Ann. Rev. Biochem. 1977. 46:669–722

ENDOCYTOSIS

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PERSPECTIVES AND SUMMARY

Endocytosis is a widespread cellular function that regulates the quantal uptake of exogenous molecules from the cell's environment via plasma-membrane–derived vesicles and vacuoles. Both soluble (pinocytosis) and

particulate (phagocytosis) substances may be interiorized, destined either for the vacuolar apparatus and intracellular digestion or transport through the cytoplasm and subsequent exocytosis. Although most, if not all, eukaryotic cells demonstrate these primitive functions, they are particularly prominent in leucocytes, macrophages, capillary endothelial and thyroid epithelial cells, yolk sac, and oocytes. Here they are involved in host defense, immunological reactions, macromolecular transport, hormone transformations, and the regulation of metabolic pathways, and perhaps in cellular nutrition as well.

Many cells generate pinocytic vesicles at constant but different rates, enclosing fluid and solutes at the concentration at which they are found in the extracellular milieu. Those solutes that bind to the plasma membrane are interiorized by adsorptive pinocytosis at rates often many thousandfold above the uptake of fluid-phase reactants. In some instances the interaction of a multivalent ligand, such as concanavalin A, with the plasma membrane leads to the stimulation of vesicle flow. Both soluble and particulate materials may bind to more specific receptors on the cell surface. These include the binding sites for ligands such as low-density lipoprotein on human fibroblasts and the immunoglobulin and complement receptors on leucocytes and macrophages. The attachment of a particle to the plasma membrane is a prerequisite for interiorization and leads to a localized perturbation of the membrane beneath the attachment site. This is characterized morphologically by the aggregation of actin-like filaments and is associated with the formation of pseudopods that enclose the particle. In order for ingestion to be completed with the formation of a phagocytic vacuole, ligands must be circumferentially distributed on the particle and make continuing contact with the plasma membrane. This insures tight apposition of particle and membrane leaflets, and leads to the subsequent fusion of the membrane and the formation of an intracytoplasmic phagosome. The mechanism by which membrane fusion occurs to form endocytic vacuoles and, later, the phagolysosome is unknown.

Endocytosis is a temperature-dependent process with a Q_{10} of approximately 3.0 and the interiorization of the fluid-phase marker, horseradish peroxidase, has an activation energy of 18 kcal/mole. Agents that depress either glycolysis and/or aerobiosis reduce the rate of endocytosis, depending upon the predominant pathway in the cell examined. The intracellular level of ATP is roughly correlated with the influence of the inhibitor employed, although exceptions are noted. The consumption of ATP may in part be related to the involvement of the contractile proteins in the endocytic event. This is suggested by the presence of actin, myosin, and an actin-binding protein in phagocytic cells, the association of actin with the plasma membrane, the ability of the cytochalasins to inhibit phagocytosis,

and the general association of these elements with membrane motility. In contrast, agents that depolymerize cytoplasmic microtubules do not appreciably alter either pinocytosis or phagocytosis.

Large amounts of plasma membrane may be interiorized through endocytosis, either continuously as in pinocytosis or in discrete phagocytic events. This may occur rapidly and through the utilization of preexisting membrane. Stereological measurements indicate that macrophages and L-cell fibroblasts interiorize 186% and 54% of their surface area, respectively, as pinocytic vesicles each hour without alteration in cell volume or surface area. Such evidence suggests that extensive recycling of membrane back to the cell surface is taking place continuously. Studies employing enzymatic iodination of the cell surface indicate that the membrane surrounding ingested latex particles is representative of the plasma membrane, whereas other investigators, using transport systems as markers, have suggested a more selective interiorization of membrane constituents. The membrane, interiorized about nondigestible latex beads, shows extensive degradation of iodinated surface polypeptides and inactivation of the plasma-membrane enzymes 5'-nucleotidase and alkaline phosphodiesterase I. Most iodinated polypeptides of the phagolysosome are degraded synchronously to the amino acid level, although a few persist for long periods in the membrane. Little is known about the composition of the pinocytic-vesicle membrane and the turnover of its components. Plasma-membrane lipid components appear to be largely conserved in the interiorized membrane.

A variety of cellular alterations occur as a consequence of phagocytosis. Peroxide formation, the hexose monophosphate shunt, oxygen consumption, and phosphatide turnover are all stimulated, whereas little change is noted in the level of cyclic nucleotides. Selected areas of macromolecular synthesis are also enhanced, as characterized by the induction of lysosomal hydrolases, heme oxygenase, apoferritin, and the secretion of a group of neutral proteinases. In addition, there is evidence that net plasma-membrane assembly occurs in proportion to the amount of membrane interiorized about nondigestible particles, but some hours after the phagocytic event.

Many of the phenomena considered in this review lack a defined biochemical base, and considerable effort will be required to unravel the complexities of the interaction between plasma membrane and cytoplasm. However, advances have been made in defining useful in vitro systems in which cells, particles, and markers are well studied and the physiology of the process delineated. Such systems are basic to our understanding of the overall role of endocytosis in the economy of cells, as well as representing useful tools for the broader analysis of the plasma membrane.

INTRODUCTION

Until recently, endocytosis has been studied by relatively small groups of biologists, e.g. those interested in microbial killing in certain white blood cells, nutrition in unicellular organisms, and transport across blood-vessel walls. It is evident, however, that endocytosis occurs in all animal-cell types, can result in the uptake of an enormous spectrum of materials, and plays essential roles in the physiology of cells and whole organisms. For example, endocytosis is involved in the transport of proteins from mother to child at various stages of development, in cholesterol metabolism, in removal of senescent and damaged cells and altered molecules, and has been implicated in the turnover of plasma membrane and in various secretory phenomena.

Most biologists have concentrated on the organelles involved in endocytosis and on the fate of ingested materials. This review focuses mainly on the interiorization process itself; it is here that important controls are exerted and information can be obtained that is useful to the related areas of membrane biology and cell motility.

Types of Endocytosis

Endocytic activity has always been divided into two categories—phagocytosis, or eating, and pinocytosis, or drinking. Most investigators use the term *phagocytosis* to describe the uptake of large particulates, i.e. those visible by light microscopy, and possibly some viruses. Uptake occurs by close apposition of a segment of plasma membrane to the particle's surface, excluding most, if not all, of the surrounding fluid (see section on factors regulating phagocytosis). The term *pinocytosis* is used to describe the vesicular uptake of everything else, ranging from small particles (lipoproteins, ferritin, colloids, immune complexes), to soluble macromolecules (enzymes, hormones, antibodies, yolk proteins, toxins), to fluid and low-molecular-weight solutes. It is likely that these materials are all interiorized in vesicles with an electron-lucent content, and it is assumed that extracellular fluid is included in this content. So the terms *phago-* and *pinocytosis* are probably accurate in distinguishing between particle- vs fluid-containing vacuoles, but it remains to be determined if these two processes differ in their initiation and metabolic requirements, or if there are many different classes of pinocytosis.

The terms *fluid* and *adsorptive endocytosis* (or pinocytosis), initially used by several workers (1–5), are useful in describing and analyzing the uptake of many biologically important materials, such as those cited above. These terms point to the fact that substances can enter the cell in the fluid content of an endocytic vesicle (fluid-phase pinocytosis) and/or be bound to the

inner aspect of the vesicle membrane (adsorptive pinocytosis). In both types, the rate of uptake is determined by the size of the vesicle and the rate of its formation, and may be influenced by the presence of the endocytosed marker. In fluid-phase endocytosis, uptake is directly related to the concentration of solute in the extracellular fluid, whereas in adsorptive endocytosis, uptake in addition depends on the number, affinity, and function of cell-surface binding sites. Adsorptive uptake is both a selective and concentrating device whereby cells can interiorize large amounts of a specific solute without ingesting a correspondingly large volume of solution.

Morphology of Endocytic Vacuoles

Endocytic vacuoles assume a wide variety of shapes and sizes in electron micrographs (Figure 1). The forming phagocytic vacuole, or *phagosome,* is readily visualized by morphologic techniques (see section on factors regulating phagocytosis). The cell surface applies closely to the particle, so that the initial phagosome usually corresponds in size and shape to that of the particle (Figure 1*a*). Sometimes the cell "nibbles" portions of larger particulates continuously or takes in small particles in tight aggregates. Pinocytic vesicles probably arise by many mechanisms, although it is harder to visualize the initial steps by which they form. Fusion of membrane folds and invaginations of spherical, tubular, or cuplike structures (Figure 1*b,c*) all occur. These different forms may result from differences in the stimuli that initiate the endocytic process or from specializations of the membranes of the cells in which they occur.

Of considerable interest is the coated vesicle (Figure 1*c*). The cytoplasmic surface of many endocytic vacuoles is smooth, but electron micrographs show that most cell types contain an additional population of vesicles in

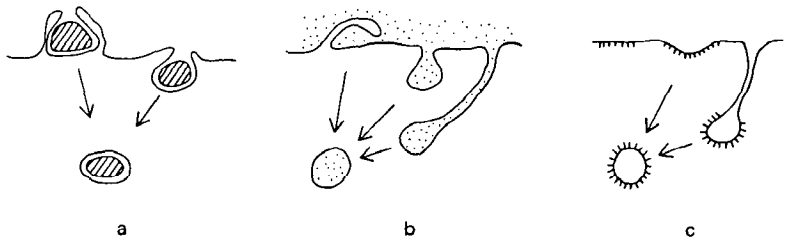

Figure 1 Endocytic vacuoles assume many shapes and sizes as they arise from the plasma membrane. In phagocytosis (*a*), the cell surface applies closely to the incoming particle. Examples of pinocytic vacuoles are shown in *b* and *c.* In thin sections, the cytoplasmic aspect of the vacuole may be smooth (*b*) or coated with tiny bristles (*c*).

which regularly spaced, short bristles protrude into the cytoplasm. From initial descriptions (6, 7), it was postulated that these coated[3] structures were involved in the selective uptake of proteins and particles by adsorptive pinocytosis. Recent work on lipoprotein uptake in human fibroblasts (8) and maternal protein uptake into oocytes (9) has supported and extended this idea. The proteins in question were conjugated to ferritin, which has an iron-laden core visible by electron microscopy. The ferritin conjugates attached predominantly to coated regions of the plasma membrane, which then seemed to invaginate (coated "pits") to form coated vesicles (Figure 1*c*). Very little conjugate bound to noncoated areas of the plasma membrane, and unconjugated ferritin or ferritin linked to nonspecific proteins did not bind. These studies suggest that receptors for adsorptive uptake may be segregated within coated regions of the cell surface, although conceivably the binding process induces clustering of the binding site and formation of the coat. In the fibroblast, coated areas account for only 2% of the cell's surface area (8), whereas in the developing oocyte, most of the plasma membrane is coated (9).

It is important to point out that coated vesicles may be involved in functions other than endocytosis. Friend & Farquhar (10) described a population of Golgi-associated vesicles that were not labeled with exogenous tracers and that stained cytochemically for thiamine pyrophosphatase, a reactivity shared with the inner Golgi saccule (10, 11). Franke and coworkers have found that coated vesicles have a secretory role in lactating mammary epithelium (12) and seem to contribute to new plasma-membrane formation during cytokinesis in plant and animal cells (13, 14), a suggestion that has also been made regarding developing nerve-growth cones (15). Rodewald's observations indicate that maternal immunoglobulin binds to the luminal plasma membrane of neonatal intestinal cells, enters these cells in smooth-surface invaginations, and is discharged from coated vesicles into the intercellular space. Presumably, these vesicles fuse with the plasma membrane on the intestinal cell's lateral borders (16).

