

The Relative Concentration of Solids in the Nucleolus, Nucleus, and Cytoplasm of the Developing Nerve Cell of the Chick

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ABSTRACT

Growing and differentiating nerve cells of the fifth cranial ganglion of the chick embryo were studied by several means. During the period of 70 hours to 11 days of incubation (Hamburger-Hamilton stages 19 to 37) average cell mass increased more than 4.5 times while cells changed from relatively undifferentiated neuroblasts to morphologically characteristic nerve cells with long processes. By making simplifying assumptions about thickness of nucleus and nucleolus, relative to cytoplasmic thickness, it was possible to calculate solute concentration of nucleus and nucleolus relative to that of the cytoplasm from measurements of optical retardations through living cells. Differences in relative solute concentration were observed in nucleolus, cytoplasm, and nucleoplasm in the approximate ratio 1.2:1.0:0.8, respectively. The ratio remained essentially constant during the growth period examined despite the fact that the cell components grow at markedly different rates. This suggests that solid concentrations are physical characteristics of nucleus, nucleolus, and cytoplasm which are maintained even during rapid growth and differentiation.

By cytochemical means it was demonstrated that mass increase in the nucleus is not associated with increase in deoxyribonucleic acid. Both ribonucleic acid and protein are in greater concentration in nucleolus and cytoplasm than in the nucleoplasm. Electron microscopy shows interruptions in the nuclear envelope as well as an approximately even distribution of electron density in nucleus and cytoplasm. It is pointed out that consistent differences in solid concentration can exist on either side of the nuclear envelope even though it contains "pores." Implications of these data are discussed.

INTRODUCTION

The application of electron microscopy to the study of the nuclear envelope of cells has revealed that its structure is not a simple uninterrupted membrane (47). Since the first description of "pores" in amphibian oocyte nuclear envelopes (8), a large number of workers have noted that the nuclear envelope of cells usually contains circular interruptions surrounded by dense annuli. (For a review see reference 46.) There is little doubt that the "pores" actually do represent holes in the bilaminar nuclear envelope, and several workers

have noted that the "pores" are filled with a dense material (*e.g.*, 27, 32, 46). This dense material within the "pores" may be part of the envelope itself because it is present even within "pores" of nuclear envelope replicas found in the cytoplasm of some echinoderm oocytes (32).

Anderson (2), among others, has pointed out that in many cases nuclear intervention in cytoplasmic events must occur across the intact nuclear envelope. Since such intervention may be highly specific (13, 23), it follows that nuclear effectors which cross the nuclear envelope to the cytoplasm probably have an appreciable molecular weight. It is tempting to consider the "pores" as routes of nucleo-cytoplasmic interchange, especially for large molecules.

The present study has been designed to investi-

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gate from a different aspect the relationship between nucleus and cytoplasm. The chosen cell was the developing nerve cell in the fifth cranial ganglion of the chick embryo. Previous evidence on nerve cells (*e.g.* 17, 24, 26) has suggested that basophilic nuclear substances pass through the nuclear envelope into the cytoplasm during the course of growth and differentiation. Such a transfer could involve free diffusion through the envelope if pores are involved. The present study is concerned with the relationship of solids on either side of the nuclear envelope with the view to better understanding the role of the nuclear envelope as a possible barrier to diffusion of solids.

Materials and Methods

Chick embryos from stage 18 to 37 (22) were removed from their shells and membranes into sterile Spratt's medium (42). The mandibular ramus of the fifth cranial ganglion was dissected out in the medium and placed in a drop of the same solution on a slide. A coverslip 0.180 ± 0.004 mm. thick was placed on the preparation and allowed to flatten it out. The edges of the coverslip were then sealed with a paraffin-vaseline mixture and the slide used for analysis of individual cells. Each preparation was observed for a period not longer than 35 minutes at room temperature. Larger ganglia from later stages were briefly treated in calcium-free Spratt's medium to loosen the cohesiveness of cells before being returned to balanced Spratt's solution and placed on a slide for spreading.

The time between cracking of the egg and initial observation was about 5 minutes but with older embryos sometimes approached 10 minutes. No observable changes occur in the cells within 30 to 45 minutes at the levels of resolution used. To test the effects of isolation in Spratt's medium, ganglia were removed as usual and then placed in a medium consisting of Spratt's medium and embryo extract (1:1) for culturing in a plasma clot on a coverslip sealed to a depression slide. Ganglion cells thus cultured showed increase in size and vigorous outgrowth of nerve processes during several days of cultivation.

Estimation of the volumes of nerve cells, nuclei, and nucleoli was accomplished by taking photomicrographs on 35 mm. film, enlarging the negatives to a standard magnification, and tracing an outline of each cell part. Since optical path measurements indicated that the average thickness of cells in all preparations was about the same, areas of cell images were determined by planimetry and considered as approximate estimates of relative volumes. The similarity of cell thicknesses in all preparations probably is due to the fact that about equal, small amounts of fluid were placed on all slides before the coverslip was added.

