hibit the cyclooxygenase pathway) increased both the rate of development and magnitude of the hypoxic response in the control lungs. Work by others has suggested that such an effect of meclofenamate is due to inhibition of prostacyclin release. The ANTU-treated rat lungs were not affected by meclofenamate. However, the threshold dose for either angiotensin II or hypoxia did not change after giving meclofenamate. Thus, while it is possible that ANTU damage to endothelium inhibited the release of a vasodilator prostaglandin during hypoxia, there probably are additional factors accounting for the enhanced vasoreactivity.

The discussion proceeded to the morphologic features of the lung vessels in rats having pulmonary hypertension from acute ANTU administration. In the capillaries, some of the endothelial cells were lifted off the basement membrane but were not gone entirely. In either case, the endothelial cell might function abnormally. Although the arterioles and venules had such injury, it seemed less extensive than in capillaries. There was also perivascular edema.

A question concerned the mechanism of acute injury with ANTU and the possible development of tolerance to repeated ANTU administration. A recent publication has implicated serotonin in the mediation of part of the ANTU injury. Also, work by Taylor has suggested that the neutrophils were involved. Although others have described tolerance, the investigators found edema, as evidenced by increase in lung wet to dry weight, following each weekly injection over four weeks in rats weighing 200 to 250 g. Age may be important in that the response was different in older rats. Further, with repeated injections, the right ventricular hypertrophy began to develop after three weeks.

Does the endothelial cell have subcellular elements indicating that it is metabolically a very active cell, and are these elements altered by ANTU? The ensuing discussion indicated that endothelial cells have by electron microscopy, extensive evidence of golgi activity. Further, with proper preparation one sees extensive networks of mitochondria, and branching is present. Thus the endothelium is probably very active metabolically, though the effect of ANTU on the metabolic elements is not known.

Did the morphologic features of the lung vessels in the pulmonary hypertensive phase after chronic ANTU administration show inflammation or intra-arterial vascular obstruction? Neither with acute nor chronic administration of ANTU did the investigators find thrombi, platelet aggregates, or swollen endothelial cells. They looked carefully for inflammatory infiltrates and found none. They did not have evidence on the effect of vasodilators. However, pressure-flow curves in lungs from the pulmonary hypertensive rats were normal, a finding compatible with the absence of fixed vascular obstruction.

**Endothelial Function in Clinical Pulmonary Hypertension**

Robyn J. Barst, M.D.;† and S. Alex Stalup, M.D.‡

The endothelium regulates the concentrations of several types of vasoactive substances that affect pulmonary vascular tone, and endothelia can oppose vasoconstriction in some circumstances by releasing vasodilators. To assess some of these endothelial functions in patients with pulmonary hypertension, we made measurements of selected vasoactive substances before and during attempts at pharmacologic vasodilatation. Studies were performed in 16 patients (14 to 23 years of age) with either idiopathic pulmonary hypertension (n = 11) or pulmonary hypertension as a consequence of unexpected early pulmonary vascular disease accompanying congenital heart defects (n = 5). In six of ten children, norepinephrine levels were elevated, and in two of the six, the concentrations of norepinephrine were greater in the aorta than in the pulmonary artery. In four out of 16 patients, thromboxane levels were increased, and in three of the four, the concentrations of thromboxane were greater in the aorta than in the pulmonary artery. These concentration gradients suggest pulmonary release of these vasoconstrictors. Identification of the contribution to pulmonary vasoconstriction made by changes in the endothelial metabolism of vasoactive substances may lead to a more fundamental understanding of the control of the pulmonary circulation, and hence lead to more specific modes of therapy for pulmonary hypertension.

