Early Stages in the Development of Collateral Circulation to the Lung in the Rat

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It has been shown repeatedly that the bronchial arteries enlarge considerably following occlusion of branches of the pulmonary artery by either embolism (Virchow,1,2 and others) or experimental ligation (Schleipfer,3 Liebow et al.,4 Ellis et al.,5 and others). The expanded bronchial arteries have been demonstrated by various anatomic methods, including bronchovascular casts4 and microscopic sections.5 Physiologic studies revealed that this bronchial collateral blood supply to the lung deprived of the pulmonary artery was able to participate in the gas exchange in this lung, and must, therefore, have reached the alveolar capillaries (Bloomer et al.6). Liebow et al.7 concluded that under these conditions a considerable amount of blood was circulating only on the left side of the circulation, entering the lung through bronchial arteries, going through the lung capillaries and pulmonary veins back to the left heart and into the aorta. This increased left cardiac output, as compared with the right cardiac output, seems, however, to be without any influence on the size and configuration of the heart in normal animals (Loring and Liebow).8

Most of the above-mentioned studies revealed that this bronchial collateral circulation developed within the first few weeks following ligation of the pulmonary artery, and continued to increase over the next months. The early stages in the development of this collateral circulation have not been explored in detail. The aim of this experimental study was therefore to obtain further elucidation of the following questions: 1. Does the collateral circulation to the lung develop simply by enlargement of pre-existing bronchial arteries or is there a formation of new vessels? 2. At what rate do pre-existing vessels enlarge? What histologic changes can be observed during this development? 3. If there is evidence for new vessel formation, are these derived from pre-existing capillaries or are they formed entirely anew? 4. How are these collateral vessels connected to the capillaries of the lung?

Methods

Although most experimental studies of collateral circulation to the lung have been carried out on dogs, the rat was chosen as more suitable for present purposes because of its smaller size. Twenty-four adult animals of the Wistar strain, weighing 300 to 350 gm., were operated upon in the following fashion: The animals were lightly anesthetized with ether. They then received sodium pentobarbital intraperitoneally in a dose of 2 mg./100 gm. body weight. A positive pressure apparatus, similar to that of Loring,9 was then applied to the head of the animal, which was allowed to breathe oxygen. A left thoracotomy was performed and the left pulmonary artery was ligated with 2 C silk ligatures. The pulmonary artery was not transected.

The animals were sacrificed in groups of 4 at 2, 5, 10, 20, 30 and 40 days after the operation and were studied by the following 2 methods:

A. In 2 animals of each group, the aorta and pulmonary artery were injected with a gelatin mass, prepared with slight modification according to Schlesinger.10 The gelatin was stained by a suspension of extremely fine particulate dyes; the color for the aorta was black and for the pulmonary artery, red. The animals were anesthetized with ether, heparinized intravenously, and sacrificed. The aorta was cannulated just below the diaphragm. A second cannula was placed into the right ventricle. Both vessels were injected simultaneously at a pressure of 25 cm. water, which was kept constant by means of a water escape valve. The injection was stopped as soon as the

coronary arteries were filled with black gelatin. The bronchial tree was injected with formalin 1:9 by gravity until the lungs filled the chest. After solidification of the gelatin, the whole chest was dissected out and immersed in formalin for further fixation. The lungs were then removed from the chest, carefully preserving adhesions to the chest wall, and cleared with methylsalicylate. The preparations were examined under a stereomicroscope.

B. The lungs of the 2 other animals of each group were fixed in 10 per cent formal saturated with HgCl₂ by inflation of the collapsed lungs through the trachea. The left lung was bisected in the frontal plane, so that the main bronchus was located entirely in the dorsal half. This part was cut into 8 horizontal slices, embedded in 1 paraffin block, and providing step sections through that lung. Serial sections were cut and stained alternately with hematoxylin and eosin, elastin-kernechtrot-pikroindigo, and the Goldner modification of the Masson stain.

Reconstructions of the course of vessels were obtained from the serial sections by tracing the contours of the vessels of each section on separate sheets of tracing paper, stacked in correct orientation. By transillumination of these in appropriate groups, the course of the vessels could easily be followed.

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Figure 1c
Left lung of rat, 10 days after ligation of the pulmonary artery. Enlargement and tortuosity of the bronchial arteries ba and of the nutrient arteries of the pulmonary vein apv. Filling of the main pulmonary artery pa from vessels surrounding silk tie at of ligature, and of peripheral branches ppa from bronchial arteries. Patchy injection of lung capillaries adjacent to bronchi ac. Compare text. X9. Pulmonary vein = pv.

An additional series of rats was operated upon in the same fashion, but they were given a subcutaneous injection of 0.001 mg. of colchicine per gram body weight approximately 6 hours prior to sacrifice. This procedure was to arrest mitoses in metaphase during this interval.

