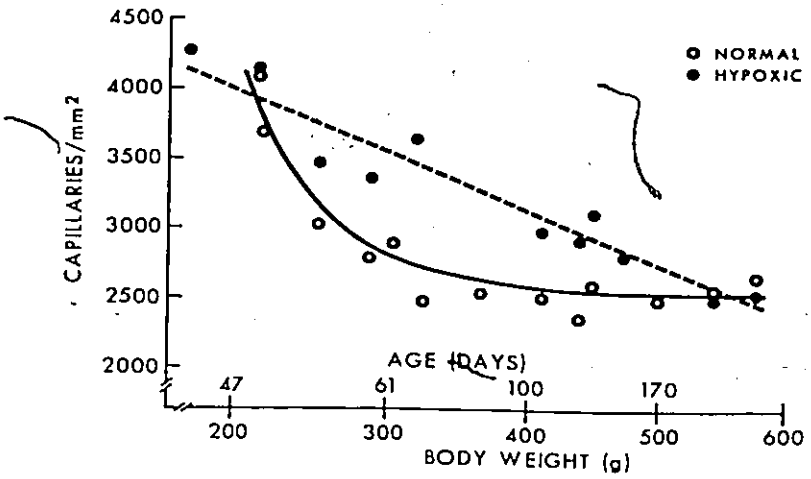
As shown in Table 1, in normoxemia, functional capillary density in normal hearts decreased with growth when the left ventricular weight was between 0.27 - 0.45 g; however, as the left ventricular weight increased further, capillary density remained relatively unchanged. The data points were well fitted with the following equation:

$$CD = 2516 + 0.7432/(LVW)^{0.3929}$$

with CD in per mm2 and LVW in gram (see Figure 5). The correlation coefficient(r) was -0.9524 when the relationship was linearized by plotting ln(CD - 2516) against ln(LVW). In hypoxemia, however, the relationship between capillary density and left ventricular weight did not show a plateau. Instead, capillary density decreased linearly with left ventricular weight throughout the entire range of left ventricular weight studied. The relationship could be expressed by the following equation:

$$CD = -4285.78(LVW) + 5229.85$$

with CD in per mm2 and LVW in gram (see Figure 5). The correlation coefficient(r) was -0.9454.



(The above abscissa is linear with respect to left ventricular weight; however, due to limited space for labelling the axis, only the age and body weight scales are displayed. The two points where the two graphs intersect correspond to left ventricular weights of approximately 0.30 g and 0.65 g.)

Figure 5: Relationship between capillary density and growth in normal hearts

These two established regression lines as shown in Figure 5 would be useful for comparing data obtained from experimental groups to that obtained from the normals. The difference in capillary density obtained under normoxemia and hypoxemia was defined as the capillary reserve. Therefore, by substracting the normoxemic capillary density from the hypoxemic capillary density using Figure 5, the relationship between capillary reserve and left ventricular weight was obtained, as shown in Figure 6.

In contrary to what Henquell et al. (1976) had found, the differences between hypoxemic and normoxemic ICD's was not constant throughout this age range. The capillary reserve varied with left ventricular weight in a parabolic relationship with a maximum, and there was no reserve when the animal was either relatively young or relatively old, as shown in Figure 6.

Figure 7, shown immediately below Figure 6, was modified from data reported by Vizek and Albrecht (1973). The comparison of the two figures is important for later discussion on the relationship between capillary reserve and left ventricular weight.

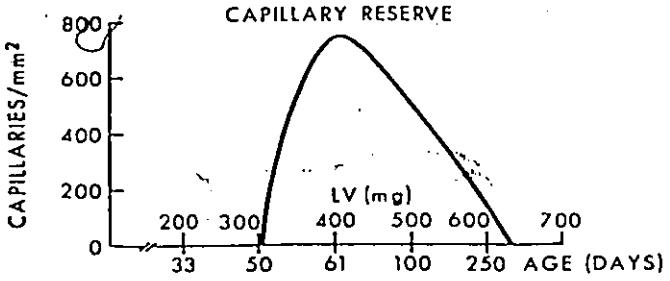


Figure 6: Relationship between capillary reserve and LVW

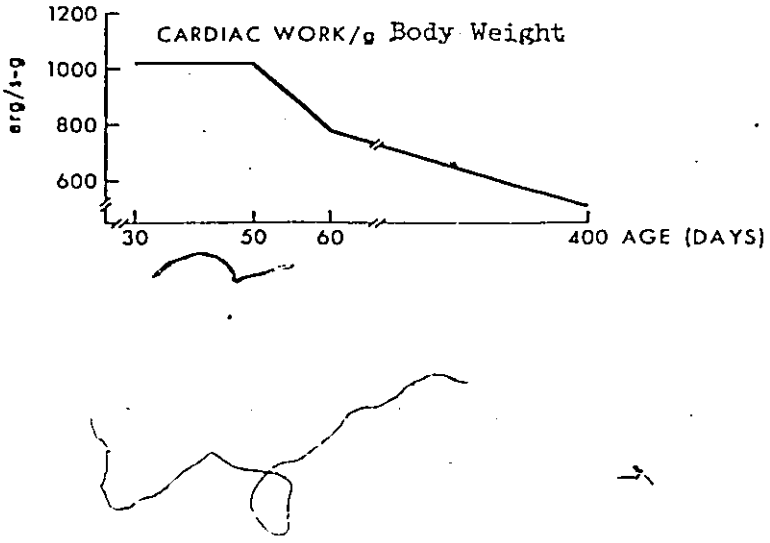


Figure 7: Relationship between cardiac pressure-volume work and age

Figure 6 shows that when the rat is young (up to 49 days), there is practically no capillary reserve. Then capillary reserve starts to appear and gradually increases and reaches a maximum of 740 capillaries/mm2 (21% of total number of capillaries) when the rat is 62 days old. Subsequently, capillary reserve begins to decline and finally approaches zero when the left ventricular weight is 0.630 g which is normally in a rat of about 560 g. The age of the rat at this body weight is difficult to estimate from the body weight because it lies on the plateau region of the growth curve, but it is probably between 150 - 180 days.

To demonstrate the appearance and disappearance of a significant capillary reserve in the rat heart, Student's t-tests are performed to compare the paired mean ICD's obtained under normoxemia and hypoxemia from rats of different ages. The results are displayed in Table 2. The t values indicate that the difference between mean ICD's obtained under normoxemia and hypoxemia is highly significant in the middle and is relatively insignificant in both extremes of left ventricular weight. Furthermore, the capillary reserves in the two largest hearts were actually negative. This "negative capillary reserve" phenomenon was a result of decreased capillary density in hypoxemia. Interestingly, some of the hypertrophied hearts show up similar tendencies; further discussion of the phenomenon will be made in section 4.4.

TABLE 2

CAPILLARY RESERVE IN NORMAL HEARTS

LVW (g)	NORMOXEMIA ICD	S.D.	N	HYPOXEMIA ICD	S.D.	N	t	CAPILLARY RESERVE
0.311	15.68	7.27	673	15.60	7.91	108	0.1048	42
0.351	18.22	7.32	2319	17.04	6.05	95	1.5496	432
0.385	18.98	8.11	336	17.35	8.37	141	1.9841	546
0.522	20.15	8.17	276	18.69	7.29	136	1.7660	400
0.526	20.56	5.63	252	18.51	6.07	191	3.6692	553
0.536	19.64	7.00	1067	17.90	6.88	158	2.9224	529
0.619	19.82	7.16	1140	19.87	6.47	258	0.1030	- 13 *
0.645	19.37	6.52	638	19.91	6.81	360	1.2363	-143 *

ICD refers to the mean ICD in um, N stands for number of observations obtained from each heart and CAPILLARY RESERVE is in capillaries/mm2
* - "negative capillary reserve ?"

3.2 EFFECT OF ONE DAY TRANSPLANTATION ON CAPILLARY DENSITY

The one day transplanted hearts in the abdomen were beating regularly although at a slower rate than the recipient hearts probably as a result of injury done to the sinoatrial node during the surgery. Capillary pattern in the one day transplanted hearts appeared basically the same as that in the normal hearts as displayed in Figure 4. Data obtained from the one day heterotopically isotransplanted hearts are shown in Table 3. The "LVW in %" refers to the ratio of actual left ventricular weight to the expected left ventricular weight. The expected left ventricular weight (LVW) was calculated from body weight (BW) using the following equation:

$$\log(LVW) = 0.745\log(BW) + 0.752$$

where LVW is in mg, BW is in g ($r=0.971$, SEE=0.066)

This relationship was established in our laboratory using over 500 rats.

