Pulmonary and Circulatory Effects of Fibrinopeptides

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With the assistance of Frank P. Szerdahelyi

ABSTRACT
Bovine fibrinopeptide B and human fibrinopeptide A given in minute concentrations to rabbits, dogs, and lambs caused pulmonary hypertension, decreased effective pulmonary blood flow, decreased lung compliance, decreased ventilatory conductance, increased frequency and volume of ventilation, and increased differences in arterial and alveolar PCO₂ and PO₂. These effects appeared immediately after injection of the peptides into the lesser circulation, increased for 15 to 30 min, and were detectable for as long as 70 min. Mean aortic pressure, heart rate, stroke volume, left and right atrial pressures, and cardiac output showed no systematic changes after injection of these peptides. On the contrary, bovine fibrinopeptide A given in equimolar doses elicited none of the pulmonary responses but induced increases in heart rate, stroke volume, and cardiac output without consistent changes in mean pulmonary or aortic pressure. These responses appeared within 3 min, increased for 5 to 10 min, and disappeared within an hour. Some possible relationships of fibrinopeptides to clinical syndromes characterized by respiratory distress, hypoxemia, pulmonary vasoconstriction, and diminished lung compliance are briefly discussed. It is speculated that the lung may be an active site of fibrinopeptide catabolism and that accelerated fibrinogen-fibrin conversion may cause pulmonary failure through fibrinopeptide-induced vasoconstriction and reduction in compliance.

ADDITIONAL KEY WORDS vasoactive peptides fibrinogen blood-clotting hyaline membrane disease pulmonary embolism pulmonary hypertension bradykinin bronchoconstriction
tween serum and plasma bioassays by pharmacologists attempting to determine the adrenaline content of blood \( (8) \). Freund \( (7) \) in his studies on the pharmacological action of autologous defibrinated blood observed that when injected intravenously this caused systemic hypotension, cardiac irregularity, and arrest in diastole in cats, rabbits, and dogs.

Brodie \( (8) \) showed that the injection of homologous and heterologous sera into the jugular vein of cats produced an immediate bradycardia and systemic hypotension, and apnea in some instances. He attributed these effects to a reflex with receptors in the pulmonary circulation since they could be abolished by division of the vagi. He believed that the blood corpuscles were essential for the production in sera of the material responsible for these changes, although he did find that some citrated plasma (which he thought might contain some corpuscles) exhibited this property when allowed to clot.

The release of a vasoconstrictor substance from platelets in the process of blood clotting has received much attention as the possible explanation for the "Brodie effect." This material, originally termed "thrombotonin," was found to be 5-hydroxytryptamine \( (9) \). Reid showed that in the anesthetized cat, 5-hydroxytryptamine had to be given in large amounts to produce systemic changes, amounts that would be released only by clotting of large volumes of blood. Other workers have reported that this substance produces reflex bradycardia, bronchoconstriction, and pulmonary vasoconstriction in the cat \( (10) \) and a reduction in lung compliance and airway conductance in anesthetized cats and dogs \( (11) \).

It is possible that histamine is released when blood clots, either from the formed elements of blood itself or from the tissues. Histamine causes marked reduction in lung compliance and airway conductance with alveolar duct constriction in cats; these functional and anatomical features are also observed after barium sulfate microembolism \( (12) \). Besides these and other substances of cellular origin, the formation of fibrin itself might yield materials that are vasoactive.

Modern development in understanding of the clotting of fibrinogen began with the demonstration that this occurs in two phases: limited proteolysis and polymerization. In the first phase, selective hydrolysis by thrombin brings about small alterations in the fibrinogen molecule \( (13, 14) \). The finding of non-protein nitrogen and ninhydrin-reacting material in the liquor from fibrin clots gave evidence that the structural changes in fibrinogen were due to release of peptide chains by thrombin \( (15, 16) \). Using bovine fibrinogen, Bettelheim and Bailey \( (17) \) showed that this non-protein nitrogen material consisted of two acidic polypeptides, which they termed peptides A and B. The further development of knowledge concerning the chemistry of the fibrinogen-fibrin transition and the physical and chemical properties of these polypeptides has been reviewed recently by Laki and Gladner \( (18) \). Blombäck and his co-workers have shown that the amino-acid sequences of the fibrinopeptides of 21 species of animals are strikingly similar and have commented on the significance of this finding for considerations of thrombin specificity and for phylogenetic theory \( (19) \). In the present paper we follow Blombäck's terminology for the fibrinopeptides.

That fibrinopeptides might have a physiological role was suggested by the findings that the liquor from clotted fibrinogen stimulated the frog heart \( (15) \) and that purified bovine fibrinopeptide B both potentiated and prolonged the bradykinin-induced contraction of isolated rat uterus \( (20) \). A similar effect was also observed with human peptide A \( (21) \) although bovine fibrinopeptide A and human fibrinopeptide AP were without effect in this system. Both human fibrinopeptide A and bovine fibrinopeptide B have a sustained systemic pressor effect in the pentolinium-treated rat \( (22) \).