A variety of interrelated vasoconstrictors and vasodilators, including prostaglandins, biogenic amines, and peptides, are known to modulate pulmonary vascular tone. Several studies have emphasized the importance of the pulmonary endothelial cell in the metabolism of these vasoactive mediators and have implicated endothelial cells in the local release of vasoactive agents. These studies suggest that modification of smooth muscle tone can occur in a number of ways by changes in pulmonary endothelial function. For example, the endothelium serves as a mechanical barrier interposed between vasoactive substances circulating in blood and the smooth muscle cell. Hence, a change in the permeability of the endothelial barrier may increase the amount of vasoactive substances reaching receptors on the smooth muscle cell. Also, endothelium selectively take up and degrade vasoactive substances with potent abilities to change smooth muscle tone, eg, norepinephrine, serotonin, and the prostaglandins E and F. Thus, it is reasonable to hypothesize that changes in the metabolism of vasoactive substances, eg, as the result of lung injury, can alter pulmonary vascular tone by altering the balance between vasoconstriction and vasodilation.
This article presents our recent studies of two mediator systems in which the balance between vasoconstrictors and vasodilators in each system influences pulmonary vascular tone: (1) the sympathetic nervous system (α- and β-adrenergic activity) and (2) the arachidonic acid cascade (thromboxane A₂ and prostacyclin). Studies were performed in 16 children and young adults (2½ to 23 years of age) with either idiopathic pulmonary hypertension (n = 11) or pulmonary hypertension as a consequence of unexpected early pulmonary vascular disease accompanying congenital heart defects (n = 5).

CIRCULATING CATECHOLAMINES IN PATIENTS WITH PULMONARY HYPERTENSION

Alpha-adrenergic stimulation is known to increase pulmonary vascular tone with the release of norepinephrine from the lung, and β-adrenergic agonists can induce vasodilatation when pulmonary vascular tone is increased. Several recent studies suggest a specific defect in the endothelial uptake and degradation of norepinephrine (a pulmonary vasoconstrictor) in pulmonary hypertension. The normal lung endothelium extracts 17 percent to 30 percent of the norepinephrine that passes through its circulation. Loss of the lung's ability to extract circulating norepinephrine in patients with pulmonary hypertension has been described. In addition, increased circulating levels of norepinephrine have been measured in some patients with pulmonary hypertension.

We measured resting plasma catecholamine concentrations in ten children (1½ to 15 years of age) with pulmonary hypertension. For the radioenzymatic assay for norepinephrine established in our laboratory (n = 26), the interassay coefficient of variation is 12 percent and the intra-assay coefficient of variation is 9 percent. The method is sensitive to <50 pg/ml. We excluded patients with evidence of congestive heart failure or severe hypoxemia. Mixed venous norepinephrine concentrations ranged from 208 to 2,900 pg/ml in the ten children with pulmonary hypertension (and were greater than 500 pg/ml in six of the ten patients); values for a control group (n = 4) ranged from 196 to 265 pg/ml. Examination of simultaneous mixed venous and systemic arterial norepinephrine concentrations showed pulmonary handling of norepinephrine that varied from a 43 percent net uptake to 54 percent net release. In two of the six patients with elevated catecholamines, the concentration of norepinephrine was ≥35 percent higher in aortic blood than in mixed venous blood, ie, more norepinephrine left the lung than entered it. This suggests pulmonary release of norepinephrine.

Several observations suggest a possible contribution of the sympathetic nervous system toward the maintenance or exacerbation of pulmonary hypertension in some patients. Although exercise and crying increase circulating catecholamines, mean pulmonary arterial pressure does not normally rise more than 10 mm Hg. However, we have observed that in patients with increased sympathetic tone and pulmonary hypertension, crying or agitation may further increase norepinephrine levels and also further increase pulmonary pressures. (For a discussion of differential effects of norepinephrine on pulmonary vascular tone in pulmonary hypertension, see the article by A. L. Hyman). Whether such increases in plasma norepinephrine levels result from increased release or decreased uptake is unknown. In any case, some pulmonary hypertension patients may respond to α-adrenergic blocking agents with a decrease in pulmonary arterial pressure. These observations suggest that elevated norepinephrine concentrations may identify patients in whom enhanced sympathetic tone contributes to pulmonary vasoconstriction. Such patients may benefit from α-adrenergic receptor blockade, β-adrenergic agonists, or both.

LUNG INJURY AND ALTERATIONS IN THE BALANCE BETWEEN PULMONARY VASOCONSTRICTION AND VASODILATION: ROLE OF ARACHIDONATE METABOLITES

There is accumulating evidence that products of the arachidonic acid cascade are involved in the control of the pulmonary circulation. Moncada and Vane suggested that the balance between thromboxane A₂ (TXA₂), a potent vasoconstrictor, and prostacyclin (PGI₂), a potent vasodilator, may be more important than their absolute concentrations in regulating vascular tone. Recent studies emphasize the importance of the products of the lipoxygenase pathway of arachidonic acid, eg, leukotrienes, in modulating pulmonary vascular tone and in interacting with the cyclooxygenase pathway, ie, by inducing TXA₂ release. Thus, it is possible that leukotrienes, to some extent, exert their actions via release of TXA₂.