TABLE 3

ONE DAY TRANSPLANTED HEARTS

	LVW (g)	EXPECTED LVW (g)	LVW in %	NORMOXEMIA ICD (um)	NORMOXEMIA CD	HYPOXEMIA ICD (um)	HYPOXEMIA CD
	0.482	0.477	101%	17.65	3210	19.22	2707
	0.487	0.451	108%	17.11	3416	17.18	3388
	0.520	0.452	115%	18.25	3002	17.10	3420
	0.556	0.463	120%	21.60	2143	20.43	2396
	0.571	0.457	125%	16.86	3518	16.64	3612
	0.587	0.524	112%	19.31	2682	20.98	2272
MEAN	0.534	0.471	114%	18.46	2995	18.59	2966
SD	0.044	0.028	9%	1.77	514	1.87	579
SEM	0.018	0.011	3%	0.72	210	0.76	236

LVW - left ventricular weight, LVW in % - ratio of found to expected LVW expressed in % when expected LVW equals 100%

Since the transplanted heart was almost empty beating and the transmission of respiratory movements was minimal, the excursions of the heart were so small that the higher power objective (22X) could be used and data from the transplanted hearts were therefore obtained with both the 22X and 11X objectives. Only the data obtained with the 11X objective have been presented in Table 3 for the following reasons. First of all, the differences between data obtained by both lenses had been found to be statistically insignificant as would be discussed in section 4.5. Secondly, the number of ICD measurements acquired with the 11X objective was higher so that the mean ICD calculated would be also more reliable. Lastly, the illumination under the 11X objective was better because of the greater numerical aperture of the lens so that the quality of the image was also better.

As shown in Table 3, the average "LVW in %" of the one day transplants was 113% ± 3% (SEM). Considering the possible difference in heart weight among different strains of rats and the inherent variability of the relationship between left ventricular weight and body weight, this degree of deviation is not statistically significant. Concerning the extent of capillary reserve in the one day transplanted hearts, paired t-tests showed that the difference between capillary densities obtained under normoxemia and hypoxemia was insignificant (paired t = 0.0797).

The averages of the above data were plotted on the background of data obtained from normal animals; they lay close to the line representing the hypoxemic values. This is shown in Figure 8.

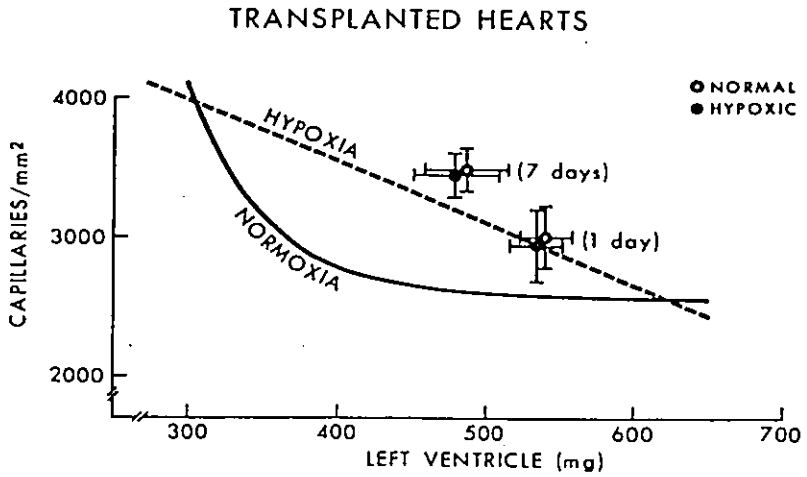


Figure 8: Capillary densities in one and seven day transplanted hearts

3.3 EFFECT OF SEVEN DAY TRANSPLANTATION ON CAPILLARY DENSITY

The seven day transplanted hearts looked essentially the same as the one day transplants except that some fibrous tissue had already grown over the pericardial surface. Consequently, focusing of the seven day transplanted hearts was more difficult due to greater reflections and backscatters of light and yield of acceptable images was smaller. However, the capillary pattern in the seven day transplanted hearts showed similar morphological characteristics as that in normal hearts as displayed in Figure 4. Again, only the data obtained with the 11X objective are presented here in Table 4 since no significant difference has been found between results obtained with 11X and 22X objectives as will be discussed in section 4.5.

TABLE 4

SEVEN DAY TRANSPLANTED HEARTS

	LVW(g)	EXPECTED LVW(g)	LVW in %	NORMOXEMIA ICD(um)	NORMOXEMIA CD	HYPOXEMIA ICD(um)	HYPOXEMIA CD
	0.435	0.483	90%	16.40	3718	17.40	3303
	0.439	0.482	91%	17.02	3452	16.04	3887
	0.482	0.455	106%	16.70	3586	17.38	3311
	0.559	0.682	82%	18.27	2996	17.55	3247
MEAN	0.479	0.526	92%	17.10	3438	17.09	3437
SD	0.058	0.105	10%	0.82	314	0.71	301
SEM	0.029	0.053	5%	0.41	157	0.35	151

LVW - left ventricular weight, LVW in % - ratio of found to expected LVW expressed in % when expected LVW equals 100%

The "LVW in %" of 92% ± 5% (SEM) as shown in Table 4 indicated that a small degree of atrophy had already occured in the transplanted hearts seven days after transplantation. It would be more interesting to study the transplanted hearts after longer period of transplantation because they would then have atrophied to about 30% of its initial weight (Dittmer and Goss, 1973). However, the growth of connective tissue on top of their pericardial surface made it impossible to study them more than seven days after transplantation.

Concerning the extent of capillary reserve in the seven day transplanted hearts, paired t-tests showed that the difference between capillary densities obtained under normoxemia and hypoxemia was insignificant ($t = 0.0025$). For comparison to the data from normal hearts, the means (± SEM) of the above data were plotted on the background of data obtained from normal animals as shown in Figure 8.

3.4 EFFECT OF HYPERTROPHY ON CAPILLARY DENSITY

Since the pressure overloaded hearts had undergone concentric hypertrophy, the apparent size of the hypertrophied heart was not changed much. It was only at the end of each experiment when the heart was dissected that the increased myocardial wall thickness of the left ventricle leading to hypertrophy could be seen. In other words, only after completing the entire experimental

procedure would one know whether the heart hypertrophied by at least +35%. This arbitrary value was selected to ensure that the average degree of hypertrophy would be great enough for observing its effect on the coronary ICD's. Furthermore, it was more difficult to work with the hypertrophied hearts than the normal ones because they were more susceptible to cardiac failure during the operation. Finally, the blood pressure in the upper body was higher due to aortic contriction so that bleeding was also more severe. Data obtained from this experimental group are shown in Table 5.

TABLE 5

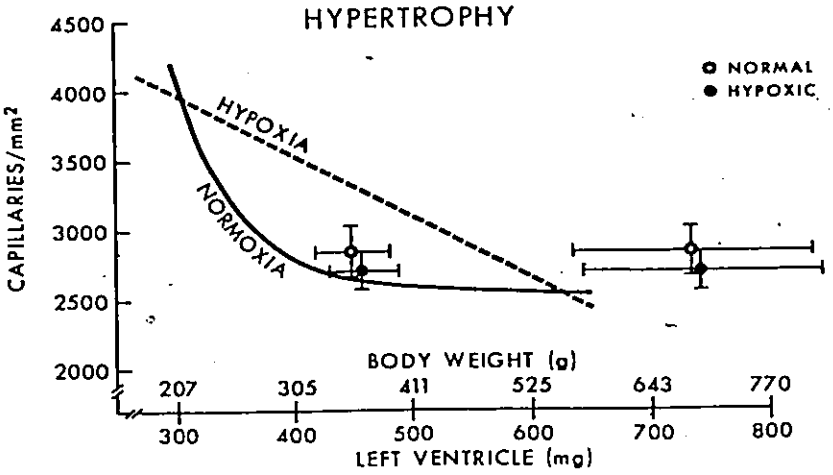
HYPERTROPHIC HEARTS

	LVW(g)	EXPECTED LVW(g)	LVW in %	NORMOXEMIA ICD(um)	CD	HYPOXEMIA ICD(um)	CD
	0.498	0.364	137%	18.12	3046	20.57	2363
	0.614	0.429	143%	16.98	3468	18.48	2928
	0.654	0.394	166%	18.80	2829	20.15	2463
	0.728	0.499	146%	20.29	2429	18.99	2773
	0.895	0.517	173%	20.74	2325	19.91	2523
	1.022	0.487	210%	18.42	2947	17.90	3121
MEAN	0.735	0.448	163%	18.89	2841	19.33	2695
SD	0.193	0.062	27%	1.40	420	1.04	295
SEM	0.079	0.025	11%	0.57	172	0.43	120

LVW - left ventricular weight, LVW in % - ratio of found to expected LVW expressed in % when expected LVW equals 100%

The "LVW in %" of 163% ± 11% (SEM) as shown in Table 5 indicated that a relatively high degree of hypertrophy developed in the hearts of this experimental group. Concerning the extent of capillary reserve, paired t-tests showed that the difference between capillary densities obtained under normoxemia and hypoxemia was insignificant ($t = 0.3341$).

The means of the above data are plotted on the background of data obtained from normal animals according to body weight and left ventricular weight respectively (see Figure 9). The pair on the right side is plotted according to average body weight (360 g) while the latter pair is plotted according to left ventricular weight (740 mg). Since the normal relationship between capillary density and left ventricular weight is disturbed by cardiac hypertrophy, this separate plotting is important for the interpretation of data as will be discussed later.



