RELATIONSHIP BETWEEN VOLUMES OF NUCLEUS AND CYTOPLASM IN NEURONS OF THE AUTONOMIC GANGLIA OF THE CAT

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Each type of cell is characterized by definite morphological and biochemical relationships between the nucleus and cytoplasm, and in particular by the ratio between the volumes of the nucleus and cytoplasm. Analysis of this ratio can be used in the performance of functional and morphological investigations.

The object of the present investigation was to study the nerve cells of the superior cervical sympathetic ganglion of the cat and also of the adjacent parasympathetic ganglion nodosum.

EXPERIMENTAL METHOD

The ganglia were fixed in Brodskii's mixture (formalin–ethanol–acetic acid in proportions of 3 :1 :0.3) and then embedded in paraffin wax. Sections cut to a thickness of 5 μ were mounted in distilled water (as they dried water was periodically added beneath the cover slip), and the unstained sections were examined under the interference microscope with a 40 x 0.65 shearing objective. The large and small diameters of the nucleus and of the whole cell were measured by means of a type MOV-1-15 ocular micrometer mounted on the interference microscope. The volumes of the nucleus and of the whole cell were calculated by the equation for an ellipsoid of rotation:

$$V = \frac{\pi}{6} Dd^2,$$

where D is the large diameter and d the small.

The volume of the cytoplasm was found from the difference between the volumes of the whole cell and of the nucleus:

$$V_{cy} = V_{ce} - V_n.$$

EXPERIMENTAL RESULTS

Before the results obtained are analyzed, two problems must be considered: 1) to what extent are we entitled to judge the ratio between the volumes of parts of cells in sections passing through different stages of histological treatment (fixation, dehydration, embedding in paraffin wax, straightening out on the slide, and so on); and 2) how closely does the true shape of the investigated cells resemble the ellipsoid of rotation chosen as the standard of reference.

In connection with the first of these problems it must be noted that the concentration of solid matter in unfixed nerve cells, according to the findings of Hyden [9, 10], is very constant, despite the difference in types. Changes undoubtedly take place in the chemical composition of the cells as a result of all stages of histological treatment. However, these changes mainly affect water and part of the lipids, provided that fixation has been adequate and has led to quantitative precipitation of the proteins and nucleic acids. Evidently the unavoidable losses of water and lipids from the cells as a result of histological treatment are of the same order of magnitude for neurons of both sympathetic and parasympathetic ganglia. This was confirmed by the results of the determination of the content of solid matter (Table 1) by means of the method of microinterferometry, the principle of which is described elsewhere [3]. It is clear from Table 1 that the optical difference of passage (in standard conditions this bears a linear relationship to the concentration of solid matter in the cell) is practically identical in the neurons of both types of ganglia.

There is also reason to suppose that the changes in volume as a result of shrinking of the cells following the extraction of some of their contents will be the same on the average in the nerve cells of both sympathetic and parasympathetic ganglia.

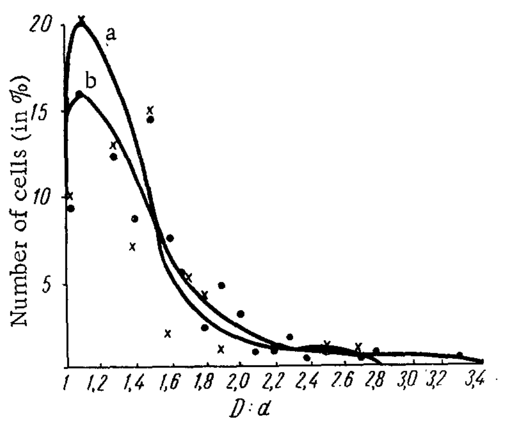
The second problem—the degree of applicability of the equation for the ellipsoid of rotation for calculating the volumes of the nerve cells—is of fundamental importance, especially in the conduct of practical quantitative cytochemical determinations by the method of cytospectrophotometry. This method only gives information concerning the concentration of a substance in the section, and the calculation of its absolute content in the cell requires knowledge of the volume of the cell or of the part of the cell to be investigated. Usually in cytophotometric investigations of neurons, the nerve cell is considered purely subjectively as the corresponding ellipsoid of rotation [1, 2, 8, 9], without quantitative calculations of any sort. Meanwhile methods have been introduced in recent years by means of which the three-dimensional shape of an object can be determined accurately from its two-dimensional sections [6]. For this purpose it is necessary to know the ratio between the large and small diameters of these two-dimensional projections of the cell body: in the case of a lenticular ellipsoid the curve of distribution of the individual sections by the size of their ratio D : d will be a curve with its convexity facing downward, and in the case of a triaxial ellipsoid the curve will be S-shaped (the first convexity facing downward, the second upward); finally, in the case of an ellipsoid of rotation the convexity of the curve will face upward.

