Chapter 1

Measurements on Microscopic Images

a) Morphometry of Arteries on Microscopic Images

It seems pertinent to begin with an example of 2-dimensional micromorphometry which was performed in the early 1960s by the team of Prof. Suwa as a part of systematized study of arteries (Suwa and Takahashi, 1971). This is for the following three reasons. First, the study presents in a typical way how, in dealing with a real biostructure, one can apply a simplified model. Usually, organs or tissues we face in a living matter have a structure too much complicated to allow direct 2-D morphometry.

Second, this study of arteries introduces a viewpoint from which one can see that the structure of an organ primarily depends on its tissue circulation. How it does, will be discussed in Chapter 3, where we deal with the structure of the liver. This led me to regard the vasculature of the liver as an adequate design that allows this organ to carry out its manifold functions. And I owe all this idea to what I previously experienced while being engaged in the morphometric studies of arteries.

Third, a brief look at the history of measurement technique is to be given. Only two measurements were involved in the study of arteries: to obtain the length and the area on microscopic level. It will be shown that all necessary measurements were practicable even in the early 60s, when no computer assist was available.

Changes of arteries in hypertension (Figs. 1-1, 1-2)

Figure 1-1 demonstrates a pair of cross-sectioned muscular arteries. Each was taken from a medium-sized subbranch of the superior mesenteric artery of an adult, about 300 μm in external diameter. The left was obtained from the autopsy of a normotensive subject, and the right, from a hypertensive of about the same age. The mean blood pressure ante mortem of the latter had been elevated by about two times the norm. Here, worthy of attention is the thickness of the media, the muscular layer of the arterial wall where smooth muscle cells are arranged so as to encircle the arterial lumen. It is obviously thickened in the hypertensive, and here too, the medial thickness appears to be about two times that of the normotensive. As will be shown, this is not considered a mere coincidence.

It is well known that systemic hypertension, when sustained for a long time, can lead to a fatal outcome of the patient such as cerebral hemorrhage, renal failure or cardiac failure. Of these, the first two diseases occur in patients in whom cerebral or renal arteries are not normal but involved beforehand in a change generally called hypertensive arterial lesion. Figure 1-2 exhibits changes of small artery found in the

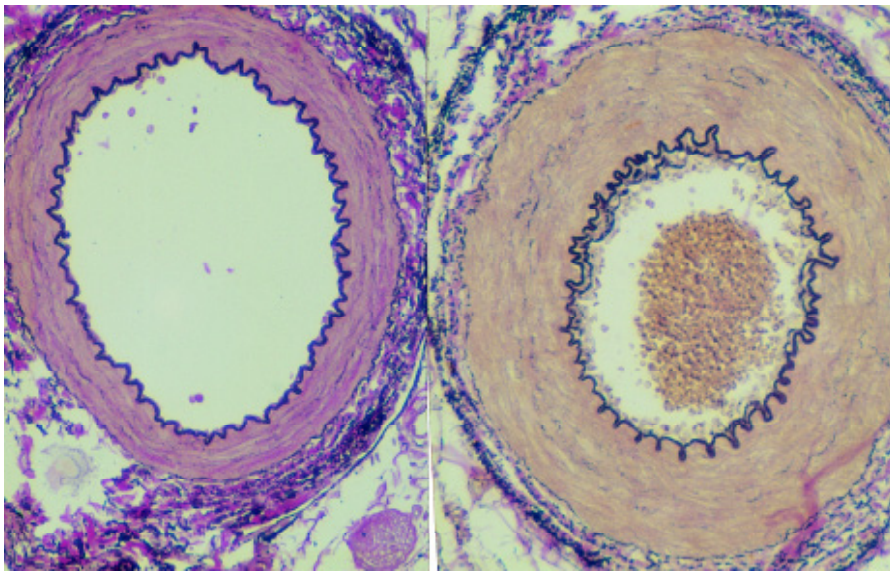

Fig. 1-1. Hypertrophy of medial smoth muscles in a systemic artery of hypertensive subject. Arterial cross sections, both from a peripheral branch of the superior mesenteric artery, about 0.3 mm in external diameter. The left is from a normotensive, and the right, from a hypertensive subject. Note the remarkably thickened medial smooth muscles in the latter. Elastica van Giesson stain.

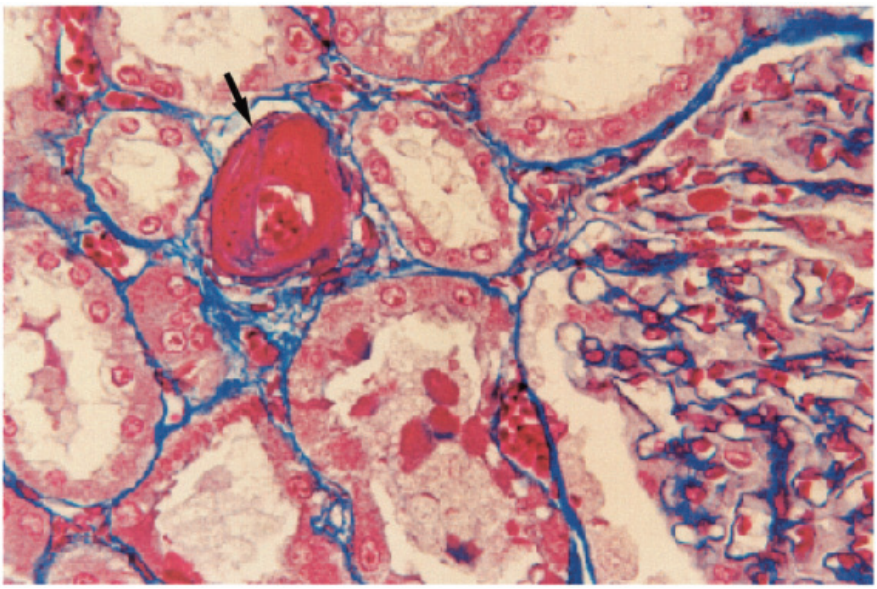

Fig. 1-2. Hypertensive arteriolar lesion (arrow) found in a glomerular vas afferens of kidney from a patient with malignant hypertension. Necrosis of vascular wall with infiltration of fibrinoid material (red). Azan-Mallory stain.

kidney of hypertensive patient, where the medial layer, uniformly reddened on Mallory's stain due to infiltration of fibrin-containing serum component, has undergone diffuse necrosis of smooth muscle cells. In the kidney of hypertensive this sort of lesion leads to luminal obstruction where the blood flow is seriously blocked. Therefore if the lesion involves a number of small arteries of the kidney, the vascular bed of the organ is significantly narrowed and chronic renal failure develops. If a hypertensive patient harbors similar lesions in the brain arteries, it means that the arteries are more or less apt to rupture at the site, resulting in cerebral hemorrhage. Now the problem we face is what hemodynamical changes dominate over the arterial circulation of hypertensives, creating such necrotic lesions in the peripheral small arteries and arterioles.

Analysis of arterial hemodynamics applying Laplace formula (Fig. 1-3)

The mechanical conditions dominating a muscular artery can be analyzed by applying Laplace formula. In Fig. 1-3, a segment of artery is represented by a thin-walled cylinder of uniform radius R. The inner pressure of the artery corresponding to the mean blood pressure is P, and this generates a tension T upon the wall along the direction vertical to the arterial axis. Then,

$$T = PR \tag{1-1}$$

which shows that the wall tension T increases in proportion to P, the inner blood pressure. Therefore when the mean pressure is elevated to two times its normotensive level, also the wall tension is doubled, so long as the arterial radius R remains unchanged. It would be reasonable to assume that in this situation, the circularly arranged smooth muscles of the media become strengthened by thickening themselves so as to antagonize the increased tension load. This explains why in hypertension, arterial changes occur in the form as in Fig. 1-1. In other words, this is an adaptation process of medial smooth muscles to elevated pressure load. The situation may be quite the same as in the left ventricular wall of hypertensive patient, where, in the form of ventricular hypertrophy, the heart muscles attain adaptation to increased mechanical load. Medial hypertrophy in arteries exposed to elevated inner pressure is also found in the expression of the third law of Thoma (1911) who says that the thickness of vascular wall is determined by tension acting transversely on the wall.

