STUDY OF BRAIN MICROVESSELS BY AUTOMATIC IMAGE ANALYSIS

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The microvascular network of the brain is very sensitive to various pathological changes in that organ, and for that reason its state may reflect the course of development of such changes. Meanwhile the course and outcome of pathological processes may themselves depend on the state of the microvessels [3]. Accordingly it is important to obtain quantitative information on the structure of the microvascular network.

Since there is no satisfactory method of solving this laborious problem as yet [1] an attempt was made to use the method of automatic image analysis for this purpose [4-6].

EXPERIMENTAL METHOD

Histological sections through the dog's brain were used as test objects. Dogs were anesthetized with hexobarbital and perfused with ink (50 ml/kg body weight, under a pressure of 16 kPa) through both common carotid arteries. After fixation of the brain in 70° alcohol it was embedded in celloidin, and alternate sections were cut to a thickness of 200 and 20 μ. Cleared 200-μ sections were used to verify completeness of filling of the microcirculatory system with ink. In the 20-μ sections, stained with cresyl violet, the cytoarchitectonic formations were outlined, and the length of fragments of capillaries was determined manually by means of an ocular grid [1]. In neighboring thin unstained sections the microcirculatory system was studied by automatic image analysis by means of the textured analysis system (TAS, Ernst Leitz, West Germany).

The image of the preparation is projected in this instrument through an optical system on the photosensitive cell of a television camera and transformed into a television image. The maximal size of the measuring field in this case (objective 25, ocular 10) is 0.016 mm^2. Pictures of microvessels filled with ink are automatically diagnosed by the system on the basis of their optical density, which is much greater than the optical density of the tissue surrounding the vessels. The ordinary television image of the vessels (Fig. 1a) is transformed under these circumstances into a double image, which is "memorized" by the apparatus (Fig. 1b) and subjected to the necessary transformations by the LSI-11 computer (Digital Equipment Corp., USA; memory capacity 64 kilobytes) to eliminate artefacts and for separating, if necessary, the details of the image connected together, and so on. Methods of mathematical morphology such as erosion followed by dilatation [6] are also used for this purpose. The "cleaned" image thus obtained is analyzed according to a specially developed program enabling the total area of fragments of the vessels, their total length and number of fragments in the field of vision, the area and length of fragments of vessels of a given diameter, the area of the field in which the measurements are made, the specific length of the vessels (per unit volume of tissue), and the mean diameter of the vessels to be determined simultaneously.

These data for many fields (hundreds and thousands) are also stored in the computer's memory, and the aggregated statistically analyzed results are printed out in digital and graphic form, especially as histograms of distribution of the area and length of the vessels de-

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TABLE 1. Results of Measurements of Sections through Cortex of Cerebellar Vermis by Different Methods (M±m)

Method of measurement	Specific length of microvessels in layer of		Time required for measurements, h
	molecular	granular	
Automatic	575±21	724±12	1/2
Manual	597±19	702±26	60—75

