Bulk Uptake of Macromolecules and Particles
Group Report

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INTRODUCTION
Bulk uptake of macromolecules is mediated by endocytic vesicles which form when segments of the plasma membrane invaginate, pinch off, and enclose a volume of extracellular fluid within the cell. Fusion of plasma membrane to plasma membrane seals the neck of the vesicle and the sites from which they invaginate. Fusion of the membrane of an endocytic vesicle with another membrane allows the possibility of transport of the contents of the endocytic vesicle to another intracellular container or to the cell exterior. Two types of endocytic vesicles have been recognized: smooth or uncoated vesicles and vesicles with coats on their cytoplasmic surfaces. The coated vesicles appear to arise from differentiated regions of the plasma membrane called coated pits, which function in selective transport of certain compounds by absorptive endocytosis. Other examples of endocytic processes described in the papers in this volume include bulk or fluid phase pinocytosis, particle uptake (phagocytosis), and vesicular transport across endothelial cells.

TYPES OF ENDOCYTOSIS
Adsorptive pinocytosis describes the endocytic uptake of macromolecules for which there are binding sites on the plasma membrane. The presence on the plasma membrane of receptors which
have high affinity for physiologically important compounds and which can be mobilized into pinocytic vesicles allows the cell to remove these compounds from the medium in a highly concentrated form with relatively little uptake of fluid. The best characterized transport system which utilizes adsorptive pinocytosis is that for uptake of low density lipoprotein (LDL). LDL binds to a high affinity receptor concentrated in coated pits on the cell surface and is taken up in coated vesicles for delivery to lysosomes. Other receptor-mediated uptake systems include those for the uptake of asialo-glycoproteins, the cobalamin carrier protein transcobalamin II, epidermal growth factor, certain immunoglobulins, and two different systems for uptake of lysosomal enzymes by fibroblasts and by macrophages. Many other sites on plasma membranes bind plant lectins or cationized markers, and uptake of these markers has been instructive in following the fate of inferiorized membrane.

Fluid phase pinocytosis describes the process by which soluble unbound markers are internalized in endocytic vesicles in proportion to their content in the medium. The fluid, electrolyte, and soluble macromolecules introduced by fluid phase pinocytosis may provide a source of nutrition for the cell, but the process is also important for uptake of large amounts of membrane and ligands.

Horseradish peroxidase at high concentrations (0.1-1.0 mg/ml) is the chief "content marker" which has been used to study fluid phase pinocytosis. At these high concentrations, it is probably internalized chiefly by fluid endocytosis in most cells. However, recent evidence (see Sly and Stahl, this volume) indicates that horseradish peroxidase is also subject to adsorptive endocytosis by rat alveolar macrophages and other reticuloendothelial cells which express the receptor for mannose/N-acetylgalactosamine-terminal glycoproteins. Thus in some situations horseradish peroxidase is not solely a content marker for fluid endocytosis.
Phagocytosis is the term used to describe the uptake of relatively large particles such as erythrocytes or latex spheres with relatively little uptake of fluid material. Phagocytosis serves a nutritional function for ciliates, a disposal or clearance function, and also participates in host defenses when microorganisms are ingested. Phagocytosis differs from pinocytotic processes in that it is clearly induced by the particle ingested, has different energy requirements than pinocytosis, and appears to be more dependent on a cytoskeleton scaffolding (is cytochalasin sensitive).

Vesicular transport across endothelial cells depends on a high-volume, bidirectional, apparently nonselective movement of vesicles and their contents between the luminal and tissue face of an endothelial cell. In this system endocytosis is followed directly by exocytosis so that the lysosomal system is largely bypassed.

AREAS FOR FUTURE INVESTIGATION
On the basis of the papers in this volume and the discussions, we identified the following generalizations and unanswered questions concerning bulk or vesicular transport of macromolecules.

1. What is the role of the ligand in adsorptive endocytosis?
   a) Do ligands induce interiorization of the membrane to which they bind or simply go along (with the possible exception of multivalent ligands that induce capping)?
   b) Is there a difference between monovalent and multivalent ligands?

2. Evidence from a number of systems indicates that recycling of some plasma membrane components through the cells occurs. The questions this conclusion raises are:
   a) Is it a selective process in terms of membrane constituents?
   b) What protects membrane components from hydrolytic attack following fusion of endocytic vesicles with lysosomes?
   c) What segregates the vesicle membranes and vesicle contents when endocytic vesicles fuse with other vesicles, discharge their contents, and their membrane components recycle?
3. Vesicular transport implies membrane flow and fusion as vesicles acquire or discharge contents. Evidence suggests that in one system or another, at least all post-Golgi membranes are capable of fusing with each other under certain conditions. Thus endocytic vesicles fuse with lysosomes, with plasma membrane, with Golgi, and with each other. However, they do not usually fuse with mitochondria or rough endoplasmic reticulum. The questions are:
   a) What determines the specificity in recognition of membranes that fuse?
   b) Do receptors or ligands confer segregational specificity?
   c) What provides the force in directional vesicular transport?
   d) How are endocytosis and exocytosis coordinated?

4. Smooth vesicles account for large amounts of vesicular transport - both in fluid phase endocytosis and in the bidirectional transport across endothelial cells. The questions are:
   a) Is the membrane composition of smooth vesicles identical to that of the plasma membrane?
   b) Do smooth vesicles have any specific receptors or do they only mediate fluid phase endocytosis?
   c) How many routes can endocytic vesicles take, and what determines their direction?
   d) What induces their formation?
   e) Is any cytoplasmic scaffolding required for their formation?

5. Coated vesicles are clearly involved in some examples of adsorptive endocytosis and also in some examples of intracellular transport of secretory products. The questions are:
   a) Are all vesicles involved in specific transport coated?
   b) Do all coated pits contain receptors for adsorptive endocytosis? Note that 90% of the coated pits seen on fibroblasts were labeled with LDL-ferritin.
   c) How many different types of receptors would a single coated pit contain?
   d) What induces formation of a coated region on a particular membrane?
   e) Is progression of a coated pit to a coated vesicle a constitutive activity or an induced activity?
f) Are the routes of incoming coated vesicles different from those of incoming smooth vesicles?
g) How is the coat lost and reassembled?
h) How is the chemical composition of the membrane in coated pits and vesicles distinctive?

6. Specific receptors in the membrane mediate adsorptive endocytosis of several physiologically important ligands. There are several important unanswered questions regarding this and other possible roles of such receptors in vesicular transport of macromolecules.
a) Does the ligand reach the lysosomal compartment still bound to the receptor?
b) Do receptors recycle through the cell with other membrane components?
c) How are receptors gathered into specialized regions like coated pits on particular membranes? Is this phenomenon general?
d) Do specific receptors exist on the cisternal face of different sets of intracellular vesicles exposed to the products of the Golgi? If they do, they may function in sorting out such products into vesicles with different functions.
e) Do specific receptors exist on the cytoplasmic face of vesicles and other membranes? If they do, do they act as binding sites for other vesicles or attachment sites for components of the cytoskeleton?
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