STEREOLOGICAL METHOD OF DETERMINING THE BULK DENSITY OF THE MYOCARDIAL ARTERIAL BED IN HISTOLOGICAL SECTIONS

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The bulk density of the intramyocardial arteries is shown to be equal to the sum of their bulk densities obtained from the results of planimetric measurements in three mutually perpendicular histological sections. To avoid systematic errors, only those vessels in which the ratio between the short (D) and long (L) diameter of the cross sections (D/L) is not less than 0.707 should be examined planimetrically. Examples of calculation of the bulk density of the intramyocardial vessels of the human heart are given. It is shown that the bulk density of the arteries in different parts of the human myocardium is not identical.

KEY WORDS: *heart; intramyocardial arteries; stereometry.*

Methods of three-dimensional stereological analysis of the structures of organs and systems are used in morphological investigations. The results thus obtained are reliable stereometric characteristics for the study of pathological processes [1, 3, 4, 6]. In particular, it is interesting to study the principles governing the organization and reorganization of the myocardial vascular bed. However, unless it is first placed on a solid and systematic basis, the stereological approach cannot be used for the analysis of the branching of the arteries of the heart, for it can be used only to study structures which have no predominant orientation or which are located with their long axis in one of the three mutually perpendicular axes of the preparation [5]. The arterial branches of the myocardium may be directed along three axes. To determine the bulk density of distribution of the vessels in histological sections, the analysis must therefore take into account the planes of orienta-

*According to Fig. 1, $\cos^2\alpha + \cos^2\beta + \cos^2\gamma = 1$ (1). In that case, the sum of the angles ($V = \alpha + \beta + \gamma$) has a minimum at about 164° 15'. In view of the continuity of the function V there is an infinite number of sets of values of the angles α, β, and γ such that each of the angles can be over 45°. Let an analysis be made at the extremum of the function $V = \alpha + \beta + \gamma$ under the conditions $\cos^2\alpha + \cos^2\beta + \cos^2\gamma = 1$; $0° \leqslant \alpha \leqslant 90°$, $0° \leqslant \beta \leqslant 90°$, $0° \leqslant \gamma \leqslant 90°$ by using Lagrange's method. Let the function: $W = V + (\cos^2\alpha + \cos^2\beta + \cos^2\gamma - \lambda)$ be formed and its derivatives: $W'_\alpha = 1 - \lambda\sin^2\alpha$, $W'_\beta = 1 - \sin^2\beta$, $W'_\gamma = 1 - \sin^2\gamma$ be found. By making these derivatives equal to zero a stationary point corresponding to equality of the three angles ($\alpha = \beta = \gamma$) is obtained. It follows from equations (1) that $\cos\alpha = \cos\beta = \cos\gamma = 1/\sqrt{3} = 0.577$, from which $\alpha = \beta = \gamma = \arccos 0.577 \approx 54°45'$. Let $\overline{n} = (\cos\alpha, \cos\beta, \cos\gamma)$ be a single vector from the first octant (see Fig. 1). Then, as we know, $\alpha + \gamma \geqslant 90°$, $\alpha + \beta \geqslant 90°$, $\gamma + \beta \geqslant 90°$; hence it follows that if any of these angles, for example $\alpha \leqslant 5°$, then $\gamma = 45°$ and $\beta = 45°$. Consequently, if the specimen is cubical and if only vessels inclined at an an angle of less than 45° to its plane are subjected to planimetry in the section, provided that the sections are mutually perpendicular, none of the vessels will be subjected to planimetry more than once.

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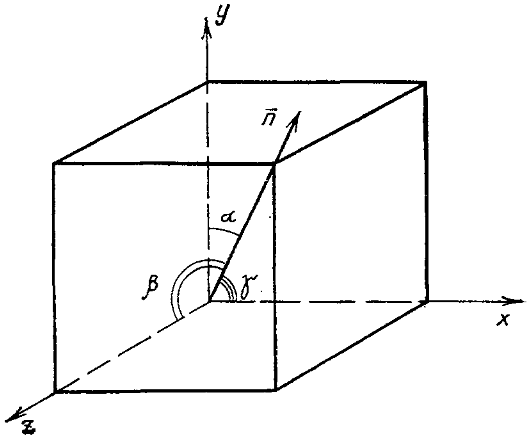

