Microcirculation in the Ventricle of the Dog and Turtle

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ABSTRACT

Phasic red cell velocity and diameters of coronary arterioles, capillaries, and venules were measured in the beating turtle and dog heart using high-speed cinematography with transillumination of the left ventricle. In the turtle, arteriolar red cell velocity was diminished during systole, but during diastole arteriolar inflow increased, especially during the rapid and the slow filling period. Capillary and venule red cell velocity was increased during systole, particularly at the time of ejection; however, during diastole red cell velocity declined and the lowest values occurred during isovolumic relaxation. In dog arterioles, capillaries, and venules, the pattern of red cell velocity was similar. Thus, in the turtle and dog, the peak arteriolar red cell velocity occurred in unison with left coronary artery inflow, and the capillary and venule flow pattern followed that of the coronary sinus. The diameters of arterioles, capillaries, and venules in the turtle ventricle all declined about 34% during systole; similar results were obtained in the dog. Capillary arrangement appeared to be predominantly parallel and cocurrent; however, capillary loops with countercurrent flow were occasionally observed. The data on microvascular phasic red cell velocity are consistent with the macroobservations of reduced coronary artery inflow and enhanced coronary sinus outflow during ventricular contraction. The results demonstrate that the shift in the flow pattern occurs at the transition from arterioles to capillaries.

KEY WORDS

red cell velocity

cardiac cycle

arteriolar flow

capillary circulation

phasic capillary flow

venule flow

ventricular capillaries

countercurrent flow

Rebatel (1) stated in 1872 that "entrance of blood into the smaller coronary vessels during systole is difficult" and that "in diastole the capillaries which have been empty of blood as a result of systolic compression, fill again from larger coronary vessels." Subsequently, numerous other investigators have confirmed the observations that coronary inflow occurs primarily during diastole and that outflow in the coronary sinus takes place mainly during systole (2-8). Consequently, there must be a point where this 180° phase shift occurs (9). However, the exact location of this shift has not been observed directly. In the atrium phasic changes in red cell velocity have been studied by high-speed cinematography with transillumination from the atrial cavity (10, 11), but this technique has never been successfully applied to the ventricle because of the thickness of the ventricular wall and the focusing problems resulting from tissue movement.

The purpose of this investigation was to detail red cell velocity in ventricular capillaries, arterioles, and venules during different phases of the cardiac cycle and to compare the pattern of ventricular microcirculation with that already described for atrial muscle. The investigation was carried out using both turtle and dog ventricles; studies on turtle hearts were performed because of the small range and the low frequency of their cardiac motion.

Methods

Freshwater turtles (Pseudemys scripta elegans) weighing 750-1,200 g and young dogs weighing 3-5 kg were used in this study.

Experiments were performed on 22 turtles in the autumn months prior to hibernation. The turtles were anesthetized with sodium pentobarbital (0.05 mg/g) given orally through a small polyethylene tube inserted into the esophagus. The plastron was removed from an area measuring about 5 × 5 cm without damaging the superficial veins, and the pericardium was incised. In
other experiments, 14 dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). The trachea was intubated and artificial ventilation was started with a Bird respirator. A left thoracotomy was then performed, and the pericardium was exposed and incised.

