Acute Pulmonary Hypertension and Lung Thromboxane Release after Endotoxin Infusion in Normal and Leukopenic Sheep

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SUMMARY. We compared alterations of pulmonary hemodynamics with the plasma concentrations of thromboxane B<sub>2</sub>, 6-keto-PGF<sub>1α</sub>, and PGF<sub>2α</sub> following intravenous Escherichia coli endotoxin infusion (1 μg/kg) in three groups of awake sheep. Group 1 served as controls. The animals in group 2 were rendered leukopenic (<1000 WBC/mm<sup>3</sup>) by nitrogen mustard treatment. Group 3 received ibuprofen (12 mg/kg) one hour before endotoxin. After endotoxin infusion, the pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) increased markedly in groups 1 and 2; however, in group 2, the increases of PAP and PVR were half those of group 1. In group 3, only minimal increases of PAP and PVR were measured. In groups 1 and 2, there was an increased plasma TxB<sub>2</sub> concentration at 30 minutes after endotoxin (group 1—0.5 ± 0.04 to 16 ± 3 ng/ml; group 2—from 0.32 ± 0.1 to 5.6 ± 1.2 ng/ml). The peak plasma concentrations of TxB<sub>2</sub> were significantly less in group 2 than in group 1. In the sheep in both groups 1 and 2, 20 minutes after endotoxin infusion, consistent pulmonary artery to aortic increases of TxB<sub>2</sub> were measured (group 1 A3.7 ± 1.1 ng/ml and group 2 A3.1 ± 0.7 ng/ml). In group 3 sheep, plasma concentrations of 6-keto-PGF<sub>1α</sub> remained below the detection limit of the radioimmunoassay. TxA<sub>2</sub> is a likely mediator of endotoxin-induced pulmonary hypertension and is generated in large quantities by cells in the lung. Circulating leukocytes contribute substantially to the peak plasma concentrations of thromboxane following endotoxin infusion. (Circ Res 50:688–694, 1982)

ACUTE transient pulmonary artery hypertension can be produced by intravenous E. coli endotoxin infusion in the baboon (Harris et al., 1980), dog (Hales et al., 1981), cat (Parratt and Sturgess, 1975), calf (Anderson et al., 1975), and sheep (Ogletree and Brigham, 1979). There is evidence that the eicosanoids (i.e., products of arachidonate metabolism) mediate the pulmonary vasoconstriction caused by endotoxin injection. Administering a fatty acid cyclooxygenase inhibitor such as indomethacin before endotoxin infusion prevents or markedly reduces pulmonary vasoconstriction in the dog (Hales et al., 1981), cat (Parratt and Sturgess, 1975), calf (Anderson et al., 1975), and sheep (Ogletree and Brigham, 1979). Previous studies of the mechanism of endotoxin-induced pulmonary vasoconstriction have focused on prostaglandins of the E and F series (Anderson et al., 1975). However, more potent vasoactive arachidonate metabolites have since been discovered.

Thromboxane A<sub>2</sub> (TxA<sub>2</sub>), a powerful vasoconstrictor and platelet aggregating agent, can be generated in large quantities by lung tissue in vitro (Hamberg et al., 1975). Prostacyclin (PGI<sub>2</sub>) is produced by the lung both in vitro and in vivo, relaxes vascular smooth muscle, and inhibits platelet aggregation (Gryglewski, 1979a; Hensby, 1979). Due to the rapid hydrolysis of both of these vasoactive compounds, it is necessary to assay their stable breakdown products, thromboxane B<sub>2</sub> (TxB<sub>2</sub>) and 6-keto-PGF<sub>1α</sub>.

Frolich et al. (1980) and Demling et al. (1981) reported that TxB<sub>2</sub> concentration in plasma and lung lymph increases following endotoxin administration in the sheep. These studies suggest that the lung is a tissue source of thromboxane. However, more direct evidence, such as a positive transpulmonary plasma TxB<sub>2</sub> concentration gradient, was not reported in either study. This was a major goal of our study in the awake unanesthetized sheep.

