

## ELECTRON MICROSCOPY OF HELA CELLS AFTER THE INGESTION OF COLLOIDAL GOLD\*†

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The process of pinocytosis has become well known through studies utilizing time-lapse phase cinematography (1-10). In this phenomenon, globules of fluid are enveloped by pseudopodia of cells and are taken into the cytoplasm. Some of the ways in which pinocytosis was considered to have possible significance were as follows: (a) Proteins or viruses might possibly be carried into cells in globules of fluid during pinocytosis; (b) It has been reported that the inclusion droplets of pinocytosis appear to develop into mitochondria (5, 6, 9); (c) Suggestions have been made that other membranous structures of the cytoplasm (5, 11-15) might be derived from the cell membrane through pinocytosis.

The successful use of silver as a tracer substance for electron microscopy (16-18) suggested that a study of pinocytosis could be carried out with fine particles of some other heavy metal. In the present study, colloidal gold was used for this purpose.

### *Methods and Materials*

*Colloidal Gold.*—The suspension of colloidal gold had been prepared as radioactive gold for therapeutic use but had decayed before we received it.<sup>1</sup> Information from the manufacturer indicated that the gold was suspended in a solution of 1 per cent gelatin and was present in a concentration of 6 to 8 mg. per ml. For estimation of the size of gold particles, the suspension of colloidal gold was mixed in our laboratory with a suspension of latex spheres<sup>2</sup> with a mean diameter of 88 m $\mu$  (s.d. 8 m $\mu$ ). In electron micrographs of gold particles and latex spheres in the same field, measurements showed the gold particles to have a mean diameter of 14 m $\mu$  (s.d.  $\pm$  5 m $\mu$ ).

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<sup>1</sup> Colloidal gold was generously furnished by Abbott Laboratories, Oak Ridge, Tennessee.

<sup>2</sup> Generously supplied by the Dow Chemical Co., Midland, Michigan.

*Tissue Culture.*—Media and methods were as used in previous work (19). In some experiments, we used the medium of Eagle (20) with 10 per cent horse serum and HeLa cells adapted for growth in horse serum.<sup>3</sup> The suspension of colloidal gold was mixed with the culture medium, and it was found that the cells grew well when 0.1 ml. of gold suspension was added to 8.0 ml. of medium. Cells were fixed, embedded, and sectioned for electron microscopy after continuous cultivation in medium containing colloidal gold for 1 to 6 days, 13 days, 4 weeks, and 5 weeks. After 6 days, 4 weeks, and 5 weeks, the nutrient medium was replaced by maintenance medium (34) with or without colloidal gold and, after further incubation for 2 to 4 days at 30°C. or 36°C., the cells were fixed, embedded, and sectioned for electron microscopy. Although intracellular particles of gold were demonstrated readily under all these conditions, no difference was noted in the location of gold particles as viewed in the sections.

*Electron Microscopy.*—Recent reports have indicated that increased resolution can be obtained by using sections of tissue extended over holes in the supporting membrane (21, 22). In the present study, we have found that the same end can be accomplished without any supporting membrane whatever by use of grids with small holes,<sup>4</sup> and that the main factor allowing the section to withstand the beam under these conditions is complete coverage of the hole by the section. It is our impression that greater resolution and clearer micrographs result from this technique, and we have used it for much of the present work.

Other electron microscopic techniques were as used previously (19, 23) except that polymerization of methacrylate was carried out at 60°C. (24), and a Porter-Blum microtome was used (25). Fixatives of Dalton (26) and Palade (27) were employed, and the cells were kept in contact with the fixative for 5 minutes. In some experiments, the cells were washed twice with balanced salt solution before fixation in order to eliminate extraneous material that might interfere with cutting the sections.

The possibility of displacement of gold particles by the knife was considered, but distortion or tearing of cytoplasm adjacent to gold particles was not evident. Also, in sections where the direction of the stroke of the knife could be ascertained by lines from defects in the cutting edge, the positions of gold particles in most cases were not those to be expected from displacement of particles by the knife.

Particles of gold in sections were compared with gold particles alone on coated grids and were identified in the sections by their great density, round shape, and occurrence in groups. Dust particles were distinguished by irregular shapes, but sections were not used unless they were practically free of dust. All locations of gold particles were confirmed by repeated observations.