Cytochemical determinations were carried out on

ganglia fixed in 1 per cent OsO_4 , buffered to pH 7.9 or in acetic-alcohol. Dehydration was followed by embedding in either *n*-butyl methacrylate or paraffin. For determination of ribonucleic acid, sections were stained in toluidine blue buffered to pH 4.0, with or without prior treatment with crystalline ribonuclease. The ribonuclease treatment was for 2 hours in 2 mg. enzyme per ml. of distilled water at 37°C. Proteins were localized with the Millon reaction, using trichloroacetic acid for demonstration of both histone and other types of proteins (39). The method creates color at the sites of non-diffusible tyrosine and possibly tryptophane. The mercuric bromphenol blue stain for proteins (29) was also employed.

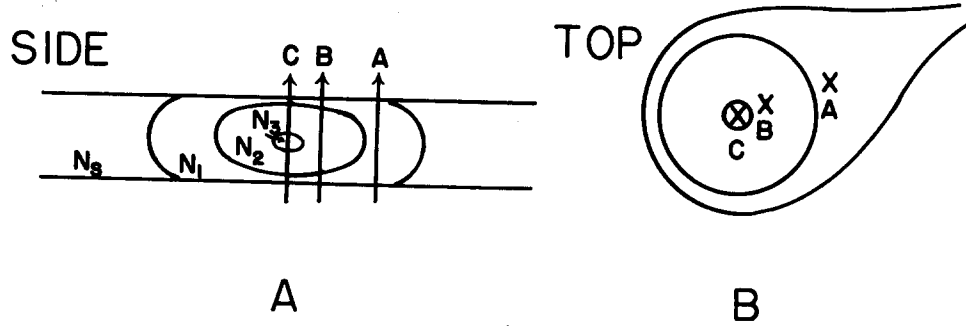
Quantitative determinations of deoxyribonucleic acid (DNA) per nucleus were made with Feulgen-stained nuclei. Total absorptions of whole nuclei were recorded with an RCA 1P21 photomultiplier tube. Light of 540 microns wave length was isolated with a monochromator. Mounting medium and nuclei had refractive indices of 1.542. The accuracy obtained with a single wave length was sufficient for the purposes of this investigation.

For electron microscopy, the ganglia were dissected out in egg fluids normally surrounding the embryo and fixed for 1 hour at 4°C. in 1 per cent OsO_4 , buffered to pH 7.9. Embedding was done in araldite (21) and thin sections observed in a Bendix-Akashi TRS-50 instrument with an objective aperture of 50 microns.

The introduction of the interference microscope to biological problems has made it possible to determine optical retardations of living cells (4, 15). Unfortunately, to measure optical retardations of cellular inclusions such as nuclei, it is necessary to know the contributing optical retardation of the cytoplasm above and below it in the optical axis (5). The case of the nucleolus is even more difficult because both nucleoplasm and cytoplasm lie above and below. For measurement of optical retardation or for calculation of concentration of cellular inclusions both the thickness of the inclusion as well as the retardation of other materials above and below must be known.

In this study it was decided that relationships of concentrations of solids in nucleolus, nucleus, and cytoplasm were important even if the various concentrations were not known in an absolute sense.

The paths *A*, *B*, and *C* of Text-fig. 1 were the locations of three interferometric measurements made on each cell. Preliminary observations on cells of all stages were made by focusing up and down with a high powered lens of short focal depth. It was concluded that there always was only a thin layer of cytoplasm above and below the nucleus. Let optical retardation measurements at *A*, *B*, and *C* be termed ϕ_A , ϕ_B , and ϕ_C and let N_s , N_1 , N_2 , and N_3 (Text-fig. 1 *A*) be the average refractive indices of Spratt's medium, cytoplasm, nucleoplasm, and nucleolus respectively. Let the thickness



TEXT-FIG. 1. Schematic diagrams of side (A) and top (B) views of a partially flattened nerve cell. A, B, and C represent points of optical path measurements while N_s , N_1 , N_2 , and N_3 represent refractive indices of Spratt's medium, cytoplasm, nucleoplasm, and nucleolus respectively.

through paths A, B, and C be called thickness a , b , and c . Because of cell flattening, total $a = \text{total } b = \text{total } c$. Let thickness of the nucleus at path B and C be called k and thickness of the nucleolus at path C be called d .

By definition, the optical paths (ϕ) through the cytoplasm, nucleus, and nucleolus are

$$\phi_{\text{cytoplasm}} = (N_1 - N_s)a \quad [1]$$

$$\phi_{\text{nucleus}} = (N_2 - N_s)k \quad [2]$$

$$\phi_{\text{nucleolus}} = (N_3 - N_s)d \quad [3]$$

The total optical path, as measured through the three areas of the cell, is the summation of the optical paths of the structures in each area through which the light passes. Thus

$$\phi_A = (N_1 - N_s)a \quad [4]$$

$$\phi_B = (N_1 - N_s)(a - k) + (N_2 - N_s)k \quad [5]$$

$$\phi_C = (N_1 - N_s)(a - k) + (N_2 - N_s)(k - d) + (N_3 - N_s)d \quad [6]$$

or rearranged and substituting the identities of Equations [1], [2], and [3].