We have found no previous reports on the effects of these peptides on the pulmonary circulation and lungs. It seems likely that they are produced continuously in vivo, and that...
they might accumulate in excessive amounts in pathological states in which fibrinogen-fibrin conversion or deposition may increase, such as thromboembolism, respiratory distress syndrome of the newborn, sepsis, endotoxin shock, oxygen toxicity, and respiratory distress following cardiopulmonary bypass.

In this communication we describe some effects of fibrinopeptides A and B on the circulation and lungs of anesthetized rabbits, dogs, and newborn lambs.

Methods

We studied 2 lambs less than 24 hours old, 12 adult rabbits, and 4 mature dogs. They were anesthetized with pentobarbital, given intravenously in rabbits (25 to 35 mg/kg) and lambs (15 to 20 mg/kg) and intraperitoneally in dogs (30 to 40 mg/kg). Rabbits were studied while they were supine, lambs and dogs while they were in the lateral position. All animals were given heparin (Invenex) (500 to 1000 units/kg) to simplify blood sampling and to minimize intravascular coagulation. Supplementary doses of pentobarbital were given as necessary during the control period to maintain a steady state judged by respiratory variables, intravascular pressures, and heart rate. The dogs were studied 10 to 14 days following left thoracotomy for implantation of a Teflon catheter in the left atrium.

The trachea was cannulated in rabbits and lambs, and intubated in dogs. The femoral vessels were exposed and catheterized with polyethylene tubes (PE 90), which were advanced into the pulmonary artery, right atrium, and thoracic aorta. A small balloon-tipped catheter was placed in mid-esophagus and inflated with 0.2 ml of air. Balloon pressure was measured with a Statham PM 13 Tc transducer. Air flow was measured with a Fleisch pneumotachygraph and a Statham PM 15 Tc transducer. Tidal volume (Vt) was obtained by electrical integration using a Grass UI-1 integrator. Tidal volume, air flow, and intraesophageal pressure were displayed on an oscilloscope (Tektronix, Inc., Type 512) and dynamic compliance (CL) and ventilatory conductance (G1) were obtained by electrical calculation.

Respiratory gas was sampled continuously from the airway through a CO2 microcatheter cell and nitrogen meter arranged in series. The heated sampling catheter and its characteristics have been described elsewhere (23).

Calibrations for end-expiratory fractions of CO2 (FECO2) and O2 (FE02) were done with gas mixtures saturated with water vapor at room temperature by bubbling through a washing tower. At least 5 gas mixtures were used for calibrations at the beginning and end of each experiment. The graphs of concentration against polygraph deflection were linear on full logarithmic paper.

Cardiac output (QICO) was determined by indicator dilution, using indocyanine green (ICG) injected into the right atrium, with a Gilson densitometer modified to have a small volume in the cuvette. Blood was drawn from the thoracic aorta through the cuvette at a constant rate (16 ml/min) with a Harvard Apparatus Company constant-withdrawal/infusion pump. With a square-wave change in dye concentration at the catheter tip the rise time for 95% response was 0.15 sec. Calibration curves were done using the same stock solution of dye and diluting it with the animal's own blood. At least 6 dilutions were used for each calibration.

Effective pulmonary blood flow (QPFCO2), that is, the equivalent amount of blood flowing through the lungs coming into equilibrium with gas in ventilated air spaces, was derived from the uptake of monochlorodifluoromethane (Freon 22, Dupont). The solubility of Freon 22 in normal blood is 0.71 ml/ml blood/atm at 37°C; its calculated permeability coefficient in tissue is about equal to that of CO2. The method is a modification of that described by Krogh and Lindhard (24), with rebreathing substituted for breathholding. It is a further development of that described by Chu et al. (23). The animal rebreathed from a sac containing a known amount (100 to 200 ml) of 100% oxygen, chosen to approximate predicted functional residual volume; the sac was connected to the pneumo tachygraph at end expiration. Freon was either added to the gas in the sac to give a concentration of 5 to 10% or was injected into the inlet tubing of a diaphragm pump connected to the airway which rapidly circulated respiratory gas through an infrared carbon monoxide analyzer (Liston-Becker Instrument Co., Model 15) and back to the airway. After the sac was attached to the airway, respiration and recirculation through the analyzer tended to rapidly equalize Freon concentration in the sac, the ventilated parts of the lung, and the analyzer. Thereafter, the fall in Freon concentration was limited mainly by the flow of blood washing it away from the lungs in the normal state, and also by the distribution of ventilation during the response to fibrinopeptide.

Rebreathing was continued for 30 to 45 sec, during which time there was no systematic alteration of any measured intravascular pressures, heart rate, respiratory frequency, or tidal volume.
Administration of Freon did not produce any consistent change in QICC.