In the course of the work, 530 micrographs were made of cells grown in media containing colloidal gold. Control studies of HeLa cells without contact with gold have utilized 475 micrographs and have included uninfected cells previously reported (19, 28).

## RESULTS

*Location of Gold Particles in Sections of HeLa Cells.*—Particles of gold were observed in the following places: (a) Within the cytoplasm in areas bounded by single membranes (Figs. 1, 4, 5); (b) in moderately dense granules of the cytoplasm (Figs. 1, 4); (c) in globoid bodies (28),<sup>5</sup> round or ovoid structures

<sup>3</sup> Obtained from Microbiological Associates, Inc., Washington, D. C.

<sup>4</sup> AEI Athene copper grids with holes 30  $\mu$  in diameter. Obtained from Smethurst High-Light Ltd., Sidcot Heaton, Bolton, Lancashire, England. Sections were placed on the shiny side of the grids.

<sup>5</sup> The term globoid body is used tentatively as a means of designation. The term inclusion droplet did not seem satisfactory to describe bodies with internal structure.

of the cytoplasm, usually bounded by single membranes and containing pleomorphic internal structure, circles, lines, etc. (Figs. 2 to 4); (*d*) in globoid bodies containing lines and resembling mitochondria (Figs. 2 to 4); (*e*) adjacent to any of the foregoing types of structures, but outside of them, in which instances the membranes of such structures were often discontinuous or absent (Figs. 1, 3, 4); (*f*) in the matrix of the cytoplasm without apparent association with any cytoplasmic organelle (Figs. 1, 3, 4). Particles of gold were not observed within or associated with typical mitochondria,<sup>6</sup> Golgi complex, granular forms of endoplasmic reticulum<sup>7</sup> (ergastoplasm), or nuclei. Granules were present occasionally in mitochondria (16, 29–33) but were distinguished easily from particles of gold by their lesser density.

*Large Amounts of Gold in HeLa Cells.*—In some sections, large masses of gold particles with or without surrounding membranes were present in the cytoplasm (Fig. 5). Occasionally, also, observation by low power of the light microscope showed a diffuse red appearance of the cells similar to the red color of the colloidal gold, and electron microscopy of such cultures revealed similar large intracytoplasmic masses of gold particles. Smaller numbers of gold particles were observed in other parts of the cytoplasm, and their location was the same as described in the preceding paragraph. Further trials showed that the ingestion of large amounts of gold occurred in the presence of some sera containing small amounts of whitish precipitate similar to that often found in stored sera.<sup>8</sup> Removal of the slight precipitates, through sedimentation by gravity or by centrifugation, resulted in serum no longer associated with ingestion of large amounts of gold.

#### DISCUSSION

Although it is most likely that particles of colloidal gold entered HeLa cells by pinocytosis in these experiments, it should be pointed out that the electron micrographs in themselves do not give evidence concerning the temporal evolution of structures in pinocytosis because various possible stages are seen after all the intervals that the cells were in contact with colloidal gold. Nevertheless, the dynamics of pinocytosis have been amply studied by others using time-lapse phase cinematography (1–10), and the phenomenon has been shown to be unusually active under conditions of tissue culture closely similar to those of the present study.

Under these circumstances, it seems reasonable to suggest that membrane-bounded areas containing particles of gold are inclusion droplets resulting from pinocytosis, although some of such formations might result from cross-sections of invaginations of the cell membrane. Moderately dense granules and globoid

<sup>6</sup> Except a single particle in one instance.

<sup>7</sup> We have used these terms as synonyms.

<sup>8</sup> No microorganisms were detected by microscopic examination or by culture.

bodies<sup>9</sup> containing particles of gold are possibly later stages of pinocytosis, and the internal structure of globoid bodies might represent accumulations of membrane material simultaneously phagocytized. Intracytoplasmic gold particles not surrounded by a membrane could be attributed to disintegration of inclusion droplets, granules, or globoid bodies.

In a recent quantitative study concerning the ingestion of radioactive colloidal gold by macrophages, the data were thought to be inconsistent with the theory that intracellular penetration of gold particles took place by pinocytosis (43). However, in addition to the present studies, electron microscopic observations of others have shown that single membranes may surround bacteria and other dense particles ingested by phagocytic cells (37, 42, 44, 45). We think that these membranes support the theory that foreign particles may enter cells by a process in which invaginations of the cell membrane are pinched off. The observations also favor the concept that pinocytosis and phagocytosis are related phenomena.

