Presence of Negative Inotropic Agents in Canine Plasma during Positive End-Expiratory Pressure

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SUMMARY Application of positive end-expiratory pressure (PEEP) will reduce cardiac output (CO). Humoral mediation of this event by circulating negative inotropic agents was examined using a rat papillary muscle bioassay. Twenty-seven dogs were anesthetized with an iv pentobarbital infusion. Plasma was obtained before and after 30 minutes of PEEP. The plasma was oxygenated in a small (4.5-ml) papillary muscle chamber using a diffusion membrane. An average PO2 of 416 mm Hg was achieved. PEEP plasma reduced developed tension (Tpd) from 2.18 ± 1.0 to 1.90 ± 1.05 g (P < 0.0001). A fall in Tpd was observed whether or not CO was maintained constant with fluid infusion. Resting tension was unchanged. The percent reduction in Tpd correlated with the fall in CO (r = 0.63, P < 0.01) when fluid was not infused to maintain CO. Reapplication of control plasma restored Tpd. Barbiturate levels in anesthetized dogs rose from 17.3 to 19.4 μg/ml during PEEP (P < 0.1). Addition of pentobarbital to normal plasma led to a slight decrease in Tpd only when the concentration exceeded 99 μg/ml. In three experiments on ex vivo perfused hearts, application of PEEP led to lowering of peak systolic pressure (PSP) within 5 minutes. Removal of PEEP restored PSP in a similar time. The results support the hypothesis that the decline in CO with PEEP is mediated in part by a circulating negative inotropic agent. Circ Res 45: 460-467, 1979

PATIENTS requiring ventilatory assistance for respiratory failure often require positive end-expiratory pressure (PEEP) to maintain an acceptable arterial oxygen tension. However, despite the improvement in oxygenation, the frequently associated decrease in cardiac output (CO) may reduce net oxygen transport (Suter et al., 1975). The mechanism of reduction in CO is debated. The explanation proposed by Cournand relates the fall in flow to a primary reduction in venous return (Cournand et al., 1948). This conclusion has gained wide acceptance and is supported by Braunwald et al. (1957) and others (Morgan et al., 1966; Kumar et al., 1970). More recently, right ventricular failure as a result of PEEP-induced pulmonary hypertension has been considered to be of importance (Elkins et al., 1974). Direct depression of left ventricular function because of impaired coronary flow secondary to high pleural pressures also has been proposed (Lozman et al., 1974).

Evidence from our laboratory supports the postulate that lung stretch during PEEP induces the elaboration of a humoral substance which depresses myocardial function (Liebman et al., 1978; Patten et al., 1978; Manny et al., 1978b). We have demonstrated depressed contractility in an isovolumetrically contracting, ex vivo perfused canine heart when PEEP was applied to the support dog (Manny et al., 1978a). The present study was designed to test further the hypothesis that PEEP induces the release of a circulating negative inotropic agent. An isolated, isotonically contracting rat papillary muscle was bathed in dog plasma obtained before and after application of PEEP. The papillary muscle preparation uses a membrane, rather than a sintered disc, for oxygenation of plasma. This avoids mechanical distortions due to foam formation.

Methods

Group I
Preparation of the Test Dog

Twenty adult mongrel dogs, weighing 20–28 kg, were anesthetized with sodium pentobarbital (25 mg/kg) and paralyzed with succinylcholine (1.5 mg/kg, iv). Anesthesia and paralysis were maintained with a 0.5 ml/min infusion of pentobarbital (750 mg) and succinylcholine (400 mg) in 150 ml of normal saline. The trachea was intubated and the dog ventilated with room air at a tidal volume of 15 ml/kg and a rate of 12 cycles/min. An external heat source was used to stabilize the body temperature at 38°C.

Arterial and 7 French thermister-tipped flow-directed pulmonary arterial catheters (Instrumentation Laboratories) were positioned via the femoral
vessels. Mean systemic and pulmonary arterial, mean pulmonary arterial wedge, and mean central venous pressures were measured with strain gauge transducers (Bentley, model 800). The pulse was monitored using the arterial wave form (Hewlett-Packard, model 78903C), and CO was determined in triplicate by the thermodilution technique (Instrumentation Laboratory, model 601). Arterial blood gases and pH were measured with standard Clark and Severinghaus electrodes (Instrumentation Laboratory, model 813), and hemoglobin and percent saturation were measured spectrophotometrically (Instrumentation Laboratory, model 282), using extinction coefficients specific for dog blood. Base excess was derived from the Siggaard-Anderson Nomogram (Siggaard-Anderson, 1963).