Recently, preparations enriched in coated vesicles have been obtained by cell fractionation. The coat or bristles seen in thin sections correspond to a "cage" or "soccer-ball–like" network on the vesicle surface (17, 18). Pearse has identified a major protein, molecular weight 180,000, which she feels makes up the cage and has termed it *clathrin* (18, 19). She proposes that the coat serves to shuttle vesicles between different membrane-limited cellular compartments.

[3]The term *coat* has also been used to describe oligosaccharide groups as well as morphologic structures (hairs, bristles) on the exterior vs cytoplasmic aspect of the plasma membrane, but this notation is not used here.

Fate of Endocytic Vacuoles

The term *vacuolar system or apparatus* is used to describe both endocytic vacuoles and the other organelles with which they may interact. It is summarized diagrammatically in Figure 2. Most incoming vesicles fuse with preexisting, membrane-bound granules called *lysosomes* (pathways 2–4 in

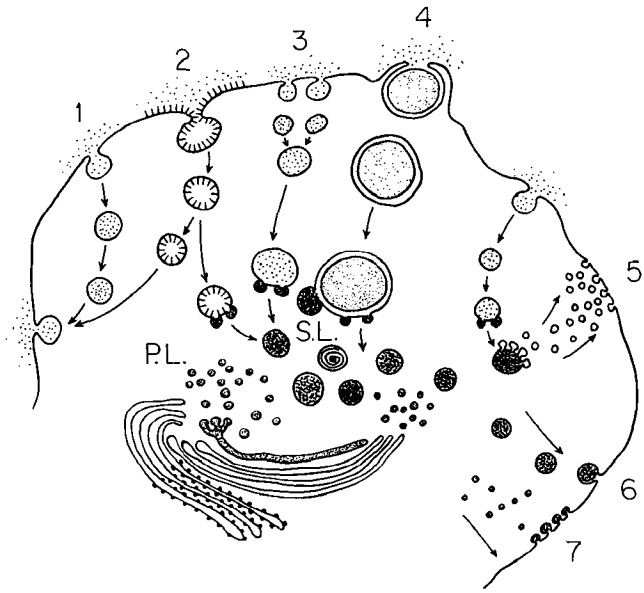

Figure 2 Diagram illustrating the various pathways for the flow and fusion of the membranes of the cell surface and vacuolar apparatus. Routes 1–4 represent experimentally defined pathways, whereas 5–7 are more speculative.

1. Fluid-phase pinocytic vesicle, which traverses the cytoplasm without fusing with lysosomes and discharges its contents on another surface of the cell. This is one of the routes followed in endothelial and mesothelial cells.

2. Adsorptive pinocytosis in which solute is initially bound to the cell surface through either specific or nonspecific determinants. After interiorization the vesicle moves through the cytoplasm and fuses either with the plasma membrane as in step 1, or with primary lysosomes (P. L.) or secondary lysosomes (S. L.). The primary lysosome is thought to arise either from the Golgi cisternae or from a region of the endoplasmic reticulum located near the inner aspect of the Golgi cisternae [a region Novikoff (11) termed GERL:Golgi—associated region of endoplasmic reticulum from which lysosomes are thought to arise].

3. Fluid-phase pinocytosis in which the vesicles fuse with each other to form a larger structure and subsequently with lysosomes to form a new population of secondary lysosomes or add substrate to existing secondary lysosomes.

Figure 2). Since the lysosome contains a broad spectrum of degradative enzymes, this pathway results in thorough degradation of the vacuolar content, e.g. iodinated proteins are digested to the level of monoiodotyrosine (20–22). The macromolecular contents of the endocytic vacuole [e.g. colloidal gold (23), inulin (Z. A. Cohn, unpublished observations), horseradish peroxidase (21, 24), bovine serum albumin (22), and polyvinylpyrrolidone (22)] are rarely if ever regurgitated intact back into the extracellular space, following fusion with lysosomes. When nondegradable materials are interiorized, as occurs when a genetic deficiency of a lysosomal hydrolase exists (25), they seem to be retained within lysosomes, which are then termed *residual bodies,* although these structures may well function like any other lysosome.

In some instances, endocytic vacuoles may traverse the cell rather than fuse with lysosomes (Figure 2, pathway 1). This route classically has been studied in vascular endothelium, where tiny vesicles appear to shuttle plasma from the capillary lumen to the tissue space (26). More recently, it has been shown that such transport need not be strictly vesicular, but rather may involve the transient formation of channels across the narrow endothelium (27). Vesicles may also transport materials across epithelial cell layers, e.g. proteins across fetal and neonatal gut (16, 28–31), and immunoglobulin A across mammary and other epithelia (32), although the mechanisms governing these processes are incompletely understood.

A third fate of endocytic vacuoles is that they remain intracellular but do not fuse with lysosomes. This occurs during pinocytosis of maternal proteins by oocytes, in which the incoming yolk protein lies within a storage granule, not a secondary lysosome (6), and during phagocytosis of certain microbes (see section on endocytosis and the penetration of intracellular parasites). We think that the failure to fuse with lysosomes is a property

4. Phagocytosis, in which a solid particle is interiorized, excluding fluid-phase constituents of the external milieu. The phagosome encloses the particle and usually fuses with primary and/or secondary lysosomes to form a phagolysosome. Exceptions to this route are described in the text.

5. A possible mechanism to explain current data on plasma-membrane interiorization and membrane recycling. Pinocytic vesicle fuses with lysosome, contributing its content to the digestive body. Membrane pinches off from the lysosome as small vesicles that exclude most of the matrix of the organelle and recirculate back to the plasma membrane.

6. The fusion of secondary lysosomes with the plasma membrane, liberating enzymes and digestion products into the extracellular environment.

7. The fusion of primary lysosomes or small secretory vesicles with the plasma membrane. The secretion of neutral proteases from macrophages and fibroblasts may follow such a vesicular pathway.

only of these specialized systems, and rarely if ever occurs in other situations. For example, reovirus particles and soluble horseradish peroxidase (HRP) are pinocytosed by cells in culture and enter lysosomes. Silverstein et al (33) noted that the entire ingested load of reovirus coat protein was degraded within this compartment, while Steinman & Cohn (21) found that 100% of endocytosed HRP was inactivated at a rate described by first-order kinetics. Both observations demonstrate that all endocytic vacuoles deliver their contents to a degradative compartment.

This summary of the vacuolar apparatus has emphasized organelles and their contents. However, endocytosis clearly brings about the interiorization of considerable amounts and/or selected portions of the cell surface (see section on membrane flow during endocytosis). Studies on the amount, biochemical composition, fate, and consequences of this membrane flow are critical to further understanding of the vacuolar system.

QUANTITATION OF PARTICLE AND SOLUTE UPTAKE

This section summarizes assays used to measure the endocytic uptake of particles and solutes. We consider measurements of the amount of surface membrane interiorized during this process in the section on membrane flow during endocytosis.

Phagocytosis of Large Particles

A number of particles have been used to follow and measure the phagocytic process; each has advantages and disadvantages. Fresh sheep red blood cells can be coated with specific antibodies and/or complement, and then used to detect specific receptors that recognize these ligands on phagocytic cells (see section on factors regulating phagocytosis). Both binding and ingestion phases of phagocytosis can be distinguished microscopically since the extracellular bound particles can be selectively lysed in hypotonic media (e.g. 34, 35). Other particles are superior to sheep red blood cells for measuring the kinetics of uptake, e.g. radiolabeled starch granules and killed bacteria (36), polystyrene (latex) and polyvinyltoluene spheres (37–39), and paraffin-oil emulsions (40, 40a). The latter two types of particles are additionally useful because of their low densities relative to other cell components. As shown initially by Wetzel & Korn (39), amoeba phagolysosomes containing low-density particulates can be prepared free of other contaminating organelles by floatation in dense sucrose and then used to analyze the composition of the vacuole content and membrane. A modification of this method has been used by Stossel et al (40, 40a) for studying the uptake of paraffin-oil emulsions in phagocytic leucocytes.

In most experiments, a particle load is administered for a short time, nonbound particles are then washed off, and the number of cell-associated particles that remain is measured. However, even though the bound material is attached specifically to a cell-surface receptor, it may not be interiorized because the cell already is filled with other particles or because the receptor to which the particle is bound does not mediate ingestion (see section on factors regulating phagocytosis). Particles binding nonspecifically to cells, to cell debris, or to the culture vessel must be distinguished from those that are ingested. A variety of approaches have been used to distinguish bound from ingested particles, e.g. treatment of cells with enzymes to release or destroy particles bound to the cell's surface. The best initial screening procedure is direct examination by light and electron microscopy.

Phagocytosis has been studied by using cells in either suspension (41) or monolayer culture (36). Both systems provide continuous and effective interaction of particles and cells, and allow measurements of maximal phagocytic capacity as well as its rate. The latter is usually linear for 10–30 min, depending on the particle. Monolayers are easily and rapidly washed free of nonbound particles and are generally superior for studies of the kinetics of uptake. They are also readily examined microscopically.

Although cells in monolayer culture behave similarly to those in suspension cultures in most phagocytic assays, significant differences have been observed. Most importantly, the surface properties of the culture vessel may cause changes in the orientation of the phagocyte's plasma-membrane constituents. As a result, the structural and functional properties of the membrane attached to the culture vessel may differ from those of the membrane exposed to the particle. Indeed, Rabinovitch et al (41a) have induced changes in Fc-receptor function of mouse peritoneal macrophages by culturing these cells on surfaces coated with antigen-antibody complexes.

Pinocytosis of Solutes and Fluid

Light microscopy was used initially to enumerate phase-lucent pinocytic vesicles in mammalian cells (42, 43) and pinocytic channels in amoebae (1, 2). When electron microscopy first suggested that most cells contain submicroscopic pinocytic vesicles (44, 45), a variety of electron-dense probes—colloidal gold and thorium (46–49), ferritin (50), peroxidases (51)—were introduced into the extracellular space. The presence of these probes within vesicles confirmed their origin at the cell's surface, and demonstrated further that incoming vesicles fuse with independently labeled lysosomes both in vitro (47, 48) and in vivo (10, 52). One difficulty with some of these probes is that they can adsorb to the cell surface; labeling of an apparently intracellular pinocytic vesicle in a thin section may really represent extra-

cellular binding to a surface invagination, a fold, or even an outgoing vesicle.

Most particulate electron-dense tracers are not useful for quantitative work, so a variety of radiolabeled and enzymatically active markers have been used instead. Again, the question of binding vs interiorization must be adequately controlled. Detection and quantitation of uptake is more difficult than in phagocytosis, because (*a*) the probes are more difficult to visualize; (*b*) the total amount of cell-associated marker in a typical culture is 1% or less of the administered load vs 50% or more in typical phagocytic systems; and (*c*) the relative amount of interiorized vs surface-bound label may be small. Controls for nonspecific binding, kinetic data on the rate of binding and uptake, and morphologic correlates are therefore important components of quantitative work. Simply measuring "bound" marker after a fixed exposure period is unsatisfactory.

At the start of a quantitative study, one should assess whether fluid or adsorptive uptake is occurring. The amount of solute accumulated by fluid-phase uptake should vary linearly with the concentration of marker in the extracellular fluid and should increase continuously with time as long as the cell is not digesting and/or releasing the label. Adsorptive uptake shows saturation with increasing loads and may exhibit a rapid early binding phase. Formation of pinocytic vesicles and hence fluid-phase uptake is not detectable at 4°C (24), whereas adsorption to cell-surface binding sites occurs at 4°C (e.g. 53, 54). Lastly, morphologic methods should substantiate and localize the surface binding step, e.g. by tagging the marker with ferritin or peroxidase for electron microscopic visualization.

