Loss of arteries reflecting loss of vascular reserve might be in the future detect loss of precapillary arteries in mental abnormalities (2), and idiopathic pulmonary hypertension. Arteries include congenital heart defects (18), lung development and conditions associated with pulmonary hypertension and loss of monectomy (17), exposure to chronic hyperoxia (11, 22), and monocrotaline and pulmonary hypertension. The authors went on to show how infusion molecule (PECAM) staining of endothelial cells or by angiogenic channels are thought to arise because of the proliferation of “apoptosis-resistant” local endothelial cells or from the seeding of the lumen with circulating progenitor endothelial cells (20).

A variety of pulmonary hypertension-inducing stimuli used in the experimental setting or related to clinical disease are associated with histological evidence of loss of distal pulmonary arteries, assessed either by platelet endothelial cell adhesion molecule (PECAM) staining of endothelial cells or by barium-gelatin infusion of the lungs. Experimental stimuli in addition to chronic hypoxia that induce loss of vessels in association with pulmonary hypertension have included injection of the toxin monocrotaline (23), monocrotaline and pneumonectomy (17), exposure to chronic hyperoxia (11, 22), and creation of aortopulmonary shunts (19). In the clinical setting, conditions associated with pulmonary hypertension and loss of arteries include congenital heart defects (18), lung developmental abnormalities (2), and idiopathic pulmonary hypertension (16). Improved resolution of current imaging techniques might, in the future, detect loss of precapillary arteries in association with pulmonary hypertension in the clinical setting. Loss of arteries reflecting loss of vascular reserve might be reflected in heightened pulmonary artery pressure and resistance with exercise or changes in pulmonary vascular impedance, which most accurately represents the total right ventricular afterload, including both steady and pulsatile right ventricular work requirements (21).

Unfortunately, only the minority of clinical or experimental studies of chronic hypoxia-induced pulmonary hypertension report whether there is loss of precapillary vessels. One of the ways in which the number of precapillary vessels is precisely determined is by barium-gelatin infusion. This barium permits radiographic assessment of the circulation and the gelatin does not allow the contrast to pass into the capillary bed. Thus it is easy to count barium-filled peripheral arteries at alveolar duct and wall level on microscopic examination of lung tissue sections. Usually the number of precapillary arteries is recorded as the number of arteries relative to 100 alveoli or per squared millimeter. Calculating arteries per 100 alveoli makes the assumption that the alveoli are normal in number and calculating squared millimeter makes the assumption that the number and size of alveoli are normal.

In addition to distensibility analysis (21), microCT (8) can be used to support loss of filling of distal arteries following exposure to chronic hypoxia using the barium-gelatin method or perfluorooctyl bromide (PFOB) as an intravascular X-ray contrast agent. With this method, isolated lungs harvested from mice are rinsed of blood and perfused with a physiological salt solution containing 5% bovine serum albumin while being ventilated with a 15% O2, 6% CO2, balance nitrogen mixture. Papavarine is added to the perfusate and recirculated prior to imaging to remove residual muscle tone. The perfusate is then replaced with PFOB, which is trapped at the precapillary level and only fills the arterial vasculature. High-resolution planar images are taken at an airway pressure of 6 mmHg for a range of intravascular pressures (between 6 and 17 mmHg).

Alternatively, one can use vWF or PECAM staining of endothelium to landmark arteries accompanying alveolar ducts and alveolar walls down to a precapillary size of 20 μm and to express those arteries relative to alveoli. This technique runs the risk of including venules in the assessment, but venules can generally be differentiated from arterioles since they are surrounded by loose connective tissue, they run in connective tissue septae in the lung, and they often have prominent branches. One of our recent studies has shown excellent correlation between the barium-gelatin and vWF immunostaining techniques to assess precapillary arteries (15). With these techniques, a reduction in the number of arteries relative to alveoli has been documented in rodents with chronic hypoxia-induced pulmonary hypertension in our laboratory (13, 14) and in that of others (3, 12).

Studies using transgenic mice have taught us that there can be tremendous discrepancies between the hemodynamic assessments of pulmonary artery pressure and resistance and the remodeling response of the distal circulation in terms of muscularization of distal vessels, hypertrophy of more proximal arteries, and loss of arteries relative to alveoli. For example, in mice with overexpression of S100A4/Mts1, a baseline increase
in pulmonary artery pressure is further augmented by chronic hypoxia relative to controls, but we were unable to identify an increase in the muscularization of distal vessels, in the loss of distal vessels landmarked as precapillary, or in the wall thickness of normally muscular arteries that would explain this discrepancy. We did, however, document marked changes in the elastin matrix (14, 15) that might have influenced the distensibility characteristics in the pulmonary circulation (9, 10).

We observed that patchy deletion of BMPR1a in smooth muscle cells and others have reported that haploinsufficiency of BMPRII results in equivalent pulmonary artery pressures found in wild-type mice exposed to chronic hypoxia, but less structural remodeling of the distal circulation (1). New studies are considering the contribution of the extracellular matrix, where an increase in aberrantly distributed elastin and collagen could be associated with reduced compliance (9, 10) and thus increased impedance even when resistance is unchanged.

