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THREE-D COMPUTATIONAL GEOMETRY: THE PATTERN OF VASCULATURE IN NORMAL AND DISEASED LIVERS AS EXPRESSED BY THE DISTRIBUTION OF DISTANCE IN SPACE

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ABSTRACT

In cirrhosis and related lesions of the liver, the pattern of microvasculature is more or less deviated from the norm. The process is called the lobular disorganization where the normally isodistant relation of the small portal and hepatic venules is gradually lost with the formation of P-C (portal-central venous) bridgings. To quantify the degree of disorganization, we defined the "architectural index": it expresses the grade of vascular isodistantism with the distribution pattern of the route length L from a portal to a hepatic venule via a point randomly located in the space. The measurement of L, on 300 to 400 points in one liver, was performed with the aid of a computer, into which the contours of venules were inputted from serial sections and stored as voxel data. Eight livers with chronic hepatitis, cirrhosis or idiopathic portal hypertension and one control liver were examined. The index proved to fully describe the grade of disorganization and work as a measure of cirrhotic changes.

Key words: disorganization of liver lobule, cirrhosis, 3-D morphometry, computer-aid.

INTRODUCTION

In applying techniques of morphometry in diagnostic or experimental pathology, we often face problems where principles of stereology are of no avail. Table 1 is a list given by DeHoff (1983) who enumerated properties of form that cannot be estimated stereologically. It includes such important features of form as the numerical density of particulate structures, connectivity in 3-D and the spatial distribution information. Of these, the

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

Number of features
Connectivity of features
Size distribution (by volume, area, diameter)
Spatial distribution information (covariograms)
Real feature shape

number of particles in the space became much easier to obtain than before, thanks to the introduction of the disector principle (Sterio, 1984). In the other problems, however, we are still in the situation in which we have to undertake direct measurements from, for example, serial sections. Breakthrough into this difficulty is hardly expectable in the near future, and we are likely to be left unrelieved from the toil of measuring the 3-D space by direct scanning. Such being the circumstances, at least the work of measurement should be minimized by introducing computer-assist as much as possible. In this paper we show an attempt at quantitatively expressing the degree of lobular disorganization which the liver tissue undergoes as it proceeds from precursor diseases to completed cirrhosis. The process involves variously altered 3-D relation between the small portal and hepatic veins, a feature we expressed with the distribution of distances to these vessels from points randomly positioned in the tissue space.

THE 3-D MORPHOLOGY OF LOBULAR DISORGANIZATION

On a 2-D section, a cirrhotic liver of man looks as if comprizing spherical nodules separately dispersed in the space. However, reconstruction from a small number of sections discloses that its 3-D structure differs from what appears on a section (Takahashi 1978). Nodules are not separate particles, but are all aggregated with the adjacent ones as in Fig. 1A. By applying a node-branch model to express their connecting relation it becomes clear that the nodules are united in the space so as to form a 3-dimensional network having many loops (see the figure). If, in another reconstruction, this network is correlated with the vascular structure of the liver as in Fig. 1B, then it is clearly visible that both the small portal and hepatic (central) veins mostly run in the interstitial sepa, where they make abundant anastomoses, giving rise to what has been called portal-central (P-C) bridgings. This vascular alteration is responsible for creating intrahepatic shunt of portal blood, a mechanism underlying the ineffective detoxication by hepatocytes, which finally leads to hepatic insufficiency. Thus, basically, the structure of cirrhotic liver consists of a vast parechymal network that 3-dimensionally intertwine with the small afferent and efferent vessels (Fig. 1C); here the vessels are also forming a network with loops, a framework created with the development of P-C bridgings. This combination of intertwining networks is the skeleton of cirrhosis, and one cannot fully understand the process of cirrhogenesis without knowing how this skeleton forms. For the present, we may assume at least that it arises as the result of hepatic necrosis developing in such a way as to connect small portal and he-

