THE NUCLEAR ENVELOPE

Its Structure and Relation to Cytoplasmic Membranes

By MICHAEL L. WATSON, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

Plates 71 to 75

(Received for publication, March 1, 1955)

Interest in the nature and function of the nuclear envelope of cells has flourished since its existence was first suspected by early light microscopists and implied by the micromanipulation studies of Kite (1) and Chambers and Fell (2). It has long been evident that if the interphase nucleus exerts fundamental control over such complex cytoplasmic activities as protein synthesis, this control must be transmitted through the nuclear membrane. Rather strong *a priori* arguments and a limited amount of experimental data support the view that such control must be mediated, at least in part, by large molecules which can pass between the nucleus and the cytoplasm (Anderson (3)). However, sparsity of unambiguous experimental data leaves the question in an unsatisfactory state (Hogeboom and Schneider (4)).

The application of the electron microscope to the study of fine structure of cells has shown rather clearly that the nucleus in interphase does possess an enveloping, membranous sheath. The early studies were made on nuclear membranes teased from the cell and examined directly on specimen grids with the surface of the membranes perpendicular to the optical axis of the microscope. Callan and Tomlin (5) in 1950 reported in an electron microscope study of oocytes of *Triturus* and *Xenopus* that the isolated nuclear envelope consisted of two layers, a continuous internal one and a porous, external layer. Bairati and Lehmann (6) and Harris and James (7) found a similar situation in *Amoeba proteus* except that they ascribed to the two layers a reversed position.

Later work utilized thin-sectioning techniques, which permitted examination of the nuclear envelope in sections normal to the nuclear surface as well as tangential to it. Hartmann (8), reporting on nerve cell nuclei, confirmed the finding of double membranes, although he failed to find pores. Bahr and Beermann (9) studying sectioned salivary glands and midgut of *Chironomus* found ring-shaped structures in sections nearly tangential to the nuclear surface, while in sections normal to it, they were able to demonstrate the presence of pores that penetrated both nuclear membranes. Gall (10) recently reported on isolated nuclear membranes from oocytes of *Triturus* dried by the critical-point method (11) and showed, in shadowed specimens, circular aggregations of spherical particles disposed as were the pores described in the previously mentioned studies. Ring-shaped structures were found in the nuclear envelopes of cells of the proximal convoluted tubules of mouse kidney by Rhodin (12) which closely resembled, in the published micrographs, the similar structures demonstrated by Bahr and Beermann (9) in tangential sections.

Studying oocytes of *Rana catesbiana*, Pollister, Gettner, and Ward (13) found thread-like structures which were continuous across the nuclear envelope from the nucleus to the cytoplasm at certain stages of egg development. These formations appeared to be associated with ring-shaped structures, revealed where the nuclear surface was inclined to the plane of the section.

In a recent note (14), the author described gaps or pores of a well defined nature in the nuclear envelopes of acinar cells of mouse pancreas. That these formations definitely penetrated both nuclear membranes could be established since they were seen in sections perpendicular to the nuclear surface. The pores appeared, in section, as gaps 200 to 400 A in width on either side of which the inner and outer nuclear membranes were joined. Recently, Palay and Palade (15) have described pores in sections normal to the surface of nerve cell nuclei.

More information about the structures mentioned can obviously contribute to a better understanding of the behavior and function of the resting cell nucleus. Accordingly, the present investigation was undertaken to determine, if possible: (1) the relation between pores and the ring-shaped structures previously mentioned; (2) generality of the pores as structures common to many if not all interphase cell nuclei; (3) the morphology of the material present within the pores and thus, perhaps, in transit through them; and (4) the relationship of the nuclear envelope to the membrane systems of the cytoplasm.

Materials and Methods

Normal, adult rats were anesthetized with ether, and a small bit of the desired tissue was removed and placed in a drop of fixative on a slab of dental wax. The tissue was quickly cut into blocks ¼ to 1 mm. on edge and transferred to cold fixative. Fixation, unless otherwise stated, was in 1 per cent OsO_4 buffered to pH 7.2 to 7.4 with veronal-acetate held at about 5°C. and lasted 2 to 4 hours. Washing for 5 minutes in the same solution without OsO_4 was followed by dehydration in graded alcohols extending over about 3 hours. The tissue was then passed through 1:1 butyl methacrylate and absolute alcohol to the monomer plus catalyst in which it remained for about 3 hours (3 changes) before polymerizing at 45°C. for 24 hours. Most of the sections used exhibited silver to silver-gold interference colors and were mounted on carbon films (16, 17).

An RCA type EMU-1 microscope was used equipped with a 10 mil condenser aperture, a 1 mil platinum objective aperture, a new standard compensated objective pole-piece, and an intermediate lens. No effort was made to maintain low beam intensities. Micrographs were made either with Eastman medium lantern slides developed 5 to 7 minutes in D-23 or with Eastman Type V-0 spectroscopic plates developed 4 minutes in D-19 at 68°C. The latter plates were found more satisfactory because of their higher contrast and possibly finer grain.

The low contrast inherent in some of the structures prompted attempts to examine sections which were unsupported by any substrate film. Collodion films were prepared by dipping glass microscope slides in 0.3 per cent collodion in butyl acetate. As the solvent evaporated, holes could be formed in the film by moisture condensed from the breath of the operator, their size and number being controlled by the rate and amount of condensation (18). Carbon was evaporated on grids coated with the porous collodion which was subsequently dissolved. A carbon film resulted with many holes 10 to 20 μ in diameter. With careful exposure to the electron beam even the thinnest sections could be micrographed through the holes. In addition to

greater contrast and resolution, a reduction of about 50 per cent in exposure time was possible. This technique was used in the micrographs in Figs. 8 and 9.

OBSERVATIONS

The nomenclature used in this paper will introduce, in general, few new terms despite the fact that we will attempt to establish a previously undescribed relationship between the nuclear envelope and the cytoplasm. The reason for this is that the origin of the nuclear envelope is not yet clearly understood. We will refer, then, to the *nuclear envelope* and include in this the *inner* and *outer* nuclear membranes and the region between them, the *perinuclear space*. The nomenclature of Porter (19), Palade and Porter (20), and Palade (21) will be followed in referring to the cytoplasmic system of cavities enclosed by the membranes of the *endoplasmic reticulum* and in referring to the *cytoplasmic particles* associated with these membranes.

Continuities between the Inner and Outer Nuclear Membranes.—The membranes which surround the nucleus do not do so continuously but appear interrupted at intervals in perpendicular sections. These interruptions in the envelope are formed by connections between the inner and outer nuclear membranes which, in some cases, may represent disk-shaped regions of fusion between the two membranes (Fig. 9), a view to be developed in more detail later. In most of the connections observed here, however, the region of fusion is missing. Thus, a pore-like opening is formed. At such points the inner and outer membranes are joined at the periphery of the pore, this junction appearing continuous at the resolutions obtained (*ca.* 30 A) (Figs. 3 and 8).

The Relationship between Pores and Rings.—In sections normal to the nuclear surface, pores in the nuclear envelope have widths ranging from about 250 to about 750 A. In any one cell type, this width is rather constant, thus, it would appear likely that the pore is circular in outline.

Rather than to attempt to establish the shape of such small structures by serial sectioning methods, it appears more fruitful to examine views in sections ranging from normal through oblique to tangential to the nuclear surface. This approach may be expected to yield more information about the shape, size, and frequency of the pores and, in particular, will permit correlation with the findings in isolated nuclear membranes examined in full-face view.

