Interpretation of the Appearances of the Small Pulmonary Blood Vessels in Animals

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Experiments on pulmonary hemodynamics in laboratory animals sometimes include the histological examination of the small blood vessels in resected-lung biopsies. In such investigations it may be important to decide whether or not the pulmonary arteries show medial hypertrophy. It is comparatively easy to detect this vascular abnormality in man, whose pulmonary arteries are thin-walled. For instance, previous studies had shown us that human "muscular pulmonary arteries" less than 300 μm in diameter taken from lungs inflated with 4 per cent formaldehyde have a media with a thickness of less than 5 per cent of the external diameter of the vessel. Furthermore, arterial vessels less than about 80 μm in diameter have virtually no muscle in their walls and are termed "arterioles." Any increase in the medial thickness of the muscular pulmonary arteries and the development of muscle in arterial vessels less than 80 μm in diameter are pathognomonic of associated pulmonary hypertension. However, pilot studies suggested to us that the detection of medial hypertrophy in the pulmonary arteries is less straightforward in some of the laboratory and domesticated mammals. These observations led us to the present investigation to determine if our criteria for hypertensive pulmonary vascular disease in man, quoted above, could be applied to lung biopsies resected from these animals.

Method

The animals studied are shown in table 1. Postmortem specimens of uninflated lung measuring on an average 15 to 20 mm. across and 5 to 10 mm. thick, in all but the smaller animals, were studied in preference to blocks taken from lungs inflated with formalin-saline. This course was taken, as it was considered that this is the form in which lung tissue is taken for examination during the course of experiments on the pulmonary circulation of laboratory animals. The blocks of lung were fixed in formalin-saline. In the case of the dog, portions of lung that had been inflated with formalin-saline were also examined. This permitted an assessment of the magnitude of the difference in medial thickness in muscular and elastic pulmonary arteries, which results from variation in the degree of inflation of the lung parenchyma. In the goat, only tissue from formalin-inflated lung was examined. All blocks of tissue were embedded in paraffin wax. Sections were stained by the Lawson modification of the Weigert-Sheridan method for elastic tissue and counterstained with Van Gieson stain to demonstrate collagen and muscle.

Results

Muscular Pulmonary Arteries

The basic structure of this class of vessel was an intima of endothelium, an internal elastic lamina, a media of circularly arranged smooth muscle, an external elastic lamina, and an adventitia of fibrous tissue (figs. 1 to 4). With the exception of the guinea pig, there was little variation in the histological structure of the media in the animals studied and the media was regular. There was, however, variation within each species as regards the thickness of the media, expressed as a percentage of the external diameter of the vessel, the range of diameters of arteries that may be considered to fall within the definition of the term "muscular artery," and the thickness of the elastic laminae.

In the guinea pig (figs. 5, 6, and 7) the media was irregular; the smooth muscle was in discontinuous masses so that some segments of the artery had what seemed to be a muscular sphincter whilst others were devoid of muscle and the wall comprised only an elastic lamina (figs. 5 and 6). In some arteries crescentic or nodular foci of smooth muscle...
Table 1

Results of Mensuration of Pulmonary and Bronchial Arteries in Specimens of Uninflated Lung in Man and Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Range of values found for thickness of media expressed as a percentage of the external diameter of the artery</th>
<th>Suggested range of diameter (in μ) of arteries classed as &quot;muscular pulmonary&quot;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>2.0-6.9</td>
<td>100-1000*</td>
</tr>
<tr>
<td></td>
<td>2.3-3.1</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>5.1-13.6</td>
<td>30-80</td>
</tr>
<tr>
<td>Dog</td>
<td>5.9-11.7</td>
<td>30-120</td>
</tr>
<tr>
<td></td>
<td>1.8-3.81</td>
<td></td>
</tr>
<tr>
<td>Fox</td>
<td>7.3-10.0</td>
<td>30-100</td>
</tr>
<tr>
<td>Goat</td>
<td>2.2-4.01</td>
<td>100-300</td>
</tr>
<tr>
<td>Mouse</td>
<td>4.4-11.7</td>
<td>20-300</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3.8-10.6</td>
<td>40-150</td>
</tr>
<tr>
<td>Rat</td>
<td>2.6-20.0</td>
<td>25-300</td>
</tr>
<tr>
<td>Sheep</td>
<td>5.2-11.8</td>
<td>30-200</td>
</tr>
<tr>
<td>Seal</td>
<td>9.1-15.8</td>
<td>20-50</td>
</tr>
<tr>
<td>Pig</td>
<td>7.4-17.6</td>
<td>25-70</td>
</tr>
<tr>
<td>Cow</td>
<td>5.0-22.6</td>
<td>30-90</td>
</tr>
<tr>
<td>Kitten</td>
<td>8.0-25.0</td>
<td>20-80</td>
</tr>
<tr>
<td>Civet</td>
<td>9.1-18.8</td>
<td>20-40</td>
</tr>
<tr>
<td>Squirrel</td>
<td>10.3-22.1</td>
<td>20</td>
</tr>
</tbody>
</table>

*Pulmonary arteries with a diameter exceeding the higher figure were classed as "elastic arteries."