If adsorptive uptake is documented, several features can be characterized quantitatively. To distinguish between the amount of adsorbed vs interiorized label, the surface pool is usually eluted selectively, e.g. by adding some competing agent, by proteolysis, or by simple reversible dissociation from surface binding sites. If the surface compartment can be measured, binding studies at different ligand concentrations will characterize the number and affinity of binding sites. Inhibition of surface binding by using a competing ligand or by using cells that lack the ligand binding site may help to differentiate between the amount of label entering in the fluid space vs that adsorbed to the vesicle membrane. Finally, an independent marker can be administered to measure and/or visualize fluid-phase uptake before and during administration of the ligand to determine if ligand binding affects this parameter.

The interaction of HRP, a glycoprotein enzyme, with tissue-culture cells illustrates all the features of fluid-phase uptake (21, 24). The enzyme does not bind to the cell's surface. Its uptake is proportional to the concentration of enzyme in the medium, proceeds continuously with time, and is blocked

at 4°C. Endocytosed enzyme is visualized cytochemically in intracellular pinocytic vesicles and lysosomes. Other markers have been used to measure fluid-phase uptake including [^{3}H]sucrose (55), [^{3}H]inulin (56), and [^{125}I]-polyvinylpyrrolidone (57). We favor HRP since its assay is easy, inexpensive, very sensitive (nanogram amounts), not complicated by nonspecific binding, and can be performed after very short exposure periods. Moreover, its intracellular route can be followed cytochemically (58).

Several materials have been shown quantitatively to exhibit features of adsorptive uptake in vitro, including gamma globulin and yolk proteins in oocytes (9), ribonuclease and other proteins in large amoebae (1, 2, 59), lysosomal enzymes (60, 61) and low-density lipoproteins in human fibroblasts (54), colloidal gold in rabbit macrophages (3, 62), altered bovine-serum albumin in rat yolk sac (22), polycation-polyanion complexes in mouse macrophages (63), lectin (64), and viruses (53, 65, 66). One of the most detailed in vitro studies of the adsorptive pinocytosis of particulate materials is that of Goldstein & Brown on the uptake of low-density lipoproteins by human fibroblasts [reviewed in this volume (66a)]. Cell-surface receptors for these lipoproteins were saturated at high lipoprotein concentrations, were not blocked by the presence of nonspecific proteins, and mediated the uptake of a much larger load of lipoproteins than fluid-phase markers. Adsorption occurred at 4°C and surface-bound lipoproteins were eluted by polyanions such as heparin and sulfated aminoglycans at both 4°C and 37°C (67). Mutant fibroblast lacking surface receptors for low-density lipoproteins do not exhibit adsorptive endocytosis of these particles.

Ashwell, Morell and co-workers [reviewed in (68)] have studied the adsorptive uptake of modified glycoproteins in vivo. They found that removal of two or more sialic acid residues from the oligosaccharides of a variety of glycoproteins, e.g. ceruloplasmin, exposes subterminal galactose residues. Glycoproteins with these galactose moieties exposed bind to a galactose-binding protein on the surface of liver parenchymal cells and are thereby rapidly and selectively cleared from the blood stream. These proteins accumulate in the lysosomes of liver cells where they are degraded.

ENERGY REQUIREMENTS FOR ENDOCYTOSIS

Cells contact their intended particulate meals either by crawling towards them or by chance encounter. In all cases, contact is followed by movement of cellular pseudopods over the particle's surface until these pseudopods meet and fuse with one another on the far side of the particle. By this act the formation of the phagocytic vacuole is completed. A cell that is actively pinocytizing shows "ruffling" movements of its plasma membrane in the region where the pinocytic vesicles seem to originate and the formation of

small-phase lucent vesicles in its peripheral cytoplasm. Frequently, structural changes accompanying pinocytosis cannot be detected with the light microscope except for the accumulation of vacuoles filled with pinocytized materials in the center of the cell.

This complex series of events involves the expenditure of metabolic energy, the movement and fusion of membranes, the displacement of cytoplasm, and a means of coordinating these activities to achieve the desired result, i.e. ingestion of particles or fluid.

Temperature

Particles bind to the surfaces of both professional[4] and facultative[5] phagocytes at 4°C, but are not ingested unless the temperature of the incubation medium exceeds some critical threshold (18–21°C) (70, 71; J. Michl and S. C. Silverstein, unpublished observation). In the case of ingestion of large particles, such as sheep red blood cells, the inhibition observed at temperatures below this threshold represents an absolute inhibition of the ingestion process and not merely a decrease in the rate of ingestion. This was determined by Michl and Silverstein (unpublished observations), who found that mouse macrophages did not ingest immunoglobulin G (IgG)–coated sheep red blood cells at temperatures below 18°C even when the incubation was continued for 24 hr. When these same phagocytes were reincubated at 37°C after 24 hr at 18°C, virtually all of the macrophages ingested four or more red cells, confirming that the macrophages remained viable throughout the 18°C incubation period. From 30% to 40% of macrophages incubated for a similar period at 20°–25°C with IgG-coated red cells ingested four or more red cells. Thus there appears to be a critical thermal transition below which phagocytosis of large particles cannot occur. Phagocytosis differs in this respect from fluid-phase pinocytosis; in the latter there appears to be no critical thermal transition below which pinocytosis ceases. Rather, Steinman et al have shown that the rate of solute uptake by pinocytosis in mouse fibroblasts shows a Q_{10} of 2.7, an activation energy of 17.6 kcal per mole, and is directly proportional to the incubation temperature from 2° to 38°C (24).

[4]In mammals there are two types of white blood cells that eat particulate materials: polymorphonuclear leucocytes and mononuclear phagocytes. Rabinovitch has called these cells *professional phagocytes* because they have made a full-time occupation of eating (69). Since much of the recent work on phagocytic mechanisms has been done on these cells, our discussion of phagocytosis focuses primarily on them.

[5]Professional phagocytes are not the only mammalian cells that eat particles. Fibroblasts, neurons, and a variety of epithelial cells also ingest particles (158). We refer to these cells as *facultative* or *nonprofessional phagocytes* (69).

ATP

Karnovsky and his colleagues applied metabolic inhibitors to cells maintained in vitro to study the roles of anaerobic and oxidative metabolism in the generation of chemical energy for particle ingestion. They [reviewed in (72–74)], and others (75–78), have shown that glycolytic inhibitors such as NaF or iodoacetic acid, but not inhibitors of oxidative metabolism [e.g. CN^- (73), dinitrophenol (73), antimycin (77)] strongly inhibit phagocytosis in most cells; and that addition of lactate or pyruvate to the incubation medium partially reverses the inhibitory effect of iodoacetic acid in polymorphonuclear leucocytes (75). On the other hand, except for alveolar macrophages (74, 80) and amoebae (37), most cells show little diminution in particle uptake in the absence of O_2 or in the presence of inhibitors of oxidative phosphorylation. As a result of these studies it has become axiomatic that phagocytosis is an energy-consuming process fueled by ATP derived from either aerobic oxidation of tricarboxylic acid cycle intermediates or anaerobic glycolysis. However, the evidence on which this conclusion is based bears further examination (see below).

NaF and iodoacetic acid inhibit the glycolytic enzymes enolase and 3-phosphoglycerate dehydrogenase, respectively. Inhibition of either enzyme decreases glucose consumption and lactate production and leads to lowered ATP production. It is generally considered that NaF and iodoacetic acid inhibit phagocytosis by lowering cellular ATP. Recently, Michl et al (81) have shown that this is an oversimplified view. These investigators have compared cellular ATP levels and phagocytic capacities of mouse macrophages incubated for 1 hr at 37°C in the presence of NaF (10^{-3} M) or 2-deoxyglucose (5×10^{-2} M). NaF caused an ~40% reduction in cellular ATP content and, as expected, inhibited the ingestion of latex or zymosan particles by over 80%. In contrast, 2-deoxyglucose (2-dG) caused an ~50% reduction in cellular ATP content, but unlike NaF did not inhibit latex or zymosan ingestion. Moreover, macrophages incubated in 2-dG for 3–4 hr continue to phagocytize latex and zymosan particles at control levels despite an ~85% reduction in cellular ATP content. In the case of NaF the block was readily reversed by removing NaF from the incubation medium (76) or by addition of pyruvate (75), confirming that lack of particle uptake was not a consequence of premorbid cellular intoxication These findings indicate that reduced cellular-ATP content is not a sufficient explanation for either the 2-dG or the NaF-mediated inhibition of phagocytosis.

Steinman et al (24) reported a similar lack of correlation between the ATP content of cells treated with metabolic inhibitors and pinocytic rate as measured by horseradish peroxidase uptake in L-strain fibroblasts. Using

confluent-monolayer cultures, they found that 5×10^{-2} M 2-dG decreased cellular ATP levels to ~40% of control levels, while pinocytosis continued at 91% of the control rate. In contrast, 10^{-3} M azide decreased cellular-ATP content only 5%, while pinocytosis fell to 57% of the control value. A rough correlation between cellular ATP content and pinocytic rate was observed when other metabolic inhibitors were used. NaF (10^{-2} M) or NaCN (10^{-3} M) reduced cellular ATP to 55–62% of control levels and caused significant decreases (38% and 69% of control levels, respectively) in pinocytic rates. Moreover, when cells were treated with both a respiratory (NaCN) and a glycolytic (2-dG) inhibitor, cellular-ATP content and pinocytic rate decreased coordinately to 15% and 18% of control levels, respectively. In contrast to these findings with mouse fibroblasts, glycolytic but not respiratory inhibitors markedly decreased pinocytosis of HRP in mouse macrophages[6] (21; R. M. Steinman, unpublished observations). Taken together, these findings are consistent with the view that pinocytosis is dependent upon metabolic energy and that this energy can be derived from either glycolytic or oxidative metabolism. However, these data do not prove that ATP is the energy source for pinocytosis.

Although all of these studies are technically sound, they suffer from two conceptual shortcomings: 1. They do not attempt to identify the energy-consuming process(es) that are involved in endocytosis. This is especially relevant in the case of phagocytosis, since both professional and facultative phagocytes exhibit metabolic alterations [increased glucose uptake, phosphorylation, and utilization (79, 83); lactate and CO_2 production, O_2 consumption, and oxidation of glucose via the hexose monophosphate shunt (72–74)] during particle ingestion, all of which are suggestive of increased ATP consumption and/or generation. Although many of these changes accompany particle ingestion, not one of them is required for it to occur; rather, they appear to be metabolic consequences of phagocytosis (see section on metabolic consequences of phagocytosis). Until the process(es) that are essential for particle ingestion are positively identified it may be difficult to distinguish those ATP-consuming events that are required for particle ingestion from those that are secondary to it. 2. They focus on cellular ATP content, and not on ATP utilization or on energy charge (84). Since ATP is being continuously regenerated, measurements of ATP levels give no

[6]Cohn & Benson (82) reported that respiratory inhibitors depress serum-induced pinosome formation in mouse macrophages, as measured by counting phase-lucent vacuoles by light microscopy. There may be no contradiction between their results and those reported by Steinman et al (24) since the rates of formation and disappearance of large phase-lucent vacuoles are not necessarily well correlated with the overall rate of uptake of HRP.

indication of the amount of ATP consumed, or available as ADP,[7] during endocytosis. Viewed from this perspective the apparent lack of correlation between ATP content and endocytic capacity may be a misleading and unreliable indicator of the amounts of ATP generated and consumed during these processes. However, even such direct measurements of ATP consumption will have to be carefully controlled since membrane perturbants initiate many of the metabolic consequences of phagocytosis in the absence of particle ingestion. The availability of inhibitors, such as colchicine (85, 86), that dissociate these metabolic consequences of phagocytosis from particle ingestion provides one means of approaching this problem.