Remarks on the Normal Histology of the Vessels of the Rat's Lung

The anatomy of the pulmonary arteries of the rat does not deviate strikingly from that of man. The major branches have the structure of elastic arteries; the smaller branches are of muscular type and show 2 distinct but delicate elastic membranes that lie inside and outside of the relatively thin muscle layer of the media. As in many rodents, the media of many medium-sized muscular branches possesses a segmented structure.11 The pulmonary veins, however, have a special structure. Their endothelium is enveloped by a thin and irregular coat of smooth muscle, surrounded by an elastic membrane. Surrounding this membrane is a thin sheath of delicate fibrous tissue and a coat of circular heart muscle fibers enveloping all veins down to a diameter of 200 to 250 μ. The heart muscle coat contains fine arterioles, venules, and capillaries. Its nutrient arteries derive from the bronchial arterial system. The venous outflow seems to go directly into pulmonary venules. In smaller pulmonary veins, the smooth muscle coat may be arranged in circular bundles. An external, but no internal, elastic membrane is typical for these vessels. The smallest veins are devoid of musculature.
Results

The collateral blood supply to the lung, after ligation of the pulmonary artery, comes from branches of the aorta and proceeds in 5 different ways: A. Pre-existing vessels enlarge. These include: (1) the bronchial arteries; and (2) the nutrient arteries of the heart muscle coat of the pulmonary veins. B. Three types of newly formed vessels that supply blood to the pulmonary parenchyma appear: (3) vessels near the ligated end of the pulmonary artery; (4) vessels in pleural adhesions; and (5) arteries in the peribronchium and in the septa surrounding the pulmonary veins.

After injection of black gelatin into the aorta of a normal control rat, the bronchial arteries appear as very fine lines, outlining the contours of the bronchus (fig. 1a). The same is seen in the nonoperated right lung of the experimental animals, serving as a control for a constant injection technique. Only the major branches of the nutrient arteries of the pulmonary veins can be seen to lead from 1 of the main bronchial arteries to the main pulmonary vein.

Two days after ligation of the pulmonary artery, only a slight increase in size of the bronchial arteries is noted (fig. 1b). Five days after operation, this dilatation is still small. In 1 case, adhesions to the parietal pleura had formed; the many vessels contained therein were filled with black gelatin.

On the tenth day after operation, marked changes in the relationship of the pulmonary vessels are noted. Figure 1c and d show that a considerable collateral blood supply to the lung has developed:

1. In figure 1c is demonstrated a very marked enlargement of the bronchial arteries, as compared with the specimens from control rats and from animals earlier in the experiments (fig. 1a and b). The originally more or less straight vessels have assumed a tortuous course. Some peripheral branches of the pulmonary artery are filled with black gelatin, as a result of “retrograde” filling from the bronchial artery (see also page 362 and fig. 9a). Black patches around the bronchi suggest that
there is direct filling of the lung capillaries from the bronchial arteries in the parenchyma adjacent to the peribronchium.

2. The nutrient arteries of the pulmonary veins can be traced half way out along the intersegmental veins (fig. 1c). The filling of the peripheral parts of the pulmonary veins is apparently due to filling from the capillaries, as suggested by the decreasing filling towards the hilum.

3. The main pulmonary artery, and its major branches, are filled with black gelatin from vessels (fig. 1c) which, coming from the scars around the ligature, pierce the wall of the pulmonary artery and thus feed blood into its lumen. This is not "retrograde" filling.
from the bronchial arteries, as can be deduced from the fact that there is no filling of vessels intermediate between the major and the peripheral branches of the pulmonary artery.

4. Figure 1d shows vessels derived from intercostal arteries that reach the visceral pleura through adhesions. The capillaries of the parenchyma of this region have become filled with black gelatin that penetrated to fill the peripheral branches of the pulmonary arteries and veins.

**A. Enlargement of Pre-Existing Vessels**

**Bronchial Arteries**

Examination of the specimens injected with black gelatin through the aorta allows an estimate to be made of the degree of enlargement of the bronchial arteries. Their slight enlargement at 10 days after ligation of the pulmonary artery (fig. 1c) has considerably increased at 20 days, as is shown in figure 2. Figure 3a represents a photograph of a thin slice of a lung 30 days after operation. Some of the enlarged tortuous major branches can be seen. The very dense filling of the lung capillaries adjacent to the bronchus, however, obscures the enlarged peripheral branches (fig. 3b). This is even more obvious at 40 days after operation, where, even in a thin block (fig. 4), only the very large tortuous main bronchial arteries can be seen, while all the peripheral branches are hidden by the densely filled lung capillaries.

A better concept of the gradual enlargement of the bronchial arteries can be obtained from the tracings of vessels in cross sections through bronchi, represented in figure 5. The bronchial arteries can generally be distinguished by the histologic characteristics and relationships described above, but where the decision is difficult, the pursuit of the vessel through the serial sections will reveal its relationship to a vessel of clearly defined type.