1Lung inflated with 4 per cent formaldehyde.

were seen between the endothelium and the internal elastic lamina; they never completely encircled the lumen but were sometimes multiple.

In general, the elastic laminae were well defined. They appeared rather thinner in the goat than in the other species examined.

A considerable range in the medial thickness of the muscular pulmonary arteries was found in all species from which postmortem specimens of uninflated lung had been taken (table 1). The mean value for the thickness of the muscular pulmonary arteries exceeded 5 per cent in all cases and 10 per cent in the majority of animals examined. In the case of the guinea pig, the arrangement of muscle was so irregular as to make estimates of medial thickness valueless. In the formalin-inflated lung from the dog and goat, the thickness of the media of the muscular pulmonary arteries was comparable to that in man, being less than 5 per cent.

Another characteristic of these animal lungs was the presence of numerous small muscular arteries with a diameter as small as 20μ, even allowing for the presence of some contraction of the arterial walls (fig. 8). These small vessels, which are in part a reflection of the small size of some of the animals, led to the extreme variation in medial thickness noted in table 1, for there was a rapid increase in the thickness of the media with progressive diminution in arterial diameter. For instance, in the rat, which was found to have a range of medial thickness of 2.6 to 26.0 per cent, the smaller figure was due to the largest muscular arteries and the higher figure was due to the smallest muscular arteries. Even in the portions of formalin-inflated lung examined in the dog, arterial vessels as small as 46μ in diameter had a distinct media with internal and external elastic laminae.

Elastic Pulmonary Arteries

As in the human lung, the basic structure of this class of vessel was similar to that of the muscular pulmonary artery, the main
Figs. 1 through 15 (See legends on opposite page)

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point of distinction being the presence of concentrically arranged elastic fibrils within the media. There was considerable variation in the thickness of the media from species to species (table 1).

Pulmonary Veins

These vessels comprised an intima of endothelium, a media of intermingled muscle, collagen, and elastic fibrils, and an adventitia of fibrous tissue admixed with elastic fibrils (fig. 9). Variations of this basic structure occurred in different species.

In most animals the pulmonary veins had predominantly fibrous walls as in the human lung. For example, we consider that collagen was the main tissue present in the pulmonary veins of the monkey, goat, dog, fox, rabbit, seal, kitten, and civet. Limiting internal and external elastic laminae were not clearly defined, and the elastic fibrils that were present were fine and arranged rather haphazardly together with the small amount of smooth muscle present.

In the rat, mouse, and squirrel there was an extension of cardiac muscle from the left atrium into the lung along the pulmonary

Figures 1 through 15

All sections were stained by the Lawson modification of the Weigert-Sheridan method for elastic tissue and counterstained with Van Gieson's stain. Cow (fig. 1). Transverse section of muscular pulmonary artery. Both the internal elastic lamina and the media are thin. The degree of medial thickness shown here and in figures 2 and 3 would be taken as evidence of hypertensive pulmonary vascular disease in a human muscular pulmonary artery, X150.

Pig (fig. 2). Transverse section of a small muscular pulmonary artery. The media and the internal and external elastic laminae are very thick, X340.

Goat (fig. 4). Longitudinal section of a muscular pulmonary artery. The media and the internal and external elastic laminae are thin as in the normal human lung, X190.

Guinea pig (fig. 5). Transverse section of muscular pulmonary artery. The internal elastic lamina is thick. The media is thick and irregular. This section has been taken through one of the sphincter-like masses of circular muscle illustrated in longitudinal section in figures 6 and 7, X340.

Guinea pig (fig. 6). Longitudinal section of muscular pulmonary artery. Above, the internal elastic lamina is thick, and circularly orientated smooth muscle forms a sphincter. Below, the wall of the arterial vessel appears to consist solely of a single elastic lamina with small subendothelial prominences protruding into the vascular lumen, X190.

