SOME DYNAMIC ASPECTS OF THE NUCLEAR ENVELOPE

R. W. MERRIAM, Ph.D.

From the Department of Zoology, Columbia University, New York. Dr. Merriam's present address is Department of Biology, State University of New York, Long Island Center, Oyster Bay, New York

ABSTRACT

Nuclei of frog oocytes were isolated, fixed in OsO_4 or $KMnO_4$, and washed. Nuclear envelopes were then dissected off, placed on grids, and air-dried for electron microscopy. Envelopes from immature oocytes at the stage of beginning yolk deposition were compared with those from mature oocytes. Envelopes from the immature stage had "pores" whose annuli contained more material and showed central globules in the center much more frequently than envelopes from mature eggs. Annuli and central globules had similar appearance and fixation properties, suggesting similar chemical composition. After fixation with $KMnO_4$, residual densities suggested that "pore" diaphragms are much more variable in thickness or composition in the younger stages. Envelopes of the immature oocytes had about 40 per cent more "pores" per unit area than mature envelopes. In crowding together, the "pores" tended to assume geometrical packing arrays in the young envelope, showing minimum center-to-center spacings of about 1530 A. Since the actual discontinuities in the membranes of the envelope are only about 950 A in diameter, this minimum distance of approach suggests that adjacent formations of the nuclear surface are associated with "pore" structure and perhaps set their limiting spacing distances. If this is true, then it can be deduced that "pore"-associated structures of the nuclear surface are probably circular in outline and about 1500 A in diameter. Isotopically labeled lysine was administered to intact, growing oocytes for 1 to 4 hours and the envelopes were subsequently isolated and fixed. Autoradiography of entire envelopes showed little or no incorporation of lysine into proteins, as compared with small fragments from other parts of the cell of roughly comparable mass. It was concluded that the isolated envelope, as seen in the electron micrographs, does not synthesize or turn over lysine-containing protein at a high rate.

INTRODUCTION

The development of information about the dynamic aspects of cellular membranes by means of the electron microscope is admittedly difficult. The nuclear envelope is no exception, and yet several ideas concerning its formation, extension, and metabolism have been advanced in the past few years.

For example, a number of studies on the formation of the nuclear envelope after mitosis (2, 18, 20, 23, 25) have produced a unanimity of opinion that the membranous elements are not formed *de novo* at the nuclear surface, but rather come from aggregation and fusion of morphologically undifferentiated membranes of the cytoplasm. Annulated "pores," which are characteristic of the envelope, apparently appear very early in this formative process (18).

A general impression that replicas of the envelope may be made at the nuclear surface has been gained from images showing concentric accumulations of membranes there. Concentric arrays of membranes, for example, may be seen

after virus infection (12) or during certain stages of oogenesis (16, 24, 29). Similarly, images showing blebbing of the membranous elements of the envelope into the cytoplasm (6, 11, 30, 32) have sometimes (*e.g.* 11, 30) been interpreted to mean that dynamic extensions from the nuclear surface establish a continuity between membranes of the envelope and other membrane systems of the cytoplasm. Indeed, it has been suggested (23) that the several membrane systems of the cytoplasm may be derived from the nuclear envelope.

Indirect bits of evidence have tended to indicate that the nuclear envelope is more than a passive structural entity. Several authors (*e.g.* 5, 7, 13, 15, 26–29) have suggested that it may actually be an agent in a metabolic or synthetic transfer of specific nuclear influences into the cytoplasm.

The present study was undertaken in the hope of gaining insight into the formation of the various structures which make up the nuclear envelope. To this end, isolated envelopes from immature oocytes were compared with mature ones, and isotopically labeled amino acids were presented to intact oocytes to see if envelope proteins (19) had an appreciable rate of incorporation.

METHODS AND MATERIALS

Oocytes from the ovaries of wintering frogs were broken in Barth's (4) solution. The isolated nucleus, cleaned of adherent cytoplasm by squirting medium over it, was immediately punctured with a glass needle to prevent envelope stretching due to nuclear swelling (19). The whole nucleus was then transferred to the fixative solution for exactly 5 minutes at room temperature. After the fixed nucleus was washed several times in distilled water, the envelope was peeled off with glass needles and placed flat on a carbon film over a grid. The preparation was air-dried and sandwiched under a layer of carbon for observation in the electron microscope.

Two fixatives were employed: 0.6 per cent aqueous $KMnO_4$ and 1.0 per cent aqueous OsO_4. Both were unbuffered, as it had previously been established (19) that buffering did not improve the image quality.

In each experiment, envelopes from fully developed oocytes were compared with those from immature oocytes from the same frog. The young cells were about 390 to 450 microns in diameter and milky in appearance, falling into Duryee's (9) stage 3 or early stage 4. All preparative procedures were kept as constant as possible, no differences between young and mature envelopes being considered significant unless demonstrated consistently in the several experiments. Altogether, 33 envelopes from seven frogs were observed.

A Philips 100 A electron microscope with an objective aperture of 25 microns and an accelerating voltage of either 40 or 80 kv was used. Pictures were taken at several settings of a continuously variable lens current. The magnification at each lens current setting was calibrated with a grating replica, but since no special measures were used, the dimensions presented must be considered close approximations.

MORPHOLOGICAL OBSERVATIONS

A cursory comparison of isolated nuclear envelopes from the two stages of oogenesis immediately reveals some differences. The dense patches containing circular fenestrae in close hexagonal packing (19) found on many mature envelope preparations (Fig. 1) are entirely missing from the immature ones (Fig. 2).

