MORPHOMETRIC INVESTIGATION OF THE KIDNEYS

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Variants of methods of morphometric and histometric investigation of the structures of the kidneys are described: measurement of the volume of cortical and medullary substance, of the pelvis and vessels, and of the number, linear dimensions, and volume of the glomeruli.

KEY WORDS: morphometric methods; cortical substance of the kidneys; renal glomeruli.

Quantitative investigation of changes in the structures of the kidney enables the adaptive and pathological changes in the parenchyma of the organ to be characterized more objectively [5]. An important aspect of this problem is the histometric study of changes in the state of the arterioles and capillaries of the glomeruli, the number of functioning and nonfunctioning glomeruli, their dimensions, total volume, and other morphological changes in the kidneys.

One of the variants of morphometric investigation of the kidneys used by the writers was as follows. After weighing of the kidney, a longitudinal incision was made through its center from the lateral surface toward the hilum. A glass plate was placed on the incised surface of the kidney and the outlines of the kidney and cortex were drawn on it (with dye or ink). These outlines were then transferred by means of tracing paper, followed by carbon paper, to thick paper (Fig. 1). The drawing of the kidney was then cut out along its outlines, the cortex was cut out separately, and the parts were weighed separately on analytical scales.

Knowing the absolute weight of the kidney without the capsule (P_k), the weight of paper corresponding to the section through the whole kidney (p_k), and that corresponding to the cortex (p_c), the proportion of the absolute weight of the kidney corresponding to the cortex (P_c) was determined (in grams):

$$P_c = P_k \cdot \frac{p_c}{p_k}.$$

The relative weight (q) of the kidney cortex was determined by weighing 1 cm^3 of cortical tissue excised by means of a special knife, consisting of two parallel razor blades fixed at a distance of 1 cm [1]. From the absolute weight of cortex and its relative weight (in g/cm^3) the volume of cortical substance of the kidney was determined:

$$V_c = \frac{P_c}{q} \text{ cm}^3.$$

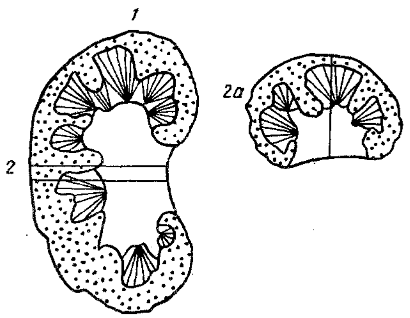

Fig. 1. Diagram of longitudinal and transverse sections through kidneys: outlines of kidney with cortex and medulla in midline longitudinal section (1); transverse block of kidney resected at level of vascular bundle (2); outlines of transverse section at level (2a).

The next stage of the investigation was the histometric study of the transverse section through the whole thickness of the kidney (at the level of the vascular bundle). For convenience of processing, two blocks were prepared (Fig. 1). The number of visible glomeruli was counted in 20 fields of vision in each section and the mean value for one field of vision determined. How-

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ever, to determine the true number of glomeruli in the kidney, data for the diameter of the glomerulus and thickness of the sections must be available.

The long and short diameters of the glomeruli were determined in two ways: 1) by means of an ocular micrometer (one division of which on the MBI-3 microscope, with a magnification of 56 times, corresponds to 14 μ); 2) with an ocular measuring grid for cytohistostereometric research [1], giving the mean number of points corresponding to one glomerulus, from which its mean area could be determined (S_{gl}). With a magnification of 56 times, each point of this grid corresponded to 4100 μ^2. The mean diameter of the glomerulus in this case was calculated bv the formula:

$$D=\sqrt{\frac{4}{\pi}\cdot S_{ge}}=1{,}1\cdot\sqrt{S_{ge}}.$$

To determine the visible number of glomeruli in 1 cm² of kidney tissue lying in the section (N_{vis}), the number of glomeruli (N_{fv}) counted in the field of vision (fv) must be multiplied by the number (n) of fields of vision studied under the magnification stated above:

$$n=\frac{1\ \text{cm}^2}{S_{fv}}=\frac{1\ \text{cm}^2}{1{,}2\ \text{mm}^2}=83\ \text{fv}.$$

$$\left(S_{fv}=\frac{\pi D^2 fv}{4}=\frac{3.14\cdot 1{,}25^2}{4}=1{,}2\text{mm}^2\right),$$

$$N_{vis}\ \text{in}1\ \text{cm}^2=N_{fv}\cdot 83.$$

The true numberof glomeruli in a section of kidney tissue 1 cm² in area is determined by the equation:

$$N_{true}\ \text{in}\ 1\ \text{cm}^2=\frac{N_{true}\cdot H}{D+H},$$

where H is the thickness of the section (in cm); D is the mean diameter of the glomerulus (in cm). The true number of glomeruli in 1 cm³ kidney tissue was calculated by the equation:

$$N_{true}\ \text{in}\ 1\ \text{cm}^3=N_{true}\ \text{in}\ 1\ \text{cm}^2\cdot\frac{1}{H},$$

where 1/H is the number of sections of that particular thickness forming 1 cm³ kidney tissue.

The number of glomeruli in the whole renal cortex was determined by the equation:

$$N=N_{true}\ \text{in}\ 1\text{cm}^3\cdot V_c,$$

where V_c is the volume of cortical substance.