Several mechanisms influence the relative proportion of constricting (thromboxane) and dilating (PGI₂, PGE) eicosanoids produced when arachidonate is released by phospholipase. A basic mechanism is the amount of arachidonate released into the cyclooxygenase pathway: low levels (as occurs in the normal lung) lead preferentially to prostacyclin formation, the higher affinity biosynthetic pathway. Increasing concentrations of arachidonate lead to increasing expression of the thromboxane synthetase pathway. Several factors influence arachidonate availability. A common endpoint is deformation of the endothelial cell membrane, as occurs with contraction of subjacent smooth muscle cells in the course of vessel constriction, and cell surface shear forces related to flow through the vessel. Also, pharmacologic stimuli, eg, angiotensin and bradykinin, induce prostaglandin release. It is generally held that the release of PGI₂ induced by angiotensin serves to oppose angiotensin-induced pulmonary vasoconstriction. PGI₂ is also released by bradykinin, and is believed to be a "second messenger" that enhances the vasodilating properties of the peptide.

Studies of endotoxia and thromboembolism suggest a role for products of the arachidonic cascade in the pathogenesis of the early pulmonary hypertension often seen in acute lung injury. In experimental thromboembolism, greater increases in TXB₂ (the stable breakdown product of TXA₂) were observed than increases in 6-keto-PGF₁α (the stable breakdown product of PGI₂). In endotoxia, rises in TXB₂ correlate well with the increases in pulmonary pressure observed. Furthermore, pretreatment with TXA₂ inhibitors prevents the early pulmonary hypertension seen after endotoxin administration.

We studied the relation between pulmonary hemodynamics and the balance of TXA₂ and PGI₂ in a 17-month-
old girl with severe pulmonary hypertension and clinical evidence of low-grade chronic lung injury of unknown cause.\textsuperscript{42} Resting measurements of prostanoids in unextracted plasma by radioimmunoassay showed an elevated TXB\textsubscript{2} to 6-keto-PGF\textsubscript{1\alpha} ratio due to increased TXB\textsubscript{2}. Intravenous (IV) infusions of PGI\textsubscript{2} reduced mean pulmonary arterial pressure (from 80 to 47 mm Hg), increased cardiac output (from 3.4 to 4.0 L/min), increased systemic arterial oxygen saturation (from 60 to 72 percent), and decreased the TXB\textsubscript{2} to 6-keto-PGF\textsubscript{1\alpha} ratio (from 6.0 to 0.2); mean systemic arterial pressure was unchanged (Fig 1). Not only did decreasing the TXB\textsubscript{2} to 6-keto-PGF\textsubscript{1\alpha} ratio by infusing PGI\textsubscript{2} reduce pulmonary pressures and increase systemic arterial oxygen saturation, but also reducing the ratio by decreasing thromboxane levels with oral nifedipine and diltiazem had the same effect (6-keto-PGF\textsubscript{1\alpha} levels did not change with administration of nifedipine or diltiazem). On chronic diltiazem therapy for 12 months, mean pulmonary arterial pressure decreased to 27 mm Hg with no appreciable change in systemic arterial pressure or flow; systemic arterial oxygen saturation increased to 90 percent and the TXB\textsubscript{2} to 6-keto-PGF\textsubscript{1\alpha} ratio decreased to 1.0 due to a decrease in TXB\textsubscript{2}. These findings support the hypothesis that the balance between TXA\textsubscript{2} and PGI\textsubscript{2} is important in regulating pulmonary vascular tone in some patients with pulmonary hypertension. Additionally, calcium-channel blockers may work independently of changes in thromboxane. Hence, TXB\textsubscript{2} may fall because pulmonary arterial pressure decreased, reducing surface shear forces (see below). The current data do not permit distinguishing between the relative contributions of these mechanisms.