Freon concentration was recorded continuously from the infrared gas analyzer. Despite the large volume of the sample cell (100 ml) the rapid flow of the recirculation system (133 ml/sec) gave a rise time for 95% response of 0.87 sec with a square-wave change in concentration. The analyzer was calibrated with injections of 1 ml of Freon into the recirculation circuit. When replotted on semilogarithmic graph paper, the calibration curve was nearly linear over the range 1.5 to 3.5% Freon. Because the analyzer was very sensitive to Freon in this system, it was possible to operate the system at low gain. Calibrations with Freon diluted in ambient air and in alveolar gas were nearly identical. Freon 22 absorbs infrared radiation sufficiently well in the wave lengths detected by the CO analyzer to give a good response, whereas CO₂ in concentrations up to 10% has little effect either alone or in combination with Freon 22.

Freon concentrations were read at 4 to 5 sec intervals, and at least four values were plotted against time on semilogarithmic paper. The points were joined and from the resultant straight line the time constant, τ, for the disappearance of Freon was determined. There was no evidence of recirculation, as judged by a change in the slope of this line, unless values taken 35 sec or longer after the start of rebreathing were plotted. During the rebreathing period, sampling through the CO₂ and N₂ analyzers was discontinued and restarted after 45 sec; N₂ concentration did not oscillate significantly with breathing at this time. Functional residual volume (FRC) was calculated from T, the volume from which Freon was absorbed, and its solubility in blood. The expression used for QPCEff is:

\[ \dot{Q}_{PC Eff} = \frac{FRC + Vs + Va}{\alpha_0 \cdot \tau} \]

where \( \tau \) is the time for Freon concentration to decrease by the factor \( 1/e \), \( e \) being the base of Naperian logarithms. Allowance was not made for the pulmonary parenchymal volume or for the solubility of Freon in this tissue. This factor, which would appear in the numerator, is small in these animals by comparison with the other volumes. When allowance was made for this, the correction of calculated \( Q_{PC Eff} \) amounted to at most 2%, and this factor has been ignored.

Comparison of simultaneously determined cardiac output by dye-dilution and effective pulmonary blood flow by this method in 17 normal, anesthetized rabbits and 4 dogs showed good correlation (Fig. 1). Probably because some venous admixture occurs in normal animals, the majority of the points fall below the lines of identity.

Intravascular pressures were measured with Statham P23 G transducers, and were recorded, as were all other variables, on a Grass Model 5 polygraph. Mean pressures were determined by electrical averaging. The positions of the catheters were checked periodically by inspection of the pulse pressure recordings and were verified at autopsy. Only mean pressures are reported here. Student's t-test was used to measure the statistical significance of changes in ventilatory and circulatory variables.

Fibrinopeptides A and B were prepared by Dr. Osbahr from highly purified fibrinogen and thrombin by the method described by Osbahr and co-workers (21). The purity of the peptides was confirmed by high-voltage paper electrophoresis and the biological activity of bovine fibrinopeptide B and human fibrinopeptide A were demonstrated in the rat uterine smooth muscle system (25). The precautions taken to remove contaminants, especially proteins of the clotting system, have been previously described (21).

The peptides were kept in the lyophilized state at −20°C until shortly before use, when aliquots were weighed at 5°C and dissolved in 1 to 3 ml of sterile 0.9% sodium chloride solution. Injections were made into either the right atrium or the pulmonary artery. Equal amounts of saline injected at the same sites were without detectable effects. At least an hour was allowed to pass between successive injections of peptide, and tachyphylaxis did not occur with these intervals.
FIBRINOPEPTIDE EFFECTS ON HEART AND LUNG

Simultaneous determinations of cardiac output by indicator dilution (Q10Q) and effective pulmonary blood flow by uptake of Freon 22 (QPC Eff) prior to administration of fibrinopeptides.

Results

Fibrinopeptide B

Rabbits. The injection of bovine fibrinopeptide B was followed by an immediate rise in mean pulmonary arterial pressure (Ppa) which reached its peak between the 7th and 15th minutes. Some elevation of Ppa persisted for 15 to 75 min, lasting longest with the largest doses. The increments ranged from 23 to 223%, and were approximately proportional to dose (Fig. 2).

The changes in mean aortic pressure (Pao),
Effective pulmonary blood flow decreased following injection of bovine fibrinopeptide B in all animals; the mean decrease was 23% (range 4.3 to 43.5%). This effect was apparent within 10 min after the injection and the nadir was reached between 15 and 30 min. This decline in $Q_{PC_{Eff}}$ lasted from 15 to 65 min, the duration being longest with the largest doses. Since indocyanine green was injected into the right atrium, the cardiac output calculated from dye concentrations of blood sampled in the thoracic aorta gives values for total pulmonary blood flow unless there are extrapulmonary shunts. The expression $Q_{PC_{Eff}}/Q_{ICG}$ is therefore the fraction of pulmonary blood equilibrated with alveolar Freon. (This expression is oversimplified; its limitations will be discussed later.) Values for this ratio are plotted in Figure 3. The mean value for the ratio before injection of bovine fibrinopeptide B was 0.98 and fell to 0.81 15 to 30 min after injection, a highly significant change ($t = 7.71; P < 0.001$). The ratio $Q_{PC_{Eff}}/Q_{ICG}$ returned to control values by the 65th min after injection.

