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Microvasculature of the Dog Left Ventricular Myocardium¹

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Abstract

One of the main branches of the left main coronary artery of normally beating dog hearts was perfused with a silicone elastomer which solidified within the vasculature. Prolonged immersion in increasingly concentrated ethanol and in methyl salicylate rendered the tissue translucent and the vasculature clearly visible. Surfaces were photographed by reflected or transmitted light microscopy, showing large groups of capillaries running parallel to muscle fibers and extending for up to a few centimeters. The arrangement of arteriolar inflows to the capillary network and venular outflows (two to four times as frequent) suggested that functional capillary lengths were 500–1000 μm . Estimates of capillary diameters, presumably at maximal dilatation, were $5.6 \pm 1.3 \mu\text{m}$. Capillary densities within muscle groups were 3100–3800/ mm^2 , giving intercapillary distances of 19–17.5 μm . With the lesser density value, the capillary surface area is estimated to be 500 cm^2/g of myocardium. Inclusion of interfascial spaces lowered the average density to about 2500/ mm^2 . Unbranched capillary lengths averaged 100 μm , with a strongly right-skewed distribution. The anatomic arrangement provides a basis mainly for concurrent flow in neighboring capillaries, and also for some diffusional exchange between inflow and outflow regions.

This study was undertaken because of the need for precise descriptions of the geometric arrangement of myocardial capillaries and of their dimensions, so that accurate values could be used for models describing the exchanges of tracers in the heart.

Wearn (1928) and later Roberts and Wearn (1941) perfused isolated and beating human, cat, and rabbit hearts with dye suspensions, and reported that there were about 5000 capillaries/ mm^2 of ventricular myocardium, a value which is too high because of their failure to correct for shrinkage with fixation. They also showed that anastomoses between capillaries were numerous and that at times the branchings were so profuse as to give the impression of single muscle fibers being completely surrounded by capillaries.

From studies in dead rabbits, Stoel (1925) stated that the capillary diameter in papillary muscle was 5 μm , the intercapillary distance was 18 μm (cylinder of influence = 9 μm in radius), and the capillary density was 3230/ mm^2 . In the same study, the capillary diameters in the adductor magnus and the semitendinosus muscles were 2.5 and 5.0 μm , respectively.

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Reynolds, Kirsch, and Bing (1958) reported values of 3–5 μm for capillary diameters in dog hearts. They also stated that these can double if the once arrested hearts were reperfused. In normally beating hearts they observed large gradients in capillary density between epicardium (1200–1600/ mm^2) and endocardium (100–340/ mm^2) which may have been related to the experimental technique of counting only those capillaries that contained erythrocytes. Although it is commonly assumed that erythrocyte and capillary diameters are similar, Krogh (1919) showed that there was considerable variation in capillary diameters of guinea pig skeletal muscle and that the mean diameter was less than erythrocyte diameters, requiring their deformation during passage. The data of Sobin and Tremmer (1972) and ours show clearly that in the dog heart, whose erythrocytes are flexible, capillary diameters are also substantially less than those of the erythrocytes; in contrast, in the goat, whose erythrocytes are stiff, Sobin and Tremmer found capillary and erythrocyte diameters to be about the same.

Shipley, Shipley, and Wearn (1937) studied capillary and fiber densities in normal and experimentally hypertrophied hearts of rabbits; they found fiber diameters of 17–19 μm and a capillary density of about 3200/ mm^2 . Martini and Honig (1969), using reflected light photography of the right ventricular surface of beating hearts of rats, found intercapillary distances of 18.0–21.4 μm , the smaller value being obtained with decreased oxygenation in the coronary inflow. This suggested that normally not all the available capillaries are open, and that hypoxia caused an increase in the number open. Myers and Honig (1964) measured the amount of labeled plasma and labeled erythrocytes in different pieces of canine hearts and reported that there existed a gradient of intravascular blood content, with higher values in the endocardium compared to the epicardium and in the apex compared to the base. They suggested that there were similar gradients of capillary density between these areas. Regional intratissue hematocrits did not differ. Hort (1968), correcting for tissue shrinkage with fixation, reported capillary density of 2000/ mm^2 , a value similar to the report of Martini and Honig, who counted only those containing erythrocytes.

From unpublished electron micrographs from Fawcett, Wittenberg (1970) found a capillary density of 2690/ mm^2 in cat papillary muscle and calculated the intercapillary distance to be 19.3 μm and fibre diameter to be 14.8 μm .

The density, diameter, and geometric arrangements of capillaries in skeletal muscle were critically reviewed by Hammersen (1968), and a cursory review of the geometry and capillary length in the heart was published by Fabel (1968). Provenza and Scherlis (1959) provided histologic descriptions of myocardial capillaries and pre- and post-capillary vessels.

The purpose of our study is to provide additional qualitative and quantitative data on canine myocardial microvasculature. Although some of the variation in capillary densities mentioned above is due to differences in species or differences in counting methods (all capillaries or only open capillaries or blood filled capillaries), we wished to obtain values for the maximum density in dog myocardium, where we are studying capillary-tissue exchange of solute molecules. The method was designed to cause full vasodilation by acute anoxia, hopefully giving filling of all capillaries. Measurements have been made from photomicrographs of cleared intact tissue after filling the vascular bed with silicone elastomer.