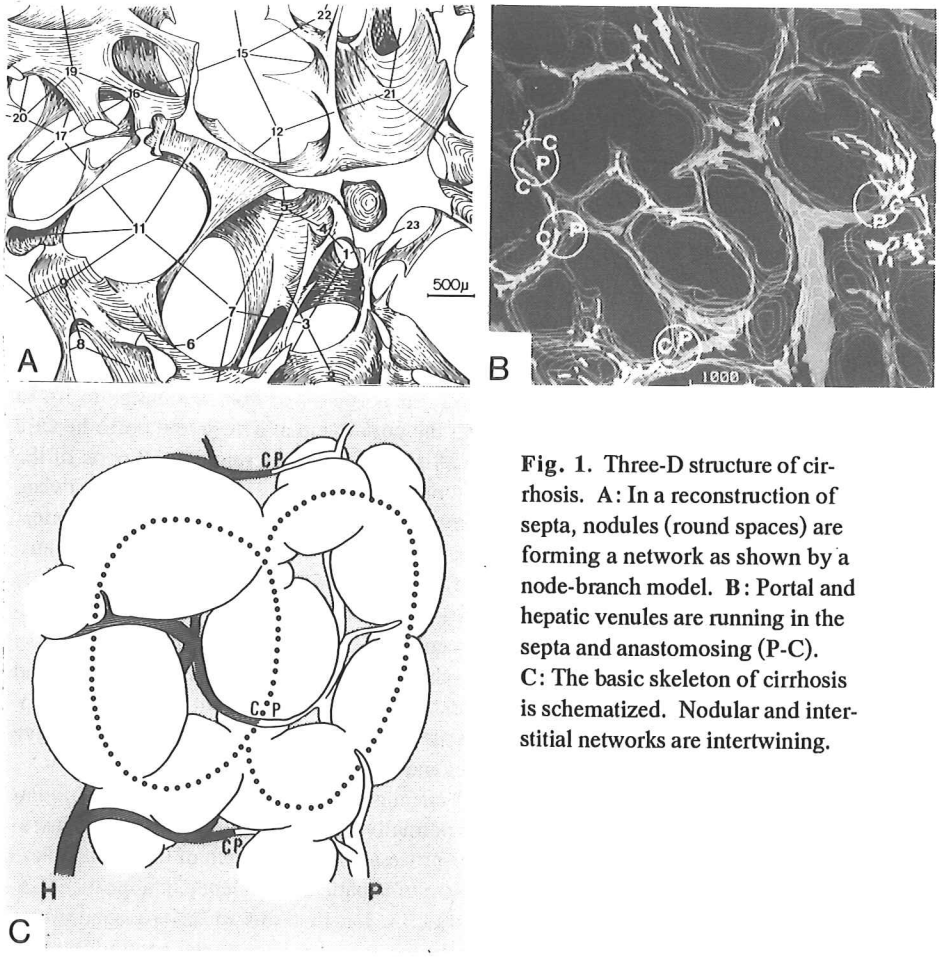

Fig. 1. Three-D structure of cirrhosis. **A**: In a reconstruction of septa, nodules (round spaces) are forming a network as shown by a node-branch model. **B**: Portal and hepatic venules are running in the septa and anastomosing (P-C). **C**: The basic skeleton of cirrhosis is schematized. Nodular and interstitial networks are intertwining.

patic veins somehow, leaving a network of interstitium; the nodules are molded only passively by the interstitial network to intertwine with it.

Every organ has its specific pattern of blood vessels. In the ordinary liver, as shown in Fig. 2, the microvasculature is characteristic in that the terminal vessels are all so arranged as to embrace a uniform distance between portal and central veins. Or, one finds in this an "interdigitating" relation in its most regular and isodistant form. In other organs, there is no longer such an impressive regularity (Takahashi, 1970; Takahashi and Chiba, 1990), with an afferent-efferent vascular relation more or less complicated and irregular. Such a difference of microvasculature among organs has a profound significance from a