Within 5 min after injection of bovine fibrinopeptide B respiratory frequency ($f$) increased in all rabbits (Table 2). Tidal volume rose or fell but the average did not alter significantly. Minute ventilation ($V_E$) increased in all animals (8 to 98%) and it increased most with the largest dose. It returned approximately to control values by 60 to 70 min.

There was a fall in dynamic lung compliance in each of 8 animals in which it was measured. The decrement ranged from 16 to 46% and reached its nadir between the 15th and 25th min after injection. The magnitude was roughly proportional to the dose of peptide. This effect persisted for 60 to 70 min, the duration being longest with the largest doses.

During the control measurements, variation in functional residual volume (FRC) was ±6%; following injection of bovine fibrinopeptide B, FRC fell by 3.8 to 21.4%. The change was proportional to the concomitant change in $C_t$, but was only 40% as great.

The fractional change in compliance after

**FIGURE 2**

Relationship of pulmonary hypertension to dose of bovine fibrinopeptide B injected into the lesser circulation of rabbits.

Heart rate, mean right atrial pressure, cardiac output, and stroke volume were variable, but on the average there was no alteration. There was no relationship between changes in these variables and the dose of peptide given.

**FIGURE 3**

Ratio of effective to total pulmonary blood flow ($Q_{PC_{Eff}}/Q_{ICG}$) in rabbits. Values of the ratio shown here were determined before injection of bovine fibrinopeptide B and afterwards at the time of maximal response.
### TABLE 1

**Circulatory Responses in Rabbits before and after Injection of Bovine Fibrinopeptide B**

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Body weight (kg)</th>
<th>Dose (nmole/kg)</th>
<th>P&lt;sub&gt;FA&lt;/sub&gt; (mm Hg)</th>
<th>P&lt;sub&gt;AS&lt;/sub&gt; (mm Hg)</th>
<th>Q&lt;sub&gt;CO&lt;/sub&gt; (ml/min)</th>
<th>Q&lt;sub&gt;CO&lt;/sub&gt;eff (ml/min)</th>
<th>Q&lt;sub&gt;AS&lt;/sub&gt; (%)</th>
<th>Heart rate (per min)</th>
<th>Stroke volume (ml)</th>
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<td>30.9</td>
<td>11 14 88 83</td>
<td>352 413 348 293</td>
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<td>284 292</td>
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<td>31.4</td>
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<td>348 403 342 242</td>
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<td>280 282</td>
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**Mean**

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<.001 <.09 <.001 <.001 <.09 <.8

* = before, p = after, Q<sub>S</sub> = percent shunt flow, P = probability. Other symbols are defined in the text. All measurements at each dose were made at the same time. Measurements made after each administration of peptide were at the time of maximum response in P<sub>FA</sub>. 
### TABLE 2

**Ventilatory Responses in Rabbits before and after Injection of Bovine Fibrinopeptide B**

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>$\dot{V_t}$ (l/min)</th>
<th>Resp. frequency (per min)</th>
<th>$V_t$ (ml)</th>
<th>PRC (ml)</th>
<th>$\dot{F}_{\text{CO}_2}$ (atm)</th>
<th>$\dot{F}_{\text{O}_2}$ (atm)</th>
<th>$C_l$ (ml/cm H$_2$O)</th>
<th>$G_l \times 10^4$ (l/sec-cm H$_2$O)</th>
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**Mean**

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<th>PRC</th>
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**P**

|                           | < .001 | < .001 | > .8  | < .001 | < .001 | < .001 | < .001 | < .001 |< .001 |

**Symbols**

Symbols are defined in Table 1 and text. Each group of measurements was made at the same time as the corresponding group in Table 1.
FIBRINOPEPTIDE EFFECTS ON HEART AND LUNG

Effects of bovine fibrinopeptide B on pulmonary blood flow and lung compliance in rabbits. Ordinates represent the ineffective fraction of blood flow (shunt), abscissae the fractional loss of compliance. Both coordinates increase with an increased response.

FIGURE 4

Injection of bovine fibrinopeptide B also correlated with that in effective pulmonary blood flow. Figure 4 shows the values 1- (Qpc_eff/Qpc_eff0) plotted against 1- (CL/CL0) at the time of maximum changes in these variables (where Qpc_eff0 and CL0 are the average values for effective pulmonary blood flow and compliance before bovine fibrinopeptide B, and Qpc_eff and CL are the minima following bovine fibrinopeptide B). The interval between measurements of Qpc_eff and CL was approximately 2 min.

Ventilatory conductance (GL) fell following injection of bovine fibrinopeptide B in each of the 5 animals in which it was measured. The decrements ranged from 16 to 50%, were proportional to the dose of peptide, and reached minimum values somewhat later than the minima for lung compliance, usually at about 25 to 30 min. The pulmonary time constant (CL × GL⁻¹) shortened initially, then lengthened at about a half hour after injection. The duration of this decrease in GL was variable, being longest with the largest doses. The effect lasted for 60 min after some large single injections.