The experiments with colloidal gold do not provide evidence that mitochondria,<sup>10</sup> Golgi complex, or the granular forms of endoplasmic reticulum (ergastoplasm) are derived from the cell wall by pinocytosis. Gold-containing areas bounded by single membranes resembled non-granular forms of endoplasmic reticulum enough to be consistent with the suggestion of others that some of such structures might be derived from the cell membrane (5, 11-15).<sup>11</sup> The presence of gold particles in globoid bodies and their absence from mitochondria do not support our earlier suggestion (28) that globoid bodies of HeLa cells may be forms of mitochondria.

Since it has been reported that colloidal gold suspended in a solution of gelatin and plasma has the same electrophoretic mobility as the alpha globulin of serum (52), it is probable that the gold particles were coated with globulin. Under such conditions, one might expect that the charge and other properties of gold particles would be such that they would be handled by cells in a manner similar to globulins or other serum proteins.

#### SUMMARY

Tissue cultures of HeLa cells were grown in media containing colloidal gold, and after various intervals, the cells were fixed, embedded, and sectioned for

<sup>9</sup> Structures having some resemblance to the globoid bodies have been described in several kinds of cells (35-39), and the suggestion has been made that some of such bodies may be the result of pinocytosis (40). Furthermore, ingested particles of mercuric sulfide and thorium dioxide have been found in moderately dense cytoplasmic granules (41, 42) similar to ones demonstrated in the present study.

<sup>10</sup> The origin of mitochondria is not known (46). Cytoplasmic structures containing particles of silver (16, 18, 46) and thorium dioxide (47) have been interpreted as possible mitochondria.

<sup>11</sup> Invaginations of the cell membrane have also been considered as possible portals of entry for the absorption of nutrient and other materials (48-51).

electron microscopy. Uncoated grids with small holes were used in many of the experiments.

Intracellular particles of gold were identified in areas surrounded by single membranes, in moderately dense granules, in globoid bodies, and in the cytoplasmic matrix. Gold particles were not found in typical mitochondria, Golgi complex, ergastoplasm (granular forms of endoplasmic reticulum), or nuclei.

The phenomenon of pinocytosis was considered to be the most likely means by which the gold particles were ingested, and the locations of gold particles appeared to have significance concerning theories that membranous organelles of the cytoplasm may be derived from the cell membrane.

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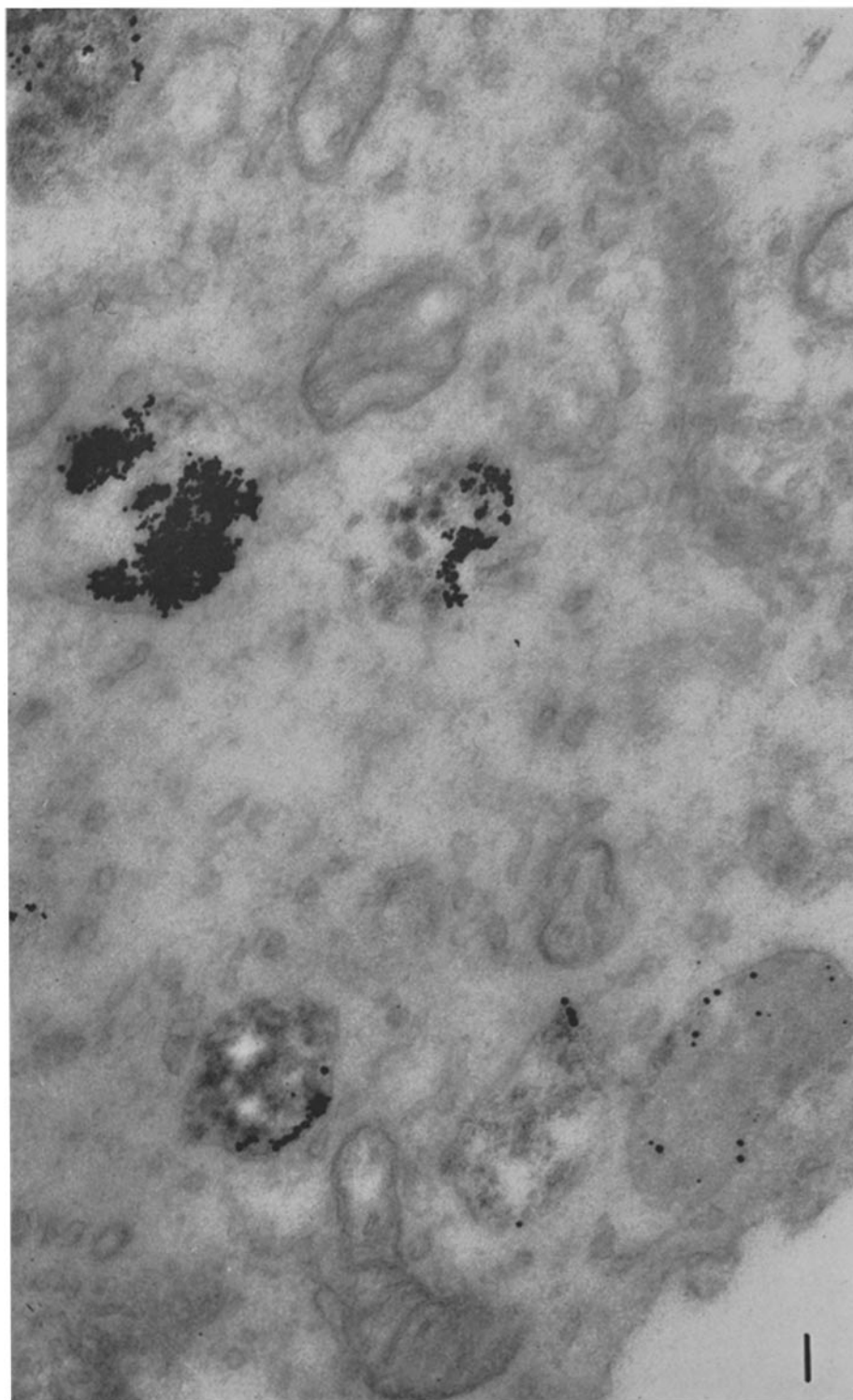
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EXPLANATION OF PLATES