This consideration provides a rationale for analyzing the hemodynamical changes

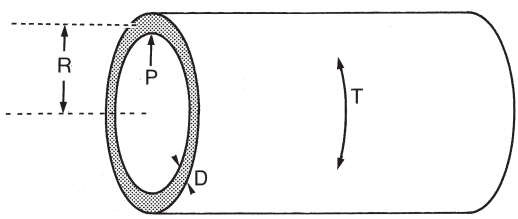

Fig. 1-3. A schema showing the application of Laplace formula to artery. *P*: mean arterial pressure dominating in the lumen. *r*: the radius of the artery. *T*: the tension exerted upon the wall. *D*: the thickness of media.

of arterial circulation in hypertensive subjects. Suppose that in normotensive subjects, we can determine in quantitative terms the medial thickness and radius of arteries supplying an organ. This is done by performing measurement at various levels from the trunk to middle to small arteries to arterioles. Then the analysis is extended to hypertensives, and if, at a level of artery, the media proved to be significantly thickened compared with the normotensive, we can conclude that at the level, the arterial wall had been exposed to an elevated pressure. Thus, the goal of this study has been to visualize how the medial hypertrophy is distributed over the arterial tree, and from this viewpoint to understand the mechanism that can produce hypertensive arterial injuries.

Standardized morphometry of arteries (Fig. 1-4)

An artery can actively change its luminal width according to the situation in which it is placed, by either constricting or relaxing its medial smooth muscles. Therefore, in cross section, an artery presents various forms between the two extremes, tightly constricted and fully dilated states. The more constricted, the thicker is its media, and vice versa, as in Fig. 1-4. The artery in the right picture corresponds to the dilated state of the non-dilated artery in the left, both with the same medial area *S* and the same perimeter length *L* of the inner elastic membrane. Therefore, if measurement is performed directly on a non-dilated artery, we obtain a medial thickness more or less larger than its dilated state. Also the measurement of radius, if performed directly, will give a varying result, depending on the state in which the artery was fixed. Thus, both the anatomical radius and medial thickness have to be defined in a state of artery standardized somehow. In this study, a given cross-sectioned artery was submitted to measurement of two quantities: the perimeter length *L* of the inner elastic lamina and the sectional area *S* of the media, as in the left figure. Then we assume that the artery was transformed into a circular state, as in the right, without changing *L* or *S*, where the media forms a circular belt of uniform thickness along the entire circumference. The anatomical radius *R* and medial thickness *D* were defined in this state, namely,

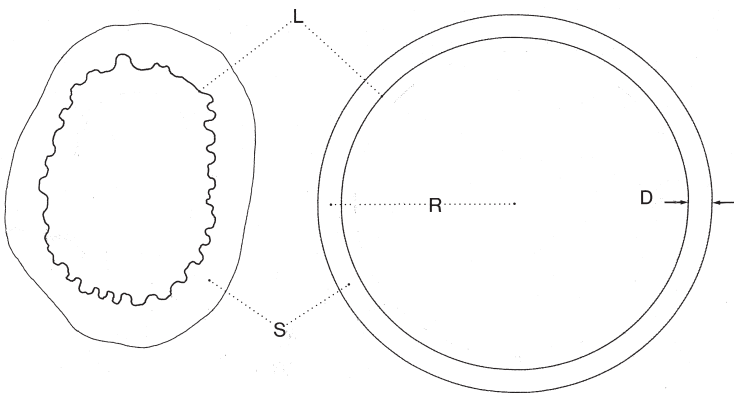

Fig. 1-4. Standardized measurement of artery. Arteries found in a real microscopic section are constricted, irregularly and to varying degree, as in the left. Here the medial area *S* and the perimeter length *L* of elastic lamina are measured, and the anatomical radius *R* and medial thickness *D* of this artery are defined in a circularly dilated state as in the right, a transformation, through which, *S* as well as *L* is kept unchanged.

$$S = 2\pi RD$$

$$L = 2\pi\left(R - D/2\right).$$

The solutions of the equations are:

$$R = \frac{S}{\sqrt{L^2 + 4\pi S} - L}$$

$$D = \frac{\sqrt{L^2 + 4\pi S} - L}{2\pi}.$$

This standardized morphometry, published by Furuyama (1962), serves as a simple geometric model of cross-sectioned arteries and found several applications. It has been employed particularly often by Yamaki (1981) who published a number of papers dealing with pulmonary vasculature and its changes in pulmonary hypertension.

Micromorphometry of arteries: measurement technique (Figs. 1-5, 1-6)

In the early 60s when our studies of arterial changes in hypertensives were under progress, we had technical difficulties in measuring the perimeter length *L* of the inner elastic membrane. In measuring *S*, the area of the medial layer, a classic-type manual

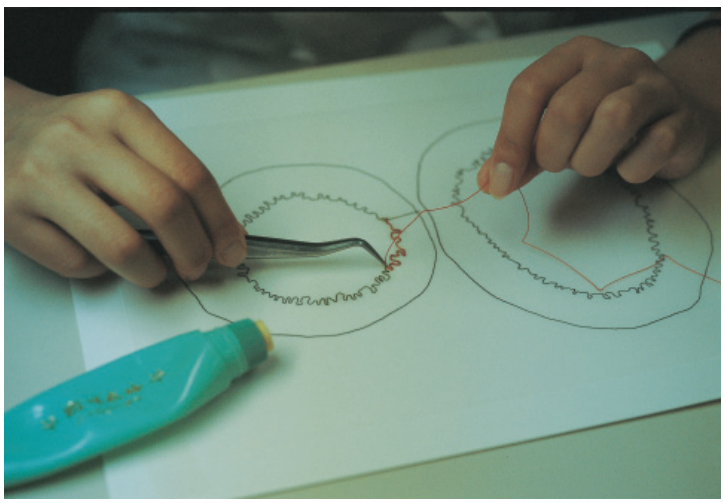

Fig. 1-5. Manual measurement of perimeter length for irregularly meandering elastic lamina. Cotton thread is stuck on the contour of elastic lamina in a drawing of arterial cross section, a laborious method employed in the 1960s when computer assist was not available.

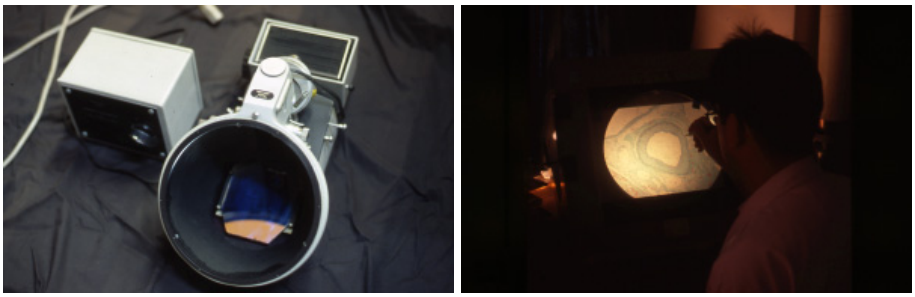

Fig. 1-6. Profile abstraction of elastic lamina from an arterial cross section (right) using a microprojector (Visopan-model, Reichert, left).

planimeter was available, though exacting time and labor. On the other hand, the irregularly indented course of the elastic lamina on cross-section of an artery refused easy determination of its perimeter length. What we employed after various attempts was a direct measurement as shown in Fig. 1-5; cotton thread was stuck on a drawing of an arterial cross section, where the finely undulating elastic lamina was faithfully followed. Today, these measurements are easily performed upon a digitizer connected with a desktop computer; a drawing of arterial profile is placed on a digitizer, where the profiles are inputted by tracing with a cursor. Figure 1-6 presents how the profile of a cross-sectioned artery was drawn on a sheet of tracing paper on which the picture was projected at an adequate magnification. Various types of microscopic projectors were used. In the figure, an artery is being drawn using a Visopan-type microprojector (Reichert). Drawing can be omitted if one manages somehow to project the image of artery directly on the digitizer. One can also make use of a software that allows to project microscopic images through CCD camera onto a computer display, and measure the length of meandering curve by following with a mouse-linked cursor.

Arterial hemodynamics in hypertension (Figs. 1-7, 1-8)

Figure 1-7 presents an example of measurement. This is from an autopsy material of a girl, aged 1 year and 4 months, dying from rotavirus enteritis, then called pseudocholera infantum. Both R, the radius, and D, the medial thickeness, were determined on the renal and superior mesenteric arteries, and the results were presented on bilogarithmic coordinates. The level of arteries subjected to measurement ranged in both arterial systems from the trunk down to the precapillary arterioles. One can see that there is a close linear regression between R and D, namely,

$$\log D = a \log R + b$$

where a and b are constants.

