AN INSTRUMENTAL METHOD OF QUANTITATIVE ESTIMATION OF THE ACTIVITY OF OXIDATIVE ENZYMES

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An instrumental method is suggested for determining the activity of oxido-reductases using tetrazolium salts as the indicator. The method is based on the properties of formazan granules, which reflect incident light to a far greater degree than the accompanying noncrystalline reaction products deposited in the tissues, and also more than the pigmented cell organoids. The experimental estimation of NADP · H_2-diaphorase activity in the intermediate lobe of the pituitary of control and experimental groups of rats demonstrates higher reproducibility of the results obtained by the proposed method than by visual assessment.

To determine the activity of dehydrogenases, diaphorases, and monoamine oxidases in cytochemistry and histochemistry, the intensity of deposition of granules of reduced tetrazolium salts is customarily estimated visually [6]. Both this method, and methods of cytophotometry in transmitted light sometimes used [1, 2, 8], have important disadvantages. For many different reasons (the wide range of dispersion of the granules, their superposition and fusion into large conglomerations, the absence of a standard shape, and so on) it is virtually impossible to count the number of granules visually. Among the disadvantages of cytophotometry in transmitted light may be mentioned the difficulty of quantitative estimation of enzyme activity in specimens which have also been stained to demonstrate cell organoids.

The object of this investigation was to develop an instrumental method of quantitative estimation of oxidative enzyme activity.

The proposed method gives much better differentiation of the formazan granules against the background of stained cell organoids, and it replaces the visual estimation of intensity of deposition of the granules by photometry. This is done by illuminating the histochemical specimen under dark field conditions and recording the light flux scattered back into the microscope objective from a microarea illuminated with the aid of a special instrument [4]. The method is based on the established property of formazan granules of scattering light to a much greater degree than stained cell organoids and than the noncrystalline products of histochemical reactions deposited in the tissue and difficult to remove [5].

Histochemical reactions for dehydrogenase, diaphorase, and oxidase using tetrazolium salts (nitro-BT, tetranitro-BT) are carried out in the usual way. For quantitative assessment of the background, in parallel sections of the histological material for testing, a substrate-free reaction is carried out (standard preparation).

The area of the section for investigation is chosen under the microscope in transmitted light. Using mixed illumination in transmitted light and under dark field conditions the diameter of a vertical incident beam of light is reduced to that of the microarea on which the granules must be counted. Later, when the

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TABLE 1. Comparative Assessment of Intensity of Histochemical Reactions by Visual and Instrumental Methods

	No. of observations	Results of measurements			Significance of difference between control and exptl. results
		background	control	expt.	
Visual assessment					
A	10	0	1,9±0,3	0,7±0,3	$t_{0,05}<t<t_{0,01}$
B	10	0	2,7±0,2	1,2±0,2	$t>t_{0,01}$
C	10	0	2,4±0,2	2,2±0,4	$t<t_{0,05}$
Instrumental measurement	10	0,22±0,01 μA	0,57±0,02 μA	0,45±0,01 μA	$t>t_{0,001}$

specimen is illuminated under dark field conditions,only the intensity of the light flux from the illuminated area is measured by converting the light by means of a photoelectric cell into an electric signal which can be recorded on a galvanometer.

The signal determining the intensity of illumination of the specimen and not induced by scatter of light of the granules is measured over identical areas of the standard preparation. The difference between the values of the electric signals from the experimental and standard specimens gives a measure of the intensity of illumination of the test area.

The suggested method was tested by comparing the results of assessment of NAD·H_2-diaphorase activity in the intermediate lobe of the pituitary obtained by parallel visual and instrumental measurements. Specimens from 10 intact male Wistar rats and 10 similar animals irradiated in a dose of 600 R 3 days before decapitation were studied (Table 1).

The visual assessment was carried out by 3 experienced histochemists using a 6-point system (activity of the standard preparation was taken as 0). The density of distribution of formazan granules, measured by the instrumental method, was reflected by the strength of the current in microamperes.

The results given in Table 1, demonstrating poor reproducibility of the results of visual assessment, reveal a high level of difference between the experimental and control values ($t > t_{0.001}$) for identical samples in the case of instrumental measurements.

A quantitative histo-cytophotometric method of instrumental evaluation of enzyme activity based on the property of formazan granules of reflecting much of the incident light has thus been developed. The method enables measurement to be made in specimens stained for cell organoids, the influence of accompanying artifacts that are difficult to remove to be allowed for quantitatively, and activity of enzymes to be investigated both over wide areas of tissue on the average or in single cells. The simplicity and accuracy (high reproducibility of the results) of the method, combined with the other advantages listed above, enable it to be recommended for wide use in histochemical investigations [3, 7].

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