A METHOD FOR THE ISOLATION OF PLASMA MEMBRANE OF ANIMAL CELLS

JOCHEN W. HEINE and CARL A. SCHNAITMAN. From the Department of Microbiology, University of Virginia, Charlottesville, Virginia 22901

The in vivo phagocytosis of polystyrene latex beads by animal cells has been observed by Schoenberg et al. (2) with rabbits and by Singer et al. (3) with mice. Wetzel and Korn (6) reported the phagocytosis of polystyrene latex beads by amebae as a means to isolate phagocytic

vesicles and their membrane. The above method was adapted to animal cells cultured in vitro for the isolation of L-cell plasma membrane.

This adaption was undertaken to provide a fast and gentle method for the isolation of very pure and enzymatically active plasma membrane. Preliminary experiments were done to determine the optimum conditions for maximum uptake and to determine the optimum size of beads. The size is important, since Wetzel and Korn (6) have shown that beads which are too small are not ingested singly. Electron microscope and biochemical quantitative results indicated that beads of 0.714 μ in diameter gave maximum uptake with maximum single ingestion during a 30 min incubation period.

L-cells were grown for 3 days in spinner culture, then 1×10^7 cells were mixed with latex beads at a ratio of 2000 per cell in 7 ml of cold medium 199, with or without serum. The cell-bead mixture was then spun onto a flat agar receiving surface at 9000 *g* for 10 min, to bring the beads in close contact with the cells. This technique was adapted from the sedimentation incubation technique used by Heine and Schnaitman (1). The tubes were then incubated for various time periods in a water bath at 37°C. At the end of each incubation period the tubes were placed in an ice bath. The cells were resuspended and washed with phosphate-buffered saline (PBS) containing 0.25 M sucrose by low speed centrifugation to remove excess beads. After several washes the cell pellet was either extracted overnight with dioxane (2 ml/10^6 cells) or suspended in a small volume of PBS, and was prepared for electron microscopy as described by Heine and Schnaitman (1), with the substitution of absolute alcohol for propylene oxide as the dehydrating agent (6). The amount of polystyrene ingested was estimated by the absorption of the dioxane solution at 259 nm (6). The kinetics of latex bead ingestion is demonstrated in Fig. 1. A control incubation tube was incubated for 60 min at 0°C to show that the uptake was temperature dependent; no uptake was noted. The uptake leveled off after about 30 min of incubation, and no difference was observed due to the presence or absence of serum in the medium. Electron micrographs of a cell containing ingested beads are shown in Fig. 2.

The number of beads ingested per cell was also calculated from electron micrographs by measuring the increase in cell volume as a consequence of bead uptake. The number obtained was 900 beads/cell, which is in good agreement with the dioxane quantitation shown in Fig. 1. This number is greater than the number of beads per ameba reported by Wetzel and Korn (6), which may be due to the larger size of beads used by those authors. When the surface area of ingested beads is related to total surface area of the cell, one finds that only about 30% of the L-cell membrane is wrapped around the beads as compared to 50% with amebae as reported by the above authors.

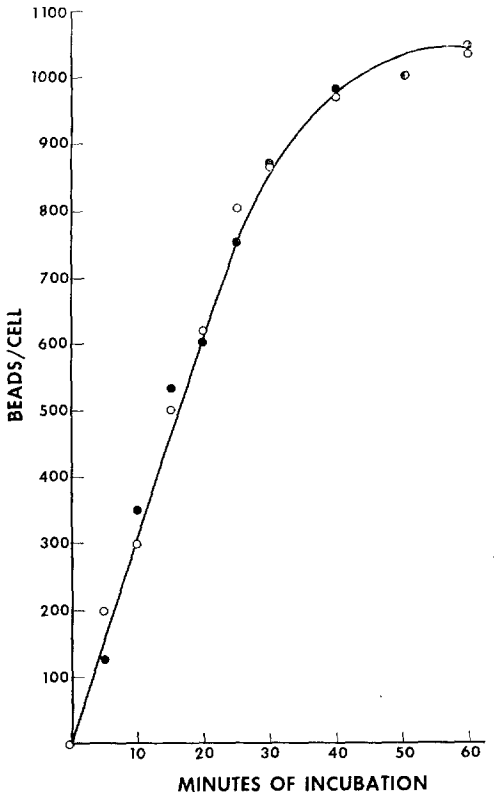

FIGURE 1 The uptake of latex beads by L-cells at 37°C. ●—●—● Medium 199 supplemented with 20% fetal calf serum; ○—○—○ Medium 199.

