EVIDENCE FROM ELECTRON MICROGRAPHS FOR THE PASSAGE OF MATERIAL THROUGH PORES OF THE NUCLEAR MEMBRANE*, ‡

By EVERETT ANDERSON, Ph.D., and H. W. BEAMS, Ph.D.

(*From the Department of Zoology, State University of Iowa, Iowa City*)

Plate 146

It has been demonstrated by a number of investigators that the nuclear membrane is not a continuous structure but is interrupted at intervals by pores, nodes, granules, or ring-like formations (1–4, 9, 11–15, 18, 20). While most of the aforementioned authors emphasize the structure of the membrane, a few have considered its porous nature as significant in facilitating the interchange of materials between the nucleus and the cytoplasm (12, 20).

It is well known from light microscope and cytochemical studies that materials traverse the nuclear membrane in certain cells either as relatively large masses or as ultramicroscopic particles (6, 19, 21). The process involving the latter has been analyzed by means of the electron microscope and serves as the basis of this report.

Material and Methods

The material used in this investigation was obtained from the reduvid bug, *Rhodnius prolixus*. Ovaries were dissected out and placed in cold osmium tetroxide solution buffered at approximately pH 7.4 with acetate-veronal buffer. They were washed, dehydrated, infiltrated, and embedded in a mixture of 72 per cent *N*-butyl methacrylate and 28 per cent methyl methacrylate. Thin sections were obtained with an International Minot rotary microtome, fitted with a glass knife, and examined with an RCA model EMU-2B electron microscope without removal of the embedding medium.

For light microscope studies ovaries were fixed in Bouin's and Carnoy's solutions and stained in Heidenhain's hematoxylin. Some of the Carnoy-fixed material was treated by the Feulgen fuchsin-sulfurous acid reagent and counterstained with fast green.

OBSERVATIONS

The ovary of *Rhodnius* is a typical hemipteran type consisting of several lobes. Each lobe is composed of an anterior part, the end chamber, which con-

* Aided in part by Grant B-301 (C) from the National Institutes of Health, United States Public Health Service. Thanks are due Dr. T. C. Evans of the Radiation Research Laboratory for kindly allowing us the use of the electron microscope.

‡ We are indebted to Dr. Paul A. Woke, of the National Institutes of Health, Bethesda, for generously supplying us with the organisms.

tains the nurse cells, and a posterior part consisting of a series of developing oocytes. For convenience of description the end chamber has been arbitrarily divided into four zones (7, 19).

In light microscope preparations treated with Heidenhain's hematoxylin the nurse cells display a densely staining spherical nucleolus usually located near the center of the nucleus. Concentrated at certain localized regions adjacent to the nuclear membrane are seen darkly stained, irregular shaped masses of material. In the cytoplasm adjoining these are also observed masses of material of the same relative tinctorial behavior. Observations on a limited number of preparations reveal the above described material, both within the nucleus and the cytoplasm, to be Feulgen-negative.

Electron micrographs through the aforementioned regions of the cell show the Heidenhain hematoxylin-staining material located both within the nucleus and the cytoplasm to be composed of granules about 200 A in diameter (Figs. 1 and 2 (*NM*)). The nucleolus appears to be made up in part of a reticulum containing granules of the same relative size (Fig. 1 (*NO*)) (*cf.* reference 5). However, it appears more dense in electron micrographs because of the relative compactness of the granules within the reticulum. Small condensations of granules of the same order of size and density are frequently seen within the region between the nucleolus and nuclear membrane (Fig. 1 (*GNS*)).