So, the following might summarize what we believe is the basis for the difference of opinion regarding hypoxia-mediated loss of distal arteries.

First, pulmonary hypertension is reversed with Rhô kinase inhibitors. However, this does not negate the loss of vessels, only that under basal conditions, the loss of distal arteries does not impair resting steady hemodynamics of the pulmonary circulation.

Second, stereology shows increased angiogenesis and increased capillary length in hypoxia. This is well documented but does not tell us about aberrant or "lost" connections between the precapillary and capillary circulation.

Third, loss of arteries is not always seen in hypoxia. Certain methodologies like barium-gelatin injection are designed to facilitate assessment of the distal precapillary pulmonary vasculature, but this method can be technically challenging particularly in murine lungs. However, PECAM or vWF immunostaining validates the loss of vessels when used in the same series of experiments and this should also be possible with microCT with PFBO.

Fourth, certain murine species may not show loss of arteries. This may be true despite the fact that other features of remodeling of the pulmonary circulation are observed. Also, we need to look beyond the vascular changes we have typically observed in chronic hypoxia-induced pulmonary hypertension into those that affect the total right ventricular afterload, including both steady and pulsatile right ventricular work.

REFERENCES


while excluding the capillary and venous beds. These latter two
method is that it only permits identification of arterial vessels
be found (6). A further problem with the barium-gelatin
used in chronically hypoxic lungs is elevated to compensate for
whether due to vasoconstriction or structural alterations of the
can be a complex function of the vascular
is fraught with difficulties, as the distance to which the barium-
completely solid and prevents further penetration. As the gelatin mixture
penetrates into the pulmonary artery at high pressure. As
and its viscosity. Increased resistance to flow of the mix,
used to examine the structural changes in the pulmonary
circulation following chronic hypoxia and have frequently
been reported as showing a loss of pulmonary vessels. However,
the problem is again that filling of the blood vessels is
in chronic hypoxic lungs is elevated to compensate for
their elevated vascular resistance, no evidence of vascular loss
can be found (6). A further problem with the barium-gelatin
method is that it only permits identification of arterial vessels
while excluding the capillary and venous beds. These latter two
segments are major sites of new vessel formation in the
systemic circulation, suggesting that their exclusion when
assessing the pulmonary circulation may be misleading (23).
Thus vascular density data obtained using barium-gelatin in-
jection must be interpreted with caution. Alternative ways of
identifying pulmonary vessels include the use of elastin stains,
immunostaining with endothelial cell markers, or the use of
resin embedding, permitting thin sectioning and reliable mor-
phometric identification. Interestingly, results obtained using
these methods frequently do not show vessel loss (3, 12–14, 22).
Once tissue sections have been obtained, the extent of the
vascular bed must then be quantified. Obtaining reliable three-
dimensional data using two-dimensional sections is not as
straightforward as it might at first appear (2, 9, 28). A com-
monly used approach has been to take a single transverse
section of the left lung at the level of the hilum, to count the
number of vessels and alveoli observed, and to compute the
ratio of these two or, alternatively, to compute the number of
vessels per unit area of the section; the resultant value has been
loosely called “vessel density.” The first problem with this
approach is that the section is not representative of the lung as
a whole. A second problem is that the number of intersections
between a section and blood vessels is not a unique function of
vessel length but also depends on the relative orientation of the
plane of section and the vessels (2, 9, 28). Thus a single section
(or multiple sections of a single fixed orientation) does not
allow reliable estimation of vessel length. Perhaps most im-
portantly, this method is not sensitive to the increases in lung
volume caused by hypoxia (4, 10, 12–14, 24, 25). For example,
vessel density as described above could remain unchanged if
the lung enlarged and grew new vessels proportionately.
Use of stereological techniques allows unbiased quantitative
analysis of the three-dimensional structure of the lung vas-
culature. Important aspects of the method are the use of system-
atic random sampling from throughout the lung, to ensure that
the data obtained are representative of the whole organ, and
quantification of changes in lung volume. This allows absolute
quantities to be measured even in circumstances where the total
lung volume changes (2, 9, 28). Use of stereology demon-
strates new vessel formation in the pulmonary circulation in
response to chronic hypoxia, not vessel loss (1, 12, 13). This
finding is supported by previous work in which the pulmonary
vascular volume, estimated by filling it with a solution con-
taining tritiated albumin, was found to be increased in chronically
hypoxic lungs (5).

Angiographic techniques form the second category of meth-
ods used to examine the structural changes in the pulmonary
circulation following chronic hypoxia and have frequently
been reported as showing a loss of pulmonary vessels. How-
ever, the problem is again that filling of the blood vessels is
influenced by pulmonary vascular resistance and is therefore
not a reliable method for identifying vessels. For example, it
has been shown that the extent of the vascular bed revealed by
such methods critically depends on the perfusion pressure (6).
The final category of evidence that is used to support the
view that structural changes underlie hypoxic pulmonary hy-
pertension is functional in nature. Once chronic hypoxic pul-
monary hypertension has become established, abrupt return to
normoxic conditions does not cause an immediate substantial
fall in pulmonary arterial pressure (7, 8). Moreover, most
vasodilator agents only produce small acute falls in pulmonary