However, the slope appears much gentler in the range of radius smaller than 0.1 mm than in the larger arteries, requiring to define the regression separately for the proximal and distal regions of R. This implies that in the peripheral region, small arteries and arterioles are equipped with a medial layer that is significantly hypertrophic compared with the larger arteries. In other words, the D/R ratio becomes higher

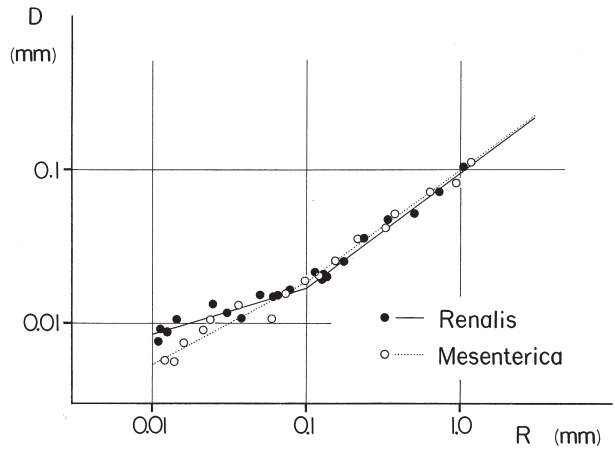

Fig. 1-7. The medial thickness D and the radius R are determined on the renal and mesenteric arteries in an autopsy case of a girl 1 year and 4 months old. The results are presented on bilogarithmic coordinates. In both the renal and mesenteric arteries there is a close linear regression, but the regression lines are gentler in the region of $R < 0.1$ mm. Reproduced from Suwa and Takahashi (1971): Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney. Urban & Schwarzenberg, München, pp. 45.

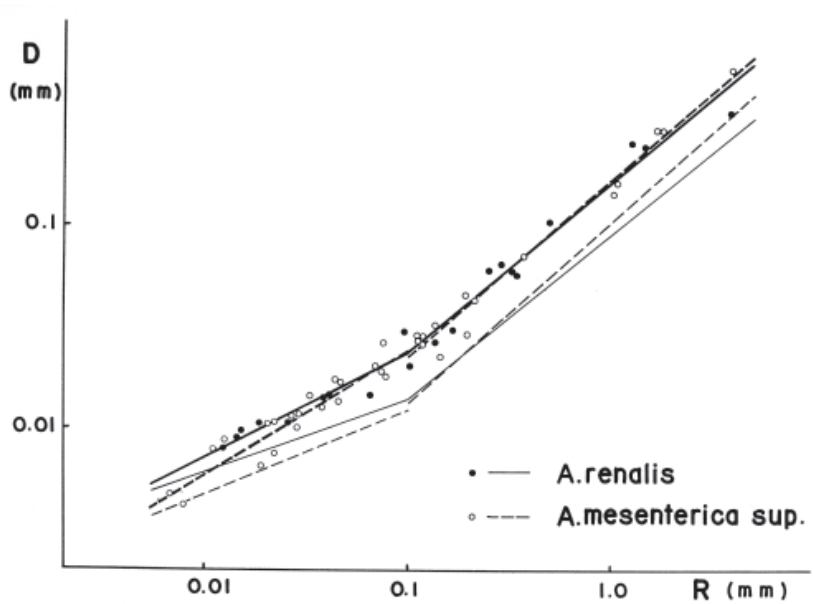

Fig. 1-8. The change of medial thickness in arterial hypertension in an autopsy case of male aged 55 years. He died of cerebral hemorrhage and his mean blood pressure was 175 mmHg. The thinner lines, solid and broken, are the regression lines from 17 normotensives of the same age group. There is a distinct medial hypertrophy in the region of $R > 0.1$ mm, whereas in the smaller arteries, D becomes growingly normalized toward the terminal region. Reproduced from Suwa and Takahashi (1971): Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney. Urban & Schwarzenberg, München, pp. 52.

toward the arterial periphery.

The measurements were performed on autopsy materials from 45 normotensive subjects so as to include various age classes as uniformly as possible. This gave us a basic data with which to compare the hypertensive cases. Shown as an example in Figure 1-8 is the result of measurement from a hypertensive patient, a male aged 55 years, who died of cerebral hemorrhage. His mean blood pressure was 175 mmHg, nearly two times higher than the control of the corresponding age group. Both the renal and mesenteric arteries are shown to have a media thickened almost double the normotensive level, at least in the range of R larger than 0.1 mm. Of the thinner lines, the solid are the regression lines with regard to renal arteries and the broken lines are those of mesenteric arteries, both calculated from the pooled data from 17 normotensive subjects of the same age group. However, the medial hypertrophy becomes ambiguous toward the arterial periphery. In the arteriolar region where R is about 0.01 mm, there is practically no medial thickening.

The result discloses a significant aspect of blood flow dynamics in hypertension. In the range of R larger than 0.1 mm, the media is uniformly thickened. This means that in this region, the media had been antagonizing a mean blood pressure elevated as high as double the normotensive level. However, at the arteriolar level of $R = 0.01$ mm, the mean blood pressure appears to have been already normalized, probably thanks to vasoconstriction of the proximal larger arteries. Now let us recall that it is in this very region of small arteries that hypertensive arterial lesions develop. Thus, development of the lesions seems to have some bearing with elevated blood pressure, but it may be safe to say that at least, the lesions are not the result of direct exposure of arterial wall to high pressure itself. We think that possibly, the development of hypertensive arterial injuries may be attributable to fluctuated blood flow and repeated ischemia in the periphery resulting from arterial constriction taking place in the proximal region.

Higher D/R in the arterioles and its physiological significance (Fig. 1-9)

Now let us come back to the normal arteries and consider the "physiological hypertrophy" of media in the peripheral region. In Fig. 1-9, the values of D/R ratio obtained from a normotensive adult is plotted against the radius R. There is a marked elevation of this ratio in the arterial periphery, particularly in the range of R smaller than 0.1mm.

Based on the Laplace formula we assumed that when P, the mean blood pressure, is elevated to two times the normal level, the tension load T upon the wall will be doubled. Consider that in this situation, the smooth muscle cells hypertrophy so as to double the medial thickness. Then an adaptation to the increased load will be attained by the medial layer now sufficiently strengthened to antagonize the wall tension T with a doubled muscular mass. Therefore, once a state of adaptation is reached, we can assume that the medial thickness D is parallel with the quantity of physical work done in a certain period by the smooth muscle cells. Thus, not only for hypertensives but for arteries in general, we can write

$$D = B \cdot T$$

where B is a constant. With this, the Laplace formula (1-1) can be re-written into

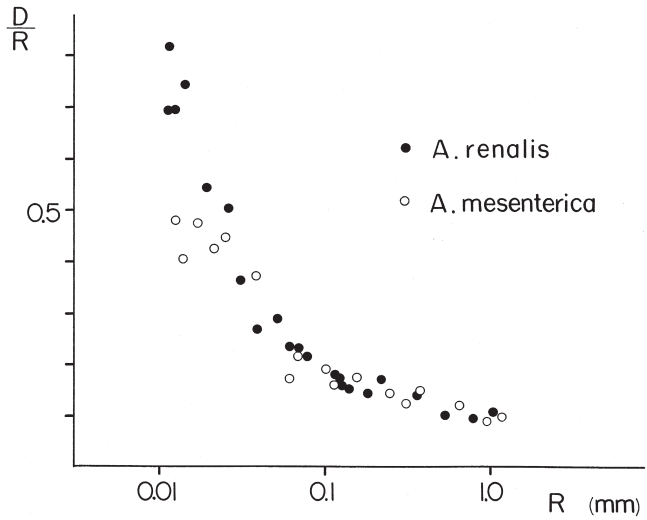

Fig. 1-9. D/R, the comparative thickness of media, rapidly rises in the arteriolar region toward the periphery, particularly in the renal artery. This physiological hypertrophy of media in the periphery cannot be understood from the quantity of physical work performed by the smooth muscle cells to antagonize the blood pressure, because the pressure must be far lowered in the peripheral region. Reproduced from Suwa and Takahashi (1971): Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney. Urban & Schwarzenberg, München, pp. 46.

$$D/R = BP.$$

According to this, the ratio D/R must be proportional to the inner blood pressure. However, of course the mean arterial pressure falls as the artery becomes smaller toward the periphery. If so, the ratio D/R might also be expected to drop from the middle to small arteries to arterioles. In reality, however, we have found quite the opposite. The ratio D/R rises with thinning arterial radius, and the highest ratio is attained at the most peripheral level of the arterial tree. Consequently, so long as the pressure-sustaining aspect of arteries is concerned, it seems as if the terminal region of arteries were equipped with a meaningless mass of extremely hypertrophic smooth muscles.

This luxury is to be interpreted in another way; it reflects the extraordinarily vigorous activities assigned to small arteries and arterioles. As well known in the domain of circulatory physiology, the peripheral arteries repeat constricting so as to regulate blood flow and distribution of blood into the tissue. Thus we realize that there are at least two different aspects in the function of arterial muscular coat, that is, to bear the tension load, and to regulate the blood flow. In the media of an artery we cannot separately assess the quantity of work done to achieve each of the two functions. However, what an unexpectedly large work is undertaken by arteries in regulating blood flow may be understood if we compare among arteries supplying different organs, as in the following.