It is evident from electron micrographs that the nucleus is surrounded by a membrane composed in part of pores having a diameter of approximately 400 A (Figs. 1 and 2 (*NP*)). In tangential sections the walls of the pores appear darkly outlined. It is difficult to determine whether or not the nuclear membrane is constructed of a single or double layer. In any case, the pores appear at times to completely interrupt it. Of special interest is the extension of the granular nuclear material into the cytoplasm through pores of the nuclear membrane (Fig. 1 (*A*) and Fig. 2 (*D*)). That is to say, in some preparations the granular material of the nucleus is continuous with that of the adjoining cytoplasm through pores of the nuclear membrane. Supporting this direct evidence for the transfer of nuclear material to the cytoplasm through pores of the nuclear membrane is a considerable amount of indirect evidence. The latter is seen in the form taken by the small masses of granules located adjacent to the openings of the pores. These often show extensions in the form of pseudopodia-like processes that are directed toward and sometimes come in direct contact with the nuclear membrane pores (Fig. 1 (*A*, *B*)). It is perhaps significant that the granules in the nucleolus (Fig. 1 (*NO*)), nuclear sap (Figs. 1 and 2, (*NM*)), and cytoplasm (Figs. 1 and 2, (*CM*)) are the same relative size and density (*cf.* references 16, 17). When aggregated they appear more dense than when isolated or in relatively small groups.

DISCUSSION

Literature from light microscope studies contains many references to the migration of material (mainly basophilic) from the nucleus to the cytoplasm (21). A striking demonstration of this condition is to be found in the nurse cells of certain insects. For example, Bonhag (6) using cytochemical methods on the nurse cells of the milkweed bug, *Oncopeltus fasciatus*, found in addition to some Feulgen-positive material, a copious amount of basophilic substance being extruded from the nucleus into the cytoplasm. Schrader and Leuchtenberger (19) studying the nurse cells of the coreid bug, *Acanthocephala*, observed the material extruded from the nucleus, unlike the nucleolus, to be largely Feulgen-positive. In the nurse cells of *Rhodnius* the masses of granular material suspected of migrating from the nucleus to the cytoplasm are largely Feulgen-negative. However, in degenerating cells, masses of Feulgen-positive material may be seen in the cytoplasm. In this connection it should be pointed out that the end chamber of the hemipteran ovary contains cells in different stages of physiological activity and cytomorphosis. Those located proximal to the ova sometimes fuse and degenerate, their contents passing into the ova as nutritive material (19). However, the cells studied here occur in the upper layers of the end chamber and are considered "normal" (*i.e.*, not degenerate), yet highly specialized.

We are cognizant of the fact that observations on the ultrastructure of nurse cells of *Rhodnius* have limitations with respect to any general interpretation of the functional relationship between the nucleus and cytoplasm. However, it is interesting to note that nurse cells reveal in electron micrographs evidence which is consistent with the cytochemical studies of Brachet (8), Caspersson (10), and others, namely, that ribonucleic acid is in part formed in the nucleolus, diffuses through the nuclear membrane, and contributes to the ribonucleic acid content of the cytoplasm. De Robertis (12) has also observed particles of the same relative size and density on both sides of the porous nuclear membrane in earthworm and frog nerve cells. He interprets this relationship as suggestive evidence for the nuclear origin of a part of the basophilic substance in the cytoplasm. As previously mentioned, other authors have observed nuclear pores in several different types of cells. However, none of the cells so far studied appears to show the material in the process of diffusing through the pores of the nuclear membrane as clearly as do the nurse cells of *Rhodnius*.

SUMMARY

1. The nurse cells of *Rhodnius* possess nucleoli that stain with Heidenhain's hematoxylin but give a negative Feulgen reaction. In localized positions adja-

cent to the nuclear membrane are seen masses of material both within the nucleus and the adjoining cytoplasm that stain with Heidenhain's hematoxylin, but, like the nucleolus, give a negative Feulgen reaction.

2. Electron micrographs of the nurse cells of *Rhodnius* reveal the nuclear membrane to contain pores approximately 400 A in diameter.

