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Size Distribution Pattern of Thyroid Follicles and Langerhans Islets Correlated with Their Hormone-secreting Activity

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The follicles of thyroid gland and the Langerhans islets of pancreas, as defined as spheres, are the basic endocrine units which are composed of hormone-secreting cells. Not only the numerical density but also the distribution pattern of these units are considered to have close bearing with the endocrine activity. Reconstruction from serial sections discloses that their size distribution can be simulated by Weibull function to a certain degree. One is required, however, to correct some errors in performing stereological analysis of these biological objects. The basic endocrine units practically have a certain minimal size. We show the theoretical treatment where the function is subjected to parallel translation so that stereologically Nvo and other quantities may be correctly estimated under this condition.

1. Introduction

Thinking about the relationship between the structure and the function of the biological organ, we can recognize three patterns of structural organization, particularly in endocrine organ.

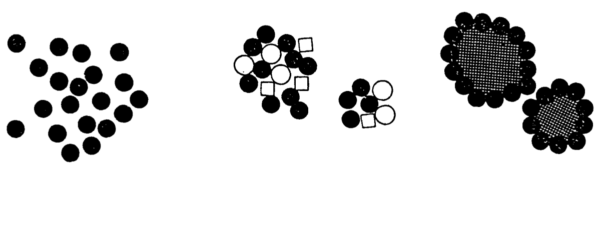


Fig. 1 Biological structures forming functional units

The first pattern, is shown in the left schema of figure 1. The individual cell itself makes a functional unit. We can find an example in parathyroid gland. In the second pattern, as shown in the middle schema of figure 1, the functional unit is the cluster of several different cells which are functionally independent from each other. This example is the Langerhans islet of the pancreas. In the right schema of figure 1, the third pattern is also the cell cluster, in which the cells are arranged specifically to store their product inside the cluster. This pattern is represented, for example, in the thyroid follicles. In the pancreas the each islets contain more than three differnt types of cells, and these are thought to regulate each other. So we can regard the pancreatic islet as a functional unit. The thyroid gland is composed of many follicles that are filled with a substance containing the thyroid hormones. In an active state the hormone contained in the substance is pumped out into the blood and the follicles are diminished in size. So the thyroid follicle can be also regarded as a functional unit. In this way, the size of the functional units is closely related to the functional state of the organ. So it is very important to correctly estimate the size distribution of functional unit as well as the numerical density.

2. The size distribution of the functional units in the biological objects

We can regard the thyroid follicles as being spherical. The distribution of the diameter of thyroid follicles is shown in figure 2 , which are appearing on the two-dimensional section as circles of various size. It has an asymmetrical distribution in which the peak has a tendency to the smaller size.

Pancreatic islets can be also regard as spheres. The diameter distribution of the pancreatic islets in three dimensional space, which is obtained from serial sections in figure 3.

Like this we can regard the size distribution of the functional unit as serial curves.

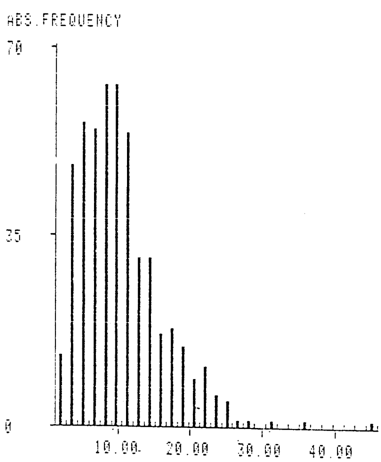


Fig. 2 Histogram of thyroid follicles on 2-dimension

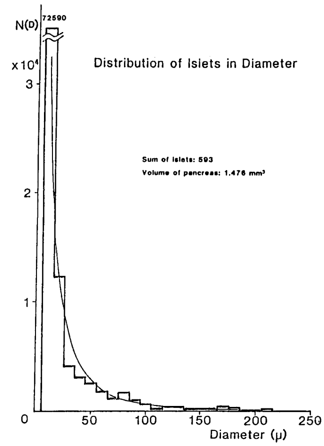


Fig. 3 Histogram of pancreatic islets on 3-dimension(Kaiho:1985)

3. Application of Weibull function to the size distribution

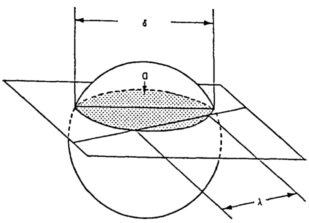
Indeed, these distribution in these three dimensional space may be expressed by a declining exponential curve, but it is better to use a Weibull function, because this function covers also the other cases showing a binomial-like distribution.

