Science on Form: Proceedings of the Second International Symposium for Science on Form,
Editor: S. Ishizaka, pp. 17–30.

THREE-D COMPUTATIONAL GEOMETRY: THE "FORM" OF VASCULAR TREE AS EXPRESSED BY THE DISTRIBUTION OF DISTANCE IN THE SPACE, ITS ORGAN DIFFERENCE AND SIGNIFICANCE IN BLOOD FLOW

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Key words: 3D reconstruction, Morphometry, Blood vessels, Human organs

Abstract. Computerized 3-D reconstruction from serial microscopic sections brings us much closer to microstructures, providing not only pictorial understanding but also 3-D quantities of them by processing the image data loaded from serial 2-D pictures. Three-D morphometry is particularly useful in studies where one is unable to rely on stereology in abstracting geometric features of living structures. Shown as an example is the analysis of the 3-D pattern of blood vessels, in an effort to correlate the organ-specific vascular arrangement with the then prevailing hemodynamics; an "architectural index" was introduced by which to reduce a vascular pattern to a distribution of distances to the nearest vessels from many test points randomly dispersed in the space. Discussion was extended over what principles dominate in the construction of an organ where several functional systems coexist including the blood vessels, each requiring its specific architecture optimal for the performance of its own function.

INTRODUCTION

The 3-D reconstruction from serial microscopic sections is a method of study long used in biomedical sciences especially histopathology, histology and embryology. Along with the recent development of computer technologies, however, we are at the dawn of a rapid diffusion of this classical technique, which is now going to involve the whole range of sciences searching for the significance of "form".

What we pathologists deal with in our research is always confined to sectional 2-D pictures from living structures. So are microscopic pictures and electron micrographs; even macroscopic examination of a whole organ is done on several cut surfaces which provide essentially 2-D pictures. Since however an organ itself is

This paper was presented at The Second International Symposium for Science on Form (October 19th–21st, 1988 at the University of Tsukuba, Japan).

mostly a 3-dimensional structure, knowledges about its spatial features are indispensable, especially if one attempts to correlate its "form" with the functional aspects. It may be desirable to find a measure by which one can reduce the various forms into some quantified geometric models, before resorting to serial section technique that exacts tremendous time and energy from the performer. Stereology, though of great help sometimes, cannot always be counted on because of algorithmic and technical limitations. Table 1 is a list given by DeHoff (1983) who tabulates the 3-D features that cannot be estimated stereologically, including connectivity, spatial distribution information, and so on. In fact, we face problems very often, where we cannot gain insight into the implication of living phenomena without coping with this methodological barrier. In the present article, we introduce an attempt at combining serial section technique with computational geometry, which provides us with 3-D quantities that are beyond the reach of stereology. This shall be illustrated on the 3-D pattern of human blood vessels, its organ difference and its functional implications.

Table 1. Properties that cannot be estimated stereologically (DeHoff, 1983).

1. Number of features
2. Connectivity of features
3. Size distribution
4. Spatial distribution information
5. Real feature shape

DIFFERENT VASCULAR PATTERNS AND THEIR SIGNIFICANCE

Let us begin by surveying what a various architectural pattern of small blood vessels human organs contain. Figure 1A presents the small vessels of the human liver reproduced from serial sections with the aid of a graphics computer, with the portal veins painted in pink, and the hepatic veins in light blue. The portal veins, through which a large amount of blood flows into the liver, correspond to arteries of other organs. Taking into account that the bar at the bottom denotes a span of 1 mm, we see that the vessels are all terminal small twigs having abundant connections with capillaries (sinusoids) that are filling the void spaces as a continuous fine network. Vasculature like this can also be visualized with a cast prepared by injecting colored resin in the vessels, after which the tissues are removed by corrosion. A cast, though looking beautiful, is a material far less useful for 3-D morphometry than a graphic reconstruction where the entire "forms" of vasculature are inputted and stored in a computer as a vast number of coordinate data sets.

Fig. 1. 3-D Microvasculature reproduced in a computer display. A (upper): Human liver. P: portal vein (pink). C: central vein (terminal twig of hepatic vein, shown in light blue). B (middle): Human lung. PA: pulmonary artery (pink). PV: pulmonary vein (light blue). The circle denotes an arterio-venous adjacency. C (lower): Human myocardium. The "interdigitated" vessels tend to arrange in a longitudinal direction, i.e., along the muscles.

