Novel vesicular extrusions during cell spreading

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Summary. During the late stages of cell spreading in vitro, the cells extrude a vesicular material into the medium. This phenomenon was observed in human glia and glioma cells as well as in human diploid fibroblasts MRC-5 and WI-38 cells. This extrusion of vesicular material is inhibited by cytochalasin-B and colcemid suggesting the involvement of microfilaments and microtubules and the active nature of this event. It appears that the cells may be excreting damaged surface components by a mechanism similar to patching, capping and endocytosis.

Morphological and molecular events occurring during cell spreading in vitro are of basic biological interest[1-3]. During the course of a study on cell spreading, we observed a novel phenomenon of vesicular extrusion by spreading cells, which has not been reported earlier.

Materials and methods. Human glia and glioma cells, and normal human diploid MRC-5 and WI-38 cells were grown under standard culture conditions in Eagle's medium containing 10% fetal calf serum (Gibco). Cells were harvested with 0.25% trypsin in $Ca^{++}Mg^{++}$ free PBS, rinsed in medium with or without serum and were layered on glass coverslips for spreading for different durations. In some cases cells were incubated in suspension in agarose-coated petri plates (do not favor cell adhesion) for 1 h in 10% serum supplemented medium or medium containing 9% fetal calf serum and 1% rabbit anti-fibronectin serum; and were then allowed to spread on glass coverslips. Cells were also incubated in the presence of cytochalasin-B (2 µg/ml), colcemid (10^{-5} M) singly or in the presence of both drugs simultaneously.

Results and discussion. Cells are spherical in suspension; cells put out filapodia and lamellipodia on contact with the substratum and begin to spread out in sequential stages as described earlier[2]. During the stage of cytoplasmic spreading, small protrusions occur on the upper surface of the nonspread nuclear region (fig. 1). These protrusions become well defined vesicular extrusions at the time of nuclear flattening (stage 4 in the cell spreading process[2] figs 1 and 2) around 40–60 min after layering. These vesicles are then slowly detached and lost into the medium by around 3–6 h after layering.

Cells that were kept in suspension in 10% serum or anti-fibronectin antiserum containing medium and then allowed to spread display this phenomenon conspicuously since the shedding of vesicles is more synchronized.

When the cells were incubated in the presence of cytochalasin-B (2 µg/ml), colcemid (10^{-5} M) singly or in both drugs simultaneously for 2 h, the vesicular release was retarded as shown in figures 2 and 3. After 2 h of spreading 20–30% of control glia cells still showed vesicles attached to the upper surface, while cells incubated with cytochalasin-B, colcemid or both retained vesicles to the extent of 48%, 63% and 90% respectively. This indicates that cytoskeletal elements such as microfilaments and microtubules are involved in the extrusion of these vesicles and, therefore, this appears to be an active process.

Human glia and glioma cells extruded vesicles with a mean diameter of 6 µm, while the vesicles from the fibroblasts

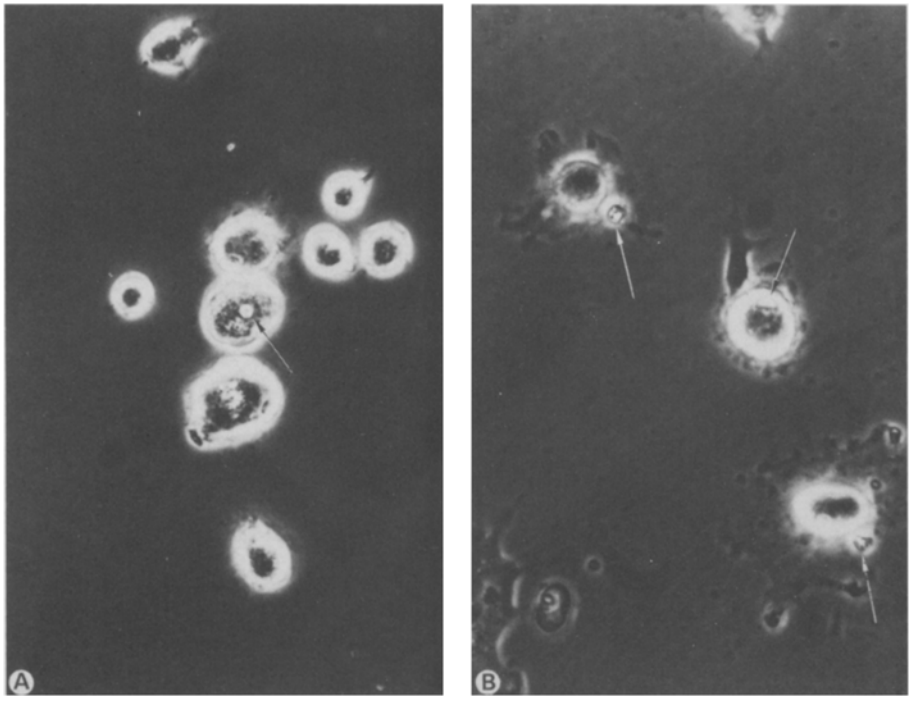

Figure 1. Vesicular extrusions in spreading MRC-5 cells, ×600. *A* Cells in the early stages of spreading. Arrow points to the vesicular protrusion. *B* Cells in cytoplasmic spreading stage (stage III) with the vesicular protrusion being very evident.