Since epillumination of the ventricle was impossible, transillumination had to be used; therefore, a 20-gauge light-transmitting needle (Glass Instruments) (Fig. 1) was inserted just underneath the superficial layer of the myocardium. A quartz rod (diameter 0.017 inches) was inserted through the lumen of the needle to transmit the light to the needle tip where a mirror angled at 45° opposed the vertical polished end of the quartz rod and reflected light upward through a small hole 0.15 inches in diameter through the ventricular muscle (Fig. 1). In this way, the superficial layers of the myocardium were transilluminated. The mirror was made by silvering a polished bit of quartz rod (diameter 0.017 inches) cut at a 45° angle; it was then fixed at the closed tip of the needle. The hole in the wall of the needle, through which light was transmitted upward, and the space between the end of the quartz rod and the mirror were filled with clear acrylic plastic to maintain a patent light path and prevent blood and tissue fluids from entering the light-conducting system. This system transmitted heat-filtered light from a pulsating xenon arc through the ventricular muscle toward the objective of the microscope (Fig. 1). The diameter of the needle (1 mm) represented about 50% of the total wall thickness in the turtle and about 12% of the total wall thickness in the dog. These data were obtained by inserting the needle, exciting the needle-containing portion of the heart in toto, and comparing the size of the needle to the thickness of the heart muscle. The thickness of the transilluminated portion of muscle covering the needle was about 500 μm, and the depth of the focused field was approximately 100 μm. These data were arrived at by observing a lens paper fiber on the surface of a platform. The depth of the focused field was then determined by moving the objective lens (turning the knob of the fine focus adjustment of the microscope) from the focus optimum to the farthest point in either direction at which subject detail was still recognizable. The number of revolutions of the fine focus adjustment knob was then correlated with the vertical displacement of the microscopic objective (one revolution = 189 μm).

A similar technique was used to determine the depth of the visualized vessels within the ventricular wall. A lens paper fiber on the surface of the heart was brought into focus. The microscope then was focused on a specific vessel within the heart muscle. The number of revolutions of the fine focus adjustment of the microscope required to refocus on the vessel was then translated into units (μm) of vertical displacement of the objective by the ratio described in the preceding paragraph. According to this method, the depth within the heart muscle of the visualized vessels varied from 60 to 200 μm. Projection of the film onto a screen with a reference scale, showed that the length of vessel in focus was approximately 160–230 μm. This length is indicated in Figures 4 and 6.

The danger of injury to the vessels which adjoined the needle had to be considered, because local vascular tone could have been affected leading to dilatation of the precapillary sphincter and diminution of capillary red cell velocity. To observe possible effects of the indwelling illuminating needle, red cell velocities in the wall of the left atrium of the cat and the dog were determined in three experiments by transillumination of atrial muscle with an intraluminal light pipe (10–14) alone and then by transillumination in conjunction with a 23-gauge needle inserted into the surface of the atrial muscle (Fig. 2). In these experiments, mean capillary red cell velocity was not significantly influenced by the intramuscular needle: mean values ± SD for the three experiments were 1.272 ± 311 μm/sec with the atrial intraluminal light pipe alone and 1.240 ± 289 μm/sec with the inserted 23-gauge needle. The needle used in these experiments was smaller than the illuminating needle used for visualization of the ventricular microcirculation (23-gauge vs. 20-gauge), but the thin left atrial muscle...
Diagram of a side view of the floating focus keeper. A contact ring floating on the cardiac surface is connected to a support tube mounted on a set of four swinging arms to allow vertical motion synchronous with the motion of the heart. The objective lens of the microscope is mounted on a sleeve with a fine-threaded nut at the opposite end, resting on top of the support tube in which the sleeve is suspended. A pin on the sleeve passes through a slot in the support tube to prevent rotation of the sleeve. Fine focusing is done without touching this system by turning the nut via a chain-sprocket mechanism around the nut.

The system is counterbalanced so that the pressure on the surface of the heart is minimized (< 450 newtons/m²). This pressure is not sufficient to impede capillary flow (14).
pictures. Despite these difficulties, it is apparent from Figure 4 that it is possible to follow the progress of an individual red cell. In practice this task is made easier because red cell velocity is determined by projecting the original film on a screen. The exposure time, using this instrument, is very short (25 microseconds), but consecutive pictures cannot be taken. Figure 4 shows that frame-to-frame determination of red cell progression is also possible in arterioles. The arrows point to the same red cell in different frames, showing its progression. The bottom of each frame illustrates that individual red cells can be recognized even in larger vessels. It is also apparent from Figures 4 and 6 that the inside diameter of individual capillaries and arterioles is recognizable. It is particularly easy to determine inside diameter when the films are viewed directly.