Despite the absence of formal proof there is abundant circumstantial evidence that endocytosis is not driven by surface forces alone (87, 88), but that it requires the expenditure of metabolic energy, which in most cases is derived from metabolism of endogenous (83) or exogenous carbohydrates (72, 74, 81, 83). Elsbach has excluded the possibility that oxidation of either exogenous or endogenous fatty acids (89, 90) fuels phagocytosis in polymorphonuclear leucocytes. The role played by fatty-acid oxidation in endo- and exocytosis (cf 91) in other cells remains unexplored. We do not know whether energy derived from reduced pyridine nucleotides can be transformed directly into this type of work (cf 92), or whether, as seems most likely, ATP is required. Nor do we have any idea whether the amount of energy expended by a cell during endocytosis is proportional to the size, surface characteristics, and number of the particles or to the amount and type of solute being ingested. Finally, we do not know which cellular organelles consume this chemical energy. One likely candidate is the contractile apparatus.

CYTOPLASMIC CONTRACTILE ELEMENTS

Microtubules

Compounds such as colchicine, which depolymerize cytoplasmic microtubules, do not inhibit phagocytosis (93–95) and cause at most a small reduction in basal pinocytic rate (21, 96) at the usual concentrations (10^{-5}–10^{-6} M). Colchicine and related alkaloids have many other effects upon cellular physiology (cf 97, 98), one of which is to alter cytoplasmic organization. In the presence of these compounds, pinosomes, which ordinarily move directly toward the cell's Golgi region, move more randomly about the cytoplasm (95).

[7]Stossel et al (83) found that ATP, ADP, and AMP levels were unchanged during phagocytosis of latex particles by polymorphonuclear leucocytes.

Actomyosin

All eukaryotic cells so far examined contain actin and myosin. The physical characteristics of nonmuscle-cell actin and myosin resemble, but are not identical to, those of the corresponding proteins derived from skeletal muscle. Moreover, there are significant differences between the cofactors that regulate the assembly and interaction of these contractile proteins in skeletal muscle and in nonmuscle cells. It is already apparent that different nonmuscle cells have adapted the fundamental molecular architecture of actin and myosin, and varied the modes by which these two proteins interact to effect different forms of cellular movement. Excellent reviews (99) and symposia (100) covering this burgeoning field have been published recently and the reader is referred to them for information concerning the role(s) of these proteins in a variety of motile cells. We confine our remarks here to the functions of these contractile proteins in phagocytosis and pinocytosis.

PHAGOCYTOSIS Phagocytic leucocytes contain actin and myosin (101–103) and there is little doubt that these proteins play a central role in the phagocytic process. Microfilaments (104), some of which have been shown to form characteristic arrowheads when treated with heavy meromyosin (105), and microfilament bundles have been identified in association with the plasma membranes of phagocytizing amoebae and macrophages. Cytochalasin B, a compound that impairs actin gelation and microfilament function (106–109), inhibits phagocytosis (110, 111), and actin, isolated from polymorphonuclear leucocytes of a patient with marked defects in leucocyte motility and phagocytosis, exhibits defective polymerization (112).

Stossel and his colleagues have examined the contractile proteins of phagocytic leucocytes in detail (102, 103, 113, 114). They have shown that actin and myosin are major protein constituents of both mononuclear and polymorphonuclear phagocytes. They have purified a high-molecular-weight actin-binding protein[8] cross-links F-actin into filament bundles, stoichiometrically induces the gelation of macrophage actin, and is released from the membrane fraction into the soluble cytoplasmic fraction during the ingestion of particles by macrophages (103). And they have identified an unstable protein (called "cofactor") that stimulates the Mg-ATPase of macrophage actomyosin up to 22-fold and, in the presence of Mg and ATP, increases the rate of contraction of gels containing macrophage actin, myosin, and actin-binding protein (113). Assuming these four proteins

[8]This substance is similar in many of its properties to filamin, a membrane-associated protein isolated from chicken gizzard smooth muscle by Wang et al (115).

constitute the major contractile and regulatory elements of phagocytic leucocytes—an assumption that admittedly may be premature—there are marked differences (summarized in Table 1) in the physical properties, relative molecular proportions, and regulatory mechanisms of the actomyosin systems in phagocytic leucocytes and skeletal-muscle cells. Some of these differences require further emphasis.

1. Skeletal muscle contains four moles of actin per mole of myosin; mononuclear phagocytes contain approximately 100 moles of actin per mole of myosin. This vast molar excess of actin suggests that actin may function independently of myosin in regulating movement in phagocytic cells [as it does in echinoderm sperm (118, 119)], and/or that actin and/or myosin redistribute within the cytoplasm as the occasion demands. If this occurs the molar ratio of actin to myosin in specific locations in the cytoplasm might approximate the 4 : 1 ratio found in skeletal-muscle cells. 2. Both muscle- and nonmuscle-cell globular actins (G-actins) form filaments under similar conditions. However, cytoplasmic-actin filaments show the unusual property of disaggregating on cooling. The finding of Storti & Rich (120) that cytoplasmic and skeletal-muscle actins exhibit differences in electrophoretic mobilities and amino acid sequences, and therefore are encoded by separate genes, further emphasizes these dissimilarities and the importance of identifying the factors, other than cooling, responsible for the reversible disaggregation of actin gels in vivo. [See Pollack & Rifkin (121) for a possible relationship between cytoplasmic actin polymerization, cell shape, and malignant transformation.] 3. Troponin-tropomyosin[9] have not been found in phagocytic leucocytes; addition of these regulatory proteins to macrophage actomyosin does not make the ATPase of macrophage myosin Ca^{2+} sensitive (113, 114), and extracellular Ca^{2+} is not required for phagocytosis (123). Thus it seems likely that the contractile proteins of phagocytic leucocytes are not regulated by the same processes that regulate the contraction of skeletal muscle (i.e. the transfer of Ca^{2+} from a bound to a soluble form).

Unfortunately, we know little about the order in which these contractile elements enter and leave the portion of cytoplasm surrounding the forming and completed phagocytic vacuole. From the previous discussion it is evident that although some of the proteins, nucleotides, and ions that regulate the aggregation and contraction of the actomyosin complex have been identified, the mechanism(s) controlling disaggregation are unknown. This is a serious drawback since any hypothesis that attempts to explain how the contraction of actomyosin causes membrane movement must, as Stossel & Hartwig (114) point out, include a mechanism for depolymerizing the actin.

[9]These regulatory factors have been tentatively identified in fibroblasts (122).

Table 1 Properties of contractile proteins of phagocytic leucocytes and skeletal muscle

Protein	Mononuclear[a] phagocytes	Polymorphonuclear[b] leucocytes	Skeletal muscle[c]
Actin			
Percentage	9–10	11	18
Concentration[d]	~210	~240	~430
Molecular weight	42,000–45,000	~45,000	45,000
Characteristics[e]	F-actin filaments in sucrose solution depolymerize to G-actin on cooling.	F-actin filaments in sucrose solution depolymerize to G-actin on cooling.	F-actin filaments do not depolymerize on cooling.
Myosin			
Percentage	0.7–1.5	?	37
Concentration	~3.7	?	~100
Molecular weight			
H chain	220,000	180,000–200,000	200,000
L chains	20,000	18,000	20,000
	15,000	15,000	18,000
		(Immunologically distinct from skeletal muscle myosin)	16,000
H:L chains[f]	1:1:1	?	2:1:2:1
Characteristics[g]	Low Mg-ATPase activity in presence of F-actin.	Low Mg-ATPase activity in presence of F-actin.	High Mg-ATPase activity in presence of F-actin.
Actin-binding protein			
Percentage	~1–1.2	Present	Absent
Molecular weight	~270,000	?	—
Characteristics	Forms 12 nm diameter hollow coils. No ATPase activity. Binds to macrophage actin, not to myosin, and cross-links F-actin into filamentous bundles. Causes gelation of actin even in absence of K^+ or presence of EGTA.	—	—
Cofactors			
Molecular weight	~70,000–90,000	?	Absent
Characteristics	Increases Mg-ATPase activity of macrophage actomyosin and speeds contraction of macrophage actomyosin gels.	?	—

[a] Data from (103, 113, 114).
[b] Data from (102, 116).
[c] Data from (99, 117).
[d] In μmole/100 g of cell protein.
[e] G-actin monomers extracted from mononuclear and polymorphonuclear leucocytes and skeletal muscle polymerize in 0.1 M KCl at neutral pH at 25–37° C to form double-helical F-actin. These filaments depolymerize upon cooling in sucrose solutions, but not in buffers containing 0.1 M KCl.
[f] Ratio in order of decreasing molecular weight.
[g] Myosins from all three cell types form bipolar filaments in 0.1 M KCl at neutral pH.

We return to this topic in the section on factors regulating phagocytosis after we have described the role of membrane receptors and particle-bound ligands in the ingestion process.

PINOCYTOSIS In contrast to the clear association of microfilaments and contractile elements with the forming phagocytic vacuole, there is no direct evidence for the association of these structures with pinocytic vesicles. The effects of cytochalasin B on the uptake of solutes and small molecules have been used as a measure of the role of the actomyosin system in pinocytosis. Cytochalasin is reported to inhibit the uptake of [^{3}H]sucrose by Chang liver cells (55) and of peroxidase by mouse macrophages (P. J. Edelson, unpublished observation), which suggests that the actomyosin system may be involved in fluid-phase endocytosis as well. However, cytochalasin is reported to have no inhibitory effect on the uptake of ferritin (124) or colloidal gold (109) by mouse macrophages, which suggests that the actomyosin system may not be involved in adsorptive pinocytosis. As noted above (in the section on quantitation of particle and solute uptake), the uptake of [^{3}H] sucrose and of peroxidase appears to us to be a much more reliable and quantitative assay of pinocytosis than uptake of ferritin or colloidal gold. In the absence of additional data we cannot assess the significance of cytochalasin-insensitive endocytosis.

FACTORS REGULATING PHAGOCYTOSIS

Surface Properties of the Particle

The charge, hydrophobicity, and chemical composition of the particle's surface are said to influence the uptake of synthetic [paraffin-oil droplets (125, 126)] and denatured [aldehyde-treated red blood cells (127, 128)] particles, and of "naturally" derived particles, such as normal red blood cells and viable bacteria (129). Unfortunately, there is at present no theory capable of satisfactorily predicting how alterations in any of these surface properties will affect the interaction of a particle with the membranes of professional or facultative phagocytes. In general, natural particles whose surfaces have not been modified, and synthetic particles coated with undenatured proteins, are poorly phagocytized, while particles whose surface proteins have been denatured or chemically modified (128) are more readily ingested.

The importance of the particle's surface chemistry in governing its ingestion is emphasized by studies of the interaction of bacteria with phagocytic leucocytes. Many pathogenic bacteria have surface structures (capsules) that inhibit their binding to and ingestion by phagocytic leucocytes. Nonpathogenic strains of the same bacteria (nonencapsulated) lack these

phagocytosis-inhibiting surface structures and are readily ingested by phagocytic leucocytes (130).

The host responds to an infection by pathogenic bacteria by producing antibodies and complement, serum proteins that coat the bacteria's surfaces and stimulate its ingestion by professional, but not by facultative, phagocytes (127). Antibodies and complement stimulate particle ingestion by interacting with receptors, which specifically bind these molecules, on the surfaces of phagocytic cells (76, 131). We summarize here information on the structure of immunoglobulins and complement pertinent to their interactions with these receptors.

