THE NUCLEAR ANNULI AS PATHWAYS FOR NUCLEOCYTOPLASMIC EXCHANGES

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ABSTRACT

Colloidal gold particles, 25 to 55 A in diameter, which had been coated with polyvinylpyrrolidone, were microinjected into the ground cytoplasm of amebas (*Chaos chaos*). At time intervals of 1 minute, 2 minutes, 10 minutes, and 24 hours after injection the cells were fixed for electron microscopy. After 24 hours, gold particles were found in both the nuclei and the ground cytoplasm, the concentration being higher in the nuclei. Colloidal particles were also present in the nuclei after 10 minutes, but at this time interval the concentration did not appear to be greater than that in the ground cytoplasm. One and 2 minutes after injection, the gold particles were located almost exclusively in the ground cytoplasm; however, individual particles were often found within the annuli of the nuclear envelope, and were located specifically in the centers of these structures. The results suggest that at least some of the gold particles which enter the nuclei pass through the annuli, and that passage through these structures may be restricted to a central channel.

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INTRODUCTION

It has been demonstrated by means of electron microscopy that macromolecules (1) and particles of macromolecular dimensions (2, 3) can pass from the cytoplasm into the nucleus in intact cells. In one study (2), colloidal gold particles 35 to 80 A in diameter, coated with polyvinylpyrrolidone, were found within the nucleoplasm 24 hours after being microinjected into the ground cytoplasm of amebas. Because of the long time interval between injection and fixation of the organisms, it was not possible to determine the specific areas of the nuclear envelope through which the particles passed.

In the experiments to be reported, the techniques employed were essentially the same as those previously described; that is, protected gold particles were injected into the ground cytoplasm of amebas and their later distribution determined by electron microscopy. The major variation in this investigation was the use of shorter time intervals between injection and fixation, to permit the detection of particles fixed in transit through the nuclear envelope. The results indicate that the annuli can serve as pathways for exchange across the nuclear envelope.

MATERIALS AND METHODS

The Culture and Treatment of Amebas

The experiments were conducted on the giant ameba *Chaos chaos* (*Pelomyxa carolinensis*). The organisms were cultured in a medium consisting of 5×10^{-4} M $CaCl_2$, 5×10^{-5} M $MgSO_4$, 1.13×10^{-4} M KH_2PO_4, and 1.5×10^{-4} M K_2HPO_4. The pH was 6.8 to 7.0. All reagents were dissolved in ion-free water. *Paramecium aurelia* were supplied as a source of food.

To insure that all experimental amebas remained in interphase and did not divide during the course of the experiments, the organisms were starved for

24 hours prior to injection, and also during the period between injection and fixation.

Preparation of Colloidal Gold

Nuclear colloidal gold sols were prepared by the method outlined by Weiser (4). 2.5 ml of a 0.6 per cent solution of chlorauric acid and 3 ml of 0.72 N K_2CO_3 were added to 120 ml of ion-free water. To this mixture was added 1 ml of an ether solution of phosphorus, prepared by diluting one part of a saturated ether solution of phosphorus with 4 parts of ether. The final mixture was allowed to stand at room temperature for 10 to 15 minutes. It was then heated until a bright red sol formed; this occurred before boiling. After heating, the sol was cooled in an ice bath and stabilized by the addition of 12 milligrams of polyvinylpyrrolidone (PVP). In the present investigation, PVP having an average molecular weight of 15,000 was used.

The stabilized colloid was centrifuged at 70,000 *g* for 30 minutes, and the precipitate was discarded. The particles remaining in the supernatant were collected by centrifuging at 100,000 *g* for 1 hour. These particles were resuspended in injection medium (see below) to remove excess PVP, and then collected in a concentrated state by centrifuging again at 100,000 *g* for 1 hour and carefully removing the supernatant. The gold particles obtained by this method were approximately 25 to 55 A in diameter, exclusive of their PVP coats. The size of the particles was measured directly from electron micrographs. Since the properties of the PVP, including its shape, average molecular weight, and chemical structure are known, it is possible, assuming that a monolayer of PVP is adsorbed on the gold, to estimate that the over-all diameter of the gold-PVP particles ranged from 60 to 100 A. Efforts to verify this by means of negative staining have been unsuccessful; however, further experiments are planned involving the use of shadowing techniques and hydrodynamic methods.