In this portion of the present work, the formations we denote as "pores" will refer temporarily to the gaps in the nuclear envelope seen in sections cut *normal* to the surface of the nucleus (Figs. 1 to 3). In contrast to these we note, on the other hand, ring-shaped structures which are seen only in views in which the plane of the specimen is substantially parallel to the nuclear surface (Fig. 4). The average minimum spacings from one center to another of the pores in normal sections and between the centers of the rings in tangential

TABLE I

Dimensions and Distributions of Pores in Nuclear Envelopes of Various Tissues

Showing tissues in which pores have been observed in the nuclear envelope in views either tangential or normal (indicated by x's) to the nuclear surface. The approximate diameters of the pores are given when known. Some of these data have been taken from the work of others which has been cited.

Source	Tissue	Normal	Tangential	Inside pore diameter
				A
Mammalian (rat)				
Ectodermal	Tongue epithelium	x		300
	Nerve (15)	x		
Mesodermal	Striated muscle—tongue	x		400
	" " diaphragm	x		
	Spleen—reticular	x	x	
	Primary spermatocyte	x		500
	Spermatogonium	x		700
	Proximal convoluted tubule cells of kidney (mouse) (12)		x	
	Collecting tubules and Henle's loops of kidney	x		
	Rat sarcoma (25)		x	600
Endodermal	Liver parenchymal	x	x	400
	Thyroid epithelium	x		500
	Pancreas acinar (mouse)	x	x	400
	Intestinal epithelium	x	x	
	Stomach epithelium—chief cell	x	x	500
	" " parietal cell	x	x	600
Amphibian	*Amblystoma* larvae (25)		x	
	Triturus oocytes (5, 10)		x	
	Xenopus oocytes (5)		x	
	Rana catesbiana oocytes (13)		x	
Insect	*Chironomus*—salivary glands (9)	x	x	500
	" midgut (9)		x	300
Protozoan	*Amoeba proteus* (6, 7)	x	x	400
	Paramecium multimicronucleatum	x	x	400
Alga	*Chlamydomonas reinhardi* (27)		x	

views have proved to be about the same (0.1 to 0.2 μ). The interior dimensions are also about the same, the rings having an inside diameter of 400 to 500 A in the liver, for example, and the pores having widths between 300 and 500 A in the same tissue (*cf.* Table I). The rings and pores may be independent,

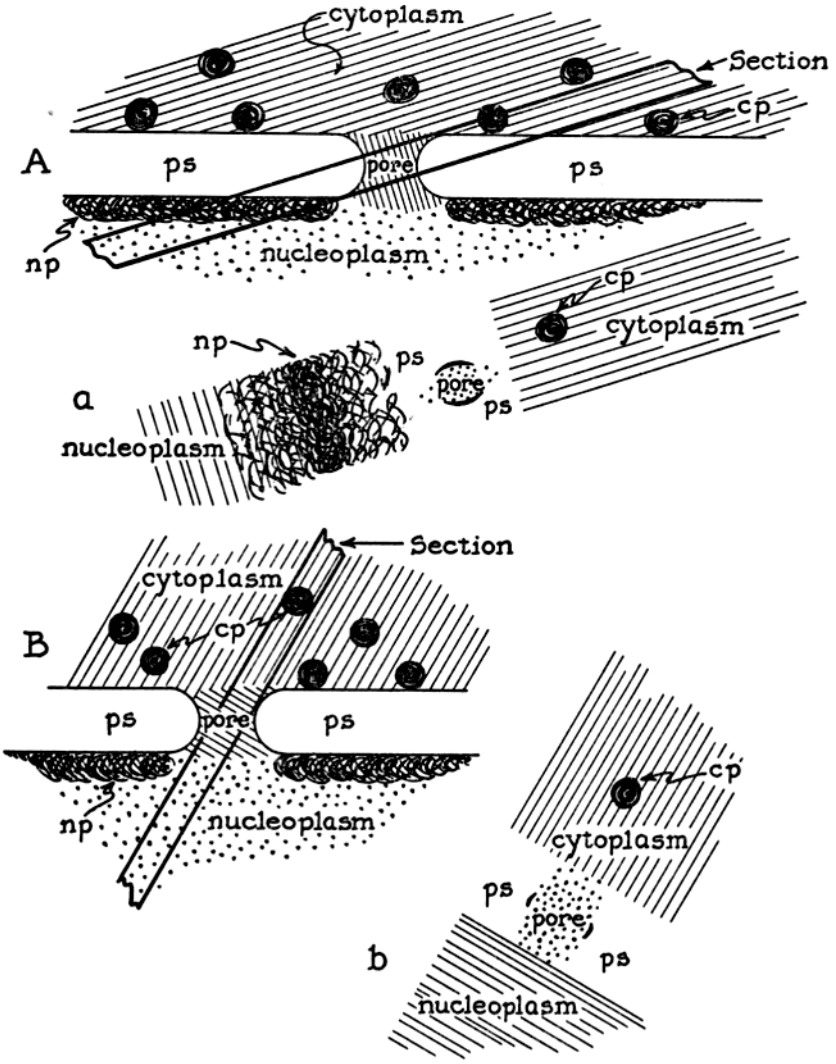
unrelated structures or they may simply be different views of the same formation. Evidence presented below strongly favors the latter interpretation.

On the assumption that the pores and rings are different views of the same structure, it is possible to make certain predictions as to their appearance in oblique section. This is done schematically in Text-fig. 1. In the micrographs (Fig. 6 *a*, *b*; Fig. 7 *a*) are found views in sections which approximate the schematic views of Text-fig. 1 *a*. The section at these points is sufficiently nearly parallel to the nuclear surface so that the ring lies within (Figs. 6 *a*, 7 *a*) or nearly within (Fig. 6 *b*) the transparent perinuclear space. Where the plane of section approaches normality to the nuclear surface, on the other hand, a picture corresponding to Text-fig. 1 *b* is obtained (Fig. 6 *c*; Fig. 7 *b*). Here, the ring is nearly invisible and instead one sees the perinuclear space interrupted by a strip of material connecting the cytoplasm and the nucleoplasm.

It thus seems that the appearance of rings and pores in sections of various obliquities to the nuclear surface can be explained on the assumption that they represent different views of a circular continuity between the inner and outer membrane covering the perinuclear space. On this basis, then, we assert that the rings and pores are views of the same structure as seen in different orientations.

Reports have been cited in the Introduction in which evidence was presented for the existence of ring-shaped and of pore-like formations in the nuclear envelopes of certain cells, these formations being considered to penetrate one or both of the nuclear membranes. From the material so far described in the present work, it seems fairly certain that, at intervals, the inner and outer nuclear membranes fuse in such a way as to allow disk-shaped regions of contact between the nucleoplasm and cytoplasm. Thus, pores are formed which definitely penetrate both nuclear membranes. These observations suggest possibilities regarding fundamental interactions between the nucleus and cytoplasm of resting cells. Before such speculations are further pursued, however, it must be known whether the pores are a general finding or whether they are only a curiosity to be noted in certain cell types. It is desirable to know, further, if there are some cells in which these formations are never found.