Since capillary plasma flow rate could not be measured, capillary red cell velocity was determined. Plasma flow rate in capillaries should be less than red cell velocity. Consequently, the findings reported in this paper cannot be extrapolated to the velocity of whole blood. Whenever possible, red cell velocity was compared between capillaries, arterioles, and venules in the same frame. However, such comparisons were not always possible, since only one layer of the capillary circulation could be observed. An average of 200–300 measurements of red cell velocity in capillaries was carried out in each preparation. Data from single measurements were then averaged, and statistical analyses were carried out using Student’s t-test (15).

Red cell velocity was correlated with aortic and ventricular pressures and with the electrocardiogram, using a time signal on the film which indicated every eighth frame. A similar signal also appeared on the hemodynamic tracings. This procedure made correlation between red cell velocity and hemodynamic measurements possible.

In the turtle, red cell velocity was measured during isovolumic contraction, rapid and slow ejection, isovolumic relaxation, and rapid and slow filling. These time intervals were obtained from the aortic and ventricular pressure curves. In the dog, only average values for systole and diastole could be obtained because of difficulty in focusing. In both turtles and dogs, inside diameters of capillaries, arterioles, and venules were measured both during systole and diastole. Vessel diameter was determined from the projected image with the same reference scale which was used to determine red cell velocity.

**Results**

Red cell velocity in capillaries of the turtle is shown in Table 1 and Figure 5. Capillaries as used in the context of this paper are the smallest blood vessels which are devoid of smooth muscle, in contrast to arterioles and metarterioles (16). Capillary diameter does not exceed that of the passing red cells (17). Consequently, in contrast to arterioles and venules, red cells course through capillaries only in single file.

Arterioles can be easily differentiated from venules because of the direction of flow at areas of bifurcation. Furthermore, in line with the observations of Ludwig (18), we also observed in both the turtle and the dog that, prior to the confluence of capillaries and postcapillaries to form a venule, the network becomes denser, the meshes shorter, and the cross bridges more frequent.

The inside diameters of arterioles in dogs ranged from 15 to 29 μm during systole and from 20 to 36 μm during diastole. The diameters of venules ranged from 11.0 to 20.0 μm during systole and from 15 to 29 μm during diastole. In the turtle, the values for arterioles ranged from 12 to 26 μm dur-
Microcirculation in the Ventricle

During systole and from 14 to 40 μm during diastole. Values for venules ranged from 9 to 15 μm during systole and from 13 to 21 μm during diastole.

Marked variations in red cell velocity between arterioles, capillaries, and venules in the turtle were noticeable (Table 1 and Fig. 5). In capillaries and venules, peak velocity occurred during systole, but in arterioles it took place during diastole. Peak arteriolar red cell velocity therefore occurred in unison with left coronary artery inflow, and the velocity pattern in capillaries and venules followed the flow pattern in the coronary sinus (3, 7).

In dogs, the pattern of red cell velocity was similar. As shown in Table 2, peak mean red cell velocity in capillaries also occurred during systole, and in the arterioles it occurred during diastole.

Capillary diameters in the ventricular muscle of turtles and dogs varied with the cardiac cycle. In the turtle, the diameters of arterioles, capillaries, and venules declined during systole to about the same degree (Table 3). In the dog, a similar change was observed.

Additional findings concern the anatomical pattern of the ventricular microcirculation in the turtle and dog. In principle, the capillary pattern in the ventricle of the turtle and the dog was similar to that previously described in the atrium of the cat. The ventricular capillaries lay on either side of the muscle fibers. Although capillary arrangement was primarily parallel and cocurrent, several interconnections between capillaries were observed at higher magnifications (Fig. 6). These intercapillary anastomoses sometimes formed interconnecting loops of different lengths. As a rule, red cell motion in different capillaries of the dog and the turtle ventricle occurred in the same direction; however, opposite (countercurrent) flow was observed in some adjoining capillaries, particularly in those joined together by large connecting loops (Fig. 6).

