Non-Hydrostatic Pulmonary Edema after Coronary Artery Ligation in Dogs
Protective Effect of Indomethacin

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With the technical assistance of C. Mark Hanna, Douglas Hollinger, and Peter Serrino

SUMMARY Pulmonary edema which develops during acute myocardial infarction is generally believed to result solely from pulmonary microvascular hypertension. However, patients with myocardial infarction and pulmonary edema occasionally are found to have normal pulmonary wedge pressure. We report data indicating that pulmonary edema develops after coronary artery ligation despite stable microvascular pressure. Four groups of open-chest dogs were studied: (1) nine dogs with left anterior descending coronary artery ligation, (2) seven dogs with sham coronary ligation, (3) seven dogs ligated after beginning an infusion of indomethacin (5 mg/kg per hr), and (4) five dogs ligated after an infusion of the drug’s vehicle was begun. Extravascular lung water and pulmonary blood volume were measured at hourly intervals during the 2 hours before and after coronary ligation or sham ligation. Gravimetric lung water was measured immediately thereafter. Changes of net pulmonary intravascular driving force (the difference of microvascular hydrostatic and oncotic pressure) after ligation or sham ligation were small and comparable in all groups. Pulmonary blood volume did not change in any group. Pulmonary extravascular water volume remained constant in the sham group but rose significantly in the ligated group. Gravimetric lung water also was significantly higher in the latter group. We interpret these results to indicate that factors other than microvascular pressure can mediate the formation of edema during acute myocardial infarction; increased pulmonary microvascular permeability may be responsible. Indomethacin infusion blocked the formation of edema after coronary ligation, even though net microvascular driving force was highest in this group. Infusion of the vehicle alone did not prevent edema. The mechanism by which indomethacin exerts this protective effect is unclear but is probably a result of its inhibition of cyclo-oxygenase or cyclic nucleotide phosphodiesterase. Circ Res 50: 301-309, 1982

PULMONARY edema is commonly categorized as "cardiogenic" or "non-cardiogenic," with the implication that the former type is mediated by pulmonary microvascular hypertension, whereas altered endothelial permeability accounts for the development of the latter form (Robin et al., 1972; Staub, 1974a, 1978). Pulmonary edema which develops in the setting of an acute myocardial infarction (MI) is generally believed to result solely from high pulmonary microvascular pressure, since depressed myocardial contractility, decreased left ventricular compliance, and peripheral venoconstriction commonly raise the pressure of the lesser circulation during acute MI. However, radiographic and clinical signs of pulmonary edema during MI are noted occasionally despite the presence of normal pulmonary wedge pressure (Nixon and Durth, 1968; Lassers et al., 1970; Kostuk et al., 1978; Timmis et al., 1981), and increased lung water (PEV) has been measured in about 15% of patients with MI and normal pulmonary wedge pressure (Biddle et al., 1974).

Recently, several investigators have presented evidence that microvascular hypertension is not the sole explanation for pulmonary edema after coronary ligation in dogs (Gee et al., 1978; Spath and Gee, 1979), cats (Massion et al., 1976, 1978), and sheep (Collins et al., 1979). These authors suggest that post-MI pulmonary edema in part results from increased pulmonary microvascular permeability.

The present study was undertaken to test the hypothesis that non-hydrostatic factors lead to the development of pulmonary edema after coronary ligation in dogs.

Methods

We studied four groups of mongrel dogs weighing 21.5-36.5 kg. Nine dogs (ligated group) underwent coronary ligation without prior drug treatment. Seven dogs (sham group) were handled identically, except that the coronary ligature was left untied. A third group of seven dogs (indomethacin-ligated group) received an intravenous infusion of indomethacin (kindly provided by Merck, Sharp & Dohme) (5 mg/kg per hr dissolved in warm 0.1 M
phosphate buffer. pH = 8.3, infused at 60–70 ml/hr) beginning 1 hour before control measurements were taken; coronary ligation was performed 3 hours after drug infusion was begun. A fourth group of five dogs (vehicle-ligated) received the phosphate buffer vehicle without indomethacin, infused at comparable rates.