Organ difference in arterial circulation (Fig. 1-10)

In Fig. 1-10, arteries supplying different organs are compared. This is from the

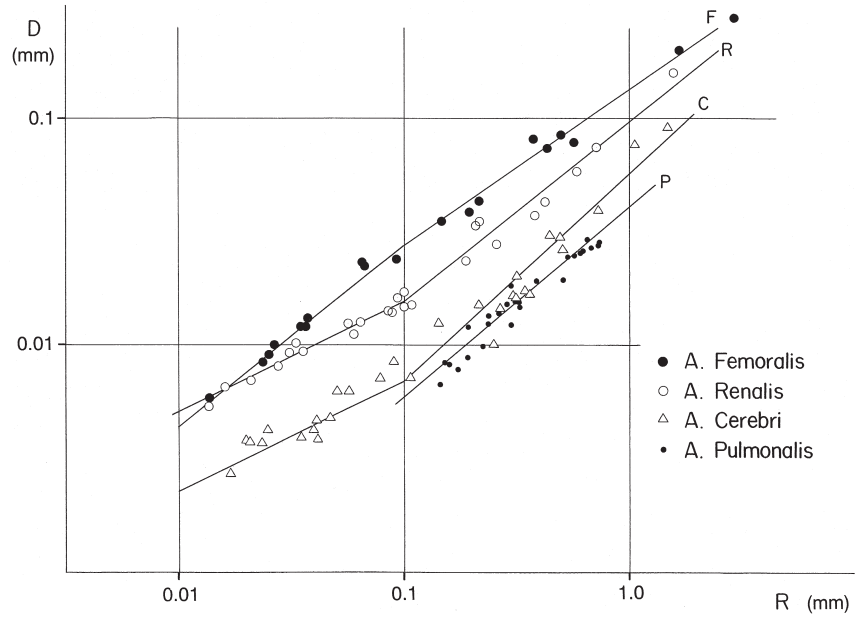

Fig. 1-10. The organ difference in the development of media is examined among the femoral (F), renal (R), cerebral (C) and pulmonary (P) arteries from autopsy material of a young male. The femoral arteries were sampled from the branches running in the quadriceps, so as to represent the arteries of skeletal muscles. Note that there is a remarkable between-organs difference. Reproduced from Suwa and Takahashi (1971): Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney. Urban & Schwarzenberg, München, pp. 46.

autopsy material of a young male aged 16 years who died of cerebral trauma. The femoral arteries are taken from arteries supplying the quadriceps muscles and represent arteries feeding the skeletal muscles; added to this are the renal, cerebral and pulmonary arteries. It may be clear at a glance that there is a great organ difference. In the pulmonary arteries, D remains at the lowest level. In this artery, the values of D keep an almost equal distance downward from the renal arteries along the entire range of R. The distance in D between the renal and pulmonary arteries corresponds, on a logarithmic scaling, to a difference of 4 to 1 along the entire range of R, and this appears to be reflecting approximately the difference of mean blood pressure between these arterial systems. Aside from this, however, the medial thickness greatly differs within arteries belonging to the systemic circulation. In the femoral artery, the arteries of skeletal muscles, D remains the highest in the middle and larger arteries, and at R = 0.1 mm, the medial thickness is about five times larger than in the cerebral arteries. Because the skeletal muscles, kidneys and the brain are belonging to the systemic circulation, it may be unthinkable that among these organs, a difference in blood pressure maximally as large as 5 to 1 should exist. From the above discussion it seems most likely that the pattern of D, which is specific of each organ, reflects different regulatory activity of arteries. For example, the extraordinarily thickened media in the arteries of skeletal muscles may be supposed to be showing that in a human who usually leads a "normal" state of living, the arteries are kept in a constricted state in most

of the time. It may be only on rare occasions that the subject is engaged in such an intensive muscular work as running full-power for a while, when the arteries may be fully open, supplying blood at a flow rate higher than 10 times "normal." On the other hand, arteries of the brain have the lowest *D* in the organs of the systemic circulation. This may be interpreted to imply that the brain comprises nerve cells so "tachytrophic" as to be vulnerable to ischemic injuries if they are placed in an environment where blood flow can greatly fluctuate as in the skeletal muscles. In the kidney, attention should be paid to the terminal region of renal arteries. Here *D* of vas afferens-class arteries is higher than in any of the other organs. Presumably, this may be related with the particularly active regulation of renal blood flow which is assumed to take place at the preglomerular arterioles. From the anatomical pattern of renal vasculature, Suwa and Takahashi (1971) theoretically concluded that in the kidney, the adequate site for blood flow regulation must be at the vasa afferentia. This is because the normal blood pressure in the glomerular capillaries is incomparably higher than in capillaries of common organs. Consider what if vasoconstriction has occurred at a proximal level, for example at the interlobular or larger arteries. The blood pressure of glomerular capillaries would fall simultaneously in a number of glomeruli below 80 mmHg, the critical height required for urine filtration, thus suspending the urine production in the area supplied by the artery. Only by alternating and intermittent constriction of vasa afferentia taking place among a large group of glomeruli, regulation of renal blood flow can be achieved while avoiding suspended urine production all at once.

The morphology of arteries, when submitted to metric analysis of radius and media, can bring about a series of insights into the functional aspects of microcirculation. This will be revisited again in Chapter 3 where the microstructure of the liver is to be related with the tissue circulation of the organ.

Application to pulmonary arteries in pulmonary hypertension

The morphometry of arteries has found other applications, of which, two studies are to be introduced.

It has been known that in interstitial lung diseases such as idiopathic pulmonary fibrosis, the vascular resistance of lung rises more or less. Although this usually has been attributed to collapse of capillary bed due to fibrosis, vasoconstriction of pulmonary arteries may be another important mechanism. On the other hand, the segments of pulmonary arteries supplying lung areas poorly ventilated due to various changes tend to constrict and suppress the blood supply to such areas. This is a mechanism called the hypoxic vasoconstriction, a response that can also occur in arteries supplying fibrotic areas of lung where ventilation is impeded. If so, overstraining of arterial wall may precipitate thickening of media in the constricted segments. Therefore if subjected to the above measurement, medial hypertrophy will reveal when and where vasoconstriction has taken place. This was undertaken by Sawai *et al*. (1994), who performed morphometry of pulmonary arteries in autopsy lungs from 21 patients dying in various stages of fibrosis due to paraquat intoxication, together with five normal lungs as control. (The paraquat-associated lung changes will be shown in the next chapeter.) The standardized morphometry described above was employed, where arteries were sampled in each case so as to cover a wide range of R from 0.05 to 1.0 mm. It was shown that the medial thickness D began to rise as early as on the 8th day of

intoxication, when, as yet, there were little fibrotic changes. Interestingly, this dating almost exactly coincides with the beginning deposition of fibrogenic matrix on alveolar wall, which will be shown in Chapter 2 (Figs. 2-25 and 2-27). Thus it seems that the medial hypertrophy is the result of hypoxic vasoconstriction triggered by the formation of matrix layer upon the alveolar septa, which causes alveolar-capillary block. In all the cases, the maximum thickening of media was found in small arteries of intraacinar level. The conclusion: hypoxic vasoconstriction of pulmonary arteries occurs in an early, pre-fibrotic stage of interstitial lung disease and contributes to elevated vascular resistance.

Application to portal veins

Another example is application to the changes of intrahepatic portal veins in patients having congenital biliary atresia with portal hypertension (also see Chapter 2). During the past decades, progress in the surgical treatment for this disease contributed to a dramatic increase in the number of long-term survivors. However, some postsurgical problems still remain, including the development of portal hypertension, a sometimes threatening complication. Nio *et al*. (1987) found that in infants with biliary atresia, smooth muscle layer emerges in the wall of medium-sized and smaller intrahepatic portal veins which normally are muscle-free. The muscular layer seemed to thicken and extend toward the periphery as portal hypertension progresses. In view of this, surgical biopsy and autopsy specimens of liver from 41 patients with biliary atresia were submitted to morphometry of intrahepatic portal veins. It was disclosed that with the persistence of portal hypertension, not only does the medial layer of proximal portal veins thicken, but extends to the periphery. In patients in whom biopsy specimens were taken at the second or third laparotomy three to five years after the corrective operation, the degree of medial hypertrophy proved to significantly correlate with the portal vein pressure intrasurgically measured. Thus, in a venous system too, medial thickening serves as a criterion for judging whether or not there is hypertension, and if there is, to what degree.

b) Morphometry of airways—what part of bronchial tree constricts in asthmatic attack ?