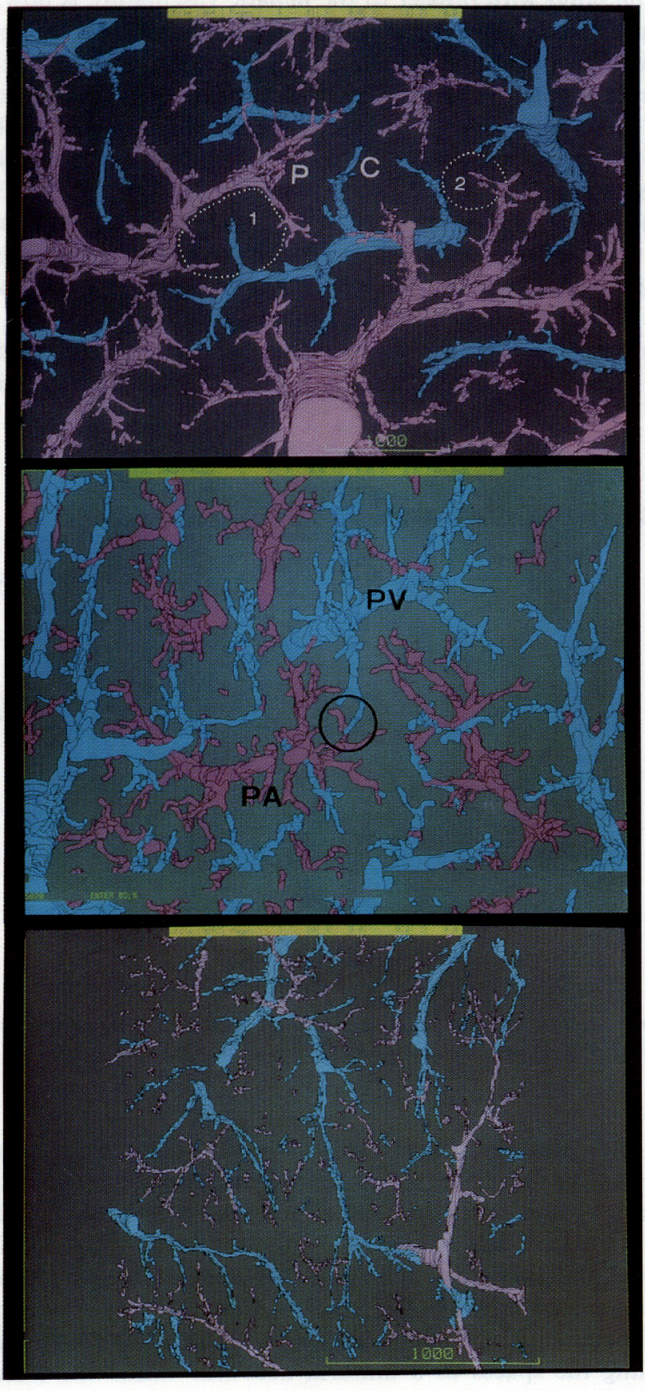

Fig. 1.

Now, the point with respect to the hepatic vasculature is how, mutually, the portal and the hepatic veins are arranged in the space. It may be clear at first sight that they are all so arranged as to embrace a remarkably uniform distance between a pair of branches. One can also say that this is a most regular example of what in anatomy is called "interdigitation", a relation like the fingers of both hands locked together. However, comparison with some other organs may readily disclose that this impressive regularity is little more than an exception. Figure 2 illustrates the small vessels of the human cerebral cortex reconstructed by a manual method at a time when no computer assist was available; the spatial relation of small inflow (artery) and outflow (vein) vessels are shown to be much more complicated and irregular as compared with the isodistance found in the liver. Thus, a pronounced organ difference does exist in the architecture of blood vessels, posing a question: What is the implication of these "forms" from a microcirculation point of view?

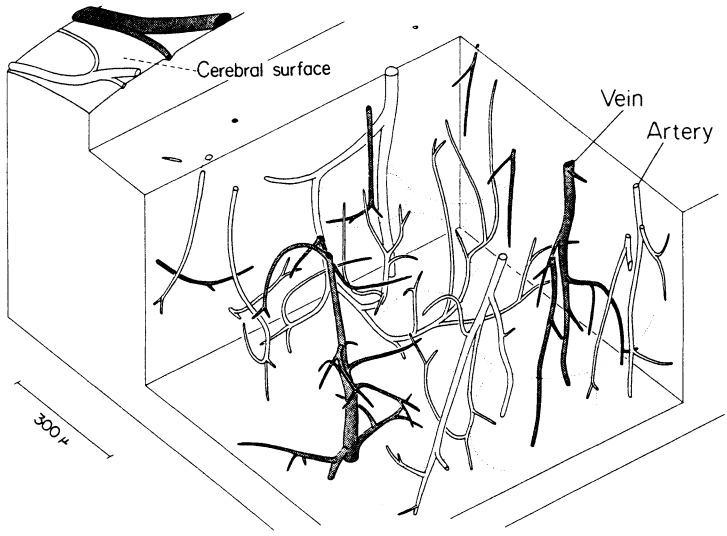

Fig. 2. Manual reconstruction of small vessels of the human cerebral cortex. The arrangement of arteries (non-shaded) and veins (shaded) is complex and appears far from isodistant.

