Pulmonary Vascular Disease in Sheffield, the Andes, Tibet, and Tanzania

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When I graduated from medical school in 1952, I determined to train as a cardiologist and was fortunate to obtain a junior clinical post at the Regional Cardiovascular Centre at Sheffield in the north of England, which was just being set up as part of the new British National Health Service. At the time I did not realize just how good that fortune was, for the first cardiologist to head the new unit was Dr. James W. Brown (Fig. 1). He was a physician from the fishing port of Grimsby who had a special interest in congenital heart disease in infants and children. In 1939 he had published a book, Congenital Heart Disease,3 long before the widespread use of cardiac catheterization or angiography in cardiological investigation. In it he described the diagnosis of various forms of congenital cardiac anomaly by purely clinical means by fingers and stethoscope at the bedside.

I Remember "J.W."

He brought to his appointment great clinical expertise and a clientele of young patients with every conceivable form of congenital cardiac anomaly who could now seek help from the rapidly developing cardiac surgery. He always regarded them as part of his family rather than as patients. At Christmas every child re-
Figure 1. Dr. James Brown, cardiologist from Grimsby who published a book on the clinical diagnosis of congenital heart disease in 1939.

cceived a signed card from him and was invited to a party with games for which prizes were awarded. J.W. cheated outrageously during these exercises so that the most cyanotic and breathless little child in his arms was able to win. He arrived for his ward rounds in a Rolls in which he took an inordinate pride. A quarter of an hour before the anticipated arrival, little faces were pressed against the windows to get the first sight. Suddenly a cheer would go up: "It's Doctor Brown!" On his instruction no special preparations were made for his visits, and the beds were incredibly untidy and littered with toys. He would carry out his ward round in a Rolls in which he took an inordinate pride. A quarter of an hour before the anticipated arrival, little faces were pressed against the windows to get the first sight. Suddenly a cheer would go up: "It's Doctor Brown!" On his instruction no special preparations were made for his visits, and the beds were incredibly untidy and littered with toys. He would carry out his ward round in a Rolls in which he took an inordinate pride. A quarter of an hour before the anticipated arrival, little faces were pressed against the windows to get the first sight. Suddenly a cheer would go up: "It's Doctor Brown!"

Hypertensive Pulmonary Vascular Disease

It began to dawn on us that the pulmonary circulation, particularly a raised pressure within it, was playing an important role in determining the clinical picture and prognosis in these patients. It appeared that there was a hitherto unexplored pathology of the pulmonary vasculature to be investigated here. From the outset it seemed probable that there were two major forms of pulmonary vascular
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disease, one involving congenital cardiac shunts and the other sustained alveolar hypoxia. By 1956 Whitaker and I had come to the conclusion that the clinical picture in congenital heart disease is often dominated by symptoms and signs characteristic of pulmonary hypertension that often mask the underlying cardiac anomaly and are often associated with definitive pathological changes in the pulmonary vasculature.21 We suggested that the term “hypertensive pulmonary vascular disease” be given to this condition in a paper we submitted to a British clinical journal. The paper was rejected on the grounds that the subject was of little medical interest or importance. When the paper was subsequently published by Circulation, we were gratified by receiving several hundreds of requests for reprints. After this, as the most junior member of the unit, I was charged with developing the study of the pathology of pulmonary hypertension. The only training I had received in histopathology was that of a medical student, so I applied for a temporary junior lectureship for one year in pathology at the University of Birmingham. This proved to be a one-way ticket, and I never found my way back to cardiology.

Soon after the commencement of my new post, two events took place that were to influence greatly my studies. I was awarded a Rockefeller Traveling Fellowship by the Medical Research Council in London to enable me to carry out research for one year in pathology at the University of Birmingham. This proved to be a one-way ticket, and I never found my way back to cardiology.

It has been known for 40 years that the Quechua Indians of the Peruvian Andes (Fig. 2) have pulmonary arterial hypertension that is mild in adults36 but more apparent in children.38 From the beginning there was evidence that this elevation of pulmonary vascular resistance was associated with structural changes in the pulmonary arterial tree. Quantitative studies2 showed that in Quechua Indians from the region of Cerro de Pasco (4,330 m), there was muscularization of a considerable number of the peripherally situated pulmonary arterioles without appreciable medial hypertrophy in the parent arteries. This was a notable contribution, for it showed that significant structural remodeling occurs in the terminal portion of the pulmonary arterial tree in the face of sustained hypobaric hypoxia in the alveolar spaces. However, this classic paper had an unfortunate title that implied that the remodeling was confined to arterial vessels and was found in all people native to high altitudes. We carried out two studies, a
Figure 2. Quechua Indian from Cuzco (3,400 m) in the Peruvian Andes.

decade apart\textsuperscript{19,25} of the pulmonary vascular remodeling that occurs in citizens of La Paz, Bolivia (3,800 m).