IMMUNOGLOBULINS Of the several immunoglobulin classes, only IgG, or 7S antibody, directly promotes phagocytosis. The Fc portion of the IgG molecule is responsible for this activity (132, 133). It does so by interacting with receptors on the leucocyte's membrane. Since the Fc fragment is the ligand for these receptors, they are known as Fc receptors. Several groups of investigators have attempted to define the subfragments or domains (151) within the Fc fragments that act as ligands for Fc receptors. All investigators agree that pepsin $[F(ab')_2]$[10] (132, 134) fragments of IgG bind to particulate or soluble antigens but do not promote the binding or ingestion of these complexes by phagocytic leucocytes. Okafor et al (135) have reported that Facb[10] fragments, produced by digestion of IgG with plasmin, are not ligands for Fc receptors since particles coated with Facb fragments do not bind to mononuclear phagocytes. $F(ab')_2$ fragments lack the second ($^CH^2$) and third ($^CH^3$) constant homology regions (the Fc fragment) of IgG, while Facb fragments are missing only the third ($^CH^3$) constant homology region (the pFc') of IgG.

Yasmeen et al (136) studied the interaction of red blood cells, coated with purified subfragments of the Fc fragment, with mononuclear phagocytes. Red cells coated with the pFc' fragment (the $^CH^3$ domain) bound to these phagocytes, while red cells coated with a subfragment comprising the $^CH^2$ domain did not.

Ciccimarra et al (137) isolated a decapeptide from Fc fragments of human IgG. This peptide inhibits binding of IgG-coated red cells of the Fc receptors of human monocytes and is identical in amino acid composition to residues 407–416 in the $^CH^3$ domain of the heavy chain of IgG $\gamma 1$ (138). These studies suggest that it is the third constant homology region ($^CH^3$) at the carboxyl end of the heavy chain that interacts with receptors for IgG on the leucocyte's membrane. However, Alexander et al (138a) have re-

[10] $F(ab')_2$ is a bivalent immunoglobulin *f*ragment capable of *a*ntigen *b*inding; Facb is a bivalent immunoglobulin *f*ragment capable of *a*ntigen and *c*omplement *b*inding.

ported that the pFc' fragment ($^{C}H^{3}$ region) of guinea pig IgG does not bind to mononuclear phagocytes, and Ovary et al (138b) have found that red blood cells coated with the Facb fragment of rabbit IgG are bound by guinea pig alveolar macrophages. These studies suggest that the $^{C}H^{2}$ domain is the ligand for Fc receptors. We can offer no explanation for the apparent contradiction in the findings of Okafor et al (135), Yasmeen et al (136), and Ciccimarra et al (137), vs those of Alexander et al (138a) and Ovary et al (138b) [see (138b) and Stanworth in (30) for further discussion of this problem].

Although the oligosaccharide chains on human IgG are contained within the Fc fragment, two lines of evidence suggest that these carbohydrate residues are not ligands for Fc receptors. 1. All of the carbohydrate on human IgG is in the second ($^{C}H^{2}$) constant homology region (138). 2. Purified pFc' fragments, presumably lacking carbohydrate, block Fc receptor activity (135).

Intact interchain disulfide bonds are required for binding to Fc receptors. IgG molecules or Fc fragments whose interchain disulfide bonds have been reduced with thiol reagents and alkylated with iodoacetamide no longer function as ligands for Fc receptors (138a, 139, 140).

Immunoglobulin M, or 19S antibody can also prepare particles for ingestion by phagocytic leucocytes. Unlike IgG, however, IgM requires the participation of serum complement to promote phagocytosis. This occurs because neither polymorphonuclear leucocytes nor mononuclear phagocytes have membrane receptors for IgM;[11] however, both cell types do have receptors for complement. Binding of IgM to a particle in the presence of serum initiates the fixation of complement to the surface of that particle.

COMPLEMENT Serum contains a group of more than a dozen proteins, which together form the complement pathway. Among these proteins C3, or the third component of complement, is the major phagocytosis-promoting molecule. C3 exists in serum as an inactive precursor molecule containing a heavy chain (110,000 mol wt) and a light chain (70,000 mol wt) joined by disulfide bridges. Specific proteases cleave a small fragment from the heavy chain of C3, converting the inactive 180,000-dalton precursor into a molecule of ~140,000–170,000 daltons called C3b (142) or C3bi (143). C3b or C3bi is the ligand that binds to the C3 receptor on phagocytic leucocytes (143–145). Further proteolytic cleavage of C3b destroys its activity as a phagocytosis-promoting ligand (76, 144). Thus C3 receptors, like Fc receptors, exhibit a high degree of ligand specificity.

[11]A macrophage membrane receptor for 8S IgM has been reported (141), but is yet unconfirmed.

Surface Properties of the Phagocyte

The membranes of phagocytic leucocytes contain receptors that are specific for the Fc fragment of IgG and for the third component of complement. We know little about the chemistry of these different receptors. They may be single molecules, groups of molecules, or domains on the cell's surface. Despite our ignorance of their molecular anatomy there is little doubt that these receptors are structurally and functionally distinct from one another.

Information about these receptors comes from studies of binding and/or ingestion of ligands or ligand-coated particles by living cells. These assays, like enzyme measurements in crude cellular homogenates, are indirect; in both cases what is measured is an activity of the receptor or enzyme and not the receptor or enzyme molecules themselves. Since changes in the conformation of a molecule or in the arrangement and charge of a particle's surface can alter its interactions with cellular membranes [see discussion in Chapter 6 of (146)], one cannot assume that binding of a given ligand or ligand-coated particle to a cell is a measure of the activity of a specific receptor. Ligand binding in the presence of competing similar and dissimilar ligands must be examined before an activity can be attributed to a specific receptor. In those cases where such measurements have been made, e.g. binding of monomeric IgG in the presence of an excess of antigen-antibody complexes (146a), or of C3b-coated particles in the presence of soluble C3, monomeric IgG, or antigen-antibody complexes (144), the receptor activity was shown to be restricted to a specific ligand.

Fc RECEPTORS The presence of two distinct types of Fc receptors on the membranes of mouse mononuclear phagocytes has been recognized only in the last year (146a, 147). For this reason it is difficult to state with certainty which of the two Fc receptors was being measured in some of the earlier literature. We omit reference to many otherwise excellent reports because of the ambiguities raised by the presence of the two Fc receptors.

TRYPSIN-RESISTANT Fc RECEPTORS Macrophages (148) and polymorphonuclear leucocytes (149) of all species express a protease (trypsin, chymotrypsin, pronase)-resistant Fc receptor that mediates the efficient binding and ingestion of IgG-antigen complexes (139, 140) and IgG-coated particles (76, 149). Particles and complexes containing IgG bind to this receptor at 4° or 37°C, in the absence of divalent cations (148), and in the presence of inhibitors of ATP generation [colchicine (129)] or microfilament function [cytochalasin B (109)]. Unkeless (146a) has shown that each mouse macrophage contains 5–8 $\times$ 10^5 of these receptors (as measured by the binding of soluble antigen-antibody complexes), and that they bind complexes with an affinity of 7 $\times$ 10^6–2.4 $\times$ 10^7 liter/mole at 37°C.

TRYPSIN-SENSITIVE Fc RECEPTORS Macrophages contain on their plasma membranes a trypsin-sensitive Fc receptor activity that selectively binds specific subclasses of human (IgG1 and IgG3) (137, 150, 151), guinea pig (IgG2) (134), and mouse (IgG2a) (152, 153) IgG. Immunoglobulins of these subclasses are called cytophilic antibodies (154, 155) because they bind with high affinity to macrophage Fc receptors in the absence of antigens. Unkeless & Eisen (152) have studied in detail the binding of mouse IgG subclass 2a to mouse macrophages. At saturation each macrophage binds 1–1.5 $\times 10^5$ IgG2a molecules with an affinity of 2×10^7 liter/mole at 37°C. [This is tenfold less than the amount of rabbit IgG reported bound to rabbit alveolar macrophages by Phillips-Quagliata et al (156) and by Arend & Mannik (157). Arend and Mannik reported that the IgG receptor they examined was trypsin resistant.] We do not know whether these trypsin-degradable Fc receptors can signal the ingestion of particles coated with cytophilic antibodies [cf Berken & Benacerraf (134) and below].

What are the differences in function between the trypsin-sensitive and -resistant Fc receptors? We simply do not know. One clue may lie in the configuration(s) of Fc fragments required for binding to each receptor. The trypsin-sensitive Fc receptor binds cytophilic antibodies as single IgG molecules uncomplexed with antigen. These immunoglobulins are easily eluted from these receptors by other monomeric immunoglobulin molecules. In contrast, the trypsin-resistant Fc receptor binds IgG's that are aggregated or complexed with antigen so that there are three or more IgG molecules in a cluster (139, 156). It is difficult to elute these multivalent ligands with monomeric immunoglobulins. Moreover, immune complexes containing three or more IgG's are rapidly cleared from the circulation in vivo (159, 160) and readily phagocytized in vitro (161). By using large and small molecules as antigens Mannik et al (159) have confirmed that the rapid in vivo clearance of antigen-antibody complexes is related to the presence of three or more IgG molecules clustered in a single complex, and not to the molecular weight of the complex per se.

Finally, it should be noted that the clearance of antigen-antibody complexes in vivo, and the phagocytosis of IgG-coated particles in vitro, occurs in the presence of whole serum, which contains a vast (>1000-fold) molar excess of monomeric IgG. Thus it seems evident that monomeric IgG is a very poor ligand for the Fc receptor that mediates immune clearance and phagocytosis, and that the interaction of antibody with antigen must induce a change in the conformation or arrangement of the Fc domains that signals ingestion of the complex. We suggest that the clustering of Fc domains that occurs when IgG binds to multivalent antigens directs their attachment to the trypsin-resistant, phagocytosis-promoting receptors. According to this view cytophilic antibodies and their trypsin-sensitive receptors merely assist

the macrophage in binding foreign antigens and particles. Once a particle coated with cytophilic antibodies binds to the cell's surface, the antibodies may cluster, dissociate from the trypsin-sensitive receptor, and then reassociate with the phagocytosis-promoting, trypsin-resistant receptor. The nearly equivalent binding constants described by Unkeless & Eisen (152) and by Unkeless (146a) for IgG's bound to the two receptors do not pose thermodynamic barriers to this mechanism.

C3 RECEPTORS The plasma membranes of polymorphonuclear leucocytes and mononuclear phagocytes contain trypsin-sensitive (148, 162, 163) receptors for C3b or C3bi (see previous section). Blockade of macrophage trypsin-resistant Fc receptors does not inhibit the ingestion of C3b-coated particles by these cells (162). Thus their sensitivity to trypsin and their continued function in the presence of Fc-receptor blockade clearly distinguish the receptor for C3b from the trypsin-resistant Fc receptor.

Several investigators (164–166) have reported that mononuclear phagocytes also express receptor activity for red blood cells coated with C3d, if the cells are prepared with purified complement components. However, mononuclear phagocytes neither bind nor ingest C3d-coated red blood cells prepared with whole serum as complement source (162–164). The reasons for this discrepancy are unknown.

C3b receptor function varies with the physiological state of the mononuclear phagocyte. Under basal conditions this receptor functions only to bind C3b-coated particles to the cell's surface, while in "stimulated" cells the C3b receptor mediates the ingestion of C3b-coated particles (162, 163). Although Fc and C3b receptors function independently of one another in binding and ingesting C3b-coated particles, the two receptors can act synergistically. Particles coated with suboptimal amounts of IgG and C3b are ingested avidly by professional phagocytes (149, 167–170), while particles coated with the same amount of either one of these ligands are not phagocytized.

The number of IgG molecules required to cause ingestion of a red blood cell by mononuclear phagocytes has been reported to vary between 150 (in the presence of C3b) and 6000 (in the absence of C3b) (167). The corresponding values for polymorphonuclear leucocytes are about tenfold greater.