After 30 minutes of ventilation at 0 cm H2O end-expiratory pressure (0-EEP) or at 15 cm H2O PEEP, hemodynamics, blood gases, and pH were measured. Twenty milliliters of arterial blood were drawn into a heparinized plastic syringe. The blood was centrifuged at 2500 rpm and 25°C for 20 minutes (Sorvall, model GLC) and the plasma separated. The papillary muscle assay was started immediately.

**Papillary Muscle Assay**

The posterior left ventricular papillary muscle from Sprague Dawley rats, weighing an average of 225 g, was used. The heart was removed after decapitation and the papillary muscle rapidly excised and immersed in a solution that had been equilibrated with 95% O2 and 5% CO2. The composition of this solution (meq/liter) was: Na+, 146; K+, 3.6; Ca2+, 4.5; Mg2+, 2.5; PO4−, 1.4; SO4−, 2.5; Cl−, 130; and HC03−, 25. Glucose concentration was 10.2 mM/liter. The final pH was adjusted to 7.40 by adding small quantities of sodium bicarbonate.

The papillary muscle length averaged 8.31 ± 2.13 mm (mean ± SD) with a range of 5.42–9.72 mm. The calculated mean cross-sectional area was 1.37 ± 0.43 mm². The muscle was suspended vertically in a 4.5-ml Plexiglas chamber using stainless steel spring clamps. The tendonous end was attached to the rigid arm of an isometric force transducer (Harvard Apparatus, model 363). The mural end was attached to the movable arm of a dial micrometer (Micrometric Instrument Co.). The chamber was mounted on a Delrin block which housed a rotating magnet and variable speed motor (Instech Laboratories, models 450/055 and 1055). A 1.25-cm diameter well in the base of the chamber contained a magnetic stirring bar which was used to ensure adequate mixing of the chamber fluid. A port at the bottom of the well allowed rapid filling and emptying of the chamber (Fig. 1).

Continual oxygenation of the electrolyte solution or test plasma was accomplished by diffusion. A Silastic membrane, 0.178 mm thick (Dow Corning), was glued with Silicone adhesive sealant (Dow Corning) onto the grooved inner face of two opposing chamber walls. The total surface area available for diffusion was 500 mm². Inlet and outlet ports in both walls allowed continuous flow of 95% and 5% CO2.

Stainless steel field-stimulating electrodes of 17.3-mm² cross-sectional area were mounted parallel to the longitudinal axis of the muscle. A 4.5-msec rectangular pulse (Grass Instrument Co., model S44), set just at suprathreshold voltage, was delivered once every 5 seconds. The stimulus voltage was increased periodically 5-fold to ensure that developed tension was not limited by excitation characteristics. The amplified signal of the force transducer was continuously displayed on an oscilloscope (Hewlett-Packard, model 78304A). Recordings were made every 15 minutes using a light beam recorder with a frequency response flat to 500 Hz (Hewlett-Packard, model 4888A), and the following variables were measured: peak developed tension, Tpd; resting tension, Tr; time-to-peak tension, TPT; and relaxation time (i.e., time for tension to fall one-half of developed tension), RT(1/2). All experiments were conducted at 26.2 ± 0.5°C.

After a 60-minute period of equilibration, length-tension curves were constructed by progressively increasing length of the isometrically contracting muscle. The length was then set so that Tpd was at the apex of the curve. Only those preparations

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**Figure 1** A schematic diagram of the papillary muscle system is shown. The stippled rectangle is the force transducer. A rigid hook used to mount the tendonous end of the papillary muscle projects from the transducer. The lower movable arm used to fix the mural end of the muscle is connected to a dial micrometer. a = Silastic membrane, b = electrode, c = gas inflow port, d = gas outflow port, e = stirring bar, f = drain port, g = rotating magnet.
which remained stable during the equilibration period were used (20 of 23). Test plasma was preoxygenated in a second chamber. The electrolyte solution was drained rapidly and the test plasma added to the muscle chamber between contractions.