We found that the changes involved are far more complex than the simple peripheral muscularization of the pulmonary arterial tree envisaged by Arias-Stella and Saldaña\textsuperscript{2}. It was easy to confirm that in the Aymara Indians hypobaric hypoxia brings about muscularization of the most peripheral portion of the pulmonary arterial tree so that muscularized pulmonary arterioles as small as 30 µm in diameter are commonplace\textsuperscript{19}. There is extension of smooth muscle even into precapillary vessels, consequently even minute arteriolar vessels of a diameter comparable to that of macrophages have a distinct muscular coat. These very small muscular vessels appear to be brought about by a hyperplasia of vascular smooth muscle cells rather than by a constriction of parent arteries. Hence they have a thick outer elastic lamina corresponding to the original single elastic fibril of the arteriole, a coat of circularly oriented smooth muscle, and a much thinner, newly formed elastic lamina\textsuperscript{25}.

An additional striking feature of the pulmonary arteries and arterioles of the Aymara is the development of longitudinally oriented smooth muscle in the intima. At first these nodules are purely muscular, but later elastic fibrils develop between the individual muscle cells, and finally the nodules and layers become increasingly sclerotic, with the muscle cells widely separated by collagen. Classically, such intimal longitudinal muscle in states of chronic hypoxia is found in association with pulmonary emphysema and has been ascribed to longitudinal stretch around abnormal air spaces such as the distended respiratory bronchioles, which occur in centrilobular emphysema. However, its development in young Aymaras, free of heart or lung disease, suggests that it is more likely due, in both high-altitude Indians and emphysematous patients, to the effects of alveolar hypoxia per se.

Another typical feature of the remodeling of the pulmonary arterial tree in chronic hypoxia is the development of inner tubes of circular smooth muscle, which come to line the intimal longitudinal muscle in pulmonary arteries and arterioles and extend into the precapillaries\textsuperscript{25}. Sometimes two or three muscular tubes are found in one pulmonary artery. In the older lesions elastin develops between the muscular tubes within the confines of the thick elastic lamina comprising the wall of the pulmonary arteriole.

The pulmonary veins and venules are also involved in the remodeling that occurs in response to the hypobaric hypoxia in the Aymara. Vascular smooth muscle cells appear in the intima, but they are widely separated by collagen. Thus it would appear that the form of the muscular proliferation in the pulmonary vascu-
lature in response to hypoxia is greatly modified by haemodynamic forces within the class of vessel in question. Overt muscular hyperplasia in the pulmonary arteries may be a response to arterial pulsation, whereas in the pulmonary veins the myofibroblasts develop more their fibroblastic features. The widespread extent of the structural changes suggests that one should conceive of the field effects of hypoxia as on individual muscle cells in small pulmonary arteries, arterioles, and venules lying adjacent to alveolar spaces rather than on only the terminal portion of the pulmonary arterial tree.

The original observations of Arias-Stella and Saldaña were made on a pure Quechua population in the Andes, and not surprisingly the muscularization of the pulmonary arterioles was found throughout the community. Our studies on the heterogeneous population of La Paz yielded different results. We found muscularization of pulmonary arterioles in 5 of 25 Aymaras and mestizos and the development of intimal longitudinal muscle in 4 of 13 Aymaras and in 5 of 12 mestizos. Inner muscular tubes were recognised in only one of the Aymaras and in none of the mestizos. We have not found hypoxic hypertensive pulmonary vascular disease in Caucasians resident at high altitude. Personal communications from Inder Anand suggest that remodeling of the pulmonary vasculature is not to be found in the native highlanders of Ladakh (3,600 m). This may be a reflection of their different genetic background from that of Andean highlanders, or it may be simply an expression of the fact that they live at lower altitudes.

Our studies on the lungs of patients with chronic obstructive lung disease, included in the study of the British Medical Research Council on the effects of long-term oxygen therapy in this condition, reveal that an identical form of remodeling with the same triad of changes (Fig. 3) occurs in emphysematous patients and to a more advanced stage than is found in Andean highlanders. This supports the view that sustained alveolar hypoxia is the basic cause of this remodeling of the pulmonary vasculature. Recent studies in my department of the ultrastructure of pulmonary arteries and arterioles in cases of pulmonary emphysema have shown that the intimal longitudinal muscle arises from the migration of smooth muscle cells from the media through gaps in the inner elastic lamina. These muscle cells closely resemble adult vascular smooth muscle cells and are unlike the migrating muscle cells found in plexogenic pulmonary arteriopathy, which are electron dense and have lost their tesselated outline. The similarity of the migrating muscle cells to adult my-
ocytes of the media may be reflected in the restriction of these cells to the intima of pulmonary arteries and the mild, benign pulmonary hypertension in patients with chronic obstructive lung disease and in native highlanders. The inner muscular tubes found in both Aymara Indians and emphysematous subjects are formed by a thickening of the layer of circumferentially situated attenuated smooth muscle cells normally found immediately beneath the endothelium. These cells secrete elastin around themselves, which forms thin inner and outer elastic laminae around the layer of circular muscle. This is the basis for the development of the inner muscular tubes of the pulmonary arteries and arterioles in states of chronic alveolar hypoxia.

