NUCLEOCYTOPLASMIC EXCHANGES DURING EARLY INTERPHASE

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ABSTRACT

Colloidal gold was injected into the cytoplasm of amebae (*Amoeba proteus*) approximately 5 min, 1 hr, and 2 hr after cytokinesis. Later, interphase cells were similarly treated. All of the amebae were fixed about 50 min after injection and were examined with the electron microscope in order to determine the distribution of the gold. It was found that for a period of 2 hr after division the uptake of gold by the nuclei was significantly greater than that during late interphase. Correlation of the gold distribution with the morphology of the nuclear envelope indicated that an inverse relationship exists between the rate of incorporation of colloid into the nucleoplasm and the degree of reconstitution of the fibrous lamina ("honeycomb" structure). These data support the view that the fibrous lamina functions in regulating nucleocytoplasmic exchanges.

INTRODUCTION

In a previous investigation (1), 30–170-A colloidal gold particles were introduced into the cytoplasm of dividing and interphase amebae (*Chaos chaos*). After study of the later distribution of the gold with the electron microscope, it was concluded that the nuclei of dividing cells incorporate substances from the cytoplasm at a significantly greater rate than interphase nuclei. It was also demonstrated that the increased uptake of cytoplasmic material by dividing nuclei is not restricted to the period of nuclear breakdown but can occur *after* re-formation of the nuclear envelope. From these experiments it would appear (*a*) that the amount of material entering the nuclei varies during the cell cycle, a factor which could be important in regulating cellular activity, and (*b*) that such variations might be dependent upon changes in the permeability of the envelope.

The two main objectives of the present investigation were to determine how long after division the increased uptake of material by the nuclei persists, and whether there are corresponding morphological changes in the nuclear envelope.

The experimental approach involved microinjection of gold particles into amebae at specific times after cytokinesis and determination of the later intracellular distribution of the colloid. It was found that gold enters the nucleus most rapidly during the first 2 hr of interphase. Furthermore, changes in the rate of uptake of gold are accompanied by changes in the morphology of the fibrous lamina, a structure which lines the inner surface of the nuclear envelope.

MATERIALS AND METHODS

Amoeba proteus was used as the experimental organism in this study. The cells were cultured in an inorganic salt solution at 18° C and were fed *Paramecium aurelia* (2). Under these conditions the cells divide about every 36 hr.

Injection Experiments

Dividing amebae, having the typical mulberry appearance, were selected from well-fed cultures and were allowed to complete cytokinesis. Colloidal gold was then microinjected into the cytoplasm of the

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TABLE I

Gold Distribution

Ameba	Total volume examined*	No. of particles in nucleo-plasm	No. of particles in cytoplasm	Particles in nucleo-plasm
	(μ^3)			%
A. Injected 0–5 min after cytokinesis‡				
1	1.4	195	82	70
2	1.4	280	132	68
3	1.7	652	244	73
4	2.9	223	81	73
5	1.1	206	150	58
Total	8.5	1556	689	69
B. Injected 1 hr after cytokinesis				
1	1.4	34	167	17
2	2.8	56	114	33
3	1.3	26	85	23
4	1.1	21	49	30
5	1.6	26	34	43
6	1.8	13	26	33
7	1.5	129	142	48
8	1.4	19	132	13
9	1.1	14	64	18
10	1.7	89	291	23
11	2.2	82	171	32
Total	17.9	509	1275	29
C. Injected 2 hr after cytokinesis				
1	1.6	25	160	14
2	1.6	36	264	12
3	1.3	18	110	14
4	1.4	28	158	15
5	1.6	44	185	19
6	0.6	6	57	10
7	1.0	2	42	5
Total	9.1	159	976	14
D. Injected during late interphase				
1	1.2	11	203	5
2	1.1	9	70	11
3	2.9	3	102	3
4	1.3	5	94	5
5	1.2	14	77	15
6	1.0	11	185	6
7	1.3	13	83	14
8	1.1	24	184	12
9	1.5	11	128	8
10	1.0	19	314	6
Total	13.6	120	1440	8

* In each case, half of the total represents nucleoplasm and half cytoplasm.

‡ In all experiments, the amebae were fixed about 50 min after injection.

daughter cells (*a*) approximately 5 min, (*b*) 1 hr, and (*c*) 2 hr after cytokinesis. Large, nondividing amebae, presumed to be in later stages of interphase, were also injected. In all instances, the amebae were fixed in OsO_4 about 50 min after injection and were prepared for examination with the electron microscope (see details below).