Plasma was tested in the following sequence: O-EEP, PEEP, O-EEP. The two O-EEP samples were drawn at the same time. Each plasma sample was allowed to bathe the muscle for 1 hour. Small aliquots were removed at 30-minute intervals to measure $P_{O_2}$, $P_{CO_2}$, and pH.

**Barbiturate** concentrations in O-EEP and PEEP plasma were determined in seven experiments by UV absorption (Goldbaum, 1948). Experiments then were conducted to assess the influence of barbiturates on contractility. Plasma was obtained from nonanesthetized dogs and was used to bathe a series of four papillary muscles. Pentobarbital sodium (Nembutal, Abbott Laboratories) was added to the test chamber in increments to achieve a concentration range of 9–200 µg/ml. The papillary muscle was equilibrated with each barbiturate concentration for 30 minutes. Records were obtained at 15-minute intervals. After exposure to the highest concentration, the chamber was emptied rapidly and refilled with oxygenated plasma without barbiturate.

**Group II**

**Preparation of the Test Dog and Papillary Muscle Assay**

Another group of experiments was conducted first, to test the effect of PEEP when CO was held constant and second, to test the effect of the removal of PEEP on the negative inotropic effect.

Seven adult mongrel dogs and the corresponding papillary muscles were prepared in a manner similar to that of group I. During ventilation at 15 cm H$_2$O PEEP, balanced salt solution was infused to maintain the CO at O-EEP$_1$ levels. After removal of PEEP, the dog again was ventilated at 0 cm H$_2$O end-expiratory pressure, (O-EEP$_2$). After 30 minutes of ventilation at O-EEP$_1$, PEEP, or O-EEP$_2$ hemodynamics, blood gases and pH were measured, and plasma was obtained from 20 ml of arterial blood. Plasma was tested in the following sequence: O-EEP$_1$, PEEP, O-EEP$_2$, O-EEP$_1$. The two O-EEP$_1$ samples were obtained at the same time. Each plasma sample was allowed to bathe the muscle for 1 hour. Small aliquots were removed at 30-minute intervals to measure $P_{O_2}$, $P_{CO_2}$, and pH.

Three improvements were made in the design of the papillary muscle bioassay apparatus used in group II experiments. First, the chamber was modified to provide a greater surface area per volume of plasma for gas exchange. Second, the stainless steel electrodes were redesigned. Each electrode was pressed onto the tinned terminal of an insulated nylon-tip jack, fitted with an outer threaded metal shell (H.H. Smith Co., model 1501). Two opposing chamber walls were drilled and tapped to accommodate the electrodes. This allowed precise positioning of the electrodes in the horizontal plane relative to the muscle. Finally, the isometric force transducer was mounted on a screw jack (Ealing, model 22-9435) which allowed longitudinal positioning of the muscle relative to the electrodes.

**Ex Vivo Perfused Hearts**

Three isolated hearts undergoing coronary perfusion from a support dog at a fixed rate of 1.4 ml/min·g heart were studied to examine the rate of appearance and disappearance of the negative inotropic effect. The details of this temperature-controlled, paced, isovolumetrically contracting preparation have been reported previously (Manny et al., 1978a). In the current experiments, the left ventricular balloon was filled with 30 ml saline, which corresponded to a diastolic pressure of 6.6 mm Hg (strain gauge transducer, Bentley, model 800). Perfusion pressure measured at the aortic arch of the isolated heart was 92 mm Hg. The changes in left ventricular peak systolic pressure in the isolated heart and mean arterial pressure in the support dog were recorded after 15 cm H$_2$O PEEP was applied or removed from the support dog.

The transit delay for the passage of substances from the lungs of the support dog to the aortic arch of the isolated heart was determined by injecting indocyanine green dye into the pulmonary artery of the support dog. At the same time, blood sampling was started from the aortic arch of the isolated heart. Blood was withdrawn at a constant rate of 20 ml/min through a cuvette densitometer (Gilson, model DTL). The mean transit time, derived from the concentration-time curve of the dye, varied from 28 to 39 seconds.

Data are presented as the mean ± se. Probability is based on Student's paired t-test.

