A COMPARATIVE STUDY OF NUCLEOCYTOPLASMIC INTERACTIONS

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It is evident that the pores of the nuclear envelope function as pathways for macromolecular exchanges between the nucleus and cytoplasm (1, 2). It is of interest, therefore, that the total pore area varies markedly in different cell types (3), as does the ultrastructure of the electron-opaque annular material associated with the pores (4). It has proven difficult, however, to determine the relative importance of these structural variations in controlling the exchange of macromolecules across the nuclear envelope.

For obtaining such structure-function correlations, a comparative study was undertaken with two cell types, amebae and oocytes. Colloidal gold was injected into the cytoplasm of the cells, and the relative rates at which the gold particles entered the nucleoplasm were determined. The results, which were taken as a measure of nucleocytoplasmic exchange, were correlated with morphological data relating to the size and number of the nuclear pores in each cell type.

The rate of incorporation of colloid into the nuclei of ameba was significantly greater than the rate obtained for oocyte nuclei. It was found that the rates of exchange were not a function of pore area, and it was concluded that the electron-opaque material associated with the pores, at least in this instance, is of primary importance in regulating nucleocytoplasmic interactions.

MATERIALS AND METHODS

The cells used in this investigation included interphase specimens of the multinucleated ameba *Chaos chaos*, and immature oocytes from frogs (*Rana pipiens*) and roaches (*Periplaneta americana*).

Amebae were cultured in an inorganic salt solution maintained at approximately 24°C and were fed *Paramecium aurelia* (1). Frog oocytes, 200–350 μ in diameter, were dissected in calcium-free Ringer's solution from ovaries that had previously been removed from the abdominal cavity. For obtaining preparations of roach oocytes, ovarioles were removed from the ovaries of decapitated animals and were placed in a solution containing 155.1 mM NaCl, 12.2 mM KCl, 4.5 mM $CaCl_2$, 4.0 mM $MgCl_2$, 1.0 mM KH_2PO_4, and 2.1 mM $NaHCO_3$ (5). The ovarioles (eight per ovary) are composed of linearly arranged oocytes and can be divided into six morphologically distinct zones; the oocytes used in this study were located in Zone IV. Since the cells can be injected while still in the ovarioles, no further dissection was necessary. A detailed description of both the ovarioles and the developing oocytes of roaches has been published by Anderson (6).

Colloidal Gold Preparations

Colloidal gold particles, ranging in diameter from 25 to 55 A and stabilized with polyvinylpyrrolidone (PVP), were prepared as described in an earlier communication (7). Prior to injection, the gold sols were dialyzed against salt solutions known to be compatible with the experimental cells (see Table I). The composition of the intracellular solutions used for amebae and frog oocytes was based on an analysis of the cytoplasm of these cells (8, 9). The intracellular roach medium, although not based on cytoplasmic analysis, was found to be compatible with the oocytes in preliminary injection experiments.

Injection Experiments

Gold sols were microinjected into the cytoplasm of the experimental cells by the techniques outlined by Chambers and Kopac (10). Amebae were easily injected and are unaffected by the procedure (1). Initially, some difficulty was encountered in penetrating the layer of follicle cells and connective tissue which surround both frog and roach oocytes. This was overcome by holding the preparations against the surface of the moist chamber with a glass rod; a micropipet was then placed adjacent to the rod and advanced toward the cells. In this way, sufficient pressure could be exerted to penetrate the surrounding tissue. Following the injections, the oocytes were carefully examined with the light microscope and any cells which appeared to be injured were discarded. Subsequent examination with the electron microscope failed to reveal any ultrastructural changes in the experimental cells.

In all instances, the cells were fixed 50 min after injection in 1 or 2% OsO_4 adjusted to pH 7.4 with Veronal-acetate buffer. The fixation times were 10 min for amebae and 20–30 min for oocytes. The subsequent procedures were the same for both cell types and involved dehydration in a graded series of alcohols followed by embedding in Araldite. Sections approximately 750 A thick were then cut and ex-

amined with either an RCA-EMU-3C or Hitachi HS-8 electron microscope.