The colloidal gold particles had diameters ranging from 30 to 170 A and were coated with polyvinylpyrrolidone (PVP). The method of preparing the colloidal gold and the microinjection procedures have been described in an earlier report (3). In that study it was also demonstrated that the injection procedures have no effect on either the structure or function of the nuclei.

The relative rates at which the gold particles entered the nuclei at different times after cytokinesis were determined by making particle counts. This was accomplished by taking electron micrographs of each experimental ameba and counting the particles in equal and adjacent areas of nucleoplasm and cytoplasm. The results are expressed as the per cent of the total particle count present in the nucleoplasm. The areas in which counts were made were selected at random. Since both the area and section thickness (approximately 1000 A) were known, the *volume* of material examined could be estimated. Further details of the counting procedure can be found in reference 1.

Statistical analysis of the results involved the use of angular transformations and multiple *t* tests. The experimental groups were considered significantly different if a probability of less than 0.01 was obtained.

TABLE II

Thickness of the Fibrous Lamina

Ameba	Time after cytokinesis				
	0–5 min*	50 min	1 hr 50 min	2 hr 50 min	Late interphase
	A	*A*	*A*	*A*	*A*
1	0	670	1340	1340	4020
2	0	670	1000	1675	3015
3	0	670	1340	1675	3350
4	0	670	1000	1340	2680
5	0	670	1340	1340	2345
6			1340	2000	3015
7			670		2345
8			1340		2345
9			1340		2680
10			1740		2680
Average	0	670	1245	1560	2850

* These measurements were made on noninjected amebae; all other cells were injected.

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Morphological Observations

The nuclear envelope of *A. proteus* can be viewed as a compound structure having a membranous portion and an elaborate honeycomb structure which extends into the nucleoplasm (4). The latter structure has been termed the fibrous lamina and may be present, in a much reduced form, in other cell types (5).

Morphological observations were undertaken to determine whether a correlation exists between the rate of uptake of gold by the nuclei following division and the degree of reconstitution of the nuclear envelope. The membranous component is probably not primarily involved in the initial, rapid incorporation of cytoplasmic material, since it reforms *before* the completion of cytokinesis (6). For this reason, emphasis was placed on the development of the fibrous lamina. Not only does this structure reform more slowly than the membranous portion, but it is also of interest since it might be related to the electron-opaque pore material (see Discussion).

The rate of development of the fibrous lamina was ascertained by measuring its thickness with the electron microscope at regular intervals after cytokinesis. For a number of reasons, including variations in the angle at which the nuclear envelopes were sectioned and possible differences in the thickness of the lamina within a single nucleus, the reported measurements represent estimates rather than exact values.

Data were obtained primarily from the injected amebae; however, noninjected cells were used to study the structure of the envelope just after the completion of cytokinesis. All amebae were fixed for 10 min in 1% OsO_4 adjusted to pH 7.4 with Veronal-acetate buffer. The cells were then dehydrated in alcohol, embedded in Araldite, sectioned at a thickness of approximately 1000 A, and examined with either an RCA-EMU-3C or a Siemens Elmiskop 1 electron microscope.

RESULTS

Injection Experiments

It can be seen in Table I *A* and in Fig. 2 that, for the first 50 min after the completion of cytokinesis, gold particles accumulate in the nucleoplasm. During the next experimental period, extending approximately from 1 hr to 1 hr 50 min