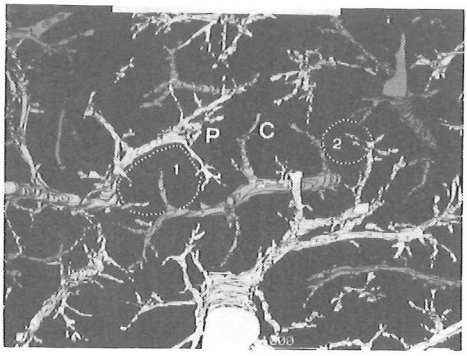

Fig. 2. The vasculature in an ordinary liver is reconstructed in a display. Note the isodistant relation between the portal (P) and central (C) venules.

microcirculation point of view. In case of liver, the portal blood flow amounting to 1/4 to 1/3 of the cardiac output is sustained through the portal vein at a very low porto-hepatic pressure difference. And yet, the portal blood is required to distribute to all parts of the voluminous organ, irrigating the parenchymal tissue as uniformly as possible. Otherwise, the liver cannot perform its task as a central organ of metabolism, where a maximal interchange of materials should take place between hepatocytes and the sinusoidal blood. Thus, from a structure-function-correlation point of view, we understand the significance of the isodistant arrangement of the portal and central veins: it ensures, with an equalized resistance to sinusoidal blood flow, to fulfil the requirement of uniform blood irrigation in an organ where the small portal veins, having no smooth muscular coat in the wall, are devoid of flow-regulation mechanism. This is a situation completely different from organs supplied only by systemic arteries, where the tissue microcirculation is susceptible of active regulation by smooth muscles of small arteries and arterioles.

Thus it may be clear that the process of cirrhogenesis, usually expressed as lobular disorganization, consists of changes from the normally isodistant to a non-isodistant vasculature with many P-C anastomoses. Since the process implies a breach of the basic pattern of microcirculation and explains the mechanism of hepatic insufficiency, it appears essential to establish a measure of lobular disorganization in terms of microvasculature. Quantification will be possible if we reduce the matter to an isodistance-contiguity problem. In the following, we propose a model.

A MODEL FOR THE QUANTIFICATION OF VASCULATURE —— THE DISTRIBUTION OF 3-D DISTANCE

Fig. 3A is a model we employed for the quantification of microvasculature. Let us take a point P randomly in the liver tissue. Suppose that we can determine L_A, the shortest distance from P to the nearest artery (in this case, the portal vein), and also L_V, that to the nearest vein (the hepatic vein). Then we define L, the sum of L_A and L_V, as the length of the shortest capillary route via P. This length changes when the point P is moved around

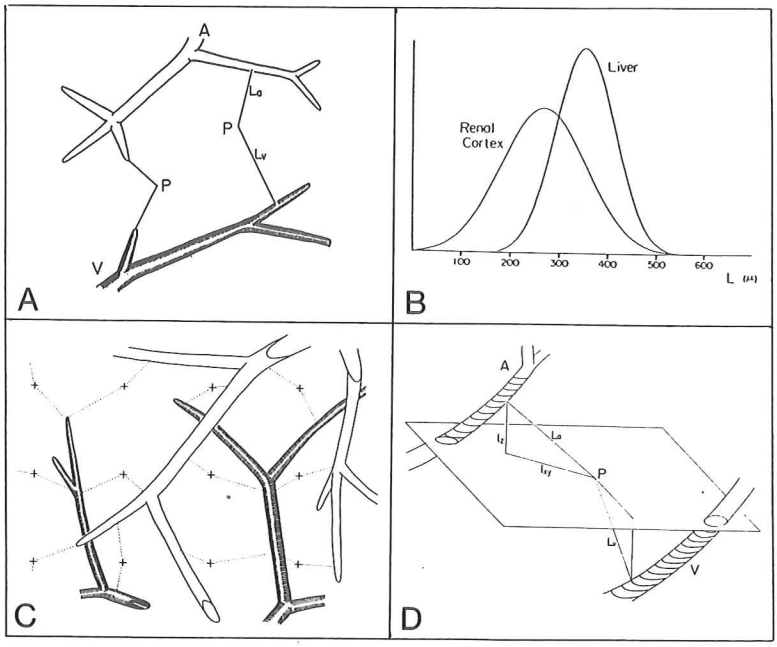

Fig. 3. Quantification of vascular pattern. **A**: The concept of L, the shortest arteriole-to-venule (A-V) length via a point P. **B**: Various dispersion of L. **C**: Setting of test points by tessellation on a section. **D**: The algorithm for the determination of L.