Our initial studies confirmed that endotoxin infusion into sheep caused profound leukopenia. This loss of white cells from the circulation has been hypothesized due to intrapulmonary sequestration of polymorphonuclear granulocytes (Coalson et al., 1979). Leukocytes are capable of thromboxane synthesis in vitro following endotoxin challenge (Spagnuolo et al., 1980), and might therefore play a role in mediating endotoxin-induced pulmonary vasoconstriction. To elucidate the role of leukocytes in the
pulmonary hypertension after endotoxin infusion, we assayed plasma TXB₂ and 6-keto-PGF₁α concentrations, assessed transpulmonary gradients, and measured pulmonary hemodynamics in sheep rendered acutely leukopenic by nitrogen mustard treatment.

Methods

Sixteen Suffolk lambs (20–32 kg) were anesthetized with halothane, intubated, and mechanically ventilated with 98% oxygen and 2% halothane. The femoral artery was cannulated with a 3-mm outside diameter (o.d.) polyvinyl chloride catheter advanced to the mid-thoracic aorta for arterial blood sampling and pressure monitoring. A #7 French flow-directed thermomodulation catheter (Elecath Co.) with a proximal injection site 12.5 cm from the tip was advanced via the femoral vein into the pulmonary artery, permitting arterial blood pressure (BP) and pulmonary artery wedge pressure (PAWP) measurements of mean pulmonary artery pressure (PAP) and pulmonary artery wedge pressure (PAWP).

Right ventricular end-diastolic pressure (RVEDP) was measured through the proximal port of a modified thermomodulation catheter. Pulmonary vascular resistance (PVR) was calculated by subtracting PAWP from PAP and dividing by the cardiac output (CO). Systemic vascular resistance (SVR) was calculated by subtracting RVEDP from the mean arterial blood pressure (MAP) and dividing by the CO. RVEDP was presumed equal to central venous pressure to calculate SVR. Pressures were continuously measured using Hewlett-Packard 1280C transducers and an 8-channel recorder (Hewlett-Packard 7798A). CO was determined by the thermomodulation method and calculated by a computer (Elecath Co., model COC 4000). Five milliliters of 0°C Ringer’s lactate were injected for each measurement. Each CO value was taken as the average of three determinations. Two to 4 hours after recovery from anesthesia and surgery, endotoxin (lipopolysaccharide W, E. coli 0111:B4, Difco Laboratories) from a single lot (#3122-25) was diluted in sterile Ringer’s lactate to a final concentration of 4 µg/ml, and infused intravenously at a dose of 1 µg/kg over a 5-minute period. Three groups of sheep were studied. Group 1 (n = 6) served as controls, receiving only an endotoxin injection. Group 2 (n = 6) was rendered leukopenic by a single intravenous injection of nitrogen mustard (1.5 mg/kg) 72 hours prior to study. Group 3 (n = 4) was treated with 12 mg/kg ibuprofen. Ibuprofen was dissolved at 50 mg/ml in sterile glycine buffer containing 0.9% wt/vol benzyl alcohol and stored in sealed glass vials (Upjohn Co.). Appropriate dilutions were made with 10 ml of Ringer’s lactate and infused intravenously at a rate of 50 µg/min one hour prior to endotoxin injection. Aortic and mixed venous blood samples for analysis of TXB₂, 6-keto-PGF₁α, and PGF₂α were simultaneously drawn every 5 minutes for the first 30 minutes after endotoxin infusion and then every 15 minutes for 2 hours. Five milliliter blood samples were drawn from the pulmonary artery and aorta with plastic syringes and transferred to glass test tubes containing 0.05 ml of 15% EDTA through a 18-g needle. The sample was immediately placed on ice, then centrifuged at 2500 g (Damon IEC PR5000) at 4°C for 10 minutes. Plasma was separated, transferred to polypropylene tubes, and frozen at −80°C.

Standard PGF₂α, tromethamine salt, 6-keto-PGF₁α, and TXB₂ were gifts from Dr. John Pike, Upjohn Co. Radiolabeled eicosanoids [³H]-PGF₂α (100–150 Ci/mmol), [³H]-6-keto-PGF₁α (100 Ci/mmol), and [³H]-TXB₂ (100–150 Ci/mmol) were purchased from New England Nuclear. Specific goat anti-rabbit γ-globulin was obtained from Clinical Assays. Specific antisera to PGF₂α was a gift from Dr. Lawrence Levine, Brandeis University. Specific antisera to 6-keto-PGF₁α and TXB₂ were purchased from Seragen, Inc. Arachidonic acid was obtained from the Sigma Chemical Co.

