DEMONSTRATION OF SURFACE ANTIGENS AND PINOCYTOSIS IN MAMMALIAN CELLS WITH FERRITIN-ANTIBODY CONJUGATES

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Ferritin, a protein of high electron scatter due to its high iron content, can be covalently conjugated to antibody and the resulting molecule employed as an "electron stain" for specific antigens (1, 2). We have conjugated ferritin to gamma globulins of antiascites tumor cell antisera, incubated the cells *in vitro* with such "ferroglobulins," and examined the thin sectioned cells in the electron microscope. Cell membrane antigens have been demonstrated by this technique, and the ferritin has also proved to be a convenient marker for studying the process of pinocytosis.

MATERIALS AND METHODS

Preparation of cells, antibodies, and incubation conditions have been reported in detail elsewhere (3, 4). Horse ferritin was coupled to rabbit immune gamma globulin by the original method of Singer (1), and 0.36 ml of the final product (ferritin, 5.6 mg/ml; gamma globulin, 6.94 mg/ml) was incubated with a washed suspension of Krebs ascites tumor cells (approximately 3×10^6 cells) in a final volume of 1 ml. After 30 minutes' incubation at 37°C with gentle shaking, the cells were centrifuged and fixed with 1 per cent buffered (pH 7.2) OsO_4. After dehydration and embedding in methacrylate, sections were cut on a Porter-Blum microtome and examined in an RCA EMU-3D electron microscope.

RESULTS AND DISCUSSION

I. *Demonstration of Surface Antigens*

Specific antibodies have been shown to induce focal zones of evagination and invagination of the plasma membrane of mammalian cells (3), and this phenomenon was again observed in these experiments. Fig. 1, taken from a cell incubated with rabbit immune ferroglobulin, illustrates the surface change; the labyrinthine folding is thought to arise as normal surface membrane movements bring separated antigenic points into sufficient proximity to be fixed successively by antibody. Higher resolution studies of such cells (Fig. 2) showed a characteristic array of ferritin molecules along the cell membrane, indicating that the conjugated antibody was fixed to surface antigens. Ferritin molecules did not appear in significant numbers on the surfaces of control cells incubated with non-immune conjugates or with uncoupled ferritin and immune gamma globulin. It may therefore be assumed that nearly every ferritin molecule associated with the cell membrane in Fig. 2 marks a site of antibody-antigen union.

This direct demonstration of the fixation of antibodies to cell membrane antigens supports the view that the cell membrane is the primary site of attack of cytotoxic antibodies and complement (4, 5). Ferritin-antibody conjugates have been employed in a more detailed study of the events of immune cytolysis (6).

II. *Pinocytosis of the Ferritin Label*

The ferritin-antibody units did not appear able to pass directly through the cell membrane into the cytoplasmic matrix. When ferritin molecules were identified in the interior of experimental and control cells, they were always contained within membrane-bounded profiles and presumably had entered the cells by the process of

pinocytosis (7–10). Cells incubated with immune ferroglobulin seemed to contain more pinocytosed ferritin than cells incubated with non-immune conjugate or with uncoupled ferritin and immune gamma globulin. This suggests that the specific fixation of the immune conjugate to the cell (11), the ferritin is incorporated into the interior, where it may be considered to lie within the system of membranes termed the endoplasmic reticulum (9). Most of the pinocytosed ferritin molecules were contained within simple vesicles of varying diameter and no internal structure