Let us attempt a model experiment (Fig. 3). The human anatomy has shown that usually, an organ receives an artery and sends off a vein, which run and divide closely in parallel with each other, not only at the entrance but in the interior of the organ (Fig. 3A). However, this close contiguity would be quite disadvantageous from a microcirculation point of view, if it is sustained down to the terminalmost vessels, for the reason shown in Fig. 3B. We assume that the vessels in this figure are arteries and veins small enough as to have abundant connections with capillaries that are filling the spaces as a uniform network. Under the close adjacency of arteries and veins, inevitably, there occurs a great inequality in the length of flow routes, creating shortcircuitted flow at the places of contiguity. Consequently, blood

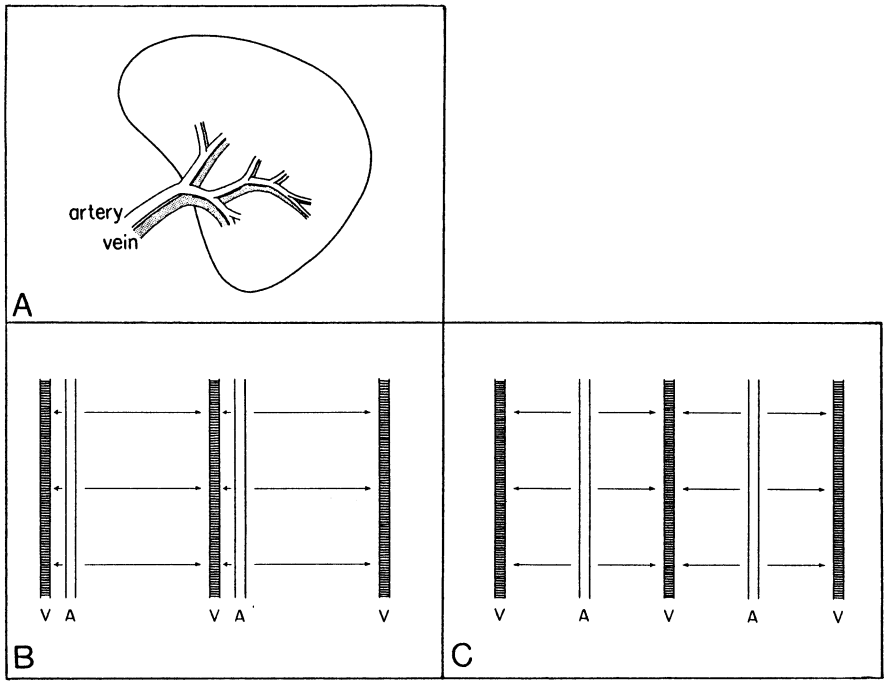

Fig. 3. A: Arterio-venous adjacency in most human organs. B and C: Vascular patterns, contiguous and isodistant. A: artery (or, inflow vessel). V: vein. For explanations see text.

cannot be distributed uniformly over the tissue, unless there is a correcting mechanism in the vessels, which actively regulates and re-distributes blood flow. It has been fully established that it is the sphincter action of smooth muscles surrounding small arteries, and also sporadically capillaries, that is responsible for this regulation. However, we also have another extreme pattern like that in Fig. 3C: The terminal arteries and veins are arranged in the space keeping a uniform distance. Clearly, under this isodistant architecture, the length of capillary routes becomes uniform, ensuring a uniform resistance to flow and, therefore, distribution of blood at a uniform density, even if the vessels are not equipped with any apparatus for regulation.

Now we see that in the tissue circulation there is an aspect, where the flow dynamics is subordinate to the arrangement of inflow and outflow vessels in the space, i.e., the vascular architecture. Under an isodistant architecture, a uniform flow in tissue is realized in the absence of regulation: In an organ with a contiguous architecture, in contrast, an active regulation of flow is indispensable. Table 2, a summary of what will be mentioned below, shows that the isodistant group includes the liver and the lung. Significantly, these are organs supplied by a vessel in which a low blood pressure predominates, namely, the portal vein supplying the liver and the pulmonary artery supplying the lung, both with a mean pressure sustained below 15 mm Hg. If in these vessels an active regulation could take place at the terminal

Table 2. Vascular architecture and the pattern of blood flow.