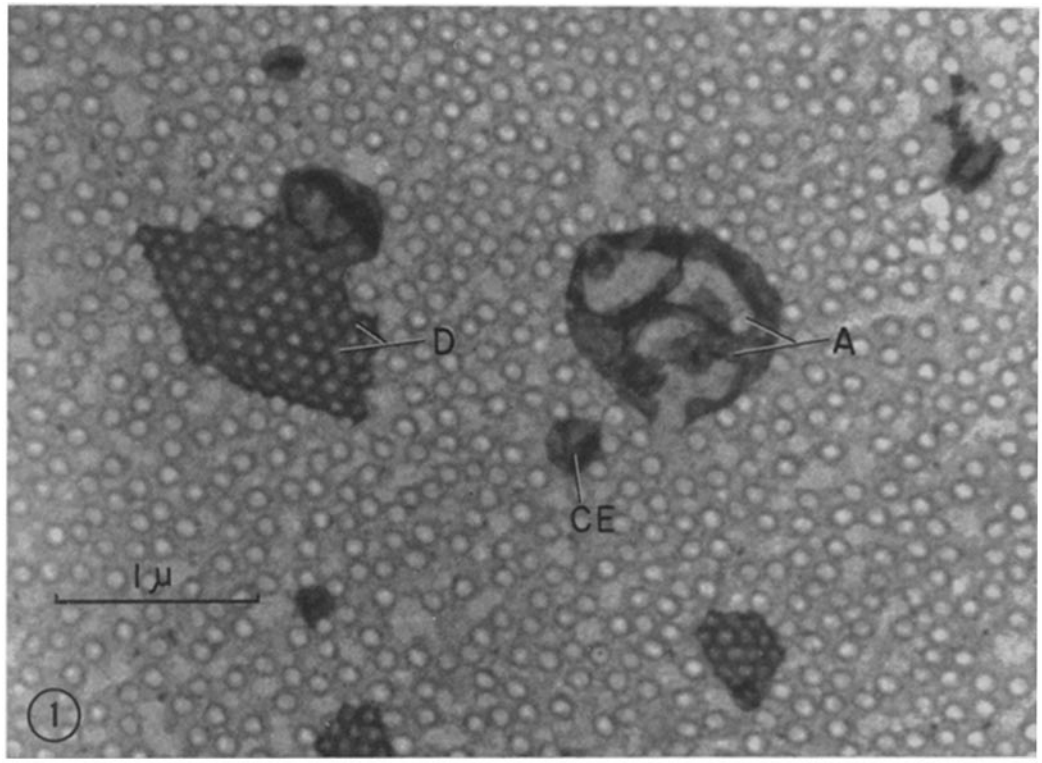
After OsO_4 fixation, the annuli of the younger states usually appear more electron opaque with respect to the surrounding membrane than do those of the mature oocyte. Moreover, the annuli of the younger cells, as measured on several

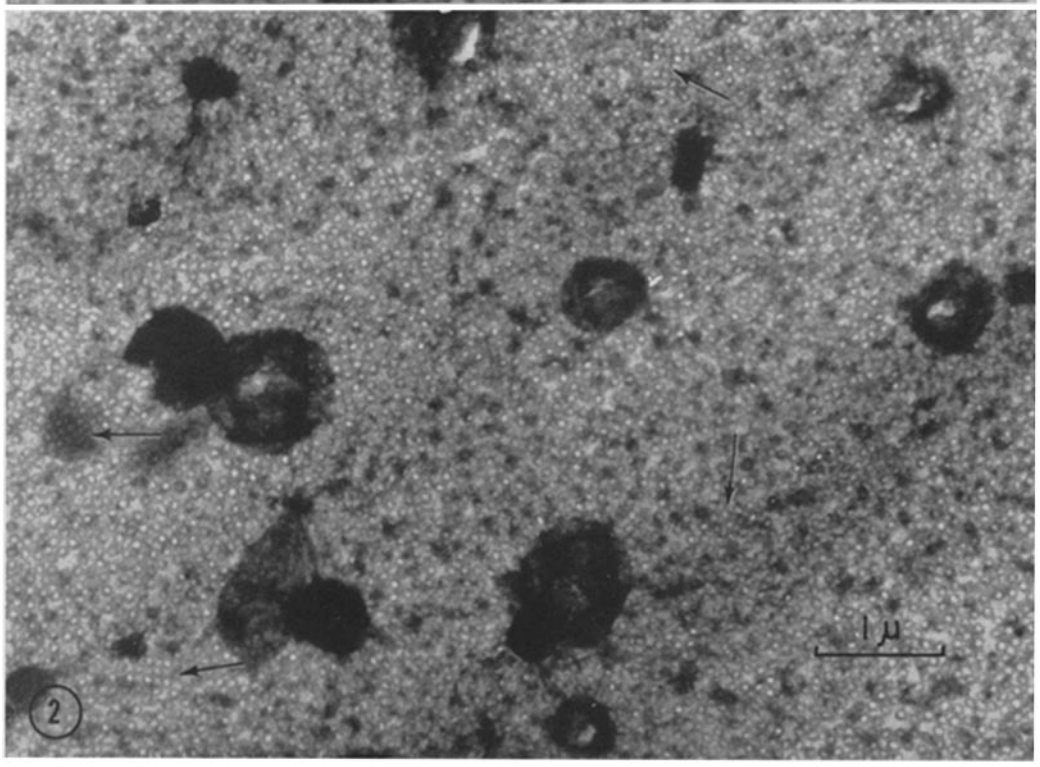
FIGURE 1

Envelope from a mature oocyte after fixation in $KMnO_4$. Superimposed on the envelope with its many annulated "pores" are patches of dense material (*D*) containing circular fenestrae in close hexagonal packing. A structural artifact due to the isolation technique can be seen at *A*, and membranous elements from the cytoplasm at *CE*. × 26,500.

FIGURE 2

Envelope from an immature oocyte after fixation in $KMnO_4$. There are characteristically no adherent dense patches, and the annulated "pores" are more closely spaced than in the mature envelope, showing localized tendencies to assume square packing arrays (arrows). × 16,500.