Presence of Nuclear Pores in Different Cell Types.—Numerous cell types representative of rat tissues derived from ectoderm, mesoderm, and endoderm were examined in sections in areas cut perpendicular to the nuclear surface. In all these tissues, without exception, pores were found in the nuclear envelopes. Results are tabulated in Table I. Pores were most apparent in those cells which had large numbers of the cytoplasmic particles of the type described by Palade (21), including liver parenchymal cells, pancreas acinar cells, and chief cells of the stomach. They were also easily visualized in primary spermatocytes, parietal cells of the stomach, and in lymphocytes. Cells in which



TEXT-FIG. 1. Schematic drawings demonstrating the appearance of pores in the nuclear envelope in sections oblique to the surface of the nucleus. In Text-figs. 1 *a* and 1 *b* is shown the appearance of pores to be expected when the section is oriented as indicated in Text-figs. 1 *A* and 1 *B*, respectively. In Text-fig. 1 *a*, the section is nearly tangential to the nuclear surface, thus, the pore appears almost in its entirety and is completely surrounded by the perinuclear space, *ps*. In Text-fig. 1 *b*, on the other hand, the section is more nearly normal to the nuclear surface, hence, only a small part of the circumference of the pore appears and a strip of material bridges the gap between the nucleoplasm and the cytoplasm. Micrographs of sections in these orientations are shown in Figs. 6 and 7. Symbols: *ps* = perinuclear space; *cp* = cytoplasmic particle; *np* = nuclear particulate matter.

pores were least distinct included striated muscle fibers, tubular epithelium in the kidney medulla, thyroid epithelium, and the epithelium of the tongue mucosa. These differences appear to be due mainly to differences in distinctness of the nuclear membranes against the surrounding matrices and to the fact that, in the former group, those in which pores could be easily discerned, there is often a wide spacing of the outer from the inner nuclear membrane on either side of the pore, whereas, this spacing is smaller in the latter group. In addition to the mammalian cells described above, pores were also found in *Paramecium multimicronucleatum* by A. W. Sedar who permitted the author to make a micrograph (Fig. 4) of his material.

Reference to Table I shows that pores are present in the nuclear envelopes of a representative sampling of mammalian tissues, including those derived from ectoderm, mesoderm, and endoderm, and also in mammalian tumor cells, amphibian tissues, an insect, protozoa, and an alga. Pores have been present in all cell types thus far examined. Thus it is clear that they are of widespread occurrence in cells not only of different embryological origin in one species, but in cells of widely different species belonging to different phyla.

A demonstration of complete absence of pores in the nuclear envelope of a particular cell type would, like all negative demonstrations, be difficult to make. However, the finding of pores generally present in the many tissues examined argues against anything except their possible transitory absence. It seems clear, then, that pores in the nuclear envelope represent a fundamental structure of resting cells. Thus, we may now appropriately ask what their function is and, since this is a morphological study, look for structures within them which may shed light on the matter.

Material within the Pores.—In almost all cases the region within the pores and immediately outside them in the nucleoplasm and cytoplasm is denser than the rest of the cytoplasmic matrix and the area between the nuclear membranes. In the liver, the dense region extends somewhat farther into the cytoplasm than into the nucleus and often appears to diffuse into a rather generalized, but less dense matrix surrounding the cytoplasmic particles (21). Details are shown in Figs. 8 and 9. Efforts to resolve the contents of the pores have not been very successful mainly because of low contrast. In these micrographs (Figs. 8 and 9), the material appears to be inhomogeneous and possibly granular. In the sections thus far examined it has not been possible to demonstrate clearly the presence within the pores of particles like those associated with the endoplasmic reticulum.

In addition to this material there exists in many pores (Fig. 9) a band across the waist of the pore. This band, presumably the profile of a diaphragm extending across the pore, is usually neither as dense nor as sharply defined as the nuclear membranes. If such a diaphragm represents an area of contact

between the inner and outer nuclear membranes, its diffuseness could be the result of dissolution of this area. This, then, would suggest a mode of formation of the pores. Reformation of the diaphragm and the subsequent separation of the nuclear membranes could result in the disappearance of the pores. It must be emphasized, however, that the presence of the band described above is the only evidence so far discovered for such a mechanism.

Relation of the Nuclear Membrane System to the Cytoplasm.—The work presented up to this point has covered the distribution and structure of the pores in the sheath surrounding the nucleus. It is now of interest to examine in detail certain other features of the nuclear envelope. The structure of the pores indicates that at intervals on the nuclear surface the inner and outer nuclear membranes are continuous with one another and define the pores. It is difficult to imagine such smooth junctions between two membranes unless they are essentially the same membrane. From this point of view, we can visualize the nuclear envelope as a thin, flattened vesicle very similar to the cisternae of the endoplasmic reticulum as it appears in certain cell types, notably the pancreas acinar cells. Such a view may appear to conflict with current ideas about the organization of the cell; however, the following material should render it more plausible.

The appearance of the nuclear membranes in sectioned acinar cells of mouse pancreas is shown in Fig. 5. There is a more or less clear space between them which is from 100 to 300 A in width. Particles of the order of 100 A in diameter are aggregated on the exposed surfaces of both membranes. Particles on the cytoplasmic side are precisely similar in appearance to the particles described by Palade (21) which are scattered throughout the cytoplasm in this cell. On the nuclear side, the particles present are more closely packed and exhibit a disorderly fine structure (22).

In many cell types, the cytoplasmic particles are found in close association with the membranes of the endoplasmic reticulum and often in characteristic patterns such as small circles, etc. (21). A similar association of particles with the outer nuclear membrane is found in some cells, the pattern of distribution of particles resembling that on the membranes of the endoplasmic reticulum in those cells (*cf.* Fig. 4).

In the mammalian tissues studied, the thickness and electron density of the inner and outer nuclear membranes (Figs. 5, 9, and 10) and of the membranes of the endoplasmic reticulum appear about the same on visual inspection of the micrographs, although the nuclear membranes are often somewhat denser. Quantitative measurements of these parameters are probably not of much value because the thickness is on the order of the resolution of the micrographs (*ca.* 30 A) and because of the difficulty of selecting points where the membrane is clearly perpendicular to the plane of the section.

In some cell types, notably nerve cells (15), the inner nuclear membrane

is noticeably thicker than the outer one. A thickening of this sort could represent a fundamental difference between the inner and outer nuclear membranes. However, the view is favored here that it indicates either an accumulation of nuclear material on the inner one or some type of differentiation of that membrane.

The contents of the perinuclear space are usually of a density different from those of the cytoplasmic matrix. Its contents appear homogeneous like the contents of the endoplasmic reticulum. In cells in which the endoplasmic reticulum is disposed in greatly flattened vesicles, as in pancreas acinar cells (22) (Fig. 5), the spacings between the nuclear membranes and between membranes of a vesicle are often about the same (*ca.* 200 to 400 A). Fenestrae in such cisternae have been described and shown in micrographs (Figs. 13 and 19 of reference 20) and although larger and irregular in shape and distribution, they resemble, in form and general construction, the pores in the nuclear envelope. In these respects, then, the nuclear envelope resembles the endoplasmic reticulum. Usually, the density of the perinuclear space is greater than that of the cytoplasmic cavities; since, however, variations in density are often apparent in the latter, they are considered as reflecting quantitative rather than qualitative differences in function.