Legend:
Right pair - plotted against body weight
Left pair - plotted against left ventricular weight

Figure 9: Capillary densities in hypertrophied hearts

3.5 COMPARISON OF CAPILLARY DENSITIES OBTAINED WITH 11X AND 22X OBJECTIVES

As mentioned in the "introduction" section, Steinhausen et al. (1978) suggested that the capillary recruitement observed by Bourdeau-Martini et al. might have been due to the greater penetration power of the optical system during hypoxemia so that more than one layer of capillaries might be observed since the theoretical depth of focus of the 11X "Ultropak" objective was 20.72 um. However, under the experimental condition, it is doubtful if the penetration of the light into the subepicardium will ever reach the theoretical depth of focus. To test this, data obtained with 11X and 22X objectives from the same heart under hypoxemia were compared. With the 22X objective one could be certain that only one layer of capillary network was filmed because the 22X objective had a theoretical depth of focus of only 5.95 um. All data shown in Table 6 were obtained from the transplanted hearts because it was impossible to use 22X objective on the hearts in situ due to their movements which are larger than the working distance (2.1 mm) of the objective. In addition, data obtained in normoxemia are also tabulated in Table 6 for comparison.

TABLE 6

ICD'S OBTAINED WITH 11X and 22X OBJECTIVES

TYPES OF HEART		HYPOXEMIA (11X) um	(22X)	DIFFERENCE (22X - 11X)
ONE DAY TRANSPLANT		17.18	19.11	+1.93
		20.98	19.59	-1.39
		19.22	18.58	-0.64
		16.64	17.33	+0.69
SEVEN DAY TRANSPLANT		16.04	15.56	-0.48
		17.40	16.24	-1.16
		17.38	16.37	-1.01
	MEAN	17.83	17.54	-0.29
	SD	1.70	1.57	1.19

TYPES OF HEART		NORMOXEMIA (11X) um	(22X)	DIFFERENCE (22X - 11X)
ONE DAY TRANSPLANT		21.60	21.18	-0.42
		17.11	18.34	+1.23
		17.65	17.56	-0.09
		16.86	17.75	+0.89
SEVEN DAY TRANSPLANT		17.02	15.80	-1.22
		16.40	16.35	-0.05
		16.70	16.59	-0.11
	MEAN	17.62	17.65	+0.03
	SD	1.80	1.79	0.81

3.6 FREQUENCY DISTRIBUTION OF THE INTERCAPILLARY DISTANCES

The large number of ICD measurements obtained from each experiment justified the plotting of ICD histograms. An interval width of 4 um, used to group the ICD's from 0 to 60 um, was chosen for two reasons, first of all, for easier comparison with data obtained by Bourdeau-Martini et al. in 1974 who grouped their data in 4 um intervals. Secondly, the average diameter of the coronary capillaries was approximately 4 um, and because ICD's in this thesis are measured from centre to centre of adjacent capillaries, the shortest distance was therefore approximately 4 um. Average ICD histograms were computed for each experimental group under normoxemia and hypoxemia by averaging the relative frequencies in each interval of histograms obtained from each experiment. The numerical results are shown in tables 7, 9, 10, 11, and the histograms are displayed in figures 10 to 13. Finally, skewness and kurtosis were computed for the two average histograms using the mid-class ICD values and the relative frequencies as shown in Table 8.

3.6.1 Normal hearts

For better comparison, frequency distributions of ICD's were computed only for those experiments from which data were obtained under both normoxemia and hypoxemia in the same heart. Table 7 shows individual frequency distributions of the ICD's obtained from experiments

performed on the 8 normal hearts under normoxemia and hypoxemia. The two average histograms (means) were calculated and are also displayed in graphical form in Figure 10. Their skewness and kurtosis are shown in Table 8. Only the skewness of the normoxic ICD histogram was significantly different ($p = 0.05$) from the ideal value of zero for normal distribution while the other differences were not significant.

TABLE 7

FREQUENCY DISTRIBUTIONS OF ICD'S IN NORMAL HEARTS

(NORMOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE													
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	
								TO								(um)
LVW(g)	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	
0.311	6	8	13	21	27	10	6	2	0	0	0	0	0	0	0	
0.351	3	3	9	20	29	14	10	4	1	1	0	0	0	0	0	
0.385	4	5	9	13	26	16	11	8	2	2	0	0	0	0	0	
0.522	0	5	5	19	25	15	12	6	6	0	0	0	0	0	0	
0.526	0	0	3	16	33	25	10	5	2	1	0	0	0	0	0	
0.536	1	2	5	19	29	16	11	7	2	1	0	0	0	0	0	
0.619	1	3	5	16	29	15	14	6	3	1	0	0	0	0	0	
0.645	1	2	8	19	26	20	12	5	2	0	0	0	0	0	0	
MEAN	2	4	7	18	28	16	11	5	2	1	0	0	0	0	0	

(HYPOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE													
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	
								TO								(um)
LVW(g)	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	
0.311	12	8	11	12	31	11	10	2	0	0	0	0	0	0	0	
0.351	4	2	8	25	35	14	6	2	0	1	0	0	0	0	0	
0.385	4	7	14	21	18	16	10	3	1	0	0	0	0	0	0	
0.522	2	5	5	21	28	15	11	5	2	0	0	0	0	0	0	
0.526	0	3	12	22	23	18	14	5	1	0	0	0	0	0	0	
0.536	1	5	10	26	22	12	11	6	0	1	0	0	0	0	0	
0.619	2	2	3	17	31	20	13	4	3	1	0	0	0	0	0	
0.645	0	1	8	16	31	17	12	7	3	0	0	0	0	0	0	
MEAN	3	4	9	20	27	15	11	4	1	0	0	0	0	0	0	

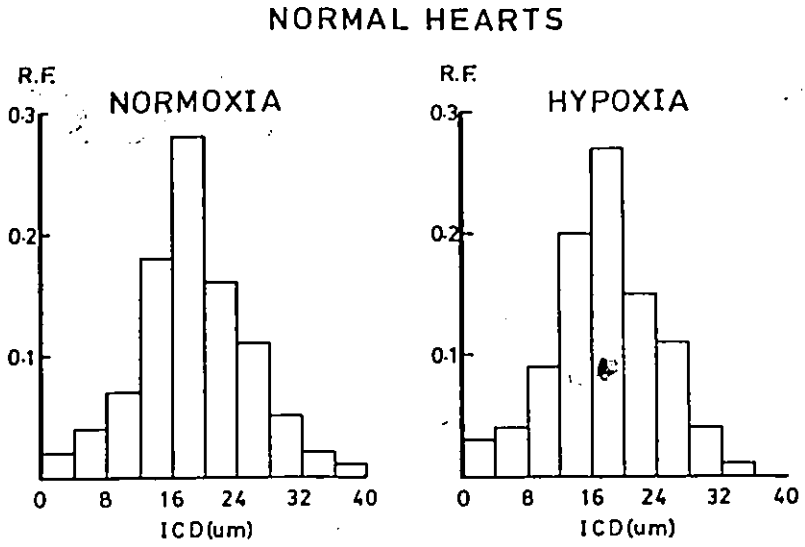


Figure 10: Average ICD histograms in normal hearts

TABLE 8

STATISTICAL PARAMETERS OF THE CORONARY ICD FREQUENCY DISTRIBUTIONS

	NORMOXIA	HYPOXIA
(NORMAL)		
MEAN	17.48 u	16.56 u
S.D.	6.71 u	6.41 u
/B1	0.5973 *	0.3653
B2	3.3549	2.9295
(ONE DAY TRANSPLANTATION)		
MEAN	17.91 u	18.13 u
S.D.	7.14 u	7.26 u
/B1	0.2056	0.2106
B2	3.1989	3.0258
(SEVEN DAY TRANSPLANTATION)		
MEAN	16.50 u	16.36 u
S.D.	6.57 u	5.61 u
/B1	0.4284 *	B1 = 0.1747
B2	3.8829 *	B2 = 3.8023 *
(CARDIAC HYPERTROPHY)		
MEAN	18.49 u	18.84 u
S.D.	6.85 u	6.06 u
/B1	0.0809	-0.1009
B2	3.4295	3.6198

/B1 = Skewness, B2 = Kurtosis.
* - statistically significant, p=0.05

3.6.2 One day transplanted hearts

Table 9 shows individual frequency distributions of the ICD's obtained from experiments performed on one day transplanted hearts under normoxemia and hypoxemia. The two average histograms (means) were calculated and are also displayed in graphical form in Figure 11. Their skewness and kurtosis are shown in Table 8. The skewness and kurtosis values of the normoxic and hypoxic ICD histograms were all not significantly different ($p = 0.05$) from the ideal values for normal distribution.