Fig. 1

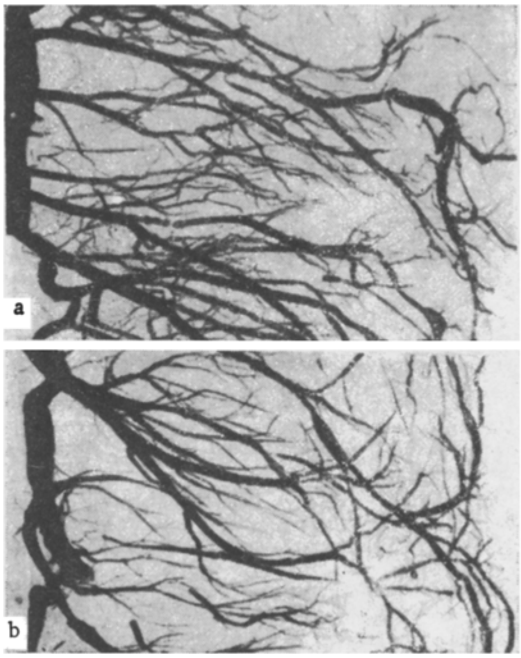

Fig. 2

Fig. 1. xoy, xoz, yoz) Mutually perpendicular planes of a block of the myocardial wall of the left ventricle; α, β, γ) angles of inclination of vessel $\bar{n}$ relative to each plane of cross section through myocardium.

Fig. 2. Roentgenograms of injected branches of coronary arteries of myocardium in two planes relative to surface of myocardium: a) horizontal section; b) vertical. Clearly the angles of inclination of all branches in the section relative to the long axis of its perpendicular branch do not exceed 45°.

TABLE 1. Values of Short and Long Diameters of Plane of Section of Vessels with an Angle of Slope of 45° relative to Plane of Section

Diameter of plane of section of vessels (in conventional units)			
short	limit of value of long diameter	short	limit of value of long diameter
1	1,41	13	18,39
2	2,92	14	19,80
3	4,24	15	21,22
4	5,65	16	22,63
5	7,02	17	24,05
6	8,49	18	25,46
7	9,90	19	26,87
8	11,32	20	28,29
9	12,73	21	29,70
10	14,14	22	31,12
11	15,56	23	32,53
12	16,96	24	33,94

tion of the vessels (x, y, z) and the corresponding bulk densities ($\varphi_x, \varphi_y, \varphi_z$), and their combined bulk density must be determined ($\varphi_x + \varphi_y + \varphi_z = \bar{\varphi}$). With this approach the same branch, depending on its angles of inclination relative to the three planes specified, can be included in the analysis twice or three times in the section (not only in one, but in two or even three planes). This results in an exaggerated combined assessment of the bulk density of arrangement of the vessels ($\bar{\varphi}$), of which there is convincing evidence.* For this reason, before the planimetric analysis by the "point" method of counting [2, 5] it carried out to estimate the bulk density of the myocardial vessels, the degree of orientation of the vessels relative to

TABLE 2. Density of Intramyocardial Vessels in Different Parts of Wall of Left Ventricle of Human Heart (M ± m)

Wall of left ventricle	Regions of wall of left ventricle		
	upper	middle	lower
Anterior	0,0201±0,00073	0,0229±0,00094	0,0161±0,00075
Lateral	0,0180±0,00069	0,0252±0,00105	0,0178±0,00073
Posterior	0,0173±0,00074	0,0263±0,00105	0,0190±0,00076

the three above-mentioned mutually perpendicular planes must be determined. If all the arterial branches without exception have an inclination relative to one of the mutually perpendicular planes of the section which is under 45°, no branch will be measured twice, or still less, three times. If, however, the angles of inclination are 45° or more, a correction will have to be made to $\overline{\varphi}$. This situation can be avoided by carrying out planimetry on only those vessels which are inclined to the section at an angle of not more than 45°. To determine these principles, investigations were carried out on roentgenograms of the horizontal and vertical layers of the wall of the left ventricle. The system of coronary arteries was filled through special cannulas with a suspension of lead carbonate in gelatin under a pressure of 140-180 mm Hg. Mutually perpendicular layers were cut from different parts of the wall of the left ventricle in three planes, to a thickness of 10-15 mm, and roentgenograms were obtained of them. These were measured and the angles of inclination of the vessels to each of the three axes of the section were determined. For this purpose, a layer of myocardium for each axis was chosen (Fig. 2).

