

# Pulmonary endothelial pavement patterns

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**ABSTRACT** The appearance of the endothelial pavement pattern was studied in the pulmonary trunk, pulmonary veins, aorta, and inferior vena cava of the rat by means of silver staining of the cell borders. The endothelial cell in each of the four blood vessels was found to have its own distinctive shape, fusiform and pointed in the direction of blood flow in the case of the aorta and larger and more rectangular in the pulmonary trunk and pulmonary veins. Detailed quantitation of the dimensions and surface area of the endothelial cells in each blood vessel was carried out by a photographic technique. Pulmonary hypertension was induced in one group of rats by feeding them on *Crotalaria spectabilis* seeds. The endothelial pavement pattern in their pulmonary trunks became disrupted with many of the cells assuming a fusiform shape reminiscent of aortic endothelium. Many small, new endothelial cells formed in the pulmonary trunk suggesting division of cells to line the enlarging blood vessels. In contrast the endothelial cells of the inferior vena cava merely increased in size to cope with the dilatation of this vein.

In this study we set out to determine if the size and shape of the endothelial cells of the pulmonary trunk differ from those of the pulmonary veins, the aorta, and the inferior vena cava. We also wanted to establish what changes, if any, occur in the endothelial cells of the pulmonary trunk exposed to severe hypertension.

## Methods

We studied 14 female Wistar albino rats whose initial mean body weight was in the range of 185 to 189g (table 1). Seven of these, which formed the test group, were fed on powdered rat food which had been adulterated by the addition of powdered *Crotalaria spectabilis* seeds to give a concentration by weight of 0.2% (2g powdered seeds per kg rat food). These seeds contain a pyrrolizidine alkaloid, monocrotaline, which induces severe pulmonary hypertension.<sup>1</sup> The other seven rats, which formed the control group, were fed on powdered rat food alone.

After periods which varied between 34 and 45 days the control and test animals were anaesthetised with ether and the outlines of the endothelial cells of the pulmonary trunk, pulmonary veins, aorta and inferior vena cava were stained by a modification of the method of Poole, Sanders, and

Florey.<sup>2</sup> The lower abdominal inferior vena cava was carefully nicked with fine-pointed scissors allow as much blood as possible to be lost. A cannula filled with 5% glucose was inserted through the incision towards the heart and tied into position using cotton thread. A small cut was then made in the lower abdominal aorta and 5% glucose was perfused through the animal by minimal manual pressure being applied to syringe attached to the cannula in the inferior vena cava. When the glucose coming out of the small cut in the aorta was blood-free, the following solutions were perfused in a similar manner: freshly-prepared 0.25% silver nitrate for 30 seconds, 5% glucose for 20 seconds, 3% cobalt bromide in 1% ammonium bromide for 20 seconds, 5% glucose, and finally 10% formalin in distilled water. The 10% formalin was perfused under hydrostatic pressure from a height of 25 cm for three hours. After fixation, the inferior vena cava, pulmonary trunk, aorta, and pulmonary veins were dissected out. After removal of adherent fat, the blood vessels were opened by

Table 1 *Body weights and ratios of left to right ventricular weight in test and control rats*

Group	Initial mean body weight (g)	Final mean body weight (g)	$\frac{LV+S}{RV}$
Control (seven rats)	189	246	4.0
Test (seven rats)	185	156	2.0

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longitudinal cut and carefully pinned to cork with fine entomological pins, with the endothelial surface uppermost. Extreme care was taken to avoid the production of preparations which had contracted or overstretched areas. While still attached to the cork, the preparations were then dehydrated in ascending grades of ethyl alcohol and cleared in xylene. The preparations were then removed from the cork and mounted between a slide and a coverslip using DPX.

After removal of the four blood vessels the heart was fixed separately in 10% formol saline. The weights of the free wall of the right ventricle (RV) and of the free wall of the left ventricle with interventricular septum (LV+S) were determined for each animal according to the method of Fulton, Hutchinson, and Morgan Jones.<sup>3</sup> Using the technique, right ventricular hypertrophy and hence by inference, pulmonary arterial hypertension, is indicated when the ratio (LV+S)/RV is less than 2.0. We used this ratio merely to confirm that in the test rats the ingestion of the *Crotalaria spectabilis* seeds had induced pulmonary hypertension.