Material and Methods

Preparations for reflected light microscopy

In this set of experiments, three adult mongrel dogs weighing between 13 and 20 kg were anesthetized with sodium pentobarbital (30 mg/kg), artificially ventilated via a cuffed endotracheal tube. A left lateral thoracotomy was performed and the fifth rib was excised to aid exposure. The left lung was removed. The pericardium was split longitudinally to form a pericardial cradle. The main left coronary artery was dissected out, and a silk thread was looped loosely around it; similarly, one of the small peripheral branches of the left anterior descending or circumflex coronary artery was cannulated in a retrograde direction with a polyethylene catheter (PE 50; i.d. = 0.58 mm; o.d. = 0.96 mm), and the catheter tip was advanced retrogradely until its tip was about 5 mm from the loop around the parent branch. The animal was then heparinized.

About 20–30 ml of the silicone rubber fluid (Microfil, MV 112, Canton Biomedical Products, Inc., Boulder, Colorado 80302) was degassed under partial vacuum and then infused manually via the polyethylene catheter while the heart was still beating. (The manufacturer reports that after the addition of catalyst the specific gravity is 1.1 and the viscosity 15–25 cP.) Just prior to the injection, the system of tubes was checked for air bubbles and then the main left coronary artery was tied off. The pressure of injection was 120–150 mm Hg at the syringe tip during the first minute of injection. The vessels appeared to be well-filled in 20–30 sec, effluence in the veins having begun in 5–10 sec. Replacement of the blood and subsequent anoxia seemed to cause full vasodilatation and complete filling. As the injectate polymerized after 1.5–2.5 min the pressure was increased to more than 300 mm Hg to overcome the resistance of the tubing. Ventricular fibrillation occurred after about 1–2 min. The silicone did not appear to flow after 3 min but was fairly soft, becoming quite well hardened only after 10–15 min.

The part of the myocardium which was filled with the white rubber-like material was cut out in a single piece and dehydrated in 25, 50, 75, 95, and 100% ethanol for 2–3 days each. It then was immersed for several days in methylsalicylate to remove the alcohol and to make the myocardial tissue transparent. The methylsalicylate was replaced repeatedly until the alcohol had been removed. Methylsalicylate has a refractive index close to that of optical glass, and the production of good photographic records is facilitated by using an objective lens with a dipping cone immersed in methylsalicylate covering the myocardium. The white silicone rubber is highly reflective to incident light, and therefore the vessels filled with this substance will contrast with the non-reflecting but still intact tissue. Pieces of the myocardium were then sliced with ordinary razor blades, and these slices were embedded in shallow plastic cradles.

Preparation for transmitted light microscopy

Because it was thought that, by using similar intravascular filling material and higher magnification of the vessels, the range of capillary diameters could be determined more precisely, a second set of experiments was performed using three other dogs (body weights, 16.5–23 kg). These were prepared as in the first experiment but bigger polyethylene catheters (PE 260; i.d. = 1.78 mm; o.d. = 2.97 mm) were used for injection into the left anterior descending or the left coronary artery and for pressure measurements in the coronary sinus. These larger catheters facilitated the injection and also gave more practical pressure measurements in spite of the high viscosity of the injectate. In one dog, the coronary sinus pressure was purposely increased to 33 mm Hg by increasing outflow resistance. The hearts were fixed by immersion in 100% alcohol and methylsalicylate as described above. Finally the tissue pieces from different parts of the perfused regions were

fixed in 10 % formalin, sliced on a microtome to 10- μ m thickness, and stained with hematoxylin and eosin.

Measurement from reflected light microscopy

The method of Prong, Bjoraker, and Harvey (1969) was used. Heart slices immersed in methylsalicylate were obliquely illuminated by means of a fiber-optic light guide coupled to a mercury lamp source. We used a Leitz microscope equipped with an ultrapak objective lens ($\times 6.5$ or $\times 11$) for immersion in the methylsalicylate, and a $\times 10$ ocular lens. The specimens and the microscope were mounted on a heavy stand to provide stability. A single-lens reflex camera was mounted above the microscope and an extensible rubber tube was placed between the camera and the microscope; this reduced the transmission of camera motion to the microscope and specimen. We used Plus X film with Kodak D19 developer, and then, from the 2.25×2.25 in. negative made 8×10 in. prints with high contrast. A scale with 10- and 100- μ m divisions was photographed with each roll of film at the same magnification as the specimen, and printed with each enlargement to compensate for any change in magnification during enlarger focusing.

A low magnification of $\times 120$ to $\times 135$ on the prints was obtained with the $\times 6.5$ objective lens to study the geometric arrangement of the vessels and their order of branching. The higher magnification of $\times 215$ to $\times 230$, obtained with the $\times 11$ objective, was used to measure capillary diameters, intercapillary distances, distances between branches along capillaries, and intercapillary connections.

For the diameter measurements by reflected-light microscopy the most profuse vessels with the smallest internal diameter in the photographic field and lacking immediate connection to vessels of 10- μ m diameter or more were regarded as the capillaries. Average diameters of groups of ten capillaries were obtained by marking a capillary internal diameter on a paper's edge with the middle of the thin pencil mark being placed at the edge of the capillary and marking the one next to it similarly until ten were marked consecutively. This total was then measured against the 10- μ m scales.