Vascular architecture	Flow dynamics	Organ examples	Inflow vessel
isodistant	non-regulatory	liver lung . . . . . brain	low pressure system
contiguous	regulatory	myocardium renal cortex	high pressure system

places, elevated resistance no longer allows blood flow to be sustained at a low pressure. The contiguous group includes as typical examples the heart muscle (myocardium) and the cortex of the kidney, and in these organs an active regulation is considered to prevail. Now, we have come to face a problem of 3-D morphometry. How can we describe in quantitative terms the pattern of vascular architecture in a given organ? What parameter can measure the grade of isodistance or contiguity?

PARAMETRIZATION OF VASCULAR PATTERNS

Figure 4A is a geometric model the author (T.T.) introduced in 1970 by which to compare among different 3-D patterns of arteries (or, inflow vessels like portal veins) and veins. A point P is taken randomly in the space. Suppose that we somehow determine La, the shortest distance from P to the nearest artery, and also Lv, that to the nearest vein. Then we can define L, the sum of La and Lv, as the length of the shortest route of capillary flow via P. Clearly, this length changes if the point P is moved around in the space. Therefore, if we set a sufficiently large number of points randomly in the space and measure L for each, then, it must become a statistical quantity (Fig. 4B). Here, if the dispersion of L is comparatively small in an organ, then the organ is likely to have an isodistant architecture. Inversely, a larger dispersion suggests that a more contiguous architecture exists. What is to be obtained by morphometry is accordingly the mean and the dispersion of L. In practice, measurement was designed to perform on hundreds of test points set by tessellation on a level of serial sections (Fig. 4C). However, apparently, we are unable to rely on stereology in this setting; our requirement involves estimation of distribution, that is beyond the scope of what Prof. Weibel expresses in this symposium as "bulk" parameters.

Manual 3-D measurement was done as the last resort. The vessels were graphically reconstructed from serial sections, during which process the shortest distance from P, for example to an artery, was determined by selecting the smallest one from distances calculated on every sequential step of serial sections (Fig. 4D). As one may easily imagine, this took as long as a full month in dealing with only a single organ. Not until recently had we been released from this grinding work, when a computer-aided measuring system was developed by one of the authors (Chiba, in literature 3). A system, originally designed for pictorial understanding of microstructures by integrating 3-D pictures (Yaegashi *et al.*, 1987), was reorganized so as

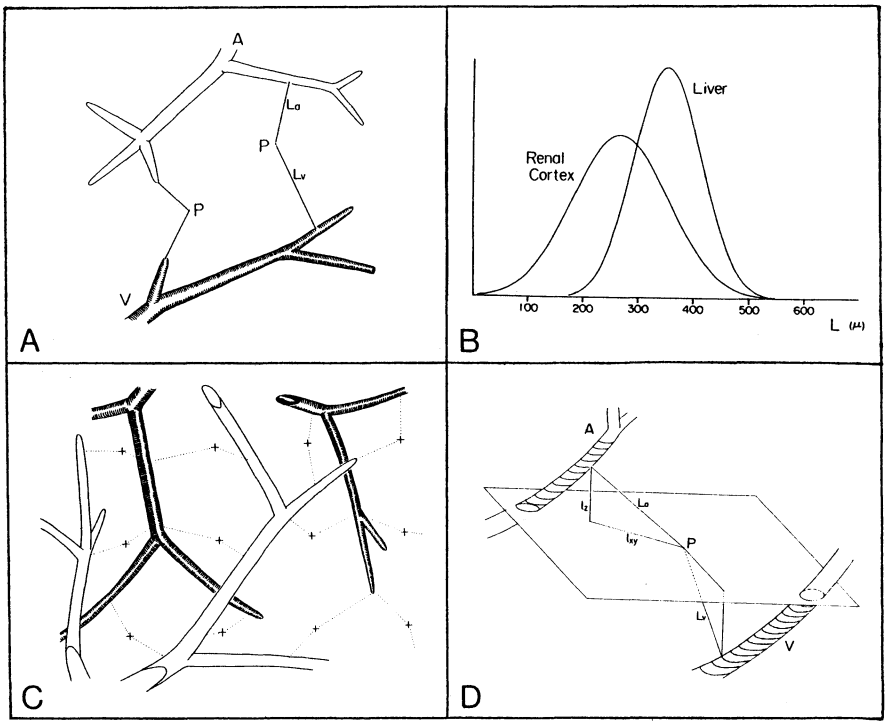

Fig. 4. A: Definition of the distance L by which to parametrize the vascular pattern. B: The less dispersed distribution of L in the liver implies a more isodistant architecture. C: Test points set by tessellation with distances to inflow (non-shaded) and outflow (shaded) vessels. D: Manual measurement of La and Lv (see text).