Plasma PGF₂α was measured directly in unextracted plasma as previously described (Levine et al., 1971; McCosh et al., 1976).

Both 6-keto-PGF₁α, and TXB₂ were assayed by similar double antibody radioimmunoassay techniques. The TXB₂ antibody was used at a dilution of 1:4000, and the absolute limits of the sensitivity of the antibody measurement were 6 and 300 pg by Rodbard’s probit analysis (Delean et al., 1978). For convenience, corrected concentrations of 50 pg/ml or less were taken as the lower limit of the plasma TXB₂ assay, as the curve is still linear at this point. Cross-reactivities at B/Bo were: arachidonic acid <0.1%, PGF₂α 0.1%, PGE₂ 0.3%, PGD₂ 2.1%, PGF₂α 4.5%, and TXB₂ <0.1%.

We conducted control experiments in which either indomethacin (25 µg/ml whole sheep blood) or ibuprofen (25 µg/ml blood) was added to EDTA-containing tubes prior to sample addition. No differences in plasma 6-keto-PGF₁α, or TXB₂ concentrations were measured in EDTA alone, EDTA and indomethacin, or EDTA and ibuprofen. Thus, significant ex vivo generation did not take place when this method of sample preparation was used.

In nine sheep, three from each group, we measured extravascular lung water. At the conclusion of the 2-hour study period, a reference blood sample for hemoglobin determination was drawn from the arterial cannula, and the sheep were killed with an overdose of barbiturate. The sternum was split and the lungs were rapidly removed after cross-clamping both lung hila. Excised lungs were passively drained of blood, weighed, and homogenized in a blender (Waring 5011) with an equal volume of water. Water content of the homogenate, the supernatant fraction of the centrifuged homogenate and whole blood, was measured by drying them to constant weight (48 hours) at 80°C. Total hemoglobin of the blood and the homogenate supernatant fraction was measured by the cyanmethemoglobin method. Calculation of extravascular lung water after correction for the intravascular blood water content was carried out as outlined by Pearce et al. (1965).

Simultaneous arterial and mixed venous blood samples were drawn to assess pH and blood gas tensions (Corning, model 175). Leukocytes were counted with a Coulter cell counter (model ZF). Platelets were counted by phase microscopy using the method of Brecher and Cronkite (1950). All data were stored in a PDP 11/10 computer. Student’s paired t-test was used to compare values before and after endotoxin administration in individual groups. An uncorrelated Student’s t-test was employed to compare values between group 1 and groups 2 or 3 (Snedecor, 1959). For single comparisons, a probability of less than 0.05 was considered to be statistically significant. For multiple comparisons in Table 1, we considered a probability of less than 0.001 to be statistically significant. All data are presented as the mean ± sem.
Results

Sheep in all three groups survived the endotoxin infusion and appeared well and hemodynamically stable at the conclusion of the experiments (i.e., 2 hours after endotoxin infusion). In group 2 sheep, pretreated with nitrogen mustard, we noted occasional episodes of diarrhea commencing 24-36 hours after the administration of the drug. Apart from this side effect, the animals appeared alert and were eating and drinking. All sheep had a normal core temperature prior to study and were infused one liter of Ringer’s lactate solution during surgery to correct any dehydration due to diarrhea.

Hemodynamic Variables

In group 1 sheep, both PAP and PVR increased significantly 20 minutes after endotoxin infusion and gradually declined, almost reaching baseline values by 120 minutes (Table 1). The time course of the pulmonary artery hypertension is shown in Figure 1A. CO remained unchanged after endotoxin infusion (baseline value 3.4 ± 0.2 liter/min) with a significant increase in heart rate (HR) from 100 ± 6 to 150 ± 11 beats/min at the time of peak PAP. HR remained significantly increased above the normal at 120 minutes (127 ± 8 beats/min). Both PAWP and RVEDP were increased significantly at 20 minutes, coinciding with the peak of PAP. PAWP increased from 6 ± 0.6 to 10 ± 0.8 mm Hg and RVEDP from 3.5 ± 0.7 to 9.0 ± 0.7 mm Hg. At that time, mean arterial BP significantly rose from 98 ± 4 to 109 ± 4 mm Hg. However, there was no significant change of SVR. The increases of PAWP, RVEDP, and BP were transient, and these values returned to baseline by 45 minutes after endotoxin infusion.

