QUANTITATIVE STEREOLOGICAL ESTIMATION OF ACCUMULATION AND LIBERATION OF INTRAFOLLICULAR COLLOID IN THE THYROID GLAND

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The biological importance of the ratio between the internal (reabsorbing colloid) surface of the follicles of the thyroid gland and the volume of colloid contained in the follicles as a characteristic of the functional state of the organ is examined. The use of the stereological method of random sections in conjunction with the linear method for the determination of this ratio is described.

KEY WORDS: thyroid gland; colloid reabsorption; stereological method.

The dynamics of the accumulation and liberation of colloid in the thyroid gland is determined by the functional state of the organ, one index of which is the ratio between the inner (reabsorbing colloid) surface of the follicles and the volume of intrafollicular colloid. Hypofunctional states are characterized by follicles of spherical shape, for which the ratio between the inner surface (S) and the volume of colloid (V) is minimal. In this state large follicles are seen as a rule. Conversely, hyperfunctional states are characterized by the small size of the follicles in the gland, and the ratio between the total reabsorption surface and the volume of colloid contained in the follicles is relatively high.

An increase in the reabsorption surface during marked intensification of colloid liberation can also take place on account of a direct increase in the area of the surface, on which numerous projections of the epithelial wall appear into the lumen of the follicles, giving them an irregular configuration; in this case the follicles maintain their previous volume for some time. Similar principles govern the changes in the ratio S/V in different species of animals known to differ in their level of thyroid activity. For instance, spherical follicles with a minimal S/V ratio are characteristic of the gland of the guinea pig; conversely, the very active thyroid gland of the hamster consists of numerous irregularly shaped follicles.

A coefficient characterizing the ratio S/V was used by Levenson [1] in the study of the rat thyroid gland; however, this worker used the concept of a "mean" follicle, for which the index was calculated. Besides the evident invalidity of the concept of a "mean" follicle used in this case (not the diameters of the follicles, but the linear dimensions of their random sections were measured), another defect of this approach is the assumption of the ideal shape of the follicles and also of the negligibly small quantity of stroma and vessels in the gland.

The writer uses a stereological method to determine the ratio S/V, which does not require any such assumption. Calculation of the index, for which the name stereological reabsorption index (SIR) is suggested, is based on the method of random sections in combination with the linear method [2].

The proportion of colloid by volume in the gland is proportional to the total length of the segments of lines intersecting the follicles (Fig. 1). The volume of colloid can be calculated by the equation $\Sigma V = h \cdot z/L$, where L is the total length of the secants (in μ), z the number of chords passing through colloid, and h their mean length. The number of intersections of the secants with the surfaces of section of the colloid, expressed as a ratio of the length of the secants, is $2z/L$. The total internal surface of the follicles is determined

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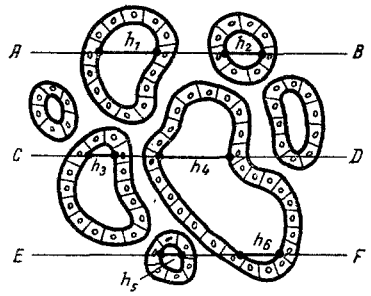

Fig. 1

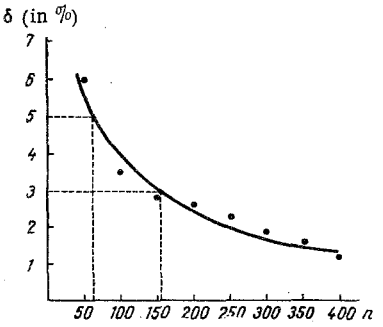

Fig. 2

Fig. 1. Determination of ratio S/V in thyroid gland by combined stereological method. AB, CD, EF) Random sections with total length L; h_1-h_6) segments of them belonging to colloid (chords). Points of intersection of colloid by lines are marked by dots.

Fig. 2. Relative error (δ) as a function of number of measurements (n) during determination of SIR. Dots represent results of determination of δ for a fixed number of measurements. Continuous line – approximating curve; broken lines illustrate method of using graph when planning the number of measurements.

by the basic equation for the method of random sections: $\Sigma S = 2 \cdot 2Z/L$. (μ^2/μ^3). By dividing ΣS by ΣV the size of the specific reabsorption surface is obtained from the volume of colloid: $SIR = \Sigma S/\Sigma V = 2 \cdot 2Z \cdot L/L \cdot Z \cdot h$ $4/h$ (μ^2/μ^3). To determine SIR, it is therefore sufficient to measure the mean length of the chords.

In practice the suggested method is carried out as follows: straight lines are drawn by means of an ocular micrometer or on a projection system in different arbitrary directions through the section of the thyroid gland, and in that way the length of the chords, i.e., parts of the scale (lines) belonging to colloid is determined. The mean length of the chords is then calculated and the result obtained expressed in microns. By substitution in the equation, the value of SIR can be determined and, as will be clear from the equation, it is directly dependent on the activity of the gland.

To determine the necessary number of measurements, it is best to plot a special graph of the relative error as a function of the number of measurements. By the use of such a graph the required number of measurements can be determined on the basis of a preassigned value of the error (Fig. 2). It will be clear from the example given that with 100 measurements the relative error does not exceed 5%. If a stricter level, for example 3%, is required, about 160 measurements will have to be made.

Model tests of the usefulness of the index under experimental conditions showed that when thyroid function was inhibited in rats by administration of exogenous thyroxine in a dose of 10 μg for 3 weeks, the value of SIR fell (on the average from 0.143 to 0.072 μ^2/μ^3).

To study the histophysiology of the thyroid gland, the best method is evidently to use the stereological index not by itself, but in combination with other morphometric estimates of the functional state of the organ. Correlation between the processes of reabsorption and accumulation of colloid, calculated by the above method, with the biochemical and histochemical criteria connected with these processes and, in particular, with the activity of certain proteolytic enzymes, such as acid phosphatase and leucineamino peptidase, is of definite interest.

LITERATURE CITED

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