For the average intercapillary distances, photographs of sections cut perpendicularly to the longitudinal axis of the capillaries were used. The cut ends were counted per 50- or 100- μ m squares of surface. The intercapillary distances were then calculated by assuming that capillaries were centered within hexagonal columns of tissue of uniform size. For a hexagonal array the intercapillary distance is the distance between parallel sides, h , and the area of the hexagon is equal to $(3)^{1/2}h^2/2$. For N capillaries, the total area, A , is $N(3)^{1/2}h^2/2$ and therefore $h = (2A(3)^{1/2}N)^{1/2}$, where both A and N are measurable experimentally.

Measurements from transmitted light microscopy

The stained sections were photographed with a fluoride oil-immersion lens (Bell and Howell, 4.3 mm, $\times 40$, which could not be used in methylsalicylate because the lens cement would be dissolved) using Eastman Kodak LS panchromatic film. The negatives were then further enlarged to 8- and 10-in. as before, providing a final enlargement of $\times 930$, as will be seen in the fifth figure.

For the measurement of the internal diameter of capillaries from the prints, a particle-size analyzer (TGZ 3, Zeiss, West Germany) was used for estimating the minor diameters of capillaries in sections cut perpendicular to the axes of the muscle fibers as in the fifth figure below. The method is to match the minor diameter with the diameter of a circular light beam, marking each capillary counted, and accumulating the count of numbers for capillaries with diameters within each 0.2 μ m interval. The distribution of the different sizes (in millimeters) and the frequencies were recorded. The 10- μ m scales photographed at the

same magnification were used to convert the millimeter readings to microns. The intercapillary distances were measured in the same manner as with incident-light photography.

Assessment of tissue and elastomer shrinkage

Observations in plastic test tubes, glass and polyethylene tubing with internal diameters of 2–8 mm, showed no detectable volume change of the silicone elastomer as it changed from fluid to solid. Shrinkage of myocardial tissue and of elastomer occurred on prolonged immersion in both concentrated ethanol and methylsalicylate. Two types of experiments were done to assess myocardial shrinkage.

Weights and volumes were measured for seven whole hearts, opened widely and immersed in the sequence of fluids, Tyrode, ethanol, and methyl salicylate, as were the experimental hearts injected with silicone elastomer. Volumes were measured by fluid displacement, initially in Tyrode solution and then in the solutions of ethanol or methylsalicylate used for clearing the tissue. Weights and volumes were measured after immersion of 3 days duration in each of the sequence of concentrations of ethanol and methylsalicylate. The results are given by the solid symbols in Fig. 1, left panel. The shrinkage was about 19 %, the final volume being 80.7 % of the initial volume.

A second experiment was done on one elastomer-filled heart. Five hours were allowed for curing of the elastomer, then the heart was cut into blocks which weighed 0.8–1.6 g initially. Substantial shrinkage occurred in these blocks, as shown by the open symbols in the left panel Fig. 1. The first method was used in this study, cutting the heart into blocks only at the time of photographing them.

We attempted to determine the direction of shrinkage in order to correct as well as possible for both capillary lengths and intercapillary distances. If shrinkage had occurred equally in all directions, the diminution in volume of whole hearts in methyl-salicylate to 81% of initial volume would be interpreted as a reduction in linear dimensions to 93 % of initial length and a reduction in cross-sectional area to 87 %. However, much of the diminution appears to have been in the direction of the myocardial fibers and the capillaries: sarcomere lengths in the transmitted light micrographs averaged $1.54 \pm 0.12 \mu\text{m}$ ($N = 380$) in these specimens instead of the usual lengths of 1.9–2.1 μm (e.g., see Fig. 5). Shortening from 1.9 to 1.54 μm is alone just sufficient to explain the observed volume change, leading us to conclude that there was little or no shrinkage perpendicular to the fiber direction and that no correction should be made on intercapillary distances. On the basis that the elastomer does shrink but still remains very compliant, we assumed that the shrinkage of the myocardium governed the lengths of the capillaries; capillary lengths should be corrected by 1/0.807. Although 19.3% shortening would suggest that the shortened capillary cylinders should increase by 11% in diameter to preserve volume, we considered that the best data on changes in capillary diameter during the clearing process would be obtained by direct observation.

Shrinkage in diameter of the elastomer filling the capillaries was assessed by measuring capillary diameters on blocks of tissue cut from one heart at each of the stages of the clearing procedure (Fig. 1, right panel). The untreated, unfixed blocks and the ethanol soaked blocks were frozen and cut, frozen into sections 7–20 μm thick, mounted in oil on glass slides, left unstained, covered and sealed. Specimens in methylsalicylate were immersed in warm liquid paraffin for 1 hr, cooled to room temperature, sectioned perpendicular to the muscle fibers at 7 μm thickness, mounted, and sealed unstained as were the others. The sections were photographed as described above for transmitted light microscopy and diameters measured using the particle-size analyzer. The average reduction was from 5.89 to 3.84 μm , a reduction to 65% of initial diameters. From this we used 1.5/1

as the ratio of true initial diameters to observed diameters in the shrunken tissue. (The shrinkage of large cylinders (1–2 mm and 1–2 cm diam) of elastomer was similar, and in some cases, more; some loss of the unenclosed superficial layers of opaque white material occurred and clouded up the solvent.)