Bronchial smooth muscles in 3-D (Fig. 1-11)

The morphometric study of arteries and the methods we developed have found another application, i.e., to the analysis of airway changes in patients of bronchial asthma. Like an artery, a segment of bronchus is a contractile tube which changes its luminal width by contracting or relaxing the smooth muscle cells circularly embedded in the wall. Figure 1-11 is a computer-aided 3-D reconstruction of a membranous bronchiole about 1mm in diameter. In reality, this is one of the images produced for the first time with the aid of the first trial system we managed to develop with which to assist serial sections technique (Takahashi *et al*., 1986; Yaegashi, Takahashi *et al*., 1987). Despite the somewhat coarse texture of image, the arrangement of smooth muscles in the bronchiolar wall is clearly visible, with the bundles of smooth muscles (shown red) surrounding in circular orientation, but strictly speaking, taking a slightly spiral course. The attack of bronchial asthma, a paroxysmal dyspnea that asthmatic

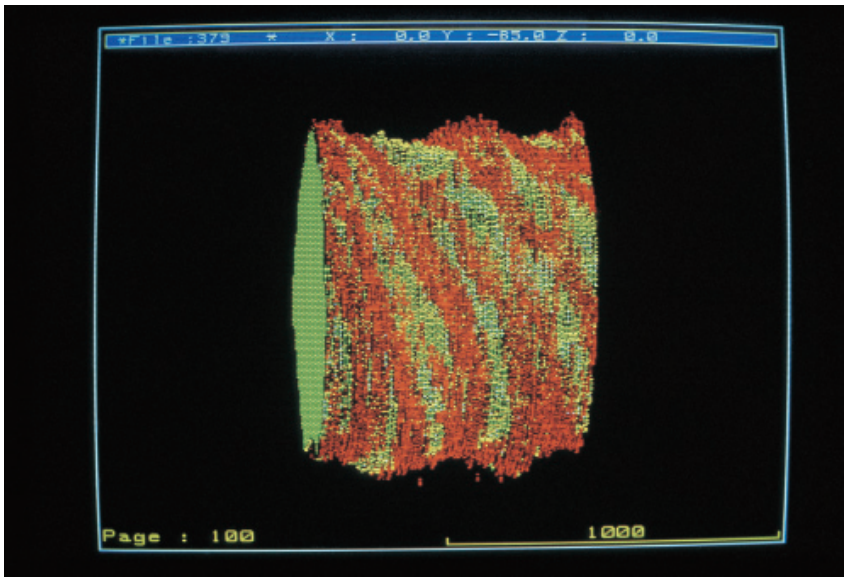

Fig. 1-11. A computer-assisted 3-D reconstruction of membranous bronchiole, about 1 mm in diameter. The lateral view is presented to demonstrate the distribution of smooth muscles in the wall (also see Fig. 4-7). The smooth muscles (red) are winding round the bronchiole, forming a gentle spiral. Reproduced from Yaegashi, Takahashi *et al.* (1986): J. Microscopy 146, pp. 57.

patients frequently suffer from, is considered to be resulting from bronchial convulsion, a tight constriction of these muscular bundles. All bronchial segments within a certain range of diameter are thought to take part in constriction more or less, but what level of airways in the bronchial tree are chiefly involved has not been clear.

Application of Laplace formula to airways (Fig. 1-12)

Also to this problem we can apply the discussion extended for the blood vessels, and here too, the mechanical forces that work on and around the bronchial wall are related in the form of Laplace formula

$$T = PR$$

where T is the circumferential tension exerted on the bronchial wall and R, the radius of a bronchial segment. However, in the case of bronchi, there is a difference from blood vessels. In this case, the pressure P does not imply the inner pressure of bronchus but denotes the force working on its wall as the elastic recoil from outside (Fig. 1-12). A segment of bronchus can keep its cylindrical shape from being collapsed, mostly with the help of the "recoil pressure." This is the force exerted upon the bronchial wall from the outside through multiple alveolar septa that contain elastic fibers and are attached at the outer surface of the bronchus, pulling it outward. The tension T generated upon the wall on account of this "elastic recoil" P is counterbalanced by the circular smooth muscles, which antagonize the recoil, keeping the bronchus from being

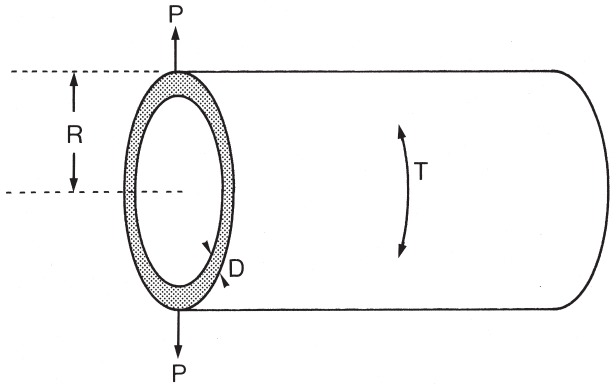

Fig. 1-12. Application of Laplace formula (Fig. 1-3) to airways. The situation differs from arteries in that, in the airways, the pressure *P* is exerted as a "recoil" pulling the wall outward. Reproduced from Ebina, Takahashi *et al*. (1990): Am Rev Resp Dis 141, pp.1326.

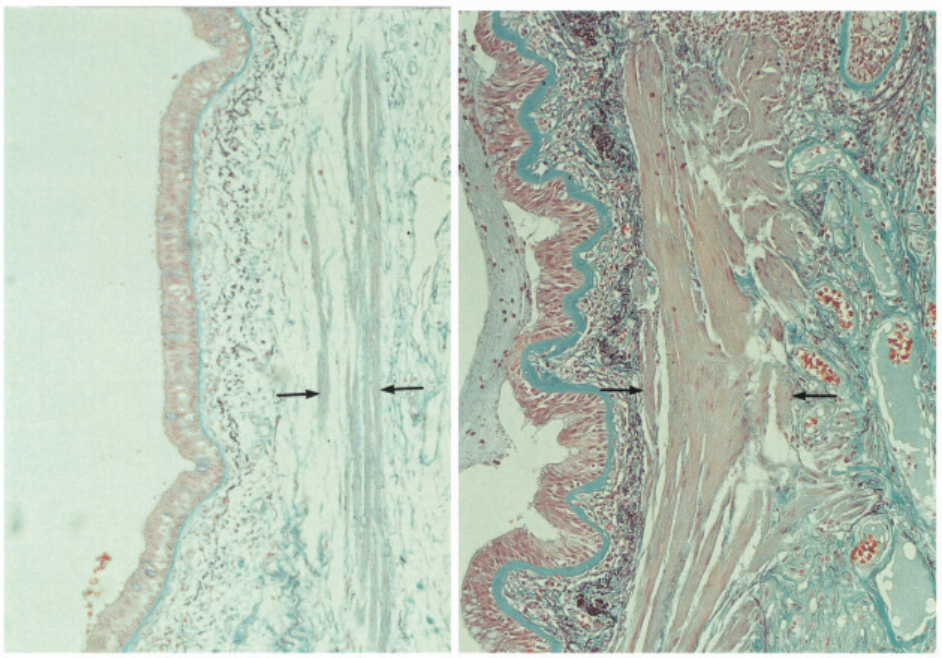

Fig. 1-13. Smooth muscle hypertrophy of bronchus in asthmatic patient. Though the muscular cells are not so densely packed (arrows) as in the medial layer of arteries, the bronchial wall of an asthmatic patient (right) appears equipped with a markedly thickened muscular layer compared with a non-asthmatic (left). Elastica-Goldner stain.

overstretched. In fact, a bronchus, when the mural smooth muscles were destroyed due to chronic inflammation, is liable to dilatation in the form of bronchiectasis, a state of bronchus which yielded to the recoil it was no longer able to resist. When an asthmatic attack occurs, the bronchial muscles constrict against this wall tension, and this implies an increased mechanical work for the smooth muscle cells. Asthmatic patients suffer from fits that occur more or less frequently and last for varying period, during which the patients endure severe respiratory distress.

Bronchial muscles in non-asthmatics and asthmatics (Fig. 1-13)

Therefore, in lungs of asthmatic patients, it seems possible that we can find another example of adaptation: work hypertrophy of bronchial muscles that have been overworked, like the medial hypertrophy of arteries in hypertension. One can see in Fig. 1-13 that in the bronchial wall of an asthmatic (the right), the smooth muscle layer is remarkably thickened compared with the left figure which is from a non-asthmatic. In this situation, as in the arteries, the thickening of bronchial muscles may be interpreted as expressing the result of adaptation of bronchi to increased mechanical work. Accordingly, if the distribution of thickened smooth muscles is visualized over the entire range of airways from the large segmental bronchi to small bronchi to bronchioles, we will be able to see at what level the airways are most susceptible to paroxysmal constriction which generates asthmatic fits. What follows is a morphometric study performed by Ebina *et al*. (1990a, b) along this context.

Standardized measurement of airways (Figs. 1-14, 1-15)

In determining the thickness of bronchial muscular layer, we face the same problem as in arteries. As in Fig. 1-14, the more dilated the bronchus, the thinner is its muscular layer, and vice versa. Therefore, the muscular thickness of a bronchial segment needs to be defined by reducing the segment to a state standardized with regard to its constriction or dilatation. There have been morphometric studies, although few in number, dealing with the thickness of bronchial muscles and its change in asthmatic patients (Takizawa *et al*., 1971; Hossain, 1973). However, ambiguities remain, which seem at least partially attributable to bias inherent in the method of study.