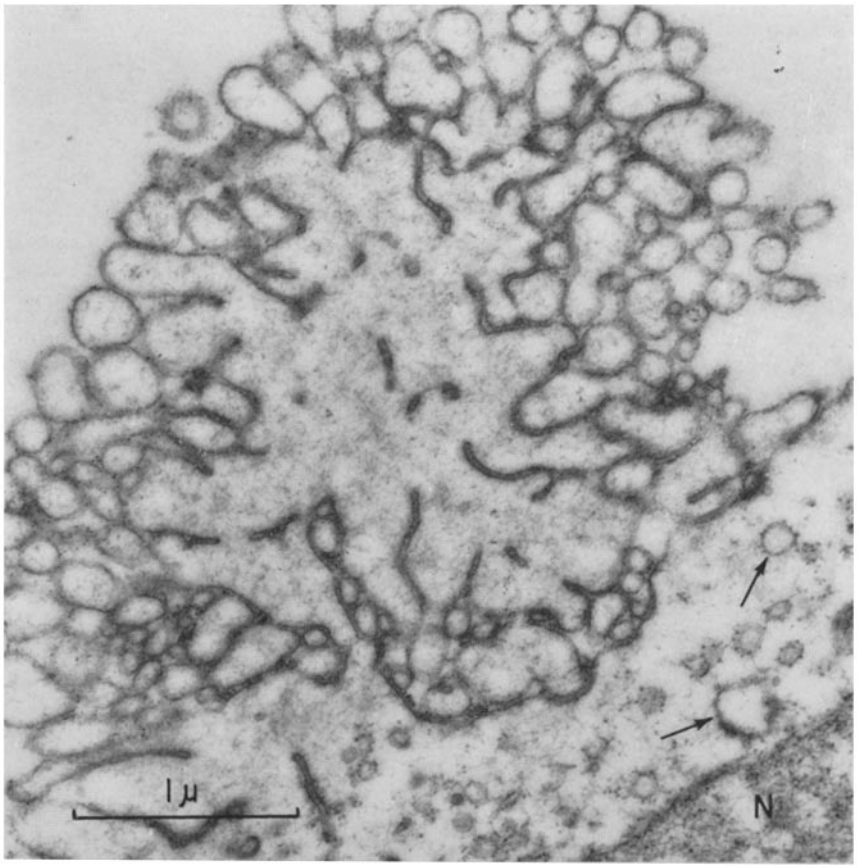

FIGURE 1

Antibody-induced surface alteration in Krebs ascites tumor cell. Note that invaginated, apposed elements of the surface membrane tend to maintain a rather constant distance of separation (100 to 300 A). In contrast, elements of endoplasmic reticulum (arrows) tend to have sectioned profiles with diameters much greater than 300 A. *N*, nucleus. × 30,000.

surface provided a greater opportunity for pinocytosis of the label to occur. Fig. 3, taken from a control cell incubated with non-immune conjugate, demonstrates a series of small vesicles extending from the surface membrane to the cell interior, with ferritin molecules present in the deepest vesicle. Presumably by such a process of invagination and vesiculation at the cell surface (Fig. 4). However, ferritin was also identified within membrane-bounded profiles exhibiting polymorphic internal organization (Figs. 5 to 7). Ferritin was not identified in every sectioned granule of this type (Figs. 8 to 10); the ferritin may have been present but outside the plane of section, or the granule may have been formed before pinocytosis of the ferritin occurred. Both

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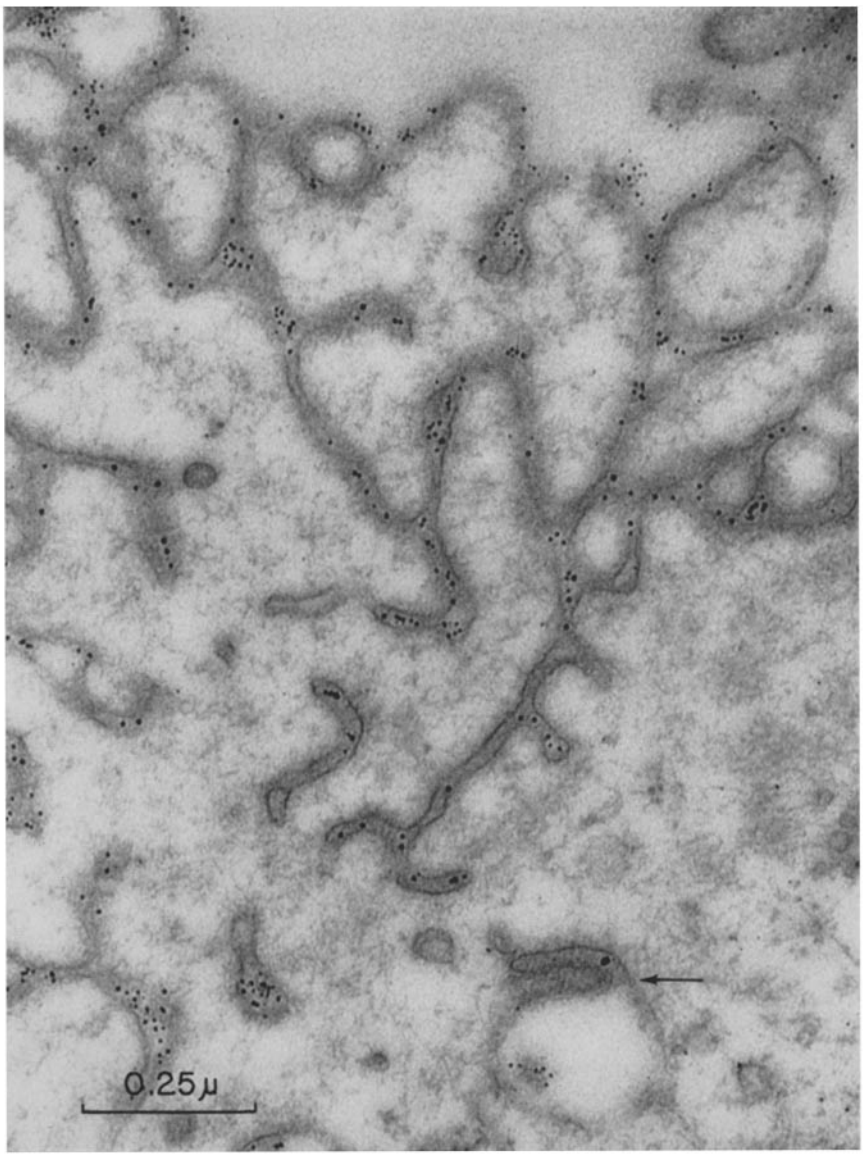