in the tissue. Therefore, when we set a sufficiently large number of points and measure L as in Fig. 3C, then it becomes a statistical quantity. Suppose that the dispersion of L is comparatively small in an organ (Fig. 3B); then the organ is likely to have an isodistant vasculature. Conversely, a larger dispersion suggests that a more contiguous vasculature exists. Cirrhotic livers are expected to belong to the latter because of the presence of abundant contiguities between the portal and hepatic venules. Therefore, in this context, what we have to obtain by morphometry is the mean and the dispersion of L.

In practice, measurement was designed to perform on 300 to 400 points set by tessellation on a level of serial sections, with a between-point distance of 100 μm. But, can we rely on stereology in estimating the mean and variance of L? Apparently, the answer is no. Our requirement includes spatial distribution information, that is beyond the scope of stereological estimation. It is for this reason that previously, the senior author had to undertake manual measurement of L from serial sections (Takahashi 1970): while graphically reconstructing the afferent and efferent vessels, the shortest distance from P to a vessel was determined by calculating the distance sequentially on serial sections (Fig. 3D). Probably

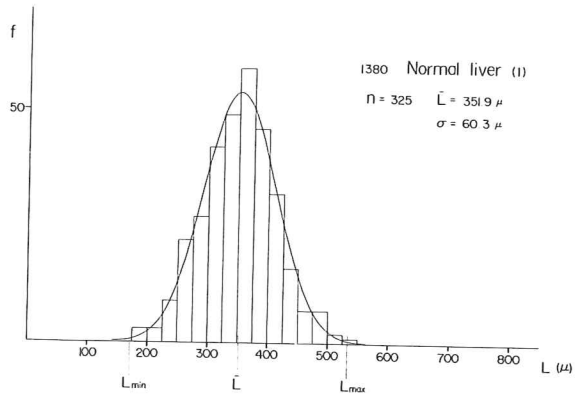

Fig. 4. The distribution of L in an ordinary liver.

one can imagine what a grinding work this requires. It is only recently that in the measurement of 3-D distance we became able to rely on the aid of a computer.

THE COMPUTER-ASSISTED 3-D MEASUREMENT

Primarily, we designed a computer system to visualize 3-D microstructures by graphic reconstruction (Yaegashi et al., 1987), but gradually we became aware that it also works as a tool for the computation of 3-D quantities. In reconstructing the liver vasculature, for example, 2-D images from serial sections are inputted by digitizing the contours of blood vessels in each section. This implies that once the inputting is finished, the whole 3-D morphology of blood vessels is being stored in the memory as a vast set of voxel data. Therefore, we can now make use of them for computation of such 3-D quantities as the distance, area, volume and so on. In the present study we used two systems: one was constructed upon a desktop computer (model 310); the other on a workstation (model 400), both Hewlett-Packard. Shown in Fig. 5 is the result of computation graphically

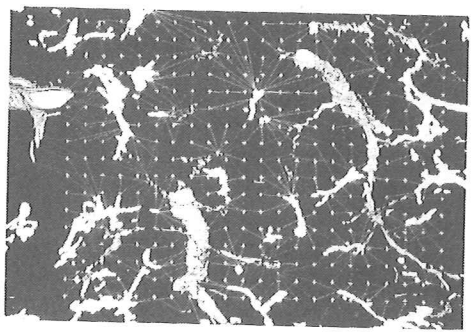

Fig. 5. The result of computation in a liver with chronic hepatitis (CAH). For 374 test points set by tessellation, the shortest routes are selected and visualized in a computer display.

visualized in a computer display, obtained from the ordinary liver.

