DETECTION AND CYTOSPECTROPHOTOMETRIC EVALUATION OF ENZYME ACTIVITY IN THYROID SECTIONS

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The relationship between the optical density of thyroid sections from rats and guinea pigs, their thickness, and the incubation time, was investigated after staining for the enzymes lactate-, succinate-, NAD·H_2-, and NADP·H_2-tetrazolium reductases, acid phosphatase, and peroxidase. Analysis of the distribution curves of the optical density values showed that the requirements of photometry are best satisfied by sections 10 μ thick with a density of 0.2-0.6 unit. The conditions under which the quantity of dye in thyroid sections increases as a linear function are described.
KEY WORDS: enzyme activity; thyroid gland.

Although the cytospectrophotometric evaluation of the intensity of enzyme reactions in sections has been shown to be theoretically possible and it has been used in model experiments [2, 4, 9-12, 19, 20], its practical application has so far been limited only to a few studies of animal tissues: the epithelium of the renal tubules [5], stratified squamous epithelium [13], the aorta [1], and the spinal cord [3].

The test object used in this investigation was the thyroid gland, and the aim was to find methods of obtaining histological preparations satisfying the requirements of photometric analysis of the activity of the enzymes lactate-, succinate-, NAD·H_2-, and NADP·H_2-tetrazolium reductases, peroxidase, and acid phosphatase, whose role in thyroid function has received the closest study [14-17]. To study this problem the degree of proportionality was examined between the activity of these enzymes and the optical density of preparations of the rat and guinea pig thyroid gland, using sections of different thickness and different incubation times. The choice of these animals was determined by the histological structure of their thyroid gland, which in the young, sexually mature rats corresponds to a high level, and in guinea pigs to a comparatively low level, of functional activity. It was thus hoped to obtain histo-enzymologic parameters suitable for the study of the thyroid parenchyma in various states.

EXPERIMENTAL METHOD

Male noninbred albino rats weighing 150-160 g and guinea pigs weighing 350-400 g were decapitated. A lobe of the thyroid gland was placed on a moist strip of filter paper and frozen in liquid nitrogen, as well as in aviation gasoline, iso-octane, propane, and Freon-22, cooled with nitrogen to a gelatinous consistency, and then transfered to a cryostat. Sections cut to a thickness of 5, 10, and 15 μ at -15, -18, and -22°C were straightened with acetone, ether, and butyl alcohol in the chamber of the cryostat, and a parallel series was similar straightened by thawing them with heat from the hand applied to the under surface of the slide. Reactions for detecting the above-mentioned tetrazolium-reductases were carried out with nitroblue (NBT), tetranitroblue (TNBT), and para-nitroneotetrazolium (PNNT) in the presence or absence of

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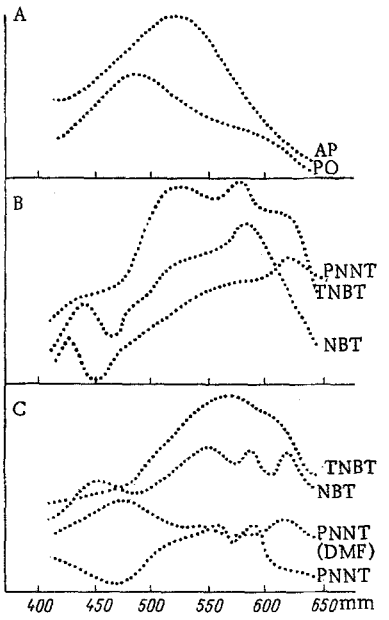

Fig. 1

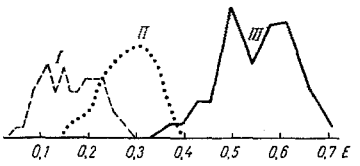

Fig. 2

Fig. 1. Absorption spectra of histo-enzymologic preparations of the thyroid gland: A) reaction for acid phosphatase (AP) and peroxidase (PO), maxima at 510-520 and 490 nm, respectively; B) reaction for NAD·H_2-dehydrogenase with different tetrazolium salts; C) the same for lactate dehydrogenase, [effect of dimethyl formamide (DMF) is shown].

Fig. 2. True curves of distribution of 250-300 measurements of optical density of TNBT-diformazan precipitate in cytoplasm of follicular epithelial cells: the curve for the 10-μ section has the most regular shape. I) 5μ, 5 min; II) 10μ, 10 min; III) 15μ, 15 min.

dimethyl formamide [6]. Acid phosphatase was determined by the azo-coupling reaction with naphthols AS–BI and AS–MX and with pararosaniline, and peroxidase by the reaction with diaminobenzidine tetrachloride [18]. The absorption spectra of the stained residues were recorded on the MUF-5 instrument within the range 400-650 nm. For the photometric analysis an instrument of original construction and working on the principle of the "plug method" was used. In each of 3 parallel sections placed on the same slide from 30-300 points of the cytoplasm were measured under 2 degrees of magnification: with objectives 40× and 70×, the area of the point examined was 2.99 and 0.785 nm^2, respectively. The numerical data were subjected to statistical analysis in the usual way.