After endotoxin infusion, group 2 sheep developed an increased PAP and PVR (Fig. 1A; Table 1). However, the increases were significantly less than group 1 sheep (Table 1). CO was unchanged from the baseline mean value of 3.1 ± 0.2 liter/min. The heart rate significantly increased from 97 ± 6 to 130 ± 9 beats/min at 20 minutes, then returned gradually to baseline at 120 minutes. As in group 1, 20 minutes after endotoxin infusion, a transient but significant increase was noted of PAWP (from 4.8 ± 0.3 to 6.5 ± 0.6 mm Hg) and RVEDP (from 3.3 ± 0.4 to 7 ± 0.9 mm Hg). BP remained unchanged from the baseline value of 86 ± 4 mm Hg. The baseline BP, CO, and SVR of group 2 was not different from group 1 sheep. In group 3 sheep, small but statistically significant increases occurred in PAP and PVR after endotoxin infusion (Fig. 1A; Table 1). There was no change of CO, PAWP, RVEDP, or HR.

Plasma TxB2, 6-Keto-PGF1α and PGF2α

Figure 1A displays the change of PAP with time and the aortic plasma concentrations of TxB2 (Fig. 1B) and 6-keto-PGF1α (Fig. 1C) after endotoxin infusion in the three groups of sheep. In group 1 sheep, plasma TxB2 increased significantly from a pre-endotoxin concentration of 0.4 ± 0.1 ng/ml to 16 ± 3 ng/ml by 30 minutes after endotoxin infusion and then gradually declined to baseline values by 120 minutes. A correlation (r = 0.87, P < 0.025) was found between the increase of plasma TxB2 and PAP. Plasma concentrations of 6-keto-PGF1α increased significantly after endotoxin infusion, but in comparison to the plasma TxB2 increase, the onset was slower and delayed, reaching peak concentrations by 45 minutes (Fig. 1C).

In four group 1 sheep, blood samples were drawn simultaneously from the pulmonary artery and aorta at precisely 30 minutes after endotoxin infusion and then assayed for TxB2 and 6-keto-PGF1α. All four sheep demonstrated significant large transpulmonary
plasma TxB2 concentration gradients. These data are summarized in Table 2.

Positive transpulmonary plasma 6-keto-PGF1α gradients were measured 30 minutes after endotoxin infusion in three of four sheep. These values are reported in Table 3. In four sheep, the aortic plasma concentrations of PGF2α increased significantly from a baseline value of 113 ± 15 to 578 ± 140 pg/ml by 25 minutes after endotoxin infusion and then gradually declined to baseline values at 60 minutes after endotoxin infusion. Figure 2 illustrates in four group 1 sheep the correspondence between the increase of aortic plasma PGF2α concentration and the PAP.

In group 2 sheep, plasma TxB2 concentration increased significantly from 0.3 ± 0.1 to 5.5 ± 1.2 ng/ml at 30 minutes after endotoxin infusion and gradually declined to baseline values by 120 minutes. Peak plasma TxB2 concentrations were significantly lower in group 2 when compared to group 1. A correlation (r = 0.91, P < 0.025) was found between the increase of plasma TxB2 and PAP. Plasma 6-keto-PGF1α concentrations also increased significantly, reaching peak values at 45 minutes after endotoxin infusion (Fig. 1c). In four group 2 sheep at 30 minutes after endotoxin infusion, the pulmonary artery and aortic blood samples were analyzed for TxB2 and 6-keto-PGF1α. Positive transpulmonary plasma TxB2 gradients were measured in all four sheep. These data are presented in Table 2. No consistent transpulmonary gradients of plasma 6-keto-PGF1α were demonstrated in these four sheep. Plasma PGF2α concentrations measured in three group 2 sheep increased from 170 ± 48 to a peak value of 480 ± 76 pg/ml at 30 minutes after endotoxin infusion.