Anesthesia was induced by intravenous thiamylal sodium (20 mg/kg) and maintained by flothane inhalation (1.5–2.5% in oxygen) through an endotracheal tube. A tidal volume of 10–15 ml/kg was delivered 10–12 times/min. The expiratory line was placed under 3–5 cm H2O. After catheters were in place, arterial PCO2 ranged from 30 to 40 mm Hg, and arterial Po2 ranged from 425 to 580 mm Hg. The heart was exposed through a thoracotomy in the left fourth interspace, and 8F polyethylene catheters with end and side holes and pretreated with 2% TDMAC-heparin (Polysciences, Inc.) were inserted into the main pulmonary artery and left atrium. Another cathether (12F) was advanced to the abdominal aorta via the femoral artery. In 12 animals (five ligated, seven sham) right ventricular end-diastolic pressure was monitored through a 5F Swan-Ganz catheter. Lead II of the electrocardiogram, left atrial mean, pulmonary arterial, and systemic arterial pressures were monitored continuously (using Statham P23AA pressure transducers and Clevite Brush Mark 260 recorder). Pulmonary microvascular pressure (Pmv) was calculated according to the equation of Gabel and Drake (1978):

\[ P_{mv} = LA + 0.5 (PA - LA), \]  

where LA and PA are end-expiratory left atrial and pulmonary arterial mean pressures, respectively. After catheters were in place, atelectatic areas of the cardiac lobe of the left lung were re-expanded, and an hour was allowed for equilibration before the study was begun.

The following were measured four times (referred to as periods I–IV) in each animal: hematocrit, total plasma protein, cardiac output (CO), pulmonary intravascular blood volume (PBV), and pulmonary extravascular water volume or lung water (PEV). Two sets of measurements were taken 1 hour apart prior to the ligation or sham ligation of the coronary artery (periods I and II). These values were averaged and served as control measurements. A third set (III) was taken 45–75 minutes after coronary ligation (or sham ligation), and a fourth set (IV) was taken 1 hour thereafter. The timing of the third and fourth sets of measurements varied somewhat, as several dogs had ventricular irritability at the planned time of study. No measurements were taken within 15 minutes of these rhythm disturbances. Blood loss resulting from the surgery or sampling was restored by arterial infusion of autologous blood harvested 1 or 2 days before the experiment. Catheters were flushed with heparin-containing saline, but no animal received more than 1000 units of heparin during the study.

After the final set of measurements was taken, the sternum was split and a filtered 4% solution of thioulnavin S (1 mg/kg) was infused through the left atrial catheter. The pulmonary hila were encircled with umbilical tape and simultaneously ligated at end-inspiration. The lungs were excised and placed in a weighed pan. An equal weight of distilled water was added, and the lungs were homogenized in a Waring Blender. Specific gravities and hemoglobin contents (cyanmethemoglobin method) of the homogenate supernatant and mixed venous blood was determined. The lung homogenate and blood were dried to constant weight, and the extravascular water was determined using the equations of Pearce et al. (1965). The heart was removed, sliced transversely, and photographed under ultraviolet light to outline the non-perfused zones as previously described (Richeson et al., 1978).

**Coronary Artery Ligation**

The left anterior descending coronary artery was dissected free immediately distal to the first diagonal branch, and a 2-0 silk ligature was passed behind it, care being exercised to exclude the accompanying vein(s). In the ligated, indomethacin-ligated, and vehicle-ligated groups, the suture was tied securely; in the sham group it was merely passed behind the vessel. The presence of ischemia was documented by the occurrence of cyanosis and hypokinesis of the involved ventricular wall, by recording S-T segment elevation on the epicardial electrogram (using a mobile wick electrode), and by inspecting the photographs of postmortem slices showing zones of non-perfusion, unstained by thioulnavin S.

**Cardiac Output**

Cardiac output was determined by injecting indocyanine green dye into the pulmonary artery, sampling dye density of aortic blood with a Gilford densitometer and amplifier (model 103IR) and a Hewlett-Packard 7100B strip chart recorder. The cardiac output was computed as an inverse function of the mean height and duration of the planimetered primary curve. Duplicate injections were performed within minutes of each other and differed by an average of 4.5%.

**Pulmonary Blood Volume**

Pulmonary blood volume was calculated as the product of cardiac output and the mean transit time difference between pulmonary arterial and left atrial injections of indocyanine green dye, both being sampled at the aorta (Yu, 1969).

**Pulmonary Extravascular Water Volume**

PEV was determined by injecting a mixture of 131I-albumin (5–9 μCi) and 3H2O (40–80 μCi) into the pulmonary artery and sampling the free-flowing stream from the femoral artery into a rack of heparinized centrifuge tubes. The exact timing of injection and of sampling was ascertained by slowly
replacing a videotape of the procedure on a split-screen monitor, one camera having been focused on the sample rack and the second on a digital timer accurate to 0.1 second. This system allowed sufficient blood flow to sample every 0.7-1.1 seconds without hemolysis. Plasma aliquots (0.3 ml) were added to 10 ml of Beckman scintillation solution, and the activities of $^3$H and $^{125}$I were determined in a Beckman LS-250 liquid scintillation counter having automatic quench compensation. PEV was calculated using the equation:

$$PEV = CO \left[ fp (1 - Hct)(MTT_w - MTT_{alb}) + frbc Hct (MTT_w - MTT_{rbc}) \right],$$

