Cardiac lymph flow and composition in acute myocardial ischemia in dogs

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FEOLA, MARIO, AND GERALD GLICK. Cardiac lymph flow and composition in acute myocardial ischemia in dogs. Am J Physiol. 229(1): 44-48. 1975.—Cardiac lymph was obtained from 12 normal dogs (group 1) for two consecutive 2-h control periods and from 7 dogs (group 2) for 2 h before and 2 h after occlusion of the circumflex branch of the left coronary artery. Lymph composition was studied with reference to pH, red blood cell (RBC) concentration, total protein content, potassium and sodium ion concentrations, and creatine phosphokinase (CPK) and acid phosphatase enzyme activities. No significant difference was noted in any variable between the two groups during the first 2-h period. In group 1, no significant change occurred in any variable as a result of the passage of time alone. In group 2, 2 h of myocardial ischemia produced increases of 53.3 ± 5.1% in lymph flow, 67 ± 5% in protein content, and 418 ± 27% in the RBC concentration, suggesting increased blood capillary permeability. Lactate rose 120.5 ± 27%, potassium concentration increased 16.9 ± 2.4%, acid phosphatase increased 30 ± 3%, and CPK rose 61.6 ± 10.9%, suggesting ischemic injury of myocardial cells. These changes in lymph were statistically significant (P < 0.05) and reflect both capillary and myocardial cell abnormalities.

Experimental myocardial infarction; myocardial enzymes; lysosomes; acid phosphatase; creatine phosphokinase; lymph stasis; myocardial interstitial edema

MYOCARDIAL INFARCTION, in pathophysiologic terms, is considered the result of insufficient coronary perfusion coupled with accumulation of products of anaerobic metabolism (38). Although experimental studies have dealt traditionally with problems related to blood flow, evidence has accumulated to suggest that changes in interstitial fluid also play an important pathophysiologic role. These changes result not only from anaerobic metabolism and myocardial cell injury, but from blood capillary alterations as well. Thus, interstitial edema, first described by Bucuier in 1939 (2), tends to aggravate ischemia by interfering mechanically with capillary blood flow (14, 17), while the drop in interstitial fluid pH tends to increase intracapillary sludging and thrombosis (25, 36). In addition, the buildup of potassium ions and lysosomal enzymes tends to enhance the vulnerability of the ischemic myocardium to fibrillation (11) and tissue destruction (39).

Venous blood draining the ischemic tissue has been reported to be a sensitive indicator of the early metabolic alterations occurring in myocardial cells (21). It provides no information, however, on the equally important changes affecting the blood capillary wall and the effects these changes may have on the development of interstitial edema. In some ways, tissue studies give a more complete picture, but they do not lend themselves to a dynamic evaluation of the ischemic process because of the traumatic effect of repeated biopsies in one small area or the possible sampling of areas with different degrees of injury.

Since the lymphatic system drains the interstitial fluid, this study was designed to investigate the feasibility of collecting cardiac lymph in experimental myocardial infarction and determining changes in its composition that would reflect both blood capillary and myocardial cell injury. Changes in lymph flow, protein content, and red blood cell (RBC) concentration were used as indices of capillary injury; changes in pH, lactate, sodium and potassium ions, creatine phosphokinase (CPK), and acid phosphatase were used as indices of cell injury. The data obtained indicate that cardiac lymph reflects the interstitial changes that occur during experimental myocardial infarction and provide information that is not obtainable by analysis of systemic venous or coronary sinus blood.

METHODS

Dogs weighing between 16 and 24 kg were anesthetized with sodium pentobarbital, 25 mg/kg, intravenously, after an overnight fast. Hematocrit values were at least 32% and plasma protein levels were above 4 g/100 ml in all the dogs. Each dog was placed on a warming mattress to keep body temperature between 37-38°C, and each was intubated and ventilated with a Harvard respirator to maintain arterial blood gases and pH within the normal range. The electrocardiogram (ECG) was continuously displayed on an Electronics for Medicine oscillograph. Polyethylene catheters were inserted through the left carotid artery into the central aorta and through the left jugular vein into the superior vena cava, and were attached to Statham P23Db pressure transducers for the monitoring of aortic and central venous pressures. Hematocrit and arterial blood gases were measured every 30 min. Ringer solution (40 ml/h) was given intravenously throughout the experiment.

The chest was opened through the left fourth intercostal space and the edges of the divided pericardium were anchored to the wound edges, suspending the heart in a pericardial cradle. The cardiac lymphatic system was visualized by injection of 0.1 ml of T-1024 dye (0.452% anhydrous salt) (Evans blue, Chilcott Laboratories) through
a 27-gauge needle into the myocardium of the apical portion of the left ventricle. The experiment was continued only in dogs in which the entire subepicardiac plexus of the left ventricle converged into one major supracardiac lymph channel; small secondary branches were ligated when present. Thus, collection of most of the lymph originating in the left ventricle was assured. The circumflex coronary artery was isolated under the left atrium, carefully avoiding the stained lymphatics running parallel to it. The isolated segment was 25–30 mm from the aorta and proximal to the obtuse marginal branch.

