THE USE OF COLLOIDAL GOLD FOR STUDIES OF INTRACELLULAR EXCHANGES IN THE AMEBA *CHAOS CHAOS*

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INTRODUCTION

In an earlier study on *Chaos chaos*, it was found that ferritin can penetrate the nuclear membrane, the outer membrane of the mitochondrion, and the membrane lining the food vacuole, after it has been introduced into the ground cytoplasm by microinjection (1). Other work has shown that ferritin and methylated ferritin can be used to analyze the mechanism of pinocytosis in amebas and to follow some of the changes which take place within the cell during the digestion and assimilation of ingested materials (2). Although ferritin and methylated ferritin have proved useful for exploring intracellular exchanges, the conclusions which can be drawn from work with a single test substance are necessarily limited. The general mechanisms of macromolecular transfers within the cell might be more clearly understood if similar experiments were done with particles of graded size, and with particles having different physical and chemical properties.

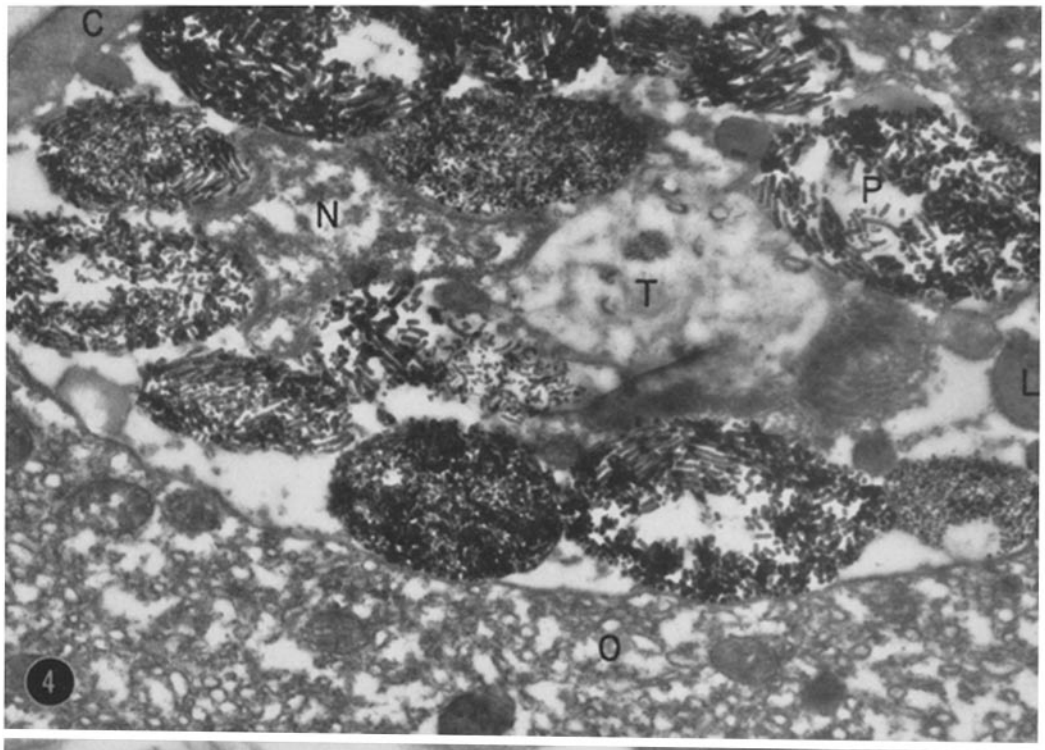
An ideal tracer substance for studies by electron microscopy of intracellular exchanges should have the following characteristics. The substance should be one which can be prepared in a wide range of particle sizes, say from 10 to 500 A. For a given experiment, it should be possible to prepare particles of nearly uniform size, and the dimensions should be measurable. The particles should

