PLANIMETRIC GRIDS FOR MACROSCOPIC AND MICROSCOPIC STEREOLOGICAL INVESTIGATIONS

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A type of grid for planimetric investigations of macroscopic and microscopic objects with equidistant nodal points of "zero" thickness is suggested. The method of performing the stereological investigation is described. Formulas are suggested for determining the necessary number of counts of points in order to enable representative data to be obtained for the quota of a structural component in the specimen studied with 95% confidence limits. Formulas for calculating the bulk density of a structural formation in a macroscopic and microscopic object with calculation of statistical indices are given.

KEY WORDS: planimetric grid; stereology; structural components.

Among known methods of planimetric analysis of macroscopic and microscopic objects in morphology, the "fields" method has achieved wide popularity (see Avtandilov [3]). The principles of probability theory on which it is based enable the results to be checked and stereometric analysis to be carried out to an assigned level of significance when determining the quota of a structural component in the specimen studied. The actual technique involves the use of special planimetric grids with nodal points. Grids (or lattices), differing in the number of intersections of lines (points), the distance between them, and the method of their arrangement, have been described [1, 3-6].

The suggested variant of planimetric grid has equidistant points. The points of this grid form the apices of black triangles, forming the boundary separating the white and black areas of the grid, where the thickness is "zero." The triangles are arranged in the plane of the grid so that the distance between the apices is the same in all directions. The planimetric grid can be used at all levels of morphometric and stereometric analysis.

For macroscopic investigations of anatomical sections of organs the grid is prepared as follows. The specimen (Fig. 1a) is photographed on the necessary scale on FT-2 technical photographic film or its outlines are traced on washed x-ray film (transparent plastic). The size of the lattice must exceed the size of the object to be analyzed and, when placed on the section, it must cover it completely (Fig. 1b). The number of points and the distance between them on the lattice can have any values depending on the size of the organ. However, since the accuracy of the "fields" method depends on the number of points counted, the distance between them is best made as small as possible. Experience has shown that in macroscopic investigations the points should not be closer than 5 mm, for if the distance is less counting is more difficult.

In all cases of macroscopic stereometric investigations it is better to use planimetric grids in which the distance between the nodal points is 10 mm.

For microscopic stereometric and planimetric investigations another grid is used (Fig. 2), which has 60 nodal points. The method of preparation of these ocular grids was described previously [2]: The specimen

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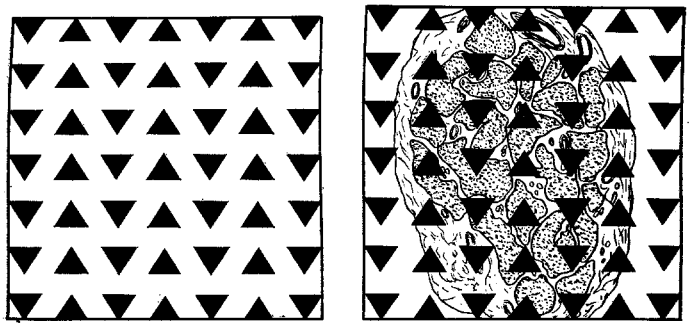

Fig. 1

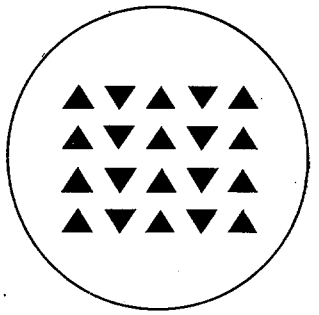

Fig. 2

Fig. 1. Planimetric grid for macroscopic stereological investigation: a) planimetric grid for macrostereometric investigation; b) grid placed on transverse section through head of dog's pancreas; points corresponding to parenchyma, stroma, vessels, and ducts are counted.

Fig. 2. Ocular insert and planimetric grid for microstereometric analysis of histological section.

is copied photographically so that its diameter does not exceed 8 mm. A positive is made on film and inserted into the ocular on the support for inserts.

In planimetric analysis by Glagolev's "fields" method the accuracy of the results depends on the number of points counted. For this reason, determination of the necessary number of counting points with which the results will be significant with the confidence limits adopted is of definite interest (in medico-biological research a 95% level of significance is usually regarded as adequate). For these purposes it is suggested that the equation (1) below be used, by means of which after the preliminary counts of a small group of points, the necessary and adequate number of counts can be determined. According to this equation, to obtain representative results N points per section must be counted:

$$N = \frac{400\,(100 - M)}{M}, \tag{1}$$

where M is the number of points corresponding to a particular structural component when the total number of points counted per section is 100. After preliminary estimation by Eq. (1), the necessary number of counts of the points is established and the investigation continued until N points have been chosen. A statistical analysis of the results is then carried out and the bulk density (V_V) of the structural components of the organ determined from the ratio between the number of points corresponding altogether to the structural component ($\overline{m}$) and to the whole section (N):

$$V_v = \frac{\overline{m}}{N}, \tag{2}$$

the standard deviation of the bulk density of the particular structural component is determined:

$$\sigma = \pm \sqrt{V_v\,(1 - V_v)}, \tag{3}$$

and the sampling error:

$$m = \frac{\sigma}{\sqrt{N}} \tag{4}$$

and the coefficient of variation:

$$C = \frac{\sigma}{\sqrt{N}} \tag{5}$$

are calculated.

If the organ is small in size and if the number of points corresponding to it is less than that required to obtain representative samples, the number of necessary points can be obtained by repeated random application of the grid to the same section of the organ or to several sections, followed by summation of all the results.

For example, if to obtain representative data no fewer than 2000 points have to be counted per macroscopic or microscopic section of an organ, and on the first application of the grid only 500 points corresponded to the particular component being studied, repeated random applications of the grid at different angles to the same section or to subsequent sections must be carried out at least four times. The results of the calculations are added separately for each structural component (vessels, glomeruli, and so on), and for the section as a whole. For instance, if the component studied corresponded to 200 of a total of 2000 points, it would be 0.1 (or 10%) of the whole volume of the organ.

The grids as described above have been tested in stereometric investigations of macroscopic and microscopic preparations and have given good results. They can be recommended for wide use in morphometric and stereometric investigations.

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A METHOD OF REPRODUCING VERTEBRAL ARTERY SYNDROME EXPERIMENTALLY

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A method of reproducing the vertebral artery syndrome in chronic experiments on dogs is suggested. Under general anesthesia two horseshoe-shaped electrodes are inserted into the vertebral canal at the selected level and fixed, and wires connected to them are brought out on the posterior surface of the neck. Rheographic and thermometric changes arising in response to stimulation by the electrodes and to measured electrical stimulation were investigated.

KEY WORDS: vertebral artery syndrome – experimental reproduction; horseshoe-shaped electrode.

The vertebral artery syndrome is frequently encountered in clinical neurology. A decisive role in its clinical manifestations is played by the action of pathologically changed surrounding structures on the artery and its sympathetic plexus. Its pathogenetic mechanisms can be studied by experimental reproduction of the syndrome.

No way of reproducing the vertebral artery syndrome could be found in the accessible literature. The only papers that were relevant dealt with the experimental study of the pathogenesis of its various symptoms in acute [1, 2, 5] and chronic [3] experiments. A method of reproducing vertebral artery syndrome has been developed by the writers in dogs. Under morphine–ether anesthesia an incision is made along the posterior border of the sternomastoid muscle. The tissues are separated and the neurovascular bundle retracted medially. The edges of the transverse processes of the vertebrae bounding the region of the vertebral artery

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