Prior to its use, the gold-PVP fraction was dialyzed against injection medium, which consisted of 0.0127 M KCl, 0.0015 M K_2HPO_4, and 0.0011 M KH_2PO_4 (1). The pH was 7.0 to 7.1. After dialysis the concentration of the particles was standardized by diluting the sol with injection medium to an optical density of 17 to 20 ($\lambda = 500$ mμ, 1 cm light path). This relatively high concentration was used to assure that the number of particles passing through the nuclear envelope in short term experiments would be sufficient to permit ready analysis with the electron microscope.

Injection Procedures

All amebas were injected once, using the methods described by Chambers and Kopac (5). In preliminary studies it was found that the injection of amounts much higher than one-tenth the cell volume caused a rupture of the plasma membrane in the area of injection, and a resulting loss of cytoplasm. This reaction began within a matter of seconds.

In actual experiments, the amounts injected corresponded to approximately one-tenth the volume of the cells, or less. Under these conditions no rupture or leakage occurred; furthermore, no long-term affects of injection could be detected (see below).

Controls

To determine the effect of injection on movement, feeding, and division, 9 amebas were fed and cultured individually following injection. For comparison, non-injected organisms were cultured simultaneously.

Preparation for Electron Microscopy

The experimental amebas were fixed for electron microscopy 1 minute, 2 minutes, 10 minutes, and 24 hours after injection. Since diffusion of the sol throughout the organisms had not occurred in the 1- and 2-minute experiments, the specific areas injected in these instances were recorded, and only these areas were sectioned and later examined with the electron microscope. In 10-minute and 24-hour experiments the areas to be examined were chosen at random. The methods for fixation and embedding were the same as those previously reported (1, 2). The results are based on the examination of 16 amebas: 8 fixed at one- and two-minute intervals, 3 fixed at ten minutes and 5 fixed 24 hours after injection.

RESULTS

Controls

Amebas which were cultured after injection were found to move and feed normally. No differences were detected between injected and non-injected amebas in their ability to divide or in their rate of division. Injected specimens,

FIGURE 1

A section through an ameba fixed 24 hours after injection. Gold particles (arrows) are present in both the nucleus (*N*) and the ground cytoplasm (*C*), but are concentrated in the nucleus. × 123,000