D
A
CE
1 μ
1


1 μ
2

prints, average 481 A between inside and outside edges, while those of the mature envelope are only 387 A (see dimensions of Table I). The differences in width of annular walls were statistically significant at the 0.5 per cent level, indicating that there is more material in annuli of the immature envelopes.

With OsO_4 fixation, the centers of the "pores" in both mature and young envelopes appear at least as dense as the surrounding general membrane (Figs. 5 and 6). With $KMnO_4$, on the other hand, the "pore" density is always less than that of the surrounding membrane and is quite uniform from "pore" to "pore" in the envelope of the mature oocyte (Fig. 3). In the younger envelope, on the other hand, there is typically a great variability in "pore" densities (Fig. 4). Apparently the diaphragm material, which is preserved by $KMmO_4$ and digestible with trypsin (19), is more variable in thickness or composition in the earlier stages of oogenesis.

The presence of globules of material in the centers of "pores" has frequently been noted in thin sections of the envelope (see *e.g.* 1, 31). It is of interest here that such central globules occur with a relatively high frequency in the immature envelope (Figs. 6 and 7) but only rarely in the mature ones (Fig. 5). Of 789 "pores" scored at random in mature envelopes, only 2 per cent had the central globule, while of 676 scored in immature envelopes, 16 per cent showed them.

Central globules and annular material are both preserved by OsO_4, but both are progressively lost during $KMnO_4$ fixation. In density and texture, images of annuli and central globules are similar (Fig. 7). Moreover, the higher incidence of central globules in the younger stages corresponds to the increased amount of annular material of these stages. For these reasons it is possible that central globules are composed of the same material as the annuli. Central globules seem to be part of the "pore" complex because they adhere to the "pore" diaphragm more tightly than to any nuclear or cytoplasmic structure from which they were parted. Specifically, they seem to be embedded in, or attached to, the diaphragm material stretching across "pores" of the envelope (19).

TABLE I

Measurement Data on "Pores" and Annuli in Osmium-Fixed Nuclear Envelope

Stage	Outside diameter of annuli			Inside diameter of annuli			No. of "pores" per μ^2		
	No. of observations	Mean	S.D.	No. of observations	Mean	S.D.	No. of areas counted	Mean	S.D.
Mature	30	1338	±75	35	563	±75	9	25.0	±2.4
Immature	33	1450	±113	26	488	±125	9	34.6	±3.1

OBSERVATIONS CONCERNING "PORE" FORMATION

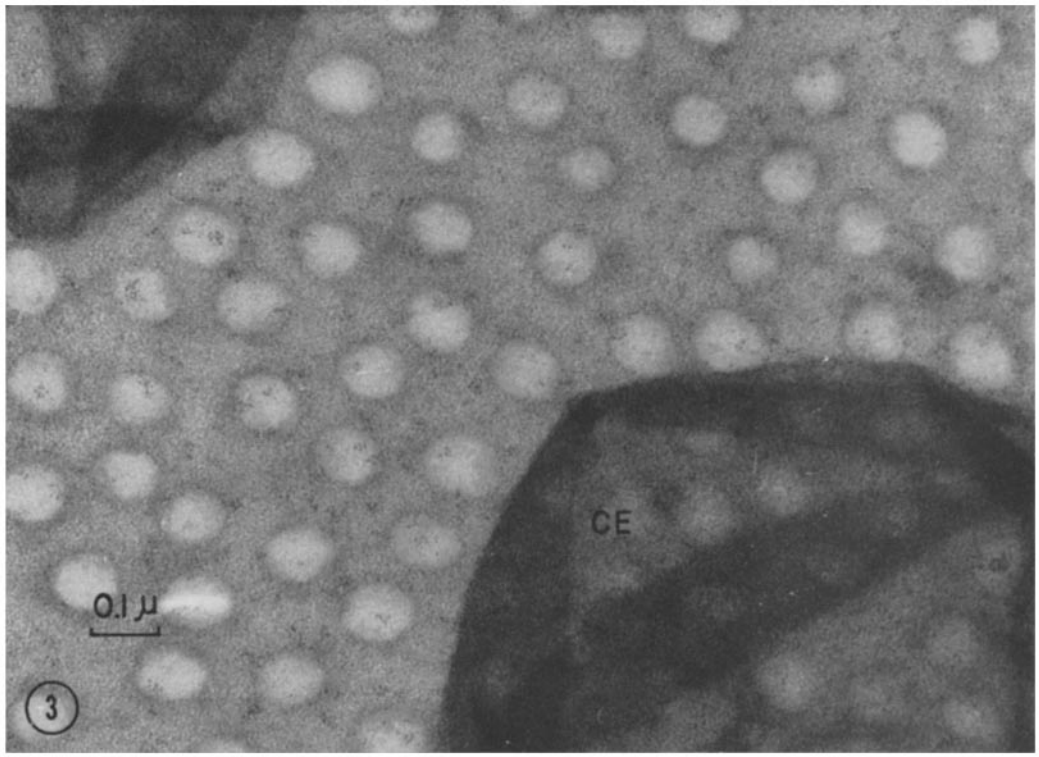
Counting the number of "pores" within known areas has revealed that in the mature nuclear envelope there are about 25 "pores" per square micron, while in the immature condition there are about 35 per square micron (see Table I). The crowding of "pores" in the immature en-