A comparison of the different stages in the tracings of figure 5 reveals again that a definite enlargement of the bronchial arteries is noted 10 days after ligation of the pulmonary artery, with a gradual increase at 20, 30 and 40 days. In addition to the increase in size of the few vessels recorded in early stages, more bronchial arteries are found in later stages. The question arises as to whether pre-existing arterioles and capillaries, too small to be recorded in the first tracings, were transformed to arteries, or whether this is due to formation of new vessels. This problem will be considered later.
A quantitative estimate of the enlargement of the bronchial arteries over a period of 40 days following ligation of the pulmonary artery can be gained from the graphs in figure 6a. The diameters of the bronchial arteries found in step sections through the lung of each instance (see page 354) were plotted against the diameter of the bronchus with which they were associated. There is a slight increase in diameters of all the bronchial arteries at 2 and 5 days. Starting with the tenth day, a marked increase in the number of vessels measuring more than 40 μ is noted. This development of large vessels goes on gradually and reaches values up to 200 μ at 30 days. It should be mentioned that only branches of the bronchial artery directly related to a bronchus were considered for this study. In figure 6b, the sums of the diameters of the bronchial arteries of 1 bronchus were again plotted against the diameter of the associated bronchus. Despite the individual variations, the gradual increase of the sums of the diameters of the vessels can be noted to be greater in larger bronchi than in smaller ones.

The statistically calculated trends of these values (fig. 6c) suggest, moreover, that 2 phases can be distinguished in this development of collaterals to the lung. At 2 and 5 days following ligation of the pulmonary artery, the slopes are almost parallel to that of the control, but slightly elevated. This slight elevation but approximate parallelism of the curves can be interpreted as instantaneously occurring diffuse general enlargement of all bronchial arteries to about the same extent. At 20, 30 and 40 days, however, the line is considerably elevated above the control and has a much steeper slope. This indicates further progress in the general enlargement of all the bronchial arteries. The steeper course of the slope results from the fact that the larger central branches of the bronchial arteries are more enlarged than more peripheral ones. The trend of the combined curves of the 2 animals studied 10 days after operation shows a somewhat intermediate position. If the slopes of the single curves are plotted separately (dotted lines) it is evident that the curve of animal 10a coincides practically with the 20 days slope, while animal 10b has a lower and flatter curve. It is not possible to decide whether this peculiar divergence indicates a kind of intermediate stage of development between 5 and 20 days, or whether it is due to other changes not related to the experiment, since animal 10b shows signs of chronic bronchitis with numerous mononuclear cells surrounding the bronchi, as is common in rats.

This quantitative evaluation indicates that after a primary initial dilatation, the most important steps in the development of the collateral circulation to the lung must take place between 5 and 10 (or 20?) days.

The injection of the aorta with black gelatin demonstrated an increase in size of the nutrient arteries of the pulmonary veins 10 days after ligation of the pulmonary artery (fig. 1c). Further examination of these lungs revealed that they enlarge at about the same rate as the bronchial arteries. Being originally very small, they will remain much smaller than the latter, even after considerable growth. While these could be traced only about halfway out along the intersegmental veins at 10 days, finer branches running along more peripheral veins are evident at 20 days (fig. 2). They seem to supply blood to the lung capillaries, as is suggested by the patchy filling of the parenchyma adjacent to the veins. Figure 3b shows remarkably large nutrient arteries of the pulmonary veins at 30 days. They have assumed a somewhat tortuous course and some branches seem to course away from the vein to reach the adjacent lung capillaries, where they cause a patchy filling with gelatin (see also fig. 10).

The enlargement of the nutrient arteries of the pulmonary veins appears very striking in the microscopic sections, as in comparing the 2 photographs of figure 7a and b. Although of arteriolar, or even capillary size in normal rats, they assume a very considerable diameter 40 days after ligation of the pulmonary artery.

The gradual enlargement of these vessels
Figure 5a

Tracings of bronchi and bronchial arteries of a control rat and of rats examined at 2, 5, 10, 20, 30 and 40 days after ligation of the pulmonary artery. Note enlargement and increase in number of bronchial arteries after 10 days. (Continued on opposite page.)

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Figure 5b

has been analyzed quantitatively in a similar fashion to that used for the bronchial arteries. The vessels measuring more than 15 μ in diameter are very few in normal rats (less than 10 per cent). This number increases steadily over the period of 40 postoperative days up to approximately 40 per cent. Since the number of vessels smaller than 15 μ remains almost constant, there is most probably a formation of new vessels associated with the enlargement.

The nutrient arteries of smaller and medium-sized pulmonary veins increase much more than those of the main veins. The explanation of this difference is that all along the bronchial tree branches of the bronchial artery reach the muscle coat of the pulmonary veins to supply blood to its vasa vasorum. When the collaterals have been stimulated, they are also used as peripheral branches to bring blood to the lung capillaries (see page 363 and fig. 10).

B. Histologic Changes Observed During the Enlargement of the Pre-Existing Arteries

The first histologic changes are noted on the fifth day after ligation of the pulmonary artery. They occur primarily in the medium-sized and smaller branches, rather than in the larger bronchial arteries. The endothelium consists of many densely arranged cells with sometimes abundant cytoplasm, protruding into the lumen of the vessel (fig. 8a). The nonundulating course of the fine internal elastic membrane indicates that this vessel is not significantly contracted, whereby it may be concluded that the increase in number of endothelial cells is the result of proliferation.