Chinese Infants at High Altitude in Tibet

Our studies in Bolivia demonstrated the histological changes in the pulmonary vasculature that occur in a fully acclimatized population of adult Aymara Indians living at high altitude in the Andes. We have found it important to distinguish in high-altitude studies between subjects who are acclimatized and those who are genetically adapted to the hypobaric hypoxia. At present, increasing numbers of lowlanders of Han origin are being introduced as residents of the Tibetan capital as part of Chinese governmental policy. This has resulted in a mix of population at high altitude of native Tibetan highlanders and lowlanders freshly arrived from low altitude. When infants of Han origin are taken up by their parents to reside in Lhasa (3,600 m), many die within months of congestive cardiac failure.

This condition was brought to the attention of Western medical circles by my colleagues Peter Harris, formerly of the National Heart and Lung Institute, London, and Inder Anand, of Chandigarh, India. They designated this condition “subacute infantile mountain sickness.” It appears to represent a failure to achieve initial acclimatization to hypobaric hypoxia and may thus be compared with brisket disease in calves being taken up for spring grazing in the Wasatch Mountains in Utah. I was privileged to examine specimens of lung brought back from Tibet by Peter Harris. The small pulmonary arteries showed severe medial hypertrophy, and there was muscularization of pulmonary arterioles. Of considerable interest was the migration of vascular smooth muscle cells from the media of the pulmonary arterioles into their lumens. This pulmonary vascular disease appeared to increase pulmonary vascular resistance, for at necropsy the infants showed hypertrophy of the right ventricle and dilatation of the right atrium and pulmonary trunk (Fig. 4).

Figure 4. Heart from a male infant of Han origin, aged 16 months, who died from subacute infantile mountain sickness 3 months after being taken up to live in Lhasa, Tibet (3,600 m). The initial clinical diagnosis was one of measles bronchopneumonia. There is pronounced right ventricular hypertrophy and dilatation of the right atrium and pulmonary trunk.
features consistent with the development of severe pulmonary hypertension.

**Pulmonary Vasculature in Indigenous Mountain Species**

During my studies of the human pulmonary vasculature at high altitude, I took the opportunity to study pulmonary arteries of indigenous mountain species in the Andes and in the Himalaya. These are mammals showing genetic adaptation to the adverse environment. Adaptation, as contrasted to acclimatization, is the development of biochemical, physiological, and anatomical features that are heritable and of genetic basis that enable the species to explore the environment of high altitude to its best advantage. Most of these studies have been carried out over the years with David Williams. In all of the species we have studied, the small pulmonary arteries have been thin walled, with a meagre amount of smooth muscle in the media and pulmonary arterioles devoid of a muscle coat. Such vessels offer a low resistance to blood flow, and consequently these indigenous mountain species do not show a significant elevation of pulmonary arterial pressure but maintain a low ratio of right-to-left cardiac ventricular weight.

We found that animals that show these microanatomical features are the high-altitude camelids of the Andes, such as the llama, the alpaca, and the guanaco. Care must be taken in interpreting these findings in camelids, for the camel family is characterized by a thin-walled pulmonary vasculature even at sea level. More reliable are similar findings in the mountain viscacha (*Lagidium peruanum*), for in general rodents respond to sustained alveolar hypoxia by muscularization of the peripheral pulmonary arterial tree. A noted example of a species adapted to the hypobaric hypoxia of high altitude is the yak (*Bos grunniens*), for the other members of the cattle family at low altitudes have a muscular pulmonary vasculature. Indeed in the mountains around Salt Lake City, calves may not achieve initial acclimatization to high altitude and then develop the potentially fatal condition of brisket disease, as noted above. In the Himalayan yak, however, the pulmonary arteries are exceedingly thin walled and the pulmonary arterioles devoid of smooth muscle. This results in a low pulmonary arterial pressure and a diminished propensity to high-altitude pulmonary hypertension.