The total number of glomeruli in the kidney was calculated separately for functioning and nonfunctioning (hyalinized and sclerosed).

The volume of one glomerulus (v) was determined by regarding it as an ellipsoid of rotation, the true dimensions of which could be determined if the difference between the long and short diameters of sections of the ellipsoid was small – $A/B \le 1.2$, where A is the long and B the short diameter of the sections – by means of the equation:

$$v=\frac{\pi}{6}\left(\sqrt{AB}\right)^3,$$

where $\sqrt{AB}$ represents the geometric mean value of the diameters (the geometric mean is equal to the square root of the product of the mean values of the long and short diameters). However, A and B are not the true diameters of the ellipsoid, but the diameters of its section, and their use in the calculations cannot reflect its true dimensions. To obtain an equation reflecting the true volume of the glomerulus of the kidney, with a correction for the plane of section, the formula for the volume of an ellipsoid must be converted to a formula for a sphere, which can easily be done by replacing the geometric mean value of the long and short diameters $\sqrt{AB}$ by D_s (the diameter of a sphere), in which case $v = (\pi/6)\cdot D_s^3$. Allowing for the relationship between the geometric mean for the long and short diameters of section of the ellipsoid (d) and the mean-square diameter of a sphere ($\bar{D}$) [4], and also the method of using inverse diameters [3], according to which $\bar{D} = (\pi/2)\cdot d$, the final equation for calculating the true volume of the renal glomeruli assumed the following form:

$$\upsilon = \frac{\pi}{6}\left(\sqrt{AB}\cdot\frac{\pi}{2}\right)^2 = \frac{\pi^4}{48}(\sqrt{AB})^3 \text{ or } \upsilon = \frac{\pi^4}{48}\cdot D_s^3$$

(the diameter being determined by means of the ocular measuring grid). To characterize the functional state of the kidney the total volume of all glomeruli was determined ($V_{tot} = v \cdot N_{true}$ in 1 cm^3).

The second method of determining these parameters which was tested also consisted of several stages. Volume analysis of the structures based on Delesse's classical method consists of dividing the kidney by horizontal parallel planes into a series of blocks not exceeding 0.5 cm in thickness. For this purpose the kidney is placed with its convex surface uppermost and the vascular bundle beneath. Each block was estimated with the aid of a modification of Glagolev's "fields" method [2], in which an enlarged variant (up to 10 × 5 cm)of the ocular grid was prepared on the film. The number of points corresponding to the whole area of section of the kidney block (n_k) and for the cortex separately (n_c) was counted. The results of investigation of all the kidney blocks were added together. The ratio between these numbers of points gives the specific volume of cortical substance (φ_c). The volume of cortical substance (V_c) of the kidney was equal to the volume of kidney (V_k), determined from the displacement of water, multiplied by the specific volume of cortical substance (φ_c). The volume of the medulla, pelvis, and blood vessels could be calculated in a similar way.

Histometry was used to determine the total number of points corresponding to all structures of the section of kidney tissue (k_k) and the number of points corresponding to the glomeruli (k_{gl}) separately, by means of the ocular measuring grid [1]. The ratio k_{gl}/k_k gives the specific volume of the glomerulus, from which the total volume of the glomeruli of the kidney could be obtained by the expression

$$V_{tot} = V_c \cdot \varphi_c.$$

Hence, the total number of glomeruli of the kidney (N) is given by

$$N = \frac{V_{tot}}{\varphi_c}.$$

It is thus desirable to use the following indices for morphometric investigations of the kidneys: 1) the weight of the kidney in grams; 2) the weight of paper corresponding to the outlines of the kidney; 3) the weight of paper corresponding to the section through the cortex; 4) the weight of cortical substance of the kidney (in grams); 5) the specific gravity of the cortex (in g/cm^3); 6) the volume of cortical substance (in cm^3); 7) the number of glomeruli in a standard field of vision; 8) the mean number of glomeruli in the fields of vision; 9) the mean long and short diameters of the glomeruli (in cm); 10) the mean area of the glomerulus in the section (in cm^2); 11) the diameter of a field of vision (in mm); 12) the area of a field of vision (in mm^2); 13) the number of fields of vision; 14) the visible number of glomeruli in 1 cm^2 of kidney tissue; 15) the true number of glomeruli in 1 cm^2 of kidney tissue; 16) the true number of glomeruli in 1 cm^3 of kidney tissue; 17) the thickness of the histological section (in cm); 18) the total number of glomeruli in 1 cm^3 of kidney tissue; 19) the volume of a glomerulus (in μ^3); 20) the total volume of all glomeruli of the kidney.

It was shown by these methods that the normal adult human kidney contains about 2.5 million glomeruli with a diameter of about 160 μ, and their volume is $8 \cdot 10^6\ \mu^3$. The total volume of the glomeruli of the kidney amounts to $20 \cdot 10^6\ \mu^3$.

Together with other methods of histological investigation of the kidneys, analysis of these quantitative parameters characterizing the state of the glomeruli makes it possible to estimate the dynamics of certain pathological processes. For example, the use of variants of morphometric investigation described above established that in different forms of hypertension the weight of the kidneys correlates in a definite manner with the volume and number of the glomeruli.

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