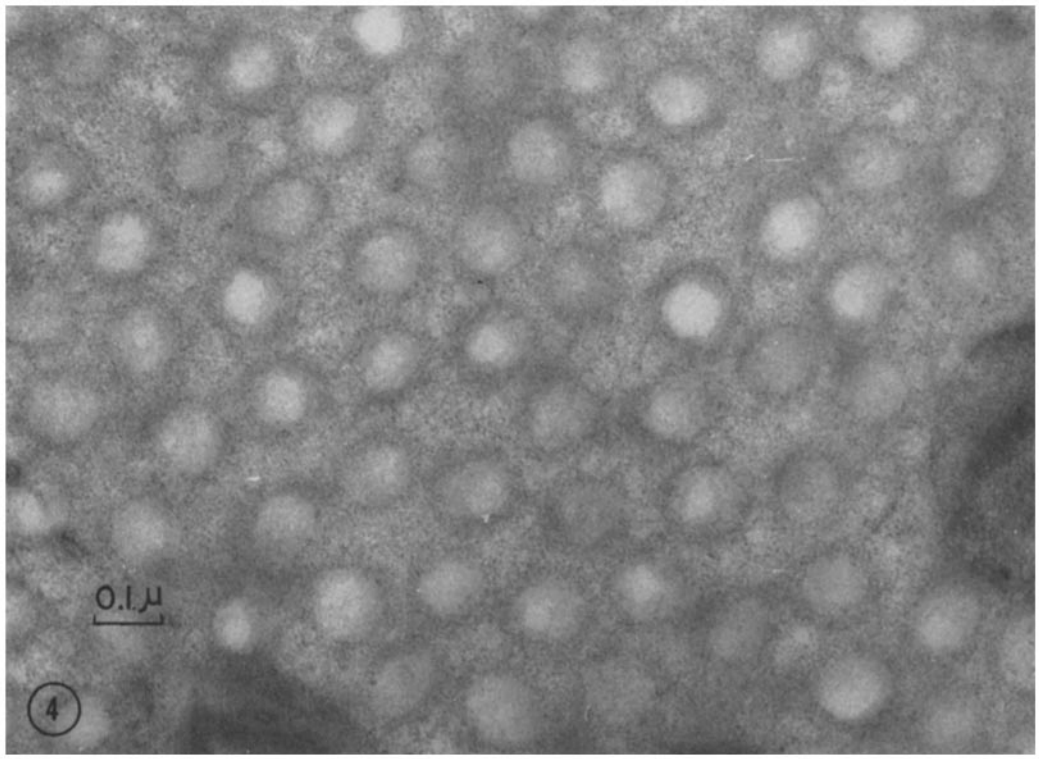
FIGURE 3

Envelope from a mature oocyte after fixation in $KMnO_4$. A cytoplasmic element can be seen adhering at *CE*. Note the relatively uniform density within the "pores" as compared with Fig. 4. × 86,000.

FIGURE 4

Envelope from an immature oocyte after fixation in $KMnO_4$. The "pores" show internal densities that range from something greater than that of the surrounding membrane to the appearance of no density at all. × 86,000.


CE
0.1 μ
3


0.1 μ
4

velope is so great that they tend to assume regular geometrical packing patterns. Such packing arrays are never extensive, seldom involving more than 4 to 10 "pores," but always in an orthogonally arranged square packing (arrows, Fig. 2).

The surfaces of both young and mature oocyte nuclei are very irregular, but approximations of comparative surface areas can be made by measuring their diameters. Taking into account the number of "pores" per unit area (Table I), one can estimate the total number of "pores" and the non-"pore" area on the surfaces of both mature and immature nuclei. From the calculations one learns that to account for the number of "pores" in the mature envelope, there must be an increase in the number of "pores" in the young nucleus by about a factor of 10. Similarly, the non-"pore" membrane must increase in area by a factor of about 13. Thus, there is little doubt that additional envelope, including "pores," must be formed as the immature oocyte grows to egg size.

If "pores" are being formed in the young stages studied, they could conceivably appear as small structures which grow to the final size or they could appear as full sized structures in unoccupied areas of the membrane. In the former situation one would expect to find a distribution of "pore" sizes ranging from small dimensions up to the definitive size. Actually, the frequency distribution of measurements on the outside diameters of annuli shows no skewness in the case of the immature envelopes. The smallest measured annulus of the immature preparations was 1188 A in diameter and the smallest of the mature envelopes was 1250 A, a difference well within the error of the measurements. It would seem that "pores," or at least the annuli which surround them, must be full sized when first appearing in the growing surface of the envelope.

When "pores" of the immature envelope are grouped in packing arrangement, it appears to be a situation of maximum crowding, the minimum distance from center to center being about 1530 A. When annular material is lost during $KMmO_4$ fixation, however, the "pore" itself measures only 930 A in diameter (19). Neglecting the annular material flattened out on the membrane around the "pore," there remains a distance of about 600 A between the edges of neighboring "pores."

As it happens, the edges of annuli appear to be nearly in contact when the "pore" centers are within 1530 A. From previous studies (19) of thin sections of the isolated envelope, however, it is known that annular material is spread out during drying, so that in life there would be appreciable membrane between the annuli even in the close packed arrays. It would seem that something else besides annuli must set the minimum approachable distance between "pores."

PROTEIN SYNTHESIS IN THE NUCLEAR ENVELOPE

Although growth of the nuclear envelope takes months and would probably be difficult to detect during hours of time by labeled amino acid incorporation, there is a possibility that the envelope might be continuously synthesizing or renewing proteins at a much faster rate (28). Such activity could presumably be due to formation or turnover of annulated "pores" or perhaps even to synthesis of proteins for nucleus or cytoplasm.