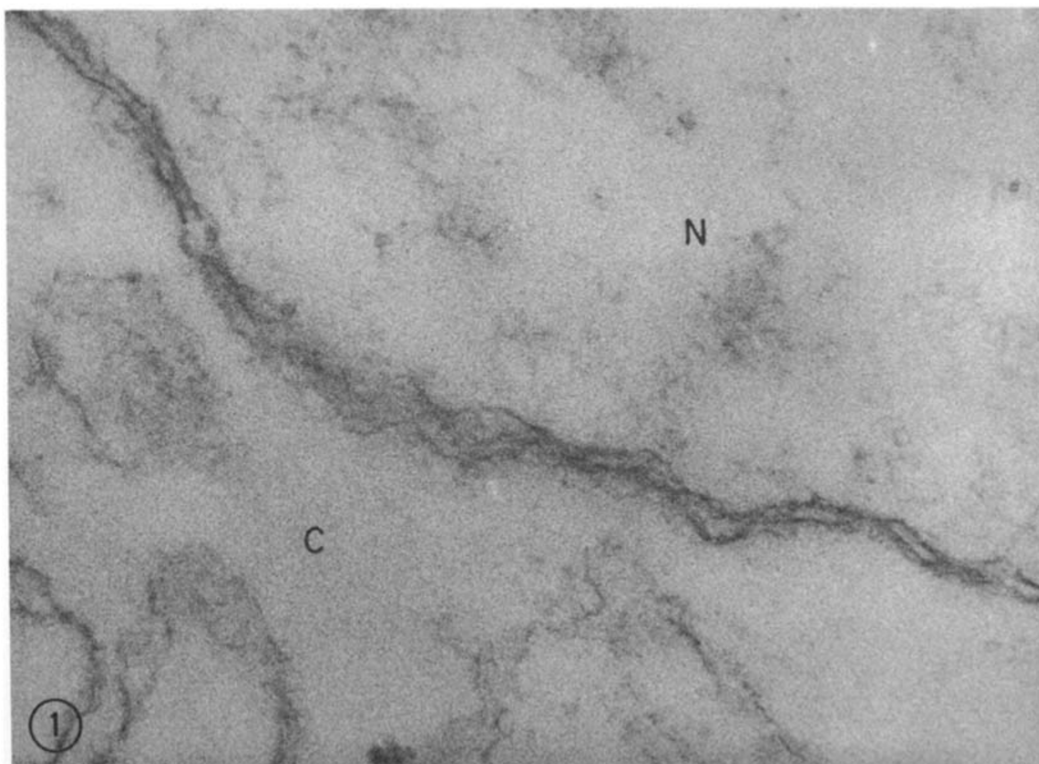

FIGURE 1 A noninjected ameba fixed just after cytokinesis. At this time, the fibrous lamina has not yet begun to re-form. *N*, nucleoplasm; *C*, cytoplasm. × 75,000.

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after division, there is a statistically significant decrease in the amount of gold incorporated by the nuclei ($P < 0.001$). The data are given in Table I *B*. A further decrease in the uptake of gold into the nucleoplasm occurs over a period ranging from 2 hr to 2 hr 50 min after cytokinesis (Table I *C*). These results are significantly lower than those obtained in the 1 hr experiments ($P < 0.01$), but they are *not* significantly different from the late interphase results shown in Table I *D* and in Fig. 3 ($P > 0.05$).

Thus, following the initial concentration of gold, there is a gradual decrease in the rate of uptake of cytoplasmic colloid by the newly formed nuclei. The rate of incorporation reaches the late interphase level about 2 hr after cytokinesis.

The size distribution of gold particles present in the nuclei at different times in the cell cycle was not studied in detail, as was the case in the earlier work on *C. chaos* (1). However, a cursory examination showed that particles as large as 130 A in diameter (exclusive of their PVP coats) enter the nuclei during the first 50 min after division. The largest particles found in late interphase nuclei had diameters of about 105 A (3). This finding, that large particles readily enter young nuclei, is consistent with previous results (1).

Morphological Observations

The initial observations confirmed the work of Roth et al. (6) concerning the early reconstruction of the membranous component of the nuclear envelope. Thus, the nuclei of cells fixed just after division are completely enclosed by double membranes containing numerous pores. With reference to diameter, the pores (approximately 650 A in diameter) are not significantly different from those present in the envelopes of late interphase nuclei (4).

The results relating to the development of the fibrous lamina are shown in Table II and Figs. 1–3. The fibrous lamina apparently does not begin to