In a number of cell types, notably the liver parenchymatous cells, primary spermatocytes, and lymphocytes, the outer nuclear membrane shows excursions or blebs proceeding away from the inner membrane into the cytoplasm. These excursions so closely resemble the endoplasmic reticulum in the features described above that they are indistinguishable from it except by their continuity with the outer nuclear membrane. Observations of this sort have led to the interpretation that these deviations of the outer nuclear membrane probably represent unfavorably oriented views of connections with membranes of the endoplasmic reticulum. It was thought that cells in which the fraction of cytoplasmic volume occupied by cavities of the endoplasmic reticulum was great, and in which the cavities themselves were large and therefore few in number would offer a better chance of demonstrating such connections. Two cell types fulfilling these conditions were found in the rat spleen, and, in them, clearly demonstrable connections between the perinuclear space and the cavities of the endoplasmic reticulum were observed. In Fig. 10 a portion of the nucleus and cytoplasm of a cell from rat spleen is shown. The cytoplasmic matrix is disposed in a relatively coarse network surrounding large cavities containing a low density material which is not evident in the micrograph (exposed to show details of the membranes). The unusually large cavities of the endoplasmic reticulum in this cell probably do not arise from distortion during preparation since neighboring cells of different types in this section were well preserved and did not exhibit this feature. These cavities are enclosed by membranes which separate them from the cytoplasmic

matrix. The membranes have associated with them at certain points on the matrix side dense cytoplasmic particles. Many of the cavities are interconnected. These features are sufficient to identify the cavities and membranes as belonging to the endoplasmic reticulum. At points indicated by arrows (Fig. 10) are two regions where the endoplasmic reticular cavities become continuous with the perinuclear space. The outer nuclear membrane is evidently continuous with, and is, indeed, a part of the membranes of the endoplasmic reticulum. Since it is also continuous with the inner membrane at points at which pores are formed (Fig. 11) it appears that the inner nuclear membrane is also part of the same system.

Connections of this type have also been observed in rat liver parenchymal cells. Such formations are convincing in their structure only in cells in which the endoplasmic reticulum is extensive and highly developed (*i.e.* pancreas acinar cells, reticular cells, liver parenchymatous cells, parotid acinar cells, etc.) and are demonstrable only in areas in which the disposition of the membrane is highly favorable.

DISCUSSION

As is well known, numerous light microscopic studies of fresh and fixed cells have suggested the existence of a visible membrane surrounding the nucleus. Micromanipulation and microdissection of cells by Kite (1) and later by Chambers and Fell (2) demonstrated the presence of a membrane about the nuclei of cells in interphase which resisted somewhat the penetration of the dissection needle. Once this barrier was passed the needle could proceed through the nucleus without further interference. Nuclear envelopes teased from cells and examined in the electron microscope were found to consist of two parallel membranes exhibiting, on the surface, numerous ring-shaped structures which were considered to be pores penetrating one or another of the membranes (5-7, 10). The orientation of the specimens under these conditions, however, made it difficult to establish definitely that the pores were patent. The application of thin-sectioning techniques to the electron microscopy of cells made possible the viewing of sections normal to the nuclear surface (8, 9). In such views gaps or breaks were reported in the continuity of the double envelope which were interpreted as pores and considered to be edgewise views of the rings seen in tangential sections. Although the evidence presented was suggestive of these interpretations, the resolutions achieved were inadequate to reveal anything of the fine structure of the pores and of the disposition of the membranes about them. Thus, it could not be positively asserted, for example, that such gaps seen in normal sections were not occasional breaks due to stresses incurred during preparation. At higher resolution, the author (14) was able to demonstrate that pores seen in sections normal to the nuclear surface of mouse pancreas acinar cells were formed

by connections between the inner and outer nuclear membranes. This provided a description of the structure of the pore and demonstrated its presence in a mammalian tissue. These electron-microscopic reports, however, were "spot" findings in a few isolated cell types and failed to demonstrate whether pores could be considered of general interest and present in most cells. Further, there has been presented no evidence known to the author indicating structural relationships between the double membranes of the nuclear envelope and membranes found in the cytoplasm.

In the present study it has been found that the nucleoplasm of cells in interphase is exposed to the cytoplasm at numerous points on its surface by the presence of pores formed by circular regions around which, in many cases, the inner and outer enveloping membranes are continuous with one another. There exists, usually, no sharply defined morphological entity covering the nuclear surface within these pores which could be described as a membrane. The pores have been found in the nuclear envelopes of a wide variety of cells with no exceptions noted, so that one seems justified in assuming that they represent a fundamental structure of most cells in interphase.

In a few cells in which the disposition of the endoplasmic reticulum was especially favorable, it has been possible to demonstrate unequivocal continuity between the endoplasmic reticulum and the nuclear envelope. Since the nuclear membranes of all cell types studied have strong morphological resemblances to one another, *e.g.* double structure, resemblance to membranes of the endoplasmic reticulum, pores, etc., and since in some cells there is definite continuity between the nuclear envelope and the endoplasmic reticulum, it seems likely that such connection exists, at least temporarily, in all resting cells. If this is accepted as true, it is an almost inescapable conclusion that the nuclear membrane system is a modified part of the endoplasmic reticulum.

There are a number of reasons for supposing that the fenestrations in the nuclear envelope may not simply be "holes" through which the cytoplasm and the nucleoplasm have "free" access to each other by simple diffusion. One is struck, in some instances, by the strong resemblance between the cytoplasmic particles of Palade and certain particles often found in large numbers in the nucleus which have been described by Porter as common in the nucleolus (*cf.* Fig. 20 of reference 23). Thus, one might consider that the cytoplasmic particles were formed within the nucleus and passed through the pores into the cytoplasm.[1] While, in the present work, it has not been possible to demonstrate particles of this size (*ca.* 150 A) and density within the pores, there has been observed a rather diffuse, low-density material almost universally present

[1] The transfer of material of unspecified morphology from the nucleolus to the cytoplasm of cells actively engaged in protein synthesis has been described by Caspersson (28) using techniques of ultraviolet microspectroscopy.

within them, and many appear to contain, in addition, an ill-defined diaphragm. Thus, we may reasonably consider the possibility that, although the pores are sufficiently large to permit the passage of the largest organic molecules, their "permeability" is under the control of mechanisms of a specific nature, mediated, perhaps, by the diffuse diaphragm described earlier.

We are led, by these observations, to picture the nucleus of the interphase cell as a large cell organelle resting in the cytoplasmic matrix, but closely covered, except at points, with a modified element of the endoplasmic reticulum. This enveloping element which we term the *perinuclear cisterna*, consists of a much flattened, membrane-enclosed cavity in close apposition to the nuclear surface. As a result of this the nucleus appears to be surrounded by a double membrane. At intervals there are circular ports or fenestrae in the perinuclear cisterna so that the nuclear surface is exposed at these points to the cytoplasmic matrix. Calculation, based on the assumptions of a pore diameter of 500 A and a spacing between pores of 1500 A, shows that between 5 and 15 per cent of the nuclear surface is thus exposed.

Thus the nucleus is in contact with its environment through two rather different channels. One-tenth of the nuclear surface has direct, though perhaps controlled, contact with the cytoplasmic matrix through the pores, while nine-tenths of its area is in indirect contact through the membrane-enclosed cavities of the endoplasmic reticulum. These differences in morphology suggest corresponding differences in function. Large molecules may, perhaps, pass, in a controlled way, through the pores between the cytoplasm and the nucleus, while inorganic ions and small molecules may be exchanged by way of the endoplasmic reticulum.