EXPERIMENTAL RESULTS AND DISCUSSION

Examination of thyroid sections obtained by various combinations of methods of freezing the tissue, temperatures of cutting the sections, and methods of mounting them on the slides showed that the follicular structure and the localization and activity of the enzymes were best preserved by the use of cold gasoline or iso-octane, with a temperature of -18°C in the cryostat, and straightening by the action of heat. The sections were then transferred quickly into a refrigerator where, as they gradually dried, they adhered firmly to the slide and were not washed from it in the incubation solution.

The photometric results showed that the activity of both the hydrolytic enzymes and the oxidoreductases was indistinguishable after keeping for 6 h at 4°C from that in freshly prepared sections. This method, moreover, preserved the exact microanatomical relations between the structural components of the thyroid gland, so that a parallel histological study of its state can be made after fixation and staining of the sections with hematoxylin-eosin.

The character of the absorption curves was affected both by the dimethyl formamide and by the character of the enzyme revealed with the aid of the tetrazolium salt (Fig. 1B, C). The latter was evidently

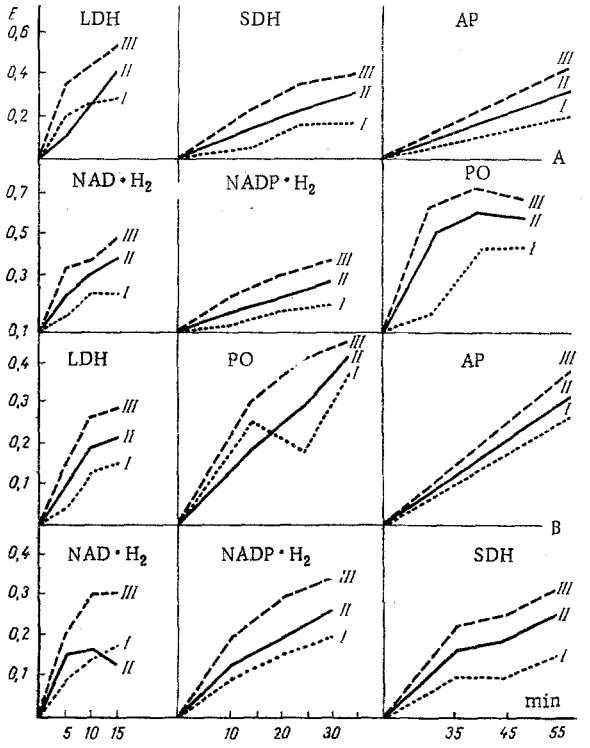

Fig. 3. Verification of validity of the Lambert–Bouguer–Beer law. Absorption (or optical density) as a function of thickness of sections and duration of incubation: A) thyroid glands of rats, B) of guinea pigs; I) 5 μ, II) 10 μ, III) 15 μ. The optimal combination of thickness and time can be chosen for each enzyme.

bound by a redox potential which differed in each reaction, as a result of which a varied quantity of the semi-reduced forms of formazan were formed. The most universal tetrazolium salt is TNBT (Chemapol), which in most reactions gives a precipitate with an absorption maximum within the range 520-580 nm. The spectral curves of the products of the azo-coupling reactions with naphthols AS–BI and of the diaminobenzidine reaction were more regular and did not create difficulties in the selection of the zone with maximal absorption (Fig. 1A). The frequency of the spectral characteristics confirms that photometric evaluation of the quantity of the colored precipitate in these reactions is possible.

During photometry of parallel sections placed on the same slide the most substantial differences were found in 5-μ preparations with low optical density. Adequate reproducibility of the results was observed on measuring the optical density of 10-μ and 15-μ sections. Considering the possibility of an incomplete reaction in the 15-μ sections [3] and also results obtained by the study of distribution curves of optical density values reflecting the level of enzyme activity (Fig. 2), it must be concluded that the requirements of photometric analysis were best met by sections 10 μ in thickness with an optical density of 0.2-0.6 unit. This conclusion was confirmed by the results of investigations carried out both on rats (Fig. 3A) and on guinea pigs (Fig. 3B). By using the curves thus obtained it is possible to choose for each thyroid enzyme the time range within which a linear relationship is observed in 10-μ sections between the increase in optical density and the duration of incubation.

The optimal size of the sample of measurements in a section was a group of 75 follicular epithelial cells. Under these circumstances it is possible to use formulas to facilitate manual processing of the data [8] and at the same time the mean values are highly reliable.

If the conditions stated above are observed, a linear relationship thus exists between the activity of the enzymes in thyroid sections and the quantity of the products of reactions by which they are revealed, as well as the between the thickness of the sections and the intensity of their staining. This state of affairs

satisfies the requirements of the Lambert–Bouguer–Beer law, so that the cytospectrophotometric analysis of histo-enzymologic preparations of the thyroid gland is possible.

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