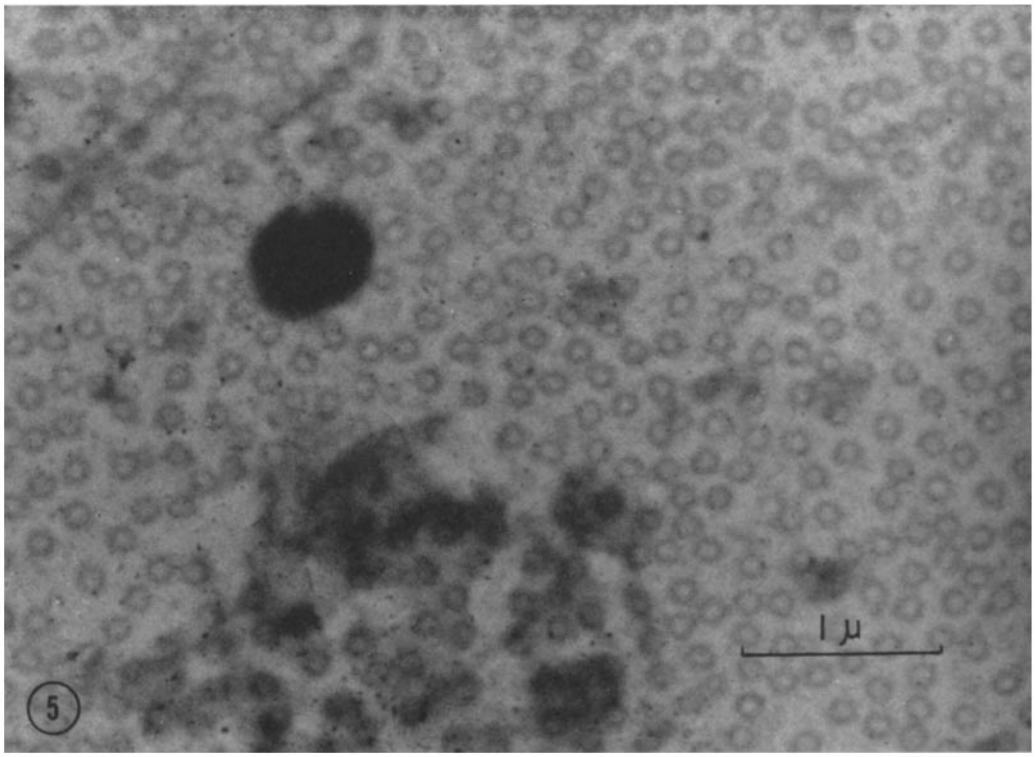
To check this possibility, half grown oocytes were taken from well fed frogs during the summer months when eggs are growing rapidly. Before each experiment the animals were force-fed for 1 or 2 days to eliminate possible starvation effects. Portions of whole ovary were removed and placed

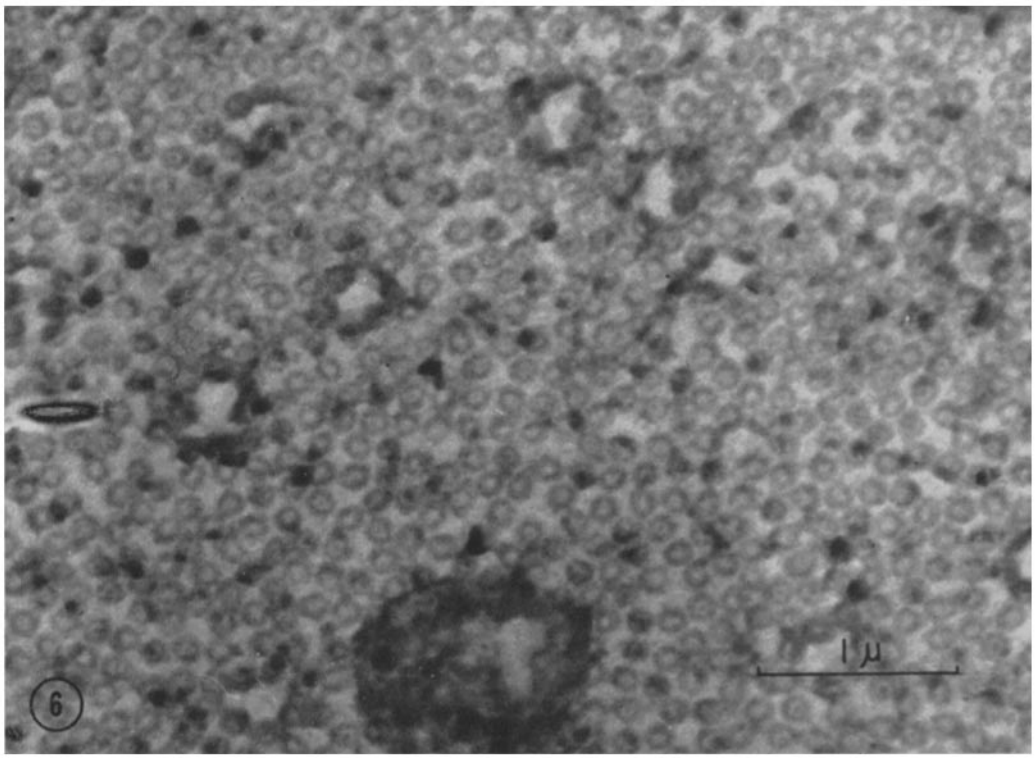
FIGURE 5

Mature envelope after fixation in OsO_4. Note the relatively less dense "pores," fewer "pores" per unit area, and fewer central globules as compared with Fig. 6. × 26,000.

FIGURE 6

Immature envelope after fixation in OsO_4. Note the relatively more variable density within the "pores," more prominent annuli, and higher incidence of central globules as compared with Fig. 5 × 26,000.


1 μ
5


1 μ
6

in Barth's solution containing 12×10^{-4} microcuries of C^{14}-lysine per milliliter (0.003 M). Under these conditions, whole oocytes incorporate the isotope into the residual solids left after hot trichloroacetic acid and lipid extractants. The amount incorporated into whole eggs or nuclei increases with time during the 1 to 4 hours of the experiments. After various times of exposure to the isotope, eggs were washed, then broken in aqueous 0.1 M KCl, and their nuclear envelopes were prepared as though for electron microscopy. Both $KMnO_4$ and OsO_4 fixations were used. Each isolated envelope was crumpled into a compact mass and deposited within a small circle etched on a glass slide, air-dried, and then covered with stripping film. After periods ranging from 30 to 70 days, the films were developed and examined for grains over the isolated envelopes.