Table 2 *Linear measurements and surface areas of endothelial cells in the four classes of vessel in the test and control animals*

	Parameter	Inferior vena cava	Class of vessel			
			Pulmonary vein	Pulmonary trunk	Aorta	
Control rats	Number of measurements	275	184	360	310	
	Length ( $\mu\text{m}$ )	50 (0.67)	48 (0.79)	30 (1.17)	55 (0.73)	
	Breadth ( $\mu\text{m}$ )	14 (0.24)	23 (0.31)	14 (0.23)	10 (0.18)	
	Ratio of length to breadth	3.5 (0.23)	2.1 (0.10)	2.5 (0.26)	5.6 (0.24)	
	Area ( $\mu\text{m}^2$ )	679 (11.63)	1022 (18.25)	426 (5.98)	476 (6.91)	
	Test rats	Number of measurements	140	175	313	175
Test rats	Length ( $\mu\text{m}$ )	64 (1.71)	47 (0.96)	40 (0.78)	46 (0.89)	
	Breadth ( $\mu\text{m}$ )	20 (0.67)	22 (0.47)	17 (0.40)	12 (0.31)	
	Ratio of length to breadth	3.3 (0.45)	2.1 (0.13)	2.4 (0.18)	3.8 (0.31)	
	Area ( $\mu\text{m}^2$ )	1235 (39.41)	1010 (29.69)	591 (14.27)	472 (10.79)	
	Statistical comparison of means between test and control rats	Length	$t=8.02$ $p<0.001$	$t=1.15$ NS	$t=7.40$ $p<0.001$	$t=7.70$ $p<0.001$
		Breadth	$t=7.99$ $p<0.001$	$t=1.40$ NS	$t=7.43$ $p<0.001$	$t=5.61$ $p<0.001$
Ratio of length to breadth		$t=0.31$ NS	$t=0.26$ NS	$t=0.49$ NS	$t=4.56$ $p<0.005$	
Area		$t=13.53$ $p<0.001$	$t=0.34$ NS	$t=10.66$ $p<0.001$	$t=0.31$ NS	

Figures in parentheses indicate the standard error of the mean. With the exception of the ratio of length to breadth all standard errors and values of  $t$  are calculated from individual measurements of endothelial cells. With the ratio of length to breadth, calculations are made from the mean values obtained from each animal.

*laria spectabilis* seeds had induced pulmonary hypertension.

#### DETERMINATION OF CELL AREAS

Photomicrographs were taken of areas of the endothelial lining of the four blood vessels in each case. The areas were selected randomly apart from the condition that they had to be stained well without undue thickening of the cell borders or obvious distortions. The magnification used was  $\times 1000$  so that dimensions measured in mm by ruler on the prints could immediately be translated into  $\mu\text{m}$  on the sections. Measurements were made on between 140 to 360 cells in individual blood vessels in the control and test groups (table 2). The length and breadth of each cell was measured. The area of each cell was determined by cutting out the image of each cell very carefully and weighing the piece of print removed. By comparing the weight with that of a known area in  $\mu\text{m}^2$  at the same magnification and printed on the same type of photographic paper, the area of the cell was calculated. From the measurements of length, breadth, and area for each cell, the mean values could be calculated for each of the four blood vessels for each rat. Further calculation yielded the mean values for length, breadth and area for each of the four blood vessels for the control and test groups as a whole (table 2).

## Results

### QUALITATIVE

#### Control rats

The endothelial cells of the aorta were long and narrow and fusiform in shape. The long axis of the cell was in the direction of blood flow (fig 1). In contrast those of the pulmonary trunk were shorter and broader and had a squat rectangular or roughly triangular shape. Their borders were tessellated (fig 2). The endothelial cells of the inferior vena cava were also rectangular but much longer and narrower; they were smooth in outline (fig 3). In the pulmonary veins the endothelial cells were large and much rounder in shape (fig 4). The shapes of the endothelial cells of the four blood vessels were so characteristic as to allow immediate identification of the vessel in question. The endothelial pavement pattern in all the blood vessels of all the control rats was strikingly uniform.

#### Test rats

In the pulmonary trunks which had been exposed to hypertension the monotonously regular endo-