$$\phi_{\text{cytoplasm}} = \phi_A \quad [7]$$

$$\phi_{\text{nucleus}} = \phi_B - (N_1 - N_s)(a - k) \quad [8]$$

$$\phi_{\text{nucleolus}} = \phi_C - (N_1 - N_s)(a - k) - (N_2 - N_s)(k - d) \quad [9]$$

For determination of ϕ_{nucleus} let us assume that the thickness of the cytoplasm above and below the nucleus is $0.1 a$. In this case $a - k$ becomes $0.1 a$ and from Equations [4] and [8]

$$\phi_{\text{nucleus}} = \phi_B - 0.1\phi_A \quad [10]$$

In the determination of $\phi_{\text{nucleolus}}$ let us further assume that vertical thickness of the nucleus and nucleolus is equal to their shortest diameter in the optical plane. Then $k - d$ can be replaced by $1 - d/k$ in which d and k can be measured in any similar units. This expression is the fraction of the optical path of the total nucleoplasm, $\phi_B - 0.1\phi_A$, lying above and below the

nucleolus. Then $(N_2 - N_s)(k - d)$ becomes $(1 - d/k)(\phi_B - 0.1\phi_A)$ and

$$\phi_{\text{nucleolus}} = \phi_C - 0.1\phi_A - (1 - d/k)(\phi_B - 0.1\phi_A) \quad [11]$$

Concentration (C) is given by

$$C = \phi/\alpha t \quad [12]$$

in which α = specific refractive increment which is taken as constant for a given system,

t = thickness.

Since it is impossible to measure thickness accurately enough, let us arbitrarily consider cytoplasmic thickness in each cell to be 1. Our assumptions now allow expression of nuclear and nucleolar thickness relative to cytoplasmic thickness. We can thus achieve values for concentration of solids (C') in the nucleus and nucleolus relative to the concentration in the cytoplasm. We are not interested in absolute values and so let $\alpha = 1$. Thus

$$C' = \phi/\text{relative } t \quad [13]$$

Then from equations [7], [10], and [11]

$$C'_{\text{cytoplasm}} = \phi_A/1 = \phi_A \quad [14]$$

$$C'_{\text{nucleus}} = \frac{\phi_B - 0.1\phi_A}{0.9} \quad [15]$$

Now thickness of the nucleolus with respect to the cytoplasm is d/a , but since $k = 0.9 a$, it can also be expressed in measurable terms as $0.9 d/k$. Then

$$C'_{\text{nucleolus}} = \frac{k[\phi_C - 0.1\phi_A - (1 - d/k)(\phi_B - 0.1\phi_A)]}{0.9 d} \quad [16]$$

Measurements were made with a shearing type, AO-Baker interference microscope with a photomultiplier tube and adjustable diaphragm mounted at a distance over the ocular. A Wratten filter gave light with an intensity maximum at 530 millimicrons. The portion

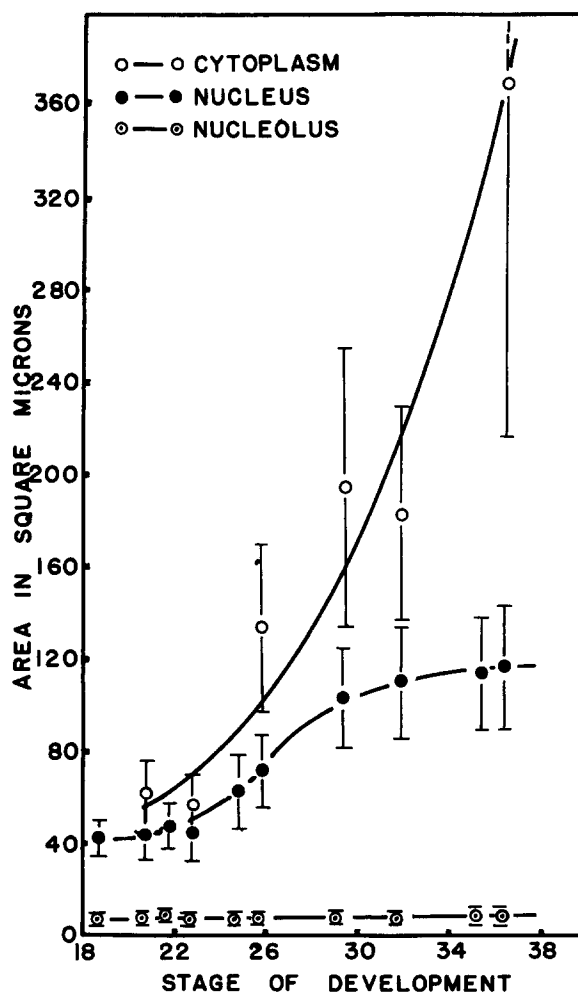
of the cell to be measured was focused at the phototube level and the diaphragm closed to delimit an area about 2 microns in diameter. Maximum extinctions were achieved by following a galvanometer needle as the goniometer was moved, each determination representing an average of at least two readings in the same area of the cell. Furthermore, care was taken to make sure that no readings were made on areas where the reference beam had penetrated an object ("ghost" image location). Preliminary measurements on finely drawn glass rod of measurable diameter and known index of refraction were made with the rods mounted in oil of known refractive index. It was found that the optical path through the center of the rod was precisely a function of rod diameter. Measured optical path at a given diameter was routinely found to be within ± 4

millimicrons (about 1/130 wave length) of the calculated optical path.