The relative rates at which the gold particles entered the nuclei in the different cell types were determined by making particle counts. Electron micrographs were taken of each experimental cell, and the gold particles were counted in equal and adjacent volumes of nucleoplasm and cytoplasm. The results are expressed as the per cent of the total count present in the nucleoplasm. Further details of the counting procedure are discussed in reference 11.

Morphological Observations

Morphological studies were performed for determining the relative number and size of the pores in amebae and oocytes. Pore counts were made from electron micrographs of sections cut perpendicularly to the nuclear envelopes of the injected cells. The lengths of envelope examined were computed with a map measurer, and the results are given as the number of pores per micron of envelope. Since the methods for fixation, embedding, and sectioning were the same for all cells studied, the values obtained should indicate *relative* differences in pore number. An alternate method of determining the number of pores would be to use tangential sections; however, such sections were difficult to obtain for amebae, probably because of the extensive folding of the envelopes. Pore diameters were measured directly from perpendicular views of the envelopes.

TABLE I

Composition of Solutions Used for Microinjection

Cell type	Concentration				
	KCl	NaCl	K_2HPO_4	KH_2PO_4	$NaHCO_3$
	(mM)	(mM)	(mM)	(mM)	(mM)
Amebae	12.7	—	1.6	1.1	—
Roach oocytes	180.0	—	—	1.0	2.1
Frog oocytes	102.1	11.1	7.2	4.8	—

RESULTS

It can be seen in Table II that the rate of uptake of collidal gold by ameba nuclei is significantly greater than either of the rates obtained for oocyte nuclei. The differences in uptake between the frog and roach oocytes are not statistically significant. Electron micrographs of injected oocytes are shown in Figs. 1 and 2. Micrographs of injected amebae have been published in previous reports (1, 7, 11).

The morphological data collected from analysis of the pores are given in Table III. The main point to be derived from these results is that the total pore area, which is a function of pore size and pore number, is *not* greater in amebae than in oocytes.

DISCUSSION

To interpret the present results, it is first necessary to consider whether the differences in the rates of gold uptake are a function of nuclear envelope permeability. Aside from permeability differences, there are three ways to account for the relatively small number of gold particles found in the nuclei of oocytes.

The first possibility is that following injection the particles were removed from solution, presum-

TABLE II

Gold Distribution

Cell No.	Total volume examined (μs)*	Particles in nucleoplasm	Particles in cytoplasm	Particles in nucleoplasm
		No.	*No.*	%
A. Amebae				
1 (9)‡	10.4	5074	4773	52
2 (9)	10.4	3618	3994	48
3 (9)	11.0	1113	709	61
4 (8)	10.4	346	201	63
5 (5)	6.1	1024	867	54
Total	48.3	11,175	10,544	52
B. Frog oocytes				
1	1.4	5	780	0.6
2	0.9	3	519	0.6
3	1.4	4	222	1.8
4	1.8	9	388	2.3
5	1.9	15	845	1.7
6	1.6	3	387	0.8
Total	9.0	39	3141	1.2
C. Roach oocytes				
1	0.9	13	687	1.9
2	0.9	10	1119	0.9
3	0.9	20	717	2.7
4	0.8	12	644	1.8
5	0.9	2	403	0.5
Total	4.4	57	3570	1.6

* Half of each value represents nucleoplasm and half cytoplasm.

‡ The values in parentheses show the number of nuclei examined in each ameba.