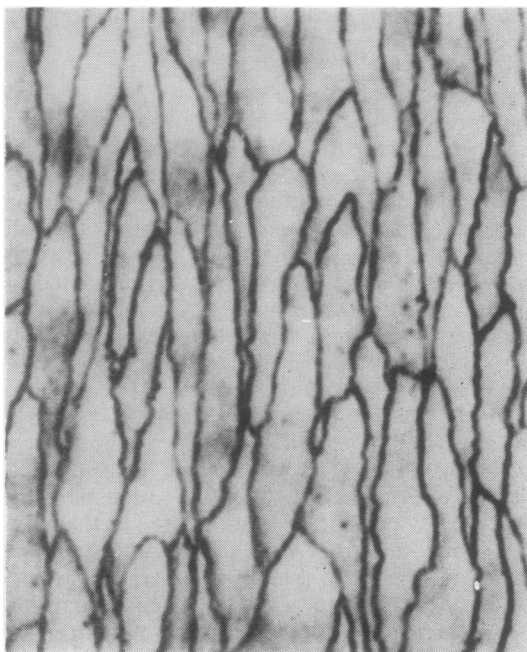


Fig 1 *Aorta of a control rat. The endothelial cell pavement pattern is strikingly uniform. Cells are small and fusiform, the greater diameter being in the direction of blood flow. In this and the other figures: silver preparation, original magnification  $\times 1000$ .*

thelial pavement pattern was disturbed (fig 5). Many of the endothelial cells lost their squat rectangular outline to assume a narrow, fusiform shape reminiscent of aortic endothelial cells. The tessellated border was lost. Thus the endothelial pavement pattern of the pulmonary trunk was quite distinct in the control (fig 2) and test groups (fig 5). In the rats with pulmonary hypertension some very small endothelial cells were found in the pulmonary trunk (fig 5).

#### QUANTITATIVE

The mean (LV+S)/RV ratio was 4.0 in the control rats but 2.0 in the test animals (table 1) thus confirming that the latter had developed pulmonary hypertension.

The mean linear measurements and areas of endothelial cells in the four classes of vessel studied in the control and test rats are shown in table 2. The endothelial cells of the aorta and pulmonary trunk were smaller than those of the inferior vena cava and pulmonary vein, the latter vessel having the largest cells. The endothelium of the inferior vena cava was somewhat elongated

in the direction of blood flow but the ratio of length to breadth was greatest by far in the aortic endothelium.

In the rats in which pulmonary arterial hypertension had been induced by ingestion of *Crotalaria spectabilis* seeds, the endothelial cells of the aorta and pulmonary vein did not change in size. In contrast, the endothelial cells of the inferior vena cava were larger with respect to length, breadth, and area. Most endothelial cells of the pulmonary trunk in the test rats were somewhat longer, broader, and greater in area but their mean increase in area was not nearly so pronounced as in the case of the inferior vena cava. Further elucidation of this anomaly was made possible by the preparation and analysis of histograms.

The graphs comprising figs 6 and 7 represent frequency distribution histograms for the areas of endothelial cells in the inferior vena cava and pulmonary trunk. The upper histogram in fig 6 shows this distribution in the inferior vena cava of control rats. It is a normal distribution with the mean and the mode coinciding at between 600–700  $\mu\text{m}^2$ . A vertical line drawn through the mode cuts the histogram into equal halves. The

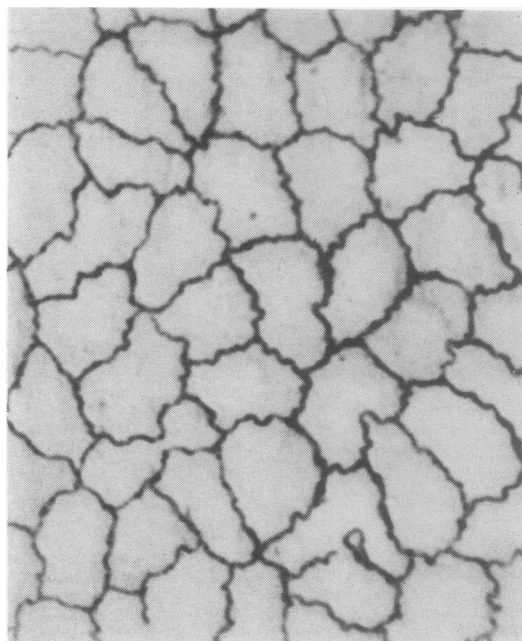


Fig 2 *Pulmonary trunk of a control rat. The pavement pattern is uniform. Cells are rectangular or roughly triangular in shape and have a tessellated border.*