(2)

where $fp$ and $frbc$ are the fractional water contents of plasma (0.94) and red cells (0.70), respectively (Chinard, 1975; Goresky et al., 1975). $MTT_w$, $MTT_{alb}$, and $MTT_{rbc}$ represent the mean transit times of water, albumin, and red blood cells, respectively. The mean transit times of water and albumin were calculated with a hand-held calculator (Texas Instruments 59), programmed to extrapolate the exponential portion of the downslope and to correct for variation in the individual sampling intervals by weighting each sample activity appropriately. The relationship between $MTT_{alb}$ and $MTT_{rbc}$ was determined in a separate group of dogs into which mixtures of $^{51}$Cr-labeled red cells and $^{125}$I-albumin had been injected into the pulmonary artery and sampled at the aorta. The mean transit times were calculated as described above. Thirty-six such measurements were performed in 10 animals. The equation of the regression line ($r = 0.997$):

$$MTT_{alb} = 1.048 MTT_{rbc} + 0.089$$

(3)

was applied to the numerical solution of Equation 2. Coronary ligation was performed on many of these animals and did not alter the relationship. Although the separation of plasma and red cell transit times is sensitive to changes of cardiac output:

$$CO \text{ (ml/kg per min)} =$$

$$-87.8 (MTT_{alb} - MTT_{rbc}) + 139 \ (r = -0.69),$$

(4)

accounting for such changes has little impact upon calculation of PEV. In the group with the greatest fluctuation of cardiac output, adjusting the separation of plasma and red cell transit times for changes in cardiac output changed the calculated PEV by only $2.2 \pm 0.4\%$.

### Protein

Total plasma protein was measured using the biuret reaction (Abbott Dichromatic Analyzer). Paired samples had a precision of $\pm 0.25\%$ (SE). Oncotic pressure was calculated using the equation of Navar and Navar (1977).

### Statistical Analysis

Because of the multiple measurements on each animal, a repeated measures model (Winer, 1971) was used to investigate the stability of the response variables over the four time periods. This model uses each dog as its own control. The responses are measured as deviations from the average response for all time periods so that the average differences among the animals are eliminated from the experimental error. Statistical comparisons were made between the average of the control measurements (periods I and II) and the post-ligation (or post-sham ligation) measurements (periods III and IV). The comparisons were made for each group of animals. Relationships among the groups of animals were investigated by using a multivariate analysis of covariance model [SAS, 1979 (Cary, North Carolina)]. The response variable was the average of the measurements at periods III and IV. The average of the control measurements was the covariate. Pairwise comparisons were made among the four groups. Gravimetric extravascular lung water among the four groups was compared using the unpaired t-test. $P$ values $<0.05$ were considered significant.

### Results

#### Sham Group

The purpose of studying this group was to assess the stability and reproducibility of the various mea-

### Table 1 Hemodynamic and Pulmonary Volume Values for the Sham-Ligated Group of Dogs ($n = 7$, except as noted)

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Periods I-II</th>
<th>Period III</th>
<th>Period IV</th>
<th>SEM</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>104.6</td>
<td>105.6</td>
<td>103.3</td>
<td>3.85</td>
<td>0.91*</td>
</tr>
<tr>
<td>Cardiac output (ml/kg per min)</td>
<td>87.1</td>
<td>80.1</td>
<td>77.1</td>
<td>2.62</td>
<td>0.05*</td>
</tr>
<tr>
<td>Systemic artery mean pressure (mm Hg)</td>
<td>92.1</td>
<td>83.9</td>
<td>87.1</td>
<td>2.30</td>
<td>0.07</td>
</tr>
<tr>
<td>Pulmonary artery mean pressure (mm Hg)</td>
<td>9.2</td>
<td>8.6</td>
<td>8.8</td>
<td>0.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Left atrial mean pressure (mm Hg)</td>
<td>3.6</td>
<td>3.7</td>
<td>3.6</td>
<td>0.4</td>
<td>0.97</td>
</tr>
<tr>
<td>Pulmonary microvascular pressure (mm Hg)</td>
<td>6.4</td>
<td>6.1</td>
<td>6.2</td>
<td>0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Pulmonary microvascular (P - $\pi$) (mm Hg)</td>
<td>$-7.2$</td>
<td>$-6.1$</td>
<td>$-5.3$</td>
<td>0.6</td>
<td>0.09</td>
</tr>
<tr>
<td>Right ventricular end-diastolic pressure (mm Hg)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.4</td>
<td>0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Pulmonary blood volume (ml/kg)</td>
<td>7.94</td>
<td>7.48</td>
<td>8.19</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>Pulmonary extravascular water volume (ml/kg)</td>
<td>3.05</td>
<td>3.09</td>
<td>3.09</td>
<td>0.12</td>
<td>0.96</td>
</tr>
<tr>
<td>PEV/PBV</td>
<td>0.39</td>
<td>0.41</td>
<td>0.38</td>
<td>0.02</td>
<td>0.52</td>
</tr>
<tr>
<td>Gravimetric extravascular water volume (ml/kg) (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td>3.72</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Periods I and II were prior to sham ligation, whereas periods III and IV were at 1 and 2 hours after sham ligation, respectively. $P$ values less than 0.05 (*) indicate that the three mean values are statistically different. The standard error of the mean (SEM) was obtained from the repeated measures model.
measurements over an extended period of anesthesia in the open-chest dog. Dissection of the coronary artery and placement of the loose suture led to no discernible color or wall motion change of the left ventricle and no epicardial S-T displacement. No perfusion defects were detected in the thioflavin S-stained slices.