## PLATE 241

FIG. 1. In the upper left corner of the figure, particles of gold are concentrated in a moderately dense granule of the cytoplasm. Slightly above the mid-portion of the figure, concentrations of gold are present in an area surrounded by a single discontinuous membrane (left) and another irregularly dense granule of the cytoplasm (right). At the bottom of the figure, there is a typical mitochondrion devoid of gold, and immediately above it three membrane-bounded areas of different densities contain particles of gold. The membrane of the central of the three areas is discontinuous and one particle of gold at the upper end is outside of the membrane-bounded area. The gold-containing area on the right is near the cell membrane and may possibly be an early stage of inclusion droplet. At the left border of the figure, a small collection of gold particles is not near any organelle. Typical mitochondria and a probable Golgi complex (upper right) do not contain particles of gold. Cells were cultivated in gold-containing medium for 13 days. Uncoated grid. Magnification, approximately 65,000.





(Harford *et al.*: Colloidal gold in HeLa cells)

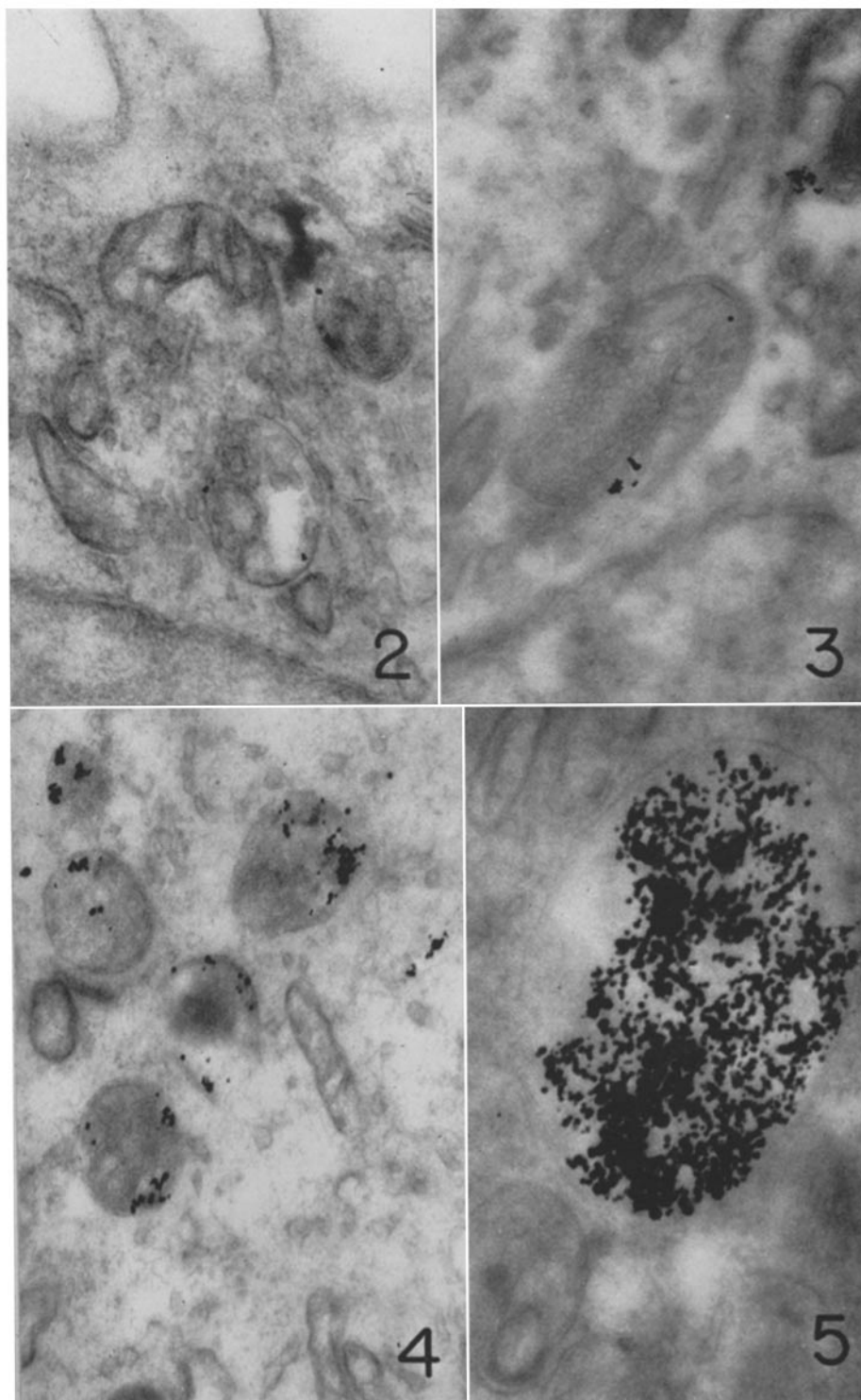
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FIG. 2. In the right mid-portion of the figure, there is a globoid body containing some curved lines and a few particles of gold. To its left, an irregular dense granule and a mitochondrion are devoid of gold. In the lower portion of the figure, there is another globoid structure containing some vesicles and a few gold particles. Large protrusions of the cell membrane are shown at the top of the figure and a small part of the nucleus at the bottom. Cells were cultivated in gold-containing medium for 2 days. Uncoated grid. Magnification, approximately 48,000.