to serve as a tool for the computation of 3-D quantities from a vast amount of image data inputted by tracing 2-D pictures on a digitizer. Figure 5 is an example visualizing the shortest routes thus determined. This is from a liver tissue, where the selected routes are indicated as lines extending from a number of test points tessellated. Once a set of serial images are inputted, the system carries out computation within several hours for one organ.

Figure 6 presents the result of measurement in a human liver, in this case on 325 test points. Apparently, L follows a normal type distribution, and so does it in all organs examined. However, the dispersion of L cannot simply be expressed by the standard deviation, because the mean L varies to a certain degree among organs. Introduced as an alternative was a method based on the normal character of the distribution, where the upper and the lower limits could be determined at the 3-σ levels (Fig. 6). An architectural index was defined by calculating the ratio of the maximum to the minimum Ls (Lmax/Lmin), which describes to what degree the arterio-venous distance is uniform in a given organ; the smaller the index, the more uniform is the arterio-venous distance, and *vice versa*. In this liver, an index of 3.1 is obtained. Liver from another patient was added to morphometry, giving an index of

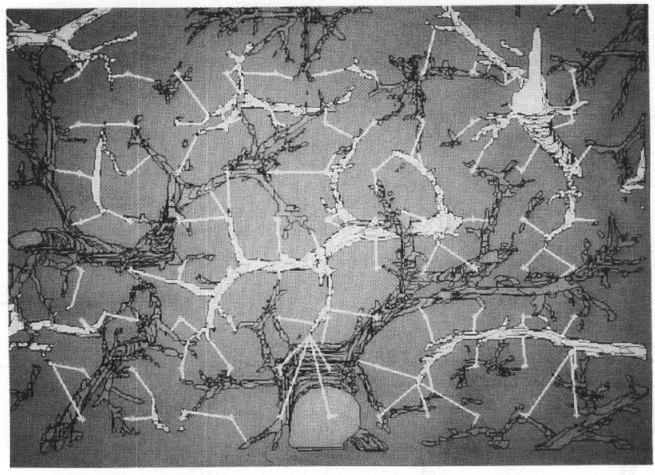

Fig. 5. The distances corresponding to La and Lv are visualized in a computer display.

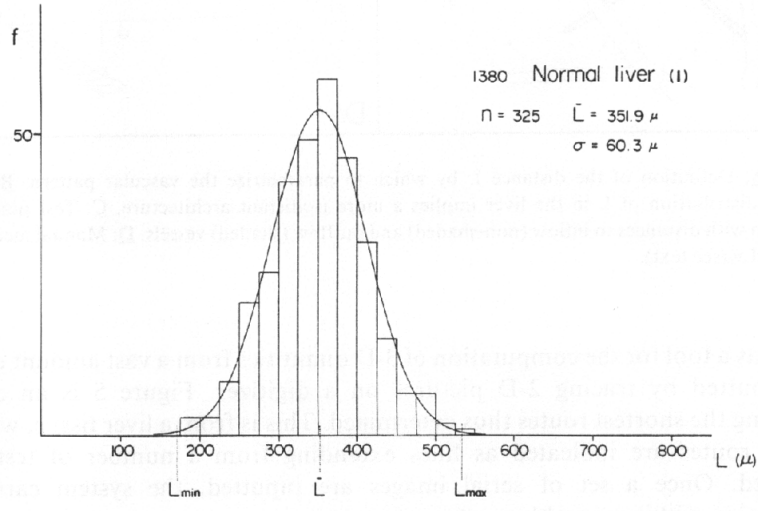

Fig. 6. The distribution of L in the liver. For Lmax and Lmin, see text.

2.9, again a value approximating 3.0. In Table 3, the indices are compared among five organs, the liver, lung, brain, heart and kidney. The value of 3.0 in the liver is by far the smallest, demonstrating that this organ is equipped with a vascular architecture prominent in isodistant character. The index exceeds 50 in the heart muscle and the kidney, organs of the systemic circulation.

Microscopically, the liver tissue of man consists of a uniformly continuing parenchymal tissue. Here the blood vessels are allowed to enjoy the greatest freedom with which they spread in the space, realizing an ideal isodistant relation. In a

Table 3. The measurement results of L.