TABLE 1. Content of Solid Matter in Nerve Cells of Autonomic Ganglia of the Cat

Type of ganglion	Number of cells	Optical difference of passage	
		in cytoplasm	in nucleus
Sympathetic	112	0.26 ± 0.01	0.27 ± 0.01
Parasympathetic	100	0.24 ± 0.01	0.30 ± 0.01

TABLE 2. Volume of Cytoplasm (V_{cy}) and of Nucleus (V_n) of Cells of Sympathetic and Parasympathetic Ganglia of a Cat

Type of ganglion	Volume of cytoplasm (in μ^3)	Volume of nucleus (in μ^3)	Ratio $V_{cy}:V_n$
Sympathetic	3.585 ± 165	371 ± 15	97 : 1
Parasympathetic . . .	8.270 ± 582	523 ± 31	158 : 1



Distribution of neurons of sympathetic and parasympathetic ganglia of a cat according to ratio between large and small diameters of the cells. D : d) ratio between large and small diameters of cells in section; a) cells of parasympathetic ganglion (individual intervals are denoted by crosses); b) cells of sympathetic ganglion (individual intervals are denoted by dots).

During the construction of these curves, two conditions must be observed: 1) the thickness of the sections must be small enough by comparison with the size of the cell body, and 2) the distribution of the bodies in space must be random. In the present case the first condition was satisfied well enough, for the thickness of the section was 5-7 μ and the diameter of the cells 20-50 μ; the second was satisfied only partially, for the arrangement of the ganglia was not completely random during preparation of the sections, but was to some degree oriented in a caudal-cranial direction.

Curves of this type were plotted for the cells of the sympathetic and parasympathetic ganglia (see Figure). They both had only a single rise, demonstrating the good approximation of the true shape of the cells of both ganglia to the ellipsoid of rotation.

TABLE 3. Logarithms of Volumes of Cytoplasm and Nucleus of Cells of the Sympathetic and Parasympathetic Ganglia of a Cat

Type of ganglion	Log V_{cy}	Log V_n	Log V_{cy} : log V_h
Sympathetic	3.45 ± 0.09	2.51 ± 0.06	1.38 ± 0.07
Parasympathetic	3.78 ± 0.09	2.64 ± 0.03	1.43 ± 0.05

We were now able with greater confidence to calculate the volume of the cells and of their nuclei from the equation for the ellipsoid of rotation, by measuring the linear dimensions and introducing the appropriate corrections for the magnification of the optical system of the interference microscope and ocular micrometer. The values obtained for the volumes of cytoplasm and nucleus of the investigated cells are given in Table 2.

It is clear from Table 2 that when the attempt was made to find a linear relationship between the values of V_{cy} and V_c no common features could be discovered between the cells of the sympathetic and parasympathetic ganglia. In this connection it is worth recalling the work of Huxley [7], who concludes that a linear relationship between the volumes of the individual parts of a cell is the exception. In most cases, in his opinion, the relationship is allometric, i.e., the linear relationship applies not to the investigated values but to their logarithms. Hence, in a general form, instead of the eq. $I = kX$, we have the eq. $I = X^k$, or $\log I = k \log X$. The linear relationship may thus be regarded as a special case of the allometric ratio in which $k = 1$.

The logarithms of the values of the volumes of the cytoplasm and nucleus were obtained for the investigated cells (Table 3). It follows from the results that the ratio between the logarithms of these values was practically identical for the two types of cells.

Hence, the nerve cells of the autonomic ganglia of the cat obey an allometric relationship as regards the volumes of their cytoplasm and nucleus. A similar conclusion was reached by authors [4, 5, 11] who investigated the cells of the spinal ganglia of the pig, chicken, and toad, the motor neurons of the anterior horns of the spinal cord of the chicken and toad, and the cells of the sympathetic ganglion of the chicken. They found that the value of the coefficient of allometry varies between limits of 0.43 and 2.30 depending on the type of cell. It was observed [5] that as a rule the value of k for the same type of cell increased with age, although the allometric type of ratio between the volumes of nucleus and cytoplasm was invariably maintained.

This type of nucleo-plasmic ratio is evidently a general rule, at least for nerve cells. No data are yet available which would indicate the principles underlying this rule. Probably the values of the volumes of nucleus and cytoplasm are directly related to the interconnected metabolic processes taking place in these parts of the cell, but further study is required of the quantitative characteristics of these processes.

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