RESULTS

In stage 19 embryos, cells of the fifth cranial ganglion have one or two prominent nucleoli and a relatively small rim of cytoplasm (Fig. 1). Nerve fiber outgrowth can be detected only in a few large cells at stage 19. The proportion of neurons with fibers increases until at stage 30 practically all neurons have processes.

The cytoplasm of developing neurons is not homogeneous. In the early stages of fibrous outgrowth, a portion of the cytoplasm near the nucleus can often be seen (24) as an area of less re-



TEXT-FIG. 2. Growth of cytoplasm, nucleus, and nucleolus. A plot showing the relationship between mean measured area and age of the embryo for different cell structures. Vertical bars represent standard deviations about the means.

TABLE I
*Relative Concentration (C') of Solids in the Cell Components**

Stage	No. of observations	Nucleolus		Nucleus		Cytoplasm		Ratio
19-22	20	0.230 ± 0.078		0.154 ± 0.050		0.178 ± 0.064		1.3:0.9:1.0
23-25	13	0.207	0.062	0.134	0.042	0.154	0.046	1.3:0.9:1.0
26-28	16	0.227	0.057	0.165	0.062	0.197	0.072	1.2:0.8:1.0
29-31	13	0.250	0.102	0.154	0.071	0.194	0.092	1.3:0.8:1.0
32-34	12	0.208	0.076	0.153	0.052	0.184	0.066	1.1:0.8:1.0
35-37	20	0.257	0.068	0.174	0.062	0.217	0.072	1.2:0.8:1.0

* Means and standard deviations.

tardation in phase contrast. This area may be associated with the development of neurofibrillae as suggested by Fig. 3. In later stages a rather large area between the nucleus and nerve cell process shows a different optical path (Fig. 2) and RNA distribution (Fig. 4) than the more peripheral cytoplasm. The functional significance of this area of the cytoplasm is unknown but cytoplasmic heterogeneity undoubtedly contributes to the variability of optical retardation measurements made through it.

Neurons show a striking increase in size during stages studied. Text-fig. 2 indicates that the average area of the cytoplasm increases at a different rate than that of the nucleus, while nucleolar size remains rather constant. On the other hand, the average optical retardation through the cytoplasm, as indicated in the cytoplasm column of Table I, does not change significantly. From this it is evident that total mass of neurons and their nuclei is increasing. In addition, it should be noted in Text-fig. 2 that there is considerable variation in cell size and that this variation becomes more pronounced as development proceeds.

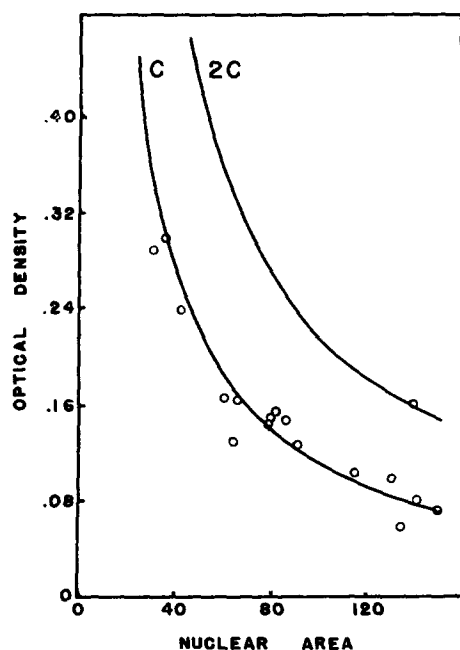
Average values of relative concentration are presented in Table I. Analysis of variance of values for each cell part at the different stages of development reveals no significant differences. We conclude that within the sensitivity of the method and over the developmental stages studied, there are no changes in nucleolar or nuclear concentration relative to that of the cytoplasm. This is also indicated by the rather constant ratios between cell parts in all groups. Thus, the data bear out the subjective observation that the nucleolus always consists of solids in the most concentrated form, the cytoplasm of solids of intermediate concentration, and nucleoplasm, including chromosomes, of solids in least concentration of all. The differences

in concentration are apparently rather constant during growth and specialization of the cell.

As already noted, the nucleus increases in size and mass during the stages studied. The question arose as to what portion of this growth could be attributed to chromosomal elements. Accordingly, a ganglion was fixed in acetic-alcohol to prevent loss of DNA (30) and stained with the Feulgen procedure (45) after determination of optimal hydrolysis time. Total Feulgen stain absorption of nuclei was estimated photometrically. A ganglion from a stage 37 embryo was chosen because at this age a whole spectrum of cell sizes can be found. Small nuclei of connective tissue elements form the lower end of a size continuum that extends to very large nuclei of the largest neurons of this study. Text-fig. 3 indicates that, in general, amount of dye per nucleus is constant from small connective tissue elements up to the largest neurons. This is taken as evidence that increase in nuclear mass is not associated with increasing chromosome number. Only one nucleus of the sample taken had twice the usual amount of stain.