Weibull distribution

$$p(r) = \frac{m}{r_0} (r/r_0)^{m-1} e^{-(r/r_0)^m}$$

The Weibull function has two parameters; this is , shape parameter, m and scale parameter, r sub zero. By changing the shape parameter this function can express variable curves from a declining exponential pattern to that like a binomial curve. So this function is very useful for our analytical studies of patterns in biological objects.

Directly determinig the distribution pattern of functional units in three dimensional space from the reconstrucion method can be done, but it is awfully laborious. So it is much better to regard the size distribution as a Weibull function. In stereology we measure the area, a of circles on section, diameter of circles, delta, or chord length, lamda obtained along a test line to estimate the numerical density of spherical bodies in three dimension.



$$F(a) = \frac{1}{\pi} \int_{\sqrt{a/\pi}}^{\infty} \frac{N(r)}{\sqrt{r^2-(a/\pi)}} dr$$

$$F(\delta) = \delta \int_{\delta/2}^{\infty} \frac{N(r)}{\sqrt{4r^2-\delta^2}} dr$$

$$F(\lambda) = \frac{\pi}{2} \lambda \int_{\lambda/2}^{\infty} N(r)\, dr$$

Fig. 4 Parameters from a sphere

The distribution of a, delta or lamda is related to the probability function of r, radius of spheres contained in a unit volume by these fomulas. The numerical distribution per unit volume with regard to radius is given by the product of the Weibull function and the number per unit volume. The easiest solution of the equation is obtained, if we rely on the measurement of chord length, lamda.

4. Some errors in proceeding of the stereological method

There are some errors in applying the stereological method for biological materials. Firstly, there is an error due to the thickness of the section. This sort of error occurs when the center of a sphere happens to be located within the thickness of the specimen. In this condition, delta is estimated to be erroneously larger than the true value.

There is the second error related to the limit of the optical resolution about the distance between two points. Though theoretically a chord can be infinitely small, we can not discriminate a chord smaller than the optical resolution, as shown in left schema of figure 5.

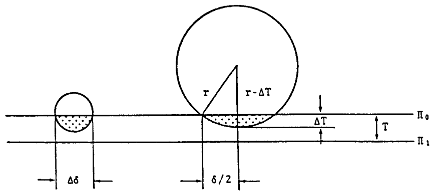


Fig. 5 Errors from optical resolution

There is another error related to the optical resolution about the thickness of cutted portion of sphere. The index from the cutted portion of sphere fails to be identified when this portion is too thin cut or too vaguely stained, like a delta T in right schema of figure 5.

5. Problem about the minimal value of the units

Most important error is this. In biological structures, the size of particles usually has a minimal value, that is, the lower limit.

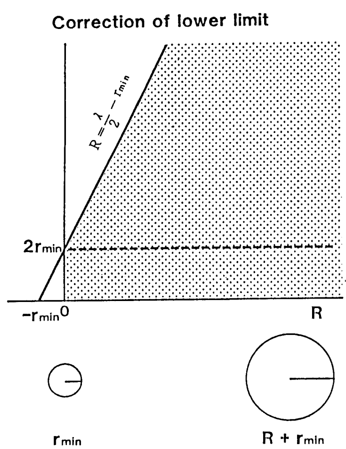


Fig. 6 Range of chord length of spheres

The abscissa shows the radius of spheres and the ordinate the chord length. This dotted area means the range in which the chord length may vary. Because the spheres in the range of smaller size is much more numerous than the other one, many shorter chord may be excessively estimated without setting the lower limit. So we must do the theoretical treatment for the correction of this error where the Weibull function is subjected to parallel translation, so that the number per unit volume, shape and scale parameters may be correctly estimated under this condition.