Results and Discussion

Epicardial region

Figure 2 shows about 1 cm² of the elastomer-filled vasculature of the left ventricular epicardial surface after clearing with methylsalicylate (1 scale division = 1 mm). The preponderance of veins on the surface is typical. Veins and arteries were distinguished at the time of the photography by following them to their branching from larger vessels identifiable from their position, as in Fig. 2. (For intermediate-sized vessels the greater wall thickness of arterioles compared to veins could be recognized by the thickness of the transparent space between the filled lumen and the filled capillaries, relative to the lumen diameter.) For example, in the left central region is a 160- μ m diam artery (left-most arrow, Fig. 2) accompanied by its two 200- μ m venae comitantes. Rather few arteriolar branches can be seen (other arrows, Fig. 2) and most are only a few millimeters long. But the surface is liberally supplied with venules, and in the lower half of the photograph these tend to parallel the muscle fibers and capillaries. Small venules (20–30 μ m diam) branch into the capillary network and are usually less than 1 mm long; their terminations are spread 0.5–1 mm apart over the whole surface.

The capillary networks are densely packed in large parallel groups of muscle fibers; two major groups are seen here, one filling the lower half of the picture, a second in the upper central region. At the junction of these regions the precise parallel arrangement is lost and the superficial venous network obscures the details. Occasionally, thin sheets of myocardium cover the larger vessels; this shows up as a thin layer of parallel capillaries, for example, just above and to the left of the left-most arrow. This is quite different from visceral pericardium where capillaries are larger in diameter and are arranged as a loose network in a sheet, such as can be seen in the upper corners of Fig. 2.

The small venules (20–30 μ m diam) of the LV epicardial surface (Fig. 3) form from capillaries in three or four steps within a distance of 50–100 μ m. No arterioles are visible; arterioles usually branch below the surface to feed the capillary network. The capillaries run parallel courses but are seen to be frequently linked both by short 10–20 μ m curved capillaries like the bar of an H, and by Y junctions. Intercapillary distances could not be measured in such photographs because one cannot readily determine the depth of a capillary in the tissue.

A subepicardial section parallel to the surface (Fig. 4) again reveals the existence of the parallel network of capillaries within the myocardium. The arteriole of about 60 μ m diam in the triad of large vessels gives off three arterioles of about 10 μ m diam, two of which are cut short, and one whose curving path can be followed for about 1 mm (arrow). The insert (same scale) shows a 160- μ m vein whose confluence from the capillary network occurs over a few hundred microns.

Capillary diameters

With reflected-light microscopy, such as in Figs. 3 and 4, 930 capillaries were measured; the mean internal diameter, uncorrected for shrinkage was 3.1 μ m (range, 2.0–4.5 μ m for the means of groups of 10 capillaries). We could not detect significant differences in diameters among left ventricles from three hearts or among epicardial, endocardial or intramural regions.

Photomicrographs of the transmitted light preparations are shown in Fig. 5. The elastomer in the capillaries appears black; these are easily distinguishable from nuclei both in cross section (left panel) and longitudinal section (right panel). Enlarged versions of these photographs were used for estimating diameters. The mean values from different sites and different hearts are given in Table 1: they ranged from 3.0 to 5.1 μm ; the average from cross sections was 3.58 μm ($N=2939$), for longitudinal sections 4.24 μm ($N = 712$), the overall average being $3.66 \pm 0.95 \mu\text{m}$ for 3651 measurements. Values obtained from longitudinal sections (subscript L in the table) were a little larger than the average presumably because of the observers' bias in avoiding measuring the diameter at points where the greater portion of a capillary had been cut away in the sectioning. These results are summarized in the histograms (Fig. 6) for cross and longitudinal sections. The frequency distributions have shapes similar to those found by Sobin and Tremer (1972).

The uncorrected 3.58 μm average for these stained cross-sectioned capillaries does not differ from the 3.84 μm average for the unstained ones (Fig. 1); both had been through the same sequence of solvents. The average of these two groups of cross-sectioned capillaries was 3.70 μm ($\text{SD} = 0.9$, $N = 5359$). These uncorrected values are similar to those reported by Sobin (1972), to the 3–5 μm seen by Reynolds *et al.* (1958) and Krogh (1919) and to the 5 μm of Stoel (1925).