The results of the investigation showed that the angle of inclination of the arterial branches to each plane of section of the myocardium was always under 45°. It could therefore be concluded that when the combined bulk density ($\overline{\varphi}$) was determined there was no need to introduce a correction if planimetry of the sections were carried out only on vessels with an angle of inclination to the plane of the section of below 45°. Such vessels are easily identified in histological sections from the results of measurement of their long (L) and short (D) diameters. If this ratio $D/L \geqq 0.707$, where D is the short and L the long diameter of the cross section of the vessels, it is measured in that particular plane of the section. Table 1, with values of the corresponding maximal short and long diameters of cross sections of the vessels acceptable for planimetric analysis, which was compiled by the authors, is recommended for these purposes. If, when the short diameter has been measured, the value obtained for the long diameter does not exceed that in the table, that vessel is suitable for planimetry, but if the ratio is below 0.707, it is not taken into consideration.

It must be remembered that the density of distribution of the vessel differs in different parts of the heart. A stereological analysis of five hearts taken from healthy subjects dying from accidents, demonstrates this heterogeneity. It was found (Table 2) that the density of the intramyocardial vessels in the wall of the left ventricle differs. For instance, in the middle part it is higher, but at the base of the ventricle and its apex it is lower. Moreover, these differences are statistically significant with 95% confidence limits. The dynamics of changes in the indices from the anterior part of the wall of the left ventricle to the posterior, through the lateral, can be studied separately in the upper, middle, and lower regions. It can be deduced from these findings that the density of distribution of the vessels in corresponding parts of the lateral and posterior regions of the wall of the left ventricle is greater than 0.05. Nevertheless, the density of distribution of the vessels and the corresponding parts of the anterior wall of the left ventricle differs from its values in the posterior and lateral wall.

The number of regions of the wall of the left ventricle which must be studied stereometrically must not be less than 6: three regions in the anterior wall and three in the postero-lateral wall. On the whole, the density of distribution of vessels in the heart can be judged on the basis of these results only by finding intergroup statistics for all six regions of the organ enumerated. In the stereometric study of the ventricle under normal conditions or in any pathological state, the method of random selection of samples can therefore be used only for each of the six individual regions separately.

Bulk stereometric analysis of the arterial bed of the heart based on the results of planimetric investigation of histological preparations must therefore be carried out on sections in three mutually perpendicular planes through the myocardium, followed by summation of these results. The basic mathematics of the method shows that the conclusion regarding the bulk density of distribution of the intramyocardial arteries purely from the results of planimetry of a section in one plane will always give rise to a systematic error, consisting of an underestimation of the bulk density of the components.

Planimetric analysis of the arterial bed of the myocardium can be used to obtain stereometric constants for different regions of the heart under normal and pathological conditions.

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QUANTITATIVE EVALUATION OF ERYTHEMA OF THE SKIN

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Special colorimetric scales for estimating erythema of the skin have been developed on the basis of general views regarding the objective nature of color perception and of colorimetric tables in a color atlas. These scales can be used to estimate common or allergic inflammation of the skin produced experimentally in the course of acute and chronic diseases. The system of the evaluation takes into account experience obtained with the assessment of inflammation of the skin on a point scale and the description of the erythema with the aid of the three physical parameters of color: its brightness (ρ,%), its wavelength (λ, nm), and its saturation (P, %).

KEY WORDS: *erythema; brightness; saturation; wavelength; color tone.*

The physiological basis of the theory of color [1, 2] enables a diagnostic feature of inflammation of the skin of such importance as erythema to be objectively assessed on the basis of E. B. Rabkin's "Color Atlas."

A technically simple method of objective evaluation of erythema of the skin in experimental animals with the aid of the colorimetric scale was described previously [3, 4]. This method is perfectly suitable for practical use in clinical and experimental research. The scale was based on a scale of different levels of increase in saturation of a pure red color tone. It covers the whole range of types of reddening of the skin likely to be observed in practice. For each chromatic object the basic characteristics are given: the wavelength λ, the brightness ρ, and the saturation P. However, considering that in experimental and clinical practice the colors to be assessed are not monochromatic but mixed colors (purple, reddish-orange) with strongly saturated and heterochromatic characteristics, three additional variants of the colorimetric scale are suggested.

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