If there is a difference in concentration of solids in cellular components, what types of solids are involved in these differences? Protein and RNA are generally considered as intimately involved in both function and structure of cytoplasm, nucleus, and nucleolus. Therefore, cytochemical staining methods for proteins and RNA were applied to sections which had been fixed and embedded in different manners. Results with all techniques were essentially similar.

With both the TCA-Millon reaction (Fig. 5) and the mercuric bromphenol blue reaction (Fig 6) for proteins, it can be seen that nucleoli and cytoplasm bind more dye per unit of volume than does the nucleoplasm. It should be noted that protein stains show a rather homogeneous distribution in



TEXT-FIG. 3. Relationship between nuclear size (area) and Feulgen stain per nucleus (optical density). Circles represent individual observations while solid lines represent theoretical values if the amount of DNA per nucleus is the same in all sizes of nuclei. The C line probably corresponds to the diploid amount of DNA while the 2 C line would represent tetraploid amounts.

the cytoplasm. With toluidine blue at pH 4.0 (Fig. 4) dye concentration is again greatest in the nucleoli, intermediate in the cytoplasm, and least in nucleoplasm. The area of the cytoplasm which shows a different phase appearance in the living cell can now be seen to be less basophilic as well. Virtually all basophilia is removable with ribonuclease. Along with previous work on other nerve cells (*e.g.* 26), these cytochemical data make it highly likely that differences in both protein and RNA contribute to the observed differences in solid concentration of the cytoplasm, nucleus, and nucleolus of the living cell.

Electron micrographs of developing ganglion cells indicate (Fig. 7) that the greater concentration of solids in the cytoplasm, as compared with nucleoplasm, is not due to greater concentration of formed elements of fine structure in the cytoplasm. Fig. 8 demonstrates that the nuclear envelopes have occasional interruptions of their bilaminar structure which might be interpreted as pores.

DISCUSSION

Two major technical variables contribute to the wide scattering of values obtained with the interference microscope. The first arises from the method of preparation of the ganglia. Ganglion cells are dissociated and spread out by placing a coverslip on the intact ganglion and letting the glass squash the cells apart. The distance between slide and coverslip is not known and will vary with the amount of medium used. If cells in different preparations are flattened to different degrees by the treatment, the absolute value of optical retardations and apparent concentration of cytoplasm will vary even though the calculated concentrations of the cell inclusions relative to the cytoplasm are correct.

Inspection of relative cytoplasmic concentrations of the various groups in Table I is informative because these values are also the measured optical retardations of the cytoplasm (Equation 14). One can see that there is only a slight tendency for values to become greater as cells become older and larger. This could mean that average cell thickness is increasing, but that solute concentration is decreasing in a reciprocal manner. It could also mean that solute concentration is remaining the same and that growing cells are becoming compressed to an increasing degree so that average thickness remains about the same. If cell thickness is increasing, despite the manner of preparation, and concentration is decreasing, then it follows that concentration of cytoplasm, nucleus, and nucleolus must decrease at differing rates. This derives from the fact that cytoplasm, nucleus, and nucleolus each grow in size at different rates while their concentrations relative to cytoplasmic concentrations maintain constant ratios. The simpler of the two hypotheses seems more likely. We feel that both absolute concentration and total thickness of preparations remain roughly constant throughout the stages studied.

The second and related technical cause of variability in the interferometric measurements obviously arises from the necessity of assumptions concerning the thickness of nucleus and nucleolus. Although observations using the fine adjustment of the microscope indicate that the nucleus always occupies almost the entire distance between slide and coverslip in the flattened cell, there obviously will be small variations in amount of cytoplasm above and below. In addition, the nucleoli are irregular in shape and no single measurement in the image plane will be a very accurate indication of their thickness in the optical axis.

Despite the considerable variability of the interferometric data, however, it is possible to say with certainty that the nucleolus has a generally greater concentration of solids than the cytoplasm, and that the cytoplasm, in turn, has a generally higher concentration than nucleoplasm. This is evident not only from the average of Table I but also from the fact that on a single cell basis the same relationship was found, with a few exceptions, for every cell measured. Furthermore, it seems reasonable to conclude from the data that the differences in concentration of the cell components remain roughly constant.

From the work of others it is apparent that many other cells also show the same differences in solid concentrations of their parts. For example, the nucleoplasm of living cells has a lower concentration of solids than the cytoplasm in echinoderm eggs (35), mast cells of the rat (38), spermatocytes of *Locusta* (40), chick fibroblasts (5), and eggs of the worm *Chaetopterus* from early until late preyolk stages (34).

Additional data from autoradiography (18) is pertinent if one assumes that fixation and embedding cause a roughly equal extraction of solids from, and a comparable shrinkage of nucleus and cytoplasm. Thus, cytoplasm of fixed, embedded, and sectioned cells has a higher mass per unit volume than the nucleus in chief cells of the gastric mucosa of the dog (14), epidermal cells of vaginal mucosa (28), and ventral horn cells of the cat (37).

The nucleolus too is known to have a higher density than the surrounding nucleoplasm from centrifugation experiments involving spinal ganglion cells of the rat (6). Interference microscopy of living chick fibroblasts (5) revealed that the nucleolus has a higher concentration of solids than either nucleoplasm or cytoplasm. A similar conclusion was reached with autoradiography of fixed nerve cells of the cat (36).