**Results**

**Group I**

The application of 15 cm H$_2$O PEEP resulted in hemodynamic changes similar to those reported in the past (Table 1) (Liebman et al., 1978; Patten et al., 1978; Manny et al., 1978a, 1978b). CO and mean arterial pressure decreased ($P < 0.001$), whereas central venous and pulmonary arterial wedge pressure increased ($P < 0.01$, $P < 0.001$). Pulse rate fell from 174 ± 14 to 159 ± 15 ($P < 0.01$). Blood gases were unchanged, but pH decreased by 0.07 units ($P < 0.05$), and the base excess fell from $-5$ to $-9$ meq/liter ($P < 0.005$) (Table 1).

**Papillary Muscle**

After addition of PEEP plasma to the papillary muscle, $T_{pd}$ decreased in 18 of 20 experiments (Table 2). After 1 hour, $T_{pd}$ had declined from an average of 1.73 ± 0.75 to 1.47 ± 0.79 g ($P < 0.0001$).
TABLE 1  Hemodynamic, Blood Gas, and Acid-Base Response to 15 cm H2O PEEP

<table>
<thead>
<tr>
<th></th>
<th>0-EEP</th>
<th>PEEP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO (liter/min)</td>
<td>3.47 ± 0.72</td>
<td>1.87 ± 0.82</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>144 ± 17</td>
<td>124 ± 18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>5 ± 3</td>
<td>11 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>4 ± 2</td>
<td>11 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P (mm&quot;H)</td>
<td>174 ± 14</td>
<td>159 ± 15</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>85 ± 10</td>
<td>93 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>33.7 ± 4.1</td>
<td>35.8 ± 9.7</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.05</td>
<td>7.27 ± 0.07</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>BE (meq/liter)</td>
<td>-5 ± 3</td>
<td>-9 ± 3</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. MAP = mean arterial pressure; PAWP = pulmonary arterial wedge pressure; CVP = central venous pressure; P = pulse; BE = base excess; and NS = not significant.

in 19 of the 20 experiments in which CO also declined. In the one dog in which CO rose during PEEP, there was an increase in Tpd. This experiment was excluded from the statistical analysis of the change in Tpd, since the PEEP plasma would not have been expected to exert a negative inotropic effect. Reapplication of 0-EEP plasma led to a slow restoration in Tpd. Only after 1 hour did the Tpd exceed the level attained during exposure to PEEP plasma (P < 0.001).

Barbiturate

In six of seven experiments, PEEP plasma contained higher concentrations of pentobarbital than 0-EEP (Table 3). The average increase was 2.1 µg/ml (P < 0.1). The addition of pentobarbital to normal plasma yielded a slight decrease in Tpd at 99 µg/ml (Fig. 2). This concentration was over three times the highest concentration measured during PEEP. Even at 200 µg/ml, only 9% depression in Tpd was observed, compared with a 14% depression with PEEP plasma.

Group II

Maintenance of Constant Flow

Infusion of balanced salt solution during PEEP resulted in maintenance of CO at levels equivalent to 0-EEP (Table 4). Mean arterial pressure decreased slightly (P < 0.01), whereas central venous and pulmonary arterial wedge pressures increased (P < 0.001, P < 0.001). Pulse rate fell, but not significantly, from 145 ± 15 to 135 ± 9. Blood gases were unchanged, but pH decreased by 0.08 unit (P < 0.005), and the base excess fell from -1 to -4 meq/liter (P < 0.001). Thirty minutes after removal of PEEP (0-EEP2), hemodynamics returned to 0-EEP levels. Blood gases were unchanged, but pH rose by 0.03 unit and the base excess rose from -4 to -3.

Papillary Muscle

After addition of PEEP plasma to the papillary muscle, Tpd decreased in seven of seven experiments. After 1 hour, Tpd had declined from an...
Addition of pentobarbital to normal plasma led to a slight decrease in Tpd at concentrations above 98 μg/ml. After testing with the highest barbiturate concentrations, reapplication of normal plasma restored Tpd. The data represent the means of data from four papillary muscle experiments.