Many cells have plasma-membrane–binding sites for proteins (171), lipoproteins [Goldstein & Brown, this volume (66a)], viruses, and a variety of other obligate intracellular parasites (see section on endocytosis and the penetration of intracellular parasites). There is no doubt that these receptors mediate the uptake of molecules and particles bearing the corresponding ligands. Viewed in this context the Fc and C3b receptors are simply two

of many ligand-specific membrane specializations adapted for the ingestion of soluble and particulate materials.

"NONSPECIFIC RECEPTORS" It is more difficult to assess whether there are cell-surface specializations governing the ingestion of particles such as latex (129), silica (172), DNA protein coacervates (48), and zymosan (35), or the phagocytosis of senescent cells (174). Both professional and facultative phagocytes ingest these particles. We will call the cell-surface factors that mediate ingestion of these artificial and denatured particles *nonspecific phagocytic receptors,* recognizing that by so naming them we have specified only an activity. In the case of professional phagocytes these nonspecific receptors can be distinguished both functionally (76) and metabolically (81) from the Fc and C3 receptors.

The plasma membranes of macrophages contain trypsin-sensitive structures that bind glutaraldehyde-treated red blood cells (175) or $F(ab')_2$-antigen complexes (161). These particles are neither bound nor ingested by trypsin-treated macrophages, while the same trypsinized macrophages ingest as many zymosan or latex particles as untreated macrophages. We do not know whether this difference reflects the presence of more than one class of nonspecific receptors or merely confirms that attachment is needed before ingestion can occur.

Professional phagocytes are not the only cells that contain Fc or C3 receptors. Lymphocytes have C3 and Fc receptors (163), and herpesvirus-infected cells express Fc receptors (176); in both cases these receptors mediate binding but not ingestion of C3- or IgG-coated particles. Similarly, unstimulated mouse macrophages bind C3-coated particles but do not ingest them. Thus the presence of Fc and C3 receptors is but one of several factors that determine whether a particle coated with the corresponding ligands will be phagocytized. Of equal importance is the presence of structures and pathways capable of relaying signals arising from these receptors to the locomotive machinery of the cell. In the case of the unstimulated mouse macrophage the C3 receptor appears not to be connected to the cell's phagocytic machinery.

Michl et al (35, 81) have described a method for experimentally uncoupling the phagocytosis promoting Fc and C3 receptors of normal and stimulated macrophages, and Boxer et al (177) have used it to inhibit Fc and C3 receptor–mediated phagocytosis in polymorphonuclear leucocytes. These workers found that 2-dG inhibits Fc- and C3-receptor–mediated phagocytosis but not phagocytosis per se. That is, the ingestion of latex and zymosan particles continues unimpaired in the presence of 2-dG. They further showed that attachment of IgG- or C3-coated particles to their corresponding membrane receptors is not inhibited by 2-dG, that the effects

of this glucose and mannose analog on phagocytosis are unrelated to its capacity to inhibit glucose utilization and ATP generation, and that its effects are rapidly reversed by glucose or mannose. Although the mechanism(s) underlying the specific inhibitory effects of 2-dG on Fc- and C3-receptor–mediated phagocytosis are unresolved, it is evident that the overall effect of this sugar is to disconnect these receptors from the cell's phagocytic machinery.

Attachment of Particles to the Phagocyte's Surface

The phagocytic process can be divided experimentally into two discrete steps: (*a*) attachment of a particle to the cell's surface and (*b*) ingestion of the particle (71, 128). Attachment of a particle to a cell bearing surface receptors for ligands on the particle's surface generally occurs independently of temperature (54, 76, 178) and the expenditure of metabolic energy. Attachment of particles for which the cell does not have specific receptors may be temperature dependent (71, 179).

Attachment does not necessarily predestine a particle for ingestion. Mycoplasma (180) and C3b-coated erythrocytes (144, 162) attach to the surfaces of unstimulated mouse macrophages but are not ingested by these cells. Addition of antimycoplasma or antierythrocyte IgG initiates the ingestion of these particles.

Ingestion is highly temperature dependent and requires active cellular metabolism (see section on energy requirements for endocytosis). Metabolic inhibitors or low temperatures (76) allow binding of particles to the cell's surface in the absence of phagocytosis. Excess or unattached particles can then be washed away and the number of particles attached can be enumerated prior to the initiation of particle ingestion. Methods such as these are advantageous since they allow the investigator, upon removing the metabolic inhibitor or raising the incubation temperature, to study a synchronous wave of particle ingestion (76, 178). However, they do not permit evaluation of the maximal numbers of particle that can be ingested. For these measurements, the methods devised by Cohn & Morse (41), Michell (36), and Stossel (40) are useful.

Segmental Response of the Cell Surface to a Phagocytic Stimulus

Griffin & Silverstein (129) examined whether the response of a cell's plasma membrane to a phagocytic stimulus is localized to the segment of membrane adjacent to the particle initiating the stimulus, or whether the stimulus spreads to a broader area of the cell's surface and elicits a general membrane response. They attached noningestible particles to the macrophage membrane. These particles served as markers for the segments of membrane to

which they were linked. The macrophages were then incubated with a second particle that was morphologically distinguishable from the membrane marker particle. Ingestion of the second particle did not prompt ingestion of the marker particle, even when the site of ingestion of the second particle was right next to the segment of membrane to which the marker particle was bound. Using this approach Griffin et al (144) also showed that particles bound to one type of membrane receptor (e.g. C3b receptors) are not phagocytized when other particles are ingested via a different receptor system (e.g. Fc receptors) on the same cell, and that Fc receptors located at different sites on the plasma membrane function independently of one another (181). They concluded that phagocytosis is a local response of a segment of a cell's surface to signals generated by specific interactions of the particle with the plasma membrane.

Roles of Ligands and Receptors in Particle Ingestion

Griffin et al (76, 181) have shown that ingestion of IgG- or C3b-coated particles proceeds by the sequential and circumferential interaction of Fc or C3b receptors on the surface of a phagocyte with ligands distributed uniformly over the particle's surface. To show this, these investigators took advantage of the observation of Taylor et al (182) that antibodies directed against lymphocyte-membrane immunoglobulins cause these cell-surface proteins, together with their attached antimembrane immunoglobulin IgG's, to cluster (cap) at one pole of the lymphocyte's surface. By using lymphocytes with IgG ligands on only one hemisphere as phagocytic test particles they determined whether the distribution of ligands on the particle's surface could exert a controlling influence upon particle ingestion. They found that phagocytes bind these capped lymphocytes via the latter cells' anti-immunoglobulin caps, that the membrane of the phagocyte spreads over the surface of these lymphocytes only in the region of the cap, and that these capped lymphocytes are not ingested. Lymphocytes diffusely coated with a similar amount of the same antibody preparation were rapidly ingested. Griffin et al (181) concluded that the distribution of ligands on a particle's surface is a critical factor in determining whether or not the particle is phagocytized. In another series of experiments these authors showed that erythrocytes diffusely coated with anti–red cell IgG were not ingested when Fc receptors lying outside the zone of attachment of these IgG-coated red cells to the phagocyte's surface were blocked (76). Similar results were obtained in studies of C3b-receptor–mediated phagocytosis of C3b-coated erythrocytes (76). Thus the initial interaction of immune ligands on the surface of a particle with receptors on the membrane of phagocytic leucocytes does not trigger the ingestion of the particle. It merely initiates a process that requires the continuous apposition of recep-

tors and ligands until the particle is fully enclosed within a phagocytic vacuole (Figure 3). Griffin et al have termed this the "zipper mechanism" of phagocytosis (76, 181).

Although the zipper mechanism accounts for the discriminatory and segmental nature of the cellular response to a phagocytic stimulus, it does not explain the mechanism by which the phagocyte moves around the particle, nor does it account for the requirement for metabolic energy. Presumably metabolic energy (e.g. ATP) is required to fuel the interaction and movement of cytoplasmic contractile elements, which are themselves responsible for cellular locomotion. Aggregation of these contractile elements is localized to the area of cytoplasm directly beneath the particle being phagocytized (114, 181). Thus in this respect, as in the behavior of the plasma membrane, the cell responds segmentally. To account for these findings, Griffin et al (76, 181) have proposed the following model of immune ligand-mediated phagocytosis (Figure 3): (*a*) Ligand-receptor interaction generates a signal [perhaps the release of actin-binding protein (113, 114) from the membrane] that initiates the aggregation of contractile

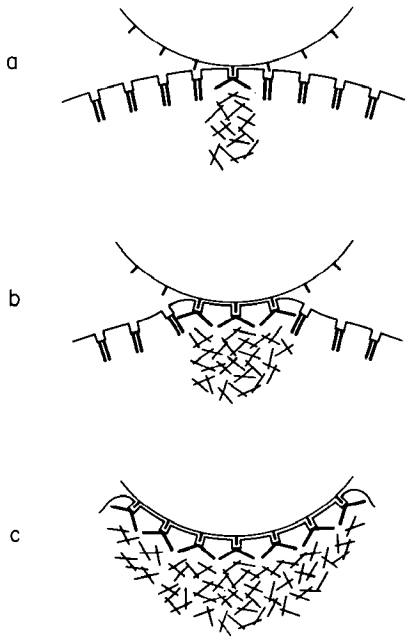

Figure 3 Model of proposed phagocytic mechanism [reproduced from Griffin et al (181) by permission of The Rockefeller University Press]. ı = IgG molecule or other ligand. ⊤ = Fc receptor or other membrane receptor in an inactive state. ⋋ = Fc receptor or other membrane receptor in an activated state. ⁂ = Cytoplasmic contractile proteins.

proteins and leads to the extension of pseudopods in the area of the attached particle. (*b*) Pseudopod extension leads to further receptor-ligand interaction and this in turn leads to further aggregation of contractile proteins. (*c*) The process may be repeated many times until the plasma membranes meet and fuse with one another, forming a phagocytic vacuole. The model does not account for the mechanism by which actomyosin contraction might cause pseudopod extension. Stossel & Hartwig (114) have suggested that randomly oriented actin filaments cross-linked by actin-binding protein could be drawn together by macrophage myosin. They note that filaments arranged in an elongated system would contract first in the shortest dimension. Were these filaments connected to the plasma membrane, contraction could result in the formation of elongated pseudopods. An alternative possibility is that actin polymerization and gelation alone causes pseudopod formation (118, 119). Actomyosin contraction may be a comparatively late occurrence and may function to squeeze the already ingested particle into the interior of the cell and/or to reduce the volume occupied by the actin gel.

Membrane Remodeling and Fusion During Phagocytosis

Scanning and transmission electron microscopic studies show that the macrophage plasma membrane is tightly apposed to the particle surface throughout the engulfment process (114, 183–185). As ingestion progresses the macrophage membrane forms a continuous collar that completely conforms to the shape of the underlying particle (185). It is evident that some change or remodeling of the cell's plasma membrane must take place in order for it to conform to the contours of the particle, but the ways in which these changes occur are unknown.

Scanning electron micrographs of particle ingestion frequently show membrane pseudopods advancing over the particle's surface. It is frequently assumed that the phagocytic vacuole forms as a result of extensive side-to-side and tip-to-tip fusion of these pseudopods. However, such fusion has not been seen when two leaflets of plasma membrane move over a single particle or when two phagocytes attempt to ingest the same particle. For these reasons we think it likely that flow and rearrangement of components in the plane of the membrane, rather than membrane fusion, is responsible for remodeling the cell's surface.