Dogs. Because of the difficulties in obtaining direct left atrial pressure in rabbits, 3 dogs were studied in which a Teflon catheter had been implanted in the left atrium. The placement was made 10 to 14 days before the experiment to allow recovery from the thoracotomy before testing of peptides.

The effects of injections of bovine fibrinopeptide B in these 3 dogs were qualitatively and quantitatively similar to those observed in rabbits, except that changes in respiratory frequency and minute ventilation were less marked.

Similar effects were seen in the pulmonary circulation, with respect both to time course and to the relationship between increment in FpA and dose. Mean left atrial pressure (P_LA) was unaltered following injection of bovine fibrinopeptide B and pressure drop through the pulmonary circulation increased in all 3 animals. Cardiac output remained unchanged in two and fell in the third dog, which received the largest dose. Calculated values for pulmonary vascular resistance increased in each animal. Effective pulmonary blood flow and the ratio Qpc_eff/Qpc_eff0 both decreased. Heart rate, stroke volume, mean aortic pressure, and systemic vascular resistance did not alter appreciably after injection of bovine fibrinopeptide B in 2 dogs. In the third (no. 1), which received the largest dose, there was a slight rise in mean aortic pressure and a fall in cardiac output; calculated systemic resistance increased.

Compliance and functional residual volume fell in all 3 animals. Ventilatory conductance decreased in each animal and, after an initial shortening, pulmonary time constant lengthened. All values had returned to within their control ranges by the 70th minute after injection.

In studies performed on 2 dogs (nos. 2 and 3) breathing room air, arterial blood was sampled for measurement of oxygen tension (P_{o2}) before and then between the 20th and 30th minutes after injection of bovine fibrinopeptide B. P_{o2} fell from 91 to 73 mm Hg after injection of peptide in dog 2 and from 89 to 71 mm Hg in dog 3. In the latter, P_{o2} rose from 43 to 51 mm Hg 20 min after injection of...
peptide, despite an increase in minute ventilation and a fall in end-expiratory Pco$_2$ from 41 to 39 mm Hg.

In these same 2 dogs, blood smears made before and 30 min after injection of bovine fibrinopeptide B were stained by Wright’s method. We did not observe any change in the formed elements of the blood after peptide administration, nor any agglutination.

Newborn Lambs. The effects of injections of bovine fibrinopeptide B in newborn lambs were similar to those seen in adult rabbits and mature dogs. In lamb 1, which received 3 doses of bovine fibrinopeptide B, indicator curves obtained with injection of dye into the right atrium and sampling from the lower thoracic aorta showed evidence of extrapulmonary right-to-left shunting that was greater.
Circulatory Responses of Dogs to Bovine Fibrinopeptides B and A

Table 1. FLA = mean left atrial pressure.

Table 2.

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Body weight (kg)</th>
<th>Dose (nmol/kg)</th>
<th>$P_{FA}$ (mm Hg)</th>
<th>$P_{FA}$ (mm Hg)</th>
<th>$P_{LA}$ (mm Hg)</th>
<th>$Q_{100}$ (l/min)</th>
<th>$Q_{100}$ (l/min)</th>
<th>$Q_{p}$ (l/min)</th>
<th>Heart rate (per min)</th>
<th>Stroke volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.4</td>
<td>58.0</td>
<td>19 36</td>
<td>122 130</td>
<td>2 4</td>
<td>1.53 1.04</td>
<td>1.51 0.67</td>
<td>0 45.6</td>
<td>146 120</td>
<td>10.2 8.7</td>
</tr>
<tr>
<td>2</td>
<td>7.7</td>
<td>29.7</td>
<td>13 19</td>
<td>59 70</td>
<td>5 3</td>
<td>1.54 1.29</td>
<td>1.50 1.05</td>
<td>2.8 26.3</td>
<td>105 108</td>
<td>14.7 12.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>54.3</td>
<td>19 28</td>
<td>140 138</td>
<td>5 4</td>
<td>0.97 1.02</td>
<td>0.96 0.73</td>
<td>1.0 18.5</td>
<td>169 174</td>
<td>5.7 5.9</td>
</tr>
</tbody>
</table>

Symbols as in Table 1. FLA = mean left atrial pressure.

Table 3.