With each envelope crumpled up, its mass was concentrated into a small area. Bits of contaminating structures with even less contrast in the phase microscope often showed high grain counts above them, indicating that other cellular solids of comparable mass contained easily detectable activity. However, $KMnO_4$-fixed nuclear envelopes uniformly showed no increase at all in grain count above that of the surrounding background. Grains over OsO_4-fixed envelopes always exceeded those of the background, but increases of similar magnitude could be noted over controls which had not been exposed to the label, presumably because of contaminating radioactive isotopes in the fixative. It was concluded that any lysine-containing proteins of the envelope are being synthesized or turned over at a rate appreciably lower than at least some other cellular solids of comparable mass. Any concept of the envelope as a center of general protein-synthesizing capacity, comparable in specific activity to

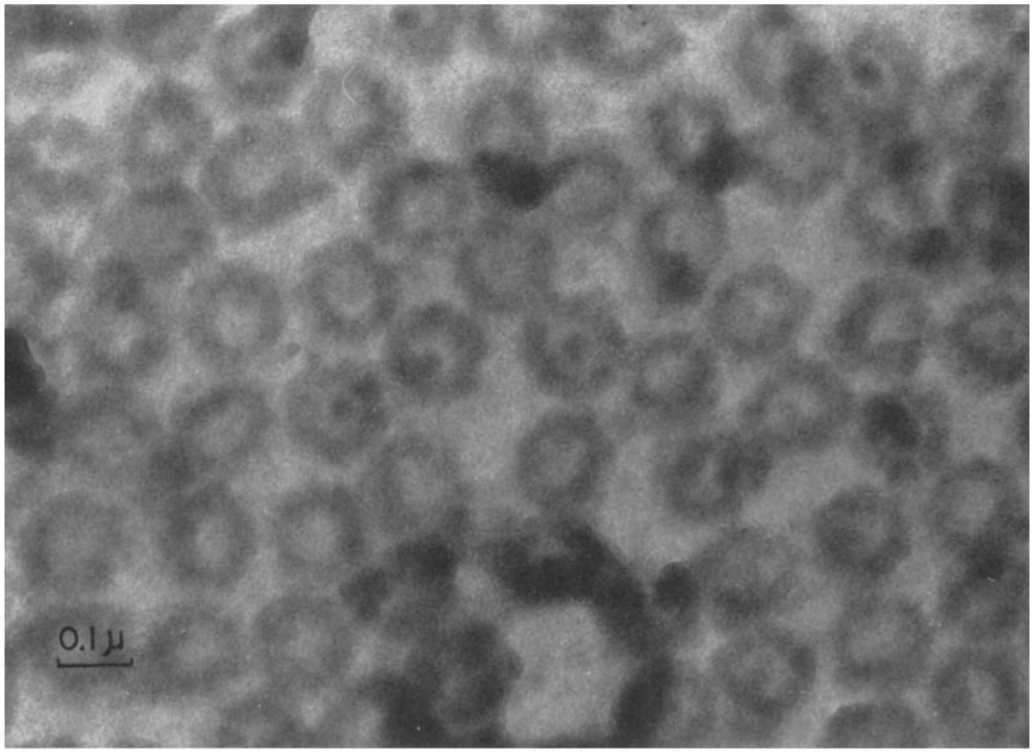


FIGURE 7

Immature envelope after fixation in OsO_4. Note the variable density within "pores" and the frequent presence of central globules. Material of the annuli and central globules is similar in appearance. × 93,000.

the ribosomes, for example, would appear to be ruled out by these crude experiments.

DISCUSSION

A. *Speculations about "Pore" Formation*

An interesting question concerning the origin of new "pores" in the expanding nuclear envelope can be raised at this point. Do they arise in a membrane which is surrounded by an essentially structureless environment, or are they reflections of structure adjacent to the membrane?

"Pores" and their associated material are not found in cellular membranes generally. They are confined to the membrane at the nuclear surface or to membranes probably formed originally at that surface (see *e.g.* 16, 29). It is also noteworthy that while "pores" are present in the membrane on the nuclear surface, extensions of the membrane into the cytoplasm do not show them (see *e.g.* 23, 32, 33). Moreover, it is difficult to think of the membranous elements of the envelope as being intrinsically special, since it has been established in a variety of materials (2, 18, 20, 23, 25) that the membranes themselves come from a general pool of cytoplasmic membranes, forming "pores" only after association with the nuclear surface (18).

On the basis of these considerations, let us make the reasonable assumption that "pore" formation is not an intrinsic property of the membrane itself but is imposed upon it by the nuclear surface. Starting with such an assumption, several things can be deduced about the two-dimensional nature of the nuclear surface.

To account conveniently for the packing arrays seen in the immature envelope where right angle measurements show a H/W ratio (Fig. 8) of 1.0, the unit structures, or plaques (a general term used here for communicative purposes), of the nuclear surface associated with "pore" formation could be circles, squares, or possibly octagons (Fig. 8 A, C). Such packing of hexagons would not give a ratio of 1.0 (Fig. 8 E).

The fenestrae in the dense patches found in association with many mature envelope preparations are circles of about the same diameter (655 ± 64 A, mean and standard deviation from 22 measurements on $KMnO_4$-fixed material) as the "pores" of the adjacent envelope (644 ± 82 A from 24 measurements) although their center-to-center spacing is only about 1119 ± 127 A (from 27 measurements). They possibly represent annulate lamellae (19) adherent to the envelope, the close hexagonal packing of their fenestrae giving an average measured H/W ratio on several prints of 0.86. From simple considerations, this could be efficiently obtained only by circles or hexagons (Fig. 8 B, F).

While it is *not* demonstrated that the same formative forces were operative in both types of structure, the most economical hypothesis would be that they were not originally formed by entirely independent means. It would then follow that circles, which could account for both types, are the most probable shape of "pore"-forming plaques at the nuclear surface (see General Discussion, below, on this point).