In this study of bronchi, the standardization technique developed in the study of arteries was applied, where the perimeter length L of the elastic lamina and the sectional area S of smooth muscles were measured. However, there was in the airways a difference from arteries in the structure of muscular layer, which required a small modification: the muscular layer of bronchus does not form a compact layer like a united belt, but is separated into several muscular bundles. Therefore the area of each bundle was separately measured, and S was given by the total, as in Fig. 1-15. By the time this study was undertaken, a system for morphometry including a microcomputer and a digitizer had been put to practical use, which greatly facilitated the measurement.

Two types of asthma revealed by morphometry (Figs. 1-16, 1-17)

Autopsy lungs from 16 patients, all dying of fatal asthmatic attack, were submitted to measurement of bronchial muscles. Figure 1-16 is the result obtained from an asthmatic patient, a male aged 71 years. As in the morphometry of arteries, the measurement data was presented on bilogarithmic coordinates, with the airway radius R in

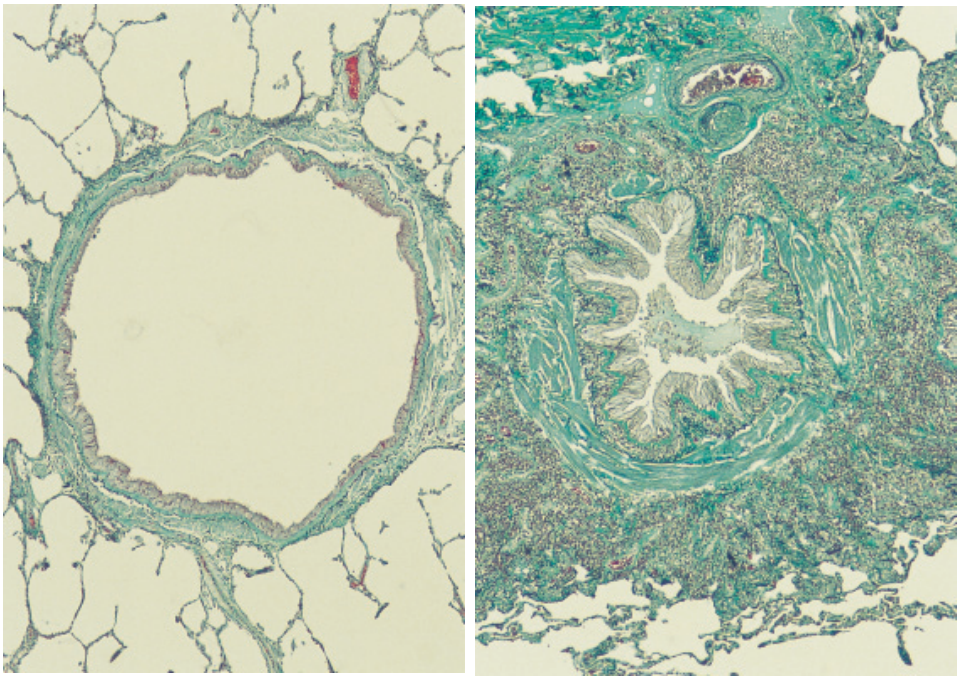

Fig. 1-14. A constricted bronchus (right) is compared with a dilated one (left). Apparently the muscular layer seems thicker in the former, making it necessary to define the muscular thickness and radius of an airway in a standardized state.

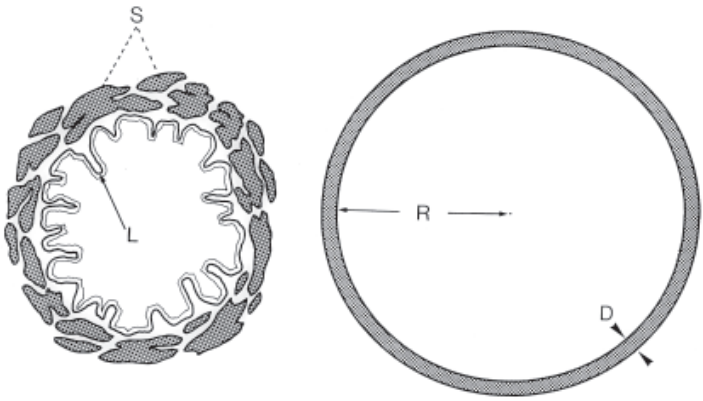

Fig. 1-15. Standardized measurement of airways. The method is basically the same as the one applied to arteries. In the bronchi however the muscular layer does not form a compact layer, requiring us to measure the area for each bundle separately.

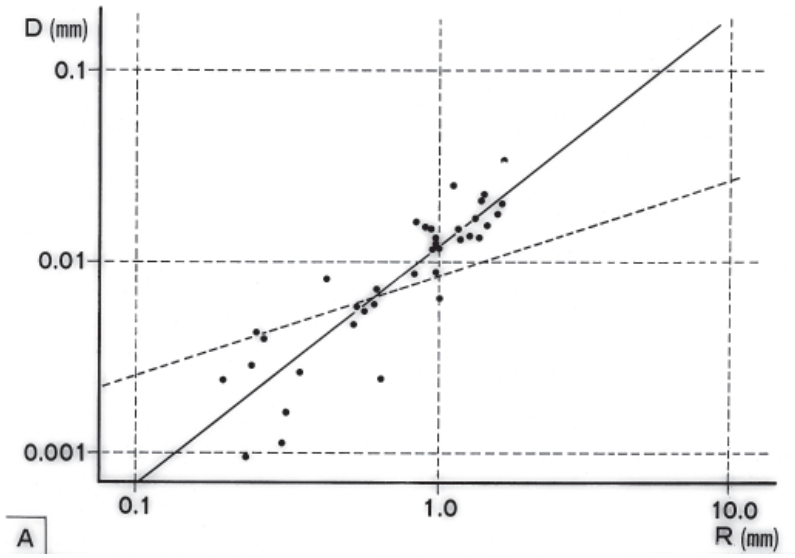

Fig. 1-16. *D*, the thickness of airway smooth muscle and *R*, the airway radius, in an asthmatic patient, Type I. Both *D* and *R* are plotted on bilogarithmic coordinates. The smooth muscle hypertrophy is found only in larger airways. Reproduced from Ebina, Takahashi *et al*. (1990): Am Rev Resp Dis 141, pp. 1330.

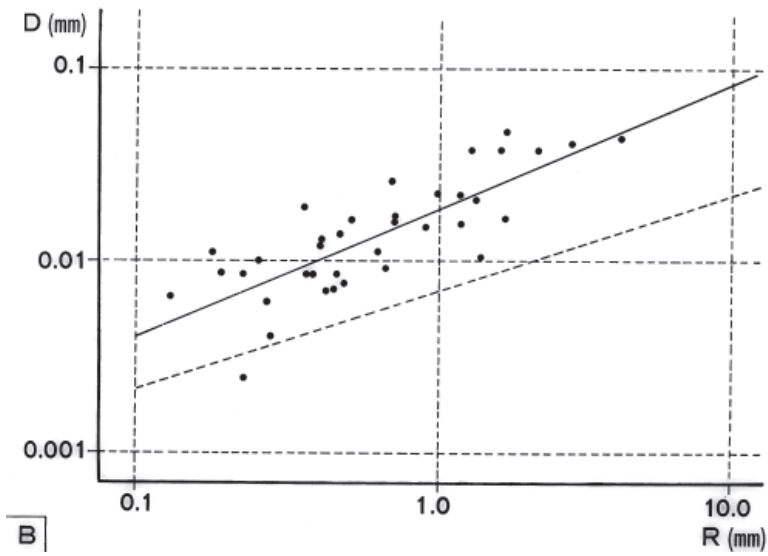

Fig. 1-17. The measurements of *D* and *R* in a patient of Type II asthma. The smooth muscle layer is significantly thickened over the entire range of *R*. Reproduced from Ebina, Takahashi *et al*. (1990): Am Rev Resp Dis 141, pp. 1330.

the abscissa, and the muscular thickness D in the ordinate. The solid line is the regression line for this patient, and the broken line is that of control lungs obtained from autopsies of 20 non-asthmatic subjects, where all the data were pooled to calculate a single regression. It is shown that in the present case of asthma, hypertrophy of muscular layer, though present, is confined to the proximal bronchi with R larger than 1 mm, while in the peripheral bronchioles the muscular layer remains as it was in a non-asthmatic state. Of the lungs from 16 cases of asthma, those from seven patients proved to have this type of distribution, and in these, airway constriction responsible for producing asthmatic attack was considered to take place in the larger range of bronchi, including the segmental and subsegmental levels. This is a result supporting what had generally been assumed (Nadel, 1980).

