THE FINE STRUCTURE OF THE NUCLEAR ENVELOPE OF AMOEBA PROTEUS

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PLATE 144

The structure of the nuclear envelope of interphase animal cells has recently been described by Watson (1) as a double nuclear membrane containing circular pores which are formed by continuities between the inner and outer membranes. These pores or gaps appear at times to permit contact between the nucleoplasm and the cytoplasm. The fine structure of the nuclear envelopes of various tissues has also been previously discussed by other investigators (2, 3, 4). Pores were first seen in *Amoeba proteus* by Bairati and Lehmann (5) in preparations of fragmented membranes, and by Harris and James (6) in sectioned material. These investigators described the nuclear envelope as composed of two layers, an outer continuous one and a thicker, coarsely porous inner layer. More recently, Greider, Kostir, and Frajola (7) also described the outer layer as continuous, although containing many pores which appear as tiny ring-like structures. The present investigation was undertaken to clarify further the relationships which exist between the two layers of the nuclear envelope.

Methods

Cultures of *Amoeba proteus* were washed free of other protozoa, and fixed in 1 per cent OsO_4 in a veronal-acetate buffer, pH 8.6 containing 0.01 per cent $CaCl_2$. The fixation period varied from 10 to 20 minutes. After dehydration, the cells were embedded in *n*-butyl methacrylate; thin sections were cut, and studied in the electron microscope (RCA model EMU-2E)

OBSERVATIONS

A portion of the nucleus of *Amoeba proteus* is seen in Figs. 1 and 2. The nucleus is cut normal (at right angles) to the nuclear envelope at *A*. At the moderate resolution of these micrographs the outer layer appears to be continuous, approximately 420 A thick, and exhibits projections running perpendicular to the nuclear surface and into the nucleoplasm (Figs. 1 and 2). These projections, seen more clearly in Fig. 2, are evenly spaced at about 1400 A, and are approximately 2800 A long.

At higher resolution (Fig. 3) the continuous outer layer is seen to consist of a double membrane containing pores with a diameter of about 640 A. The edges

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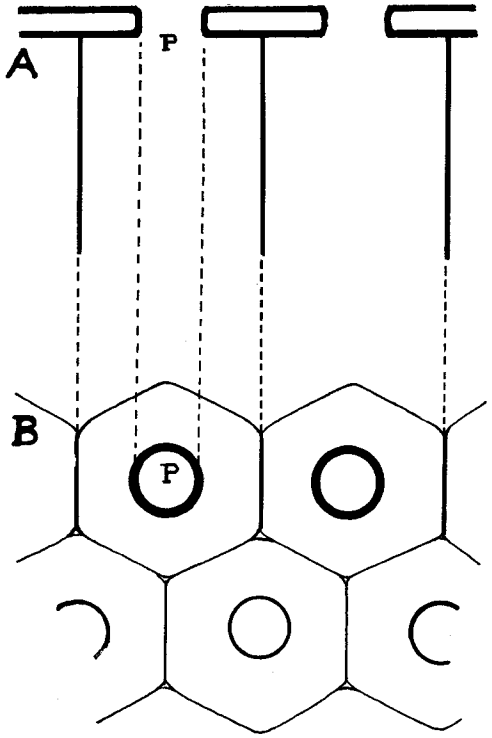
of the pore indicated in the micrograph appear as a continuity of the inner and outer membranes, and a dark band or diaphragm is seen across the opening of this pore.

At *B* in Fig. 1, the nucleus is cut nearly tangential to the nuclear envelope. Here is seen a close-packed array of hexagons with a distance between sides of



TEXT-FIG. 1. A diagrammatic representation of the postulated structure of the nuclear envelope. Sectioned views perpendicular (*A*) and tangential (*B*) to the nuclear envelope are seen. The pore (*P*) represents discontinuities formed by the confluence of the inner and outer membranes. In the tangential view (*B*) the pores are centrally located, one within each hexagonal profile. When tangential sections are cut below the double membrane surface, the pores do not appear in the hexagons. (See *B* in Fig. 1, and *b* in Fig. 4).