Table 1 presents the hemodynamic and pulmonary volume data collected prior to sham ligation (average of periods I and II), and 1 and 2 hours afterward (periods III and IV). Mean systemic arterial pressure and cardiac output tended to fall from control levels, although only the change in cardiac output 2 hours after sham ligation was significant. Calculated $P_{mv}$ was constant, although the net intravascular outward driving force (the difference between intravascular hydrostatic and oncotic force, $P - \pi$) rose slightly, owing to a slight but progressive fall of plasma protein. Heart rate, right ventricular end-diastolic pressure, mean pulmonary artery and left atrial pressures, PBV, and PEV remained constant (see Fig. 1).

**Ligated Group**

Within several minutes of coronary ligation, epicardial S-T segment elevation was demonstrable over a variable portion of the anterior wall of the left ventricle: usually this region was noted to be cyanotic and hypokinetic. A zone of thioflavin S non-perfusion was observed in heart slices of all animals in this group. The extent of the perfusion abnormality was quite variable but always involved the anterior free wall of the left ventricle and anterior portion of the left ventricular apex. The extent of subendocardial involvement was always wider than that of the subepicardial region, and the latter often showed islands and peninsulas of tissue with normal or nearly normal fluorescence. The interventricular septum was spared, except in three dogs in which perfusion defects were seen in the anterior and left side of the septum. A small portion of the anterior free wall of the right ventricle was involved in one dog; however, right ventricular end-diastolic pressure remained constant in this dog.

Ventricular arrhythmias developed in about one-half of the ischemic animals, usually appearing 25-30 minutes after ligation. Arrhythmias were not treated, and all abated spontaneously 15-45 minutes after their appearance. Although the severity and duration of arrhythmia were not quantified, there was no obvious relationship noted between their occurrence and subsequent hemodynamic or pulmonary vascular and extravascular volume changes.

Hemodynamic and pulmonary volume values for the ligated group are presented in Table 2. The ligated dogs were comparable to sham-ligated dogs in all respects at the outset. Cardiac output fell significantly after coronary ligation. Mean systemic artery pressure was variable after coronary ligation but fell significantly in the group of dogs. Left atrial mean pressure rose minimally but consistently. $P_{mv}$ did not change significantly throughout the experimental period. The greatest change in left atrial mean pressure at the study times was a rise of 3.5 mm Hg. During episodes of ventricular arrhythmia, prominent "v" waves were occasionally present in the left atrial pressure tracing and the left atrial mean pressure was slightly higher than that recorded in Table, but even at these times the mean pressure was usually only 1-2 mm Hg higher than the recorded pressures. The greatest rise of left atrial mean pressure during arrhythmia was 4 mm Hg. Pulmonary artery mean pressure was unchanged after ligation. Oncotic pressure fell progressively in the ligated group; hence the net outward intravascular driving force ($P - \pi_{mv}$) rose significantly (i.e., the difference between $P_{mv}$ and $\pi_{mv}$ diminished). However, the degree of change, was small (1.9 mm Hg) and was nearly identical to that of the sham group. Right ventricular end-diastolic pressure (measured in five dogs) did not change after ligation, and was similar to that of the sham group.

PBV in the ligated group did not change significantly. However, PEV rose significantly, being 20% and 27% higher than control at periods III and IV, respectively. The ratio of PEV/PBV also rose significantly after ligation. The change of PEV in this group was not highly correlated with changes in systemic artery pressure ($r = 0.05$), cardiac output ($r = 0.14$), $P_{mv}$ ($r = 0.29$), oncotic pressure ($r = 0.41$), or ($P - \pi_{mv}$) ($r = 0.31$).