\textit{Interaction Between Mechanical and Metabolic Factors}

Several studies\textsuperscript{28,31} suggest that arachidonate metabolism by pulmonary endothelial cells is altered by changes in flow dynamics, and that endothelial cell deformation in response to shear stress can increase the release of arachidonate, with normal healthy endothelial cells increasing PGI\textsubscript{2} release. These studies support the hypothesis offered by Rodbard\textsuperscript{34} that vascular shear stress controls vessel diameter. But since an increase in shear stress (from high flow and a large pressure drop across the vascular bed) can damage the endothelium,\textsuperscript{34} and injured endothelial cells may fail to release PGI\textsubscript{2}, the increased arachidonate released by the increase in shear stress may be diverted into the thromboxane synthetase pathway. This may lead to a vicious cycle of increased vasoconstriction, increased shear force, and increased damage, with perpetuation of the pulmonary hypertension.

In three patients with idiopathic pulmonary hypertension, concentrations of TXB\textsubscript{2} increased concomitantly with increases in pulmonary blood flow during IV infusions of PGI\textsubscript{2}. Aortic TXB\textsubscript{2} concentrations were greater than pulmonary artery concentrations, suggesting pulmonary production of TXA\textsubscript{2}. In the data obtained from the patient depicted in Figure 2, a modest degree of pulmonary vasodilatation was achieved with PGI\textsubscript{2} infusion. The question posed by this patient is whether complete vasodilatation could have been achieved if thromboxane had not been released in the lung. We have observed other patients in whom PGI\textsubscript{2} infusion increased pulmonary blood flow concomitant with increased pulmonary arterial pressure and with increased release of thromboxane. Failure of PGI\textsubscript{2} to vasodilate the pulmonary vascular bed in these patients may have resulted from the simultaneous release of thromboxane. It is possible that pulmonary hypertension can result from both decreased production of vasodilators such as PGI\textsubscript{2} (as in endothelial cell injury) and excess production of vasoconstrictors such as thromboxane. If excess production of vasoconstrictors is the cause, administration of exogenous vasodilators is unlikely to be of benefit unless the stimulus to increased release of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Relation between pulmonary hemodynamics and the balance of TXA\textsubscript{2} and PGI\textsubscript{2} (measured as their stable metabolites, TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha}, respectively) during IV PGI\textsubscript{2} infusion or sublingual nifedipine in a 17 month-old girl with idiopathic pulmonary hypertension. (Data modified from reference 30.)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Pulmonary release of TXB\textsubscript{2} concomitant with increased pulmonary blood flow during IV PGI\textsubscript{2} infusion in a five-year-old boy with idiopathic pulmonary hypertension.}
\end{figure}
vasoconstrictors can be overcome. These patients suggest that altered endothelial cell function (perhaps related to cell injury) may modify arachidonate metabolism and exacerbate and perpetuate pulmonary hypertension in some patients. The pattern of arachidonate metabolites that we measured (increased TXB₂ concentrations and/or the release of thromboxane when pulmonary blood flow increased) is consistent with changes in arachidonate metabolism seen in experimental models of lung injury.⁷⁸

Common to studies of several models of lung injury is damage to the endothelium, concomitant with decreased uptake of biogenic amines (serotonin and norepinephrine) and prostaglandins (PGE and PGF₂α), decreased metabolism of vasoactive peptides (angiotensin I and bradykinin), and the release of TXA₂ and PGI₂. Identification of the contribution to pulmonary vasconstriction made by changes in the endothelial metabolism of vasoactive substances may lead to a more fundamental understanding of the control of the pulmonary circulation, and hence to more specific modes of therapy for clinical pulmonary hypertension.

ACKNOWLEDGMENTS: The authors thank Dr. Allen Cato of the Burroughs Wellcome Company for his critical evaluation of this manuscript. Epoprostenol sodium (Flolan®), is synthesized by The Upjohn Company and formulated by Wellcome Foundation Limited. The Burroughs Wellcome Company graciously supplied the prostacyclin (epoprostenol sodium) used in these studies. This work was supported in part by NIH Clinical Investigator Award HL 01311 (Dr. Barst) and NIH research grant HL 14218 (SCOR).