A polyethylene catheter (PE-50) previously filled with dilute heparin solution (10 U/ml) was inserted into the supracardiac lymph channel with the aid of a Zeiss dissecting microscope. The spinal column of the animal was elevated and the draining catheter was exteriorized through the right fourth intercostal space to assure a dependable flowing system.

Aortic and central venous pressures and the ECG were recorded at regular intervals. Mean pressures were obtained by electrical integration. Heart rate was derived from the ECG signal. Cardiac lymph, after a flush period of 15 min, was allowed to flow by gravity, under a layer of mineral oil, into a graduated, heparinized tube packed in ice and placed 5 inches below the level of the lymph channel. Lymph flow was measured every 30 min. At the end of the collection period, a sample was taken for determination of RBC concentration; then the collecting tube was centrifuged at 2,000 × g for 30 min at 4°C and the supernatant was stored at −15°C until the time of analysis. Coronary sinus blood was obtained by direct puncture of the coronary sinus at a site about 1 cm from its entrance into the right atrium. Systemic venous blood was sampled from the superior vena cava.

Lymph composition was studied with reference to pH, RBC concentration, total protein content, sodium and potassium ion concentrations, and CPK and acid phosphatase enzyme activity. pHs were measured on a blood gas analyzer (Instrumentation Laboratories, Boston). Protein concentration was determined by the biuret method (20) and content was calculated as (concentration × lymph volume)/100. Sodium and potassium were measured by flame photometry. Lactate was determined by use of the Sigma kit no. 826 (Sigma Tech. Bull. 726/826 UV, p. 5, 1966). CPK was measured by Natelson’s method (20) and expressed in units; acid phosphatase was measured in Bodansky units (20). Because erythrocytes contain a phosphorouoesterase which reacts with the substrate used in the determination of acid phosphatase and because the cardiac lymph became grossly blood stained during ischemia, the hemoglobin content of the lymph sample was assayed by spectrophotometry and the acid phosphatase values were corrected for the contribution of hemolysis by using a hemoglobin-enzymie calibration curve after the method of Sutherland et al. (29). These measurements required a total of about 3 ml of lymph.

To obtain adequate amounts of lymph, we collected lymph for two consecutive 2-h periods. Dogs were assigned alternately to two groups; group 1 in which the circumflex artery was occluded but not occluded and group 2 in which the artery was occluded at the end of the first 2-h period. This occlusion was done by the two-step method of Harris (10). At the end of each 2-h period, samples of systemic and coronary sinus blood were obtained for the same determinations carried out on lymph.

Each dog served as its own control. In group 1, we assessed the effects of the passage of time alone and these data were compared to those obtained in group 2, thereby allowing us to elucidate the changes produced by ischemia. Statistical analysis was carried out using the Student t test.

At the end of the experiment, the anatomical site and extent of myocardial ischemia were delineated by the intravenous injection of fluorescein (Fluorescein 5%; 1 ampul = 10 ml; Alcon Laboratories, Fort Worth) and examination under UV light. Fluorescein (28), on reaching the coronary circulation, diffuses uniformly through the capillary wall and gives a brilliant yellow-green fluorescence of the normal myocardium. In contrast, the ischemic area remains unstained. The margins of this area were marked with Evans blue. After excision of the heart, the volume of the left ventricle and of the ischemic zone were estimated by water displacement. Sections of the posterior papillary muscle were taken for histologic examination.

RESULTS

Twelve dogs comprising group 1 remained under stable physiologic conditions for the entire 4-h period of lymph collection. Of the 12 dogs assigned to group 2, four developed ventricular fibrillation after occlusion of the circumflex artery and died without any attempt at resuscitation being made and one was excluded because of lack of development of ischemic changes. Data, therefore, were obtained from seven dogs subjected to myocardial ischemia.