Such circles, as judged by minimum spacings in the immature envelope, would have a diameter of about 1530 A. Presumably only their central

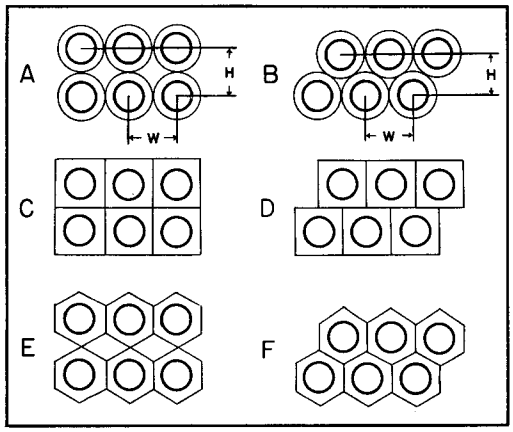


FIGURE 8

Schematic representations of possible packing arrays of "pores," in which the central dark circles represent the actual "pores" and the surrounding lighter lines the limits of associated nuclear structure, as postulated in the text. The H/W ratio, as used in the text, is graphically defined in A and B.

A, B. Two types of packing of circles which give H/W ratios of 1.0 and 0.86 respectively. These were the ratios actually measured in the arrays of the immature envelope and in the dense patches adherent to mature envelopes (Fig. 1).

C, D. Arrays of squares where both arrangements give an H/W ratio of 1.0, unlike the situation in the two types of measured arrays.

E, F. Arrangements of hexagons which both give ratios deviating from 1.0, unlike the situation in the two types of measured arrays.

parts would be concerned with the formation of "pores" 930 A in diameter.

During growth of the germinal vesicle in the frog oocyte, the data suggest that such circular plaques on the nuclear surface would form *de novo*, full size, wherever sufficient area was available. The pattern of two-dimensional distribution being generally random (Fig. 2), they probably arise structurally independent of neighboring circular areas.

During later stages of nuclear growth, "pores" become more widely spaced as the general membrane between them increases in relative area. They lose their tendency to form regular arrays, suggesting that underlying nuclear structures associated with them do not increase in area concomitantly. If they did, the "pores" could be expected to maintain packing arrays with an increased minimum spacing between centers. Further, if the presumed plaques do not increase in area but simply are pushed apart, it follows that nuclear surface structure is characterized during later stages of growth by a decrease in the rate of formation of new 1500 A plaques relative to the rate of increase in the surface area of intervening non-plaque material or structures.

B. *General Discussion*

With respect to the structural nature of the nuclear surface, it is interesting to recall that in amebae there is a cortical layer just under the nuclear envelope which can be seen by the usual techniques of electron microscopy (8, 21). It appears as a spongy layer about 2800 A thick and is divided, in this case, into hexagonally shaped areas about 1400 A across with a nuclear envelope "pore" centered in each.

Within nuclei from rat tissue also, Watson (31) has reported intranuclear "channels," circular in cross-sectional outline and about 1500 A across, which abut against the nuclear envelope at the locations of the 1000 A "pores." In these cells, two nuclear phases can be roughly distinguished: dense material, presumably chromatin, and a less dense phase consisting of the intranuclear "channels" and connected areas within the nucleus.

The term "channel," as used by Watson, suggests that its material moves through the denser chromatin to or from the "pore." Alternatively, one could think of the nucleus as a structural entity (less dense phase) in which the chromosomes are embedded. This would suggest that chromatin material was excluded from the regions of the "pores" because of structures there, approaching the envelope only between them. Such an interpretation would not interfere with ideas about substances moving through the "pores," but would strongly support the suggestion in this paper about the nuclear surface as a complex *structure*, even to the dimensions deduced. The nuclear plaques of this paper would correspond to the ends of Watson's intranuclear channels where they abut on the nuclear envelope. (For considerations about the relationship between structural or non-diffusible mass of the nucleus and diffusible mass, see reference 17.)

As early as 1954, Pollister, Gettner, and Ward (22) and Gall (10) reported central globules within the "pores" of amphibian oocyte nuclear envelopes. These observations were repeated in echinoderm eggs (1) the next year and in other materials later. Watson (31) has located the central globule at the waist of the "pore" in thin sections cut at right angles to the envelope. The observations of the present study also demonstrate that it sticks to the "pore" diaphragm even after the envelope has been torn away from its adjacent environment. This supports the data (19) on the existence of a "pore" diaphragm in frog oocytes and suggests that in other tissues as well, it may be located at the waist of the "pore" because it is attached to, or part of, a diaphragm.

Both the annuli and the central globules of "pores" are lost when fixation is in $KMnO_4$. Both are preserved by OsO_4. At high magnifications the dense materials of annuli and central globules have the same textural appearance. Furthermore, there exists a rough positive correlation between numbers of central globules and the amount of material in the annuli during the course of oogenesis. For these reasons, it is quite possible that annuli and central globules are composed of the same substance(s) and perhaps have the same function(s). A relationship between amount of annular material around "pores" and cell type has been reported previously (3), indicating that annular morphology may reflect function.

Mature oocytes in the ovaries of wintering frogs are in a state of suspended development as far as increase in mass or size is concerned. Unlike the mature oocytes, the immature ones show a

wide range of sizes, from about 400 microns in diameter to very small sizes, suggesting a slow but continuous increase in size. Still, there is no certainty that "pores" of the immature oocytes were actually in the process of formation. The fact that no decreasing series of "pore" sizes could be found in the immature envelopes should therefore be regarded with caution. By itself, the observation may not actually rule out the possibility that "pores" grow from small to definitive size. One should recall, however, that when new "pores" are appearing in the envelope forming about telophase nuclei of dividing *Chaetopterus* eggs (18), only the characteristic size of "pore" could be found. Taken together, the evidence does indeed suggest that "pores" arise as full sized entities.

The experiments to detect lysine incorporation into proteins of the nuclear envelope suffer from several limitations. Only one amino acid was used; its specific activity was relatively low; the irregular crumpling of the envelope and the difficulty of knowing whether the entire envelope was deposited made accurate quantitation of grain counts per unit area or per whole envelope impossible.