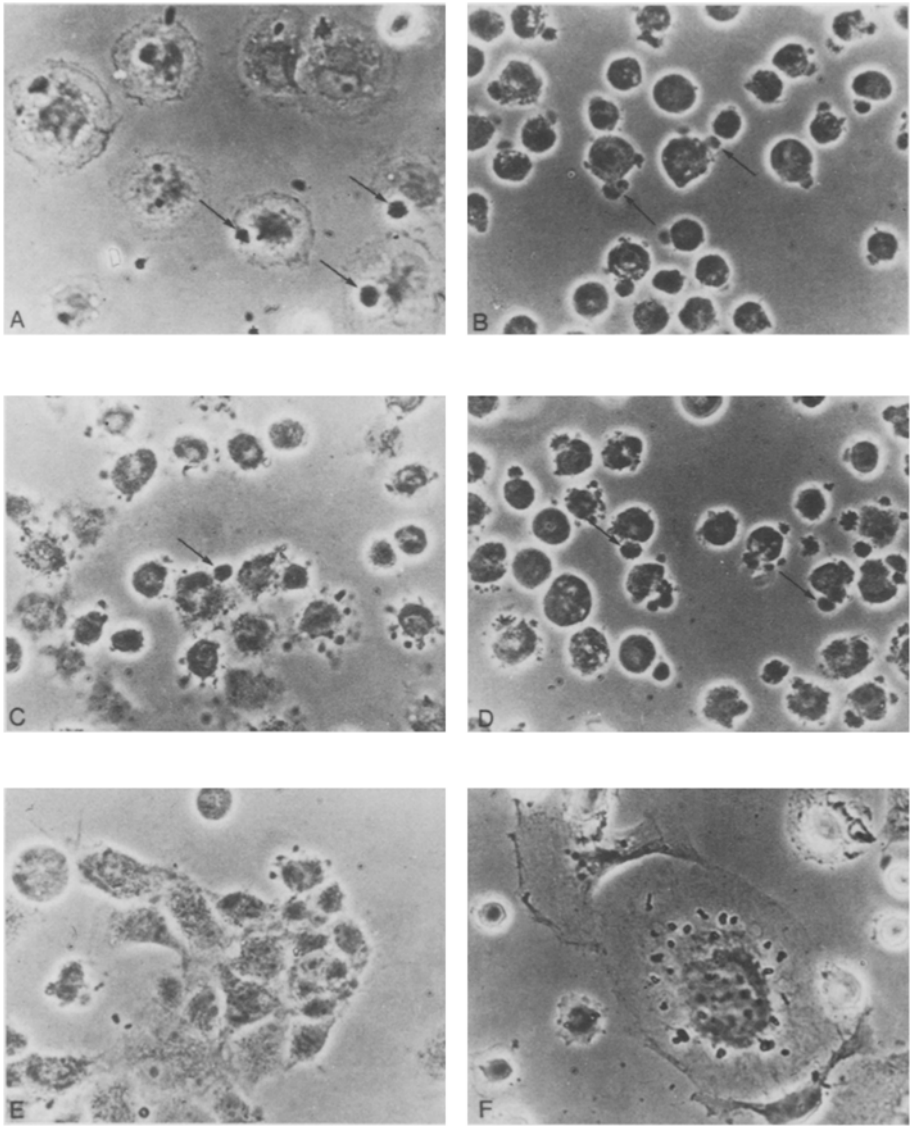

Figure 2. Vesicular extrusions in spreading human glia cells; ×300. *A* Cells spread for 2 h; note the dark phase vesicular extrusion bodies on cells that are in the advanced stage of spreading. *B* Cells incubated with 2 µg/ml cytochalasin-B for 2 h; note the vesicular extrusions on each cell. *C* Cells incubated for 2 h in the presence of 10^{-5} M colcemid showing partial spreading and vesicular extrusions. *D* Cells incubated for 2 h with 2 µg/ml cytochalasin-B and 10^{-5} M colcemid simultaneously showing no residual spreading. *E* Cells spread for 6 h; the vesicular bodies have been detached and lost into the medium. *F* A giant cell showing numerous tiny vesicular bodies in the perinuclear area.

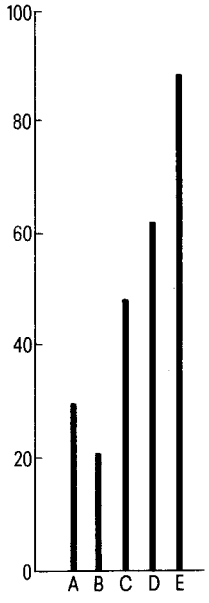

Figure 3. Histogram showing percent glia cells with undetached vesicles 2 h after layering. Abscissa: different treatments. A) Cells layered in the absence of drugs; B) cells layered in 0.5% dimethylsulfoxide (DMSO) (control for cytochalasin-B treatment); C) cells layered in the presence of 10^{-5} M colcemid; D) cells layered in the presence of 10^{-5} M colcemid and 10 µg/ml cytochalasin-B simultaneously.

MRC-5 and WI-38 cells were of a mean diameter of 4 µm. Since glioma cells also excreted these vesicles, this phenomenon is displayed by both normal and neoplastic cells and therefore, appears to be a basic cellular phenomenon. The role of this vesicular shedding is not known. One explanation might be that the cells are packaging and discarding all the damaged molecules during exposure to trypsin in a manner somewhat similar to patching, capping and endocytosis. Patching of surface components due to ligand independent events has been reported[4]. A role in excretary function for microfilaments and microtubules has been postulated earlier[3].

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