

ELECTRON MICROSCOPY OF THE NUCLEAR MEMBRANE OF AMOEBA PROTEUS*, †

BY MARIE H. GREIDER, WENCEL J. KOSTIR, AND WALTER J. FRAJOLA, Ph.D.

(From the Herman A. Hoster Research Laboratory of the Department of Medicine and
the Department of Zoology, The Ohio State University, Columbus)

PLATE 147

In 1952 Bairati and Lehmann (1) described two layers of isolated nuclear membrane of *Amoeba proteus*, an inner porous or honeycombed layer and an outer thin continuous layer. Later in that year Harris and James (2) corroborated these findings with micrographs obtained from thin sections. As a result of recent refinements in thin sectioning techniques a new ultrastructure of the nuclear membrane of *Amoeba proteus* has been observed. The outer layer contains ring-shaped differentiations or annuli arranged in parallel rows. The inner layer consists of a series of parallel, tube-like structures arranged perpendicularly to the nuclear surface. These observations necessitate a reinterpretation of the structure of this membrane in relation to the structure of the nuclear membrane of other species.

Procedure

Amebas were fixed for 15 to 30 minutes in 1 per cent osmium tetroxide solution, buffered at pH 7.7 or pH 8.3 with sodium acetate-veronal buffer. Washing, dehydrating, impregnating, and embedding were performed essentially according to the procedures described by Borysko and Sapranauskas (3) for tissue culture cells.

OBSERVATIONS

In sections normal to the nuclear surface (Fig. 1) the structural aspect of the outer layer is that of a double membrane between which are alternating bands of greater and lesser density. Discontinuities between the membranes in the less electron-dense bands exist in some areas. Annuli, whose diameters are equal to the width of the more dense bands, appear in oblique sections of this layer (Fig. 2). These annuli or ring-shaped structures have an outside diameter of approximately 80 μ . When observed in a section tangential to

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the surface of the nuclear membrane the annuli are arranged in parallel rows (Figs. 3 and 4). The distance from the center of one annulus to the next is approximately 200 to 225 $m\mu$.

In sections normal to the nuclear surface (Fig. 1) the inner layer appears to be composed of "canals," which in oblique sections (Fig. 2) have the appearance of pores. In Fig. 3 this difference in appearance can be observed in several areas of the same section. Therefore, it is believed that the inner layer consists of short tube-like structures with adjacent structures sharing the same wall. Their major axes are parallel to one another but perpendicular to the nuclear surface.

The thickness of the inner layer, as determined by the length of the "canals" or tube-like structures, is approximately 300 $m\mu$. The outer diameter of the pores is approximately 200 $m\mu$.

DISCUSSION

The electron micrographs presented by Pappas (4) at this meeting also illustrate this structure of the nuclear membrane of *Amoeba proteus*. Several of his micrographs of sections normal to the surface showed very clearly the structure of the membrane at the sites of the annuli of the outer layer. Many of the electron micrographs of other cell types, published by other authors (5-8), have illustrated elements in the nuclear membrane similar to those observed in the outer layer of the nuclear membrane of *Amoeba proteus*. Thus only the outer layer of the nuclear membrane of *Amoeba proteus* seems to correspond to the nuclear membranes of other cell types.

The presence of the inner layer with its large porous structure appears to be unique to *Amoeba proteus*. Bairati and Lehmann (1) and Harris and James (2) characterized this layer as honeycombed. This is in agreement with the interpretation given above because it is assumed that the walls of the canals form the circular outlines of the pores.

SUMMARY

An electron microscope study of the nuclear membrane of *Amoeba proteus* by thin sectioning techniques has revealed an ultrastructure in the outer layer of the membrane that is homologous to the pores and annuli observed in the nuclear membranes of many other cell types studied by these techniques. An inner honeycombed layer apparently unique to *Amoeba proteus* is also described.