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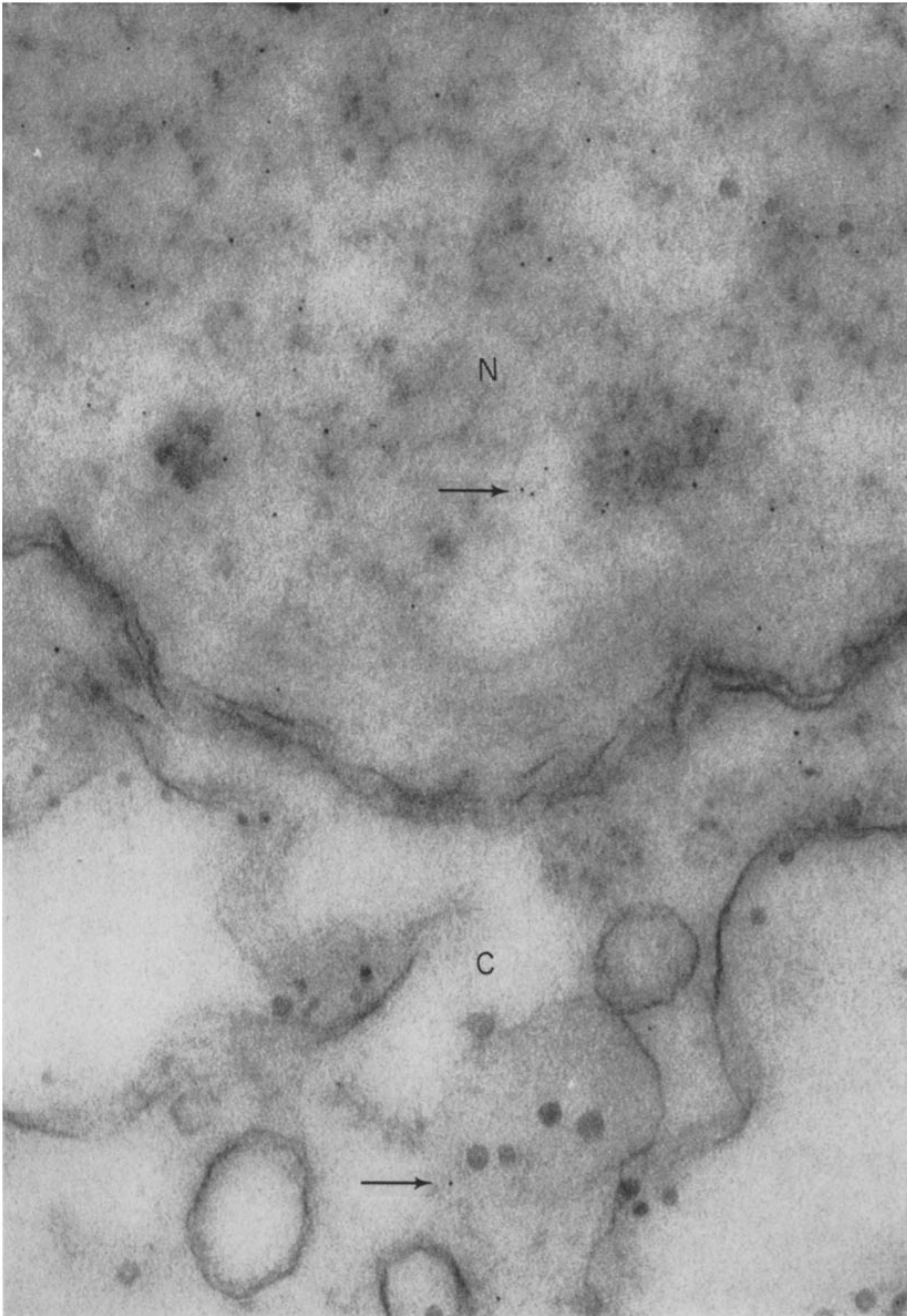

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examined with the electron microscope, exhibited no structural changes in the nuclei or other organelles. These results indicate that neither the structure nor the normal functions of the amebas are affected by the injection procedures.

24-Hour Experiments

The distribution of the gold particles used in these experiments was the same, after 24 hours, as the larger particles used previously (2). The gold particles were found in both the nuclei and the ground cytoplasm. The highest concentration was in the nuclei (Fig. 1).

10-Minute Experiments

Gold particles were found in the nuclei after 10 minutes. At this time interval, the concentration of particles in the nuclei appeared equal to, but not greater than, the concentration in the ground cytoplasm.

1- and 2-Minute Experiments

In cells fixed 1 and 2 minutes after injection, the gold particles were found almost exclusively in the ground cytoplasm (Fig. 2). Only occasionally were particles found within the nucleoplasm. In all cells examined at these time intervals, gold particles were frequently found in the annuli of the nuclear envelopes, and were, in almost every instance, located specifically in the centers of these annular structures when seen in tangential sections (Figs. 5, 6). In sections cut perpendicularly to the nuclear envelope gold particles were found, in some instances, at the mid-point of the annular axis, and in other instances on either the nuclear or cytoplasmic side of that point. Occasionally, 2 or 3 particles were found within a single annulus; in such instances, the particles were located either side by side in the centers of the annuli (Fig. 5) or along the annular axis (Figs. 3, 4). In the latter instances, the colloidal particles appeared to be caught by fixation at different points of penetration within a central channel. In addition to those particles found in the annuli, others occasionally appeared to lie between the two membranes of the nuclear envelope (within the perinuclear space, Fig. 3).

DISCUSSION

The presence of colloidal gold in the nuclei at 10 minutes and 24 hours after injection, demonstrates that gold particles, initially coated with PVP, having a negative charge, and having an over-all diameter estimated at 60 to 100 A, can cross the nuclear envelope. The particles used in this investigation were smaller than those used previously (2), and were coated with a PVP preparation of lower molecular weight. The present results support the conclusions already drawn regarding the passage of gold-PVP particles into the nucleus over longer periods of time.