Corrected for shrinkage and shortening by multiplying by 1.5, the cross-sectional diameters averaged $5.5 \pm 1.3 \mu\text{m}$ ($N = 5359$) (data of Figs. 1 and 6). These corrected diameters for the myocardial capillaries are somewhat less than erythrocyte diameters, and are only slightly larger than Sobin's (1972) estimates. Direct observations and high-speed filming have shown that erythrocytes are deformed during traversal of the capillary bed (Microcirculatory Society Film Archives) suggesting that capillary diameters are less than 7 μm . It seems likely that the capillaries in our preparation are maximally dilated: a raised outflow pressure (heart A of the table) produced no increase in diameter, as Nichol *et al.* (1951) observed in mesentery; the oxygen deprivation that must accompany perfusion with Microfil can be expected to cause full vasodilatation (Detar and Bohr, 1968). The diameters observed in rat hearts by Martini and Honig (1969) were closer to 4 μm . Reynolds *et al.* (1958) observed in continuously perfused hearts that KCl-induced arrest, producing a relaxed myocardium, resulted in increased capillary diameters; Majno (1970) observed capillary dilation with increased outflow pressure in cremaster muscle. Our conclusion is that, with the correction for shrinkage, the diameters have not been underestimated, and were almost certainly slightly larger than myocardial capillaries *in vivo*. The smallest diameter capillaries probably did not permit passage of erythrocytes, but from these data we cannot estimate whether the distribution of diameters is the same as *in vivo*, or what fraction might have been open to erythrocyte or plasma transport.

Endocardial region

In Fig. 7 is a methylsalicylate-cleared specimen deep within the myocardium sectioned perpendicular to the endocardial surface. (It is unusual in that it shows the beginning of a Thebesian sinusoid which drains into the ventricular cavity. Left ventricular Thebesian vessels are very readily apparent on the endocardial surface of injected hearts, cleared or uncleared, appearing to the unaided eye like white spider nevi. Only one or two dozen are to be found per left ventricle. Drainage into Thebesian vessels is from capillaries, as seen in this figure.) The network of capillaries is as dense as in the epicardium. Typically, triads, consisting of a small arteriole with two larger venules, penetrate this region. To the left of center is an atypical triad, unusual in having the arteriole placed to one side rather than between the venules. This section is cut a little obliquely; one can see the cut ends of the capillaries as brightly illuminated spots. The capillaries are grouped, just as are the muscle fibers. Through the cleared areolar tissue between the muscle bundles, the capillaries and

some of their relatively infrequent branches can be seen. The uniformity of intercapillary spacing is quite evident. As in the epicardium, no vessels larger than capillaries can be seen joining arterioles and venules, and careful searching revealed no direct links between arterioles and sinusoids. As with the veins in epicardium and endocardium, the interbranch distances in the segments between capillaries and sinusoids are very short.

The diffusion distance for oxygen and other solutes between the inflowing arteriolar blood and effluent blood in the accompanying venules is quite short, as shown in Figs. 2, 4, and 7, and it seems likely that there might be countercurrent exchange of rapidly diffusible substances over the 15–100 μm separating the lumina of the vessels. This anatomic arrangement may explain the apparent diffusional shunting seen in the heart for water (Yipintsoi and Bassingthwaighe, 1970) and xenon (Bassingthwaighe *et al.*, 1970). Another anatomic site where this might occur is in the epicardium where the inflowing arterioles most often are a few hundred microns deep to the surface but outflowing veins are almost wholly superficial. Since the arterial source and venous drainage are side by side (as in Fig. 2), the blood flow streams must form a loop with blood flowing from deep arterioles to the capillaries farthest from the large vessels and returning in the opposite direction in superficial capillaries and venules. With distances of even 100 μm and a diffusion coefficient for water in the myocardium of $7.4 \times 10^{-6} \text{ cm}^2/\text{sec}$ (Suenson *et al.*, 1973) only a small amount of diffusional shunting of water could be expected in both epi- and endocardial regions at normal or lowflows, as was observed by Yipintsoi, but would be undetectable at high flows, as was the case.

Capillary densities and intercapillary distances

In cross-sectional views from different locations in the hearts (such as Fig. 7 which shows subendocardium), we counted the number of cut capillary ends in a specified area and estimated intercapillary distances and capillary densities. The choice of regions influences the results. Choosing regions of dense capillarity within muscle bundles, in seventeen 50- μm squares there were 11.5 ± 1.5 capillaries (mean and standard deviation), and in fourteen 100- μm squares, there were 36.6 ± 7.6 capillaries. Without using correction for shrinkage in area, these numbers give intercapillary distances (using a hexagonal model) of 15.8 and 17.8 μm , and an average capillary density of 3880/ mm^2 , which should be considered as a maximum density observed in selected regions. In other photographs, we divided the field into a matrix of $90 \times 90 \mu\text{m}$ squares. Some squares included large vessels and clear spaces separating muscle groups (as seen in Fig. 7) and others included only muscular tissue without large vessels or interfascial spaces. From 112 squares including interfascial spaces, a total of 2294 capillaries were counted giving densities of $2530 \pm 610 \text{ mm}^2$ and intercapillary distances of $21.4 \pm 4.3 \mu\text{m}$. In 76 squares within muscle fascia, 1956 capillaries were seen, giving densities of $3180 \pm 650/\text{mm}^2$ and intercapillary distances of $18.8 \pm 4.0 \mu\text{m}$. Similar results were found by two independent observers.