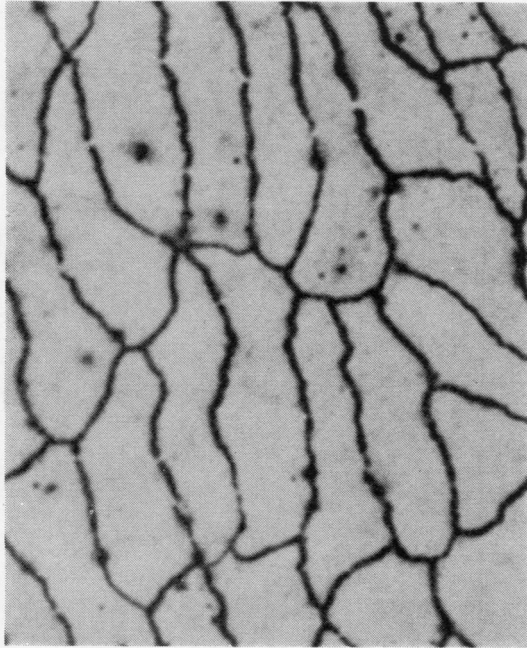


Fig 3 *Inferior vena cava of a control rat. The pavement pattern is uniform. Cells are rectangular, being elongated in the direction of blood flow.*



Fig 5 *Pulmonary trunk of a test rat. The endothelial cell pavement pattern is irregular with considerable variation in the size of the cells, some of them being very small. There are groups of fusiform, pointed cells (between arrows) reminiscent of those seen in the aorta of control rats shown in fig 1.*

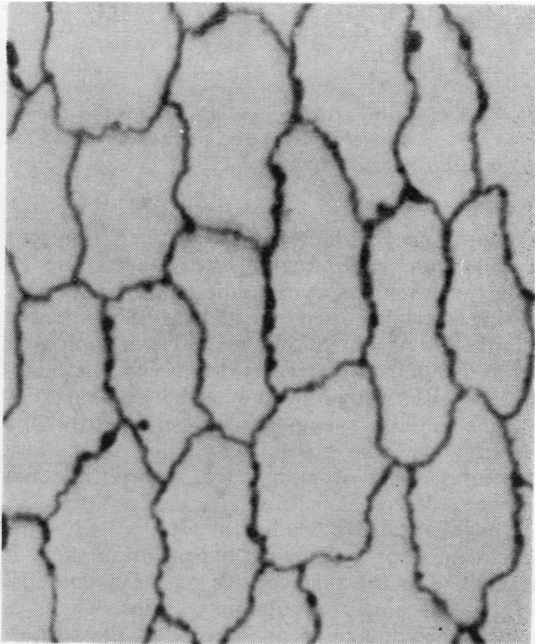


Fig 4 *Pulmonary vein of a control rat. The pavement pattern is uniform. Cells are more rounded.*

lower histogram shows the distribution of areas in the inferior vena cava of test rats. This too approximates to a normal distribution although the spread of values is broader than in the control rats. The mean and mode are roughly coincident at 1100–1300  $\mu\text{m}^2$ . A downward extension of the vertical line through the mode of the upper graph cuts the lower graph into two unequal parts, such that the area of the graph to the left of this line is only 0.084 of the total area. The trend in the inferior vena cava has thus been for all the endothelial cells to enlarge with a consequent displacement of the histogram to the right. The situation is analogous to inflation of a flexible tube.

In fig 7, similar histograms of the pulmonary trunk are shown. In the control rats the distribution is normal with the mean and mode lying between 400–500  $\mu\text{m}^2$ . In the test rats, however, the distribution of areas is a poor approximation to normal since it is skewed sharply to the left. The mode is between 300–400  $\mu\text{m}^2$  but the mean is nearly 600  $\mu\text{m}^2$ . An extension of the line through the mode of the upper graph cuts the

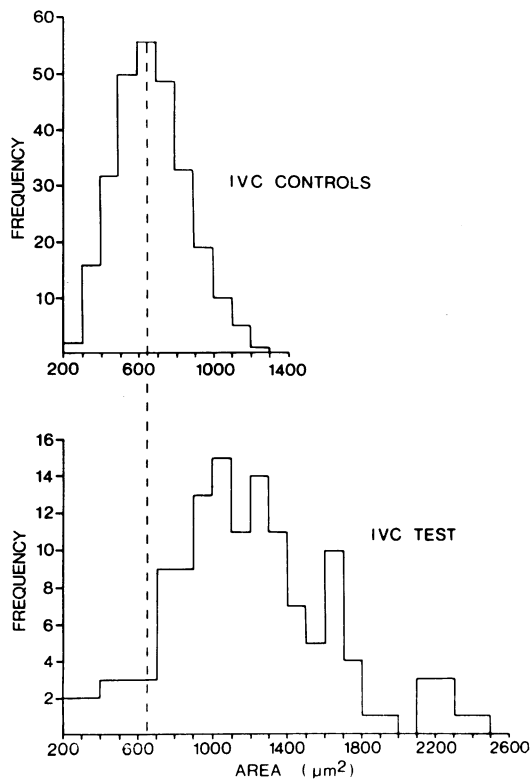


Fig 6 Frequency distribution histograms for the areas of endothelial cells in the inferior vena cava of the control and test rats.

lower graph into two unequal parts. In this vessel, however, as much as 0.35 of the total area is to the left of this line. The pulmonary trunk thus reacts differently from the inferior vena cava to an increased intraluminal pressure. Only a proportion of cells enlarge as in the inferior vena cava, the remainder staying at control areas thereby skewing the histogram to the left.