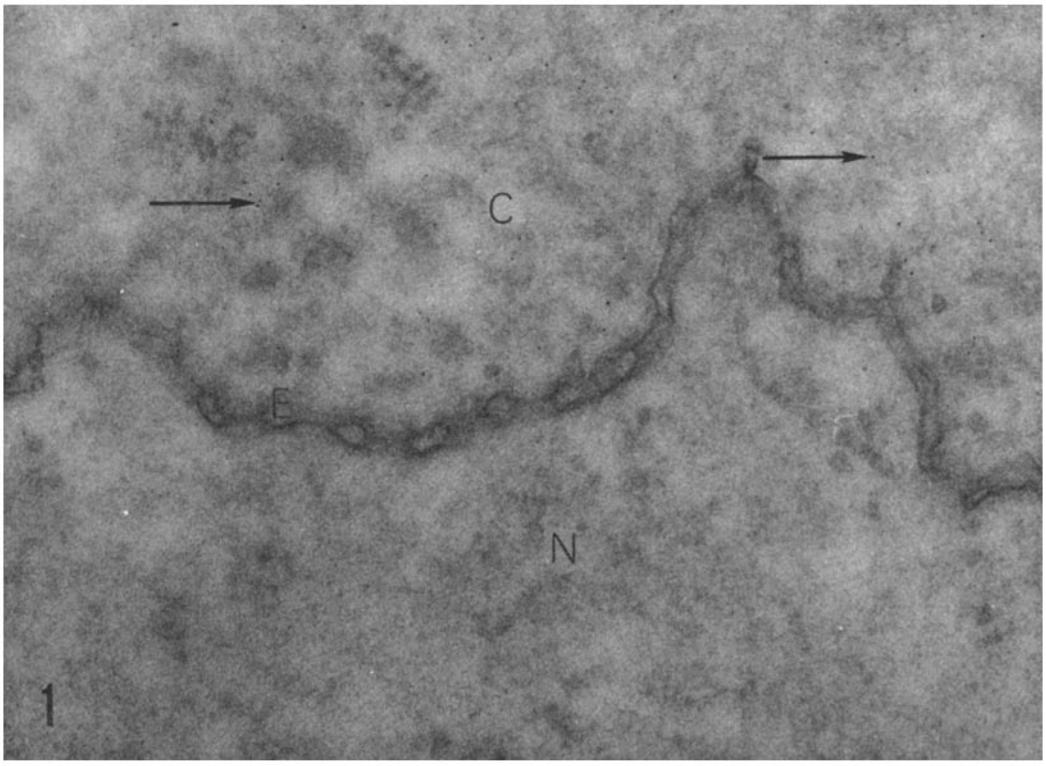

FIGURE 1 An electron micrograph of an injected frog oocyte. The gold particles (arrows) are located almost entirely in the cytoplasm (*C*). *N*, nucleoplasm; *E*, nuclear envelope. × 84,000.

ably by binding to a cytoplasmic component, and were not available to the nuclei. This, however, is not consistent with the observation that the particles located within the cytoplasm were randomly distributed and were frequently in contact with the outer surface of the nuclear envelope. In addition, PVP remains osmotically active after being injected into the cytoplasm of immature frog oocytes (12). This would not be the case if the PVP were removed from solution. Since the gold particles were coated with PVP and take on the properties of this substance, it can be assumed that the gold sols would also remain suspended in the cytoplasm.

The second way of explaining the present results involves a mechanism by which the colloidal particles could be pumped out of the nucleoplasm, with the rate of removal varying in different cells. Although no experimental evidence could be obtained regarding this possibility, it seems highly unlikely that such a specific mechanism would exist for the removal of a foreign substance.

The third possible explanation for the observed gold distributions is based on the fact that the large, spherical nuclei of oocytes have a greater volume-to-surface area ratio than ameba nuclei. Thus, even if the permeability of the envelopes to colloidal gold was the same in both cell types, the concentration of colloid in the oocyte nuclei would be lower due to increased dilution. The significance of such a dilution factor was determined by calculating the volume-to-surface area ratios. It was found that the ratios for roach and frog oocyte nuclei were, respectively, five and seven times greater than the ratio for ameba nuclei. These values, however, are much too low to account for the 33–44-fold differences in gold uptake.