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>$V_{E}$ (l/min)</th>
<th>$V_{E}/K_{V}$ (l/min)</th>
<th>Resp. frequency (per min)</th>
<th>$V_{T}$ (ml)</th>
<th>$F_{HbO_{2}}$ (atm)</th>
<th>$F_{HbO_{2}}$ (atm)</th>
<th>$P_{RC}$ (ml)</th>
<th>$C_{l}$ (ml/cm H$_2$O)</th>
<th>$G_{l} \times 10^5$ (l/sec cm H$_2$O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.12</td>
<td>0.91</td>
<td>0.18 0.14</td>
<td>14 12</td>
<td>80 76</td>
<td>0.073 0.062</td>
<td>0.137 0.138</td>
<td>207 212</td>
<td>28.4 13.9</td>
</tr>
<tr>
<td>2</td>
<td>1.64</td>
<td>1.92</td>
<td>0.21 0.23</td>
<td>30 32</td>
<td>54 60</td>
<td>0.072 0.060</td>
<td>0.138 0.150</td>
<td>173 161</td>
<td>19.8 11.4</td>
</tr>
<tr>
<td>3</td>
<td>1.50</td>
<td>1.95</td>
<td>0.29 0.39</td>
<td>25 30</td>
<td>60 65</td>
<td>0.061 0.055</td>
<td>0.149 0.155</td>
<td>168 146</td>
<td>15.4 12.2</td>
</tr>
</tbody>
</table>

Symbols as in Table 3. Each group of measurements was made at the same time as the corresponding group in Table 3.
with larger doses of peptide B and that reversed spontaneously on all three occasions. Shunting occurred either at the foramen ovale or the ductus arteriosus, more probably the former since PPA was always less than mean aortic pressure. Postmortem examination showed that both the foramen ovale and the ductus arteriosus were patent. In lamb 2 a small right-to-left shunt was demonstrated with the larger dose of bovine fibrinopeptide B.

The changes in ventilatory mechanics in the lambs were greater in relation to the dose given than in the rabbits and dogs. Changes in lung compliance were greater, especially in lamb 2, and there was a tenfold reduction in ventilatory conductance in this lamb. Following the injection of the largest dose, apnea occurred in 12 min in lamb 2 and in 30 min in lamb 1; it persisted for 1 to 2 min after which slow, non-rhythmic respiration began. Respiratory frequency and P*PA returned to within control range by 30 min following the apneic episode, but ventilatory mechanics were still grossly abnormal. Oxygen was administered both during and after the apneic period. Lung compliance, ventilatory conductance, and effective pulmonary blood flow increased thereafter and stabilized at about 1 hour at approximately 70 to 80% of their control values.

**Discussion**

The chemistry of the fibrinogen-fibrin transition and the physical and chemical properties of the fibrinopeptides have been extensively investigated (18, 19), but the physiological properties of these polypeptides have received little attention. It has been reported that uterine smooth muscle (20, 21) is affected by bovine fibrinopeptide B and human fibrinopeptide A, that rabbit carotid artery strips are affected by bovine fibrinopeptide B (20, 26), and that pentolinium-treated rats (22) show a systemic pressor effect after administration of these peptides.

We have found that bovine fibrinopeptide B causes pulmonary vasoconstriction which occurs quickly, reverses spontaneously, and is nonetheless prolonged. Our data do not clearly demonstrate the site of constriction, but it is likely that the pulmonary arterioles are responsible. On 6 occasions in our experi-

### Table 5

**Circulatory Effects of Bovine Fibrinopeptide B in Two Newborn Lambs**

<table>
<thead>
<tr>
<th>Lamb no.</th>
<th>Body weight (kg)</th>
<th>Age (hr)</th>
<th>Dose PPA (nmol/kg)</th>
<th>PPA (mm Hg)</th>
<th>Q (ml/min)</th>
<th>Q*PA (ml/min)</th>
<th>Heart rate (per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5</td>
<td>24</td>
<td>25.8</td>
<td>32 41</td>
<td>40 42</td>
<td>621 788</td>
<td>191 210</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48.7</td>
<td>35 41</td>
<td>41 44</td>
<td>703 719</td>
<td>210 216</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>73.4</td>
<td>29 36</td>
<td>43 39</td>
<td>622 630</td>
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<td>4.2</td>
<td>22</td>
<td>35.7</td>
<td>20 22</td>
<td>50 53</td>
<td>— 483</td>
<td>114 120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47.4</td>
<td>15 18</td>
<td>50 53</td>
<td>— 483</td>
<td>120 128</td>
</tr>
</tbody>
</table>

Symbols as in Table 1.

Increases in cardiac output and effective pulmonary blood flow in both animals, and these changes were approximately equal when expressed as percentages. Calculated stroke volume also increased. The cardiac acceleration was obvious within 3 min of injecting bovine fibrinopeptide A and cardiac output was increased by the 2nd to 6th min. Maximum cardiac output was observed by the 6th to 10th min after injection and had returned towards control values by the 30th min.

Following injection of bovine fibrinopeptide A, respiratory frequency, tidal volume, ventilation, dynamic lung compliance, ventilatory conductance, and pulmonary time constant showed little change.

**Fibrinopeptide A (dogs) (Tables 3 and 4; Fig. 5)**

Bovine fibrinopeptide A produced no consistent change in intravascular pressures when given to 2 dogs, in doses approximately equal on a molar basis to effective doses of bovine fibrinopeptide B. Heart rate increased in both dogs, although no acceleration was observed in dog 3 with the smallest dose.
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mean pulmonary arterial pressure was above 35 mm Hg for long intervals, yet there was no pre- or post-mortem evidence of edema. Pulmonary venoconstriction, therefore, is not an attractive possibility.