For the isolation of membranes, L-cells (10^9 cells) prepared as described above were suspended with latex beads at a multiplicity of 1000 beads/cell in 25 ml of medium 199 with 15% serum in a polyallomer centrifuge tube (1 × 3½ in.). The large number of cells suspended in the small quantity of fluid facilitated a close contact between cells and latex beads. The cell-bead mixture

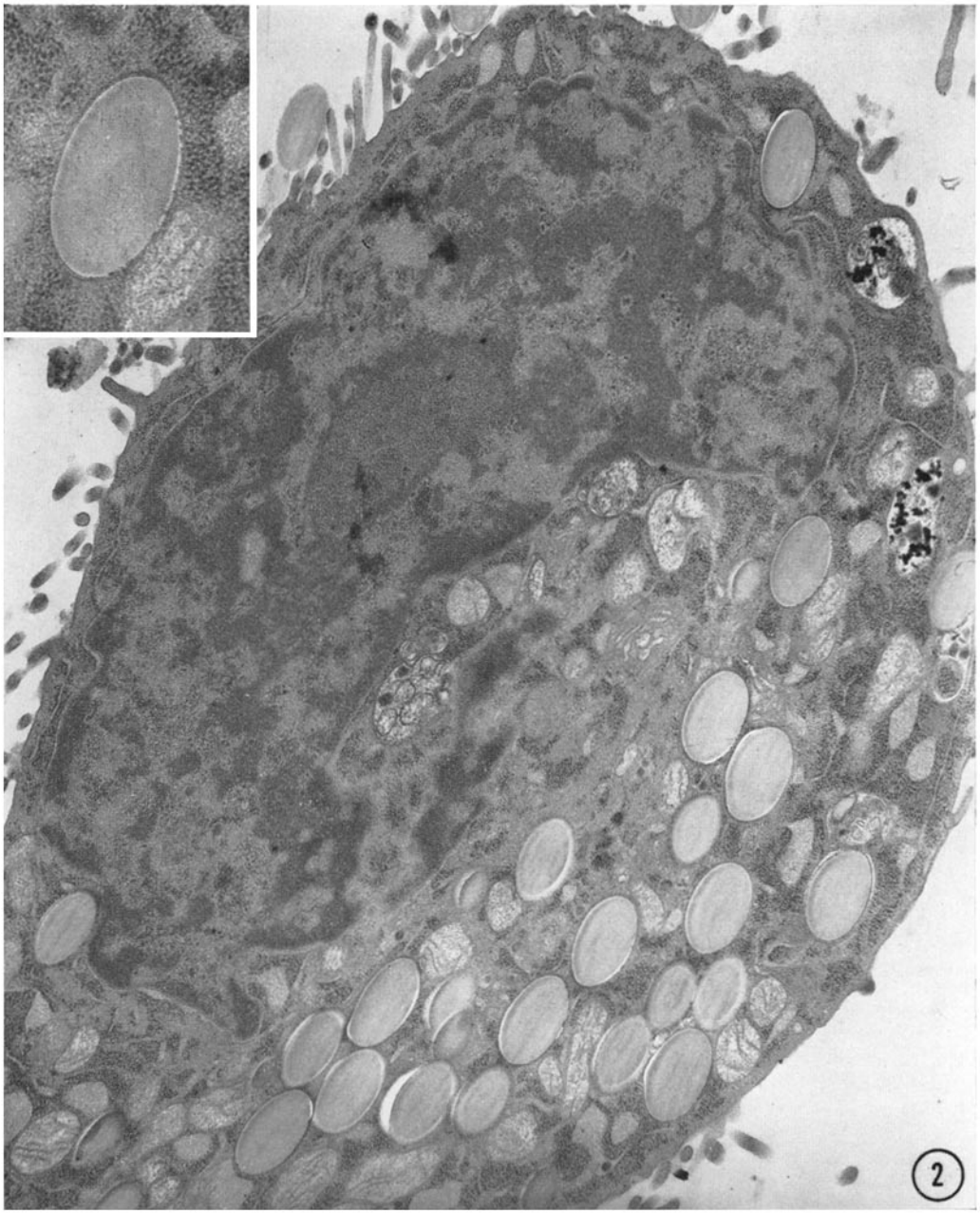

FIGURE 2 Typical cross-section of an L-cell with ingested latex beads after 30 min incubation at 37°C. The insert shows an ingested bead enclosed by membrane. × 15,000. *Insert*, × 33,800.