The presence, in 1- and 2-minute experiments, of gold particles in the annuli, and particularly the location of these particles on the nuclear side of the annuli indicate that at least some gold particles enter the nuclei through these structures. The possibility of the annuli serving as pathways for nucleocytoplasmic exchanges has been suggested by various investigators (*i.e.*, 6, 7). Previous evidence supporting this thesis has been provided by Anderson and Beams (8), who observed electron-opaque material extending through the annuli of nurse cell nuclei.

Several explanations might account for the finding that the gold particles observed within the annuli were located, in almost every case, in the centers of the annuli. The simplest explanation is that there exists, within each annulus, a thick-walled tube of some material which the gold particles cannot penetrate. This would restrict the passage of material to a central channel. In support of this idea there is evidence that tubular structures are associated with the nuclear annuli of certain cells (9–11). Although no such structures were observed directly in the present study, the regions within the annuli often appeared more electron-opaque than surrounding areas (Figs. 3 to 5). This suggests the presence of an osmiophilic substance, which could conceivably represent the walls of a tube-like element.

FIGURE 2

One minute after injection the gold particles are found almost entirely in the ground cytoplasm (*C*). Two of the particles (arrow) which are present in the nucleus (*N*) give the appearance of having just penetrated an annulus. × 123,000.

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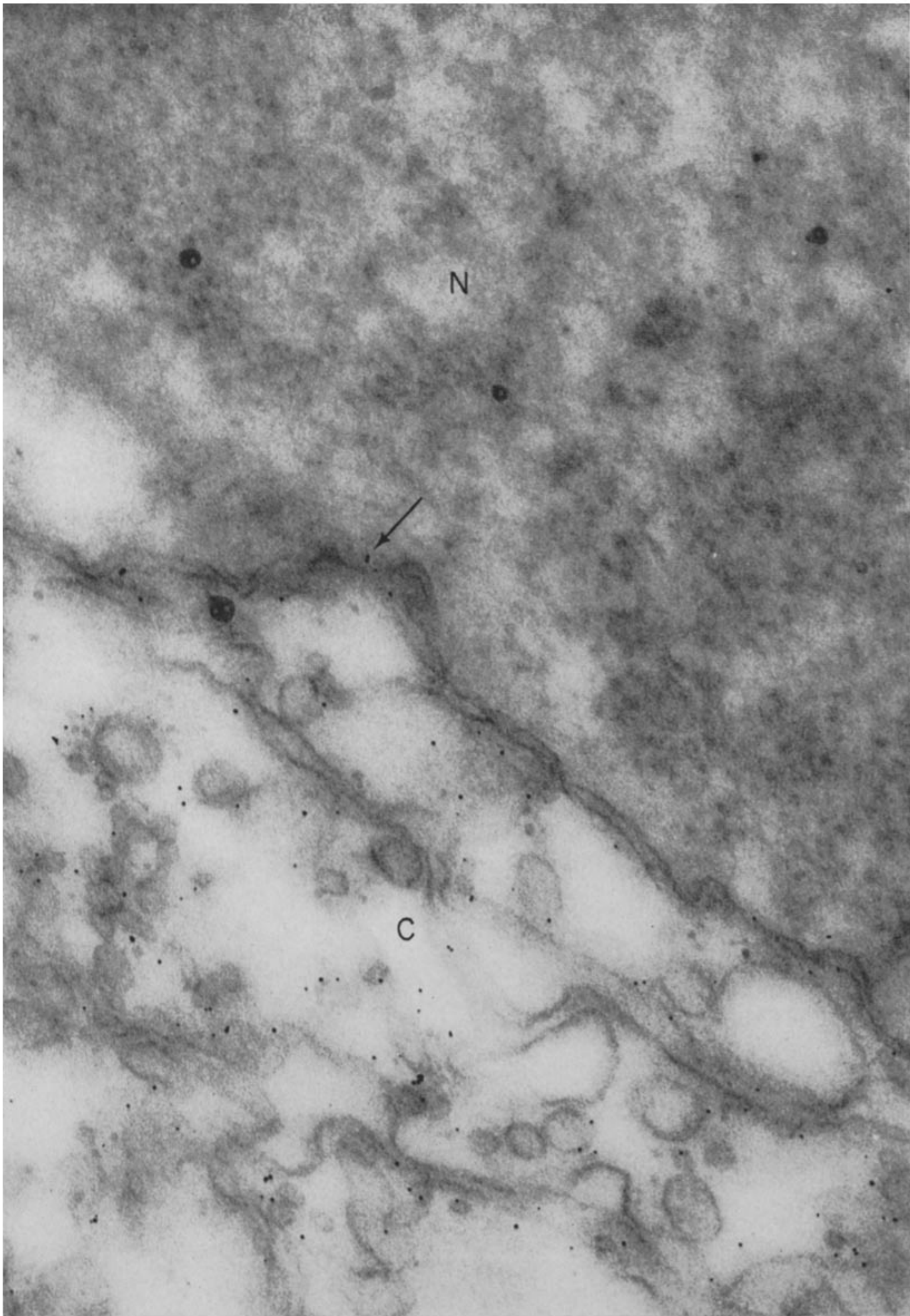