**Indomethacin-Ligated Group**

Indomethacin infusion brought about prompt hemodynamic changes which were sustained throughout the experiment: mean systemic artery pressure

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**Figure 1** Pulmonary extravascular water volume (PEV) is plotted against time in the four groups of dogs. The sham group is represented by open circles (O), the ligated group by closed circles (9), the indomethacin-ligated group by triangles (△), and the vehicle-ligated group by squares (■). Asterisks indicate that the value is significantly different from control values.
rose 9.6 ± 6.2 mm Hg, pulmonary artery mean pressure rose 1.8 ± 0.4 mm Hg, left atrial mean pressure rose 1.3 ± 0.3 mm Hg, and cardiac output fell 8.2 ± 7.4 ml/kg per min. Consequently, at the time periods before coronary ligation, the indomethacin-ligated group differed from the other groups in that mean systemic arterial, left atrial and pulmonary arterial pressures, and $P_{mv}$ were significantly higher and $(P - \pi)_{mv}$ was significantly less negative. Cardiac output was lower in the indomethacin-ligated group, although the difference was not not statistically significant.

Hemodynamic and pulmonary volume data for this group are summarized in Table 3. Mean systemic artery pressure and cardiac output fell significantly after coronary ligation. Pulmonary artery and left atrial mean pressure, and $P_{mv}$ rose minimally, and the net outward driving force, $(P - \pi)_{mv}$, became slightly less negative. Despite the fact that net driving force was highest in this group, PEV, PBV, and PEV/PBV remained unchanged throughout the experiment.

Fluorescence photographs of the postmortem heart slices showed substantial zones of thioflavin S hypoperfusion, similar in distribution to those of the ligated group. Although no quantitative evaluation was performed to assess what fraction of the zone at risk was made ischemic, there was no noticeable difference between the extent of ischemia in this group and that of the ligated group.

### Vehicle-Ligated Group

During the pre-ligation periods, the vehicle-ligated group of dogs was similar to the sham and ligated groups, except that mean systemic artery pressure was significantly lower in this group. With continued infusion of the alkaline vehicle, systemic pressure rose slightly and was comparable to that of the sham and ligated groups.

As in the other groups, cardiac output fell significantly after coronary ligation, left atrial mean pressure and $P_{mv}$ rose marginally, and net outward driving force became slightly less negative. PEV and PEV/PBV rose significantly after ligation (see Table 4). The degree of change of PEV and of PEV/PBV was similar to that of the ligated group.

Postmortem slices showed ischemic zones similar in extent and distribution to those of the ligated and indomethacin-ligated groups.

### Gravimetric Analysis

Gravimetric lung water was measured in all but four ligated and two sham dogs. Group values are listed in Tables 1-4. Pulmonary extravascular volumes of the sham and indomethacin-ligated groups were similar. In both the ligated and vehicle-ligated groups, gravimetric extravascular water was significantly higher ($P < 0.025$) than in the other two groups. The fraction of gravimetric lung water measured by the indicator-dilution technique (at period

### Table 2 Hemodynamic and Pulmonary Volume Values for the Ligated Group of Dogs (n = 9, except as noted)

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Periods I-II</th>
<th>Period III</th>
<th>Period IV</th>
<th>SEM</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>109.2</td>
<td>110.7</td>
<td>105.0</td>
<td>2.57</td>
<td>0.30</td>
</tr>
<tr>
<td>Cardiac output (ml/kg per min)</td>
<td>94.5</td>
<td>74.1</td>
<td>71.9</td>
<td>4.27</td>
<td>0.003*</td>
</tr>
<tr>
<td>Systemic artery mean pressure (mm Hg)</td>
<td>90.1</td>
<td>80.7</td>
<td>82.2</td>
<td>2.48</td>
<td>0.04*</td>
</tr>
<tr>
<td>Pulmonary artery mean pressure (mm Hg)</td>
<td>10.3</td>
<td>10.0</td>
<td>9.9</td>
<td>0.4</td>
<td>0.75</td>
</tr>
<tr>
<td>Pulmonary microvascular pressure (mm Hg)</td>
<td>3.6</td>
<td>4.6</td>
<td>4.3</td>
<td>0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Pulmonary microvascular (P - \pi) (mm Hg)</td>
<td>-6.1</td>
<td>-4.5</td>
<td>-4.2</td>
<td>0.3</td>
<td>0.002*</td>
</tr>
<tr>
<td>Right ventricular end-diastolic pressure (mm Hg) (n = 5)</td>
<td>1.6</td>
<td>0.9</td>
<td>1.1</td>
<td>0.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Pulmonary blood volume (ml/kg)</td>
<td>7.25</td>
<td>6.70</td>
<td>7.15</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Pulmonary extravascular water volume (ml/kg)</td>
<td>3.05</td>
<td>3.65</td>
<td>3.87</td>
<td>0.12</td>
<td>0.001*</td>
</tr>
<tr>
<td>PEV/PBV</td>
<td>0.43</td>
<td>0.56</td>
<td>0.55</td>
<td>0.02</td>
<td>0.001*</td>
</tr>
<tr>
<td>Gravimetric extravascular water volume (ml/kg) (n = 5)</td>
<td>5.04</td>
<td></td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See footnote, Table 1, for definition of symbols.