### Discussion

Our results show that each of the four blood vessels is lined by endothelial cells with their own characteristic size and shape. It seems likely that the configuration of these cells is related to the haemodynamic conditions obtaining within the vessels so that in general, the higher the intravascular pressure the smaller and more pointed the endothelial cell. In contrast the endothelial cells of veins are larger and more rounded.

The endothelial cells of the pulmonary trunk (fig 2) have a characteristic squat rectangular or

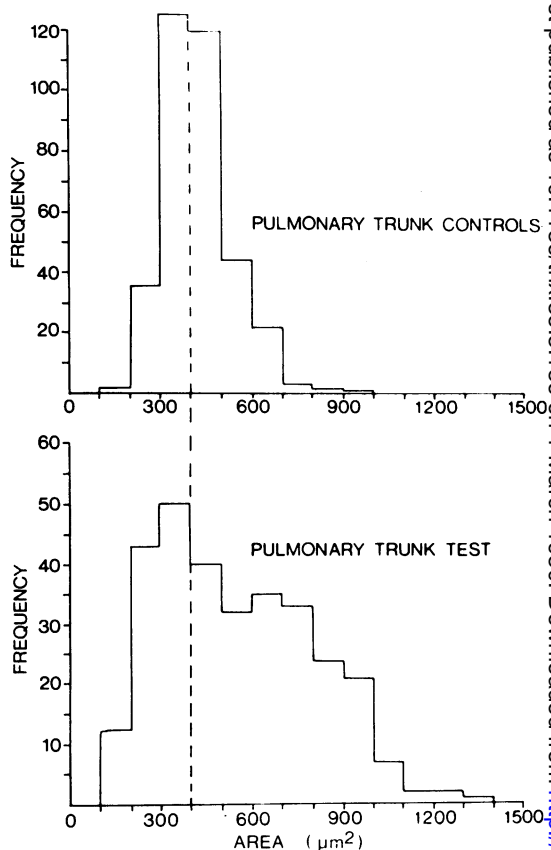


Fig 7 Frequency distribution histograms for the areas of endothelial cells in the pulmonary trunk of the control and test rats.

triangular shape which is quite distinct from that of pulmonary veins (fig 4) or the aorta (fig 1). With the development of pulmonary hypertension, however, striking changes occur in the pulmonary endothelial pavement pattern. The monotonous regularity of the pattern is disturbed. The endothelial cells change in the shape so that they become initially triangular and later narrow and fusiform like the aortic endothelial cells. This structural effect of pulmonary hypertension has not been described before.

Our quantitative studies show that with the development of pulmonary hypertension there is no change in the surface area of the endothelial cells of the aorta or pulmonary veins. There is, however, an unequivocal increase in the surface area of the endothelial cells of the inferior vena cava in the test rats. This is without doubt an expression of the rapid dilatation of this vein

which occurs during the terminal onset of congestive cardiac failure secondary to the development of pulmonary hypertension.

The behaviour of the endothelial cells of the pulmonary trunk is not so easy to interpret and cannot be explained by simple distension as in the inferior vena cava. Qualitatively, the endothelial cells in the pulmonary trunk of test rats showed considerable variation in size, with numerous small cells scattered among cells several times their surface area. A possible explanation for the skewed distribution (fig 7) is that endothelial cells in the pulmonary trunk have differing elasticity so that some expand but others resist expansion. It seems unlikely that such striking variations in physical properties of cells could exist over the small distances covered by the photographic prints. A similar effect could be obtained if the endothelial cells were to overlap one another. A more likely explanation is that endothelial cells undergo division as a response to an increase in circumference of the pulmonary trunk. The new, small cells would be responsible for skewing the size distribution to the left. Presumably with the passage of time the daughter cells would mature, restoring the distribution to

normal should the animal survive the pulmonary hypertension.

The difference in reaction of endothelial cells between the inferior vena cava and pulmonary trunk is probably a reflection of the differing time during which the vessel walls are stretched. In the inferior vena cava a raised intraluminal pressure occurs briefly during congestive heart failure and endothelial cells, therefore, have insufficient time to divide.

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