On the basis of the above discussion, it seems reasonable to assume that the observed differences in nucleocytoplasmic exchanges are due to variations in nuclear permeability. As already indicated, the pores of the nuclear envelope represent important pathways for the passage of macromolecules between the nucleoplasm and cytoplasm. The pores, however, are not simply unobstructed

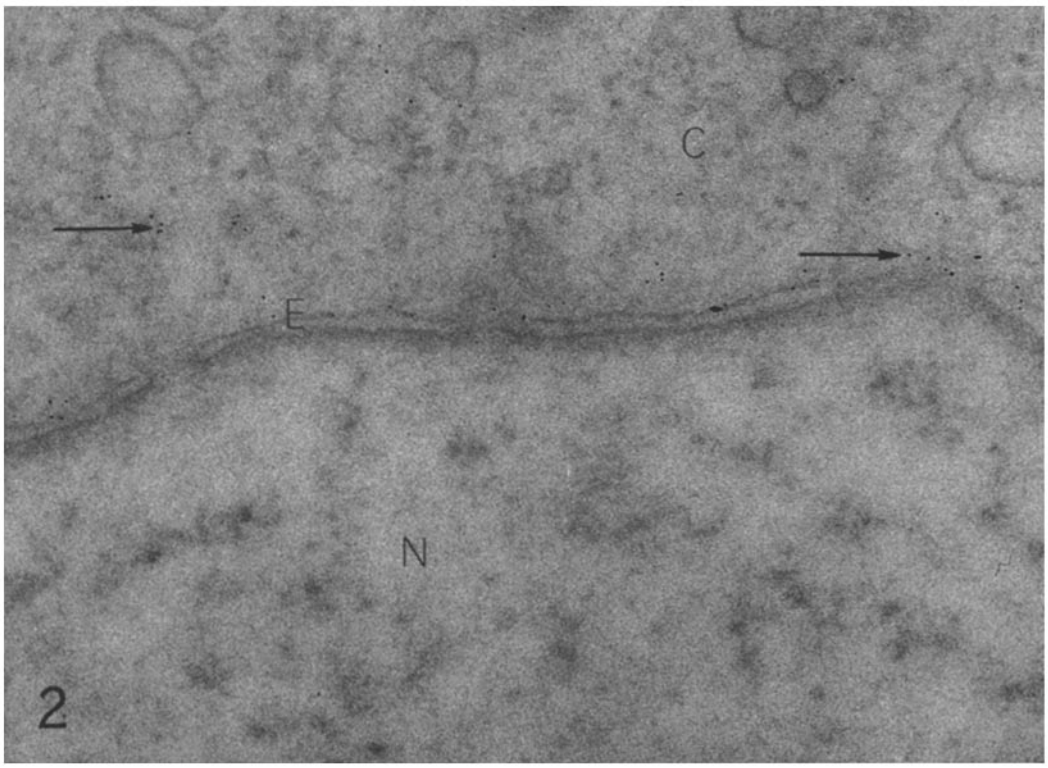

FIGURE 2 A section through an injected roach oocyte. The gold particles (arrows) are concentrated in the cytoplasm (*C*). *N*, nucleoplasm; *E*, nuclear envelope. × 84,000.

TABLE III

Analysis of the Number and Size of the Nuclear Pores

Cell type	Pore size		Pore number	
	No. of pores measured	Mean pore diameter ± SE	Length of envelope examined	Mean No. of pores per μ of envelope ± SE
		A	μ	
Amebae	10	615 ± 19	43 (15)*	1.8 ± 0.1
Frog oocytes	48	655 ± 7	49 (6)	2.9 ± 0.2
Roach oocytes	11	660 ± 12	14 (5)	1.8 ± 0.3

* The values in parentheses indicate the number of nuclei examined.

spaces, but contain an electron-opaque annular material which can affect exchanges across the envelope (7). It follows that nucleocytoplasmic interactions can be controlled in at least two ways: first, by variation of the number of pores, and second, by variation of the composition or amount of the annular material. Both of these mechanisms probably contribute to the regulation of nucleocytoplasmic interactions, but the present results suggest that the nature of the annular material is of primary importance. This conclusion is based on the observation that the nuclei of ameba are more permeable than oocyte nuclei, but do not have a greater pore area.