Such data lead one to the conclusion that the differences in concentration of solids between nucleolus, nucleus, and cytoplasm, as noted in this study, may be rather common in animal cells. There are exceptions, however, which indicate that the nucleus may sometimes have a higher concentration than cytoplasm. For example, dividing staminal hair cells of *Tradescantia* (16) have nuclei with a higher concentration than cytoplasm, as do *Amoebae* (1), and desquamated oral epithelial cells (35). With regard to these cases, it should be noted that dividing cells, cells with multitudinous

small nucleoli, and cells approaching a terminal pycnosis may represent special cases.

The concentration differences noted in this study probably reflect fundamental physical differences in the structural organization of the cell. For example, the nucleolus has never been shown with the electron microscope in this (34) or in any other material to have an investing membrane. Since the concentration of solids within the nucleolus is consistently higher than that of the surrounding nucleoplasm, we conclude that a substantial part of nucleolar solids are non-diffusible or structurally bound.

The difference in concentration between nucleus and cytoplasm, on the other hand, is not so readily comprehensible because there is a nuclear envelope between the two. If we assume that the "pores" in the nuclear envelope are really not pores but only areas of different structure and permeability characteristics, and if we further assume that the total nuclear membrane is not generally permeable to large molecules, then it is possible to understand differences in solid concentration on each side of the envelope in a straightforward way. If, however, the "pores" in the envelope are actually holes through which large molecules may diffuse, then once again we are forced to the conclusion that a substantial fraction of the solids of the cell are not capable of free diffusion but are structurally bound.

The most direct evidence for distinguishing between the two alternatives is that concerning the permeability of the nuclear envelope to large molecules. The use of fluorescent antibodies and other labeling techniques to determine intracellular sites of specific proteins has given some evidence that the nuclear envelope in the living cell is permeable to large molecules. Thus, Coons (11) found a protein antigen, injected into the whole animal, deposited in nuclei of several tissues. Gitlin *et al.* (20) found several blood plasma proteins in nuclei of different cell types of human tissue. Hemoglobin has been found in erythrocytic cell nuclei (9, 43). Radioactive ovalbumin, injected into rabbits, has been detected subsequently in the nuclear fraction of homogenates of liver and spleen (12). Isolated nuclei of various kinds also show the ability to pass large molecules through the nuclear envelope (3, 25, 33, 44) although data from isolated nuclei do little more than suggest the condition in the intact cell.

On the other hand, there are reports that large molecules do not pass through the nuclear envelope

in the intact cell. Callan (7) reports that such molecules as plasma albumin, gum acacia, and glycogen will not pass through the nuclear envelope of *Triturus* oocytes. A carefully characterized albumin labeled with aminofluorescein, has been used to study the distribution of the protein after injection into whole rats (41). Contrary to the findings of others the authors in this instance could find no evidence of the fluorescent dye in nuclei of any tissue studied. Likewise I¹³¹-labeled rabbit serum albumin, when injected into chickens, has been found by autoradiography only in the cytoplasm of immature plasma cells and not in nuclei (10). It seems impossible at present to make generalizations about the permeability of the nuclear envelope to large molecules.

If the envelope is permeable to large molecules, the data presented in this study indicate that an appreciable fraction of cellular solids are non-diffusible. The electron micrographic evidence for the cells of this study indicates that solids *other than* those of membranes or granules are involved. A picture emerges in which a three-dimensional framework of considerable mass, including the whole cell, is bathed in a fluid phase whose dissolved contents form a continuum from nucleus to cytoplasm *via* pores in the nuclear envelope.

If the nuclear envelope is not freely permeable to large molecules then it becomes necessary to postulate a special transport mechanism for large molecules. This might involve the "pores" in the envelope or the continuity between the endoplasmic reticulum and the perinuclear space (46). Another possibility would consist of an actual synthesis of specific mediators by the envelope itself (31) under influence of nuclear or cytoplasmic agents which themselves could not pass. If such were the case, it would become easier to understand the rather elaborate fine structure of the "pores" and their surrounding annuli (19, 32, 47, 48) and the intimate morphological association of chromosomes with the nuclear envelope (*e.g.* 36) which has classically been observed by cytologists in a variety of cells.