In photographs of the cleared specimens such as Fig. 7, the nonmuscular avascular clear spaces comprised 12–16% of the total area of the photographic field. Using the hexagonal model and excluding the spaces from our calculations will result in average capillary densities of 3100/ mm^2 (4959 capillaries) and intercapillary distances, h , of 19.3 μm . Standard deviations are inversely related to the size of the region observed: for 100- μm squares, the standard deviation of density was about 700/ mm^2 and was much less for 1-mm squares. If one considers the region to be composed of squares with central capillaries, the shortest intercapillary distance would be the length of the side, S , of the square, the relationship to h being $S = 0.93h$, but the distance to the corners is longer. For nonoverlapping circles arranged hexagonally, the diameter is h , but 9% of the area of the hexagon lies outside of the circles. Since this is not very different from the avascular fraction of cross-sectional area (about 15%), one could justifiably consider such an array of

circles to be roughly descriptive of the anatomic arrangement, which gives the same average value of $19\text{ }\mu\text{m}$ for intercapillary distance.

Similar measurements from histologic sections (e.g., Fig. 5, left panel) on wholly muscular-capillary areas of $100 \times 100\text{ }\mu\text{m}$ which were free of avascular spaces, gave a density of $3800/\text{mm}^2$ (on 1480 capillaries counted) and intercapillary distances averaging $17.4\text{ }\mu\text{m}$ (range $13.6\text{--}25.4\text{ }\mu\text{m}$).

All of these measurements were made on cross sections as close to perpendicular to the capillaries as possible. Any obliquity results in lower estimates of density: sections at 80° and 70° give values 98.5 and 94% of the true values. Some preference was therefore given to measurements from cleared specimens (such as Fig. 7) where any marked obliquity could be recognized. This was not possible with the histologic sections. The above numbers for density and intercapillary distance are uncorrected for shrinkage, on the basis that all of the shrinkage was longitudinal and therefore none occurred in cross-sectional area. Assuming full vasodilatation, our estimates should represent maximal densities, minimal intercapillary distances.

Capillary branching and functional lengths

Assessment of branching of capillaries was attempted from longitudinal sections. Figure 3 shows a good example of parallel capillaries in superficial epicardium with the triad of larger vessels directed almost normal to the capillaries. Figure 8 is a section of left ventricular myocardium cut parallel to the surface and viewed from the endocardial side. Capillaries can be followed for several hundred microns on such sections. By viewing several adjacent regions one can gain some appreciation of the possible sites of inflow and outflow and of the lengths of the flow streams through the capillary network.

From prints like Figs. 3, 4, and 8 we estimated the relative frequencies of different types of capillary branching. Our classification was: (1) “H” or “K” branch taking off at right angles and appearing to go around a muscle fiber to join a parallel capillary on the other side of a muscle cell (as the horizontal bar of an “H,” which may look like a “K” if the branch is very small); (2) “Y” or “T” branch—simple binary branching; (The arms of the T often become parallel so that the junctional regions appear as a yoke around a muscle fiber); and (3) “fork” or multiple branches occurring at short intervals. This type is common at a confluence giving rise to a venule and is also seen in the thin layer of visceral pericardium. Using these criteria, of 433 branches distinctly seen, 66 % were of the “Y” or “T” type, 28% were of the “H” type, and 6% were in the multiple-branching group. The data are inadequate to show any differences between endocardial and epicardial regions.

The capillaries were very long; it was possible to follow a single capillary in the photographic prints for 1 or 2 mm, and despite the difficulties of cutting sections parallel to the fibers, many of $400\text{--}600\text{ }\mu\text{m}$ lengths can be seen in Figs. 3, 4, and 8. The length of individual capillaries in the heart is indefinite, for they seem to extend the whole length of the muscle bundle. Each receives inflow either directly or via other capillaries from a few arterioles spaced widely along its course, and drains, again either directly or via other capillaries, into venules spaced more closely. Thus it is difficult to ascertain in a completely filled bed, the length of capillary that an erythrocyte might traverse in going from artery to vein. Even the direction of flow may not be predictable, except in those capillaries connecting directly to an arteriole or venule, and it seems likely that in many the direction could change under the control of arteriolar vasomotion.

To obtain an approximate idea of functional capillary lengths, we perfused the left anterior descending coronary artery of two hearts with the elastomer while the circumflex coronary

artery remained intact and blood-perfused. The pressure of the elastomer injection was approximately aortic pressure. The regions at the junction of the cognate beds supplied by the two arteries were examined; the lengths of groups of capillaries from the edge of an elastomer-filled region to the nearest venules ranged from 300 to 1200 μm . Distances to the arteriolar supply were more difficult to determine, but many were greater than 1 mm. Quite often a clear line of flow from an arteriole through a single capillary into a venule was not apparent; rather, arteriolar blood entered a broad network of parallel and “interconnecting” capillaries such that the general direction of blood flow in the network must have been partly parallel and partly diagonal or perpendicular to the capillaries. One can conceive of a broad flow-path beginning as parallel concurrent flow within a group of capillaries arising from an arteriole, then traversing the parallel network of capillaries by utilizing the H and Y branches in a meandering fashion where the average direction of flow can be even perpendicular to the parallel capillaries, then returning via the superficial capillaries with relatively concurrent flow to a confluence forming a venule within 100–300 μm of the originating arteriole. This arrangement would provide roughly parallel concurrent flows locally in all parts of the flow path, and would avoid countercurrent flows in adjacent capillaries, which would be an undesirable situation resulting in diffusional shunting of oxygen from inflow to outflow and depriving more distant parts of the capillary and tissue of oxygen. However, some diffusional shunting, short-circuiting the broad loop of the flow path, is quantitatively reasonable, just as was argued concerning the epicardial regions, and would be compatible with the observations on tritiated water dilution curves (Yipintsoi and Bassingthwaighe, 1970). An unfortunate corollary might be that when flow is slowed by arterial obstruction any potential diffusional short circuit for oxygen leads to further deprivation of the tissue O_2 supply and which might be not recognized at the arteriolar level where presumably the regulation occurs.