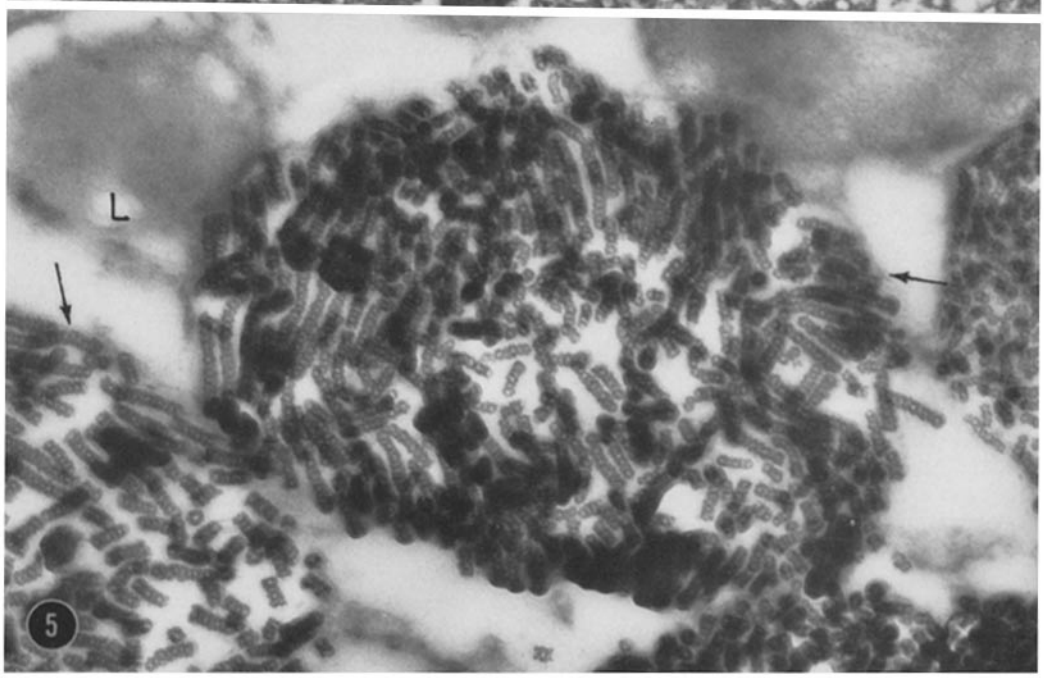
FIGURE 4

Test cell (*T*) filled with dense, oval masses of pigmented material (*P*) characteristically present late in oogenesis. Nucleus (*N*), oocyte (*O*), vitelline membrane (*C*), homogeneous inclusions (*L*). × 15,000.

FIGURE 5

Higher magnification of pigment at stage similar to that shown in Fig. 4. Note long chains of spherical units containing dense periphery and lightly osmicated central region. Limiting membrane visible at arrows. Homogeneous inclusion (*L*). × 48,000.





consist of an electron-scattering core surrounded by a "coat" the properties of which could be varied widely at will.

These considerations led to a study of gold sols of different particle sizes, stabilized by a variety of different coating substances. Many substances of biological interest, including different polypeptides, polynucleotides, polysaccharides, and organic polymers are effective as stabilizers, and the sols can be prepared in a wide range of particle sizes by the classical methods of colloid chemistry (3). In addition, fractions of graded particle size can be prepared from such sols by differential centrifugation.

The purpose of this communication is to report results obtained with the first such preparation, which consisted of gold particles 35 A to 80 A in diameter, stabilized by polyvinylpyrrolidone (PVP). The particles were introduced into the ground cytoplasm by microinjection, and the distribution of the particles was determined by electron microscopy. The distribution of gold-PVP is compared with that of ferritin, and significant differences are noted and analyzed.

MATERIALS AND METHODS

Colloidal gold was prepared by reducing gold chloride with phosphorous (3). The sol was stabilized by adding polyvinylpyrrolidone, of average molecular weight 40,000, and was fractionated by the following procedure: the sol was centrifuged at 40,000 *g* for 15 minutes, to remove the larger particles. The supernatant was centrifuged at 80,000 *g* for 30 minutes. The loosely packed pellet from this step was suspended in injection medium (see below) to remove excess PVP, centrifuged again at 80,000 *g* for 30 minutes, and collected in a concentrated state by carefully drawing off the supernatant. The pellet prepared in this fashion consisted of spherical gold particles ranging in size from 35 to 80 A, exclusive of their PVP coats. Particle diameters were measured from electron micrographs.

A concentrated suspension was dialyzed against the injection medium, which contained 0.0016 M K_2HPO_4, 0.0011 M KH_2PO_4, and 0.0127 M KCl (pH 7.0 to 7.1). The ionic composition of the injection medium is approximately that of the ground cytoplasm of *Chaos chaos* (1). Following dialysis, the concentration was standardized by diluting the sol with injection medium to an optical density of 10.5 (λ = 500 mμ, 1 cm light path).