The importance of the presence of the nucleus for the existence of the cell in interphase is shown by the observation of Chambers and Fell (2) and many others, that though enucleated cells may survive for a time, they will eventually succumb. Thus, it appears that, in the resting cell, the nucleus is not simply a repository for genetic information, but is actively involved in cell metabolism. A specific function for the nucleus has been demonstrated by Hogeboom and Schneider (24) who reported that an enzyme involved in the synthesis of coenzyme I (diphosphopyridine nucleotide) is present only in the nuclear fraction of liver homogenates. Since DPN is active in the cytoplasm, this finding indicates that either this enzyme in very low concentrations or DPN, itself, must be transferred from the nucleus to the cytoplasm in the resting cell.

We have presented here morphological evidence for two substantially different passageways between the nucleus and cytoplasm. The microdissection and biochemical evidence for an active interphase nucleus cannot be ignored. The morphological evidence here presented suggests that this activity involves the independent transfer of both small and large molecules.

SUMMARY

An electron microscope study of thin sections of interphase cells has revealed the following:—

Circular pores are formed in the double nuclear envelope by continuities between the inner and outer membranes which permit contact between the nucleoplasm and the cytoplasm unmediated by a well defined membrane.

The pores, seen in sections normal to the nuclear envelope, are profiles of the ring-shaped structures described by others and seen in tangential section.

The inner and outer nuclear membranes are continuous with one another and enclose the perinuclear space.

The pores contain a diffuse, faintly particulate material.

A survey of cells of the rat derived from the embryonic ectoderm, mesoderm, and endoderm, and of a protozoan and an alga has revealed pores in all tissues examined, without exception. It is concluded that pores in the nuclear envelope are a fundamental feature of all resting cells.

In certain cells, the outer nuclear membrane is continuous with membranes of the endoplasmic reticulum, hence the perinuclear space is continuous with cavities enclosed by those membranes. There are indications that this is true for all resting cells, at least in a transitory way.

On the basis of these observations, the hypothesis is made that two pathways of exchange exist between the nucleus and the cytoplasm; by way of the perinuclear space and cavities of the endoplasmic reticulum and by way of the pores in the nuclear envelope.

The author wishes gratefully to acknowledge the value of many discussions with Dr. George Palade and Dr. Keith Porter of The Rockefeller Institute pertaining to the work and to the preparation of this manuscript.

BIBLIOGRAPHY

1. Kite, G. L., *Am. J. Physiol.*, 1913, **32,** 146.
2. Chambers, R., and Fell, B., *Proc. Roy. Soc. London, Series B*, 1931, **109,** 380.
3. Anderson, N. G., *Science*, 1953, **117,** 517.
4. Hogeboom, G. H., and Schneider, W. C., *Science*, 1953, **118,** 419.
5. Callan, H. G., and Tomlin, S. G., *Proc. Roy. Soc. London, Series B*, 1950, **137,** 367.
6. Bairati, A., and Lehmann, F., *Experientia*, 1952, **8,** 60.
7. Harris, P., and James, T., *Experientia*, 1952, **8,** 384.
8. Hartmann, J. F., *J. Comp. Neurol.*, 1953, **99,** 201.
9. Bahr, G. F., and Beermann, W., *Exp. Cell Research*, 1954, **6,** 519.
10. Gall, J. G., *Exp. Cell Research*, 1954, **7,** 197.
11. Anderson, T. F., *Tr. New York Acad. Sc.*, 1951, **13,** series 2, 130.
12. Rhodin, J., Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954, 111.

13. Pollister, A. W., Gettner, M., and Ward, R., *Science*, 1954, **120**, 789 (abstract).
14. Watson, M. L., *Biochem. et Biophysic. Acta*, 1954, **15**, 475.
15. Palay, S. L., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.
16. Bradley, D. E., *Brit. J. Appl. Physics*, 1954, **5**, 65.
17. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 183.
18. Jaffe, M. S., *J. Appl. Physics*, 1948, **19**, 1191 (abstract).
19. Porter, K. R., *J. Exp. Med.*, 1953, **97**, 727.
20. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100**, 641.
21. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
22. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7**, 393.
23. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2**, 346.
24. Hogeboom, G. H., and Schneider, W. C., *J. Biol. Chem.*, 1952, **197**, 611.
25. Porter, K. R., unpublished data.
26. Dalton, A. J., and Felix, M. D., *Am. J. Anat.*, 1954, **94**, 171.
27. Sager, R., and Palade, G. E., unpublished data.
28. Caspersson, T. O. Cell Growth and Cell Function. A Cytochemical Study, New York, W. W. Norton and Co., Inc., 1950, 101.