		$\bar{L}$	σ	Lmax	Lmin	$\frac{Lmax}{Lmin}$
normal liver	(1)	352	60	533	171	3.1
	(2)	419	69	625	214	2.9
lung		218	50	368	69	5.4
cerebral cortex		165	45	299	31	9.7
myocardium		168	54	330	6	51.1
renal cortex		267	86	525	10	54.7
cirrhosis	(1)	522	127	905	140	6.5
	(2)	488	138	902	73	12.3

microscopic section, the liver tissue also appears as if composed of small unitary structures called liver lobules (Fig. 7). There has been a long historical debate on whether it is possible to define such a unit in anatomical as well as geometric terms, and in what way if it is possible (Elias, 1949; Rappaport *et al.*, 1954). We think that the seemingly discrete units are nothing but a 2-D expression corresponding to a 3-D architecture with extremely isodistant relation of the inflow and outflow vessels.

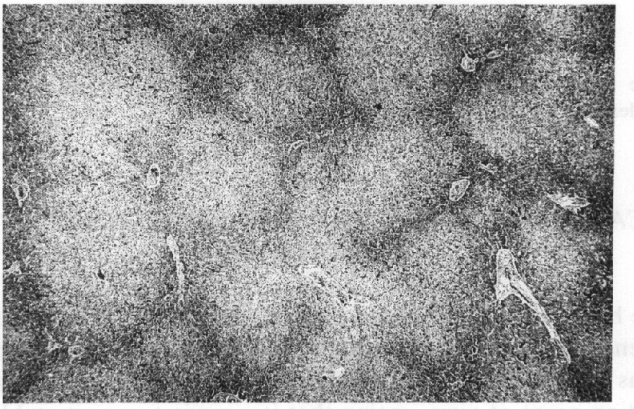

Fig. 7. Microscopic appearance of the human liver, composed of several lobules, the areas delimited by the dark zones. ×20.

THE INTENSITY OF ACTIVE REGULATION OF BLOOD FLOW

The basic assumption of this study has been that under a contiguous arrangement of small vessels, the blood flow has to be regulated. The regulation is performed by constriction of smooth muscles surrounding small arteries as their muscular coat. Therefore, wherever the blood flow is subjected to an active and incessant regulation, the small arteries are expected to have correspondingly

strengthened muscles. In fact, an organ difference appears to exist in the degree to which the muscular coat is developed, which was studied by a morphometric method devised and applied to arteries by Suwa and Takahashi (1971). The results are illustrated in Fig. 8 where the ordinate is the mean muscular thickness D of small artery at 100 μm in radius, and the abscissa the architectural index. $D=0$ in the liver because the intrahepatic portal veins that are the main inflow vessels of this organ are usually devoid of mural smooth muscles. Though the number of data is not very large, one can find a sort of correlation, lending support to the view that the larger the architectural inequality, the more active the regulation.

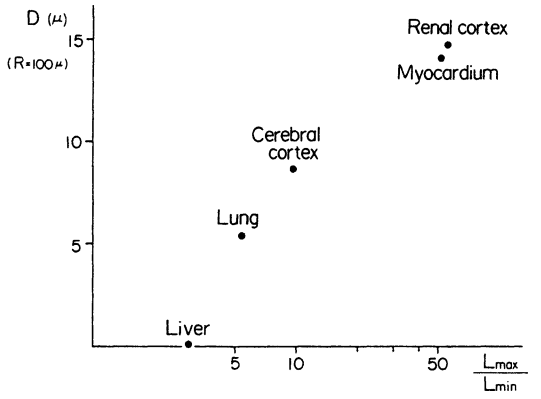

Fig. 8. D, the mean thickness of arteriolar muscular coat, is correlated with Lmax/Lmin, the architectural index.

ANATOMICAL BACKGROUND FOR THE DIVERGENCE OF VASCULAR PATTERNS

Still, we have a question unanswered. What is responsible for the variety and organ difference in the architectural pattern of vessels? Undoubtedly, the regular isodistance as found in the liver must be a relation most advantageous from a microcirculation point of view, seeing that it promises a most stable and uniform distribution of blood. Nevertheless, it is only in the liver, and less typically in the lung, that one can find this vascular pattern. On what ground is it accountable that this regular vasculature is confined to these organs, while the pattern apparently ensures the most smooth microcirculation in tissue? In reality, the background can be understood in the organ structure itself.