Following addition of PEEP plasma, RT\textsubscript{1/2} fell slightly from 0.18 ± 0.05 to 0.16 ± 0.03 seconds (\(P < 0.05\)). Neither O-EEP\textsubscript{2} plasma nor reintroduction of O-EEP\textsubscript{1} plasma could restore this value to baseline. PEEP plasma did not alter Tr or TPT.

Modification in electrode design, positioning, and additional experience with the preparation enabled us to achieve consistently higher values for Tpd in the experiments of group II than in group I. Modification in the chamber dimensions provides a greater surface area per volume of plasma for gas exchange. This results in a higher average \(P_{\text{O}_2}\) of 519 ± 41 mm Hg compared with group I. \(P_{\text{CO}_2}\) and pH were comparable to group I (35.4 ± 4.3, 7.39 ± 0.08).

**Ex Vivo Heart**

Within 15–20 seconds after application of PEEP to the support dog, there was a 10– to 20-mm Hg fall in mean arterial blood pressure. Soon after, a transient increase in peak systolic pressure (PSP) in the isolated heart was observed. This was followed by a decline in PSP, which reached a minimum in 3–5 minutes (Fig. 3). After removal of PEEP, recovery of PSP took 5–10 minutes.

**Discussion**

The reduction in CO that attends the use of PEEP usually is attributed to mechanical events. The most prominent of these events is an increase in pleural pressure, which is thought to impede venous return (Suter et al., 1975). Such mechanisms are likely to be operative under many circumstances but need not always be invoked to explain flow reductions with PEEP. Thus, the application of 15 cm H\textsubscript{2}O PEEP to a series of animals whose chest walls had been excised and in which pleural pressure remained constant led to dramatic falls in CO (Liebman et al., 1978). Further, the flow reduction was greater than in closed-chested animals treated with PEEP (Manny et al., 1978b). This was not due to right ventricular failure, since elevation of the pulmonary artery pressure during O-EEP in these open-chested dogs to levels equivalent to PEEP did not affect CO.

### Table 4  Hemodynamic, Blood Gas, and Acid-Base Response to 15 cm H\textsubscript{2}O PEEP

<table>
<thead>
<tr>
<th></th>
<th>0-EEP\textsubscript{1}</th>
<th>P</th>
<th>PEEP</th>
<th>P</th>
<th>0-EEP\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO (liter/min)</td>
<td>3.65 ± 0.77</td>
<td>NS</td>
<td>3.74 ± 1.01</td>
<td>NS</td>
<td>3.99 ± 0.76</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>144 ± 7</td>
<td>&lt; 0.01</td>
<td>137 ± 7</td>
<td>NS</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>7 ± 2.4</td>
<td>&lt; 0.001</td>
<td>14 ± 2.7</td>
<td>&lt; 0.01</td>
<td>9 ± 2.7</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>5 ± 3.5</td>
<td>&lt; 0.001</td>
<td>13 ± 4.8</td>
<td>&lt; 0.005</td>
<td>8 ± 3.8</td>
</tr>
<tr>
<td>P (min\textsuperscript{-1})</td>
<td>145 ± 15</td>
<td>NS</td>
<td>135 ± 9</td>
<td>NS</td>
<td>135 ± 7</td>
</tr>
<tr>
<td>(P_{\text{O}_2}) (mm Hg)</td>
<td>107 ± 16</td>
<td>NS</td>
<td>105 ± 11</td>
<td>NS</td>
<td>102 ± 13</td>
</tr>
<tr>
<td>(P_{\text{CO}_2}) (mm Hg)</td>
<td>32.8 ± 3.5</td>
<td>NS</td>
<td>40.0 ± 7.7</td>
<td>NS</td>
<td>35.4 ± 6.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.04</td>
<td>&lt; 0.005</td>
<td>7.33 ± 0.03</td>
<td>NS</td>
<td>7.36 ± 0.03</td>
</tr>
<tr>
<td>BE (meq/liter)</td>
<td>-1 ± 3</td>
<td>&lt; 0.001</td>
<td>-4 ± 3</td>
<td>NS</td>
<td>-3 ± 2</td>
</tr>
</tbody>
</table>

\(P\) is probability of a difference between adjacent columns. NS = not significant.