REFERENCES
4 Bakhle YS, Vane JR. Pharmacokinetic function of the pulmonary circulation. Physiol Rev 1974; 1007-45
10 Gillis CN, Greene NM, Cronau LH, Hammond GL. Pulmonary extraction of 5-hydroxytryptamine and norepinephrine before and after cardiopulmonary bypass in man. Circ Res 1972; 30:666
32 Rodhard S. Vascular calibrator. Cardioiology 1975; 60:4-49
33 Fry DL. Acute vascular endothelial changes associated with increased blood velocity gradients. Circ Res 1968; 22:165-97
DISCUSSION

The discussion opened with a question of how the norepinephrine (NE) concentrations correlated with the severity of pulmonary vascular disease. There is evidence that in patients with high pulmonary vascular resistance, the lung either decreased its removal of NE or increased its production, or both. In addition, when the children became aggrivated during the heart catheterization, agitation or anxiety which would not ordinarily increase pressure in normal individuals increased pulmonary arterial pressure and caused NE to be elaborated from the lung. Some pressure rises could be partially inhibited by alpha blockade. This suggests that NE was in part responsible for moment-to-moment exacerbations of pulmonary hypertension.

Given the inability of the patients and the errors inherent in the analyses, discussion ensued as to the reliability of interpreting findings derived from single time points. The coefficient of variation (SD/mean x 100) of the enzymatic assay (REA) for NE was 11 percent which meant that a difference between arterial and venous samples needed to exceed 20 percent to reliably show a gradient. However, at least two other laboratories, using other techniques, have reported that pulmonary hypertension interferes with the NE uptake by the lung. In the present study when serial samples from a single site showed changes exceeding 11 percent with corresponding changes in pulmonary arterial pressure, the measurements were deemed relevant and valid.

However, the problem of establishing cause and effect was complex. During the catheterization, should the child become upset, there appeared to be a sympathetic discharge which amplified the pulmonary hypertension and may have helped sustain it. In some of the patients, when they were sedated, the pulmonary arterial pressure fell. However, these transient pressure and NE increases have not constituted sufficient rationale for chronic alpha adrenergic blocking therapy since the contribution of increased sympathetic tone to chronic pulmonary hypertension remains unknown. Also, in patients with heart failure, high sympathetic tone may be necessary to sustain systemic arterial pressure, perhaps at the expense of increased pulmonary artery pressure. Alpha blockade in these patients may have a therapeutic effect in the pulmonary circulation while producing systemic arterial hypotension.

The question was raised as to whether NE was a very effective pulmonary pressor agent given that published work done with stellate ganglion stimulation has shown relatively small increases in pulmonary arterial pressure. It seems likely that in the normal pulmonary circulation with its low resting tone, sympathetic stimulation might have little effect. However, when tone is already high, a further sympathetic constrictor stimulus may produce a disproportionate increase in pressure. Further, although circulating levels of NE do reflect total sympathetic tone, they bear little relation to regional tissue levels because not all of the NE released locally in tissue gets into the blood and then it becomes substantially diluted.

The question was raised as to whether the elevated thromboxane levels seen in two of the patients originated from endothelial cells or from platelets, and whether the thromboxane was contributing to the pulmonary hypertension. The observation was made that administration of nifedipine was followed by a fall in pulmonary vascular resistance and a fall in thromboxane, raising the possibility that the nifedipine had inhibited the release of thromboxane. One possibility was that the thromboxane was liberated from endothelial cells. Alternatively, the high thromboxane reflected release from platelets which is a calcium dependent event, and hence may be affected by calcium antagonists.

Whether thromboxane was contributing to a major extent to pulmonary hypertension was also not clear. On the one hand, it is a potent pulmonary constrictor and it has been implicated in elevations of pulmonary arterial pressure following administration of some vasoactive agents and in some animal models of lung injury. However, in other models of lung injury, thromboxane does not mediate the pulmonary hypertension. Thus, while a beginning has been made that implicates endothelial cell dysfunction in clinical pulmonary vasoreactivity, major questions remain as to the separate contributions made by specific cell functions. These include the role of impaired uptake of vasoconstrictor compounds, an altered profile of cyclooxygenase products when the cell is injured, and injury-related alterations in the interaction of endothelium with leukocytes and platelets.

Calcium Kinetics in Vascular Smooth Muscle*

George B. Weiss, Ph.D.

The accumulation, binding, and mobilization of Ca++ in vascular smooth muscle directly affects intracellular free Ca++ levels and contractility. Techniques have been developed to delineate Ca++ uptake and efflux parameters in isolated vessels. Similar Ca++-related components are present in different types of vessels, but their relative importance for induction and maintenance of tension differ.

*Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, N.J.
Reprint requests: Dr. Weiss, Cardiopulmonary Research, Ciba-Geigy, 556 Morris Avenue, Summit, New Jersey 07901.