TABLE 9

FREQUENCY DISTRIBUTIONS OF ICD'S IN ONE DAY TRANSPLANTED HEARTS

(NORMOXIA)

LVW (g)	RELATIVE FREQUENCIES IN PERCENTAGE														(um)
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	
	TO														
	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
0.482	2	4	10	25	27	14	8	4	1	0	0	0	0	0	0
0.487	6	6	15	25	20	8	3	6	2	3	0	0	0	0	0
0.520	2	4	8	23	28	14	9	3	1	1	0	0	0	0	0
0.556	3	4	4	10	21	15	21	10	3	2	0	0	0	0	0
0.571	3	5	10	27	28	11	8	2	1	0	0	0	0	0	0
0.587	1	3	5	16	33	18	10	4	2	1	0	0	0	0	0
MEAN	3	4	9	21	26	13	10	5	2	1	0	0	0	0	0

(HYPOXIA)

LVW (g)	RELATIVE FREQUENCIES IN PERCENTAGE														(um)
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	
	TO														
	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
0.482	2	4	7	12	25	17	18	8	2	1	0	0	0	0	0
0.487	3	5	15	27	17	11	8	5	1	0	0	0	0	0	0
0.520	4	5	9	25	28	11	9	3	1	0	0	0	0	0	0
0.556	2	4	7	12	25	17	18	8	2	1	0	0	0	0	0
0.571	2	7	10	28	26	12	5	2	1	0	0	0	0	0	0
0.587	2	2	5	8	29	20	16	6	4	2	1	0	0	0	0
MEAN	3	5	9	19	25	15	12	5	2	1	0	0	0	0	0

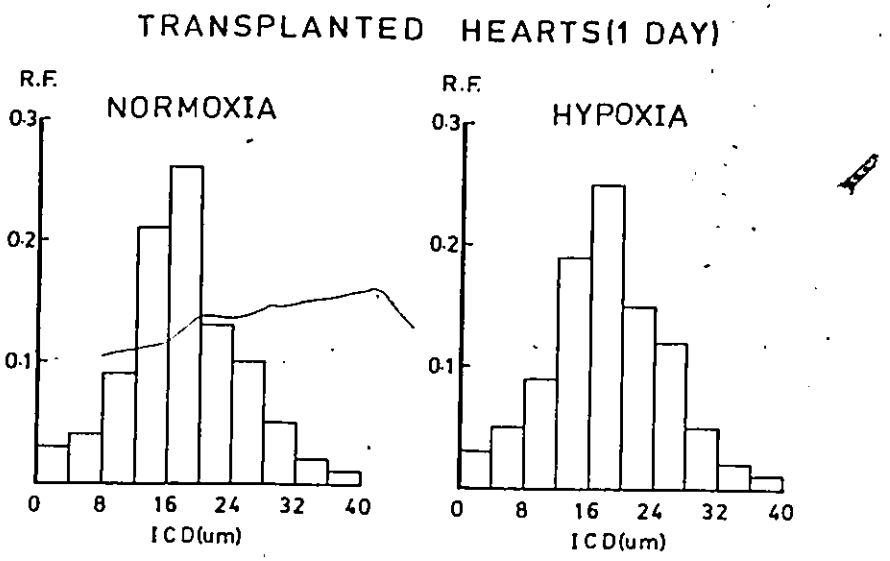


Figure 11: Average ICD histograms in one day transplants

3.6.3 Seven day transplanted hearts

Table 10 shows individual frequency distributions of the ICD's obtained from experiments performed on seven day transplanted hearts under normoxemia and hypoxemia. The two average histograms (means) were calculated and are also displayed in graphical form in Figure 12. Their skewness and kurtosis were calculated and is shown in Table 8. The skewness and kurtosis values of the normoxic ICD histogram were both significantly different from the ideal values for normal distribution. On the other hand, only the kurtosis value of the hypoxic ICD histogram was significantly different ($p = 0.05$) from the ideal value for normal distribution.

TABLE 10

FREQUENCY DISTRIBUTIONS OF ICD'S IN SEVEN DAY TRANSPLANTED HEARTS

(NORMOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE														
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56
LVW (g)	4	8	12	16	20	24	28	TO 32	36	40	44	48	52	56	60 (um)
0.435	2	5	13	28	27	11	5	2	1	0	0	0	0	0	0
0.439	3	4	11	30	25	11	6	3	1	0	0	0	0	0	0
0.482	2	3	8	33	29	14	4	2	0	0	0	0	0	0	0
0.559	3	7	12	23	17	12	10	4	2	2	0	0	0	0	0
MEAN	3	5	11	29	25	12	6	3	1	1	0	0	0	0	0

(HYPOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE														
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56
LVW (g)	4	8	12	16	20	24	28	TO 32	36	40	44	48	52	56	60 (um)
0.435	4	4	18	18	31	5	8	1	0	0	0	0	0	0	0
0.439	1	4	10	38	29	8	5	1	0	0	0	0	0	0	0
0.482	1	2	6	30	34	16	6	1	0	0	0	0	0	0	0
0.559	1	5	7	32	27	10	7	2	2	1	1	0	0	0	0
MEAN	2	4	10	30	10	7	1	1	0	0	0	0	0	0	0

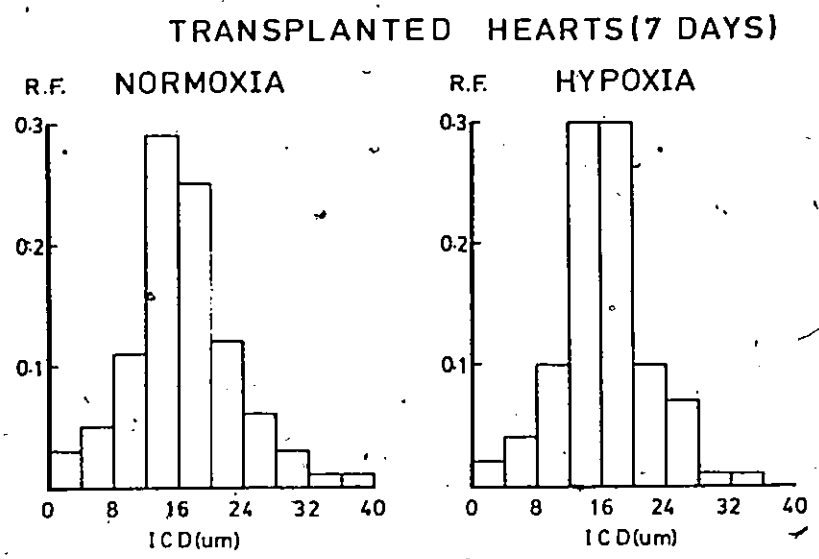


Figure 12: Average ICD histograms in seven day transplants

3.6.4 Hypertrophied hearts

Table 11 shows individual frequency distributions of the ICD's obtained from experiments performed on hypertrophied hearts under normoxemia and hypoxemia. The two average histograms (means) were calculated and are also displayed in graphical form in Figure 13. Their skewness and kurtosis were calculated and are shown in Table 8. The skewness and kurtosis values of the normoxic and hypoxic ICD histograms were all not significantly different ($p = 0.05$) from the ideal values for normal distribution.

TABLE 11

FREQUENCY DISTRIBUTIONS OF ICD'S IN HYPERTROPHIED HEARTS

(NORMOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE														
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56
								TO							
LVW (g)	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	(um)
0.498	3	4	10	24	26	14	9	3	1	1	0	0	0	0	0	
0.614	4	3	9	26	30	11	10	2	1	0	0	0	0	0	0	
0.654	3	3	6	19	27	19	11	6	1	0	0	0	0	0	0	
0.728	2	3	7	16	24	16	11	9	3	2	0	0	0	0	0	
0.895	1	2	3	13	27	22	18	6	2	0	0	0	0	0	0	
1.022	2	2	4	20	38	17	9	3	1	0	0	0	0	0	0	
MEAN	3	3	7	20	29	17	11	5	2	1	0	0	0	0	0	

(HYPOXIA)

	RELATIVE FREQUENCIES IN PERCENTAGE														
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56
								TO							
LVW (g)	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	(um)
0.498	1	1	3	14	35	18	12	3	3	0	0	1	1	0	0	
0.614	2	3	6	14	37	24	7	2	0	0	0	0	0	0	0	
0.654	3	3	3	15	26	20	14	7	3	0	0	0	0	0	0	
0.728	5	2	7	21	23	21	7	7	3	1	0	0	0	0	0	
0.895	1	2	4	16	30	21	15	4	1	1	1	0	0	0	0	
1.022	2	2	5	25	36	15	8	2	1	0	0	0	0	0	0	
MEAN	2	2	5	18	31	20	11	4	2	0	0	0	0	0	0	

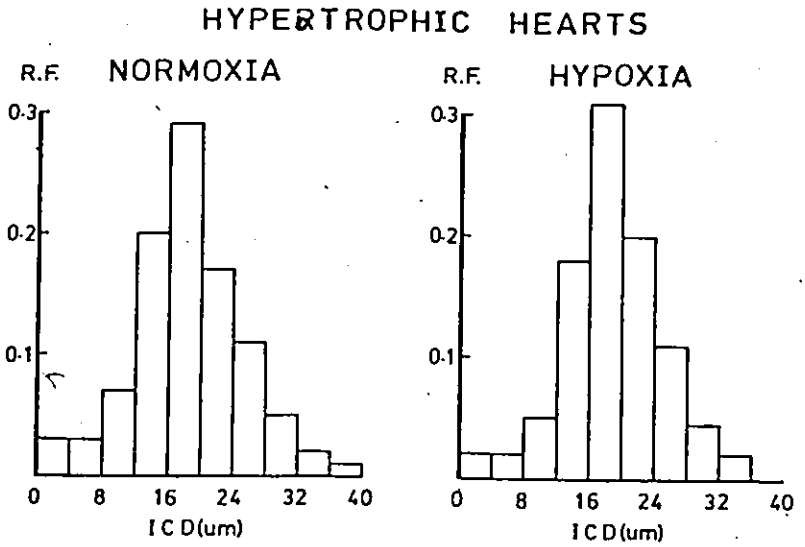


Figure 13: Average ICD histograms in hypertrophied hearts

Chapter IV

DISCUSSION

4.1 EFFECT OF GROWTH ON CORONARY CAPILLARY DENSITY

The present finding of an almost negative exponential relationship between normoxemic coronary capillary density and left ventricular weight is rather unexpected as compared to reported data. In 1976, Henquell et al. used in vivo cinematography (similar to the present method) to study the effect of left ventricular growth on coronary ICD in the rat heart. They found an inverse linear relationship between coronary ICD and left ventricular weight throughout the age of 40 to 400 days. When their ICD's were converted to capillary densities, the relationship between coronary capillary density and left ventricular weight was still approximately linear. In our case, in rats between the age of 49 days to 62 days, we observed a decrease of functional capillary density from about 4000/mm2 almost negative exponentially to a plateau of 2500/mm2. On the other hand, they found a linear decrease of functional capillary density from about 3300/mm2 to 2600/mm2 in this age range. Since they did not tabulate all their numerical results, it is difficult to offer any explanation for the difference between our and their findings.