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EXPLANATION OF PLATES

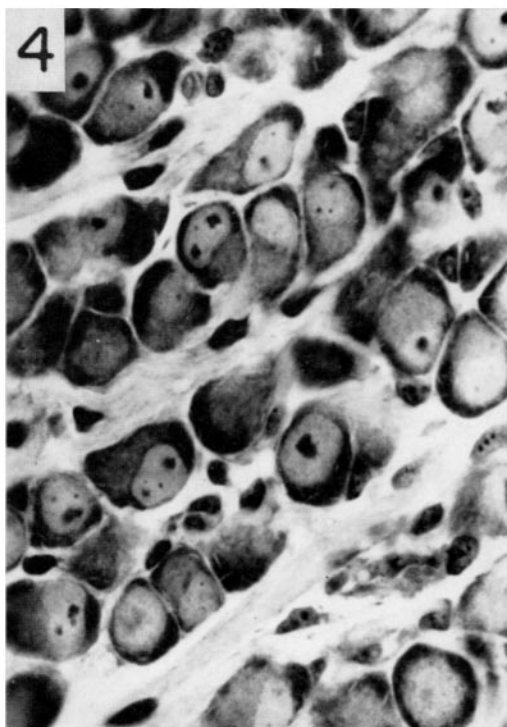
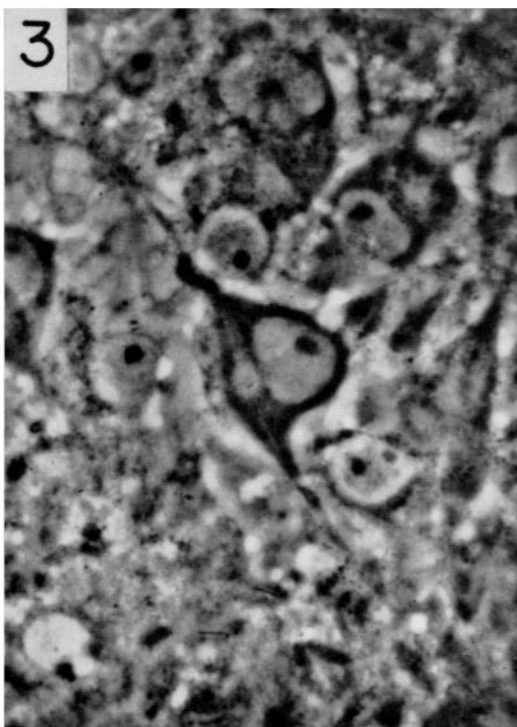
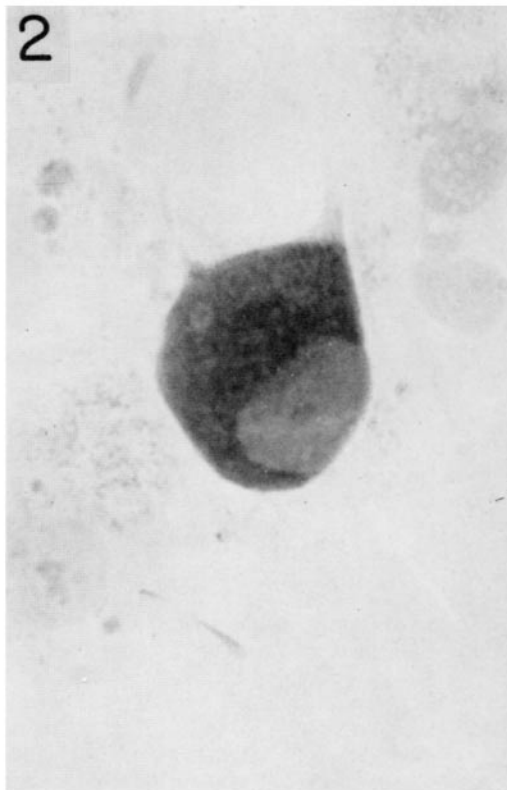
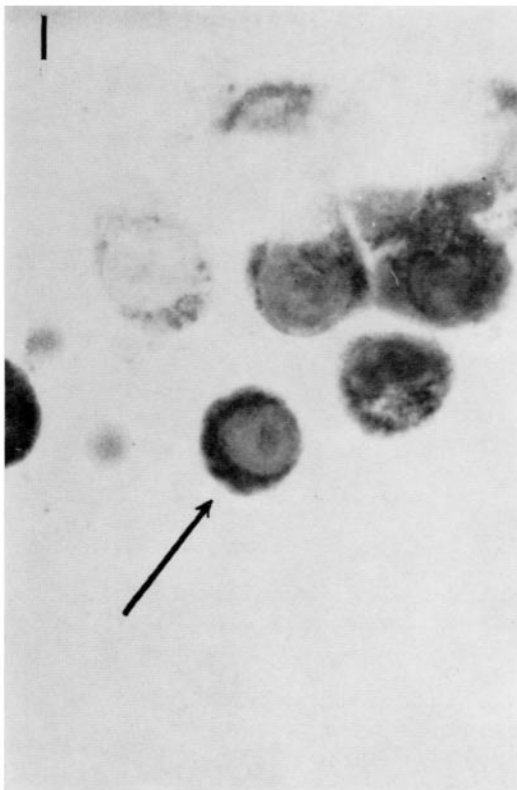
PLATE 73

FIG. 1. Living neuroblast from a stage 19 chick embryo (at the arrow). Interference microscope. $\times 1,200$.

FIG. 2. Living nerve cell from a stage 35 chick embryo showing a juxtannuclear area of slightly different optical retardation than the peripheral cytoplasm. There are two nerve cell processes. Interference microscope. $\times 1,200$.

FIG. 3. Living nerve cell from a stage 29 chick embryo showing a juxtannuclear area of different phase contrast in apparent continuity with fibrillar elements of the cytoplasm which are oriented toward the two cell processes. Phase contrast. $\times 880$.