Discussion

Most of the experiments on phasic red cell velocity were carried out in the turtle ventricle (Pseudemys scripta elegans), since the range and the frequency of cardiac motion are considerably less than they are in mammalian hearts (heart rate at room temperature 20–26 beats/min). The outer layer of the turtle heart (compacta) could be used for these experiments, since it is supplied from the coronary arteries which originate from the right aortic arch (19, 20). In the compacta, the capillaries are elongated, but in the deep layers of the turtle heart (spongiosa) few capillaries are seen. The
spongiosa's blood supply is mainly derived from blood in the cardiac chamber directly (20). According to Ostadal and Schiebler (21), the finer structure of the coronary capillaries of several species of turtles is not different from that of fish or mammals. In the dog, a total of 1,589 individual measurements of red cell velocity was made. The observations of phasic red cell velocity in dogs agreed with those in turtles except that, in systole and diastole, capillary red cell velocity exceeded that in the turtle (Table 2). Since red cell velocity was determined only in subepicardial layers, no conclusions on capillary circulation in other areas of the heart can be drawn. Tschopp et al. (22) and Rakusan et al. (23) have demonstrated variation in capillary flow between right and left ventricular muscle. Variations in regional myocardial blood flow have also been demonstrated by Hoffman et al. (24), Downey (25), and Kirk and associates (26, 27). The latter discovered that systole inhibits blood flow in the endomyocardium more than it does in the epicardium. Winbury et al. (28) have found nonuniformity of partial pressure of oxygen, perfusion, and blood content between the epicardial and the endomyocardium.

The finding of maximal flow in arterioles during diastole is in line with results on blood flow in coronary arteries, and the speedup in red cell velocity in capillaries and venules during systole is in agreement with observations on coronary sinus flow (3, 7).

The question arises as to the cause of this 180° phase shift from the arterioles to the adjoining capillaries. It is possible that the causes for this phenomenon can be found in an interplay of systemic blood pressure, intramural pressure, and vascular cross-sectional area and capacity. During systole, ventricular contraction throttles coronary inflow due to high intramural pressure and counteracts blood pressure in coronary arterioles and arteries. Therefore, red cell velocity in the arterioles decreases. At the same time, the capillaries are compressed and the blood contained in them is massaged towards the venules, increasing capillary red cell velocity. This phenomenon may lead, in the latter part of systole, to a depletion of capillary blood volume so that capillary red cell velocity decreases (Fig. 5). However, objective evidence for such a redistribution of capillary blood volume is not available.

During early diastole, the arterioles and capillaries dilate as intramural pressure decreases (Table 3). Therefore, in the presence of the diminished arteriolar resistance which exists in conjunction with a relatively high aortic pressure, red cell velocity in coronary arterioles and arteries increases to a peak in the middle of diastole; it diminishes again as the aortic pressure falls to its

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Systole</th>
<th>Diastole</th>
<th>( P ) (systole vs. diastole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillaries (10)</td>
<td>3150 ± 201</td>
<td>1428 ± 277</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Arterioles (10)</td>
<td>1168 ± 389</td>
<td>3391 ± 214</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Venules (9)</td>
<td>3495 ± 254</td>
<td>1624 ± 518</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

All values are means ± SD; number of dogs on which experiments were performed is given in parentheses.