In group 3 sheep pretreated with ibuprofen, the plasma TxB2 concentration increased slightly from levels undetectable by radioimmunoassay to 240 ± 80 pg/ml at 30 minutes (Fig. 1B). Plasma 6-keto-PGF1α

### Table 3

<table>
<thead>
<tr>
<th>Pulmonary Artery and Aortic Plasma Concentrations of 6-Keto-PGF1α in Four Group 1 Sheep following Endotoxin Infusion</th>
</tr>
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<tr>
<td></td>
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<tr>
<td>PA A</td>
</tr>
<tr>
<td>Pre-endotoxin</td>
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<td>30 minutes</td>
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1, 2, 3, 4 are individual sheep. PA = pulmonary artery; A = aorta. Plasma 6-keto-PGF1α concentrations are expressed as pico-grams per milliliter.

### Table 2

Transpulmonary Plasma TxB2 Gradients (ng/ml) in Four Control and Four Leukopenic Sheep following Endotoxin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary artery</th>
<th>Aorta</th>
<th>ΔTxB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-endotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.34 ± 0.1</td>
<td>0.33 ± 0.08</td>
<td>-0.01 ± 0.1</td>
</tr>
<tr>
<td>Leukopenic</td>
<td>0.40 ± 0.1</td>
<td>0.41 ± 0.1</td>
<td>+0.01 ± 0.2</td>
</tr>
<tr>
<td>30 Minutes post-endotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.6 ± 2.2*</td>
<td>12.3 ± 3.3*</td>
<td>+3.7 ± 1.1*</td>
</tr>
<tr>
<td>Leukopenic</td>
<td>2.5 ± 0.8*</td>
<td>5.9 ± 1.1*</td>
<td>+3.1 ± 0.7*</td>
</tr>
</tbody>
</table>

* P < 0.01, values differ significantly from pre-endotoxin plasma TxB2 concentration by paired t-test.
† P < 0.01, the aortic TxB2 concentration differs significantly from the pulmonary artery concentration.
concentrations always remained below the detection limit of the radioimmunoassay (Fig. 1C).

Arterial Blood Gas Tensions and pH

After endotoxin infusion, both group 1 and group 2 sheep developed signs of respiratory difficulty with rapid, shallow, and labored breathing. The severity of distress (e.g., tachypnea) appeared more pronounced in group 1 animals. In both group 1 and 2 sheep, the $P_{aO_2}$ fell significantly 20 minutes after endotoxin infusion (Table 1). This decline of $P_{aO_2}$ was significantly less in group 2 sheep when compared to group 1 (Table 1). Group 3 sheep appeared unaffected by the endotoxin infusion and their $P_{aO_2}$ was unchanged (Table 1). There was no significant change of $P_{aCO_2}$ or $pH$ in any group.

Leukocyte and Platelet Concentrations

In group 2 sheep, nitrogen mustard treatment reduced the circulating leukocyte concentrations to less than 1000/mm$^3$ (Table 1). Platelet count was unchanged in this group when compared to group 1 and 3 sheep. There was no change of platelet concentra-

tion in any group following endotoxin infusion (Table 1). A significant reduction of leukocyte concentration was measured in group 1 and 3 sheep, and was most pronounced between 20 and 30 minutes after endotoxin infusion (Table 1).

Postmortem Lung Water Determination

Extravascular lung water 2 hours after endotoxin infusion was 78 ± 0.6% in group 1 ($n = 3$), 77.8 ± 0.7% in group 2 ($n = 3$), and 79.1 ± 0.5% in group 3 ($n = 3$). In this laboratory, previous determinations of extravascular lung water of seven control sheep of comparable size averaged 78.1 ± 0.6% (Delaney et al., 1979). Thus, at 2 hours, there was no significant difference of extravascular lung water between any group and the control.