Such looping flow streams, which should be viewed as potentially variable in form, are possible only by utilizing the cross branches between capillaries. These cross branches are close together relative to the size of region (>1 mm) supplied by an arteriole. Observed lengths were corrected for shrinkage by dividing by 0.807. Corrected lengths of unbranched capillaries averaged 102 μm , ($N = 190$); of these, 64% were less than 62 μm , 10% were 62–124 μm , 18% from 124 to 250 μm , and 8% longer than 250 μm . Overestimates would occur if a branch was hidden on the side of the capillary away from the camera.

Venules and arterioles

Venules are shown in Figs. 2–4 and 7–9. They are more frequent, and branch more frequently than arterioles, and are smooth walled, often ovoid, but seldom flat. (Sinusoids were usually flattened, lying between large muscle groups.) Arterioles were defined as inflow vessels of 10–200 μm diam and were usually conclusively identified by their origination from an artery, but also, after developing one's capabilities for pattern recognition by following many to their origin, by their relationship to clearly identifiable venules, lying between a pair of venules (left-most arrow, Figs. 2 and 4) or closely parallel to venules (other arrows, Fig. 2). Arterioles were fewer than venules (Figs. 2–4, 7–9), but relatively fewest on the superficial epicardium; the paucity implies that they must branch quickly into capillaries and that a few capillaries deliver flow to many others—thus one would anticipate that blood velocities in these arteriolar capillaries are high compared to those nearer the venous end of the flow stream.

The venule in the insert in Fig. 4 appears as if it is slightly compressed by circular bands of smooth muscle. The two superficial venules in Fig. 9 show dilated regions just beyond their origins, but no circumscribing indentations. Branching of these venules into capillaries of the lacy areolar, visceral pericardium can be seen on both at the region of constriction as well as farther out from the major vein. The main arteriole and venule are deep to the

endothelial layer. The arteriole lies deep to the venular branches; it is unusual for the venules to cross arterioles (and little need to, since they are usually paired); where crossings occur, the veins lie superficially as in this figure, avoiding compression during cardiac contraction. Another example is seen in the center of Fig. 1. Intervenous communications are common; the branch of the lower venule in Fig. 9 going diagonally upward and right joins the upper venule about 1 mm away.

Figure 9 also shows the rather large tortuous capillaries of the visceral pericardium. Some capillaries are unfilled and corrected intercapillary distances of 70–200 μm are common. Although visceral pericardium forms a sheet like the mesentery does, the anatomy of the local microvasculature is quite different, no modular units of the form described by Frasher and Wayland (1972) being seen.

Functional implications

Quantitative descriptions of the anatomic arrangements and dimensions of capillaries and small vessels in normal and pathophysiological states are essential to the detailed understanding of blood–tissue exchanges of small solute molecules, nutrient substrates, and blood gases. This study presents only a small fraction of the required data, much of which needs to be acquired *in vivo*. For example, while our data give an idea of the maximal capillary, *in vivo* experiments such as those of Martini and Honig (1969) on the influences of $p\text{O}_2$ and $p\text{CO}_2$, on the functioning fraction of the capillary bed are vital to the computation of intercapillary diffusion distances, intratissue concentration gradients, and solute delivery in various physiological states.

What this study documents is that intercapillary distances can be short enough to completely justify an assumption that concentration gradients perpendicular to the capillary are negligibly small for small hydrophilic solute molecules, an assumption which vastly simplifies the mathematical modelling of blood–tissue exchanges (Bassingthwaighe, 1974). The anatomic arrangement is conducive to maximizing the extent of concurrent flow patterns, and minimizing diffusional shunting which tends to deprive the deeper regions of highly diffusible consumable gases and substrates (Bassingthwaighe and Yipintsoi, 1969). The observed distances between inflow and outflow vessels are compatible with the observed diffusional shunting of water (Yipintsoi and Bassingthwaighe, 1970) but substantially more experimental data on various solutes, on the anatomy, and more complex analyses are required before the tracer solute data can be interpreted firmly in terms of functional diffusional and convective routes of transport within the myocardium.