We found that cardiac catheterization in the yak is not without its interests and complications. Prior to our expedition to the Himalaya, Peter Harris and David Williams carried out a reconnoitre to Whipsnade Zoo in the United Kingdom. They found the yaks in their paddock very muscular and aggressive. Their keepers volunteered the information that they would be unwilling to enter the paddock to feed them, let alone carry out a cardiac catheterization. A major surprise awaited us on our arrival in Ladakh, for here the yaks under the scrutiny of the village head man were tranquil and cooperative. It was clear to us that, if one wished to see wild yaks, the place for it was England rather than the Himalaya. An exotic example of an indigenous high-altitude species that we have studied is the Tibetan snow-pig (*Marmota himalayana*) (Fig. 5). It also

![Tibetan snow-pig (*Marmota himalayana*)](image)
has thin-walled pulmonary arteries and a low pulmonary arterial pressure.

**Plexogenic Pulmonary Arteriopathy**

When I carried out research under Jesse Edwards at the Mayo Clinic in the year from the autumn of 1957, we studied the histopathology of the pulmonary circulation and devised a grading system for the changes we saw.\(^{12}\) This has pleased some but not others. The central feature of the pathology was a striking lesion combining vascular and cellular proliferation that Jesse had termed the “plexiform lesion”. This proved to be of physiological and clinical interest as well as of histopathological interest. Its presence was found to be an indicator of pulmonary blood flow falling below the normal range and a rapidly rising pulmonary arterial pressure.\(^{14}\) It appeared to be a useful histological marker in lung tissue of a shift in the nature of pulmonary vascular resistance from one determined largely by functional factors to one of fixed organic basis. This in turn suggested an immediately irreversible pulmonary hypertension that would not be susceptible to benefit from closure of a congenital cardiac septal defect. The implication was that the plexiform lesion was of importance in indicating whether a patient with a congenital cardiac anomaly and associated pulmonary hypertension should be referred for corrective heart surgery.\(^{15}\)

In 1973 I was attending a World Health Organization meeting on primary pulmonary hypertension in Geneva with Kees Wagenvoort. This meeting had been called in response to the epidemic of the disease that broke out in Western Europe during the period of 1967–70. At the end of one of the morning sessions the chairman charged the two of us with producing a name for the pulmonary vascular pathology underlying the clinical syndrome of primary pulmonary hypertension. It was to be ready by the opening of the afternoon session, we were told. Professor Wagenvoort and his wife, Noek, and I repaired to the restaurant, and over lunch with red wine had an urgent discussion to come up with an appropriate term. We concluded that the designation must indicate that the essential feature of the disease was the plexiform lesion, which need not, however, be present in every case. In other words, the condition was “plexogenic” rather than “plexiform”. The term was accepted by the meeting in the afternoon, and the disease became known as “plexogenic pulmonary arteriopathy.”\(^{11}\) That is how the term was born, and it has not been loved by all clinicians, who, once having read this account, may conclude that the drinking of wine should be banned on such occasions.

**Vascular Smooth Muscle Migration**

Early studies of the pathogenesis of plexogenic pulmonary arteriopathy suggested that constriction of small pulmonary arteries was of central importance, particularly in the formation of the plexiform lesion.\(^{45}\) However, our studies over the years have demonstrated that tissue proliferation is also of considerable importance, and this is demonstrated well by electron microscopy.\(^{41}\) Thus the pulmonary arteriopathy of primary pulmonary hypertension, congenital cardiac shunts, and rare examples of cirrhosis of the liver or portal vein thrombosis should be regarded as not entirely vasoconstrictive in nature but in part a manifestation of abnormal cell growth. This concept has considerable relevance to the use of pulmonary vasodilators in primary pulmonary hypertension, which cannot be expected to reverse overgrowth of cellular tissues.

In our original description of the grading system\(^{12}\) we paid scant attention to
Grade 2, and at that time we did not appreciate its significance. We referred to it as a “cellular intimal reaction” and suggested it might be due to endothelial cells. Further studies over the years by light and electron microscopy have revealed that it is due to migration of smooth muscle cells from the media into the intima, where there is a transformation into myofibroblasts, which proliferate in the vascular lumen obstructing it. Subsequent investigation of the ultrastructure of plexogenic pulmonary arteriopathy has shown that, early in the disease, the muscle cells in the inner half of the media show increased electron density\(^{18}\) (Fig. 6). They lose their tesselated outline, become smooth, and can be detected in the act of passing through gaps in the inner elastic lamina to reach the intima.