However, in the remaining 9 patients of asthma, the result of measurement was quite different. As shown in Fig. 1-17, which is from a male asthmatic 54-years-old, the muscular layer is significantly thickened over the entire range of airways from the segmental bronchi of 4 mm in radius to respiratory bronchioles of 0.2 mm. It is likely that in these nine patients, the asthmatic attack occurs as the result of airway constriction that involves not only the larger bronchi but the terminal and even the respiratory bronchioles. While comparing among the data, we noticed that in each of the 16 patients of asthma, the pattern of muscular hypertrophy belonged to either the former (Fig. 1-16) or the latter type (Fig. 1-17), and there was no patient showing an intermediate pattern. Therefore it was considered that according to what level of airways are involved in paroxysmal constriction, the patients of asthma are classifiable into two types; the patients with hypertrophy in the larger bronchi, and those with overall hypertrophy. The former was designated as Type I asthmatics and the latter, as Type II, respectively. Thus, there appears to be a significant number of patients in whom airway hyperreactivity is distributed over the whole bronchial tree, although so far, patients corresponding to Type I had been considered to be typical asthmatics. Some factors, in either pathological or clinical aspect, seemed likely to be underlying the difference between the two types. However, up to now, we have not managed to focus on a small number of factors to which the between-groups difference may be ascribed. Given the large number of factors potentially responsible for airway hyperreactivity, it may be necessary to study a larger population for clinical as well as pathologic abnormalities.

c) Standardized morphometry of airways in normal and diseased lungs

Pulmonary emphysema and chronic bronchitis (or bronchiolitis) are diseases associated with chronic airflow obstruction, and are classified as chronic obstructive pulmonary disease (COPD). However, COPD is an entity mostly established upon studies of respiratory function, while the airway obstruction itself has not always been defined on a firm pathological basis. In emphysema, it is the loss of alveolar septa that has been considered responsible for producing impaired expiratory function (see Figs. 2-17 and 2-19).

On the other hand, in chronic bronchitis or bronchiolitis, obstruction is considered attributable to inflammatory processes that advance in the airway wall and produce various grades of irreversible luminal narrowing. However, in such cases, we are

still far from the state in which we can correlate the functional impairment (the reduced expiratory efficiency) with the morphological changes of airways. What level of airways in the bronchial tree are involved in obstruction? To what degree the whole airway bed is narrowed? Although these are questions to be answered only by morphology, the studies on the part of pathology are retarded, which seems attributable at least partially to the following difficulty.

In a microscopic section of lung, one can find a number of airways with different calibers. Imagine that in a biopsy specimen of lung we found an airway, 1mm in inner diameter. In this situation, can we determine whether the airway we are looking at has become narrower or wider than its previous state? Clearly, the answer is no. So long as information is lacking about the original dimension of the airway in question, we cannot say whether the airway at our hand is in a stenotic or dilated condition. Even if it seemed thinner than normal, one cannot deny that it had been a small segment in the first place. To overcome this, Yaegashi and Takahashi (1994ab) devised a method of standardized morphometry of airways.

Airways and their collateral pulmonary arteries (Figs. 1-18, 1-19)

The method of standardization for airways was designed so as to make use of their close arrangement with pulmonary arteries, both running in parallel from the hilar large trunks down to the terminal branches. Figure 1-18 is a computer-assisted 3-D reconstruction of lung visualizing airways (green), small pulmonary arteries and arterioles (pink) and pulmonary veins (blue) where one can see segments of airways and pulmonary arteries running closely in parallel. On account of this arrangement, airway usually emerges in a microscopic section attended by an artery running in its close proximity, as in Fig. 1-19. In chronic bronchitis or bronchiolitis, the collateral

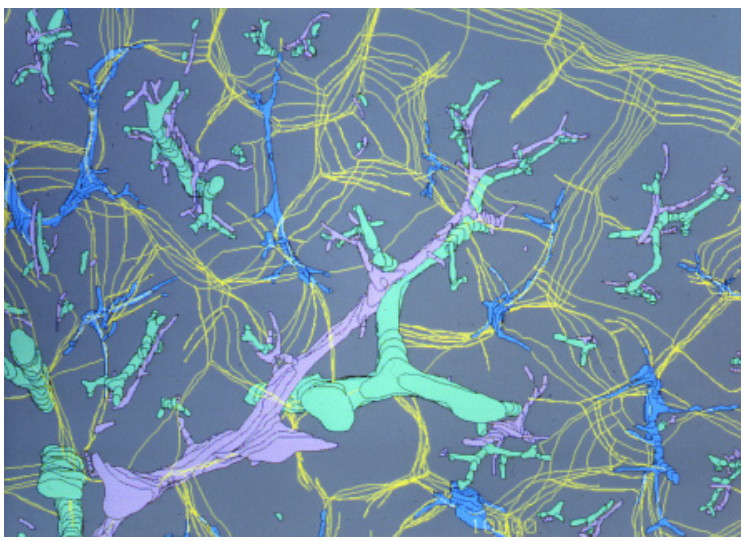

Fig. 1-18. Computer-assisted 3-D reconstruction of peripheral airways (green), pulmonary arteries(pink) and veins (blue). The yellow wireframes express the interlobular septa. Note that most of the airway segments are attended by a collateral pulmonary artery.

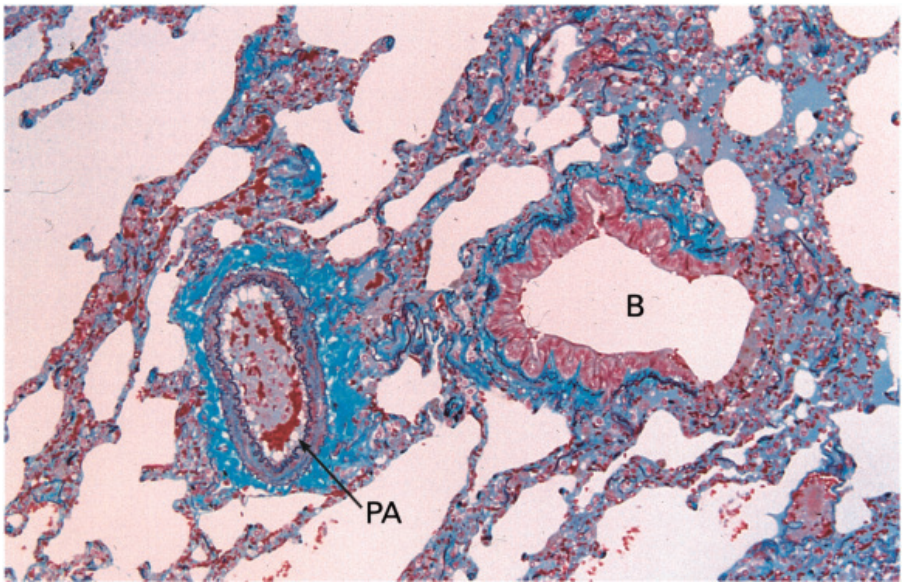

Fig. 1-19. Cross section of an airway (membranous bronchiole, denoted by B) and its collateral pulmonary artery (PA) in a microscopic section of lung. Elastica-Goldner stain.

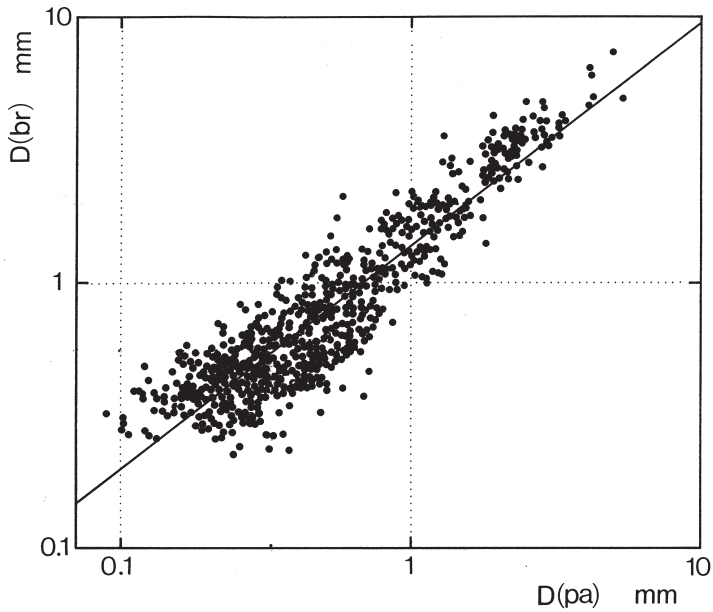

Fig. 1-20. *D*(br), the diameter of bronchi and bronchioles, and *D*(pa), that of pulmonary artery running parallel with the airway, measured on 695 pairs from 17 normal lungs. Note the close linear regression between *D*(pa) and *D*(br) on bilogarithmic coordinates. Reproduced from Yaegashi and Takahashi (1994): Arch Pathol Lab Med 118, pp.972.

pulmonary arteries, far more resistant to inflammation than the airways, retain their original structure and dimension until late in the course of disease. Hence, if there is a close correlation in the caliber between airways and their attending pulmonary arteries, we will be able to know from the dimension of collateral artery to what degree an airway is being narrowed or dilated. In view of this, autopsy lungs from 17 patients with neither clinical nor pathological finding suggesting lung disease were submitted to measurement of diameters for airways and attending pulmonary arteries. The age of the patients ranged from 10 to 78 years. A total of 695 pairs of airways and arteries were sampled. For the airways as well as the arteries, the diameter was defined in a standardized state in which both the epithelial basement membrane of airways and the inner elastic membrane of arteries were stretched into a circle. Airways were sampled including the whole ranges from the segmental bronchi down to the respiratory bronchioles.