In several places, indeed, endothelial cells are found in mitosis (figs. 8b and c). The cells are rounded, contain abundant cytoplasm, and protrude into the lumen of the vessel; the nuclei show mitoses in various phases. The mitoses are particularly prominent in the rats treated with colchicine prior to sacrifice (see page 355). After completing division, the cells rearrange themselves in the endothelial layer (fig. 8c). The texture of the media appears somewhat loosened. Many of the muscle cells have lost their long spindle shape and have become oval or even somewhat rounded. As a sign of proliferation, several muscle cells are found in various stages of mitosis (fig. 8d); their cytoplasm has lost its fibrillar structure but has retained the staining properties of smooth muscle cells. The marked mitotic activity of these smooth muscle cells is again impressively demonstrated in the rats that received colchicine. The same changes are visible 10 days after operation. The original main bronchial arteries of a bronchus, located near the pulmonary artery and vein and accompanied by a large nerve, show a relatively thin media, the cells of which are spindle shaped. Other large vessels, accompanied by a small nerve, certainly represent enlarged previously small bronchial arteries. Their endothelium contains many cells; the internal elastic membrane is very thin, often even not recognizable as such. The media is composed of oval, or almost rounded, muscle cells, which are irregularly arranged. Mitoses are still found in the endothelium and in the media.

At 20 days, all the bronchial arteries are enlarged and have a similar appearance. The endothelium is still highly cellular. The internal elastic membrane is very fine and consists of loosely arranged longitudinal elastic fibers. The muscle cells of the media are oval and irregularly arranged; the interstitial collagenous and elastic fibers of the media are...
increased, as compared with the situation in normal arteries.

At 30 days, the structure of the vessel wall has not changed except for some increase in the elastic fibers. Forty days after operation, the endothelium appears less prominent. The internal elastic membrane is more definite, but still not as strongly developed as in normal arteries. The muscle cells of the media begin to assume, once again, more or less of a spindle shape, but are still somewhat irregularly arranged, with increased interstitial collagenous and elastic fibers.

The histologic changes observed in the nutrient arteries of the pulmonary veins, during their enlargement, correspond to those described for the bronchial arteries.

C. How are the Enlarged Pre-Existing Vessels Connected to the Lung Capillaries?

According to Verloop, no anastomoses between the bronchial and pulmonary arteries exist in the rat’s lung. The examination of control animals confirmed this finding in the present series.

After ligation of the pulmonary artery, however, in some injected specimens occasional peripheral branches of the pulmonary artery seemed to become filled from bronchial arteries through tiny precapillary anastomoses (fig. 1c, 9a). In figure 9a, a thin tortuous branch of the bronchial artery is shown to connect with a branch of a pulmonary artery 10 days after ligation. It seems, therefore, that bronchopulmonary arterial anastomoses develop consequent to the ligation of the pulmonary artery. In the serial sections, however, this was very difficult to establish. Only a few direct communications of the bronchial with the pulmonary artery were found and these were mostly located close to regions of pleural fibrosis. Figure 9b indicates, moreover, that the 2 arteries are not connected by an anastomosing vessel, as is common in other species after ligation of the pulmonary artery. The markedly dilated bronchial artery rather runs along the bronchus in very close relationship to the branch of the pulmonary artery. The walls of the 2 vessels are separated from each other by very little connective tissue. At 2 points, close together, the lumina of the 2 vessels communicate over a short distance. Beyond these anastomoses, the bronchial and pulmonary arteries again run separately.

Direct communications from the enlarged bronchial artery to the capillaries of alveoli adjacent to the peribronchium are much more...
prominent than these arterial bronchopulmonary anastomoses. Figure 9b demonstrates a small vessel leaving the bronchial artery and opening into alveolar capillaries in close relationship to the anastomosis described above. Another example is documented in figure 9c, where a smaller branch leaves a larger bronchial artery in order to reach lung capillaries. Starting 10 days after ligation of the pulmonary artery, this is quite commonly encountered in all our experimental animals. Since we could not identify similar vessels in normal control rats with certainty, it can be assumed that these vessels connecting bronchial arteries with lung capillaries are newly formed, consequent to the ligation of the pulmonary artery (see page 369 and fig. 13).

As demonstrated in figure 10, the nutrient arteries are directly connected with lung capillaries, adjacent to septa in which the veins are located. A branch of the enlarged nutrient artery of a small pulmonary vein runs towards the lung parenchyma, where it opens into capillaries. Another branch of the same vessel follows the thin heart muscle coat of the pulmonary vein.