For the minimum ICD, that is, the hypoxemic ICD, assuming that all precapillary sphincters are open under this degree of hypoxemia (PaO_2 < 50 mmHg), there has been many reports on the effect of cardiac growth on the anatomical coronary capillary density and our present finding of an inverse linear relationship between maximum coronary capillary density and left ventricular weight basically agrees with the findings of most previous workers in this field. In 1963, Rakusan and Poupa studied the anatomical coronary ICD changes in the rat heart during development and reported an similar inverse linear relationship between anatomical coronary capillary density and heart weight. They found a similar decrease of capillary density (3800 - 2700/mm2) to ours (3900 - 2500/mm2) for the similar span of age. Since they were using histological techniques, their capillary density should reflect the total anatomical capillary density of the heart. This therefore gives positive support to our assumption that the hypoxemic capillary density represents the entire anatomical capillary density. Rakusan et al. (1967), using I^{131}-Albumin as an intravascular tracer, found that the maximal coronary vascular capacity decreased linearly with body weight. Henquell et al. in 1976 used in vivo cinematography (similar to the present method) to study the effect of growth on capillary density and found a linear relationship between minimum ICD and left ventricular

weight. When their ICD's were converted to capillary densities, the relationship between coronary capillary density and left ventricular weight was still approximately linear. However, throughout the similar span of left ventricular weight to ours, they found a decrease of capillary density from 4630 to 3300/mm2. Interestingly, their decrement in capillary density (1330/mm2) is similar to ours (1400/mm2); therefore, it appears that our regression line and theirs are different only in the intercepts. In fact, the regression of their coronary capillary density on left ventricular weight gives a very similar slope (4135) to ours (4286). The difference in intercepts may be due to the fact that they used female Sprague-Dawley rats while we used the males.

The relationship between capillary reserve and left ventricular weight is obtained by subtracting the normoxemic curve from the hypoxemic line as displayed in Figure 5. This parabolic relationship between capillary reserve and left ventricular weight derived from Table 2 and illustrated in Figure 6 can perhaps be explained in terms of a relationship between age dependent morphological and functional development of precapillary sphincters, growth of individual muscle fibres and changes in oxygen consumption of the myocardium per unit of its mass (MVO2/g).

In the first period of life the precapillary sphincters might not have been present or if present they

were inactive, consequently all capillaries were open all the time. Alternatively the MVO2 of the myocardium might have been so high that all capillaries were open under normoxemic conditions even if sphincters were present and functional.

The initial part of the second period when the capillary reserve started to appear and gradually increased could have been due either to anatomical development of precapillary sphincters or their activation. This led to closure of some of the capillaries under normoxemia due to the age dependent decrease of cardiac MVO2/g. In the latter part of the second period after the recruitement capacity peaked the growing diameters of the myofibres pushed the existing capillaries further apart thus increasing the diffusion pathway. Since the length of the diffusion distance is a major determinant of oxygen delivery, more capillaries had to stay open under normoxemia, which led to decreased recruitement in hypoxemia. When a critical distance was reached all capillaries remained open even under normoxemia in order to supply the myocardial tissue because of a long diffusion pathway, irrespectively of the existence of functional precapillary sphincters.

Although the existence of precapillary sphincters in the heart is still controversial, there is histological evidence suggesting that precapillary sphincters do exist in the coronary microcirculation (Provenza and Scherlis, 1959).

In the following paragraphs, the initial rise of capillary reserve with left ventricular growth from zero to the maximum reserve of 740 capillaries/mm2 will be discussed in more detail and summarized. This will be immediately followed by the discussion on the subsequent exhaustion of the capillary reserve with further ventricular growth.

The initial increase of capillary reserve with left ventricular growth was unexpected because the increase in muscle diameter associated with growth would move the existing capillaries further apart, resulting in prolonged intercapillary distances and decreased capillary density. Therefore the heart would be expected to recruit more capillaries even in normoxemia to maintain an optimal capillary density to avoid tissue hypoxia. However, the contrary was found, more capillaries were actually kept as reserve as the heart increased in size. There are two possible explanations. Firstly, the recruitement apparatus, that is, the precapillary sphincters, may be not available when the animal is relatively young so that the heart operates with maximal capillary density even though it does not need it. Later in life as the precapillary sphincters develop, some of the capillaries then become inoperative and are kept as a reserve. The second possible explanation is that the heart may indeed require such a high capillary density for adequate oxygen supply when the animal is young so that all the anatomically available capillaries are

functional. But with advancing age, it then does not need such a high capillary density and the excess capillaries are kept as a reserve. In other words, it is speculated that when the animal is relatively young, myocardial oxygen consumption per gram tissue (MVO2/g) may be so high that the heart necessitates all capillaries to be functional in order to achieve an adequate oxygen supply. With advancing age, MVO2/g decreases so that less capillaries are required and the excess ones are kept as a reserve.

The first hypothesis is not very probable because at the age when the capillary reserve starts to appear, that is, the 7th week, the rat is already a young adult with a body weight of around 200 gms and all its physiologic functions have fully developed; therefore, it seems unlikely that development of the capillary sphincters is the cause of capillary recruitement at this stage. The second hypothesis seems to be more probable because of the following. First of all, the relationship between cardiac pressure-volume work and age in the rat as shown in Figure 7 provides indirect support since the age of 7 weeks at which cardiac work decreases dramatically coincides with the first appearance of capillary reserve. Secondly, at this age, proliferation of coronary capillaries ceases (Rakusan et al., 1965) which suggests that capillary proliferation and capillary recruitement may be closely related.

Based on the above, the second hypothesis is advanced and the following events are postulated. Shortly after birth, MVO2/g first increases with age probably as a result of neurohumoral influence (Gold et al., 1968) in order to cope with the increase in the whole body oxygen consumption (Brody, 1945) associated with growth. This increase in MVO2/g somehow triggers the proliferative activity of the coronary capillaries perhaps as a result of transitional tissue hypoxia. Up to this stage, the functional capillary density is the same as the anatomical capillary density with no capillary reserve because the heart necessitates all the existing capillaries to avoid tissue hypoxia. As the animal grows further, MVO2/g starts to decline; consequently, transitional tissue hypoxia no longer occurs under normal conditions and capillary proliferation stops in this stage. As the MVO2/g declines further, the heart will be oversupplied with oxygen with the existing high number of capillaries so that some of the capillaries are closed and kept as a reserve. The closing of capillaries continues with the decrease of MVO2/g until the MVO2/g reaches a more or less plateau minimum and the coronary capillary reserve reaches its maximum. Recently, Olivetti et al. (1980) showed that the mean intercapillary distance was reduced from 32.0 um when the rat was one day old to 17.6 um when the rat was 11 days old. Since the MVO2/g is still increasing in the 1 to 11 days postnatal period in the rat,

the decrease of ICD in this period therefore gives further support to our MV02/g increase triggered capillary proliferation hypothesis. However, this finding of lack of coronary capillary reserve in young animals seems to contradict the fact that young animals are actually more tolerant to hypoxia. Since oxygen supply depends on blood flow and diffusion, young animals therefore probably have mechanisms to substantially increase myocardial blood flow to compensate for this limitation. The mechanism is likely to be related to the overall resistance of the coronary network.

This paragraph suggests an explanation for the subsequent recruitement and final exhaustion of the capillary reserve with age. In order to explain the decrease of capillary reserve with left ventricular growth, it is important to note that there are two opposing forces determining the myocardial tissue oxygen tension during growth. One of them has been discussed in the last paragraph which is the decrease in MVO2/g resulting in a relative increase of tissue oxygen tension. The opposing force is the increase in diameter of cardiac myocytes associated with growth, moving capillaries further apart to decrease tissue oxygen tension. Since it has been assumed that capillary recruitement is controlled by tissue oxygen tension, capillary reserve will therefore decrease or increase according to the balance between the two forces. Accordingly, the following events are postulated for the subsequent exhaustion of capillary reserves. After the MVO2/g decreased to a low level later in life, the main determinant of the myocardial tissue oxygen tension is the increasing diffusion pathway due to hypertrophy of existing myocytes which will move the capillaries further apart. The progress of this process may cause tissue hypoxia and this may cause recruitement of more capillaries under normoxemia.