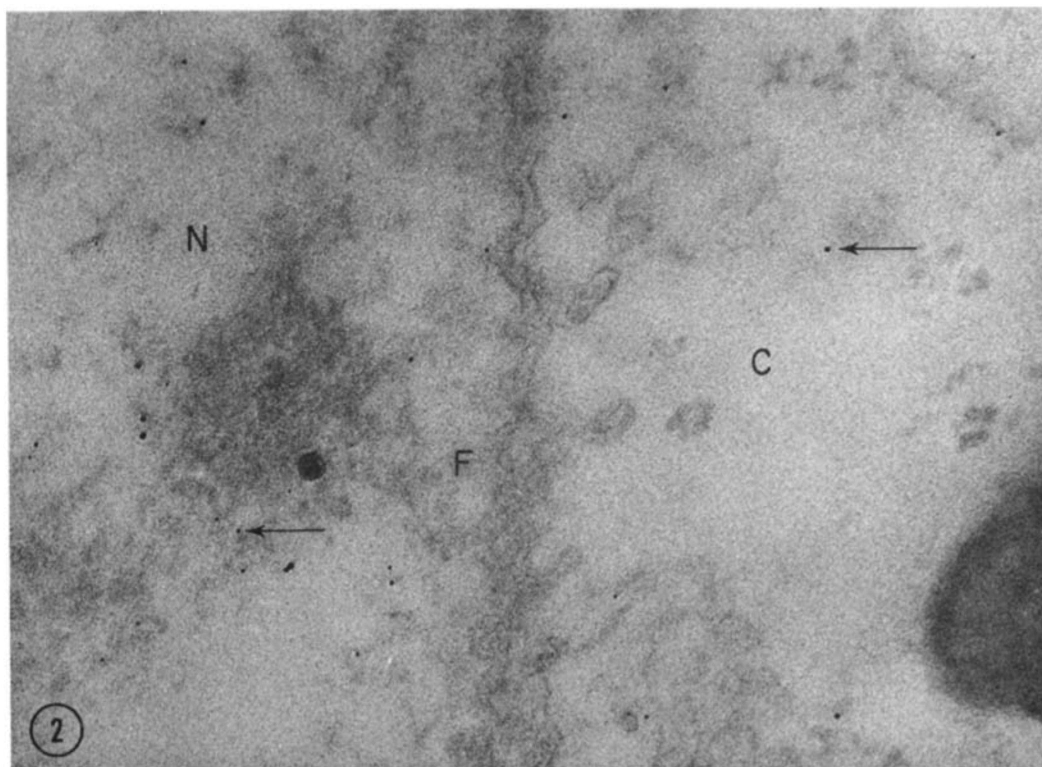

FIGURE 2 An electron micrograph of an ameba injected within 5 min after cytokinesis and fixed 50 min later. It can be seen that the gold particles (arrows) are more concentrated in the nucleoplasm (*N*) than in the cytoplasm (*C*). The fibrous lamina (*F*) is in an early stage of reconstitution. × 75,000.

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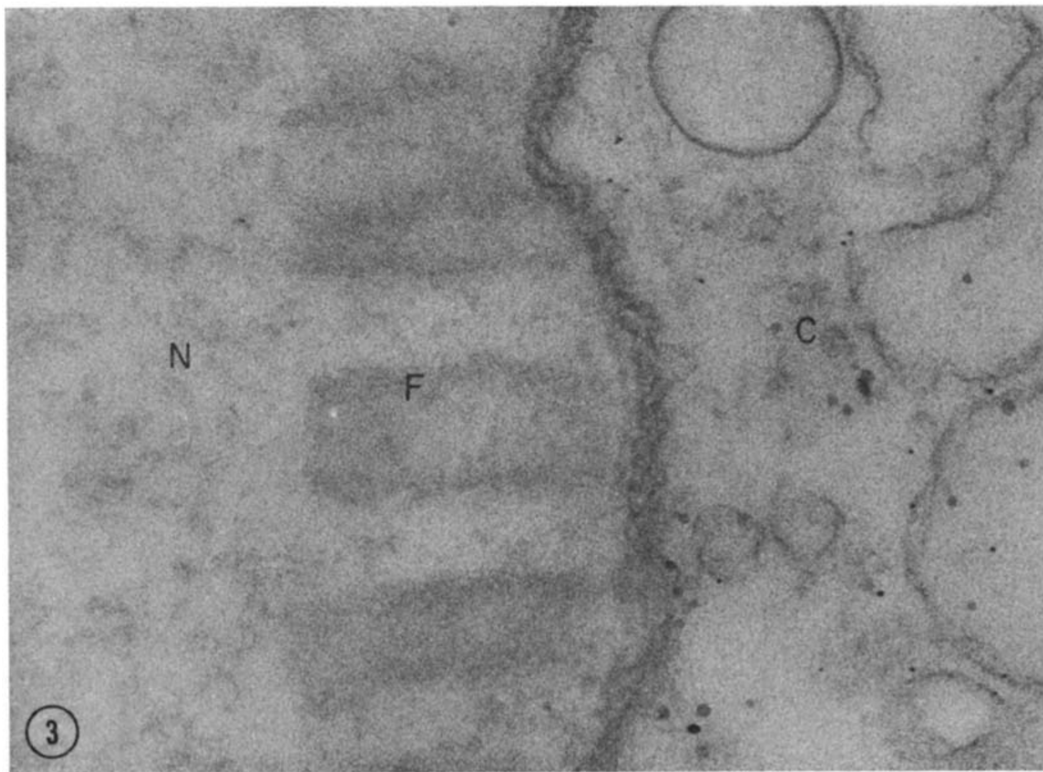