D. Formation of New Vessels Supplying Arterial Blood to the Lung

Figure 11a represents a higher magnification of the site of ligation of the pulmonary artery in the lung of a rat sacrificed 10 days after operation (see also fig. 1e). The scar that formed around the silk ligature contains many fine vessels filled with black gelatin from the aorta. They represent branches of mediastinal arteries. Some are branches of the main bronchial arteries. A few of these vessels are seen to connect with the distal portion of the

Figure 6b and c

b: Sums of the diameters of the bronchial arteries per cross-section of bronchus, plotted separately for each animal. Compare with figure 6a and text. Slopes tend to increase gradually with time. c: Trend of the curves in figure 6b, calculated statistically for the combination of the 2 curves of 1 experimental period. Dashed lines: Individual trends of the 2 animals with diverse values examined at 10 days after ligation.
ligated pulmonary artery, thus filling the lumen of the main pulmonary artery and its major branches with black gelatin (see page 357).

The communication of these vessels with the lumen of the pulmonary artery beyond the ligature can also be recognized in serial microscopic sections. In figure 11b, the part of the pulmonary artery just distal to the ligature is met twice in section. In the more proximal transection on the left, the lumen is found to be narrow; the vessel wall shows a folded media and marked intimal proliferation. The more distal section of the same vessel on the right has a widely patent lumen with an unaltered vessel wall. The pulmonary artery is surrounded by a plexus of tortuous arteries. One of them is seen to penetrate through the wall of the pulmonary artery, and the pursuit of these vessels in the serial sections indeed reveals that several of them do so. Figure 11b is representative: The ligated pulmonary artery is narrowed or even completely occluded by folding of the media and intimal proliferation up to the point where the collateral vessels enter its lumen. From there on the lumen remains patent. It is, therefore, most likely that these penetrating vessels supply blood to the pulmonary artery in such quantity as to ensure a significant blood flow through its branches, which remain patent and serve as distributors of this blood to the lung capillaries.

The study of the development of these vessels reveals that 2 days after ligation, the connective tissue sheath surrounding the pulmonary artery just distal to the ligature is infiltrated by hemorrhage, and contains many polymorphonuclear leukocytes, lymphocytes and histiocytes. In some places, the wall of the pulmonary artery is interrupted by a narrow channel (fig. 12a). At higher magnification (fig. 12b) this channel is seen to be lined by an immature endothelium in continuity with the endothelium of the pulmonary artery. This channel communicates with a wide vessel, likewise lined by an immature endothelium. This seems to be missing in some places, or to consist of rounded cells resembling histiocytes (fig. 12b).

At 5 days after operation, these vessels display an irregular muscle coat. Occasional mitoses are found in cells lying directly adjacent to the still immature thick endothelium (fig. 12c), which also can show mitoses. Ten days after ligation, the arteries surrounding and
Figure 7b

Low-power view of pulmonary vein pv 30 days after ligation of the pulmonary artery. Some of the nutrient arteries apv are markedly enlarged. An enlarged branch of the bronchial artery ba is seen to supply blood to the nutrient arteries. ×120.

Communicating with the pulmonary artery have a more definite muscle coat, which is still irregular; a very thin internal elastic membrane can be recognized, becoming stronger at 20 days. It consists of fine longitudinal elastic fibers. The media of these collateral vessels contains more elastic fibers than that of regular arteries. At 30 and 40 days, the muscle cells of the media are spindle shaped and are more or less circularly arranged. The internal elastic membrane is stronger, but not yet as strong as that of normal arteries.

Vessels in Pleural Adhesions

After thoracotomy and ligation of the pulmonary artery, adhesions of the lung to the parietal pleura can form. As figure 1d illustrates, these adhesions carry blood vessels which, coming from the body wall, grow into the lung.

Two days after operation, there is simply a layer of fibrin containing some erythrocytes and free round cells. Mesothelium is absent, but the connective tissue layers of the visceral pleura are preserved. The adjacent parenchyma shows some hemorrhage. The connective tissue of the septa and peribronchium of these regions contains many free round cells resembling histiocytes.

Ten days after operation, the fibrin has become replaced by a layer of delicate connective tissue, in part covered by mesothelium and containing many thin-wall blood vessels. Separated from this layer by the original elastic membrane of the pleura, a zone of delicate subpleural fibrosis is found in the adjacent parenchyma. Peripheral branches of the pulmonary artery and vein are recognized here by their histologic features. Many smaller and larger thin-walled channels are found in close relationship to these pulmonary vessels. They communicate with the vessels in the adhesion. It is not yet possible to make a distinction between arteries and veins. There was evidence in these preparations that the angiogenesis here proceeds in a manner similar to that described below for new vessels in the peribronchium (see page 369).
At 20 days after operation, an irregular muscle coat is characteristic for the arteries among the newly formed vessels. Also a thin and irregular internal elastic membrane has developed.

At 40 days, the thickness of the wall of these arteries corresponds approximately to that of a normal artery, but there is a slightly irregular arrangement of the smooth muscle cells, and interstitial elastic and collagenous fibers are more numerous than in ordinary arteries.