Discussion

This study demonstrates that intravenous infusion of endotoxin into sheep causes transient pulmonary artery hypertension with the subsequent release of thromboxane and prostacyclin from the lung. The plasma concentration of $TxB_2$ rises within minutes after the increase of pulmonary artery pressure (Fig. 1). The pulmonary hypertension appears mediated by prostaglandins and thromboxane, since pretreatment with ibuprofen, a cyclooxygenase inhibitor (Gryglewski, 1979b), prevents the rise of pulmonary artery pressure, as well as the increase of plasma $TxB_2$ and $PGF_{2\alpha}$ concentrations. Leukopenia induced by nitrogen mustard therapy prior to endotoxin infusion attenuates the pulmonary hypertension and reduces by half the peak plasma concentrations of $TxB_2$. This suggests that, in addition to lung tissue, leukocytes are a major contributing source of endotoxin-stimulated thromboxane synthesis.

Following intravenous endotoxin infusion, thromboxane synthesis could occur in lung cells (Gryglewski et al., 1976), platelets (Moncada and Vane, 1978), leukocytes (Spagnuolo et al., 1980), and possibly cells in other organs. We measured large transpulmonary blood gradients of $TxB_2$ in both control (group 1) and leukopenic (group 2) sheep indicating a major pulmonary source exists of this vasoactive compound (Table 2). By application of the Fick principle, at a cardiac output measured at 3.2 liters/min with a pulmonary arteriovenous $TxB_2$ plasma difference of 3.7 ng/ml, the peak pulmonary $TxB_2$ release rate into the systemic circulation in group 1 sheep 30 minutes after endotoxin injection amounted to 12 pg/min. This was accompanied by marked pulmonary hypertension but without a change of the systemic vascular resistance. The lack of generalized systemic vasoconstriction might be explained, if, following intrapulmonary synthesis and pulmonary vasoconstriction, thromboxane $A_2$ diffused into the pulmonary circulation and was rapidly converted to the weak vasoconstrictor $TxB_2$. In addition, systemic and pulmonary vessels may have different sensitivities of response to circulating thromboxanes (Schuette et al., 1982).
Although we have no direct evidence we believe that the pulmonary hypertension following endotoxin infusion is the result of pulmonary vasoconstriction rather than mechanical obstruction of the lung’s circulation by platelet or white cell aggregates. Platelet counts did not change after endotoxin, making platelet microembolism unlikely. Pulmonary vascular resistance after endotoxin increased markedly in sheep rendered neutropenic, making major white cell vascular obstruction unlikely. In addition, in normal sheep, ibuprofen treatment abolished the increase of pulmonary vascular resistance and hypertension after endotoxin but not the transient leukopenia. Leukocytes disappear from circulating blood, but it is unlikely that they are a primary obstructive cause of the reversible pulmonary hypertension.

Endotoxin infusion into either control or ibuprofen treated sheep was associated with a marked 70% decrease in circulating leukocyte concentrations. Since human granulocytes release TxB₂ when incubated in vitro with E. coli endotoxin (Spagnuolo et al., 1980), we hoped to reduce the quantity of thromboxane synthesized by circulating leukocytes by inducing leukopenia with nitrogen mustard treatment. In leukopenic sheep, the peak plasma concentrations of TxB₂ were significantly decreased when compared to controls (Fig. 1B). This suggests a role for circulating sheep leukocytes as a source of thromboxane production after endotoxin infusion. However, despite a 90% reduction of circulating leukocyte concentrations, the lung released thromboxane after endotoxin infusion, as evidenced by consistent transpulmonary plasma gradients of TxB₂ (Table 2). The attenuated pulmonary hypertension of leukopenic sheep in comparison to the control parallels their reduced peak plasma concentrations of TxB₂ (Fig. 1).

Our results contrast with the study of Heflin et al. (1979), who did not measure a reduced pulmonary hypertension following endotoxin infusion in sheep made neutropenic with hydroxyurea. Nitrogen mustard therapy may interfere with arachidonic acid metabolism in various cells (e.g., platelets, white cells, etc.) or reduce pulmonary vascular reactivity to eicosanoid vasoconstrictor compounds. Although there was no significant difference in platelet concentration between our normal and leukopenic sheep, nitrogen mustard may bind to platelet receptor sites, alter arachidonic acid metabolism, or induce other alterations of platelet biochemistry.

