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THE PRACTICAL USE OF STEREOLOGY IN CHARACTERISING CAPILLARY NETWORKS

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ABSTRACT

This study shows how to quantify capillary networks using older and recently developed stereological methods: 1) The surface area of capillaries may either be derived from vertical sections using cycloid grids or from isotropic, uniform random sections or estimated on sections with arbitrary orientation if the capillary network is isotropic. 2) The length of capillaries may either be estimated using isotropic, uniform random sections or using sections with arbitrary orientation if the capillary network is isotropic. 3) Diameter of capillaries may be calculated from length and surface area if the capillaries are much longer than their diameter. 4) The 0-dimensional number of capillaries may be estimated with normal "disectors" by using a topological definition of a capillary unit.

Key words: capillaries, diameter, length, number, stereology, surface area.

INTRODUCTION

Capillaries in organisms constitute a very dynamic and important system of tubes providing a large surface area for exchange of gases and solutes in tissues. The tubular structure of capillaries is ideal for efficient transportation and distribution of fluids throughout tissues. This structure and function of capillaries also determines their overall topology: capillaries are all connected and form a complicated network.
This report aims at showing methods by which to obtain reliable quantitative information on the structure of capillary networks using stereological methods. Glomerular capillaries were chosen as an example for this report because they constitute a well-defined and complicated capillary network that reacts quickly to different environmental conditions, e.g., diabetes.

MATERIALS AND METHODS

Animals

Five-week old normal rats and streptozotocin-induced diabetic rats treated with ~ 1 IU of long-acting, heat-treated Ultralente insulin (Novo-Nordisk, Bagsværd, Denmark) were observed for 50 days. Rats were given i.p. pentobarbital (50 mg/kg) and the right kidneys were perfusion-fixed retrogradely through the aorta for 5 min with a phosphate-buffered solution containing 1%

glutaraldehyde and 3% paraformaldehyde. The kidneys were coded in order to evaluate them without prior knowledge of experimental grouping.

Sectioning

The whole kidney was cut into 1.5 mm slices and every third slice was sampled systematically random. A uniform distribution of tissue blocks was punched out from the cortex of the kidney using a transparent plastic disc, perforated with equidistant spaced holes, and a biopsy needle with a diameter of 1.5 mm. Three blocks of tissue samples were stained *en bloc* with uranyl acetate, dehydrated and embedded in Epon. In order to obtain isotropic, uniform random (IUR) sections of the tissue samples the isector (Nyengaard and Gundersen, 1992) was used. Three consecutive sections with a thickness of 1.5 mm were cut from each Epon block and stained with toluidine blue.

Stereological background

The estimation of surface area density of capillaries requires that either the capillary network is isotropic or that the orientation of test lines in space is isotropic which can be ensured by generating IUR test lines by vertical sections (Baddeley et al., 1986) in conjunction with cycloid grids or by generating IUR test planes using the orientator (Mattfeldt et al., 1990) or the isector. All methods estimate the surface area density of glomerular capillaries, $S_V(cap/glo)$, by counting intersections per line length, I_L, between the test lines and the capillary surface area in a sampled glomerular profile:

$$S_V(cap/glo) = 2 \bullet I_L \quad (1)$$

The 1-dimensional structure length density of capillaries may be estimated using IUR test planes in case the capillary network does not appear as isotropic. One requirement for this estimation is that the tubule under study is much longer than its diameter which is nearly always the case for capillaries. As mentioned above, IUR test planes may be generated by the orientator or the isector. The estimation of length density of glomerular capillaries, $L_V(cap/glo)$, is performed by counting capillary profiles per area, Q_A, in a sampled glomerular profile:

$$L_V(cap/glo) = 2 \bullet Q_A \quad (2)$$

Calculation of diameter of capillaries, $\bar{d}(cap)$, from surface area density and length density is conditioned by the requirement that capillaries are cylindrical tubes. This requirement cannot be completely fulfilled in organisms, however, it is best facilitated if the capillaries are perfusion fixed. Under these circumstances the mean diameter of glomerular capillaries is calculated as:

$$\bar{d}(cap) = \frac{S_V(cap/glo)}{L_V(cap/glo) \bullet \pi} \quad (3)$$

The estimation of numerical density of capillaries requires a definition of what one capillary unit is: a topological definition of a structural unit in a capillary network facilitates this task by estimating the contribution to the Euler number (Euler-Poincare' characteristic) of the capillary network (Nyengaard and Marcussen, 1993). A physical disector (Sterio, 1984) is the means by which the topological events of the capillaries in the sampling section are evaluated. The Euler number can be estimated directly on sections with arbitrary edge effects (Bhanu Prasad et al., 1989) thus providing a method for estimating capillary number on all capillary systems irrespective of edge effects. The 0-dimensional Euler number is independent of isotropy of either disectors or of the capillary network: the Euler number may be defined as a counting measure estimated in arbitrarily oriented windows with arbitrary, regular edges which only requires the strong additivity property (Hadwiger, 1957, pp. 236-240) and uniform sampling. In a glomerulus the estimation of numerical density of capillaries becomes much easier when using complete