The connections of these pleural vessels with those of the lung are quite complex. There are end-to-end anastomoses with small peripheral branches of the pulmonary artery and with enlarged bronchial arteries. Many vessels of the organization tissue are directly connected with the capillaries of adjacent alveoli. It is more difficult to establish their
Figure 9a
Left lung of rat 10 days after operation. Thin peripheral block. Peripheral branches of the pulmonary artery pa are injected with black gelatin from the tortuous bronchial arteries ba. X19. Compare text.

relationship to the pulmonary veins. The injected specimens suggest direct, possibly end-to-end, anastomoses. This can, however, not be proved with certainty in the serial sections because of the practical difficulty in distinguishing the peripheral branches of the pulmonary veins from the various collateral vessels.

Formation of New Vessels in the Peribronchium

The number of bronchial arteries per bronchus and the number of nutrient arteries of the pulmonary vein has definitely increased at 30 and 40 days following ligation of the pulmonary artery (see page 358, figs. 5, 6a and 8d). Moreover, direct connections of these vessels to the lung capillaries are rarely found in normal lungs, but are quite frequent after ligation of the pulmonary artery (see page 362, figs. 9b, 9c, and 10). These 2 findings suggest strongly that formation of new vessels must occur in regions of these lungs where no granulation tissue is formed postoperatively.

Figure 9b (below)
Anastomosis A between enlarged bronchial ba and pulmonary artery pa, in rat 20 days after ligation of the pulmonary artery. The lumina of the 2 parallel vessels communicate over a short distance. A small branch be of bronchial artery goes directly to lung capillaries. X480.
**Figure 9c**

Direct connection of bronchial artery ba to lung capillaries by means of a thin-walled vessel bc. ×400.

**Figure 10**

Nutrient artery apv of pulmonary vein pv sends branch bc to alveolar capillaries ac. Another branch bm goes to the heart muscle coat hm of the pulmonary vein. ×270.
Signs of new vessel formation indeed can be seen from 10 to 40 days after ligation of the pulmonary artery.

As a first stage in the process of angiogenesis, solid cords of round cells are encountered in the connective tissue of the peribronchium and of the pulmonary septa, often in connection with bronchial vessels (fig. 13a and b). A wide variation in the size of these cords is noted; they can be 2 to 4 cells thick in one place and 5 to 8 cells thick in another. Occasional mitoses are found in these cells.

As a next step in this development, an outer layer of cells envelops the solid cord. These cells are either somewhat spindle shaped (fig. 13c and e) or they can be more numerous and more densely arranged, resembling an epithelioid gland-like structure (fig. 13b and d). This tube is often eccentrically shaped, thick on one side and thin on the other (fig. 13e). The original compact cell cord is found, here, as a core within this cell tube (fig. 13b to f) and separated from it incompletely by a gap, which may be an artifact. Multiple cytoplasmic bridges connect the 2 structures. Mitoses are found in the core and in the tube.

The further development of these precursors of blood vessels cannot be traced with absolute certainty in the present material. It seems, most probable, however, that the external cell layer represents the anlage of the media, while the compact cell core will form the endothelium. Figure 13e suggests this process, in that the core possesses a groove, the edges of which are in connection with the enveloping cell tube. Figure 13f can also be interpreted in this sense. In vessel (1) the cell core is still compact; the cells are arranged in form of a rosette. Vessel (2) shows a lumen which is surrounded by a garland of cells resembling the cells of the core of vessels (1) in arrangement. Both vessels are enveloped by a sheath of spindle-shaped cells, most probably representing the anlage of the media; those of vessel (2) look more mature than those in vessel (1).

The formation of the vessels connecting the branches of the bronchial arteries and of the nutrient arteries of the pulmonary vein with the lung capillaries passes through the same stages of angiogenesis, as described above, for the formation of new arteries in the peribronchium. First, there is a solid cord of round cells, leading from an artery or from a vessel in formation, towards the parenchyma (fig. 13b). The connection of this cord to the capillaries is established by a group of densely arranged cells, similar to those forming the cord, and often aligned in 2 to 3 layers along the edge of the parenchyma. They are directly connected with adjacent capillaries of the alveolar wall (fig. 13c) and

Figure 11a
Site of ligature of pulmonary artery 10 days after ligation. Injection of aorta with black gelatin caused injection of the pulmonary artery pa beyond the ligature through vessels sv surrounding the silk tie t, which have grown into the lumen of the pulmonary artery. Compare figure 1c and text. ×19.
Section through the pulmonary artery $pa_1$, just beyond the ligature on the left, 40 days after operation. The pulmonary artery is cut twice: The proximal section $pa_1$ shows a narrowed lumen, folded media and intimal proliferation. The distal portion $pa_2$ is widely patent. One of the surrounding collateral vessels $sv_1$ penetrates through the thick wall of the pulmonary artery. Compare text. $\times 85$.

are often found at the base of an interalveolar septum.

As figure 13c demonstrates, these primary vessel precursors also become enveloped by an external layer of somewhat spindle-shaped cells, remaining closely connected with the still solid core. Finally a lumen forms, surrounded by an endothelium and a primitive media.