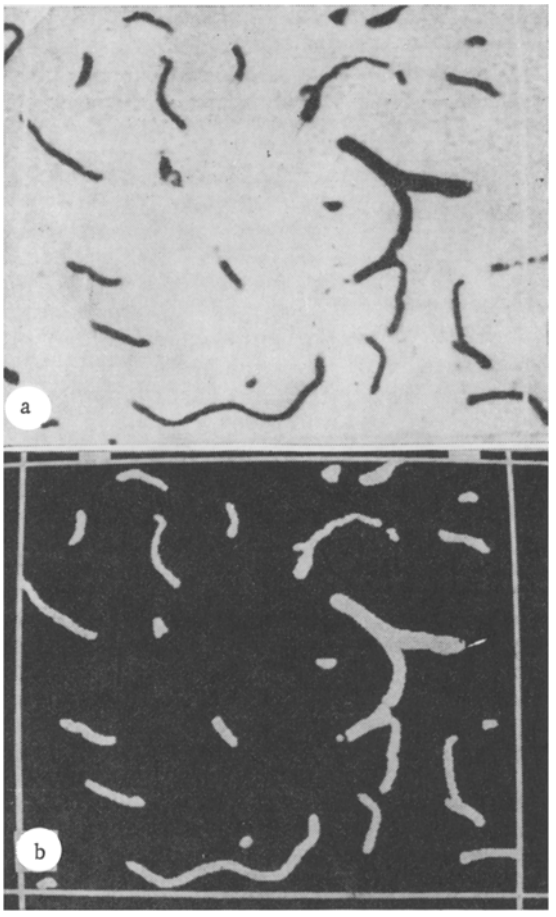

Fig. 1

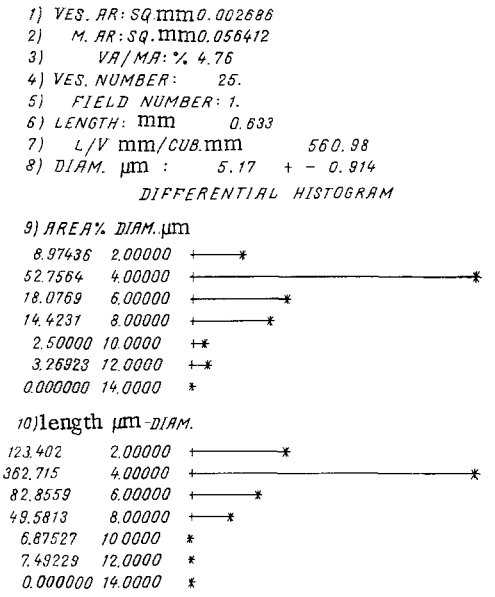

Fig. 2

Fig. 1. Microvessels of dog's brain: a) original television image; b) double image of microvessels led into instrument's memory. Injection with ink. Photographed from screen of monitor, objective 25, ocular 10.

Fig. 2. Digital print-out from PAS system giving results of determination of morphometric parameters of vessels illustrated in Fig. 1. 1) Total area of microvessels (in mm^2), 2) total area of fields in which measurements are made (in mm^2), 3) ratio of total area of vessels to area of fields measured (in %), 4) number of fragments of vessels, 5) number of fields, 6) total length of vessels (in mm), 7) specific length of vessels (length per unit volume of tissue) (in mm/mm^3), 8) mean diameter of vessels and error of means (in µ), 9, 10) histograms of distribution of area (in %) and length (in µ) of vessels depending on their diameter.

pending on their diameter (Fig. 2). The time required to perform the measurements does not exceed 5 sec.

EXPERIMENTAL RESULTS

Differences in the state of the microvascular network can be detected by using quantitative parameters which are undetectable by visual assessment and qualitative description. For instance, if regions of the microvascular system in the molecular and granular layers are compared visually, the density of the vascular network in each appears the same. It is equally difficult to detect visually differences in the density of distribution of vessels in the surface areas of the cortex and on the floor of the fissures. Detection of differences of this type is particularly important in the study of pathology, for different areas of the microvascular system respond differently to different disturbances, so that it is necessary to differentiate between anatomical differences and changes arising as a result of pathological processes.

Investigations of the angioarchitectonics of the cerebellum by the automatic image analysis method showed that the specific length of fragments of the microvessels in the cortex increases with increasing distance from the apices of the gyri toward the floor of the fissures, and the length of visible microvessels in the granular layer of the cortex is greater than in the molecular layer (Table 1). These data confirm conclusions drawn previously by manual measurements [2], and they indicate that the results obtained by the new method adequately correspond to those obtained by traditional methods of counting. To determine the degree of quantitative correlation between results obtained by the two methods, measurements were made in the same areas of sections. As Table 1 shows, the results obtained by manual and automatic methods coincide quantitatively with a sufficiently high degree of accuracy, for differences between them are not statistically significant.

The question of whether it is worthwhile using the new method and the associated expense may arise. To solve this problem, besides other factors it is essential to take into account the time required to carry out the measurements (Table 1). Systematization and statistical analysis of data obtained manually, with construction of histograms (which the apparatus does at once, while actually printing out the results of the measurements) requires additional expenditure of time. On average the efficiency of work with the automatic analyzer is 100-150 times higher. Moreover, it is unrealistic for practical purposes to consider counting the whole section manually, for this would require about 10 working days. In practice, three or four sample fissures are counted and the results are extrapolated to the whole section, and this naturally reduces the reliability of the final results. It is also important to note that in a given time the instrument provides up to 10 parameters immediately, whereas with manual counting only one or two parameters can be obtained.

The use of the automatic image analysis method to obtain morphometric parameters of the microvascular system of the brain thus leads to a considerable increase in the efficiency of the research and, no less important, in the objectivity of the measurements.

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