Figure 1B is the vessels of the lung demonstrated to compare with those of the liver. The circulation prevailing in the lung also appears to have much in common, with the pulmonary arteries having low blood pressure and being inert in regulatory activities. Corresponding to these functional features, reconstruction of the lung vessels discloses an isodistant pattern simulating the liver vessels. There is however a difference. Often, we encounter such arterio-venous contiguities among pairs of

terminal twigs that never proved to exist in the liver, and this suggests a mild degree of irregularities remaining. A deviation from the highest regularity is reflected in the distribution of L, where the architectural index is 5.4, somewhat larger than 3.0 of the liver. The background underlying this difference reveals itself in the microstructure of lung as in Fig. 9, a sketch from a microscopic picture of this organ, in which one can see spaces dividing into small chambers. There are terminal air spaces from small bronchi (respiratory bronchioles) to alveolar ducts and alveoli, that are organized into a tree, following a principle that provides the lung with a structure optimal for ventilation and gas exchange (Weibel, 1963). On the other hand, the blood vessels have to be subordinate to the structure of the airways because they are confined within the wall of the air spaces. In other words, in the lung, the degree of freedom assigned to the architecture of blood vessels is correspondingly reduced.

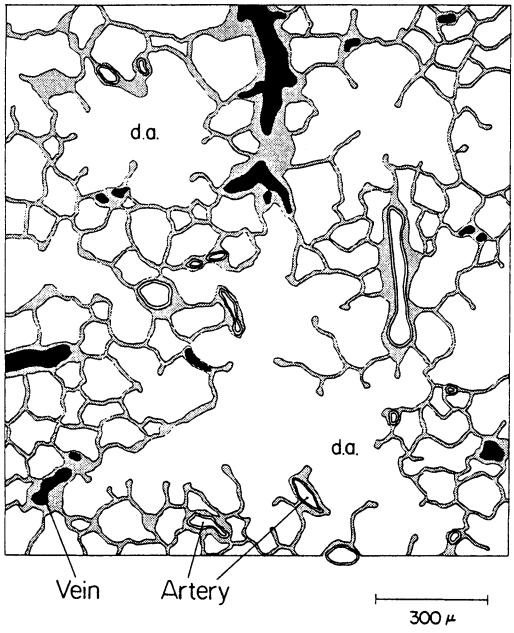

Fig. 9. A sketch from a microscopic picture of human lung. There are spaces including alveolar ducts (d.a.), dividing into small chambers, the alveoli.

The circumstances are the same in any organ. Figure 10 is a model of the left ventricle of the heart showing how the heart muscles are organized (Tezuka & Takahashi, 1975). This strikingly regular arrangement is likely to have close bearing with the mechanical performance of the cardiac pump. However, this very regularity deprives the coexisting blood vessels of the freedom to form an isodistant relation; the small vessels tend to align along a certain direction as in Fig. 1C, that means, along the bundles of heart muscles, making the architecture far remote from a regular isodistance.

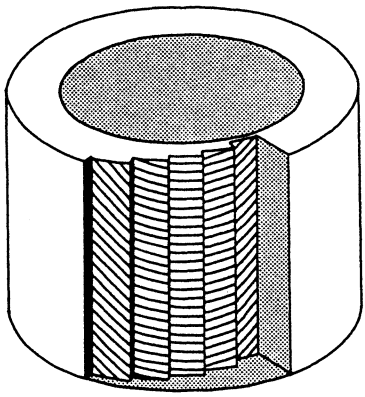

Fig. 10. A cylindrical model for the left ventricle of heart, showing the regular transition of heart muscle fibers with myocardial depth.

Now we have come to a conclusion. Generally speaking, an organ comprizes two or more functional systems. The heart has the muscles and blood vessels, the lung the airways and blood vessels, and also the liver, the blood vessels and a gland system secreting bile. However, each system requires its own architectural principle which allows it to work at the highest efficiency. Thus, the heart muscle requires a spiral arrangement that appears to meet a requirement for highest mechanical efficiency, the air spaces of the lung a regular arborescence ensuring an efficient gas flow, and the blood vessels a regular isodistance as their ideal architecture. However, not every requirement can always be satisfied, because, only a limited space is allowed to an organ, in which the systems have to coexist somehow (Fig. 11). For example in the heart, we see the architecture of muscles being given a great preference; the very reverse is in the liver, where the blood vessels are given the largest degree of freedom. This is probably reflected in the fact that the gland system of this organ is of quite a peculiar type, i.e., an amorphous gland (netlike gland) requiring little if any specific architecture.