Discussion

The left lung of the rat consists only of 1 lobe, corresponding proportionately in size to the left upper lobe of other species. The ligation of the left pulmonary artery, therefore, reduces the respiratory volume of the lung only by approximately $\frac{1}{4}$ to $\frac{1}{5}$. It is well known that the remaining portion of the lung is more than sufficient to sustain life. Oximetric studies on dogs, after interruption of the pulmonary arterial supply to a part of the lung, indicated that the remaining lung was able to maintain full oxygen saturation of the arterial blood. Bloomer et al., had to produce arterial desaturation by deep anesthesia in order to determine the oxygen absorption by bronchial blood in the lung of the dog deprived of its pulmonary artery. The intact portion of the lung being sufficient for the respiratory requirements of the body, the developing systemic collaterals do not seem to be of functional importance to the organism. Since all the available vessels enlarge to a considerable extent, and since new vessels form, this collateral circulation seems to be far beyond reasonable nutrient needs of this relatively small organ.

The question of what mechanisms are involved in the development of collateral circulation has repeatedly been discussed. In the systemic circulation, the assumption of Holman and others of a "simple mechanistic action" seems to be best supported by evi-
end today. This would be due to changes in pressure gradients, which could result in dilatation of pre-existing collateral channels. Winblad and associates' occluded the superficial femoral artery in dogs and found a reappearance of blood pressure and pulse in the distal portion of the occluded vessel after a few minutes. Since perfusion of the limb with blood saturated with oxygen did not change the result, the authors consider this partial compensation as due to the mechanical opening of pre-existing collaterals. Liebow and associates have similarly interpreted the conditions occurring in the lung following ligation of the pulmonary artery: The interruption of blood flow in the pulmonary artery causes a reduction of the counter-pressure to the inflowing bronchial arterial blood in the peripheral meshwork of capillaries, where the 2 arterial systems meet. Thus, the flow in the bronchial arteries increases and causes enlargement of the bronchial arteries. The same group, however, were forced to conclude that other than mechanical forces must also be at work, since when both pulmonary arteries and veins are ligated, newly formed arterial and venous transpleural collaterals invariably found their connections with pulmonary arteries and veins respectively, an observation that cannot be explained on a mechanical basis.

Analyzing the enlargement of the bronchial arteries quantitatively (Page 359; fig. 6e), it was possible to divide the process of enlargement of the bronchial arteries following ligation of the pulmonary artery into 2 phases. At 2 and 5 days after operation, all the bronchial arteries are slightly but generally enlarged. This seems to occur almost at once. The structure of these vessels appears almost unchanged on microscopic examination. These early changes seem purely of hemodynamic origin, i.e., dilatation of the bronchial arteries. An altered pressure gradient could represent the hemodynamic factor. It would correspond to the instantaneous partial compensation for
the vascular occlusion observed by Winblad and associates.\textsuperscript{13}

At 5 days, however, microscopic examination indicates beginning proliferative changes in the endothelium and media of the bronchial arteries without further dilatation of the vessels. That true proliferation of the vessels takes place is demonstrated by the occurrence of mitoses in endothelial and smooth muscle cells. In this respect, it might be of interest that mitotic proliferation of smooth muscle cells has been found in arteries of rats also about 6 days after application of external traction to an artery.\textsuperscript{15}

The changing slopes of the curves in figure 6c, at 10, 20, 30, and 40 days, suggest strongly that a second phase of proliferative enlargement of the bronchial arteries succeeds the phase of dilatation. This seems to be associated with the formation of new arteries. In their study of the development of collateral circulation in the mouse's ear, North and Sanders\textsuperscript{16} also found evidence for a proliferative
enlargement of collateral arteries, with a similar time sequence. It seems that the growth of the vessel wall and the "dilatation" of the vessel lumen proceed "hand in hand," rather than one ahead of the other.

The conclusion, therefore, seems justified that a collateral circulation to the lung following ligation of the pulmonary artery develops in two steps: 1. Hemodynamic conditions, as changes in pressure gradients, cause an initial dilatation of the pre-existing systemic arteries in the lung. 2. This is succeeded by a gradual proliferative enlargement of these vessels, apparently not caused by simple hemodynamic factors.

This latter statement is supported by the observation of signs of new vessel formation in regions of these lungs where no formation of granulation tissue could stimulate such a process. It was demonstrated that the primary anlage of newly forming arteries consists in a solid cord of round cells (fig. 13) in connection with smaller branches of the bronchial artery. Before developing a lumen, this cord becomes enveloped by a tube of somewhat spindle-shaped cells, probably representing the anlage of the media. Secondarily, a lumen forms within the internal solid cord, the cells of which form the endothelium. This observation indicates that the formation of these new arteries is not caused by mechanical factors. Rather, it suggests the activity of some chemical "angiogenetic factors" occurring in these tissues following ligation of the pulmonary artery. Furthermore, it appears surprising that the vessel precursors receive an anlage of the media before the endothelial cord becomes canalized.