### Table 3 Hemodynamic and Pulmonary Volume Values for the Indomethacin-Ligated Group of Dogs (n = 7)

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Periods I-II</th>
<th>Period III</th>
<th>Period IV</th>
<th>SEM</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>104.3</td>
<td>114.3</td>
<td>109.9</td>
<td>2.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Cardiac output (ml/kg per min)</td>
<td>81.7</td>
<td>65.4</td>
<td>56.9</td>
<td>4.3</td>
<td>0.005*</td>
</tr>
<tr>
<td>Systemic artery mean pressure (mm Hg)</td>
<td>108.4</td>
<td>105.9</td>
<td>98.7</td>
<td>2.2</td>
<td>0.02*</td>
</tr>
<tr>
<td>Pulmonary artery mean pressure (mm Hg)</td>
<td>11.6</td>
<td>12.0</td>
<td>12.2</td>
<td>0.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Left atrial mean pressure (mm Hg)</td>
<td>6.8</td>
<td>7.9</td>
<td>7.4</td>
<td>0.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Pulmonary microvascular pressure (mm Hg)</td>
<td>9.2</td>
<td>9.9</td>
<td>9.8</td>
<td>0.4</td>
<td>0.45</td>
</tr>
<tr>
<td>Pulmonary microvascular (P - \pi) (mm Hg)</td>
<td>-3.1</td>
<td>-2.2</td>
<td>-1.9</td>
<td>0.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Pulmonary blood volume (ml/kg)</td>
<td>7.07</td>
<td>7.37</td>
<td>7.09</td>
<td>0.28</td>
<td>0.70</td>
</tr>
<tr>
<td>Pulmonary extravascular water volume (ml/kg)</td>
<td>2.99</td>
<td>3.12</td>
<td>2.91</td>
<td>0.14</td>
<td>0.55</td>
</tr>
<tr>
<td>PEV/PBV</td>
<td>0.44</td>
<td>0.44</td>
<td>0.41</td>
<td>0.02</td>
<td>0.65</td>
</tr>
<tr>
<td>Gravimetric extravascular water volume (ml/kg)</td>
<td></td>
<td></td>
<td>3.39</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

See footnote, Table 1, for definition of symbols.
IV) varied considerably, but this fraction (0.81 ± 0.04) was not significantly different among the four groups.

Comparison of Group Responses to Coronary Ligation or Sham Ligation

A multivariate analysis of covariance was performed to compare the responses of the four groups of dogs; the average of the control measurements (periods I-II) was used as the covariate. The group responses for most variables were comparable, although the fall of cardiac output of the indomethacin-ligated group was greater than that of the vehicle-ligated group (P < 0.05), and the change of PEV/PBV in the vehicle-ligated group was greater than that of the ligated group (P < 0.05). The most striking differences of group responses are the changes of PEV and PEV/PBV. The sham and indomethacin-ligated groups demonstrated similar responses of PEV and PEV/PBV, and the responses of the ligated and vehicle-ligated groups also were similar. However, the response of PEV in the ligated group differed significantly from the response of the sham group (P < 0.01) and the indomethacin-ligated group (P < 0.01), and the PEV response of the vehicle-ligated group differed from the response of the sham group (P < 0.01) and the indomethacin-ligated group (P < 0.01). The rise of PEV/PBV in the ligated group differed from the responses of the sham group (P < 0.001) and the indomethacin-ligated group (P < 0.001), and the PEV/PBV response of the vehicle-ligated dogs differed from those of the sham group (P < 0.01) and indomethacin-ligated dogs (P < 0.05).

Discussion

According to the Starling relationship, the rate and direction of fluid movement (Qf) across the microvascular endothelium is determined by the conductance of the endothelial barrier (Kf) and the driving forces on either side of the endothelium:

\[ Q_f = K_f [(P_{mv} - P_{pmv}) - \sigma(\pi_{mv} - \pi_{pmv})] \]

where \( P_{mv} \) and \( P_{pmv} \) denote microvascular and perimicrovascular hydrostatic pressures, respectively, and \( (\pi_{mv} - \pi_{pmv}) \) represents the oncotic gradient across the capillary endothelium, modified by the reflection coefficient \( \sigma \).

The pulmonary transudation that often occurs after acute myocardial infarction is generally believed to result solely from elevated pulmonary microvascular pressure. Although microvascular hypertension is a frequent cause of pulmonary edema in this setting, occasionally the two do not co-exist. Discrepancies between pulmonary wedge pressure on the one hand and lung water measurements (Biddle et al., 1974), clinical (Nixon and Durth, 1968; Timmis et al., 1981), and radiographic (Lassers et al., 1970; Kostuk et al., 1978) evidence of pulmonary edema on the other usually have been attributed to a phase lag between lowering of pressure and water clearance from the lung. Nonhydrostatic mediation has not generally been suspected.