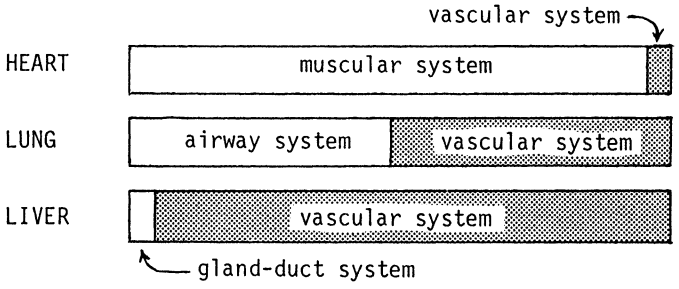

Fig. 11. A schema illustrating the structural principle of organs. The span of each functional system expresses the degree of freedom assigned to its architecture.

COMMENTS ON RECONSTRUCTION TECHNOLOGY

Before finishing, some technical aspects of 3-D visualization and analysis are to be mentioned about. Reconstruction includes several processes; 1) preparation of serial sections, 2) abstraction of necessary profiles from serial 2-D pictures, 3) inputting of the profiles into a computer, 4) 3-D visualization, and 5) computation of 3-D quantities. Of these, the last two tasks belong to computer engineering. With respect to the second and the third tasks, that are the abstraction of necessary images from microscopic pictures and their inputting, we have scarcely been successful in computerizing. This is because of the presence in the pictures of human tissues of a variety of noise images that, complicating all facets of microscopic appearance, impede it from being purified into a set of components to be reconstructed. Therefore in our laboratory, these steps are still performed by manual method, time-consuming as they are. Also the first task, the preparation of serial sections, is another daunting work requiring highly sophisticated skill. It is in these first three tasks that we expect the possibility of introducing and applying a confocal microscope or other new tools that might relieve us from consuming too much time and energy.

In closing, we would like to stress the difference between the value of 3-D visualization and that of 3-D quantification in biomedical research. As one of the authors (T.T.) pointed out at the First International Symposium for Science on Form (1986), if one attempts at visualizing a 3-D material by reconstruction, it instantly becomes clear that what is produced is always the surface of the material. If he continues reconstructing, he only obtains renewed surfaces. This is the sort of frustration we often feel while making scanning electron microscopy. Our ability to recognize the three dimensions appears to be quite a limited one, so long as we are relying on expression in a 2-D graphics display. In performing a research, what we really need is not a sophisticated picture but some information, such as the skeleton of structure, its topology, distribution information, or 3-D quantities that allow us to gain insight into the functional implications. In the example of blood vessels we introduced, reconstruction has been performed simply to obtain one quantity, that is, the architectural index, while the 3-D pictures were merely by-products. Thus, we are looking always forward to having a much extended possibility of computational geometry with the introduction of new tools for microscopy, which will give us a great aid in establishing a "bridge" between the structure and function.

REFERENCES

1. DeHoff, R. T.: Quantitative serial sectioning analysis: preview. *J. Microscopy* **131**: 259–263, 1983.
2. Takahashi, T.: Lobular structure of the human liver from the viewpoint of hepatic vascular architecture. *Tohoku J. Exp. Med.* **101**: 119–140, 1970.
3. Takahashi, T., Chiba, T., Yaegashi, H.: Three-D reconstruction of biostructures and its computerization—A possible extension of stereology. *Acta Stereol* **6/III**: 723–731, 1987.
4. Yaegashi, H., Takahashi, T., Kawasaki, M.: Microcomputer-aided reconstruction: A system designed for the study of 3D microstructure in histology and histopathology. *J. Microscopy* **146**: 55–65, 1987.

5. Elias, H.: A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary systems. *Am. J. Anat.* **85**: 379–456, 1949.
6. Rappaport, A. M., Borowy, Z. J., Lougheed, W. M., Lotto, W. N.: Subdivision of hexagonal liver lobules into a structural and functional unit; role in hepatic physiology and pathology. *Anat. Record* **119**: 11–34, 1954.
7. Suwa, N., Takahashi, T.: *Morphological and Morphometrical Analysis of Circulation in Hypertension and Ischemic Kidney.* Urban & Schwarzenberg, München, 1971, pp. 40–60.
8. Weibel, E. R.: *Morphometry of the Human Lung.* Springer-Verlag, Berlin, 1963.
9. Tezuka, F., Takahashi, T.: Pathology of cardiac hypertrophy in pressure overload. *Jap. Circ. J.* **40**: 1111–1118, 1976.
10. Takahashi, T., Iwama, N., Yaegashi, H.: The 3-D microstructure of cancer and its topological properties. In: Science on Form—Proceedings of the First International Symposium for Science on Form (Ishizaka *et al.* ed) KTK/Reidel Publishing Co., Tokyo, Dordrecht, 1986, pp. 543–552.