A similar observation is reported by Benninghoff, who found in the premenstrual en-
Figure 13b, c, d and e

b: New vessel formation. First stage: Solid cord of cells without lumen sc. First signs of formation of external layer of flat cells ec. Cord is in connection with alveolar wall a. Another sprout in more advanced stage is seen on the right sc. First signs of formation of external layer of flat cells ec. Second stage: Formation of an envelope of flat or spindle-shaped cells ec around solid cord sc. Sprout in connection with alveolar capillaries a. c: New vessel formation. Cross section of vessel sprout consisting of core of densely arranged round cells sc enveloped by a sheath of cuboidal or flat cells ec. Second stage: Formation of an envelope of flat or spindle-shaped cells ec. d: New vessel formation. Cross section of vessel sprout consisting of core of densely arranged round cells sc enveloped by a sheath of cuboidal or flat cells ec. e: New vessel formation. Solid cell core sc shows groove g. Enveloping cells ec have assumed a somewhat spindle shape. X750.

dometrium arterioles which resembled vessel (1) in figure 13f. Instead of an endothelial tube, he found a solid cell cord, surrounded by a few spindle-shaped cells. Benninghoff could not decide whether these vessels were in formation or in regression. Werthemann finds that newly forming vessels in pleural adhesions possess first an undifferentiated envelope of primitive fibroblastic cells, secondarily differentiating variously to pericytes, smooth muscle or adventitial cells. In our preparations, the cells enveloping the still solid endothelial cord also appear rather poorly differentiated. They assume a more definite muscle.
structure only when the lumen is present (fig. 13f). Thereafter, hemodynamic factors seem to influence the differentiation of the accessory wall structures of these vessels. This process of angiogenesis, however, needs further investigation before final statements can be made.

The formation of new vessels in regions where tissue has been damaged is not surprising. It is, however, interesting that vessels growing through pleural adhesions meet peripheral branches of the pulmonary artery and vein with which they can form end-to-end anastomoses. Vessels form also in the granulation tissue in the region of the ligature. The examination of the injected specimens and of the serial sections reveals that some of these vessels penetrate through the thick wall of the pulmonary artery into its lumen. These vessels can assume a size large enough to permit an abundant flow of stained gelatin into the pulmonary artery after injection of the aorta. Such fillings have certainly often been misinterpreted as "retrograde injection" of the pulmonary artery from peripheral bronchopulmonary anastomoses.

Direct anastomoses between the enlarged bronchial arteries and the branches of the ligated pulmonary arteries could be demonstrated only relatively rarely in these rats within the first 40 days. This stands in contrast to the findings in dogs in identical experiments, where these anastomoses are commonly found after several months.4-14 Although Verloop11 demonstrated the absence of arterial bronchopulmonary anastomoses in normal rats, previous experimental ligations of the pulmonary artery in this animal suggested a secondary formation of such connections postoperatively with higher frequency than found here.5,8 These statements were based chiefly on the appearance of material injected into the aorta in larger and medium-sized branches of the pulmonary artery. Anastomoses were demonstrated in vinylite casts.8 Some material could, however, have reached the pulmonary artery through vessels penetrating into the distal pulmonary artery at the site of ligature (see figs. 1c and 11a). On the other hand, it is possible that such anastomoses developed in later stages than were examined in this present study, from capillaries connecting the 2 vessels peripherally.

In these rats, it is striking that the enlarged bronchial arteries and the nutrient arteries of the pulmonary veins are directly connected to the capillaries of the parenchyma by multiple small branches. These are found to develop in the same angiogenetic process as was described above for the formation of new arteries.

Summary

The early stages of the development of collateral circulation to the lung following experimental ligation of the pulmonary artery were studied in adult rats. Five different types of vessels were found to be involved:

1. The bronchial arteries enlarge. Quantitative gross and microscopic studies indicated that this is first due to hemodynamic factors leading to dilatation of the vessels. After 5 days, true growth of the vessels occurs, associated with proliferative changes in the vessel wall, including mitoses in endothelial and smooth muscle cells.

2. Pre-existing nutrient arteries of the heart muscle coat of the pulmonary veins enlarge by the same processes.
3. New vessels form in the granulation tissue at the ligature. They can grow through the thick vessel wall into the lumen of the main pulmonary artery to form a considerable portal of entry for blood into this vessel.

4. Vessels form in pleural adhesions and can supply blood to the lung from intercostal arteries.

5. New bronchial arteries form in regions where no granulation tissue formation could stimulate their development. The primary anlage consists of a solid cell cord, soon surrounded by an envelope of spindle-shaped cells, representing the anlage of the media. The cell cord differentiates secondarily to an endothelial tube by forming a lumen.

The enlarged systemic arteries are directly connected to the lung capillaries by means of multiple small branches, which are also newly formed arteries or arterioles. Arterial bronchopulmonary anastomoses are not as commonly found early in the development of collateral circulation in the rat, as late in this process in dogs. It may be concluded that the enlargement of collateral arteries to the lung following ligation of the pulmonary artery may be initiated by hemodynamic conditions, such as changed pressure gradients. After 5 days, however, other, probably humoral factors must be responsible for further stimulation of the growth of pre-existing vessels and for the initiation and guidance of new vessel formation.

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Early Stages in the Development of Collateral Circulation to the Lung in the Rat

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