The final event in engulfment is the closure of the phagocytic vacuole, an event that does require membrane fusion. Scanning electron micrographs show that frequently the last connection of the phagocytic vacuole with the extracellular space is a small circular opening or channel at the center of the enveloping membrane collar. Closure of this channel requires only a very limited point of membrane fusion, analogous to the point of fusion seen when a pinocytic vesicle fuses with the cell surface (186). Restriction of

membrane fusion to such very small and discrete sites could account for the lack of fusion between adjacent phagocytic cells, and the infrequency with which fusion of membranes in actively phagocytizing cells is seen by electron microscopy.

Little is known about the molecular dynamics of membrane fusion. Metabolic energy and divalent cations appear to be required in some systems (187), and the possible role of lysophosphatides as mediators of the process has been discussed (90, 188, 189). In the last few years Oates & Touster (190) have developed a model system for studying fusion of phagolysosomes in vitro, Scheid & Choppin (191) have identified a protein responsible for the hemolytic and cell-fusing activities of paramyxoviruses, and several groups of investigators (192–195) have reported that unilamellar liposomes fuse with the plasma membranes of cells in culture. Thus systems may now be available for studying this process in detail.

METABOLIC CONSEQUENCES OF PHAGOCYTOSIS

Oxidative Metabolism

During particle ingestion professional phagocytes show a two- to threefold increase in CN^--insensitive O_2 consumption, a marked increase in H_2O_2 production, and a two- to tenfold increase in glucose oxidation via the hexose monophosphate shunt (72–74). Several lines of evidence indicate that these metabolic alterations are not required to provide the energy for particle ingestion: 1. O_2 consumption and hexose monophosphate shunt activity are unchanged during particle ingestion by facultative phagocytes (77). 2. Leucocytes incubated with colchicine or in the absence of O_2 ingest particles normally but do not exhibit increased O_2 uptake and hexose monophosphate shunt activity (85, 86). 3. Membrane perturbants (e.g. low concentrations of detergents, surface-bound immune complexes) stimulate oxidative metabolism in the absence of particle ingestion (196–198).

Most workers in this field now agree that this burst of oxidative activity results from the activation of a plasma-membrane–linked NADH (199) or NADPH (200) oxidase that converts O_2 to H_2O_2 via superoxide anions (198) and that it is H_2O_2 that drives the hexose monophosphate shunt. The role of this pathway in the production of oxygen metabolites (superoxide, singlet oxygen, hydroxyl ions, and H_2O_2) constitutes a new and exciting chapter in biochemistry; the interested reader is referred to several excellent recent reviews (198, 200–202) for more detailed information. Suffice it to say that these oxygen metabolites appear to be important effectors of the microbicidal activities of phagocytic leucocytes (198, 201, 202) and individuals genetically deficient in the enzymes of this pathway (203) have severe recurrent bacterial infections.

Phospholipids and Cholesterol

Several effects are associated with phagocytosis: increased incorporation of lysophosphatides into phosphatidyl choline and phosphatidyl ethanolamine (204, 205), enhanced uptake of ^{32}P into phosphatidic acid and phosphatidyl inositol (206), and an increased flux of fatty acids from cellular triglycerides to lecithin (89). In the case of phagocytizing polymorphonuclear leucocytes the amount of phosphatidyl choline formed from lysophosphatides in the medium can amount to as much as 5% of total cellular lecithin (204). Most of this newly synthesized lipid is incorporated into the plasma membrane, and about half of it is transferred from the plasma membrane to phagocytic vacuoles, presumably as part of the vacuolar membrane, during particle ingestion (83). Despite all of these findings, evidence is lacking that increased phospholipid synthesis is obligatory for phagocytosis. Granulocytes (90), L cells (207), and macrophages (76) all ingest particles in the absence of an exogenous lipid source. In the absence of selective inhibitors of phospholipid synthesis and incorporation it is impossible to assess the role of endogenous lipid pools in this process.

Although exogenous cholesterol is not required for particle ingestion, its presence is required for membrane renewal in mouse macrophages. Werb & Cohn (208) found that these cells exhibit a marked increase in total cellular cholesterol and phospholipid 6–10 hr after ingestion of latex particles. The size of this increase is proportional to the number of latex particles ingested and is strictly dependent upon the presence of exogenous cholesterol. Morphologic and functional analyses of these macrophages suggest that the increases in cellular phospholipid and cholesterol are related to new plasma-membrane synthesis, and that the availability of both newly synthesized proteins and exogenous cholesterol is required for membrane synthesis to occur.

Secretion of Neutral and Acid Hydrolases

Endocytosis stimulates the secretion of acid and/or neutral hydrolases in a variety of cell types. Polymorphonuclear leucocytes release some of their acid hydrolases into the surrounding medium in response to particle ingestion. The amount of enzyme released is proportional to the number of particles ingested. Ignarro et al (209, 210) have shown that for any given amount of particle ingestion, enzyme release from phagocytizing polymorphonuclear leucocytes is enhanced by compounds that elevate cyclic GMP (cGMP) levels and is inhibited by removal of Ca^{2+} from the extracellular medium. These findings demonstrate that enzyme release is not required for particle ingestion to occur. Similar findings regarding the role of cGMP in enzyme secretion have been reported by Zurier et al (211). In polymorphonuclear leucocytes, the hydrolase-containing granules fuse with the

membrane of the forming phagosome before its separation from the cell's surface (212). For this reason it has been suggested that lysosomal enzymes "leak" out around the particle during its interiorization. However, the uptake of fluid-phase markers, such as HRP, in mouse macrophages is not stimulated by particle ingestion (P. Edelson, unpublished observations). This result indicates that the vacuolar membrane forms a tight seal with the surface of the particle and suggests that the leakage concept may be an oversimplified view of the mechanism of lysosomal-enzyme release during phagocytosis.

The secretion of neutral proteases, such as plasminogen activators, collagenase, and elastase, is closely coupled to particle ingestion in fibroblasts and macrophages [see (213) for review]. Endocytosis of indigestible particles, such as latex beads, by an appropriately primed cell (214) causes maximal and sustained stimulation of neutral protease secretion, while the ingestion of digestible particles such as red blood cells increases secretion only transiently. In the latter instance an increase in secretory rate is maintained only until the particle is degraded. In both cases very little of the enzyme is stored intracellularly. Thus secretion appears to be an accurate reflection of de novo enzyme synthesis.

Particle ingestion per se does not appear to be an obligatory prerequisite for protease secretion since colchicine (213) and tumor promotors, such as phorbol myristate acetate (173), all stimulate the release of one or more of these enzymes.

Induction of Lysosomal-Enzyme Synthesis

In contrast to the effects of the endocytosis of indigestible materials on enzyme secretion, the endocytosis of digestible materials appears to regulate the levels of intracellular lysosomal enzymes. Phagocytosis of red blood cells or pinocytosis of serum proteins induces the synthesis of lysosomal enzymes in mouse macrophages and leads to a tenfold increase in their intracellular content (215). The uptake of latex or sucrose has no corresponding stimulatory effect, which suggests that the products of intracellular digestion are responsible for this increase in enzyme synthesis (215).

Cyclic Nucleotides

Most investigators have found no change in cyclic AMP (cAMP) levels in phagocytizing polymorphonuclear leucocytes (83). In a few cases increased cAMP levels have been reported to accompany particle ingestion, but upon further examination the major proportion of this increase was attributed to changes in cAMP levels in nonphagocytizing mononuclear cells (probably lymphocytes) present in the culture (216). Seyberth et al have reported a small and transient increase in cAMP levels in phagocytizing alveolar

macrophages (217) and Ignarro and his colleagues (210) have found substantial increases in cGMP levels during particle ingestion in polymorphonuclear leucocytes. In the latter case, omission of Ca^{2+} from the medium prevented the phagocytosis-stimulated increase in cGMP levels (210). Since phagocytosis proceeds normally in the absence of extracellular Ca^{2+} (123), it seems unlikely that the ingestion process itself is regulated by changes in cGMP levels.

Muschel et al (218) have studied mutant clones of SV40-transformed mouse macrophages that bind but do not ingest IgG-coated red blood cells. Addition of cAMP to the medium stimulated the ingestion of these IgG-coated cells. Although the mechanism of this stimulation remains unknown, these interesting observations and those on the inhibitory effects of 2-dG on immune-ligand–mediated phagocytosis (35, 81, 177) may presage a new chapter in defining the molecules that regulate phagocytosis.

FACTORS REGULATING PINOCYTOSIS

In contrast to the detailed information known about the mechanism of ingestion of large particles, the factors governing pinocytic activity are poorly understood. At this time we can list three categories of pinocytosis (detailed below), but we do not know whether they have common or different control mechanisms.

Pinocytosis of Ligands Bound to the Cell Surface

Binding of ligands to cells can stimulate pinocytic activity. Classically this has been studied in large amoebae that form pinocytic channels upon encountering a variety of charged substances, from ions to macromolecules, all capable of binding to their surface. These substances are all interiorized while bound to the vesicle membrane (219, 220).

Pinocytosis in mammalian cells is also stimulated by membrane-bound ligands. Edelson & Cohn (64) found that concanavalin A (conA) increased the uptake of fluid-phase markers threefold in mouse macrophages and that mannose and glucose blocked the lectin's effect. They identified the conA on the cell's surface and on the inner aspect of the endocytic vacuole membrane. The fact that conA is bivalent or possibly multivalent in its interaction with cell-surface saccharides seems to be important. Steinman (unpublished observations) has noted that succinylated conA, which behaves as a univalent ligand in several mouse cells, does not stimulate pinocytosis even though it is interiorized in an adsorptive fashion.

B lymphocytes rapidly pinocytose anti-immunoglobulin molecules bound to their cell-surface immunoglobulins (182, 221, 222); again, the bivalent nature of the anti-immunoglobulin is important since univalent anti-

immunoglobulin fragments are not rapidly interiorized (182, 221). Roth et al (9) found that maternal-yolk proteins, which bind to specific receptors or the oocyte plasma membrane, stimulate uptake of a fluid-phase marker. Two other examples are known in which presumptive surface ligands stimulate pinocytosis (although the fate of the ligand itself has not been documented): the stimulation of pinocytosis in mouse macrophages by a 19S hemagglutinin (223) and in toad bladder mucosa by antidiuretic hormone and its analogues (224).

It is not established yet if other materials interiorized by adsorptive endocytosis (see section on quantitation of particle and solute uptake) stimulate their own uptake. Presumably some do, but an alternative possibility is that pinocytosis of some or all parts of the plasma membrane proceeds continuously (see section on "constitutive" pinocytosis below), and adsorption simply allows the ligand to be included in the incoming vesicle.

Pinocytosis Following Secretion

Neurons (225–229), exocrine (230) and endocrine (231) pancreatic cells, salivary gland (232), anterior (223–229, 233, 234) and posterior (235–237) pituitary cells, and adrenal medullary cells (229), to name a few, exhibit an enhanced rate of fluid-phase pinocytosis for minutes to hours following a burst of secretory activity, as was originally predicted by Palade (238). In most of these cases pinocytosis is detected morphologically, e.g. by the formation of intracellular vacuoles filled with exogenous markers like HRP. Baker et al (236) measured fluid uptake in whole, stimulated rat pituitary, and estimated that the volume of fluid endocytosed was similar to the volume of the secretory-granule content. De Camilli et al (239), using freeze-fracture techniques, provided evidence that the endocytosed membrane is similar in appearance to that added to the cell surface during secretion. He found that the plasma membrane of salivary-gland cells is rich in intramembranous particles, whereas the secretion-granule membrane is particle poor. Following secretion, the cell's luminal surface is greatly amplified and contains a mosaic of particle-rich and particle-poor regions. The two types of regions never mix; rather, the particle-poor zones selectively disappear, which suggests that the secretory-granule membrane is completely retrieved, presumably by endocytosis. A more specialized example of direct retrieval of secretory-granule membrane was found by Hausmann & Allen (240), who showed that trichocyst-granule membrane is endocytosed immediately after its fusion with the surface of *Paramecium.*

"*Constitutive*" *Pinocytosis*

Some cells pinocytose at characteristic and constant rates for long periods of time (days). This has been demonstrated quantitatively in cultures of

nondividing mouse macrophages (21) and of growing fibroblast lines (24). In vivo, the vesicles and lysosomes of many epithelial cells, e.g. kidney (51, 52) and vas deferens (10), are readily filled with pinocytic tracers, which suggests that they too are endocytosing continuously. Factors controlling this constitutive pinocytosis have not been identified; conceivably it is secondary to ligand binding and/or secretory activity.