Several recent clinical studies (Carlson et al., 1979; Fein et al., 1979) have attempted to differentiate "hydrostatic" and "permeability" pulmonary edema by measuring the ratio of protein concentration in edema fluid to that of plasma. A higher ratio would denote abnormal protein flux and thus altered endothelial integrity. The authors of these reports did not characterize the pulmonary edema fluid of myocardial infarction patients per se; however, in both studies the edema fluid: plasma protein ratios from patients with acute myocardial infarction were intermediate between the high ratios of patients with permeability edema and the low ratios of patients with left ventricular failure without acute myocardial infarction. In one of these studies (Carlson et al., 1979), the number of patients with acute infarction was sufficient that their protein ratios differed significantly from those of the group with heart failure without myocardial infarction (P < 0.01) and from those of the group with permeability edema (P < 0.05). These data suggest that, in patients with acute myocardial infarction, both microvascular hypertension and altered permeability may be causes of their pulmonary edema.

Massion et al. (1976, 1978) presented indirect evidence that microvascular permeability may be altered by proteases liberated from or activated by
the acutely ischemic myocardium, since the protease-inhibitor aprotinin prevented pulmonary edema in cats after coronary ligation. Gee and Spath (1978) measured greater protein flow through the right lymphatic duct in volume-loaded dogs with circumflex artery ligation than that in a sham group of dogs. Myocardial contribution to lymph protein could account for these findings in part, however. Dogs after coronary ligation have higher gravimetric lung water than control dogs with comparable P_mv (Spath and Gee, 1979). Lung lymph flow and pulmonary protein clearance in anesthetized sheep rises shortly after coronary ligation despite minimal change in P_mv (Collins et al., 1979).

One purpose of the present study was to test whether pulmonary edema would develop after coronary ligation in the presence of normal and unchanging pulmonary microvascular pressure. The model employed (open-chest, anesthetized, mechanically ventilated dog) is far from physiological; however, the stability of the lung water in the sham group indicates that these factors are not responsible for the changes observed in other groups.

The assumptions upon which the indicator-dilution technique is based, and the fact that it defines an operational, rather than an anatomic or physiologic space, require that studies using this method be interpreted with these reservations in mind. Chnard (1975) and Staub (1974b) have reviewed the limitations of this method. The reliance of the current study upon directional changes of sequential measurements in the same animal circumvents many of these potential objections. The principal limitation of the indicator-dilution technique is that it measures only a fraction of the gravimetrically determined lung water. The higher gravimetric lung water in the ligated and vehicle-ligated dogs argues against the notion that the post-ligation rise of PEV merely reflects a greater measured fraction of true lung water. It was not possible, using these techniques, to measure the filtering surface area of the pulmonary microvascular bed, but PBV remained stable in all groups. In those groups in which a rise of PEV was observed (ligated and vehicle-ligated), a weak correlation was present between PEV and PBV (r = 0.35, P < 0.05); however, changes of PEV and PBV were not highly correlated (r = 0.28). This and the minimal change of P_mv suggest that additional vascular recruitment cannot account for the change of PEV.

Our data show that shortly after coronary ligation in anesthetized dogs, pulmonary edema develops in the face of a stable P_mv. Although the change of outward driving force was significant only for the ligated group of dogs, the degree of this change was small (1.9 mm Hg), and pairwise comparisons (using multivariate analysis of covariance) of outward driving force showed that the response of this variable was similar in all four groups of dogs.

We have assumed that pulmonary vascular resistance was equally distributed proximal and distal to the microvascular bed, as found in normal dogs by Gabel and Drake (1978), and remained so throughout the experiment. If, in fact, such were not true, P_mv might rise, even with stable pulmonary artery and left atrial pressures. In the extreme case, if all of the resistance were to shift to sites distal to the filtering bed, P_mv would rise to equal mean pulmonary artery pressure (a rise of 2.8 mm Hg in the ligated group). Such a small change would seem incapable of inducing pulmonary edema; thus redistribution of vascular resistance provides an unsatisfactory explanation of our data.

The constancy of right ventricular end-diastolic pressure in the ligated group assured us that bronchial venous and pulmonary lymphatic hypertension were not responsible for the rise of PEV (Miller et al., 1978).