For several reasons, it is likely that TxA₂—which we measured as its stable breakdown product, TxB₂—mediated the endotoxin-induced pulmonary hypertension. TxB₂ itself is only a weak vasoconstrictor (Friedman et al., 1979). However, TxA₂ is a potent vasoconstrictor produced by the metabolism of arachidonate and 50- to 100-fold more vasoactive in vitro than PGI₂ (Hamberg et al., 1975). After endotoxin, increases of plasma PGI₂ concentration also paralleled the rise in PAP, but the maximum PGI₂ concentration was many times smaller than the peak plasma TxB₂ concentration (Fig. 2). Infused PGI₂ is a weak pulmonary vasoconstrictor in sheep and far less potent than the cyclic endoperoxide, PGI₂, which is the obligatory precursor of both thromboxanes and prostaglandins (Bowers et al., 1979). It is unclear whether the pulmonary vasoconstrictor effects of PGI₂ are due to its rapid conversion to TxA₂.

The increase of plasma TxB₂ after endotoxin infusion correlated (r = 0.87) with the increase of PAP, although the peak PAP consistently preceded the peak TxB₂ concentration by 5 to 10 minutes. This time lag may be explained if TxA₂ is synthesized in the lung, induces local vasoconstriction, and gradually diffuses into the bloodstream as the stable breakdown product TxB₂. Very small local concentrations of endoperoxides, thromboxanes, and prostaglandins may cause pulmonary vasoconstriction long before plasma TxB₂ concentrations rise to high levels.

The peak plasma TxB₂ concentrations we measured are higher and the values for 6-keto-PGF₁α are lower than those reported by Demling et al. in sheep. These differences may be due to differences in the radioimmunoassay techniques, as well as the greater quantity of endotoxin they infused.

The hemodynamic effect of the elevated plasma concentration of 6-keto-PGF₁α is unclear. Prostacyclin is a major product of arachidonate metabolism in vascular endothelium, a vasodilator and an inhibitor of platelet aggregation (Gryglewski, 1979b). We measured the transpulmonary release of 6-keto-PGF₁α in three of four group 1 sheep, suggesting a pulmonary vascular endothelial source for this compound (Table 3).

Endotoxemia in the sheep is characterized by an increased lung vascular permeability (Brigham et al., 1979), transient hypoxemia, and profound peripheral leukopenia. Despite increased lung transvascular fluid filtration due to increased hydrostatic forces and, subsequently, increased lung vessel permeability, we did not measure an increased extravascular lung water content 2 hours after endotoxin infusion. The efficiency of the pulmonary lymphatics in clearing excess interstitial fluid and the small dose of endotoxin we infused explain this finding. The transient hypoxemia that occurs after endotoxin infusion (Table 1) is probably best explained by a ventilation-perfusion inequality. Severe bronchoconstriction with a decreased lung compliance occurs after endotoxin infusion in sheep (Peter Hüttemeier, unpublished observations). Bronchoconstriction has been attributed to the actions of both the prostaglandins, thromboxanes, and leukotrienes such as SRSA (Gryglewski et al., 1976; Sirois et al., 1980). In addition, the pulmonary artery hypertension may redistribute pulmonary blood flow to alveoli that are poorly ventilated. The decline of Pao₂ parallels the severity of pulmonary hypertension and was abolished by ibuprofen and significantly attenuated in leukopenic animals.

The precise mechanism by which endotoxin stimulates pulmonary production and release of thromboxane and prostacyclin is poorly understood and probably complex. Many stimuli can cause prostaglandin release from the lung and other tissues (Piper and Vane, 1971). Cooper at al. (1980) have demon-
Stratified that activated complement infusion can trigger thromboxane release in the sheep. Since endotoxin activates complement both directly and indirectly, it may be that this is a stimulus to both the release of thromboxane and the leukopenia (Muller-Eberhard, 1975; McKay, 1973; Craddock et al., 1977). Clearly, further studies are necessary to determine the precise mechanism triggering lung cells to synthesize and release thromboxanes and prostaglandins following endotoxin infusion.

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Dr. Peterson is an Established Investigator of the American Heart Association.

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