3. In electron micrographs the nucleolus is seen to be composed of a reticulum containing tightly packed granules. Between the centrally located nucleolus and the nuclear membrane are observed relatively small bunches of granules of the same relative size as those occurring in the nucleolus. Aggregated at certain positions adjacent to the nuclear membrane both within the nucleus and in the adjoining cytoplasm are irregularly shaped masses of granules. Certain of these masses within the nucleus are seen to be continuous with those in the cytoplasm through narrow isthmuses of material extending through pores of the nuclear membrane. Other masses of granules show evidence of preparing to enter the pores by projecting tongues of material toward and into them. In the adjacent cytoplasm pear-shaped masses of granules are seen in front of and in contact with the pores which suggests that they were fixed in the process of or just after completing passage through the pores.

BIBLIOGRAPHY

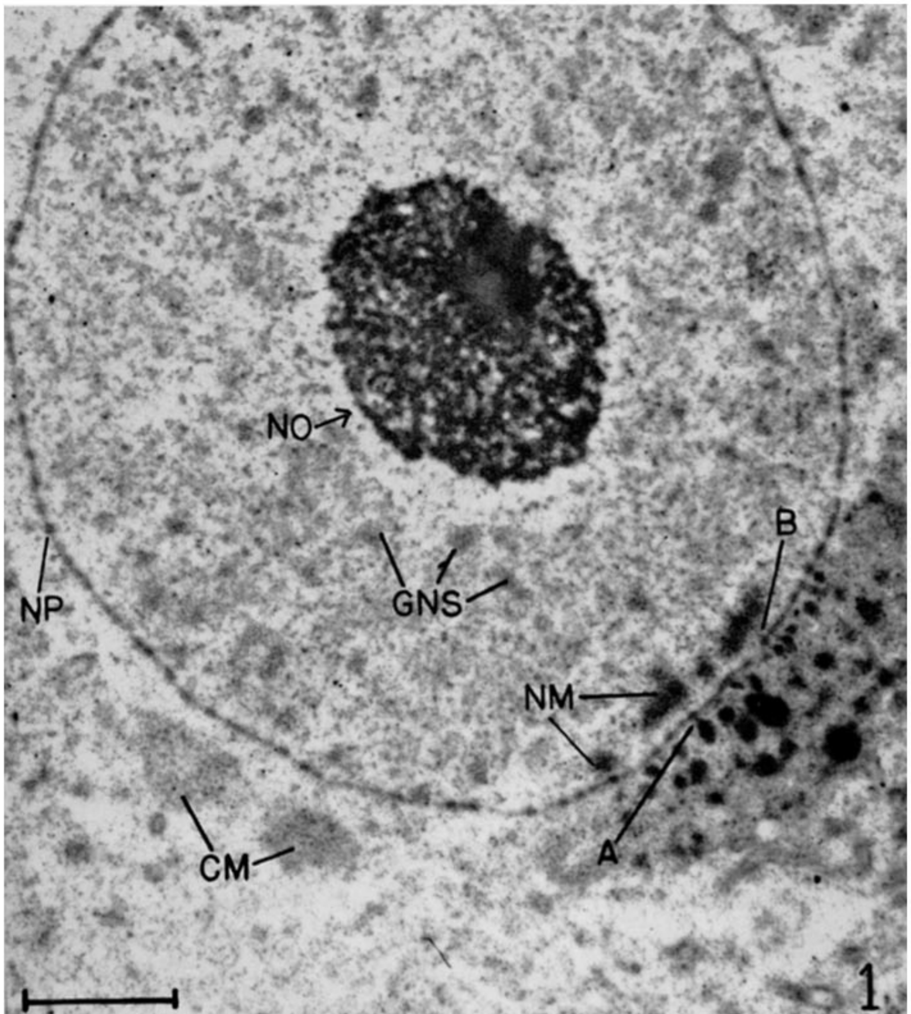
1. Afzelius, B. A., *Exp. Cell Research*, 1955, **8,** 147.
2. Bahr, G. F., and Beerman, W., *Exp. Cell Research*, 1954, **6,** 519.
3. Bairati, A., and Lehmann, F. E., *Experientia*, 1952, **8,** 60.
4. Beams, H. W., Tahmisian, T. N., Anderson, E., and Devine, R. L., *Proc. Soc. Exp. Biol. and Med.*, 1956, **91,** 473.
5. Bernhard, W., Bauer, A., Groop, A., Haguenau, F., and Oberling, C., *Exp. Cell Research*, 1955, **9,** 88.
6. Bonhag, P. F., *J. Morphol.*, 1955, **96,** 381.
7. Bonhag, P. F., *J. Morphol.*, 1955, **97,** 283.
8. Brachet, J., Chemical Embryology, New York, Interscience Publishers, Inc., 1950.
9. Callan, H. G., and Tomlin, S. G., *Proc. Roy. Soc. London, Series B*, 1950, **137,** 367.
10. Caspersson, T., Cell Growth and Cell Function, New York, W. W. Norton Company, 1950.
11. Dawson, I. M., Hassack, J., and Wyburn, G. M., *Proc. Roy. Soc. London, Series B*, 1955, **144,** 132.
12. De Robertis, E., *J. Histochem. and Cytochem.*, 1954, **2,** 341.
13. Gall, J. G., *Exp. Cell Research*, 1954, **7,** 197.
14. Gay, H., *Proc. Nat. Acad. Sc.*, 1955, **41,** 370.
15. Harris, P., and James, T. W., *Experientia*, 1952, **8,** 384.
16. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
17. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2,** 345.