FIGURE 2

Higher resolution study of zone of surface folding in cell incubated with immune ferroglobulin. Ferritin molecules are arrayed along the cell membrane and mark sites of antibody-antigen bonding. Arrow marks transition between apposed surface membranes and a large spherical profile. Its distance from the cell surface and its large diameter suggest that the profile is an element of smooth surfaced endoplasmic reticulum. × 93,000.

the clear vesicles and the profiles with internal structure were bounded externally by a smooth 70 A membrane. Ferritin was not identified within cavities of rough surfaced endoplasmic reticulum nor within mitochondria.

It thus appeared that ferritin, originally enclosed within invaginated segments of the plasma membrane, eventually became segregated in the cell interior in structures bounded by a smooth membrane and exhibiting varying stages of

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organization. It might be assumed that the latter structures were in at least intermittent continuity with the system of smooth surfaced endoplasmic reticulum, and they may represent further specializations of that system.

Studies by Parks *et al.* with Kupffer cells (12), by Odor with mesothelium (13), and by Harford *et al.* with HeLa cells (14) have also shown that pinocytosed colloidal particles appear in clear vacuoles and in cytoplasmic granules of varying size, density, and structure. Brandt's phase and fluorescence microscopic studies on the amoeba

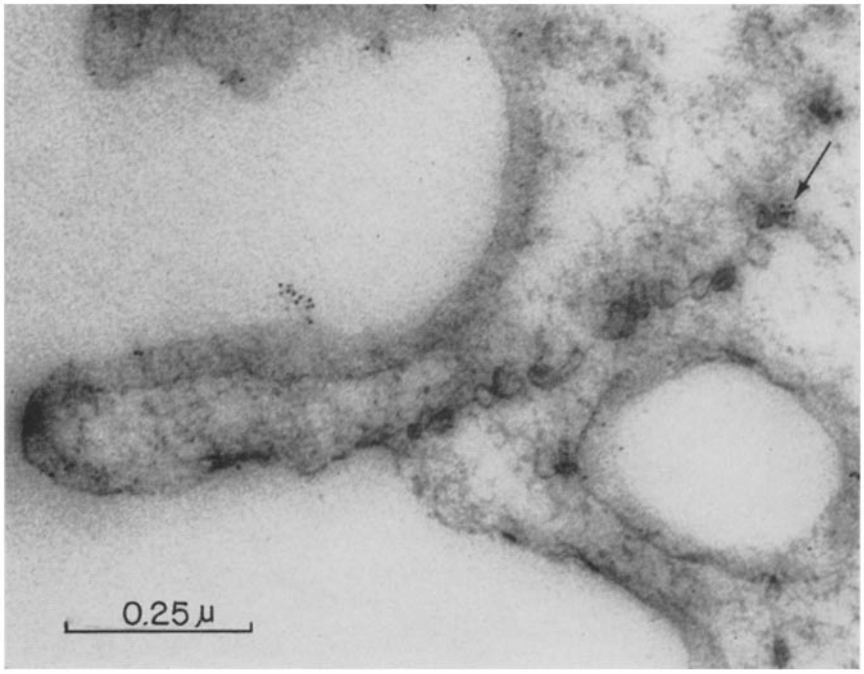

FIGURE 3

Cell incubated with non-immune ferroglobulin. A series of small vesicles extend from the cell surface into the cytoplasmic matrix. Ferritin molecules (arrow) are identified in deepest vesicle. × 98,000.

FIGURE 4

Ferritin molecules within spherical profiles lacking internal organization. × 68,000.

FIGURES 5 AND 6

Ferritin molecules segregated within profiles possessing internal folds or vesicles. *N*, nucleus. × 60,000.

FIGURE 7

Ferritin within large profile with polymorphic internal organization (compare with Fig. 9). × 75,000.

FIGURES 8 TO 10

Additional examples of cytoplasmic bodies (arrows) that are thought to be lysosomes (see text). × 68,000.