This may be the reason why further growth of myocytes is accompanied by exhaustion of the capillary reserve.

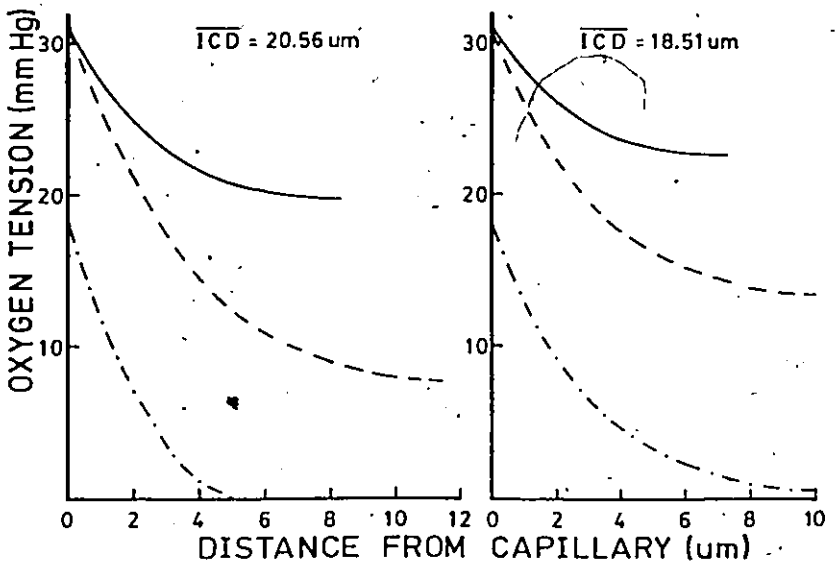
In summary, therefore, the initial increase of capillary reserve with left ventricular growth is believed to be due to the dramatic decrease of MVO2/g during the first period. The subsequent recruitement of spare capillaries under normoxemia and final exhaustion of the capillary reserve is believed to be due to the continual increase in the diameter of cardiac myocytes associated with further growth of the heart.

The special relationship between functional coronary capillary density and left ventricular weight as discussed above may also explain the controversy about the existence of capillary reserve in the heart. Recalling Figure 5, the capillary densities under normoxemia and hypoxemia both varied extensively with left ventricular weight. Since Bourdeau-Martini et al. in 1974 were using different animals for normoxemic and hypoxemic experiments, there was a possibility that their hypoxemic capillary density (4400/mm2) might be obtained from relatively young animals while their normoxemic capillary density (2250/mm2) might be obtained from relatively older animals because they were using rats with a relatively wide range of body weights (180 - 300g). These together thus magnified the capillary reserve to 2150 cap/mm2 as reported.

Steinhausen et al. in 1978 were using animals with relatively small range of body weights (250 - 300 g) and performed both the normoxemic and hypoxemic experiments on the same animals. Interestingly, the coronary capillary densities reported by them lay closely to our normoxemic curve. Therefore, it appeared that capillary recruitement was handicapped in their preparation. This could be due to at least two reasons. Firstly, hypoxemia in their case might not be severe enough to cause capillary recruitement. Since they did not mention for how long their animals were breathing 5% oxygen, the degree of hypoxemia was uncertain. Secondly, it was also possible that their heart restrainer might have interfered with the recruitement mechanism of the coronary capillaries.

Finally, to show the functional significance of the capillary reserve of 553/mm2 established from our data (Table 2), Krogh-Erlang's equation was applied to calculate the profile of the oxygen tension around the venous end of a capillary under theoretical normoxemia and hypoxemia. The venous end was chosen because this is where anoxic zones were most likely to occur. Since the ICD's reported in Table 2 referred to the mean values only, the oxygen profiles were also calculated for tissue cylinders with an arbitrary ICD of 30% above the average value to represent profiles in areas with longer actual ICD's. However, since the coefficient of variation of the ICD's in our experiments

was around 30%, no more than 16% of capillaries were separated by more than 1.3 times mean value of ICD. Therefore, the oxygen profiles calculated should be representative of the lowest pO2 profiles for at least 84% of the coronary capillaries. Finally, normoxemia was represented by a venous pO2 of 31 mmHg and hypoxemia was represented by a venous pO2 of 18 mmHg. These values were taken from a study by Holtz et al. in 1977. For the other variables of the Krogh-Erlang's equation, capillary diameter of 4 um was assumed (Sobin and Tremer, 1972), Krogh's diffusion coefficient of $2.1*10^{-5}$/min*mmHg was adopted (Kety, 1957) and lastly, the oxygen consumption of the rat heart was taken as $6.5*10^{-3}$ ml/g*sec (Honig and Bourdeau-Martini, 1973, Duvelleroy et al., 1976, Kissling, 1980). The calculated oxygen tension profiles around the venous ends of the capillaries are displayed in Figure 14.



Legend:
Solid line : R = mean ICD, PvO2 = 31 mmHg
Broken line : R = 1.3*(mean ICD), PvO2 = 31 mmHg
Broken line with dots : R = 1.3*(mean ICD), PvO2 = 18 mmHg

Figure 14: Oxygen tension profiles surrounding the venous end of capillary

The profiles of oxygen tension displayed in Figure 14 clearly show that the capillary reserve of 553/mm2 (changing the mean ICD from 20.56 um to 18.51 um) is indeed important for the oxygen supply to the myocardium. Under normoxemia (venous pO2 31 mmHg), both the longer and shorter ICD's are adequate for satisfying the oxygen demand of the heart. However, under hypoxemia (venous pO2 18 mmHg), the longer ICD is not adequate for satisfying the oxygen demand of the heart, resulting in an anoxic zone occupying over half of the radius of the tissue cylinder, whereas under the shorter ICD, a minimal tissue oxygen tension of 0.4 mmHg is still maintained which is barely sufficient for oxidative phosphorylation in the mitochondria (Wittenberg, 1970).

Besides facilitating diffusional transport as illustrated above, capillary recruitement is also important in decreasing the overall coronary vascular resistance since it has been shown that one-third to one-half of the minimum coronary vascular resistance is due to capillary resistance (Archie, 1978b). In other words, capillary recruitement facilitates both the bulk and diffusional transport of oxygen to the tissue by decreasing the coronary vascular resistance and the intercapillary distance.

4.2 EFFECT OF ONE DAY TRANSPLANTATION

Since the one day isotransplanted heart was essentially a denervated heart performing very little work,

the lack of reserve as shown in Table 3 suggested that the surgical operation might have strong influence on the capillary recruitement mechanisms either through denervation or as a general surgical trauma. Since the average "LVW in %" was within normal range and the appearance of the one day transplants was not different from normal, the causal factor seemed likely to be cardiac denervation.

This was contrary to the concept that capillary recruitement was primarily mediated through local humoral factors (Honig et al., 1970; Granger et al., 1975, Henquell and Honig, 1978; Belloni, 1979). However, there was also evidence that neural mechanism was important for capillary recruitements in intestines (Lautt and Graham, 1977), skeletal muscles (Hudlicka and Renkin, 1968) and the heart (L'Abbate et al., 1976). There were also reports showing that oxygen and lactate utilization increased after cardiac denervation (Drake, 1976); this could be due to the increased availability of substrate and oxygen as a result of opening of all coronary precapillary sphincters after denervation since capillary exchange was believed to be the bottleneck of myocardial oxygen consumption (Kammermeier and Kammermeier, 1975). Also, myocardial tissue oxygen tension had been shown to be strongly influenced by sympathetic stimulation (Peigl, 1975).

Anatomically, nerve terminals had been found in proximity to precapillary sphincters (Kisch, 1958, Uchizono,

1964, Forbes et al., 1975, Forbes et al., 1977). Therefore, the closing of precapillary sphincters appeared to be mainly if not entirely under neural control since present data showed that complete denervation could cause the opening of all capillary sphincters. There may be similar tonic sympathetic discharges to the precapillary sphincters as to the arterioles under normoxia. This is not to mean that local factors do not have a role in capillary recruitement; rather, they may act by modifying the effect of this tonic sympathetic stimulation. Cobbold et al. (1963) suggested that vasodilator metabolites, when in high concentration, could actually overcome the sympathetic constriction of precapillary sphincters. Therefore, regarding the opening of coronary precapillary sphincters, the possible participation of local factors can not be excluded.

4.3 EFFECT OF SEVEN DAY TRANSPLANTATION

Since it had been shown that 8 days after transplantation, the transplanted rat heart was still completely denervated (Lund et al., 1978), the seven day transplanted heart could be considered a denervated heart in the early stage of the process of atrophy. The lack of capillary recruitement in these transplants as shown in Table 4 could then be explained in terms of cardiac denervation as in the previous section. Furthermore, as shown in Figure 8, the means of the data lay above the

hypoxemic line, indicating an increase in capillary density in the seven day transplants. This suggested that seven days after transplantation, the preexisting capillaries were not obliterated so that the decrease in the diameter of the cardiac myocytes associated with cardiac atrophy simply moved the capillaries closer, resulting in a higher capillary density.