### Table 5  Changes in Developed Tension (g) during Exposure to 0-EEP\textsubscript{1}, PEEP, and O-EEP\textsubscript{2} Plasma

<table>
<thead>
<tr>
<th></th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-EEP\textsubscript{1}</td>
<td>2.96 ± 0.74</td>
<td>3.00 ± 0.81</td>
<td>3.04 ± 0.66</td>
<td>3.04 ± 0.66</td>
</tr>
<tr>
<td>PEEP</td>
<td>2.96 ± 0.74</td>
<td>2.93 ± 0.72</td>
<td>2.90 ± 0.72</td>
<td>2.86 ± 0.76</td>
</tr>
<tr>
<td>0-EEP\textsubscript{2}</td>
<td>2.98 ± 0.91</td>
<td>3.01 ± 0.87</td>
<td>3.09 ± 1.03</td>
<td>3.11 ± 0.95</td>
</tr>
<tr>
<td>0-EEP\textsubscript{1}</td>
<td>3.07 ± 0.94</td>
<td>3.09 ± 0.93</td>
<td>3.10 ± 0.79</td>
<td>3.10 ± 0.81</td>
</tr>
</tbody>
</table>
NEGATIVE INOTROPISM AND PEEP/Grindlinger et al. 465

not lead to a fall in CO (Liebman et al., 1978).

Reduction in left ventricular contractility during PEEP was suggested by a rising left ventricular filling pressure despite a falling CO (Liebman et al., 1978; Manny et al., 1978b). Support for humoral mediation of this event was provided by a series of cross-circulation experiments in which application of PEEP to one animal led to decreasing CO in both (Patten et al., 1978). This humoral hypothesis was strengthened by a series of studies on ex vivo perfused isolated hearts (Manny et al., 1978a). When PEEP was applied to the support dog that provided coronary flow to the heart, there was a decrease in contractility. This fall in contractility still was observed after infusions were given to the support dog to prevent the PEEP-induced reduction in CO. The event that appeared to stimulate release of a negative inotropic agent or cause a reduction in levels of a positive inotropic agent was lung stretch, since when PEEP was applied, but the functional residual capacity was kept constant, the isolated heart was not depressed. Since the lungs are active metabolic organs, capable of elaborating and metabolizing a variety of vasoactive substances (Said et al. 1975; Heineman et al., 1977), it is reasonable to postulate that lung stretch can alter pulmonary metabolic activity such that there is a resultant depression in cardiac contractility. It also could be argued that lung stretch acts via a neural arc (possibly vagal) causing release of substances at a site distant from the lung.

This study demonstrates that plasma from a PEEP-treated dog will exert a negative inotropic effect on rat papillary muscle. The decrease in contractility is based on a reduction of Tpd at constant preloads. This negative inotropic effect was reversed following replacement of PEEP plasma with O-EEP; or O-EEP\textsubscript{2} plasma.

Papillary Muscle Characteristics

The newly developed papillary muscle bioassay system is ideally suited for testing small volumes of plasma. Substitution of a membrane oxygenator for the usual sintered disc system or bubble oxygenator eliminates mechanical distortions induced by plasma foam. Our most recent experience has indicated that a plasma Po\textsubscript{2} above 500 mm Hg may be obtained routinely after 10-15 minutes of equilibration.

The findings of a reduced Tpd with PEEP plasma is consistent with a negative inotropic effect but also may be related to an alteration in excitation, excitation-contraction coupling, contraction, and relaxation processes. We found no evidence that the threshold for excitation was influenced by PEEP plasma. Increasing the stimulation voltage 5-fold did not reverse the depression in Tpd. Since stimulating frequency was far below the physiological rate, it is unlikely that the refractory period played a role. These considerations make it probable that Tpd was independent of the characteristics of stimulation.

The observed fall in Tpd was not related to variations in parallel elastic compliance since Tr was unchanged. Further, these experiments were conducted near the apex of the length-tension curve, where small changes in Tr would not be expected to influence Tpd significantly (Sonnenblick and Skelton, 1971). It is possible that an increase in series compliance could account for the fall in Tpd (Hill, 1951). However, this event should also delay the occurrence of peak tension (Sonnenblick, 1964). Our observation was that TPT was either unchanged or reduced.