We can estimate the parameter of the Weibull function calculating the moments of lamda. Because of the presence of a certain minimal size of spheres, we must consider two formulas applied separately the range of index, lamda, under and above the minimal value. Using the monents of the lamda, we can determine the two parameters (m, r sub zero) of the Weibull function and numerical density (Nvo).

$$In(\lambda) = \int_0^\infty \lambda^n F(\lambda)\, d\lambda$$

$$= \int_0^{2 r_{min}} \int_0^\infty \frac{\pi}{2} \lambda^{n+1} N(R)\, dR\, d\lambda + \int_{2 r_{min}}^\infty \int_{\lambda/2 - r_{min}}^\infty \frac{\pi}{2} \lambda^{n+1} N(R)\, dR\, d\lambda$$

$$= \frac{\pi}{2} \int_0^\infty N(R)\, dR \int_0^{2(R + r_{min})} \lambda^{n+1}\, d\lambda$$

$$N(R) = Nvo \frac{m}{Ro} \left(R/_{Ro}\right)^{m-1} e^{-(R/Ro)^m}$$

$$m \quad Ro \quad Nvo \quad \Leftarrow \quad Io(\lambda) = \overline{N}_{\lambda o} \quad I_1(\lambda) = \Sigma\lambda \quad I_2(\lambda) = \Sigma\lambda^2$$

The size distribution of pancreatic islets which was determined in this way with correction of lower limit is shown in figure 7. Because the lower limit of the islet corresponds to the size of single endocrine cell, we could directly measure this size from a small number of serial sections. The lower limit is 4 micrometers in this case. And using this value as r-mini, minimal radius of the islet, we estimated the parameters of the Weibull function.

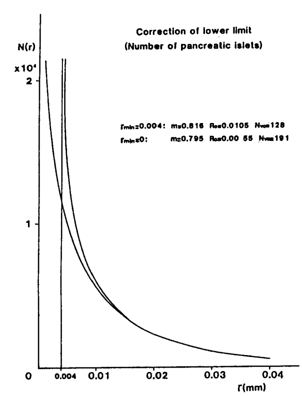


Fig. 7 Size distribution of pancreatic islets on three dimension

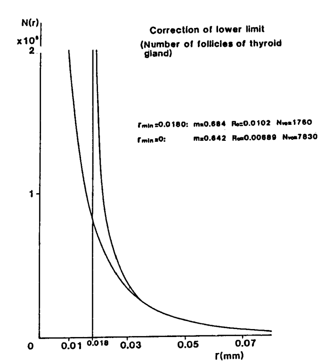


Fig. 8 Size distribution of thyroid follicles on three dimension

Comparing the results with and without setting the lower limit, we can find only small differnce in shape parameter: m, but there is a big difference concerning the number of islets per unit voluve. The number estimated with correction of the lower limit is much smaller.

6. Theoretical determination of the minimal value

For the pancreatic islets, we can easily measure the lower limit of size from serial sections. However, for the thyroid follicle, the minimal size is too large to be measured directly. In this case, we can theoretically determine the minimal value by calculating four moments of lamda.

$$In(\lambda) = \int_0^{\infty} \lambda^n F(\lambda)\, d\lambda = f(m,\ Ro,\ Nvo,\ r_{min})$$

$$Io(\lambda) = \bar{N}\lambda_O \quad I_1(\lambda) = \Sigma\lambda \quad I_2(\lambda) = \Sigma\lambda^2 \quad I_3(\lambda) = \Sigma\lambda^3$$

We could obtain the lower limit of folliclear radius to be 18 micrometers. Figure 8 shows two distribution curves of thyroid follicles with and without setting of the lower limit. There is a marked difference in the number of follicles per unit volume between the two condition.

7. Conclusion

In applying stereological methods to biological objects, we shoud be careful of some errors, especially those, occuring when we neglect the presence of the lower limit.

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