EXPLANATION OF PLATES

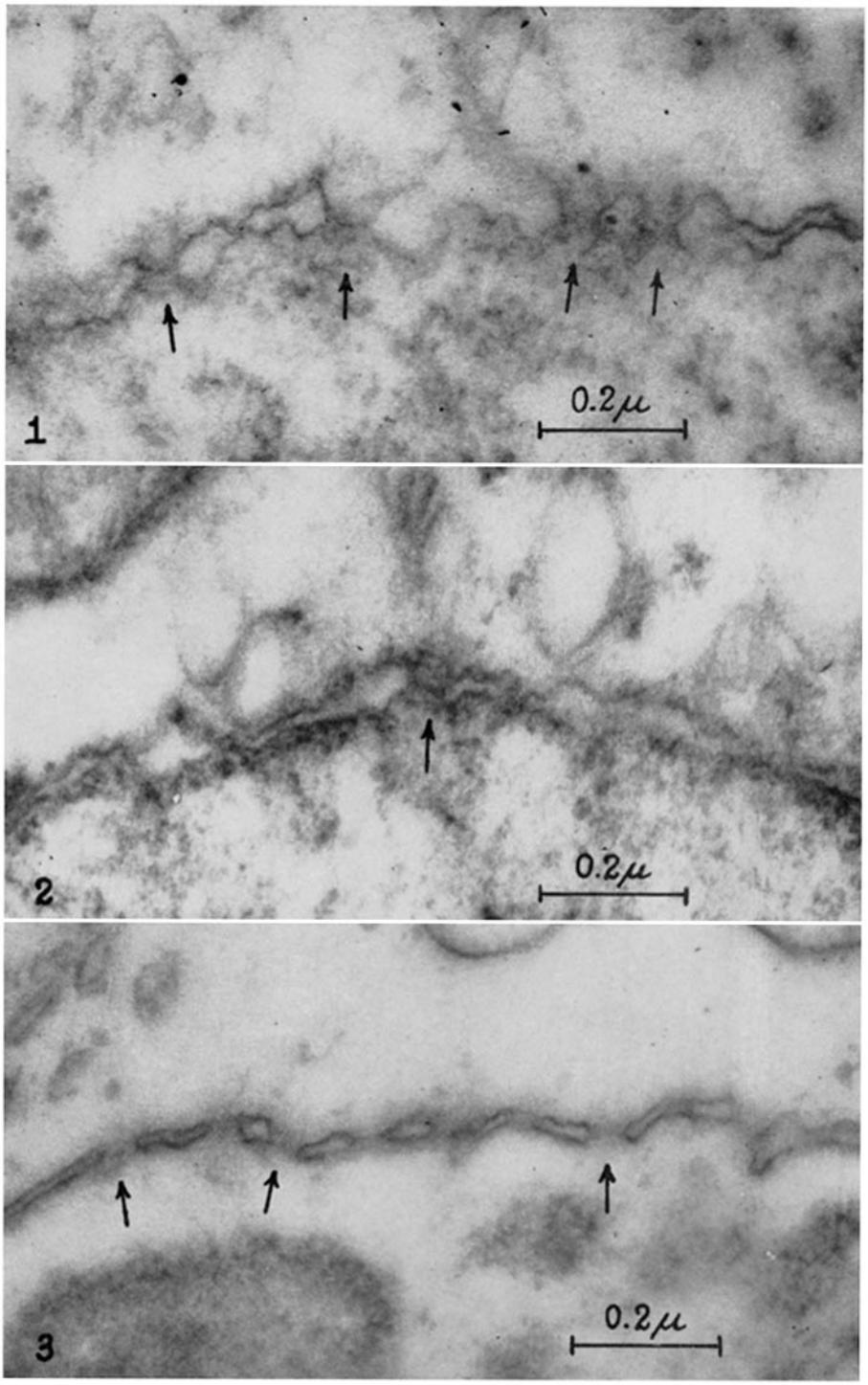
PLATE 71

The appearance of the nuclear envelopes of three different cell types as seen in sections normal to the nuclear surface. The nuclear envelope is a double structure formed by two membranes spaced apart by about 300 A and surrounding the contents of the nucleus. Between the membranes is the perinuclear space. The two membranes in section can be followed horizontally across the center of each micrograph. At points indicated by arrows, gaps or pores are formed where the inner and outer membranes are joined with one another. In favorable views, these connections between the membranes appear to be continuous (Figs. 1, 3, and 8).

FIG. 1. Primary spermatocyte of the rat. The wide fluctuations in spacing between the membranes in this cell account for their frequent indistinctness where they are inclined to the plane of the section. × 100,000.

FIG. 2. Parietal cell from rat stomach. × 100,000.

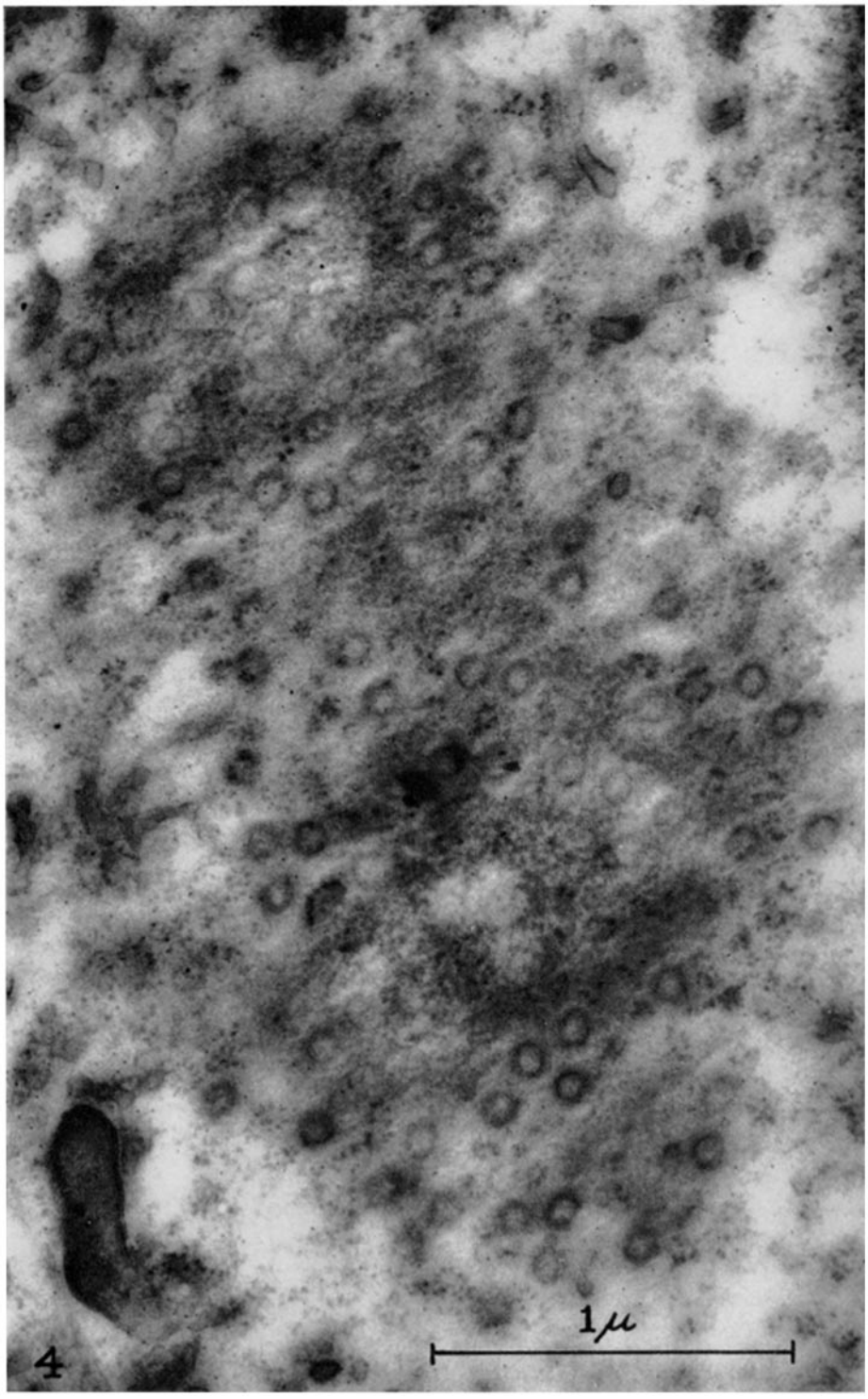
FIG. 3. Macronucleus of *Paramecium multimicronucleatum*. Pores are possibly more numerous and are much more easily visualized in the *Paramecium* than they are in most mammalian tissues. × 100,000.



(Watson: Nuclear envelope)

PLATE 72

FIG. 4. Section tangential to the surface of a rat liver parenchymatous cell. Numerous ring-shaped structures which represent pores in tangential views (see text) are scattered over the nuclear surface. Most of these profiles appear to be dark rings with an inside diameter of about 500 A and an outside diameter of 1000 to 1200 A. A few of the rings appear to be made up of or encrusted with closely spaced granules resembling the cytoplasmic particles (21). Near the periphery of the tangentially sectioned nucleus, cytoplasmic particles are present, arranged in circles. In the liver cells such circular aggregations of particles are found not only on the nuclear membrane, but throughout the cytoplasm as well (not present in this micrograph). These findings suggest that circular arrangements of spherical particles reported by Gall (10) on isolated nuclear membranes (see Introduction) may arise from formations similar to those described above. × 50,000.