was incubated for 30 min in a water bath with continuous stirring at 37°C in an atmosphere containing 5% CO_2. The excess beads were removed by washing several times by centrifugation at 500 *g* for 5 min in PBS containing 0.25 M sucrose, 0.5 g/l BSA, and 10^{-3} M EDTA. The cells were then ruptured by rapid decompression from a nitrogen bomb (Parr Instrument Co., Moline, Ill.) after equilibration for 20 min at 800 psi of N_2 at 4°C (4).

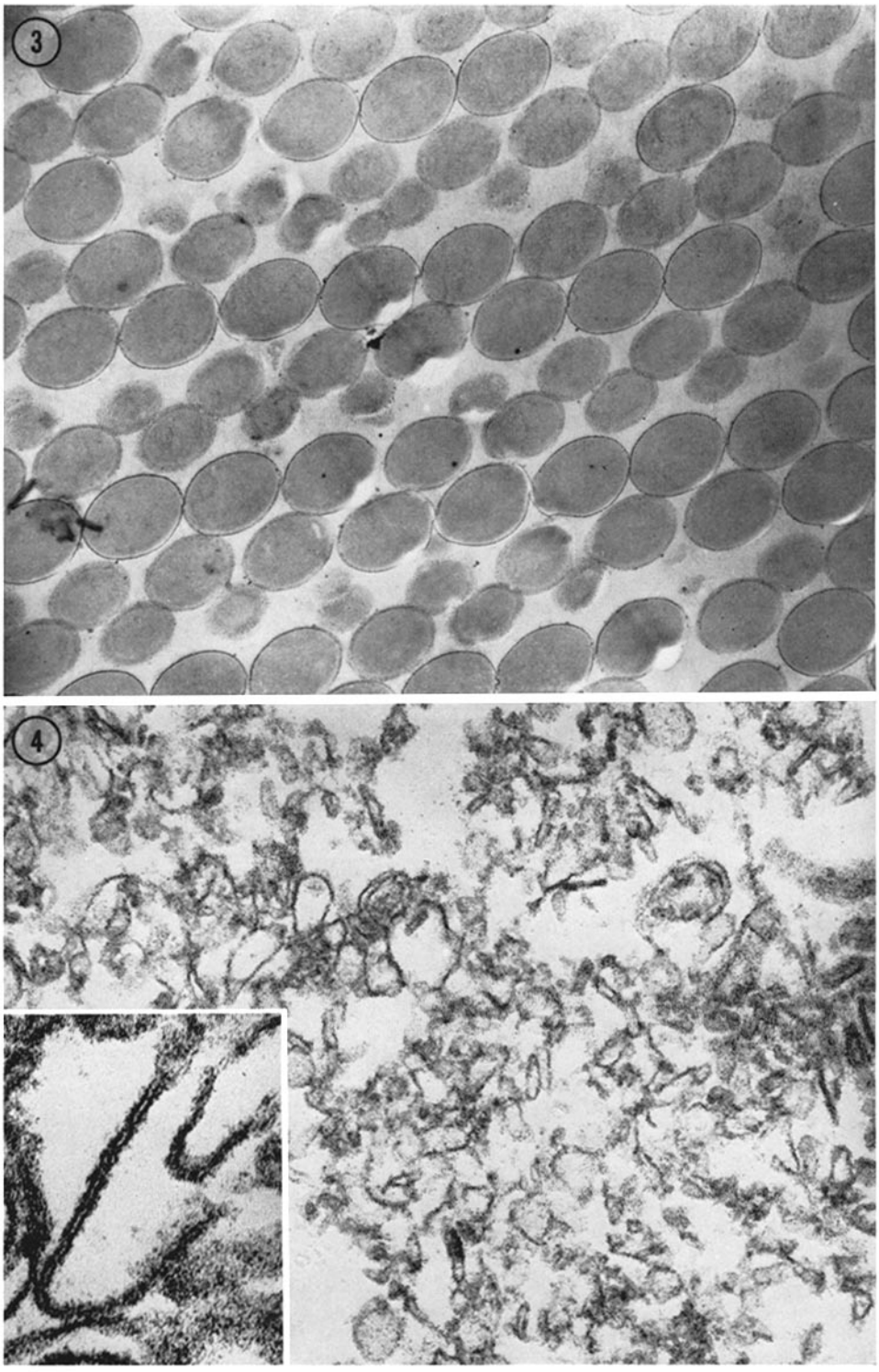

FIGURE 3 Membrane-enclosed beads from 10–20% sucrose interphase of gradient. All beads are singly enclosed. × 20,000.