Changes in the active state may be related to temperature (Edman et al., 1974), oxygenation (Tyberg et al., 1970), or acid-base (Johannsson and Nilsson, 1975) characteristics of the bathing medium. These were held constant and are therefore not likely to be related to the fall in Tpd. It is possible that lactate levels rose in the support dog secondary to a PEEP-induced fall in the CO of group I or redistribution of flow in group II (Manny et al., 1979). We did not measure lactate but did observe a significant decrease in base excess. Lactate ion will reduce contractility (Gimeno et al., 1966) but only in the presence of acidosis. In the absence of acidosis, lactate will increase Tpd by prolonging the duration of the active state, despite a small reduction in contractility parameters (Pannier and Wayne, 1970). We observed a slight and nonsignificant reduction in TPT, which is inconsistent with a lactate effect. Furthermore, in group II dogs in which CO was maintained constant during PEEP, Tpd fell despite only modest decreases in base excess.

Finally, a barbiturate infusion was used for anesthesia. The metabolism of this known myocardial depressant could have been decreased during the low flow state that accompanies PEEP. The measured barbiturate levels did indeed rise, although only slightly. The highest levels found were below those reported to decrease cardiac contractility.
In Vivo Negative Inotropic Effects

These considerations make it likely that PEEP plasma contains a negative inotropic agent (or reduced levels of a positive inotropic agent). The decrease in contractility is not caused by changes in $P_{O_2}$, pH, temperature, lactate, or barbiturate. This negative inotropic effect of PEEP has been demonstrated previously in intact, as well as in ex vivo perfused hearts (Patten et al., 1978; Manny et al., 1978a). The present study does not demonstrate an in vivo decrease in contractility, since no direct measurements of contractility parameters, such as end-systolic or end-diastolic fiber length, were made. However, the inference that negative inotropic did occur in vivo is supported by the direct relationship between the in vivo percent decrease in CO and the in vitro percent decrease in Tpd (Fig. 4). The equation describing the least square fit of this relationship is % fall Tpd = 36% fall CO - 0.19. It is possible, but not proven by our data, that these are causally related events. If the latter is true, then, in theory, the slope of 36% is an approximation of the role of contractility a possible underestimate.

Studies on the ex vivo perfused heart indicate that the negative inotropic effect appears and disappears within minutes (Fig. 3). Application of PEEP leads, at first, to an increase and, eventually, a net decrease in contractility. Removal of PEEP is followed by restoration of contractility and a transient positive inotropic effect. These events are consistent with a combination of positive and negative inotropic influences. Thus, the hypotension occurring immediately after PEEP is likely to have stimulated the sympathoadrenal system. In previous studies, bleeding the support animal, used to perfuse the isolated heart, to achieve a CO and arterial pressure equivalent to values observed during PEEP, led to an increase in PSP (Manny et al., 1978a). In other experiments, preventing the PEEP-induced decline in flow and pressure with volume infusions led to significant falls in PSP.

The delayed recovery of Tpd after the reappllication of O-EEP plasma to the papillary muscle, compared with the rapid restoration of PSP in the perfused heart, is consistent with the presence of a negative inotropic agent. It would be expected that the capacity for metabolism of a newly formed agent might be reduced in vitro. Any negative inotropic agent still attached to the cellular membrane after exchange of plasmas might require spontaneous degradation for inactivation.

The negative inotropic agent has not been characterized chemically. The fact that arterial levels of the prostaglandin, PGF$_{2\alpha}$, and its metabolite, 15-keto-13,14 dihydro—PGF$_{2\alpha}$, were not changed by PEEP (Manny et al., 1978a) does not exclude the action of other prostaglandins. These lipids are attractive mediators, since lung stretch will alter the pulmonary processing and metabolism of these agents (Berry et al., 1971). Further, many of the prostaglandins are vasoactive (Lee, 1976), and at least one of them is thought to possess negative inotropic properties (Fitzpatrick et al., 1978). It remains possible that nonlipid agents may be involved. For example, several peptides have been found in the effluent of lungs following mechanical stimulation (Said et al., 1975; Berry et al., 1971). Further, the lungs recently have been shown to secrete plasminogen activator in response to pressure breathing and PEEP (McLoughlin et al., 1978).

Whether the release mechanisms for the negative inotropic agent share common properties with vasoactive substances is unknown. The resolution of these questions requires further study.

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