Such migration of smooth muscle cells from the media into the intima through gaps in the inner elastic lamina is reminiscent of the process that occurs in patients with emphysema or in native highlanders and leads to the characteristic layers of intimal longitudinal muscle in states of chronic alveolar hypoxia. However, in plexogenic pulmonary arteriopathy the process is different in ways that have great pathological and clinical implications. Here the muscle cells are electron dense and have smooth outlines. Having reached the intima the myocytes become transformed into myofibroblasts which replicate vigorously in the intima and give rise to other changes such as fibrosis and elastosis. They then proliferate in the lumens of arteries and arterioles, occluding them and elevating pulmonary arterial resistance. This leads to a severe pulmonary hypertension, which may prove rapidly fatal. This is a very different outcome from the mild, benign pulmonary hypertension of the native highlander. It is as though in plexogenic pulmonary arteriopathy the smooth muscle cells have had their nature and behaviour changed by something like a growth factor permeating the inner half of the media, perhaps from the vascular lumen or the endothelium.

**Figure 6.** Electron micrograph of longitudinal section of a muscular pulmonary artery from a woman of 23 years with primary pulmonary hypertension. The adventitia (a) consists of a discontinuous external elastic lamina and a loose collection of collagen fibrils. The media contains smooth muscle cells sectioned transversely. Those in the outer media (m1) are pale with numerous peripheral attachment points between which the cytoplasm bulges outward. Smooth muscle cells in the inner media (m2) are denser and lack a ruffled border. Two of them can be seen in the process of sending out cytoplasmic extensions between gaps in the internal elastic lamina (el) into the intima (i). Here they are associated with elongated cytoplasmic processes from other smooth muscle cells (arrow). Scale line = 13 \(\mu\)m.

Pulmonary Endocrine Cells, Peptides, and Pulmonary Vascular Disease

Further investigation of this migration of smooth muscle cells from the media of pulmonary arteries has shown an interesting association with pulmonary endocrine cells and the peptides they contain. Through the kindness of Professor Magdi Yacoub, we gained access to speci-
mens of lung he removed at combined heart-lung operations at Harefield Hospital in London. The two major peptides found in human pulmonary endocrine cells are calcitonin and bombesin, and their functions are unknown. We found that pulmonary endocrine cells increased in number and prominence in only one form of hypertensive pulmonary vascular disease, namely, plexogenic pulmonary arteriopathy, be it primary or secondary (Fig. 7).

The peptide concerned was gastrin-releasing peptide, the human counterpart of that of amphibian skin, bombesin. There is, moreover, an interesting association between the proliferation of neuroendocrine cells containing this peptide and the stage of plexogenic pulmonary arteriopathy that has been reached. Thus they are numerous when classic cellular plexiform lesions are present, and their numbers fall off when these dilatation lesions become mature with wider vascular channels. The most interesting feature is that they are most numerous in the preplexiform stage, before the plexiform lesions have developed, when the major component of the pathology is migration of vascular smooth muscle cells from media to intima.7,27 The basis for this association remains obscure. Increased prominence of pulmonary endocrine cells containing bombesin is also found in cases of subacute infantile mountain sickness in Tibet.13

Muscular Evaginations

When smooth muscle cells constrict, they do not simply become shorter and thicker but become covered in bulbous extrusions.6 This was originally shown by scanning electron microscopy of the smooth muscle cells of the stomach wall of Bufo marinus. We wondered if these extrusions could be demonstrated by transmission electron microscopy, for if they could, they would indicate a very early stage of constriction in pulmonary hypertension, long before the migration of smooth muscle cells from the media or their proliferation in the intima. Our investigations confirmed that they could indeed be found in the pulmonary trunk and small pulmonary arteries of rats exposed to hypoxia40 and in the pulmonary arteries and arterioles in this species following the administration of pyrrolizidine alkaloids.39 Such prominences were found to be due to evaginations of the cytoplasm of the smooth muscle cells between attachment points in the plasmalemma, which act as points of anchorage for the intracellular fibrils of actin and myosin. The clear cytoplasm of the evaginations is devoid of myofilaments and organelles (Fig. 8).
When vascular smooth muscle cells constrict, the evaginations squeeze through deficiencies in the adjacent inner or outer elastic lamina of the wall of the blood vessel to extend into the intima or adventitia, respectively. Because deficiencies in the pulmonary arteries occur naturally in the outer lamina, muscular evaginations in these vessels tend to be into the adventitia. In contrast, deficiencies in pulmonary veins occur mainly in the inner elastic lamina so that in these vessels the evaginations are found in the intima. In this situation the surface of the muscular evagination presses on the undersurface of the endothelial cells. Because the cytoplasm of muscle and endothelial cell comes into such intimate contact, substances may pass between these two cells, which raises interesting physiological possibilities. Only lungs that have been fixed in distension are satisfactory for a search for muscular evaginations, for they are also readily produced by collapse. These evaginations are the earliest structural changes detectable at ultrastructural level in animals that indicate pulmonary vasoconstriction.