The gold sol was introduced into the ground cytoplasm of amebas (*Chaos chaos* or *Pelomyxa carolinensis*) by microinjection. The amebas were starved for 24 hours before injection and during the period between injection and fixation. Under these conditions the amebas remained in interphase during the course of the experiments. Each ameba was injected either one or two times; the amount of each injection corresponded to approximately one-tenth the volume of the cell. Amebas which were injected once were fixed either immediately (within 5 seconds) or 24 hours after injection. Amebas which were injected twice were fixed 24 hours after the final injection. The cells were fixed for 8 minutes in 1.0 per cent buffered osmium tetroxide at pH 8.5, dehydrated in alcohol, and embedded in epoxy resin. Thin sections were cut and examined with an RCA-EMU-3C electron microscope. A total of eleven cells, seven singly injected and four doubly injected, were examined.

To determine the effects of the injection procedures on various physiological processes, seven amebas were injected twice, then fed, and cultured individually. These were compared with non-injected specimens which were cultured simultaneously.

The design of the experiment and the techniques used were essentially the same as those described in earlier work with ferritin (1).

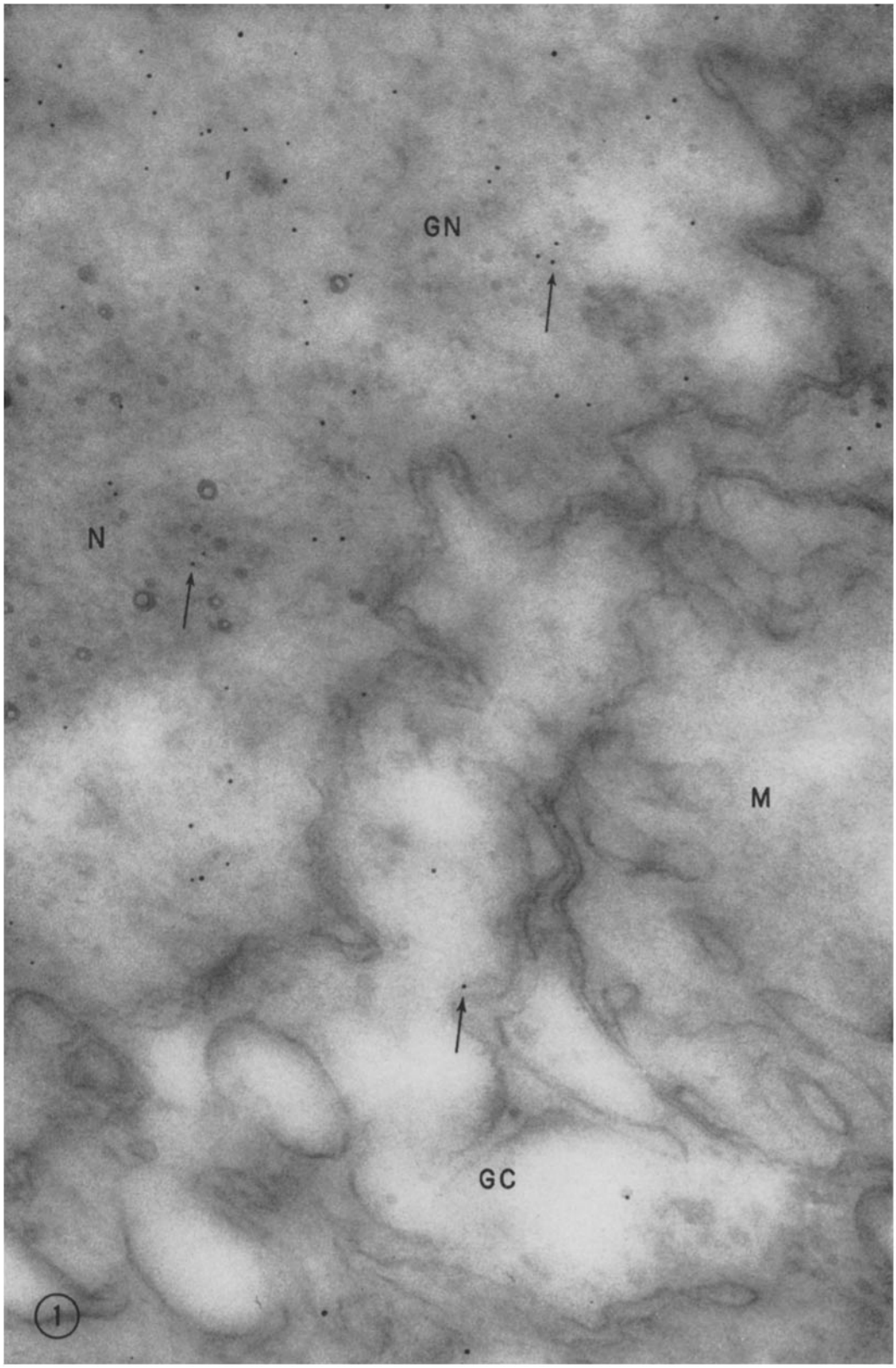
RESULTS

Effects of Injection: When amebas which had been cultured following injection were compared with non-injected specimens, no differences were detected in movement, feeding, ability to divide, or rate of division. These observations demonstrate that the injection procedures did not affect the normal functioning of the organisms. Also, no structural changes were detected by electron microscopy in nuclei or mitochondria in sections of injected amebas.

Twenty-four-Hour Experiments: In amebas fixed 24 hours after being injected, colloidal gold particles were found dispersed throughout the ground cytoplasm, the nucleoplasm, and the

FIGURE 1

A section through an ameba fixed 24 hours after a single injection. Colloidal gold particles (arrows) are scattered throughout the ground cytoplasm (*GC*), ground nucleoplasm (*GN*), and the nucleolus (*N*). No particles are present in the mitochondrion (*M*). Magnification, 116,000.



nucleoli (Fig. 1). The concentration of gold particles was consistently higher in the nucleoplasm than in the cytoplasm. Gold was found in mitochondria only exceptionally; the particles in mitochondria were found between the outer and inner membranes, never within the inner matrix. Aggregates of gold particles were found frequently in food vacuoles. The results described were the same for both singly and doubly injected amebas.