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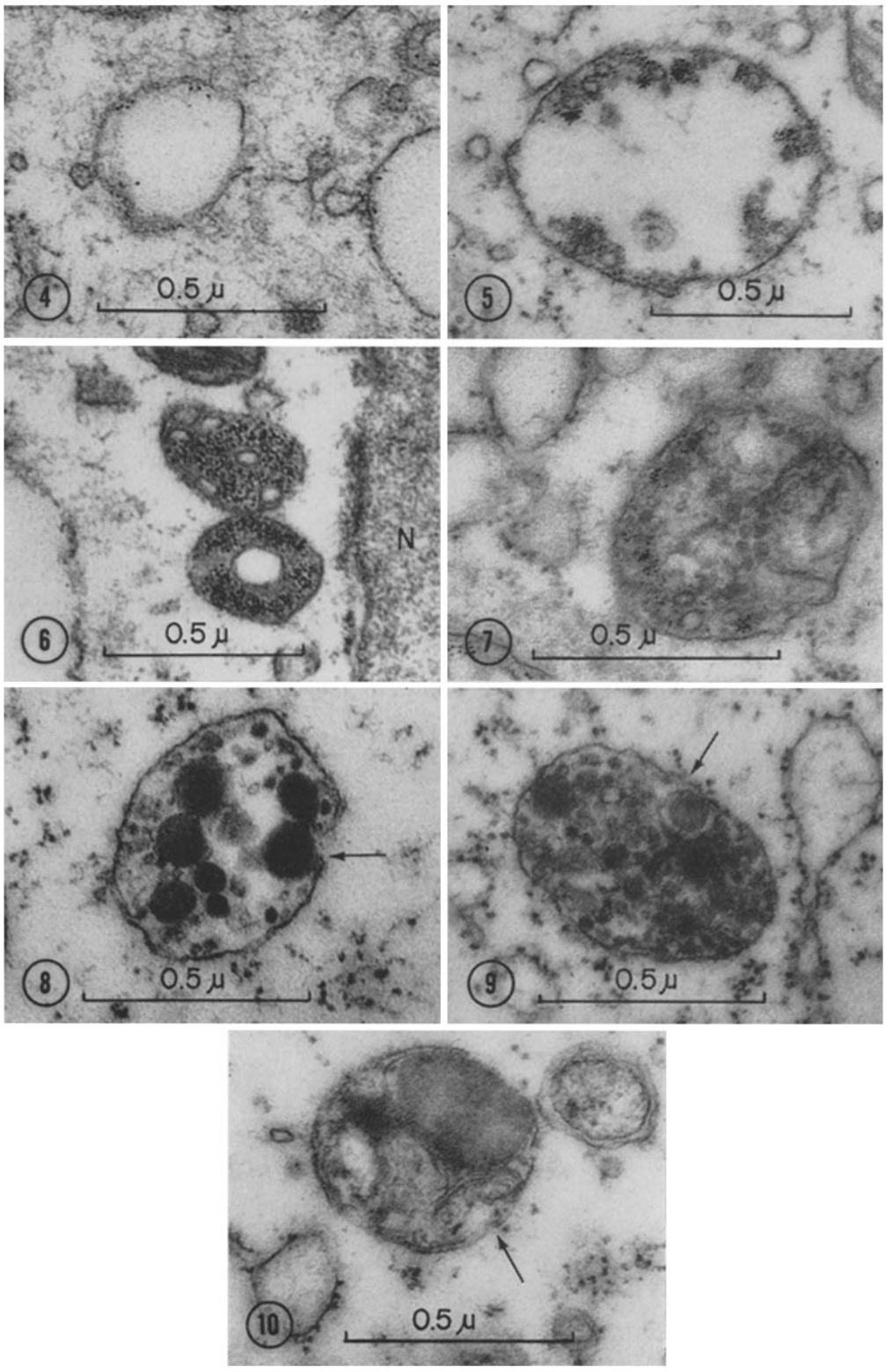

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Chaos chaos have indicated that pinocytotic vacuoles may develop "internal structure" by virtue of infoldings of the limiting membrane of the vacuole (15).

Evidence has accumulated in recent years to suggest that cytoplasmic inclusions containing segregated phagocytosed or pinocytosed "foreign material" ("phagosomes," "digestive vacuoles") belong within the functional class of particles designated as lysosomes (16–22). Particles so designated, because of their complement of acid hydrolytic enzymes or content of segregated foreign material, exhibit a range of variation in size, density, and details of internal structure. The morphologic details seem to vary with the cell type and the functional state of a given cell.

We suggest that the structures illustrated in Figs. 5 to 10 of this paper can be considered to be lysosomes. Some of the granules clearly are involved in the pinocytotic process, and within the noted range of variation they appear similar to lysosomes from other cell types (21, 23–26).

This investigation was supported by grants from the United States Public Health Service (PX-204-8-S 1, E-4140, GSF-424, C-3249, and SF-319) and by the Lillia Babbit Hyde Foundation.

Received for publication, July 20, 1961.

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