In a recent experiment we subjected Wistar albino rats to a reduced barometric pressure of 490 mmHg to simulate an altitude of 3,550 m. They developed numerous muscular evaginations in muscularized pulmonary arterioles, but, when some of the rats were allowed to recover in room air, the evaginations disappeared within a week. Clearly they represent an acute vasoconstrictive response soon lost after removal of the hypoxic stimulus. As far as I am aware muscular evaginations of pulmonary arteries have not been described in man.

**Venous and Parenchymal Changes in Plexogenic Pulmonary Arteriopathy**

Studies of plexogenic pulmonary arteriopathy have largely been restricted to the histopathology and ultrastructure of the pulmonary arterial tree. In 1987 I began receiving considerable tissue from Harefield Hospital transplantation cases, which included cases of plexogenic pulmonary arteriopathy, both primary and secondary to congenital cardiac septal defects. As a result of this experience, my concept of the pathology of this disease has been modified. It has become apparent to me that in plexogenic pulmonary arteriopathy changes occur in the pulmonary veins and venules and in the lung parenchyma, together with a considerable accumulation of lung mast cells.

In a study of the histopathology of 36
cases of plexogenic pulmonary arteriopathy,\textsuperscript{5} we found intimal proliferations in pulmonary veins in all but 5 cases, 4 of the exceptions being children 6 years of age or less. However, three children of 5 to 7 years of age showed widespread intimal fibrosis of pulmonary veins, one of whom had a luminal obstruction of 18.8\%, defined as the average thickness of the intima expressed as a percentage of the internal diameter of the vein. Three adults with primary arteriopathy who had luminal venous obstructions of greater than 28\% had an appreciably more cellular type of intimal thickening. Embedded within the collagenous matrix of the thickened venous intima, cells were identified that closely resembled myofibroblasts, and in some areas bundles of mature smooth muscle were seen. Similar changes were found in 5 cases of secondary plexogenic pulmonary arteriopathy. Electron microscopy confirmed the identity of the cells within the intima as myofibroblasts.

Some of the intimal fibrosis in pulmonary veins in plexogenic pulmonary arteriopathy may be an expression of age change or a consequence of the increased levels of pulmonary blood flow found early in the life history of pre- and posttricuspid congenital cardiac shunts. In some instances, however, the myofibroblasts in the pulmonary venous intima appeared to be transformed smooth muscle cells that had migrated from the media, thus resembling the migration of myocytes in the pulmonary arteries that occurs in plexogenic pulmonary arteriopathy. This recalls the remodeling of pulmonary veins of the Aymara Indians. Associated with this intimal proliferation in pulmonary veins and venules, one finds a whole range of parenchymal changes in the lung in plexogenic pulmonary arteriopathy. These include focal haemorrhages, proliferation of granular pneumonocytes, accumulations of alveolar macrophages, dystrophic calcification, small osseous nodules, periarterial accumulations of lymphocytes, and even groups of cells resembling meningocytes around pulmonary venules.\textsuperscript{5}

In view of the venous and parenchymal lesions in plexogenic pulmonary arteriopathy, we were prompted to study the population of lung mast cells in this disease.\textsuperscript{26} Mast cells were found to abound in the lung parenchyma, with perhaps a greater tendency to accumulate in the adventitia of pulmonary arteries. They occurred equally commonly in the primary and secondary forms of the arteriopathy, and their numbers did not appear to be closely related to the stage of the disease reached. If anything, they were somewhat more numerous in association with cellular plexiform lesions and even more so in the preplexiform stage, when muscle cells were migrating into the intima. They were less common with mature plexiform lesions. When Ehrlich discovered mast cells in the last century, he noted at the outset that they were abundant in the lung in the brown induration of mitral stenosis.\textsuperscript{37} We were subsequently able to confirm this finding in mitral stenosis and chronic left ventricular failure\textsuperscript{20} and in association with the similar parenchymal changes induced in the lung by metabolites of monocrotaline.\textsuperscript{28} It is probable that in plexogenic pulmonary arteriopathy the accumulation of lung mast cells is also part of the parenchymal changes found in the lung substance. An intriguing possibility is that the periarterial mast cells are secreting histamine, which in man appears to have a dilating effect on pulmonary arteries in which H-2 receptors seem to be dominant.