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Centrally located granules have been found to occur normally within the nuclear annuli of oocytes (9), salivary gland cells in *Drosophila* (12), mammalian somatic cells (11), and amebas (13). In such instances, however, it was not possible to determine whether this material was passing through the annuli or was actually part of the structure of the annuli. On the basis of this study, it seems possible that the granules observed in previous investigations were in the process of penetrating the annuli.

In the present investigation, occasional gold particles seemed to be located in the perinuclear space. In many of these instances, however, there was the possibility that the particles were actually within annuli, but that these structures could not be recognized due to the thickness of the sections. Further experiments with thin serial sections would be of interest, particularly in view of the results of Moore *et al.* (3) which were interpreted to indicate that colloidal iron particles can pass directly across the double membrane of the nuclear envelope in somatic cells.

The present results indicate clearly that the passage of particles of macromolecular dimensions across the nuclear envelope occurs, at least in part, through the annuli. Furthermore, it appears likely that passage is restricted to central channels within the annuli. Several questions concerning the mechanisms controlling such movements are suggested by the results. For example, does the fact that gold particles were so often observed in the centers of the annuli mean that the particles are adsorbed in these regions? If passage were solely by free diffusion, it seems unlikely that particles would have been caught in transit through the nuclear envelope with such frequency. If it can be established that adsorption does occur during passage through the annuli, one could then consider seriously some mechanisms which, at present, can only be suggested. Two such mechanisms are: (1) The particles may be adsorbed to the substance which forms the walls of the channels, and may then be transported by an inward flow of the "carrier" substance. (2) The substance lining the channels may be static, and particles may move inward by successive steps of adsorption and desorption from binding sites on the channel wall. In determining whether adsorption does occur, it will be of interest to study the behavior of positively charged particles. A second question is whether the passage of macromolecules, of a particular type, from the ground cytoplasm into the nucleus

FIGURE 3

A section cut perpendicularly to the nuclear envelope of an ameba fixed 1 minute after injection. Three gold particles can be seen extending through the center of an annulus (A). Some colloidal particles appear to be present in the perinuclear space (*P*). *C*, ground cytoplasm; *N*, nucleus. × 216,000.

FIGURE 4

A portion of an ameba fixed 1 minute after injection. In this section, perpendicular to the nuclear envelope, two annuli can be seen, both containing centrally located gold particles (*G*). *C*, ground cytoplasm; *N*, nucleus. × 216,000.

FIGURE 5

A tangential section through the nuclear envelope of an injected ameba fixed after 1 minute. Two annuli are shown. One (A_1) contains a single centrally located gold particle, the other (A_2) contains two particles located side by side within the center of the annulus. × 216,000.

FIGURE 6

In this tangential section cut through the nuclear envelope of an ameba fixed 1 minute after injection, four annuli can be observed. In one (A_1) no centrally located particles are present. In two of the annuli (A_2, A_3) centrally located gold particles can be seen. In the fourth annulus (A_4) an electron-opaque body, of the type normally found within some of these structures, is present. × 216,000.