FIGURE 4 Isolated membranes. × 54,000; *Insert*, × 161,000.

The procedure used for the isolation of membrane-coated beads and membranes was that of Wetzel and Korn (6), with slight modification. The solution released from the nitrogen bomb was mixed with an equal volume of 60% (w/v) sucrose in 0.02 M Tris buffer containing 10^{-3} M EDTA at pH 6.8, and was placed into polyallomer centrifuge tubes. It was then overlayered with 25, 20, and 10% sucrose made up in the above buffer and was centrifuged in a Spinco SW 25.1 rotor at 79,000 *g* for 90 min. The band at the interphase of 10–20% sucrose was collected and diluted with Tris-EDTA buffer as above, and centrifuged at 20,000 *g* in the SW 25.1 rotor for 15 min. The membrane-coated beads were resuspended in the above buffer and subjected to two 30-sec pulses of sonication (Blackstone Ultrasonics, Inc., Sheffield, Pa.) at maximum intensity with 1 min intervals between pulses. The beads were then pelleted in an SW 50 rotor at 15,000 *g* for 15 min and the supernatant was centrifuged at 180,000 *g* for 60 min. The membrane pellet was resuspended in PBS for electron microscope and/or chemical analysis.

Micrographs prepared from cells with ingested beads and isolated membrane-enclosed beads show only singly ingested beads (Figs. 2 and 3). The band between the interphase of 10 and 20% sucrose contained primarily beads enclosed tightly and individually by plasma membrane. Multiple ingestion (more than one bead/vesicle) was observed with an incubation period of 40 min and longer. Fig. 4 shows the isolated membranes which appear free of glycogen and microsomal contamination.

Preliminary chemical analysis showed a cholesterol-to-phospholipid ratio of 0.7, in good agreement with the value reported for plasma membrane by Weinstein et al. (5). This method has also been used for the isolation of chick embryo plasma membrane, for which the ratio of cholesterol to phospholipid was 0.6 (McSharry and Wagner, in preparation). Further studies on the chemical and enzymatic properties of this preparation are in progress.

This investigation was supported by grant AI-00102 from the National Institute of Allergy and Infectious Diseases, grant CA-10387 from the National Cancer Institute, grant E-617 from the American Cancer Society, and by grant GB-8142 from the National Science Foundation.

Received for publication 22 July 1970.

REFERENCES

1. HEINE, J. W., and C. A. SCHNAITMAN. 1969. Fusion of vesicular stomatitis virus with the cytoplasmic membrane of L-cells. *J. Virol.* **3**: 619.
2. SCHOENBERG, M. D., P. A. GILMAN, V. R. MUMAW, and R. D. MOORE. 1961. The phagocytosis of uniform polystyrene latex particles by the reticuloendothelial system in the rabbit. *Brit. J. Exp. Pathol.* **42**:486.
3. SINGER, J. M., L. ALDERSBERG, E. M. HOENIG, E. ENDE, and Y. TCHORSCH. 1969. Radiolabeled latex particles in the investigation of phagocytosis *in vivo*: clearance and histological observations. *J. Reticuloendothelial Soc.* **6**:561.
4. WALLACH, D. F. H., and V. KAMAT. 1964. Isolation of cytoplasmic membranes from Ehrlich ascites tumor cells. *Proc. Nat. Acad. Sci. U.S.A.* **52**:721.
5. WEINSTEIN, D. B., and J. B. MARSH. 1969. Membranes of animal cells. IV. Lipids of the L-cells and its surface membrane. *J. Biol. Chem.* **244**: 4103.
6. WETZEL, M. G., and E. D. KORN. 1969. Phagocytosis of latex beads by *Acanthamoeba costellanii* (NeFF). III. Isolation of phagocytic vesicles and their membranes. *J. Cell. Biol.* **43**:90.