The pulmonary edema in the ligated and vehicle-ligated groups of dogs must have resulted from an alteration of either lymphatic drainage or one of the unmeasured elements of the Starling relationship: perimicrovascular hydrostatic or oncotic pressure, or microvascular permeability. The stability of lung water in the sham group indicates the effect of anesthesia upon lung lymphatic pulsatile flow (Staub, 1974b) does not account for the change observed in the ligated and vehicle-ligated groups. Perimicrovascular hydrostatic and oncotic pressures were not measured in the current study. It is possible that increased filtration may have resulted from changes of these variables, but it seems unlikely that myocardial ischemia would exert a primary change upon the pulmonary interstitium.

Although the above possibilities cannot be completely refuted by our data, we believe that alteration in microvascular permeability is the most tenable explanation for the observed rise of extravascular water after coronary ligation.

Although several laboratories have reported similar conclusions using different methods (Massion et al., 1976; Gee et al., 1978; Collins et al., 1979), the mediator(s) of this rise in permeability has not been identified. Berger and co-workers (1976) have shown that after coronary ligation in the anesthetized dog, there is release of prostaglandins E and F from the ischemic myocardium. Spath and colleagues (1980) suggest a role for prostacyclin in the mediation of pulmonary edema, as their volume-expanded dogs demonstrated a rise of prostacyclin levels in right lymphatic duct protein-rich lymph after coronary ligation. No such rise was seen in sham-ligated animals. It is possible that the prostacyclin was formed as a response to edema rather than being responsible for it. Indeed, Demling et al. (1981) have shown that prostacyclin infusion attenuates the increased permeability of the pulmonary vasculature during edema caused by endotoxin. Moreover, Brigham (1978) cites salicylate-induced pulmonary edema as evidence that prostaglandins...
have a protective effect upon the pulmonary vascular endothelium.

Our results show that the indomethacin infusion blocks the formation of post-myocardial infarction pulmonary edema in this model. Since infusion of the drug's vehicle alone at a comparable rate did not prevent edema formation, indomethacin itself appears to be responsible for the salutary effect. Since the fall of cardiac output in this group was greater than in the other groups, it is possible that a derecruitment of filtering surface area played a role in this protective effect. However, the intravascular net driving force was highest in the indomethacin-ligated group, and the extent of myocardial ischemia was comparable to that of other ligated animals. Thus it seems unlikely that the protective effect was hydrostatic or due to myocardial preservation.

The mechanism whereby indomethacin prevented edema is unclear. Cyclo-oxygenase inhibition is the most fully studied action of indomethacin (Flower, 1974); thus interference with prostaglandin, prostacyclin, or thromboxane synthesis is a leading consideration. Whereas the vasomotor effects of this group of agents are well-known, the ability of prostaglandins to alter microvascular permeability is not fully understood. Infusion of arachidonate (Ogletree and Brigham, 1980), or the cyclic endoperoxide PGH-2 or its 9-methylene ether analogue (PGH2-A) (Bowers et al., 1979) into sheep with lung lymph fistulas leads to enhanced flow of protein-poor lymph. Microvascular permeability is not altered. Prostacyclin is capable of increasing lung lymph flow and protein clearance into the lymph without altering pulmonary artery pressure (Ogletree and Brigham, 1978); however, this is now thought to reflect a change in filtering surface area rather than an increase in permeability (Ogletree, 1981).

In the peripheral circulation, prostaglandins may promote edema indirectly, by potentiating the alteration of permeability caused by other phlogistic agents such as histamine (Komoriya et al., 1978) or bradykinin (Peck and Williams, 1978). The potentiating effect of prostaglandins upon other mediators in the lung has not been studied.

Among the other actions of indomethacin, cyclic nucleotide phosphodiesterase inhibition could also explain the salutary effect of indomethacin in this study. The rise of cyclic AMP which would result from phosphodiesterase inhibition might stabilize lysosomal membranes and prevent release of other phlogistic mediators (Weiss and Hait, 1977).

In conclusion, this study has shown that extravascular lung water increases shortly after coronary ligation in the dog in the face of trivial changes in intravascular driving force. Of the non-hydrostatic mechanisms which could explain this, we believe that an alteration of microvascular permeability is the most likely. The degree of pulmonary edema in the ligated dogs was not great, probably reflecting only interstitial accumulation. However, if altered permeability is the cause, the significance of this finding becomes progressively greater at higher microvascular pressures, as is frequently the case after human myocardial infarction. Pre-ligation infusion of indomethacin prevented edema in this model, although the mechanism of this protective effect is not clear.

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References

Nixon PGF, Durrh MB (1968) Pulmonary edema with low left ventricular diastolic pressure in acute myocardial infarction. Lancet 2: 146-147
Ogletree ML (in press) Pharmacology of prostaglandins in the pulmonary microcirculation. Ann NY Acad Sci
Ogletree ML, Brigham KL (1978) Prostacyclin (PGL2) and PGE1 produce opposite effects on sheep lung vascular permeability (abstr) Fed Proc 38(II): 1266
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