Fig. 4 is the result of measurement in an ordinary human liver, in this case on 325 points (*Ps*). Apparently *L* follows a normal distribution, and so does it not only in every ordinary organ we examined but also in cirrhosis and its precursor states of the liver. As mentioned, the aim of this study is to obtain the dispersion of *L*. In this case, however, the standard deviation does not serve as an appropriate parameter of dispersion, because the mean *L* varies to a certain degree among various conditions: it markedly enlarges in cirrhosis where the internodular parenchyma continues to regenerate and becomes hyperplastic. Therefore we employed another method. Since the distribution is normal, we can define its upper and lower limits at the 3-σ levels. If, then, the ratio of the maximum to the minimum *L*s was calculated, it serves as an index of architecture, describing to what degree the porto-hepatic venous distance is uniform. The smaller the index, the more uniform is the distance, and *vice versa*. In an ordinary liver we obtained an index value of 2.7. This is by far the smallest among the five organs examined, the liver, lung, cerebral cortex, myocardium and kidney, and demonstrates that certainly, the liver is equipped with an architecture prominent in isodistant architecture (Takahashi, 1970; Takahashi and Chiba). The index exceeds 50 in some organs of systemic circulation.

The liver tissue of man comprises a uniformly continuing parenchyma. Here the blood vessels enjoy the greatest freedom with which they spread in the space, realizing an ideal isodistant relation. We think that the hepatic lobules, while in a 2-D section they seem to exist as a unitary structure of the organ, simply correspond to an extremely isodistant architecture in the 3-D.

Application to chronic liver diseases

Based on the above, we are now able to compare among livers with more or less advanced lobular disorganization. Shown in Fig. 5 is the result of computation graphically visualized in a computer display, obtained in a case of chronic hepatitis (CAH). Microscopically, and also in the result of reconstruction, there appeared to be a moderately disorganized lobular structure with abundant P-C bridgings, but the parenchymal nodulation appeared not to have advanced so far. One can see in the figure 374 test points, from each of which the route of the minimum distance to a portal venule and that to a hepatic venule are selected and visualized. The analysis was extended to eight livers with chronic diseases including three with chronic hepatitis (CAH), two with cirrhosis and three with idiopathic portal hypertension (IPH). Cases with IPH were examined because in Japan where the disease has been studied intensively under the name of Banti disease, there have been arguments on the part of pathologists on whether these livers represent a precirrhotic condition, or simply, a hepatic fibrosis of non-cirrhotic character.

The results of measurement in the nine livers including one control are shown in Table 2. A remarkable finding is that in the two cirrhotic livers, the architectural indices are so much elevated as to exceed 10.0. In both cases, cirrhosis was of the posthepatitic type of Gall (1960), with fully completed and coarsened nodules. The index values are so elevated as to be comparable with those obtained in some systemic organs, suggesting that the liver

Table 2: The result of 3-D measurement.

	mean L	SD (log)	L_{max}	L_{min}	L_{max}/L_{min}
Control liver	413 (μm)	.073	684 (μm)	250 (μm)	2.7
Chronic hepatitis (1)	377	.129	919	155	5.9
(2)	350	.129	853	144	5.9
(3)	577	.135	1466	227	6.5
Cirrhosis (1)	528	.175	1770	158	11.2
(2)	572	.181	1995	164	12.2
Idiopathic PH (1)	648	.111	1390	300	4.63
(2)	639	.129	1560	262	5.95
(3)	660	.137	1702	256	6.65

microcirculation in these cases have been subjected to a severe deviation from the norm. The livers diagnosed histopathologically as chronic hepatitis all proved to have an intermediate value, a result sufficiently coinciding with the microscopic appearances where the dis organization of tissue architecture appears also halfway. In IPH cases it derserves attention that there also is a mild to moderate disorganization, showing a process common to that toward cirrhosis is under way: this will serve in re-examining its pathology, particularly in Japan where patients with this disease are not uncommon.

Conclusion

We introduced an application of what is called the technique of computation geometry to studies of human pathology. We find in this a promising technique though there still remains a difficulty: its being time-consuming. We think however that we are now in the phase in the history of pathology in which we can expect developments in technology which will provide us with devices for light microscopic tomography of tissues, such as the scanning microscopes. We are looking forward to having more extension in this aspect of pathology.

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