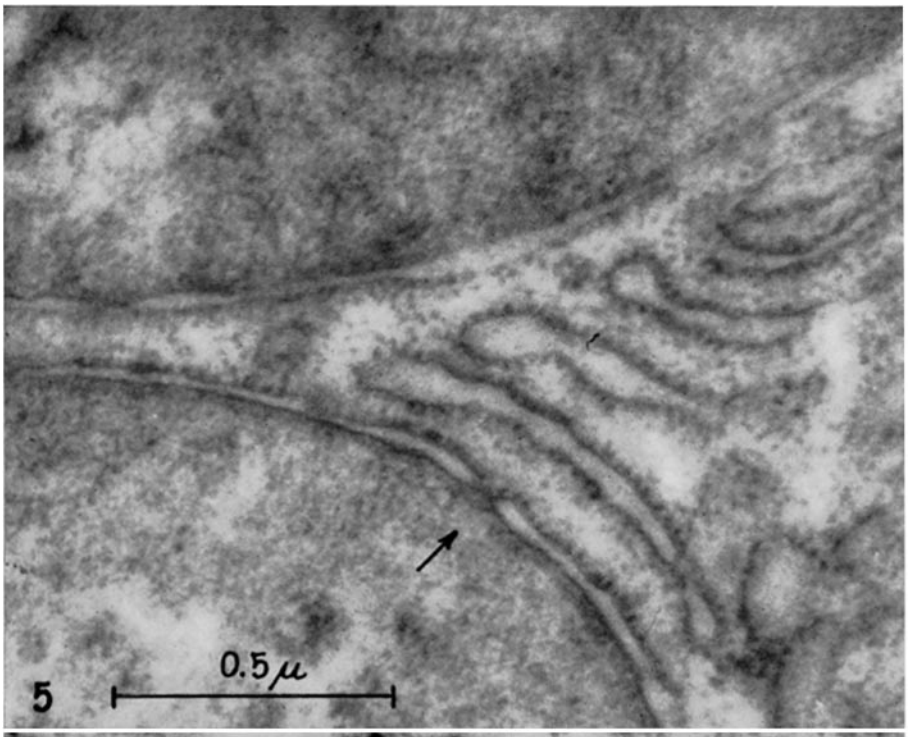


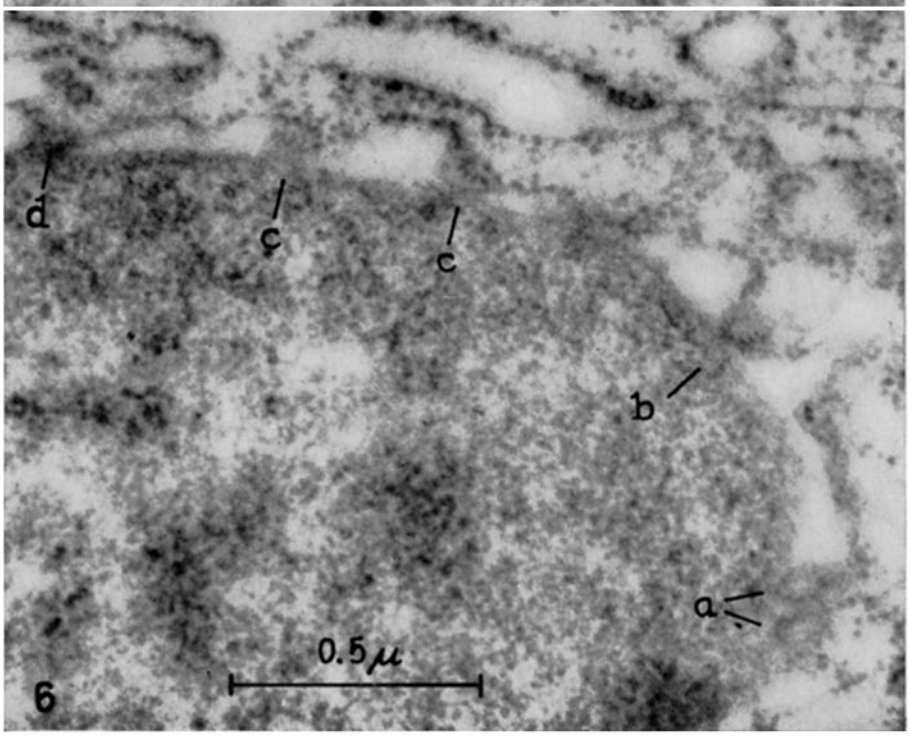
(Watson: Nuclear envelope)

PLATE 73

FIG. 5. Section normal to the surfaces of two nuclei of a binucleate, acinar cell of rat pancreas. The presence of cytoplasmic particles along membranes of the endoplasmic reticulum and along the outer nuclear membrane is clearly evident. The appearance of the outer nuclear membrane and its associated particles makes it essentially indistinguishable from membranes of the endoplasmic reticulum. A pore in one nuclear envelope is indicated by the arrow. Similarities of this sort were pointed out by Dalton and Felix (26) in the epithelial cells of the epididymis and led Sjöstrand and Hanzon (22) to consider the outer nuclear membrane of rat pancreas acinar cells as a single member of the system of otherwise double cytoplasmic membranes. × 75,000.

FIG. 6. Section oblique to the surface of the nucleus of a chief cell of rat stomach. At *a*, elliptical profiles of pores "tilted" with respect to the plane of the section are seen and at *b*, *c*, and *d* are views as the plane of the section approaches more nearly perpendicularity to the nuclear surface. Comparison with Text-fig. 1 will help to explain this appearance. Views of this sort indicate that the gaps in sections normal to the nuclear surface and rings seen in tangential sections are simply different aspects of the same formation. × 66,000.





(Watson: Nuclear envelope)