glomerular profiles because there are no edge effects to take into consideration and the disector ensures unbiased sampling of the topological events in the third dimension. If t denotes section thickness, $\Sigma\chi$(cap) denotes the Euler number of the capillary network and Σa(glo) denotes area of the sampled glomerular profile then the numerical density of glomerular capillaries, W_V(cap/glo), is estimated as:

$$W_V(cap/glo) = \frac{-\Sigma\chi(cap)}{2 \bullet t \bullet \Sigma a(glo)} \quad (4)$$

In order to obtain the above-mentioned structural densities per "organ", i.e. per glomerulus, the mean volume of the "organ" or glomerulus should be estimated. The main stereological tools for estimating number-weighted mean glomerular volume are the Cavalieri-method and the disector-method. In this report we use a combination of fractionator (Gundersen, 1986) sampling of cortical kidney tissue and disector sampling of glomeruli in order to estimate numerical density of glomeruli, N_V(glo/cor). The estimation of volume density of glomeruli in the cortex of the kidney, V_V(glo/cor), is accomplished by point-counting glomerular profiles and cortex of the kidney. The mean volume of glomeruli is calculated as:

$$\bar{v}_N(glo) = \frac{V_V(glo/cor)}{N_V(glo/cor)} \quad (5)$$

A uniform sample of glomerular volume is the set of all 2-D glomerular profiles on a random thin section. When further subsampling is performed, these profiles are considered as individual, countable profiles of which a constant number, 2, is sampled uniformly. Since a varying number of glomeruli, m, is hit by the sections, the result is a varying sampling fraction, of which the inverse, m/2, is used as the weight for calculating the average densities of surface area, length and number of capillaries.

Statistical analysis

Coefficient of error, CE_{ste}, was estimated as the SEM divided by the arithmetic mean using formula 9 given previously (Kroustrup and Gundersen, 1983). An unpaired, two-tailed t-test with a level of significance of 0.05 was used to test differences between groups.

RESULTS

Some of the results have previously been published in Nyengaard and Rasch (1993).

CE_{ste} of mean glomerular volume is 0.05; CE_{ste} of total number of capillaries per glomerulus is 0.13; CE_{ste} of total length of capillaries per glomerulus is 0.09; and CE_{ste} of total surface area of capillaries per glomerulus is 0.07.

The mean glomerular volume increases from 1.14±0.11 (mean ± SD) 10^6 μm^3 in the control rats to 1.67±0.11 10^6 μm^3 ($2p<0.05$) in the diabetic rats. The total number of capillaries per glomerulus is 215±29 in the controls and increases to 316±45 ($2p<0.05$) in the diabetics. The total length of capillaries expands from 12.5±2.2 mm in the controls to 19.4±3.0 mm ($2p<0.05$) in the diabetics. The total surface area of capillaries is 291±42 10^{-3} mm^2 in the controls and is increased to 469±70 10^{-3} mm^2 ($2p<0.05$) in the diabetics. The diameter of glomerular capillaries in control rats 7.50±0.82 μm does not differ from 7.72±0.51 μm in the diabetic rats ($2p>0.05$).

DISCUSSION

This report do not find any significant vasodilation of glomerular capillaries in diabetic rats, however, we find an increase in surface area and length which is mainly caused by generation of

new capillaries. This is quite remarkable because the formation of a new capillary is a complicated process and the results suggest that the number of glomerular capillaries is an important variable in glomerular hypertrophy.

It has not before been known whether the complicated glomerular capillary network dilated, lengthened or generated new capillaries following glomerular hypertrophy. Three-dimensional reconstruction of the complete capillary network was previously (before 1988) the only method to obtain information about capillary number whereas estimation of length and surface area by stereological techniques have been possible for many years. Using a topological definition of a capillary structural unit in combination with the disector-principle this problem is solved without making any assumptions about shape and size of the capillaries. Although somewhat more difficult, the capillary number estimator may also be used on other capillary systems with arbitrary edge effects. It is also important that the capillary number estimator is independent of isotropy of the capillaries or isotropy of the sections. It should, however, be mentioned that all the estimates are dependent on dimensional changes of the capillaries due to histological processing but the use of plastic in this report should minimise this problem.

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