Guinea pig (fig. 7). Longitudinal section of muscular pulmonary artery. The internal elastic lamina is thick. Sphincter-like masses of circularly orientated smooth muscle are seen at intervals along the artery. Between these masses the wall of the artery is thin, X190.

Pig (fig. 8). Transverse section of a very small muscular pulmonary artery. Internal and external elastic laminae and a thick muscular media are seen clearly. Such appearances in an arterial vessel of this small size in the human lung is pathognomonic of hypertensive pulmonary vascular disease, X340.

Pig (fig. 9). Transverse section of large pulmonary vein. In contrast to the pulmonary artery there is no thick internal elastic lamina. The media consists of circularly arranged smooth muscle with concentric elastic laminae. Many of the elastic laminae are crowded together at the junction between media and adventitia, X190.

Rat (fig. 10). Transverse section of large pulmonary vein. The internal elastic lamina is thick. The media is composed of cardiac muscle, X75.

Rat (fig. 11). Part of the transverse section of large pulmonary vein shown in the preceding figure, at higher magnification to show the cardiac muscle of the media, X340.

Pig (fig. 12). Longitudinal section of small pulmonary vein. The vessel has a beaded appearance due to the discrete fibromuscular masses protruding into the lumen, X340.

Pig (fig. 13). Transverse section of small pulmonary vein. There is a thick elastic lamina, and internal to this is one of the fibromuscular prominences shown in the preceding figure, cut in transverse section. The protruding mass gives the vessel the appearance of being partially occluded, X340.

Guinea pig (fig. 14). Transverse section of bronchial artery. A very thick media composed of circularly arranged smooth muscle is lined on its inner aspect by a thick, internal elastic lamina, X340.

Pig (fig. 15). Transverse section of bronchial artery. There is an outer layer of circularly orientated smooth muscle, a thick elastic lamina internal to this, and a thick inner layer of longitudinally arranged smooth muscle, X340.
veins. Hence there was a sharply defined media limited internally by a thick, often reduplicated, internal elastic lamina. The media was thick and composed almost entirely of cardiac muscle, with very little elastic tissue or collagen (figs. 10 and 11). In the rat this muscle was demarcated into a poorly defined inner circular and an outer longitudinal layer. An external elastic lamina was not seen in the rat, but fine, discontinuous fibrils were occasionally present.

In the cow and the pig (fig. 12) the pulmonary veins had a beaded appearance in longitudinal section owing to the presence of discrete fibromuscular masses protruding into the lumen (fig. 12). The muscle present was of the smooth variety. Transverse sections of some of the smaller pulmonary veins appeared almost totally occluded by these fibromuscular masses (fig. 13). Others showed a thin rim of this material. Focally the endothelium was in contact with the external elastic lamina. The internal elastic lamina was very thin or absent, and the external lamina was thick and frequently reduplicated. The arrangement of the muscle in these small pulmonary veins was always eccentric in contrast to the regularity of the media of arteries.

In all instances the small pulmonary veins approximated the fibrous type in structure and had a media composed mainly of collagen and fine, haphazardly arranged elastic fibrils not delineated by well-defined internal and external laminae. The smallest pulmonary veins, about 100μ in diameter, contained very little smooth muscle and elastic tissue. The pulmonary venules consisted of an endothelial lining over a single elastic lamina.

Bronchial Arteries

Bronchial arteries in the animals examined showed the same variation in structure as described in the human lung by Verloop.4 In many instances, usually in the walls of bronchi, they were composed of an intima of endothelium, a thick internal elastic lamina, a regular media composed of circularly arranged smooth muscle, and an external elastic lamina that was thin or absent (fig. 14). It is on bronchial arteries of this type that measurements of the medial thickness were carried out and included in table 1. In other sites, usually in the pleura or interlobular septa, they had in addition a layer of longitudinal muscle internal to the circularly arranged smooth muscle, as in figure 15.

Discussion

There was a close similarity between the histological structure of the small pulmonary blood vessels from the formalin-inflated lungs of the goat, the dog, and man. In both animals the medial thickness of the muscular pulmonary arteries was less than 5 per cent of the external diameter of the vessel, as in the human lung.2 The structure of the elastic pulmonary arteries and pulmonary veins was also very similar. In the goat, the transition from muscular pulmonary artery to arteriole devoid of muscle occurred at a diameter of about 80 to 100μ. However, in the dog, even in formalin-inflated material, small arterial vessels less than 50μ in diameter had a distinct media.