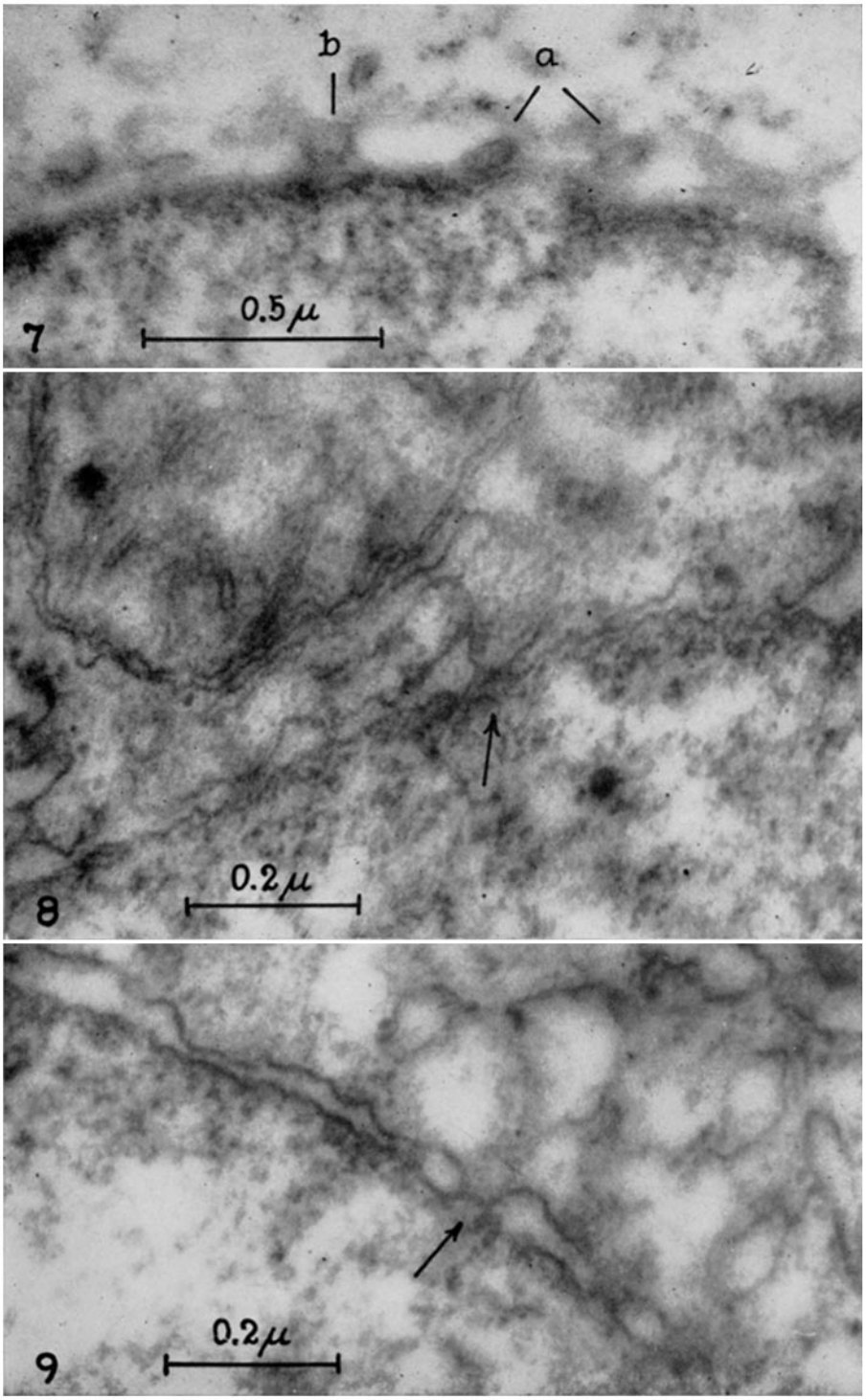
PLATE 74

FIG. 7. Section oblique to the surface of the nucleus of a rat liver parenchymatous cell. At *a* the angle of sectioning is sufficiently nearly tangential so that elongated images of two pores are seen in their entirety. At *b* the angle of sectioning is more nearly perpendicular to the nuclear surface. The material bridging the perinuclear space between the cytoplasm and the nucleoplasm can be seen. The two sides of the bridge at the perinuclear space are slightly darkened and represent a small portion of the ring to which the plane of the section is inclined (*cf.* Text-fig. 1 *b*). × 66,000.

FIGS. 8 and 9. Sections normal to the nuclear surface of rat liver parenchymatous cells. In these views, material can be seen within the pores which are indicated by arrows.

FIG. 8. The pore contains a moderately concentrated material which reaches greatest density near the waist of the pore. This material which is inhomogeneous and exhibits a finely granular structure, diffuses gradually into the matrices of the nucleoplasm and cytoplasm. The inner and outer nuclear membranes are smoothly joined on either side of the pore. × 120,000.

FIG. 9. A material similar to that described above is present within the pore and extends for a distance into the cytoplasm and the nucleoplasm. This pore is bridged in section by a diffuse, dark band which may represent a diaphragm. × 120,000.

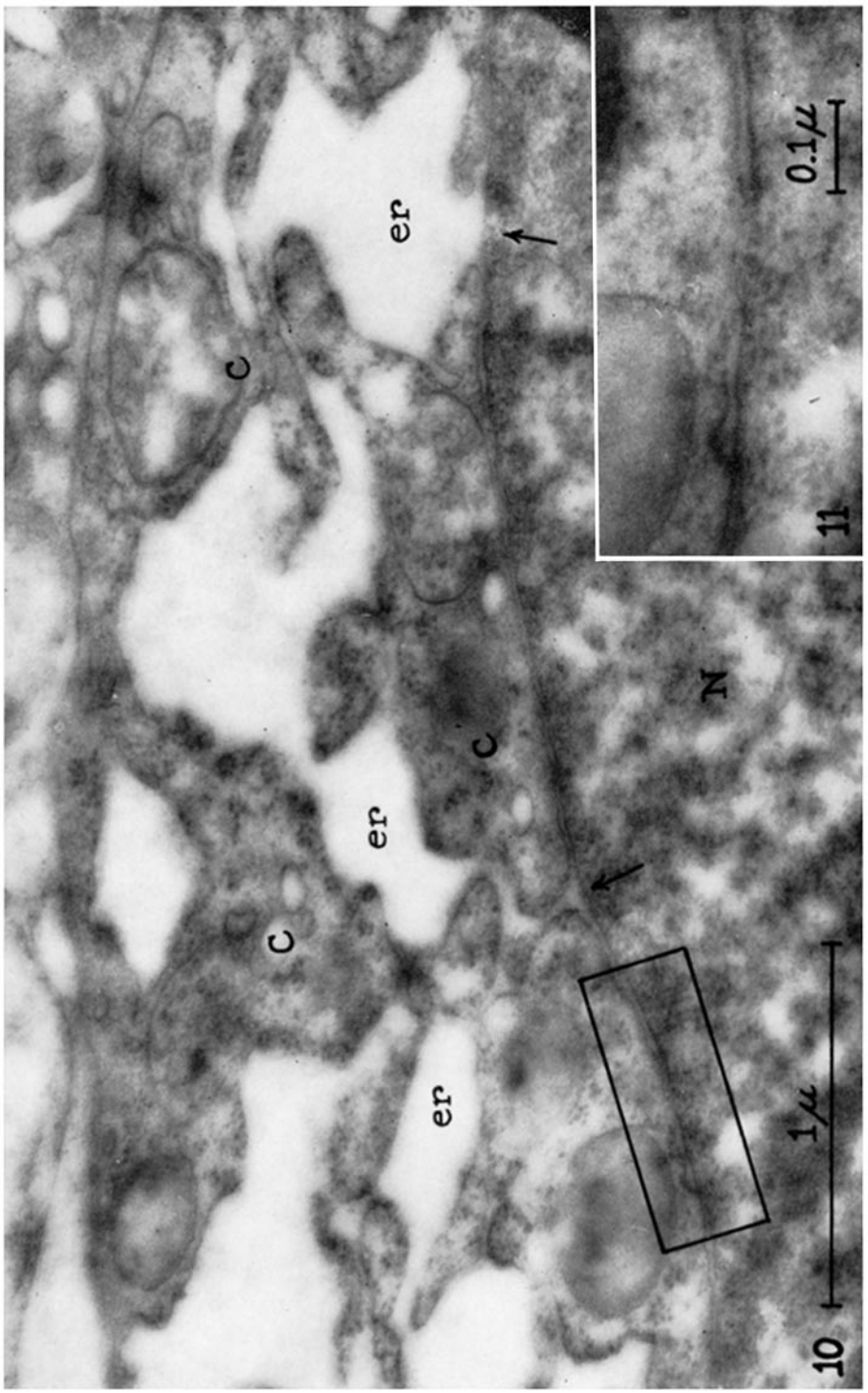


(Watson: Nuclear envelope)

PLATE 75

FIG. 10. Section normal to the surface of the nucleus (*N* in micrograph) of what is tentatively identified as a reticular cell from rat spleen. The endoplasmic reticulum (*er* in micrograph) of this cell is coarse in structure and occupies about as much area in the section as does the cytoplasmic matrix (*C* in micrograph). This arrangement offers an excellent chance of finding connections between the nuclear envelope and the endoplasmic reticulum. Two such connections are present in the micrograph and are indicated by arrows. At these points, the outer nuclear membrane leaves the surface of the nucleus and proceeds without interruption into the cytoplasm to form membranes of the endoplasmic reticulum. The inner and outer nuclear membranes are joined at a number of pores, one of which is shown in Fig. 11 at higher power. × 50,000.

FIG. 11. An enlargement of the area enclosed by the rectangle in Fig. 10 to show a pore in the nuclear envelope. The presence of this pore and of others in Fig. 10 indicates that not only is the outer nuclear membrane connected to the endoplasmic reticulum, but it is also connected to the inner nuclear membrane. Thus the *perinuclear cisterna*, comprising both nuclear membranes and the perinuclear space, appears to be a modified part of the endoplasmic reticulum. × 100,000.



(Watson: Nuclear envelope)