This peptide also caused a fall in dynamic lung compliance, functional residual volume, and ventilatory conductance. Although there was some increase in the rate of breathing, especially in rabbits, this fall in lung compliance was probably not due to frequency dependence, since the decrease in ventilatory conductance was only 4% and the time constant was approximately one eighth the duration of a respiratory cycle. Measurements of quasi-static compliance will be necessary in order to test this conclusion unambiguously.

The decrease in effective pulmonary blood flow corresponded with the change in lung compliance (Fig. 4). The fall in QpCEf is probably the result of decreased ventilation: blood ratios in some regions of the lungs, since diminished Freon exchange occurred at a time when ventilation had increased by 10 to 90% over control levels, and since the changes in CL and QpCEf paralleled each other closely throughout each experiment.

Our technique for estimating effective pulmonary blood flow employs rebreathing and hence minimizes inequalities in alveolar gas concentration. The sensitivity of the method to unevenness of ventilation is thus reduced as compared to that of the single-breath method. The quantity $1 - (Q_{p_{CEf}}/Q_{ICC})$ therefore gives a minimum measure of "venous admixture." Nonetheless, large discrepancies between effective pulmonary blood flow and cardiac output occurred during the action of bovine fibrinopeptide B. Values for venous admixture were near zero during the control observations and again when the effects of bovine fibrinopeptide B had disappeared, but increased to as high as 52% during the action of the peptide.

From a theoretical point of view, Freon uptake could be reduced during the action of bovine fibrinopeptide B by other mechanisms, such as decreased diffusing capacity of the
lungs or increased proportional blood flow through intrapulmonary or extrapulmonary anatomical shunts. The evidence we possess does not permit us to discard these explanations summarily but does make us doubt them. It does not seem reasonable to attribute to decreased pulmonary diffusing capacity a 50% decrease in uptake of the highly diffusible Freon in the presence of normal cardiac output and increased ventilation, especially as the animals were only mildly hypoxemic breathing room air. We have no reason to suspect that extrapulmonary anatomical shunts develop and regress under the influence of bovine fibrinopeptide B, except in the newborn lambs, and it was only in these animals that indicator dilution curves gave evidence of such shunting. The possibility that intrapulmonary arteriovenous (anatomical) shunts are present and can be opened by bovine fibrinopeptide B seems remote to us, and all the more so because its net action is to increase total pulmonary vascular resistance.

The reasons why bovine fibrinopeptide B caused acceleration of respiration in some instances and apnea in others remains to be elucidated. We have not yet used chemical or surgical ablations that would help in demonstrating what extrapulmonary mechanisms might be involved in the responses to fibrinopeptides.

Although the results presented in this paper were obtained with bovine fibrinopeptides, we have also examined the action of human fibrinopeptides in dogs in a few experiments. Human fibrinopeptide A in equivalent doses produced responses similar and can be opened by bovine fibrinopeptide B. Human fibrinopeptide AP (containing phosphoserine in place of the serine in human fibrinopeptide A) failed to cause changes in any of the variables that we were observing when given in equivalent dosage. Likewise, rat uterine muscle is affected in vitro by human fibrinopeptide A and bovine fibrinopeptide B, but not by human AP or bovine A. It is of interest that the amino-acid sequences are similar in the regions near the supposed active sites of human fibrinopeptide A (21) and bovine fibrinopeptide B (20). The sequence in human fibrinopeptide A (Ala-Asp-Ser-Gly-Glu-) is also especially close to that in several hydrolases (18, 20, 27), notably thrombin and acetyl cholinesterase, and this fact may well be related to the mode of its physiological action.

The onset and duration of the effects of bovine fibrinopeptide B on both pulmonary circulation and ventilatory mechanics differ from those of other known vasoactive peptides such as bradykinin, angiotensin, and vasopressin. The onset of the bradykinin (28) and angiotensin (29) effects is very rapid and these effects last less than 3 min after a single injection. The vascular effects of vasopressin wax and wane more gradually but these too are more rapid than those of bovine fibrinopeptide B.

In vitro studies suggest that bovine fibrinopeptide B might act as a potentiator of bradykinin-induced contraction of smooth muscle (20, 21). Although such a mode of action might explain some of the effects of bovine fibrinopeptide B in the intact rabbit and dog, the systemic hypotension (28), bradycardia, and increase in cardiac output (30) usually observed following injection of bradykinin are absent. Possibly bovine fibrinopeptide B and human fibrinopeptide A may have exerted effects in all our experiments by modifying the action of endogenously produced bradykinin. The information we possess at present does not rule out this or other explanations for their actions. They may alter the response of smooth muscle cells to many kinds of stimuli, both natural and artificial, or they may have a direct contractile effect of their own.