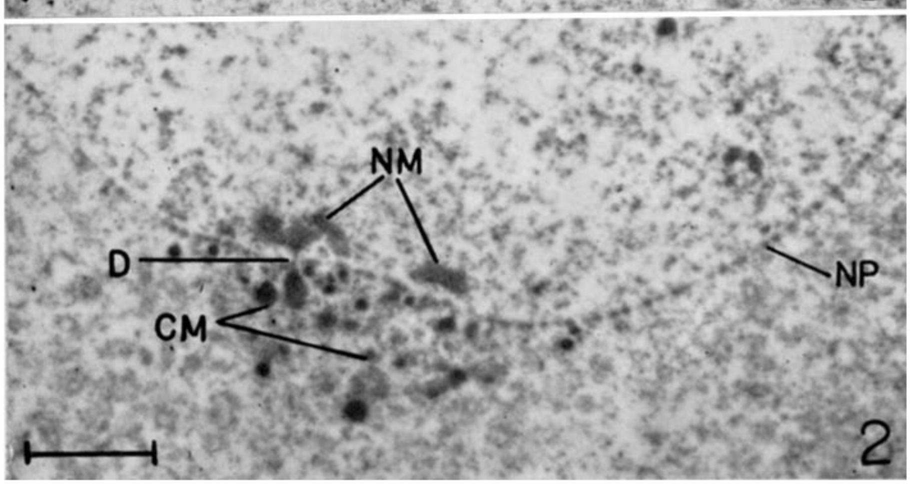
18. Rhodin, J., Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954, 1.
19. Schrader, F., and Leuchtenberger, C., *Exp. Cell Research*, 1952, **3,** 136.
20. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 197.
21. Wilson, E. B., The Cell in Development and Heredity, New York, The Macmillan Company, 1925.

EXPLANATION OF PLATE 146

FIG. 1. Electron micrograph of a nurse cell showing the granular nucleolus (*NO*), granules in the nuclear sap (*GNS*), nuclear pores (*NP*), and granules aggregated adjacent to the nuclear membrane some of which are in contact with the pores of the membrane (*B*). In the adjacent cytoplasm may be seen granules of the same relative size and density (*CM*). Small pear-shaped masses of these appear in contact with the nuclear membrane pores (*A*).

FIG. 2. Electron micrograph of nurse cell showing the granules within the nucleus (*NM*) and cytoplasm (*CM*). In addition, the granules of both the nucleus and cytoplasm may be seen continuous through a pore of the nuclear membrane at *D*.





(Anderson and Beams: Passage of material through nuclear membrane pores)