The average increase in capillary density was 7% which was in good agreement with the expected increase (6%) calculated from the average decrease in left ventricular weight. This is contrary to what Rakusan et al. had found in 1967 since they reported decreased vascular capacity in atrophied hearts. However, cardiac atrophy in their case was induced by protein deprivation instead of decreased working load as in our case, and the degree of atrophy was more severe in their case (-18%), so that it is rather difficult to compare the present finding with theirs.

4.4 EFFECT OF CARDIAC HYPERTROPHY

The position of the mean capillary densities plotted against - left ventricular weight as shown in Figure 9 suggested that capillary densities at the subepicardium of the hypertrophied hearts had not changed significantly from normal. There are two possible hypothetical explanations. Firstly, capillary proliferation might have occured during the process of hypertrophy so that the final capillary

density was maintained at the normal level. The second possibility is that the subepicardium might not be representative of the entire myocardium during the process of stimulated growth. In other words, perhaps the degree of hypertrophy was less in the subepicardium than the average so that the capillary density at the subepicardium remained relatively unchanged.

The first explanation is not very likely since it is generally accepted that capillary proliferation does not exist in the adult rat hearts. On the other hand, the second hypothesis is supported by the following evidence. Firstly, it had been calculated that the subepicardium was the layer in the heart where wall stress and pressure were approaching zero (Streeter et al., 1970, Hikman-Coffelt et al, 1979). If muscle hypertrophy was caused by increased stress and tension, then it will be expected that the subepicardium will hypertrophy to a lesser degree. Secondly, theoretical calculation indicated that, in concentric cardiac hypertrophy, which was the case in pressure overload cardiac hypertrophy, the subepicardium should hypertrophy to a lesser degree than other layers of the heart. Lastly, the second hypothesis was also supported by experimental studies (Dowell, 1977, Lund and Tomanek, 1978, Hatt, et al., 1979) demonstrating a lesser degree of hypertrophy in the subepicardium. Based on the above, the second hypothesis is advanced and consequently it is

expected that the subepicardium will also have a smaller decrease in capillary density than other layers of the heart.

If the subepicardium was indeed nonrepresentative of the entire hypertrophic myocardium, then perhaps plotting subepicardial capillary density against the hypertrophied left ventricular weight was not justified. This led to plotting of subepicardial capillary density against body weight because the body weight, like the subepicardium, was relatively less affected during the process leading to cardiac hypertrophy. The two average data points were plotted against the body weight which is shown as the right pair in Figure 9. This time, they were well below the hypoxemic line which suggested that cardiac hypertrophy induced a decrease in anatomical capillary density in the subepicardium assuming that all available capillaries were open under hypoxemic conditions. Furthermore, assuming harmonic growth of cardiac myocytes (Korecky and Rakusan, 1978), the expected decrease in capillary density was 28%; however, the actual decrease in capillary density in the hypertrophied hearts was only 17%. This therefore further supports our hypothesis that cardiac hypertrophy induces a smaller decrease in capillary density in the subepicardium than other regions of the heart. Since Henquell et al. (1972) reported similar findings on the hypertension induced hypertrophied hearts, pressure-overload cardiac hypertrophy

induced by hypertension and aortic constriction therefore appeared similar in terms of exhaustion of capillary reserve and capillary density changes in the subepicardium.

As shown in Table 5, capillary reserve was absent in the hypertrophied hearts since the difference between capillary densities obtained under normoxemia and hypoxemia was insignificant. This implied the recruitement of all existing capillaries even under normoxemia in the hypertrophied hearts. If capillary proliferation did stop after the 7th postnatal week (Rakusan et al.,1965), then it would not be surprising that, even under normoxemia, the hypertrophied hearts needed to have all the capillaries open to sustain an adequate oxygen supply. However, the recruitement patterns in individual hypertrophied hearts were actually quite inhomogeneous. Paired data points are shown in Figure 15.

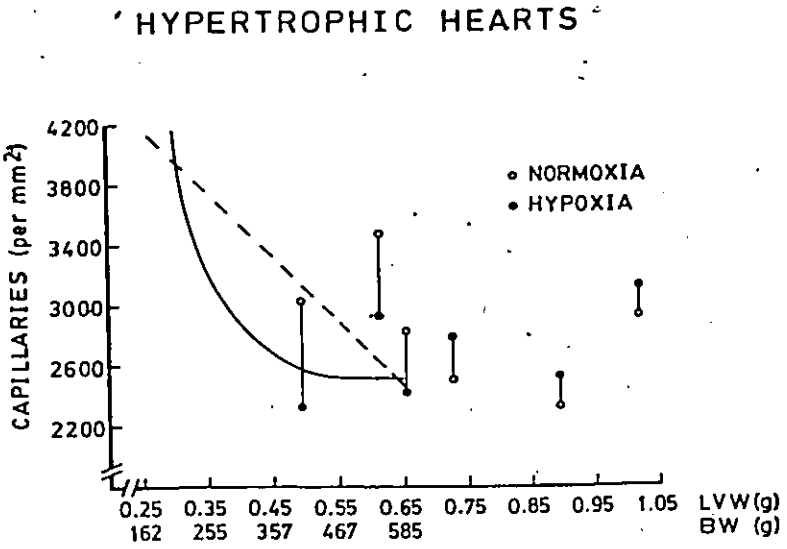


Figure 15: Individual data from hypertrophied hearts

As shown in Figure 15, in the smaller hearts in terms of left ventricular weight, the direction of capillary density changes in hypoxemia was actually reversed, that is, coronary capillary densities in these hearts actually decreased in hypoxemia. But as the heart hypertrophied more, the difference in capillary density became smaller and finally returned to the normal direction, that is, increase in capillary density in hypoxemia. However, capillary reserves in these hearts were very small and were statistically insignificant.

The decrease in capillary density in hypoxemia in smaller hearts might be a continuation of the trend with further growth as displayed in Figure 5 and Table 2. This might be due to coronary steal from the subepicardium by other layers of the heart. In fact, coronary steal in case of coronary occlusion had been well documented (Wichmann et al., 1978). Intramyocardial steal had also been shown to exist in the heart (Reves et al., 1977). The mechanism behind this stealing phenomenom might be due to different vasodilatory capacity in different layers of the heart (Flohr et al., 1977, Archie, 1978a). It had been shown that the SUBENDO/SUBEPI perfusion ratio increased under sympathetic stimulation (Schwartz and Stone, 1977). Therefore, it was probably the dilation of the coronary vessels in the inner layer of the heart which caused the collapse of the subepicardial capillaries.

As shown in Figure 15, coronary steal did not occur in all hypertrophied hearts; capillary reserve was simply absent in hypertrophied hearts of larger size. This might be explained as follows. It was thought that coronary steal from the subepicardium could occur only if the subepicardium could afford it, in a sense that it would not cause severe tissue hypoxia. As the size of the hypertrophied hearts increased more, the changes in the subepicardium would also become more significant such as increased intercapillary distances and increased systolic compression; so that, to avoid further impairment of oxygen supply, coronary steal could no longer be permissible.

In summary, there was no capillary recruitement in hypoxemia in the hypertrophied hearts. When the size of the hypertrophied hearts were relatively small, coronary steal occurred in the subepicardium; when the size of the hypertrophied hearts increased more, coronary steal could no longer occur and capillary reserves were simply absent.

4.5 COMPARISON OF CAPILLARY DENSITIES OBTAINED WITH 22X AND 11X OBJECTIVES

As mentioned in the introduction, capillary recruitement observed by Honig et al. could have been an artifact resulting from the use of optical systems with low magnification and deeper penetration. Therefore, data obtained by low power (11X) and high power (22X) objectives under normoxemic and hypoxemic conditions from the same

animals as shown in Table 6 were compared using paired t-tests but the differences were found to be insignificant (normoxemia, t=0.0370; hypoxemia, t=0.2437), indicating that there was no difference between capillary densities obtained with 11X or 22X objectives. Since the 22X objective had a theoretical depth of focus of only 5.95 um which could cover only one layer of capillary network in the subepicardium, it was concluded that data obtained with the 11X objective under the outlined experimental conditions was also a valid representation of one layer of capillary network in the subepicardium despite the fact that the depth of focus of the 11X objective is greater than that of the 22X objective. In other words, the increase in depth of focus achieved by the 11X objective did not bring into focus any more capillaries. This implies that capillaries are indeed arranged predominantly in layers across the myocardium. Therefore, an increase in depth of focus less than one intercapillary distance will not cause the focusing of any more capillaries since there are probably only few capillaries situated between the predominant layers. This also implied that the observed capillary reserves did indeed exist in normal hearts and could not be explained as an artifact. In fact, besides the report from Bourdeau-Martini et al.'s group, various indirect studies have also suggested the presence of capillary recruitement in hypoxemia (Duran et al., 1977, Marsicano et al., 1977, Kreutz et al., 1978; Weiss and Cohen, 1978, Briden et al., 1979; Martin et al., 1979). Our data therefore gives further support to the existence of capillary reserve in normal hearts.