The average weight of the animals was essentially the same in both groups. No significant differences were noted in any variable between the animals in group 1 and those in group 2 under control conditions. Lymph flow and most lymph constituents were within ranges previously reported from this and other laboratories (5, 16, 19, 33, 34). The differences between lymph and serum of systemic venous and coronary sinus blood were also within previously described ranges (34). In group 1, no significant change occurred in any variable as a result of the passage of time alone. The changes observed in group 2 in the lymph collected during 2 h of ischemia clearly indicated both blood capillary and myocardial cell injury. Thus, lymph flow increased 53.3 ± 5.1 (SE) %, total protein content rose 67 ± 5 %, and RBC concentration increased 418 ± 27 %, suggesting increased capillary permeability; increases in lactate averaged 120.5 ± 27 %, potassium concentration 16.9 ± 2.4 %, acid phosphatase 30 ± 3 %, and CPK 61.6 ± 10.9 %, indicating anaerobic metabolism and increased cell membrane permeability. Concurrent with the increase in lactate concentration, the pH of the lymph declined from 7.55 ± 0.01 to 7.31 ± 0.01. As potassium concentration increased in the lymph, sodium concentration decreased, suggesting a failure of the sodium pump at the level of the cell membrane. These changes in lymph were all statistically significant (P < 0.05) in contrast to the nonsignificant changes that occurred, with the exception of CPK, in systemic venous and coronary sinus blood. The data are summarized in Tables 1 and 2.
In five dogs, the changes in the volume of lymph flow were correlated with hemodynamic changes at 30-min intervals (Table 3). During the first 30 min of ischemia, lymph flow decreased concomitantly with a drop in mean aortic pressure and heart rate. It increased $235 \pm 15\%$ during the following period, when the central venous pressure started to rise and blood capillary permeability increased, as shown by the lymph becoming blood stained. It remained high in the following periods, as central venous pressure continued to rise (up $291 \pm 10\%$ from control) and mean aortic pressure and heart rate tended to recover.

The development of myocardial ischemia was evidenced by ST-segment elevations in leads II, III, and aVF of the electrocardiograms. Altered contraction of the posterolateral wall of the left ventricle was also evident. Fluorescein continued to rise (up $291 \pm 10\%$ from control) and mean aortic pressure and heart rate tended to recover.

**Discussion**

It was the primary purpose of this investigation to determine the feasibility of evaluating changes in the interstitial compartment of the heart during experimental acute myocardial infarction by the collection and analysis of cardiac lymph. A further objective was to compare the sensitivity of lymph versus systemic and coronary sinus blood in their ability to detect such changes.

In these experiments, lymph flow increased $53.3 \pm 5.1\%$ during the 2-h period after ligation of the circumflex coronary artery. We attribute this increase to the ischemic process, since other, potentially important factors such as hypoxia (18), hypoproteinemia (19), and fluid infusion (35) cannot account for our findings, and since in the animals of group 1 no change occurred as a result of time alone.

The changes we observed in the volume of lymph flow during acute myocardial infarction are explicable in terms of the Starling equilibrium (27) as it has been recently interpreted and modified by Guyton et al. (8) and Taylor et al. (31). As they indicate, the net flow of fluid out of the blood capillary into the interstitial space depends on: 1) the hydrostatic pressure difference between the capillary and the interstitial space, 2) the colloid osmotic pressure difference between the plasma and interstitial fluid, and 3) the permeability of the capillary membrane as it is influenced by the filtration coefficient of the capillary and the reflection coefficient of the plasma proteins. The further movement of interstitial fluid into the lymph capillary is regulated by similar factors as they exist between the interstitial space and the lumen of the lymphatic. Additional factors that have been postulated to promote the propulsion of lymph include an active lymphatic pump (9), a tissue pump, which in the present experiments would be the consequence of cardiac contraction (5, 15), and an effect of arterial pulsations similar to the vis a tergo present in the venous system (22).

With these concepts in mind, it seems reasonable to postulate that the initial decrease in lymphatic flow we observed during the first 30 min after coronary artery ligation probably resulted from the decrease in aortic pressure that ensued, with consequent decreases in coronary perfusion pressure and effective capillary filtration pressure. It also seems likely that the propulsive force produced by cardiac contraction decreased. During this period, we do not have any evidence that changes in capillary permeability or in colloid osmotic pressure gradients occurred.

After the first 30 min of ischemia, however, the volume of lymph flow increased greatly. This increase in flow resulted undoubtedly from several factors. First, the recovery toward control levels that occurred in aortic pressure...
cardiac lymph flow and composition

Table 3. Hemodynamic data and lymph flow in five dogs with acute myocardial ischemia

<table>
<thead>
<tr>
<th></th>
<th>Control, min</th>
<th>Time postocclusion, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-30</td>
<td>30-60</td>
</tr>
<tr>
<td>IHR</td>
<td>153 ± 5.8</td>
<td>138 ± 5.5*</td>
</tr>
<tr>
<td>MAP</td>
<td>108 ± 5</td>
<td>95 ± 4*</td>
</tr>
<tr>
<td>CVP</td>
<td>4.4 ± 0.1</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>LF</td>
<td>1.09 ± 0.02</td>
<td>0.74 ± 0.003*</td>
</tr>
</tbody>
</table>

Values are means ± SE. IHR, heart rate (beats/min); MAP, mean aortic pressure (mmHg); CVP, central venous pressure (mmHg); LF, lymph flow (ml). * P < 0.05 relative to preceding interval. † P < 0.05 relative to first postocclusion interval.