In contrast to this state of affairs in the inflated lung, in the uninflated postmortem material the muscular pulmonary arteries in the dog and the other animals studied appeared comparatively thick-walled. Although we found a considerable range in the medial thickness of individual arteries, even the lowest values found for most species exceeded 5 per cent, which is the figure we have found in formalin-inflated material in human cases of cor pulmonale.2 In most instances the highest values found for medial thickness exceeded 10 per cent, a figure characteristic of the muscular pulmonary arteries in formalin-inflated material in human cases of mitral stenosis or congenital heart disease with pulmonary hypertension.2 While this appearance of muscularity in the small pulmonary arteries in the animals was no doubt due in part to partial collapse of the lung parenchyma surrounding the vessels, there appears to be a real difference in the amount of muscle in the media compared to man. We have found in recent studies that even
in uninflated postmortem specimens of normal human lung the thickness of the media of small muscular pulmonary arteries less than 300μ in diameter ranges from 2 to only 6.9 per cent.

The muscular appearance of the small pulmonary arteries in animals does not appear to be related to the existence of a pulmonary arterial pressure appreciably higher than that found in the human lung. Doyle and his associates, for instance, have found the range of pulmonary arterial pressures in a small number of cows to be 24/17 to 80/20 mm. Hg. When the small pulmonary arteries are normal in dogs, the mean pressure in the pulmonary circulation is of comparable magnitude to that found in the human lung.

In reptiles this generalization does not hold, for the existence of thick-walled pulmonary arteries in the lungs of the crocodile and turtle may well be related to the anatomy of the heart in these primitive animals, which exposes the pulmonary circulation to hemodynamic stresses similar to those found in human congenital heart disease. For instance, in the crocodile and alligator the pulmonary trunk and second aorta both arise from the right ventricle. As pointed out by Edwards, functional phenomena have been demonstrated in the turtle similar to those of the Eisenmenger complex in man, including the presence of pulmonary hypertension.

In the human lung the finding of large numbers of small muscular vessels less than 80μ in diameter is pathognomonic of hypertensive pulmonary vascular disease, but our observations show that this is a normal finding in the lungs of many laboratory and domesticated mammals, especially if the animals are small. Such muscular vessels with a diameter as little as 20μ are merely the smallest muscular pulmonary arteries and have no pathological significance, unless there are added histological features such as intimal fibrosis or plexiform lesions.

On this account we think that, when histological studies are carried out on the pulmonary vessels in a lung biopsy from an animal after it has been subjected to perfusion or other studies of pulmonary hemodynamics, published data on mensuration of the human pulmonary vessels such as those quoted above should not be used as a yardstick. If they are, the small pulmonary arteries and arterioles of the animal will appear hypertensive, even when they are in fact normal. It would also seem likely that conclusions drawn from experiments on the pulmonary circulation of such animals, particularly concerning vasomotor responses, should be applied to human disease critically.

Apart from the quantity, the type and arrangement of muscle in the pulmonary vessels of these mammals may differ from those of man. An example of this is the presence of striated muscle of cardiac type around the larger intrapulmonary veins in the rat, squirrel, and mouse. Brenner was aware that human pulmonary veins outside the lung have prolongations of cardiac muscle from the left atrium along them, but there is no intrapulmonary extension of these. Clearly, such intrapulmonary cardiac muscle probably contracts rhythmically in life and introduces the possibility of hemodynamic factors and reflexes not present in the human lung.

Also noteworthy were the thick-walled muscular pulmonary veins with irregular fibromuscular prominences projecting into the lumen in the cow and the pig. The peculiar arrangement of smooth muscle in the media of the muscular pulmonary arteries of the guinea pig suggests that these vessels may have different physiological potentialities from human thin-walled pulmonary arteries.

**Summary**

A study was made of the histology of the small pulmonary blood vessels in uninflated postmortem specimens from the monkey, dog, fox, mouse, rat, rabbit, squirrel, sheep, pig, cow, seal, kitten, civet, and guinea pig. The small pulmonary arteries appeared unusually muscular compared to those of man, and the increased medial thickness was reminiscent of the medial hypertrophy found as an early change in the human lung in diseases associated with pulmonary arterial hypertension.
This suggests to us that the histological criteria usually employed for grade 1 hypertensive pulmonary vascular disease in man are invalid when applied to lung biopsies from animals used for experiments on the pulmonary circulation. There is a sphincter-like arrangement of the muscle of the media of the muscular pulmonary arteries of the guinea pig. In the rat, mouse, and squirrel there is an extension of cardiac muscle along the larger intrapulmonary veins. Fibromuscular prominences project into the vein lumen in the cow and the pig.

References
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