The same general conclusion can be arrived at by using a different set of data. Merriam (13),

studying whole-mount preparations of nuclei isolated from immature frog oocytes, found that there are 35 pores per square micron of envelope. If one uses a value of 655 A for the diameter of the pores, it can be calculated that these structures occupy 11% of the envelope. Obviously, the observed differences in the rates of nucleocytoplasmic exchange cannot be explained simply in terms of total pore area, since it would be impossible for ameba nuclei to have 44 times more open area than frog oocyte nuclei.

Detailed morphological studies of frog oocytes have shown that the annular material in these cells forms a dense diaphragm-like structure which appears to extend across the pores (14, 15). The presence of such a structure could account for the low rate of macromolecular exchange. The factors which effect the exchanges of macromolecules, however, do not necessarily influence other molecular species, as demonstrated by the fact that inorganic ions freely diffuse through the nuclear pores of amphibian oocytes (16). A further discussion of the passage of ions across the nuclear envelope can be found in reference 17.

In considering the function of the annular material, it is interesting to compare the rates of nucleocytoplasmic exchange with the over-all activity of the cells studied. The fact that oocytes are less active than amebae (with regard to growth rate, movement, division, etc.) and also have lower rates of exchange is consistent with the view that the annular material might regulate cellular activity by controlling the passage of macromolecules across the nuclear envelope. Furthermore, the composition of the annular material is not necessarily fixed in a given cell type, but may vary depending on the physiological state of the cell. For example, it has been shown that the ultrastructure of the pore complex changes during different growth phases of *Tetrahymena* (3). There are also indications that the permeability of the nuclear envelope varies during the division cycle in amebae, and that these variations are related to changes in the annular material (18). Of course, considerably more information will be necessary before the importance of such a regulatory mechanism can be established.

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REFERENCES

1. FELDHERR, C. M. 1962. *J. Cell Biol.* **14**:65.
2. STEVENS, B. J., and H. SWIFT. 1966. *J. Cell Biol.* **31**:55.
3. WUNDERLICH, F., and W. W. FRANKE. 1968. *J. Cell Biol.* **38**:458.
4. KESSEL, R. G. 1968. *J. Ultrastruct. Res. Suppl.* 10.
5. TREHERNE, J. E. 1961. *J. Exp. Biol.* **38**:315.
6. ANDERSON, E. 1964. *J. Cell Biol.* **20**:131.
7. FELDHERR, C. M. 1965. *J. Cell Biol.* **25**:43.
8. FELDHERR, C. M. 1962. *J. Cell Biol.* **12**:159.
9. BIALASZEWICZ, K. 1929. *Protoplasma.* **6**:1.
10. CHAMBERS, R. W., and M. J. KOPAC. 1950. *In* McClung's Handbook of Microscopical Techniques. R. McClung Jones, editor. Paul B. Hoeber, Inc., New York. **3**:492.
11. FELDHERR, C. M. 1966. *J. Cell Biol.* **31**:199.
12. HARDING, C. V., and C. M. FELDHERR. 1959. *J. Gen. Physiol.* **42**:1155.
13. MERRIAM, R. W. 1962. *J. Cell Biol.* **12**:79.
14. MERRIAM, R. W. 1961. *J. Biophys. Biochem. Cytol.* **11**:559.
15. WARD, R. T., and E. WARD. 1968. *J. Cell Biol.* **39**:139A. (Abstr.)
16. KANNO, Y., and W. R. LOEWENSTEIN. 1963. *Exp. Cell Res.* **31**:149.
17. WIENER, J., D. SPIRO, and W. R. LOEWENSTEIN. 1965. *J. Cell Biol.* **27**:107.
18. FELDHERR, C. M. 1968. *J. Cell Biol.* **39**:49.