Without intending to imply that we understand fully the mechanisms of action of the fibrinopeptides, we may still attempt to summarize our present opinions about them. Bovine fibrinopeptide B and human fibrinopeptide A appear to cause smooth muscle contraction in the pulmonary vessels and around the airways. These peptides did not increase smooth muscle tone in systemic vessels so far as we could detect in our experiments. Such
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an action might become prominent under different experimental circumstances. Bovine fibrinopeptide A did not stimulate smooth muscle in any way that was apparent by our observational techniques, but it did increase the frequency and volume of the heart beat. In a broad view, therefore, the effects of B appear deleterious to cardiopulmonary function, whereas those of A are not, at least in the concentrations that we have used.

In order to put our results into better physiological perspective, we observe that the doses of bovine fibrinopeptide B required to produce marked changes in the pulmonary circulation and ventilatory mechanics were small, 10 to 93 nmole/kg. We calculate that these amounts of peptide would be released by clotting of 0.75% to 2% of the blood volume in either rabbits or dogs. It has been suggested from half-life estimations (31, 32) using isotopic labels that fibrinogen is lost from the circulation at the rate of 17 to 24% per day. Synthesis rates for fibrinogen in both humans and rabbits indicate that 14.4 to 29.5% of total plasma fibrinogen is replaced per day (33). We calculate that the amounts of peptide used in these experiments are equivalent to the amount normally produced in vivo in 1 to 5 hours.

Robin et al. (34) have obtained an extract of the calf's lung by endobronchial lavage. This extract causes pulmonary hypertension when injected intravenously into dogs and calves. The physical, chemical, and pharmacologic properties of the material, termed pulmonary arterioconstrictor substance, were not fully characterized in the report. It passed through a 5-μ Millipore filter but was retained by an ultrafiltration membrane. Whether more than one kind of particle might carry the hypertensive activity in the lung washings was not established, but presumably the molecular weight of the active substance or substances exceeded 1000. Bovine fibrinopeptide B, with a molecular weight of approximately 2400, would not be excluded, therefore, from consideration as a possible component of the active species. If the lungs normally sequestered fibrinopeptide from the circulating blood, one might find some of it in endobronchial washings. The chemical nature of the pulmonary pressor materials as obtained in washings will have to be established before a relationship between fibrinopeptides and these materials can be critically examined.

Robin et al. attempted to exclude intravascular clotting and pulmonary embolism as the mechanism of the hypertensive response. Microscopic examination of the lungs after intravenous administration of the extract did however reveal fibrin thrombi in occasional pulmonary capillaries and pulmonary arterioles in some animals. The lung is rich in thromboplastic material and injection of lung extract might cause intravascular clotting even in the heparinized animal. The information we possess at present does not permit us to exclude the possibility, therefore, that lung washings induce pulmonary hypertension through formation of fibrinopeptide from the recipient's own fibrinogen, either within the pulmonary vasculature, in other circulatory beds, or both.

The cardiopulmonary effects that we have observed following injection of bovine fibrinopeptide B resemble those seen in experimental pulmonary embolism (35). Halmagyi and Colebatch observed hyperventilation, arterial hypoxemia, pulmonary hypertension, bronchoconstriction, and a fall in lung compliance following embolism with small doses of barium sulfate in sheep, and they concluded that these changes were probably caused by the release of an unknown substance. Halmagyi and co-workers (36) showed in cross-circulation experiments between pairs of sheep that pulmonary embolism, produced both by barium sulfate and by autologous blood clots in the donor, produced in the recipient a rise in pulmonary arterial pressure, a fall in lung compliance, and an increase in ventilation. These effects could be reversed by interrupting the unilateral carotid-jugular cross-circulation. It seems possible that fibrinopeptides could be one type of humoral agent responsible for the pulmonary vascular and airway changes associated with pulmonary microembolism.

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Excessive amounts of fibrinopeptides may also be produced in those pathological states where there is considerable fibrinogen-fibrin conversion, such as respiratory distress syndrome of the newborn and in overwhelming bacterial infections. In the former, reduced compliance, decreased effective pulmonary blood flow and hypoxemia are outstanding functional changes (37) and in the latter, respiratory distress and cyanosis are frequent clinical features. It has recently been reported that serum from cord blood of newborn infants contains a material of molecular weight 160,000 which reacts with antiserum to human fibrinogen (38). This material, termed split products of fibrinogen, is detectable in larger amounts and for a longer time post partum in infants with respiratory distress syndrome and in others with low Apgar scores than in normal infants. How this finding may relate to the respiratory distress syndrome and to fibrinopeptide formation remains to be determined.

The observations of Bainbridge and Evans that the heart-lung preparation was able to removed vasoconstrictor material from defibrinated blood suggest an important role for the lung parenchyma in removing from the blood certain vasoconstrictors, possibly the fibrinopeptides, which may be continuously forming. One might speculate that within a certain range of peptide production the lung is able to destroy the material that reaches it, but that if its capacity for such "detoxicaion" is exceeded both pulmonary and systemic circulatory changes may occur.

References


Pulmonary and Circulatory Effects of Fibrinopeptides
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