Immediate Fixation Experiments: In amebas which were fixed within 5 seconds after injection, the gold particles were found only in the ground cytoplasm (Fig. 2). This result shows that the gold particles which were present in various organelles 24 hours after injection did not enter those organelles during injection of the cytoplasm or during fixation.

DISCUSSION

It is concluded that, in *Chaos chaos*, 35 to 80 A gold particles, initially coated with PVP, can penetrate the nuclear membrane, the food vacuole membrane, and, less commonly, the outer membrane of the mitochondrion. Somewhat similar results were obtained with ferritin, but the distribution of gold-PVP differed from that of ferritin in several respects. The first was that gold-PVP particles were present in the nucleoplasm after 24 hours in higher concentration than in the cytoplasm. Ferritin particles were found after 24 hours in the nucleoplasm, but were not specifically concentrated there. A second point of difference was the higher concentration of gold-PVP in the nucleoli, where ferritin particles were rarely found. Finally, gold-PVP particles were found only infrequently in mitochondria, where ferritin particles commonly appeared.

Assuming that a monolayer of PVP is adsorbed on the gold, and is not exchanged or added to in the cytoplasm, one may estimate that the over all diameter of the gold-PVP particles used in this study averages 100 to 200 A. The diameter of the ferritin molecule is about 96 A (4). Both types of particles are negatively charged at neutral pH. The larger size of the gold-PVP particles could explain the difference between the two substances as regards entry into mitochondria, but would not readily explain the higher concentration of gold in the nucleus. Therefore, although differences in particle size may account for a part of the results, differences in the surface chemistry of the particles seem also to be important.

Studies are under way in which gold particles of different sizes, stabilized with PVP and with other types of coating agents, will be used to explore the relative importance of particle size and particle reactivity. The possibility that coated particles may be modified after injection into the cytoplasm will be tested by *in vitro* studies with bulk cytoplasm obtained from mass cultures.

Colloidal gold and other types of electron-scattering colloids have been used fairly widely in electron microscopy, (*e.g.*, 5, 6, 7), but little attention has been paid to control of particle size and surface reactivity. The present study suggests that colloidal gold may be used in a rational way to determine experimentally the factors which control the passage of macromolecules between cell compartments.

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FIGURE 2

A portion of an ameba fixed immediately after injection. Gold particles (arrow) are found in the ground cytoplasm (*GC*) but not in the ground nucleoplasm (*GN*). Magnification, 116,000.