For these reasons, the experimental design rules out only the possibility that the oocyte envelope is a highly active agent in protein synthesis such as is suggested by the data of Sirlin and Elsdale (28). If the present findings in oocytes can be generalized, it probably means that the activities detected in sections of embryonic tissues by Sirlin and Elsdale represent nuclear or cytoplasmic solids closely associated with the nuclear envelope.

The findings in this study do not rule out the possibilities that envelope structures may be synthesizing a non-lysine-containing protein(s) in large quantities or that lysine-containing proteins may be synthesized at only moderate rates.

A final point may be mentioned. The mature oocyte not only has an unusually large nucleus with a maximum number of envelope "pores," but presumably it also has a maximum potential for genetic response to environmental factors. A progressive reduction in genetic potential, which occurs during cellular differentiation (14), is accompanied by progressive reduction in total number of "pores" per nucleus as nuclear size and surface area are progressively reduced during cleavage.

This work was done under a postdoctoral fellowship of the United States Public Health Service, Genetics Training Grant 2G-216(C2).

Received for publication, May 4, 1961.

BIBLIOGRAPHY

1. AFZELIUS, B. A., The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope, *Exp. Cell Research*, 1955, **8**, 147.
2. AMANO, S., and TANAKA, H., Electron microscope study on the disappearance and regeneration of the nuclear envelope during mitosis, *Acta Haematol. Japon.*, 1957, **20**, 319.
3. BAHR, G. F., and BEERMAN, W., The fine structure of the nuclear membrane in the larval salivary gland and midgut of *Chironomus*, *Exp. Cell Research*, 1954, **6**, 519.
4. BARTH, L. G., and BARTH, L. J., Differentiation of cells of the *Rana pipiens* gastrula in unconditioned medium, *J. Embryol. and Exp. Morphol.*, 1959, **7**, 210.
5. BERNHARD, W., Ultrastructural aspects of nucleo-cytoplasmic relationship, *Exp. Cell Research*, 1959, **6**, Suppl., 17.
6. BRANDT, P. W., and PAPPAS, G. D., Mitochondria. II. The nuclear-mitochondrial relationship in *Pelomyxa carolinensis* Wilson, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 91.
7. BRENNER, S., The chromatic nuclear membrane, *Exp. Cell Research*, 1953, **5**, 257.
8. COHEN, A. I., Electron microscopic observations of *Amoeba proteus* in growth and inanition, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 859.
9. DURYEE, W. R., Chromosomal physiology in relation to nuclear structure, *Ann. New York Acad. Sc.*, 1950, **50**, 920.
10. GALL, J. G., Observations on the nuclear membrane with the electron microscope, *Exp. Cell Research*, 1954, **7**, 197.
11. GAY, H., Nucleo-cytoplasmic relations in salivary gland cells of *Drosophila*, *Proc. Nat. Acad. Sc.*, 1955, **41**, 370.
12. GREGG, M. B., and MORGAN, C., Reduplication of nuclear membranes in HeLa cells infected with adenovirus, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 539.
13. KAUFMANN, B. P., and GAY, H., The nuclear membrane as an intermediary in gene-controlled reactions, *in The Nucleus*, 1958, **1**, 57.
14. KING, T. J., and BRIGGS, R., Changes in the

nuclei of differentiating gastrula cells, as demonstrated by nuclear transplantation, *Proc. Nat. Acad. Sc.*, 1955, **41**, 321.

15. MERRIAM, R. W., The nuclear envelope as a possible agent in specific synthetic events in the cytoplasm of sand dollar eggs, *Biol. Bull.*, 1958, **115**, 329.
16. MERRIAM, R. W., The origin and fate of annulate lamellae in maturing sand dollar eggs, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 117.
17. MERRIAM, R. W., Permeability and structural characteristics of isolated nuclei from *Chaetopterus* eggs, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 353.
18. MERRIAM, R. W., Nuclear envelope structure during cell division in *Chaetopterus* eggs, *Exp. Cell Research*, 1961, **22**, 93.
19. MERRIAM, R. W., On the fine structure and composition of the nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 559.
20. MOSES, M. J., Breakdown and reformation of the nuclear envelope at cell division, *Proc. 4th Internat. Conf. Electron Micr.*, Berlin, 1958, 230.
21. PAPPAS, G. D., The fine structure of the nuclear envelope of *Amoeba proteus*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 431.
22. POLLISTER, A. W., GETTNER, M., and WARD, R., Nucleocytoplasmic interchange in oocytes, *Science*, 1954, **120**, 789.
23. PORTER, K. R., and MACHADO, R. D., Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 167.
24. REBHUN, L. I., Electron microscopy of basophilic structures of some invertebrate oocytes. I. Periodic lamellae and the nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 93.
25. ROTH, L. E., Mitotic events in nuclei of *Pelomyxa* as observed by electron microscopy, *Anat. Rec.*, 1959, **134**, 631.
26. RUTHMANN, A., Basophilic lamellar systems in the crayfish spermatocyte, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 267.
27. SCHULTZ, J., Interrelations between nucleus and cytoplasm: Problems at the biological level, *Exp. Cell Research*, 1952, Suppl. 2, 17.
28. SIRLIN, J. L., and ELSDALE, T. R., Rates of labelling of RNA and proteins in cell components of the amphibian myoblast, *Exp. Cell Research*, 1959, **18**, 268.
29. SWIFT, H., The fine structure of annulate lamellae, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 415.
30. WATSON, M. L., The nuclear envelope. Its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
31. WATSON, M. L., Further observations on the nuclear envelope of the animal cell, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 147.
32. WHALEY, E. G., MOLLENHAUER, H. H., and KEPHART, J. E., The endoplasmic reticulum and the Golgi structures in maize root cells, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 501.
33. WHALEY, W. G., personal communication.