Probably improved tissue perfusion through collateral channels and restored the via a tergo to some degree. Second, quite likely as a result of hypoxia, capillary permeability increased, as evidenced by the blood staining and the increase in the protein content of the lymph. Third, capillary permeability must have also increased on a hemodynamic basis as a result of the increase in central venous pressure. Such an increase in venous pressure, by increasing the average capillary hydrostatic pressure, would augment fluid filtration into the interstitial tissue increasing the average capillary hydrostatic pressure, venous pressure. Such an increase in venous pressure, by increasing the average capillary hydrostatic pressure, would augment fluid filtration into the interstitial tissue increasing the average capillary hydrostatic pressure, venous pressure. Such an increase in venous pressure, by increasing the average capillary hydrostatic pressure, would augment fluid filtration into the interstitial tissue.

Fourth, actual forward propulsion of the lymph through the lymphatic circulation was undoubtedly augmented by the rise in interstitial fluid pressure (8). Indeed, interstitial edema with distention of lymph channels was evident in the histologic sections of the ischemic posterior papillary muscle. This interstitial edema developed despite the increase in cardiac lymph flow. This increase in flow, however, was slight when compared to the 20-fold increase that occurs in some tissues as a result of rises in venous pressure (6). Under the conditions of our experiments, the increase of approximately 53% in lymph flow during ischemia did not provide enough of a safety factor to prevent formation of edema (7). The importance of such a factor has been demonstrated by Kline et al. (13) and Pick et al. (23), whose studies showed an aggravation of myocardial ischemic damage in dogs subjected to lymphatic obstruction.

The changes we noted in the composition of cardiac lymph are in general agreement with the results of others. Thus, the elevations in RBC concentration and protein content indicate increased capillary permeability with edema formation (2, 14, 17); the drop in pH concurrent with the rise in lactate concentration, the increase in potassium levels coupled with the decrease in sodium, and the increases in CPK and acid phosphatase enzyme activities relate to anaerobic metabolism (26) and increased lysosomal and cell membrane permeability (3, 4, 24).

The lack of significant changes in systemic venous blood was an expected finding, but the lack of detectable changes in the coronary sinus blood was somewhat surprising. However, the inadequacy of coronary sinus sampling in evaluation of ischemic myocardial alterations in the dog has been demonstrated previously. Thomas et al. (32) evaluated the early changes in local venous blood as compared to coronary sinus blood after ligation of the left anterior descending coronary artery, which produced a left ventricular lesion of 21%. Whereas local venous potassium rose 150%, coronary sinus serum potassium failed to change significantly. Similarly, Szabo et al. (30) failed to find any elevation of myocardial enzymes for periods of 2 h after ligation of the left anterior descending artery. It seems likely that this inability to detect changes in coronary sinus blood after insults to the myocardium is related primarily to the dilution of a relatively small amount of circumflex artery drainage by a much larger amount of anterior descending artery drainage. In contrast, the bulk of the increase in cardiac lymph drainage probably originates from the ischemic area and, therefore, a greater percent of the collected lymph will reflect the changes induced by ischemia, i.e., rather than seeing a dilutional effect, we see a magnifying effect. Furthermore, diffusion through the interstitial space may extend beyond the ischemic region and, consequently, the ions and enzymes have a chance to be transported by lymphatic channels originating in relatively normal areas, a mechanism which is less likely to influence the composition of the venous drainage.

Other mechanisms may also account for the lack of detectable changes in the composition of coronary sinus blood. Anatomically, a rich plexus of venous veins anastomoses is usually present in the canine heart bridging the posterior and middle cardiac veins to the anterior descending veins, which empty separately into the right atrium thereby bypassing the coronary sinus. For some ischemic metabolites, local venous blood determinations produce curves that are characterized by early elevations (first 15-30 min) with subsequent decreases (21). Thus, it is possible that although some changes might have occurred in the coronary sinus blood during the early period of ischemia, these no longer apparent at the time of sampling. The lymph determinations, on the other hand, were carried out over the 2-h collection period so that even transient changes could be detected.

It has been observed (30) that the concentrations of the enzyme LDH, SGOT, and CPK rise in the cardiac lymph within 1–2 h of ischemia, whereas corresponding rises of serum activity can be detected only after 2–6 h. The increases in enzyme activity after coronary occlusion are always much higher in cardiac lymph than in serum (30). Thus, it is possible that the transport of myocardial enzymes into the circulation may take a preferential route via the lymphatics.

In summary, the results of this study show that analysis of alterations in cardiac lymph flow composition gives a more reliable picture than analysis of systemic or coronary sinus blood. Furthermore, such analysis allows deductions to be made with respect both to capillary and myocardial cell abnormalities.

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