**Dietary Pulmonary Hypertension**

In the 1960s Michael Kay and I became aware that dietary factors could lead to severe pulmonary hypertension in animals. Lalich and Merkow\textsuperscript{34} and Lalich and
Ehrhart had observed that prolonged oral administration of Crotalaria spectabilis seeds or their active principle, the pyrrolizidine alkaloid monocrotaline, to rats induces in them medial hypertrophy and arteritis in the pulmonary arteries with associated cardiac hypertrophy. Initially we confirmed that the seeds of this plant, which originated in India and came to be used as a cover crop in the southern states of the United States, were capable of inducing pulmonary hypertension in rats. The elevation of pulmonary arterial pressure was found to have an organic basis in pulmonary vascular disease characterized by medial hypertrophy of small pulmonary arteries, which in a minority of cases progressed to necrotising arteritis. There was associated muscularization of the pulmonary arterioles and of the pulmonary trunk. There was, however, no migration of muscle cells from the media to lead to any form of intimal proliferation, and no plexiform lesions developed. An important aspect of monocrotaline pulmonary vascular disease was hypertrophy of the intimal muscular pads of pulmonary veins. This venous obstruction became associated with a wide variety of exudative lesions in the lung parenchyma, ranging from pulmonary haemorrhage, osseous nodules, aggregations of pulmonary macrophages, and proliferation of granular pneumonocytes, to accumulations of mast cells.

It soon became apparent that other members of the genus Crotalaria and its alkaloids were capable of inducing pulmonary hypertension in animals. Fulvine is a pyrrolizidine alkaloid contained in the foliage and seeds of Crotalaria fulva. This leguminous plant is one of several that was used in the West Indies for the preparation of bush teas, consumed by the indigenous population for medicinal and social purposes. Fulvine and the other alkaloids contained in bush tea were known to cause veno-occlusive disease of the liver in the Caribbean, but there was no suggestion that their ingestion led to pulmonary veno-occlusive disease. Fulvine is closely related chemically to monocrotaline, and Professor Bras was so kind as to send us some of the alkaloid from Jamaica. We were able to show that fulvine produces in rats hypertensive pulmonary vascular disease identical to that produced by Crotalaria spectabilis.

We found that the genus Senecio was also effective in inducing pulmonary hypertension and associated pulmonary vascular disease in animals. There is a common belief in the United Kingdom that any natural substance is “good for you,” an idea encouraged by commercial television. Ragwort, sometimes called “stinking nanny,” (Senecio jacobaea) is a common herb of the English countryside and preparations of it are freely available from herbalists and so-called health stores in Britain, where they are recommended for the treatment of various ailments (Fig. 9). In fact, the seeds and foliage of Senecio jacobaea contain at least six pyrrolizidine alkaloids. Dr. Burns, on our staff, visited a store to buy a bagful of this health food. It was given to rats in their diet, and within the month all had died of congestive cardiac failure secondary to hypertensive pulmonary vascular disease of a type similar to that produced by Crotalaria spectabilis seeds.

An important question left to be answered was whether pyrrolizidine alkaloids could induce hypertensive pulmonary vascular disease in man. For some years I acted as external examiner for medical students in Tanzania. On one occasion I was shown lung sections from a young man of 19 years, who had died from congestive cardiac failure in Muhimbili Hospital in Dar-es-Salaam. They showed the typical features of plexogenic pulmonary arteriopathy. The patient came from Kilwa, an area where witch doctors practised, administering herbal concoctions to their customers. Growing in profusion around Kilwa was a leguminous plant,
Figure 9. Advertising material supplied with a packet of ragwort obtained at a "health store". Said to be efficacious for gouty and arthritic joint pains, the plant led to the death from hypertensive pulmonary vascular disease of Wistar albino rats when added to their diet.

*Crotalaria laburnoides*, which has a widespread distribution in East Africa, occurring in Tanzania, Kenya, and Uganda (Fig. 10). It is sometimes called *C. bagamoyensis* because it grows in the coastal area around the small township of Bagamoyo, an important centre of the African slave trade in the last century and well known to the missionary-explorer David Livingstone. We were unable to establish that this boy had been given any herbal infusion containing this plant species. Nevertheless, I collected its seeds and brought them back to Liverpool where they were administered over a period of 2 months to Wistar albino rats. A proportion died in congestive cardiac failure secondary to hypertensive pulmonary vascular disease of the type associated with pyrrolizidine alkaloids.\(^\text{17}\)

All of these studies confirmed that the pyrrolizidine alkaloids contained in the genera *Crotalaria* or *Senecio* were capable of inducing pulmonary hypertension and associated pulmonary vascular disease in a variety of animals. There was no proof that dietary factors were involved in the aetiology of pulmonary hypertension in man. The concept of dietary pulmonary hypertension had been born, but at that time it did not seem to have a clinical application. These investigations appeared to be a classic example of "ivory tower" research, for the amusement and indulgence of professors protected behind the walls of their academic institution.