Regression in diameter between airways and their collateral arteries (Fig. 1-20)

Figure 1-20 demonstrates the result of measurement, where all the data from the 695 pairs were pooled, with *D* (pa), the diameter of pulmonary artery in the abscissa, and *D* (br), that of the airways in the ordinate, both expressed on logarithmic coordinates. There is a correlation close enough to allow to estimate the diameter of airway from that of the attending pulmonary artery, even in lungs with COPD in which airways can strongly be stenosed or dilated. Although there are practically no age changes in the regression equation, the ranges for bronchi, membranous bronchioles and respiratory bronchioles proved to move during the growth period.

Peculiar disease of airways: small airways disease? (Figs. 1-21, 1-22, 1-23)

Here to be shown is an autopsy case where the standardized measurement of airways contributed to the elucidation of chronic airflow obstruction. The patient was

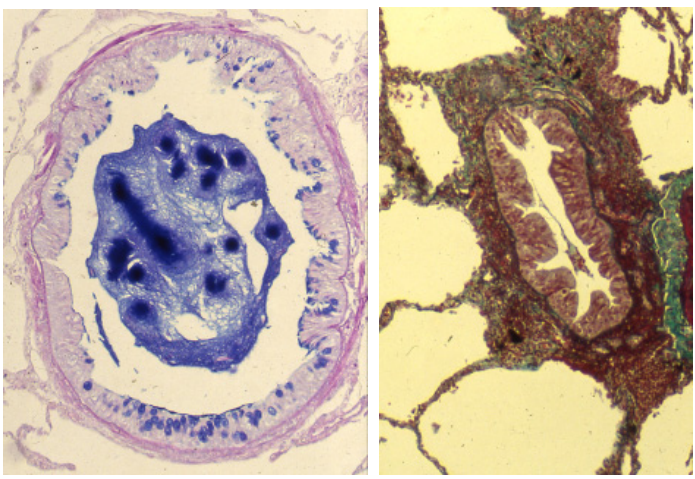

Fig. 1-21. The small airways in a peculiar case of chronic airway obstruction, perhaps corresponding to "small airways disease." Right: a membranous bronchiole with the lumen somewhat narrower than normal, and with mild fibrosis in the surroundings (elastica-Goldner stain). Left: luminal plugging with mucus, with goblet cell metaplasia of lining epithelia (alcian blue-PAS stain).

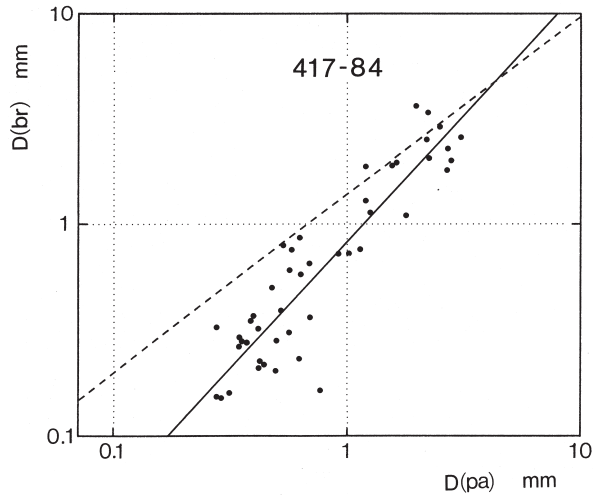

Fig. 1-22. *D*(pa) - *D*(br) regression in the case of "small airways disease" shown in Fig. 1-21. The airway diameter is significantly lower than the regression line of normal lungs (the broken line), particularly in the periphery. Reproduced from Yaegashi and Takahashi (1994): Arch Pathol Lab Med 118, pp. 981.

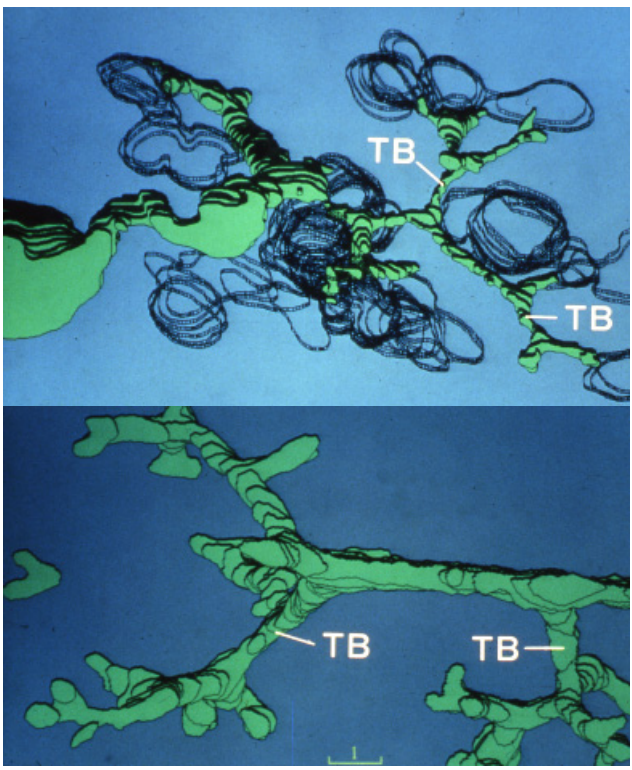

Fig. 1-23. Upper: computer-aided 3-D reconstruction of peripheral bronchioles in the lung with "small airways disease." The small airways are apparently thinner than in a normal lung (lower). TB: terminal bronchiole. Reproduced from Yaegashi and Takahashi (1994): Arch Pathol Lab Med 118, pp. 981.

a male aged 68 years, who had been treated for 25 years under varying diagnoses including asthma, emphysema or chronic bronchitis, and died of respiratory insufficiency. Microscopically, emphysematous changes were minimum if any. The membranous bronchioles seemed to have lumina somewhat narrower than normal (Fig. 1-21 right). Signs of chronic inflammation were found in the wall of bronchioles with sparse infiltration of mononuclear cells and slight fibrosis, and there were goblet cell metaplasia of bronchiolar epithelia, sometimes with luminal plugging with mucus (Fig. 1-21 left). None of these, when acting alone, appeared to be serious enough to narrow the lumen by themselves. However, as in Fig. 1-22, the standardized morphometry revealed an overall airway narrowing that was distributed fairly uniform and tended to increase toward the periphery. The narrowing is of such a degree as to fully explain the development of airflow obstruction, independently of inflammatory changes. Without quantifying the luminal diameter with the standardized technique, this sort of bronchiolar obstruction would never be assessed for certain.

In the upper part of Fig. 1-23, the above lung was subjected to computer-aided 3-D reconstruction to visualize the peripheral airways. The figure, produced at an equal magnification as in the control lung shown below, discloses a marked luminal narrowing uniformly involving the whole range of membranous bronchioles, where TB denotes the terminal bronchioles. Thus, the case appears to be a sort of lung disease not definable within the scope of conventional categories of COPD, shedding light on the presence of bronchiolar disease that previously has not been defined in clear pathological terms. Besides the luminal narrowing, there were in the membranous bronchioles signs of mild chronic inflammation and hypersecretion of mucus as shown by goblet cell metaplasia and mucus plugging, suggesting that chronic irritation of bronchiolar mucosa had been lasting. The luminal narrowing seems to be the result of peribronchiolar fibrosis due to mild but recurring inflammation. This may be a state corresponding to the bronchiolar version of chronic bronchitis, the latter involving the larger bronchi and having been regarded as a response to chronic irritation rather than as an inflammation in the strict sense of the term. Thus, study of the case reminds us of the concept of small airways disease proposed by Hogg *et al.* (1968). However, there has been ambiguities about what pathological changes actually progress in the bronchioles in "small airways disease." We think that in the present morphometry and 3-D visualization, the pathological features corresponding to this condition has become a little clearer.