### TABLE 3

<table>
<thead>
<tr>
<th>Capillaries</th>
<th>Arterioles</th>
<th>Venules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turtle</td>
<td>Dog</td>
<td>Turtle</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Systole</td>
<td>5.2 ± 0.8</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>(n = 75)</td>
<td>(n = 56)</td>
<td>(n = 45)</td>
</tr>
<tr>
<td>Diastole</td>
<td>8.1 ± 1.0</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>(n = 81)</td>
<td>(n = 67)</td>
<td>(n = 79)</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

All values are means ± SD; \( N \) = number of measurements.
FIGURE 6
Capillary pattern of the turtle ventricle. This picture was obtained using a Hasselblad camera (exposure time 25 μseconds). The capillary arrangement was primarily parallel and cocurrent, but some intercapillary anastomoses were present. 100 μm = 25.9 mm.

preejection value. In contrast, red cell velocity in capillaries decreases rapidly during early diastole because of the transient effects of increasing capillary cross-sectional area and capacity. The influence of capillary cross-sectional area and capacity on capillary red cell velocity may be expressed by applying the equation of continuity:

\[ A_1 V_1 = A_2 V_2 \]  
\[ V_2 = A_1 V_1 / A_2 \]  

where \( A_1 \) is the cross-sectional area of arterioles during diastole, \( A_2 \) is the cross-sectional area of capillaries during diastole, \( V_1 \) is the red cell velocity in arterioles during diastole, and \( V_2 \) is the red cell velocity in capillaries during diastole. Thus, in diastole, capillary red cell velocity \( (V_2) \) decreases as capillary cross-sectional area \( (A_2) \) increases. However, as Table 3 shows, both \( A_1 \) and \( A_2 \) increase proportionally. Consequently, the observed increase in \( A_2 \) is compensated for by a rise in \( A_1 \). It is, however, within the realm of possibility that \( A_2 \) increases, not because of the greater diameter of individual capillaries during diastole, but because of a rise in the total number of perfused capillaries (recruitment). Such a phenomenon may be present in deeper layers of the myocardium; however, our observations do not establish its occurrence.

Perusal of the data in Tables 1 and 2 indicates a ratio of aggregate capillary cross-sectional area to aggregate arteriolar cross-sectional area in the turtle of 0.87 during systole and 3.65 during diastole compared with 0.37 during systole and 2.38 during diastole in the dog. These ratios show that the aggregate cross-sectional area of arterioles is large compared with that of capillaries during systole and suggest the possibility, proposed by Duling and Berne (29), that oxygen is lost from the blood in the arterioles prior to its entrance into the capillaries.

Our measurements of microvascular inside diameters taken from single high-speed movie frames are generally in agreement with those of other investigators (17, 30). The data on capillary inside diameters of the dog ventricle for instance are in the same range as those of Bing et al. (30) (3–5 μm) and Sobin and Tremer (17) (3–4 μm).

The anatomical pattern of the capillaries observed in the ventricle of the dog and the turtle (Fig. 6) closely resembles that previously described in atrial muscle. In both, capillary arrangement appears to be predominantly parallel and cocurrent; however, some countercurrent flow is visible particularly in those vessels that are joined together by connecting loops. Using barium gelatin pigment injection for visualization of human coronary vessels, Fox and Hutchins (31) noticed that the capillaries were located between fibers in a ratio of approximately one capillary to each fiber. The fibers or vessels branch and interconnect among others to form two intermeshing three-dimensional networks. Dynamic events such as co- or countercurrent flow could of course not be observed in these post mortem preparations. The presence of countercurrent flow has also been noticed by Grote et al. (32) and Huhmann et al. (33) in the perfused rat heart.

The role of countercurrent flow in the oxygenation of heart muscle is still questionable. Whether countercurrent flow results in bypass of tissue and a short-circuit in oxygen diffusion is also not clear. Luebbers (34) is of the opinion that neither the cylinder model nor the cone model of the capillary circulation accounts for maintenance of adequate oxygen transport in heart muscle; rather he thinks that asymmetry of capillary origin best accounts for adequate oxygenation. In addition, the data of Grabowski and Bassingthwaighte (35) on osmotic transients in the myocardium are inconsistent with any significant role of countercurrent diffusion in blood-perfused hearts.
References


MICROCIRCULATION IN THE VENTRICLE


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