FIG. 4. Ganglion from a stage 37 chick embryo, fixed in OsO_4 , buffered to pH 7.8, embedded in methacrylate, sectioned at 1 micron, and stained with toluidine blue at pH 4.0. All staining is removable by prior digestion with RNase. $\times 700$.



(Merriam and Koch: Concentration of solids in nerve cell)

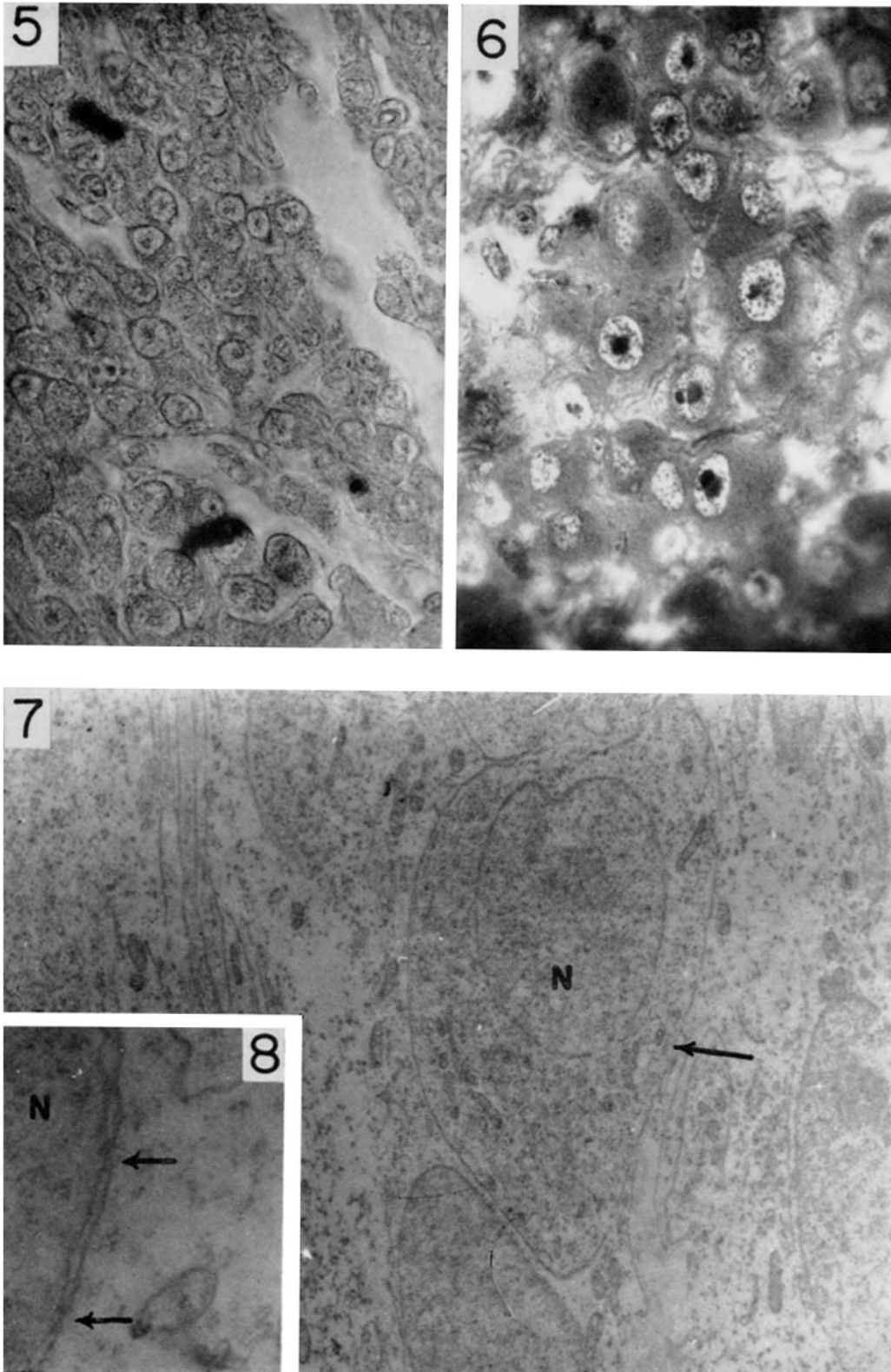
PLATE 74

FIG. 5. Ganglion from stage 37 chick embryo, fixed in acetic-alcohol, embedded in paraffin, sectioned at 8 microns, and stained with the TCA-Millon reaction for protein-bound tyrosine and tryptophane. $\times 800$.

FIG. 6. Ganglion from a stage 37 chick embryo fixed in acetic-alcohol, embedded in methacrylate, sectioned at 4 microns, and stained with mercuric bromphenol blue for protein. $\times 890$.

FIG. 7. Low power electron micrograph of a thin section through a nerve cell body from a stage 28 chick embryo (at the arrow). There is little difference between the average electron density or concentration of fine structure of the nucleus (*N*) and cytoplasm in this osmium-fixed material. About $\times 11,000$.

FIG. 8. Electron micrograph of the bilaminar nuclear envelope of a nerve cell from a stage 28 chick embryo. "Pores" can be seen at the arrows. About $\times 50,000$.



(Merriam and Koch: Concentration of solids in nerve cell)