1400 A. Thus we see that on the inner surface of the nuclear membranes there lies a honeycomb-like layer, the axes of whose elements extend perpendicularly to the nuclear surface and into the nucleus for a distance of about 2800 A. When sections of the nuclear envelope are cut obliquely so that the outer double membranes as well as the inner honeycomb structures appear (Fig. 4), then ring-like profiles of the nuclear pores at *a* as well as hexagons of the honeycomb at *b* are observed.

Sections may be obtained tangential to the surface of the nucleus, showing

both the pores in the nuclear membranes and the hexagonal lattice of the honeycomb structure (Fig. 5). Here, one can see each pore centered at the base of a hexagonal prism. Thus, the honeycomb structure bears an intimate relationship to the array of pores in the nuclear membranes. The pore is about 640 A in diameter and therefore about half the diameter of the hexagon (*ca.* 1400 A).

A schematic drawing of these structures (Text-fig. 1) shows views both perpendicular and oblique to the surface of the nucleus. In this drawing, the pores (*P*) are centered in the one case (*A*) between the walls of the longitudinally cut hexagonal prisms, and in the other case (*B*), within the hexagonal profiles. Although the perpendicular view shows the elements of the honeycomb connected to the inner nuclear membrane, the reality of this connection is not definite. The walls of the hexagonal prisms are less distinct in section than are the nuclear membranes. Thus it appears that the walls are not identical in structure to the nuclear membranes.

DISCUSSION AND CONCLUSIONS

This study has shown that the nuclear envelope of *Amoeba proteus* consists of two membranes, containing pores which are formed where continuity between the inner and outer membranes occurs. In this respect, the structure of the nuclear envelope of *Amoeba proteus* is similar to that of mammalian (1, 8), amphibian (9, 10), and invertebrate cells (11).

However, unlike other animal cells, the nuclear envelope of *Amoeba proteus* is associated with an additional structure consisting of a thick (2800 A) layer of closely packed hexagonal prisms, each prism terminating at the nuclear envelope in a precisely centered pore. Corresponding to the inner structure, the pores are also arranged in a hexagonal array. This study shows that the "continuous" outer membrane of *Amoeba proteus* described by previous investigators (2, 3, 4) in reality possesses pores some of which at least may be patent, and that the "porous inner membrane" is the honeycomb structure described above.