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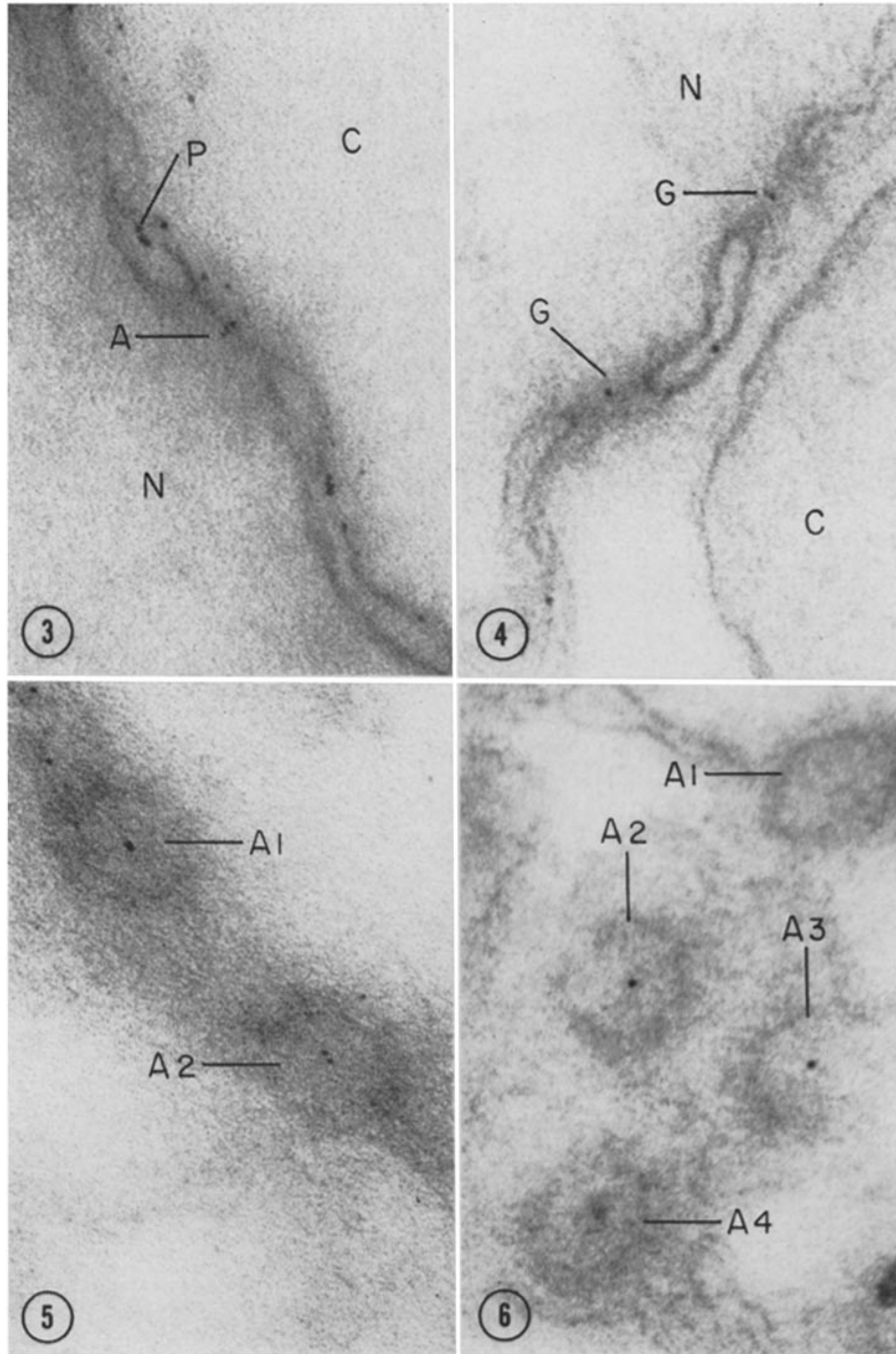

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is a one-way process. The 10-minute experiments carried out in this investigation indicate that gold particles can enter the nucleus at a fairly rapid rate. This being the case, one could explain the fact that colloidal gold is concentrated in the nucleus after 24 hours by the hypothesis that, after entering the nucleus, the particles cannot return with equal facility to the ground cytoplasm. The work of Goldstein and Plaut (14) on *Ameba proteus* indicates that such a process may work in the reverse direction. They found that RNA or a precursor of RNA can leave, but not reenter, the nuclei.

The results suggest that it should be possible in future experiments, using the methods employed here, to answer these and other questions concerning nucleocytoplasmic exchanges.

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