FIG. 3. In the lower mid-portion of the figure, there is a large globoid body containing lines, circles, and some particles of gold. In the upper right portion of the figure a cluster of gold particles is near another globoid body. Cells were cultivated in gold-containing medium for 8 days and then kept in maintenance medium for 2 days at 30°C. Magnification, approximately 55,000.

FIG. 4. Shows five ovoid structures containing concentrations of gold particles. The lowest gold-containing globoid body contains lines that have some resemblance to cristae of mitochondria. The globoid body at the upper right has faint linear internal structure and nearby on the outside are two to three gold particles. It is possible that the gold particles near this body were carried out of it by the stroke of the knife, but there is no visible tear in the cytoplasm. The cluster of gold particles at the right margin of the figure is not near the globoid bodies. In this section, the direction of knife cut is not known. In the right mid-portion of the figure is a typical mitochondrion without gold particles, and throughout the figure, there are agranular forms of endoplasmic reticulum devoid of gold. Cells were cultivated in gold-containing medium for 13 days. Uncoated grid. Magnification, approximately 28,000.

FIG. 5. An unusually large collection of gold particles is located in an ovoid area bounded by a single membrane. A mitochondrion in the upper left corner and a globoid body in the lower left portion of the figure are lacking in gold particles. Cells were cultivated in gold-containing medium for 3 days. Uncoated grid. Magnification, approximately 65,000.



(Harford *et al.*: Colloidal gold in HeLa cells)