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EXPLANATION OF PLATE 147

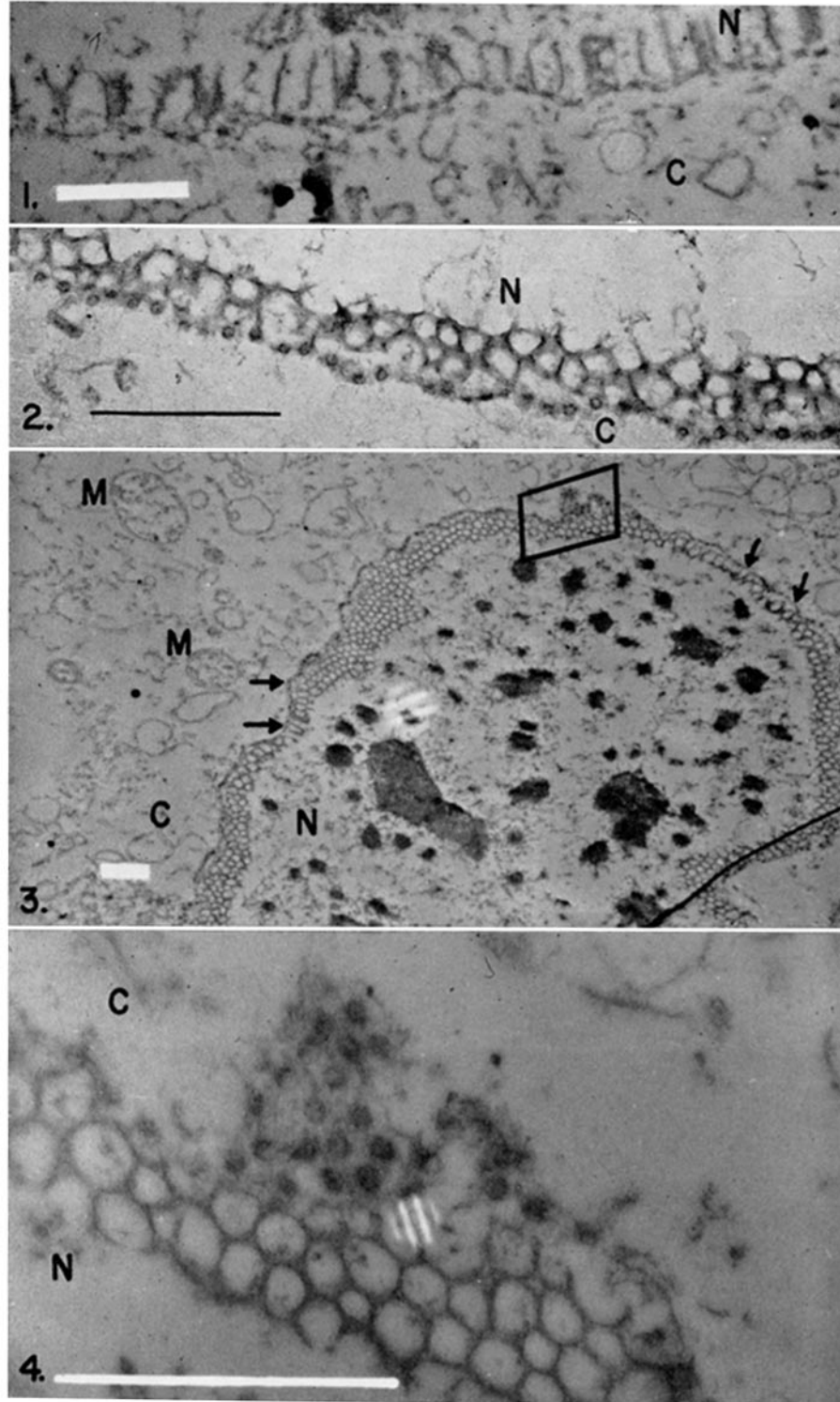
All micrographs represent thin sections of the nuclear membrane of *Amoeba proteus*, with the nuclear or inner area indicated by *N* and cytoplasmic or outer area by *C*. The length of the bar in each figure represents 1 micron.

FIG. 1. In this section (normal to the surface of the nuclear membrane) the outer layer consists of a double membrane in which are alternating light and dense areas. The inner layer is an area containing a series of dense lines approximately parallel to one another but perpendicular to the double membrane of the outer layer.

FIG. 2. In this section (oblique to the surface of the nuclear membrane) the outer layer consists of small annuli while the inner layer has a honeycombed appearance.

FIG. 3. In this micrograph the arrows mark adjacent areas having the appearance of pores and canals. This denotes changes in the profile of the nuclear surface such that a given section can contain areas sectioned both obliquely and normally. Two mitochondrial profiles (*M*) are illustrated. The outlined portion is shown in higher magnification in Fig. 4.

FIG. 4. In this section (almost tangential to the surface of the nuclear membrane) several rows of annuli are present in a portion of the outer layer.



(Greider *et al.*: Nuclear membrane of *Amoeba proteus*)