A value for S_{cap} , the capillary surface area per gram of myocardium, is needed for the interpretation of permeability–surface area products obtained from physiological experiments using tracer or osmotic techniques. This can be calculated directly from our data independent of the capillary arrangement within the tissue:

$$S_{\text{cap}} = \frac{2\pi R_c N}{\rho A}.$$

where the density, ρ , is taken to be 1.063 g/ml (Yipintsoi *et al.*, 1972) and R_c is the capillary radius, the other symbols being as previously defined. From the average corrected diameter of 5.5 μm , R_c is 2.75 μm ; using an average capillary density of 3100/mm², N/A , we calculate

$$S_{\text{cap}} = \frac{2\pi(2.75 \times 10^{-4} \text{ cm})}{1.063 \text{ g/ml}} \times 310,000/\text{cm}^2$$

$$= 504 \text{ cm}^2/\text{g myocardium}.$$

Assuming a random relationship between local capillary densities and diameters, and using a standard deviation of densities of $250/\text{mm}^2$ and of radii of $1.3 \mu\text{m}$, the standard deviation of S_{cap} in local small regions might be about $125 \text{ cm}^2/\text{g}$ or 25 %.

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Fig. 1. Shrinkage on immersion plotted as a function of sequentially higher concentrations of ethanol and methylsalicylate. A: Shrinkage of myocardium in the form of 1–2 ml pieces (closed symbols). Ordinate is M/M_0 , measured mass divided by initial mass of the same heart or piece, or V/V_0 , volume over initial volume. Bars give $\pm$ SD. (Densities of 100% alcohol and methylsalicylate are 0.79 and 1.17 g/ml. The density of the whole hearts decreased from an initial average of 1.06 g/ml to 0.88 in 100% ethanol and in methylsalicylate increased to 1.18 g/ml.) B: Diameter of columns of Microfil within capillaries.

Fig. 2. Vasculature of the left ventricular epicardium filled with Microfil. Scale divisions are 1 mm. The top is toward the base of the heart. The capillaries form dense parallel networks in the middle upper region running 30° to the right of the ventricular axis and in the lower half running 30° to the left. Most of the larger vessels are veins. The arrow tips mark arterioles.

Fig. 3. Venules and capillaries on left ventricular epicardial surface. Scale divisions are 10 and 100 μm . (Different heart from that shown in Fig. 2.) No arterioles are present.

Fig. 4. Subepicardial vasculature. Section parallel to epicardium, 1 mm deep. Scale divisions 10 and 100 μm . A 60- μm arteriole, accompanied by two venules, gives rise to three 10- μm arterioles, two short and one long (at arrow). The insert (same scale) shows a 160- μm vein giving rise to small venules and branching rapidly into the parallel capillaries.

Fig. 5. Sections of ventricular myocardium near apex stained with hematoxylin and eosin (heart C). Scale shows 10- μ m divisions. Sarcomere lengths are distinctly shortened. Note the separations between individual fibers and groups of fibers.

Fig. 6.

Frequency histogram of uncorrected capillary diameters measured on histologic sections by transmission microscopy. The ordinate is the percentage of observations per 1- μ m class width so that the area under each curve is unity. Class width for plotting = 0.2 μ m. (Numbers of observations are given in parentheses.) Measurements from cross sections, dashed line, where minor diameters are taken from any elliptical sections (e.g., left panel, Fig. 5), give lower estimates than those from longitudinal sections (e.g., right panel of Fig. 5), dotted line, where one tends to avoid measuring at points which might be too thin because of sectioning partly through the capillary itself. All data = large dots.

Fig. 7. Cross section of endocardial region of left ventricle. At right are a 15- μ m arteriole (and a branch cut off from it) with two other vessels, presumably venules. Scale divisions are 10 and 100 μ m. Overall capillary density here was about $3000 \pm 600/\text{mm}^2$ in 90- μ m squares, but was higher ($3300 \pm 530/\text{mm}^2$) in smaller wholly muscular regions.

Fig. 8.
Apical left ventricular myocardium in longitudinal section viewed from the endocardial side.
An arteriole and branching venule are visible within the tissue lying toward the epicardial surface.

Fig. 9.

Two venules and visceral pericardium of the left ventricular surface. Scales are 10 and 100 μm . An arteriole lies deep. The diagonal branch at the lower right is an intervenous connection joining these two venules. The figure is a composite of two photographs of adjacent regions. (There is an artefact in the left lower corner that should be ignored.)

TABLE 1
Uncorrected Capillary Internal Diameters^a

Heart	Site ^b	N	Mean (μm)	SD
A	Mid	130	3.83	0.71
	Mid	385	3.83	0.85
	Mid	75	3.99	0.74
	Apex	230	3.31	0.67
	Mid _L	120	5.14	0.83
B	Septum	113	3.83	0.81
	Septum	276	4.19	0.80
	Endo	307	3.80	0.97
	Endo	188	3.50	0.75
	Septum _L	260	4.08	1.03
	Apex _L	72	4.20	0.80
	Septum _L	212	3.94	0.88
C	Apex	607	3.03	0.73
	Apex	111	3.30	0.87
	Apex	362	3.73	0.83
	Apex	155	3.33	0.76
	Apex _L	48	4.27	0.53

^a Measured by transmitted light method with particle-size analyzer; all sections were from left ventricles. Mean coronary sinus pressures (mm Hg) during elastomer perfusion were: A, 33; B, 5; C, 12.

^b Measurements were made mainly of vessels cut in cross section or oblique section (where minor diameter of ellipse is used). The subscript L denotes longitudinally cut sections, where silhouette diameters were used.

Mid = area between subepicardium and subendocardium; Endo = subendocardium including papillary muscle.