FIGURE 3 A section through an ameba injected during late interphase and fixed after 50 min. In this instance the gold concentration is higher in the cytoplasm (*C*) than in the nucleoplasm (*N*). The fibrous lamina (*F*) is fully developed. × 75,000.

re-form until after the completion of cytokinesis. Within the first 1 hr 50 min, it gradually attains an average thickness of about 1245 A. By 2 hr 50 min its thickness averages approximately 1560 A, just over half of the late interphase value which is estimated to be 2850 A. The latter value is consistent with that reported by Pappas (4).

The following correlations can be drawn between the development of the fibrous lamina and the uptake of colloidal gold by the nuclei. First, for approximately 2 hr after cytokinesis there is an inverse relationship between the thickness of the lamina and the incorporation of gold. Second, the rate of uptake of gold decreases to the late interphase level before the fibrous lamina has fully re-formed.

DISCUSSION

It can be concluded from the above results that cytoplasmic material enters the nucleus most rapidly during the early stages of interphase. A similar conclusion could be drawn from the work of Prescott and Bender (7). These investigators found that a normally slow migrating nuclear protein fraction, which is released into the cytoplasm of *A. proteus* during mitosis, rapidly returns to the nucleus after cytokinesis. Rao and Prescott (8) established that complete return of the protein requires about 3 hr.

The simplest explanation for the present results which demonstrate changes in both the concentration and the size of particles present in the nuclei is that newly reconstructed nuclear envelopes are more permeable than those of older interphase cells. This would account for an increased uptake of gold during early interphase. An alternative explanation is that the uptake of gold into the nucleoplasm remains constant during the cell cycle, but that the rate of outflow varies. This possibility is less likely, however, since it does not readily account for the observed differences in the size of

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particles present in the nucleoplasm at various times after division.

The initial accumulation of gold in the nucleoplasm does not necessarily imply that a unique transport mechanism is functioning just after cytokinesis, or that the newly formed nuclei have a special affinity for the colloid. Similar accumulations have been observed in late interphase nuclei when the time between injection and fixation was 24 hr (2). In both instances, the accumulation might be due to binding within the nucleoplasm, the limiting factor being the ability of the gold to cross the envelope.

With regard to the permeability of the nuclear envelope, the following points should be kept in mind. First, the nuclear pores are probably the major pathways for the passage of large substances into the nucleoplasm (2, 9). Second, the pores are not simply spaces which permit free communication between the nucleoplasm and cytoplasm, but they contain an electron-opaque material which most likely plays an important role in regulating exchanges across the envelope (3, 10).

Fawcett (5) recently examined the ultrastructure of the nuclear envelope in a variety of cells and suggested that a fibrous lamina, comparable to that found in *A. proteus* but much less extensive, might be a normal component of vertebrate cells. In his electron micrographs, portions of the fibrous lamina are seen extending across the pores, and they appear to contribute to the electron-opaque pore material. Considering its morphology, Fawcett implied that the lamina might be involved in controlling nucleocytoplasmic interactions.

In the present study, an inverse relationship, lasting about 2 hr, was found to exist between the uptake of colloidal gold by the nuclei and the thickness of the fibrous lamina. These results do not prove that the lamina is involved in controlling nuclear permeability, but they are consistent with this view. The relationship, however, is complicated by the fact that the rate of uptake of gold reaches equilibrium before the fibrous lamina has completely reformed. One might hypothesize that only a portion of the lamina functions in regulating permeability, presumably that component occupying the pore area.

In future experiments, an attempt will be made to determine whether variations in nucleocytoplasmic exchanges, such as those described in this report, are of significance in regulating cellular activity.

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