4.6 FREQUENCY DISTRIBUTIONS OF THE CORONARY INTERCAPILLARY DISTANCES

One of the advantages of the method used in this project is that it allows for the computation of the frequency distribution of the coronary intercapillary distances. The numerous intercapillary anastomoses will

help distributing the oxygen suppy evenly to different regions of the heart by collateral circulation; however, if the ICD distribution is extremely skewed, focal hypoxic areas will be expected to exist. By comparing the coronary ICD frequency distributions of the paired data from normoxemia and hypoxemia, it is also possible to understand how the change of mean intercapillary distance is attained, that is, which range of ICD's has actually changed.

The following paragraphs describe the changes in the coronary ICD frequency distribution from normoxemia to hypoxemia in the normal and the different experimental groups of hearts.

4.6.1 Normal hearts

Several points were apparent in comparing the two average ICD histograms in Figure 10 . First of all, the range of ICD was from 0 to 40 um for normoxemia and only 0 to 36 um for hypoxemia, indicating that some extremely long ICD's actually disappeared. Secondly the relative frequencies for the shorter ICD's increased in hypoxemia; the obvious ones were the intervals of 8 - 12 um and 12 - 16 um. These two observations together demonstrated the recruitement of capillaries in hypoxemia, changing the longer ICD's into shorter ICD's. To examine it quantitatively, skewness and kurtosis of the two average histograms, as shown in Table 8, are compared. The

difference in skewness between the two average histograms indicated that there were more longer ICD's under normoxemia than hypoxemia and that the frequency distribution of ICD's under hypoxemia was closer to complete symmetry than that under normoxemia. Furthermore, the ICD frequency distribution under hypoxemia had a kurtosis value closer to the ideal value of 3 for normal distribution. Therefore, it appeared that the anatomical coronary capillary network was structured in such a way that the resulting intercapillary distances were normally distributed. Under normoxemia, some capillaries were closed and kept as capillary reserves, resulting in a slightly skewed ICD frequency distribution. However, the degree of skewness was small so that the numerous intercapillary anastomoses could probably still maintain the even distribution of oxygen supply across the layer of myocardium (Goresky and Goldsmith, 1973). This was basically in agreement with what Bourdeau-Martini et al. (1974) had found, that is, roughly normal ICD frequency distributions under both normoxemia and hypoxemia.

4.6.2 One day transplanted hearts

As shown in Figure 11, the frequency distributions of the coronary ICD's under normoxemia and hypoxemia were very similar with a mixture of slight positive and negative deviations in both sides of the modal interval. To examine it quantitatively, skewness and kurtosis of the two

histograms, as shown in Table 8, are compared. Both skewness and kurtosis of the two distributions were very similar. Furthermore, the values of skewness and kurtosis were also not significantly different from the ideal values for normal distribution. As shown in Figure 8, it had been found that the mean capillary densities of the one day transplants lay practically on the hypoxemic level; now the frequency distributions were also similar to the ICD frequency distributions of the normal animals in hypoxemia. It apeared therefore that the anatomical arrangement of the capillaries in the one day transplanted hearts was the same as that in normal hearts.

4.6.3 Seven day transplanted hearts

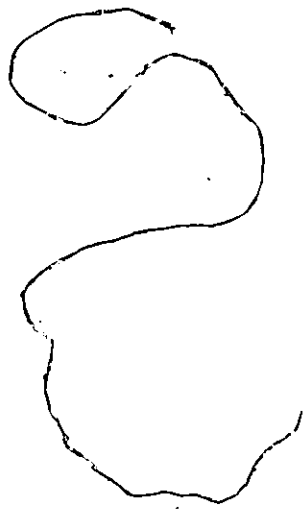
As shown in Figure 12, the two average ICD histograms appeared quite similar. Again the skewness and kurtosis of the two histograms, as shown in Table 8, are compared. The skewness and especially the kurtosis values of the two histograms were very similar. The slight difference between the two skewness values was probably due to artifacts in histogram grouping since the mean ICD's were around 16 um which happened to be the common boundary of the 12 - 16 um and 16 - 20 um intervals. Comparing the skewness and kurtosis values of the seven day transplants to that of the one day transplants, the major difference was the kurtosis. The kurtosis value for the 7 day transplants was

significantly different from the ideal value of 3 for normal distribution. This indicated that the coronary ICD frequency distribution of the 7 day transplants was more peaked than the normal distribution, or slightly leptokurtic. This might be explained in the following way. The seven day transplanted hearts had already started to atrophy; however, with the same degree of atrophy, the decrease in diameter would probably be more for the larger myocytes than for the smaller myocytes. Similarly, the decrease in ICD would also probably be more for the longer ICD's than for the shorter ICD's. The disappearance of a substantial number of the long ICD's together with a moderate increase in the number of extremely short ICD's would make the middle ICD classes very peaked, resulting in a leptokurtic distribution. This might also explain the low frequencies of the extremely long ICD classes 32 - 36 um and 36 - 40 um.

4.6.4 Hypertrophied hearts

To explain the difference between the two average histograms as shown in Figure 13, the coronary steal phenomenon had to be taken into account because the coronary steal phenomenon became especially prominent after the averaging process. Comparison of the two histograms indicated that, in average, hypoxemia actually caused the reduction of the relative frequencies of shorter

intercapillary distances. To examine the phenomenon quantitatively, skewness and kurtosis as shown in Table 8 are again compared. The most noticeable difference was the change of direction of skewness. The coronary ICD frequency distribution changed from positive skewness (modal ICD < mean ICD) under normoxemia to slightly negative skewness (modal ICD > mean ICD) under hypoxemia. This therefore further illustrated the coronary steal phenomenon as described before, that is, the increase of the relative frequencies of longer ICD's in hypoxemia.



Chapter V

SUMMARY

Functional coronary intercapillary distances (ICD's) under normoxemia and hypoxemia were obtained using the technique of stop-motion cinematography of the surface of the beating rat heart in situ.

Between the age of 49 days and 200 days, the coronary ICD under normoxemia increased from 15.68 um to 19.37 um while the coronary ICD under hypoxemia increased from 15.60 um to 19.91 um. When these ICD's were converted to capillary densities, the capillary density under normoxemia decreased almost exponentially with left ventricular weight from 4070/mm2 to a plateau of 2670/mm2 while under hypoxemic conditions, it decreased linearly with left ventricular weight from 4110/mm2 to 2520/mm2. Consequently, the capillary reserve varied with left ventricular weight, appearing when the rat reached 49 days of age, reaching a maximum of 740/mm2 which was 21% of the total capillary density when the rat was 62 days old and disappearing at 200 days of age. The initial increase of capillary reserve with age was explained in terms of the rapid decrease of myocardial oxygen consumption per gram tissue in the early postnatal period. The subsequent decrease of capillary

reserve with age was explained in terms of myocytic hypertrophy associated with cardiac growth.

Cardiac denervation and subsequently cardiac atrophy was induced by heterotopically isotransplantation of a donor rat's heart onto the abdominal vessels of an inbred recipient rat. In the short term (1 day) heterotopically isotransplanted hearts, there was no significant difference between the normoxemic (18.46 um) and hypoxemic (18.59 um) mean ICD's. In addition, these average values lay almost exactly on the graph representing the hypoxemic data of normal hearts, suggesting that the closing of coronary precapillary sphincters was primarily neurally mediated. In the longer term (7 days) heterotopically isotransplanted hearts, cardiac atrophy had already started; the average decrease of left ventricular weight was 8%. There was no significant difference between the normoxemic (17.10 um) and hypoxemic (17.09 um) mean ICD's. In addition, these average values are above the hypoxemic line for normal hearts, indicating an increase in capillary density in the seven day transplants. This then suggested that seven days after transplantation, the preexisting capillaries in the transplanted heart did not obliterate and that due to decrease in diameters of cardiac myocytes associated with cardiac atrophy, capillaries were closer to each other, resulting in a higher capillary density.

Cardiac hypertrophy was induced by subdiaphragmatic aortic constriction. The average increase in left ventricular weight was 63%. There was no significant difference between the normoxemic (18.89 um) and hypoxemic (19.33 um) mean ICD's. Furthermore, these two average values were below the hypoxemic line of normal hearts, suggesting that cardiac hypertrophy caused an increase in ICD and that coronary capillary reserve in the hypertrophied heart was exhausted even under normoxemia. However, the decrease of capillary density in the subepicardium (17%) was smaller than the calculated overall average decrease (28%) in the heart, suggesting that the degree of hypertrophy might have been less in the subepicardium than else where.

Capillary densities obtained with 11X and 22X objectives were compared in the same heart under various conditions but no difference was found. Since the 22X objective had a depth of focus of only 5.95 um, data obtained with the 11X objective were therefore also representative of one layer of coronary capillaries. The existence of capillary reserve was therefore confirmed and the cause of the controversy suggested.

Finally, frequency distributions of ICD's were computed from data obtained under normoxemia and hypoxemia in all the above groups of hearts. They were all approximately normal. The skewness and kurtosis values indicated that the anatomical coronary capillary network in

the normal rat heart was structured in such a way that the resulting intercapillary distances were normally distributed. Under normoxemia, some capillaries were closed and kept as capillary reserves, resulting in a slightly skewed ICD frequency distribution.

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