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EXPLANATION OF PLATE 144

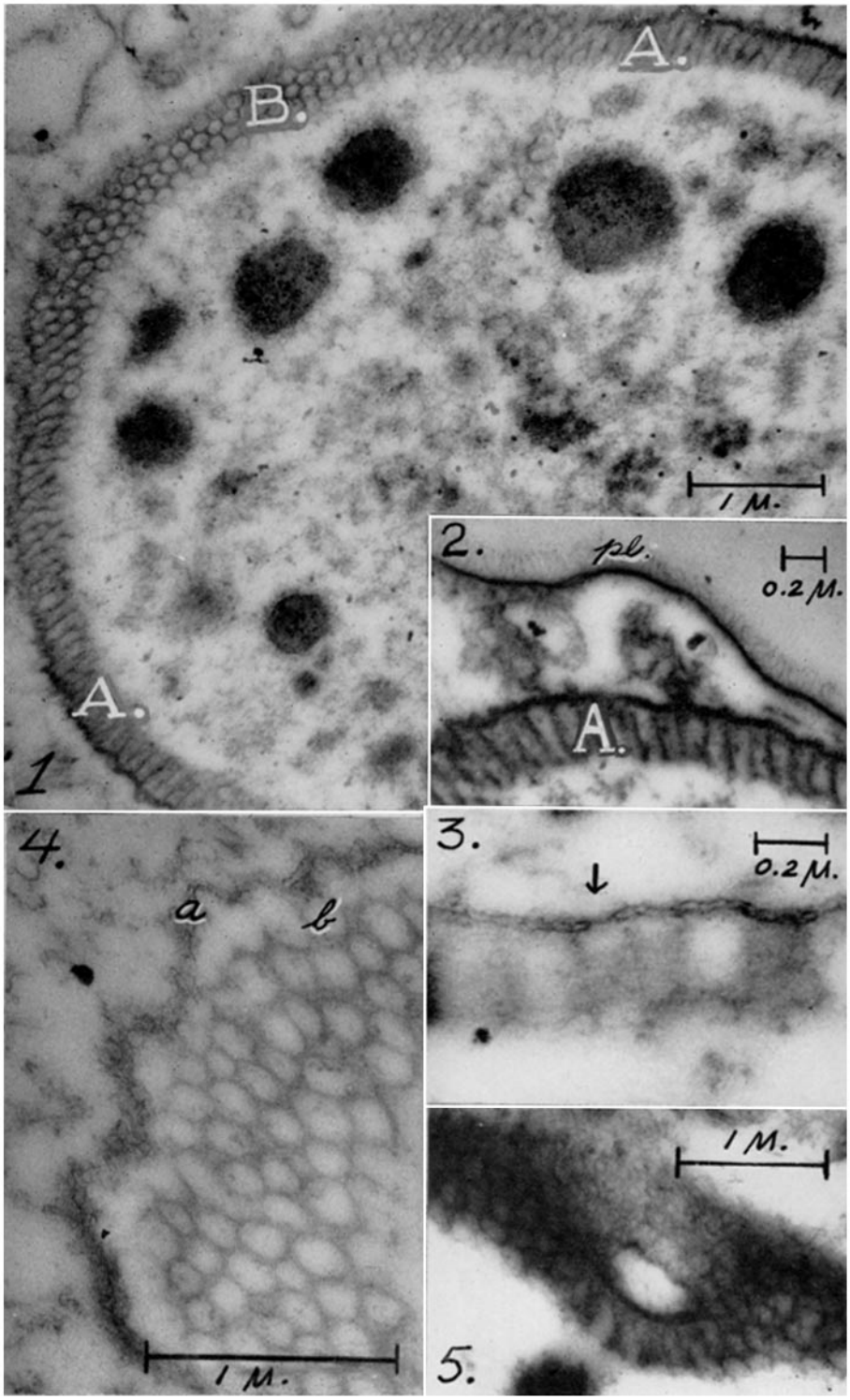
FIG. 1. Electron micrograph of a section of the nucleus of *Amoeba proteus*. The nucleus is cut normal to the nuclear envelope at *A*. At *B* the nucleus is cut tangential to the envelope. Note honeycomb appearance of the envelope at *B*. × 19,000.

FIG. 2. Section normal to the nuclear envelope (*A*), showing evenly spaced projections extending inwards from the nuclear surface about 1400 A apart and approximately 2500 A long. The plasmalemma (*pl*), the outermost limiting membrane of the ameba, shows its characteristic fine filamentous extensions. × 29,000.

FIG. 3. At higher magnification the outermost layer of the nuclear envelope is seen to consist of two membranes. A pore (at arrow) is formed where the inner and the outer membranes are joined with one another. The diameter of such pores is approximately 640 A. A dark band, or diaphragm, seems to cover the opening of this pore. × 47,500.

FIG. 4. An oblique section through the nuclear envelope. Along *a*, the double membrane of the nuclear envelope, the pores appear as ring-like structures. Hexagonal profiles representing the inner honeycomb structure are seen at *b*. × 35,000.

FIG. 5. Section cut tangential to the surface of the nuclear envelope. Note the location of the nuclear pore in the centers of the hexagonal profiles of the honeycomb structure. × 22,000.



(Pappas: Nuclear envelope of *Amoeba proteus*)