Figure 10. *Crotalaria laburnoides*, growing in swampy ground near the coast of Tanzania.
Menocil Pulmonary Hypertension

In 1967 the attitude toward such experiments changed dramatically, and the concept of dietary pulmonary hypertension to humans was considered seriously when a sudden 20-fold increase in the incidence of primary pulmonary hypertension was observed in a Swiss medical clinic. A considerable number of the patients concerned had taken the appetite-suppressing drug aminorex, 2-amino-5-phenyl-2-oxazoline, with the commercial name of Menocil, which was available in Switzerland from November 1965 to October 1968. A similar increase in the incidence of this disease occurred in Austria and West Germany, where the drug was available. I found myself involved in this epidemic in two ways. First, I was called on to visit various centres in the three countries seeking to establish the nature of the pulmonary vascular disease emerging in Europe. The pathology proved to be that of plexogenic pulmonary arteriopathy, indistinguishable histologically from that found in classic primary pulmonary hypertension. Second, with colleagues in my department I undertook experimental studies to ascertain whether the drug could induce pulmonary vascular disease in animals. In fact, high oral dosage of aminorex for 43 weeks in rats, as we have seen above, a species susceptible to dietary pulmonary hypertension, and for 20 weeks in dogs failed to reveal any evidence of hypertensive pulmonary vascular disease. Grover and Byrne-Quinn failed to produce pulmonary hypertension by injecting aminorex daily for a month into calves in which the pulmonary vasculature was sensitized by chronic hypoxia induced by living at an altitude where the barometric pressure was 625 mmHg.

Twenty years after these events there appears to have been a general acceptance of an association between the ingestion of aminorex fumarate and the development of plexogenic pulmonary arteriopathy to an extent that the diagnosis “menocil pulmonary hypertension” has entered medical parlance. Yet the present position with regard to aminorex is that, although statistical epidemiological evidence linked this drug to pulmonary hypertension in humans there was no direct proof by animal experimentation that aminorex causes plexogenic pulmonary arteriopathy in patients. The caveat remains that failure to produce pulmonary vascular disease in animals by the drug may merely reflect a species difference in the reactivity of the pulmonary arteries.

An Animal Model for Primary Pulmonary Hypertension

Many workers have sought an animal model for primary pulmonary hypertension, but our studies over the years convince us that none is satisfactory. A widely used model is the administration of monocrotaline to rats, either by injection of the alkaloid or administration of Crotalaria spectabilis seeds to the diet. Fatal pulmonary vascular disease is induced readily enough, but it differs in important respects from that found in humans. Thus it readily induces hyperplasia of vascular smooth muscle cells, and in about 30% of cases leads to fibrinoid necrosis and an acute inflammatory reaction. However, it does not bring about migration of myocytes into the intima so that there is no intimal fibrosis or elastosis, concentric laminar intimal proliferation, or development of plexiform lesions, the hallmarks of plexogenic pulmonary arteriopathy in man. Our later studies have revealed that in rats showing medial hypertrophy or fibrinoid necrosis in their pulmonary arteries induced by monocrotaline, bombesin cannot be demonstrated in the pulmonary endocrine cells. This is in striking contrast to what occurs in humans, where the migration of muscle cells...
cells from the media into the intima and the formation of plexiform lesions are associated with an increase in the numbers and prominence of bombesin-containing cells in the terminal bronchioles. Apparently the rat is not capable of developing intimal proliferation in the pulmonary arteries, and in some way this may be related to absence of bombesin-containing pulmonary endocrine cells.

The pulmonary vascular remodeling brought about by exposing rats to hypobaric hypoxia in a decompression chamber also forms a disappointing animal model for what occurs in humans. In rats, many of the muscular evaginations referred to above develop in the pulmonary arteries. They indicate intense vasoconstriction in response to the hypoxic stimulus and remain for about a week. Such evaginations have not been reported in human lung specimens. In rats, exposure to hypobaric hypoxia and the development of pulmonary vasoconstriction with muscular evaginations and right ventricular hypertrophy is rapidly fatal.

In humans the remodeling of the pulmonary arterial tree in response to hypoxia comprises the formation of intimal longitudinal muscle and tubes of circular muscle internal to it. Such complex remodeling of longitudinal and circular muscle is not seen in rats. In native highlanders and emphysematous patients, such remodeling is hardly more than a marker of chronic hypoxia; it appears to be benign and not to reduce longevity. Clearly the rat provides a poor animal model for pulmonary hypertension and vascular disease in man.

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References
13. Heath, D., P. Harris, G. J. Sui, Y. H. Liu, J. Gosney, E. Harris, and I. S. Anand. Pulmonary blood vessels and endocrine cells in...


41. Smith, P., D. Heath, M. Yacoub, B. Mad-