Pinocytic activity can be increased to a new steady state in some of the above examples—although again the control mechanisms are unknown. Edelson et al (241) found that mouse macrophages pinocytose several times more actively when obtained from inflammatory exudates. Steinman et al (24) and Kaplan (242) have also observed that some cell lines pinocytose 2–4 times faster upon reaching confluence. Since confluent populations contain a larger proportion of cells in the G_1 phase of the cell cycle, this finding may be related to that of Quintart et al (243), who noted that synchronized hepatoma cells interiorize HRP most actively in G_1.

MEMBRANE FLOW DURING ENDOCYTOSIS

Rate of Plasma-Membrane Influx—Morphologic Studies

The area of membrane interiorized during endocytosis—both absolute and relative to plasma-membrane area—can be estimated, particularly by stereology (244, 245). Stereology is a statistical approach for obtaining three-dimensional information from two-dimensional micrographs. For example, the surface-to-volume ratio of the cell is related to the number of times a grid of test lines intersects the cell surface per unit length of line in randomly selected micrographs. This ratio can be converted to an absolute surface area if cell volume is known. Steinman et al (246), using this approach, found the surface area of mouse macrophages and L-cell fibroblasts to be 825 and 2100 μm^2, respectively, values that are three times the area of smooth spheres of equivalent volume.

The area of membrane interiorized during large particle uptake can be estimated in two ways: (*a*) by counting the number of spherical particles ingested or (*b*) by stereology. Method *a* requires that the membrane of the phagocytic vacuole be closely apposed to the particle surface, that the particles be of uniform and defined size, and that each is taken up in a separate vacuole. These requirements were met in Hubbard's & Cohn's study (207) of the uptake of 1.1-μm diameter latex spheres in L cells. Each cell ingested an average of 170 particles having a total surface area of 646 μm^2, some 31% of the total plasma membrane area of 2100 μm^2. Using stereology, method *b,* even with particles of nonuniform size and shape one can compare the relative areas of phagosome and cell surface by counting the number of times a grid of test lines intersects vacuolar membrane vs

plasma membrane. The absolute amount of interiorized membrane can be obtained if the surface area is known (see above). This technique has the added advantage of estimating cell-surface area before and after phagocytosis.

Membrane influx during pinocytosis is more difficult to measure since the incoming vesicles are small, hard to identify, rapidly fuse with one another and with lysosomes, and appear to decrease in surface area following their interiorization. Steinman et al (246) examined the rate of surface interiorization in macrophages and L cells using the following method. (*a*) They estimated the rate of fluid uptake per minute by obtaining quantitative data on the rate of solute (HRP) uptake. (*b*) They identified the incoming, cytochemically reactive (HRP-containing) pinocytic vesicles, and showed that the rate at which this compartment expands in volume is similar to the predicted rate of fluid influx. (*c*) They measured the surface area of the incoming cytochemically reactive vesicles, relative to plasma-membrane area, using stereology. The equivalent of 3.1% of the macrophage and 0.9% of the L-cell surface were interiorized each minute. The total cell-surface area remained constant throughout a 3-hr period. Even higher rates of membrane influx (5–50 cell-surface equivalents per hour) have been estimated for pinocytosis in *Acanthamoeba castellani* (56).

Fate of the Interiorized Plasma Membrane— Morphologic Studies

FUSION WITH LYSOSOMES In most cells, endocytic vacuoles rapidly fuse with lysosomes. Silverstein & Dales (65) found reovirus particles in lysosomes within 15 min (the first time-point tested) of the onset of endocytosis, and by 60 min, most of the surface-adsorbed virus was in lysosomes. Steinman et al (246) showed that HRP enters the entire pinocytic vesicle compartment of cultured macrophages and L cells within 5 min, and gradually (45–60 min) saturates the preexisting lysosome compartment. Morphologic techniques can also be used to show that ingested macromolecular contents remain with the lysosome and do not leak out into the cytoplasm, e.g. colloids (47) and HRP (247). Some microorganisms are exceptional in that they do escape the vacuolar confines (see section on endocytosis and the penetration of intracellular parasites).

Following particle uptake, electron microscopy shows only that the vacuolar membrane shrinks in size as the particle is digested, but is at all times closely applied to the content (e.g. 161). The shrinkage process is slow (hours to days) and there is no information on the fate of the ingested membrane.

Using phase-contrast microscopy, fluid-filled pinocytic vesicles can be observed to shrink very rapidly (42, 47). Steinman et al (246) obtained quantitative data on the rapidity and extent of the shrinkage process. Using HRP as a marker, they found that macrophages (qualitatively similar data were obtained in L cells) pinocytose the equivalent of 25% of their cell volume and 186% of their cell-surface area each hour. Yet throughout the 3-hr period studied, cell volume and area remained constant, and both the pinocytic vesicle and secondary lysosome compartment occupied a constant volume and area, equivalent to 2–3% and 15–20% of the whole cell volume and area, respectively. Thus the membrane area of vesicles entering the cell hourly is some ten times the steady-state dimensions of vesicle and lysosome compartments. Presumably an efflux of pinocytosed fluid and low-molecular-weight solutes occurs following fusion with the lysosome, which facilitates the shrinkage process, but this is still speculative.

RECYCLING The idea of recycling of plasma membrane was initially proposed by Palade (44) following his initial electron microscopic observations of the endoplasmic reticulum. Until recently, it has been difficult to obtain evidence supporting this concept. The findings of Steinman et al (246) provide experimental support for the membrane-recycling hypothesis. Steinman et al showed that the total surface area of plasma membrane, pinocytic vesicle membrane, and secondary lysosomal membrane of mouse fibroblasts each remains constant despite extensive interiorization of the cell's surface membrane during pinocytosis. These results led Steinman et al to propose that interiorized membrane is recycled back to the cell surface intact (246). Extensive degradation and resynthesis—at least for most membrane components—were considered unlikely in view of the rapidity of the process, the considerable metabolic load this would impose, and the failure to detect such rapid degradation in most studies of plasma-membrane turnover (see below). The mechanism of the proposed recycling process is unknown, e.g. does it involve disassembly into membrane constituents vs return of fully assembled membrane. Two sorts of membrane-bound organelles can be implicated: 1. tiny vesicles, often seen in association with pinocytic vesicles and lysosomes, that may originate from them (see pathway 5, Figure 2), and 2. the Golgi apparatus. Incoming fluid-phase HRP does not enter Golgi saccules in most cultured cells (21, 24). However, there are three examples (233, 234, 248) in which HRP initially is associated with the plasma membrane and later can be identified in the inner lamellae of the Golgi cisternae (Figure 2), as well as endocytic vacuoles and lysosomes. Perhaps HRP in these instances is tracing the path of recycling plasma membrane. It may be relevant that extensive shrinkage and possible recy-

cling of secretory-granule membrane also occurs in the Golgi region, e.g. when condensing vacuoles mature into zymogen granules (249).

The idea of membrane recycling in pinocytosis is reminiscent of the membrane-retrieval hypothesis in secretory cells (see section on factors regulating pinocytosis), i.e. it is likely that membrane added to the cell surface during fusion of secretory granules is recaptured by pinocytosis. The fate of retrieved membrane is not clear, but in several studies fusion with lysosomes has been documented (e.g. 229).

Freeze fracture has been used to look at the composition and fate of endocytosed membrane. In *Acanthamoeba* (250), the membrane of the interiorized phagosome has a threefold increase in intramembranous-particle density relative to the plasma membrane of the forming phagocytic vacuole. In *Paramecium* (251), the food-vacuole membrane exhibits marked changes in intramembranous-particle number and size during its intracellular life; these changes occur concurrently with other events visible in transmission microscopy, e.g. fusion with lysosomes, pinching off of putative recycling vesicles (252). These freeze-fracture findings are striking, although too preliminary to interpret mechanistically.

Composition of the Interiorized Plasma Membrane—Biochemical Studies

Is endocytosed membrane a representative or selected sample of the plasma membrane from which it arises? The selective notion was born with the observations of Tsan & Berlin (253), who found that the ingestion of particles by neutrophils and macrophages did not reduce their ability to transport certain purines and amino acids into the cells. Neither the K_m nor the V_{max} of the surface-transport sites was altered, even though an estimated 30–50% of the cell surface had been ingested (the transport assay could detect a 7% decrease in V_{max}). They suggested that transport sites in the plasma membrane were excluded from the forming phagocytic vacuole. The authors ruled out the possibility that the cells replaced interiorized transport sites from an intracellular pool. They used a nonpenetrating thiol reagent to inactivate surface-transport sites prior to phagocytosis and showed that transport activity was not restored when the treated cells phagocytosed latex. Subsequently Ukena & Berlin (97) found that particle uptake did lead to the expected loss of transport sites if cells were treated with colchicine prior to ingestion. They proposed that the exclusion of transport sites from the vacuole or membrane is regulated by microtubules.

These interesting observations are not easily interpreted. It is possible that transport sites are both interiorized and replaced during phagocytosis, but that the replacement process itself is sensitive to the thiol-reactive drug. Moreover, neither the area of plasma membrane interiorized nor the surface

area of the cells before and after phagocytosis were measured. Intracellular pools of plasma-membrane components have been suggested to exist in other systems, e.g. 5'-nucleotidase in liver (254, 255) and cultivated macrophages (256), insulin receptors in liver (257), and acetylcholine receptors in muscle (258). Hopefully these transport experiments can be extended by direct analysis of the transport molecules themselves and their distribution in normal and colchicine-treated cells.

Two studies of pinocytosis have suggested that the endocytic-vacuole membrane is selective in its composition. (*a*) Following administration of anti-immunoglobulins, the surface of B lymphocytes is cleared of its immunoglobulin binding sites, but binding of antibodies to other surface components, like histocompatibility antigens, is not perceptibly altered (182). Since the anti-immunoglobulin is pinocytosed (some may also be shed from the cell), there appears to be selective uptake of cell-surface immunoglobulin. However, it is known that both the cell-surface immunoglobulins and the anti-immunoglobulin probes can redistribute (patching, capping) along the cell surface prior to their endocytosis (182, 221). This movement, and not the interiorization step, may be the selective process, and the interiorized membrane may otherwise be identical to the plasma membrane from which it is derived. (*b*) Schneider et al (259) report that specific anti–plasma-membrane antibodies are not interiorized into lysosomes, as assessed by cell fractionation and fluorescence microscopy, but the same cells do endocytose nonspecific immunoglobulins. Exclusion of the anti–plasma-membrane binding site from the pinocytic vesicle may explain these findings.

In contrast to these indirect approaches, there is direct evidence that components of the plasma membrane are included in endocytic vacuoles in amounts proportional to the total area of membrane interiorized. Plasma membrane and the latex-phagolysosome membrane have been compared in the same cells with respect to the following components:

ENZYMES Hubbard & Cohn (207) and Werb & Cohn (208) found a substantial (30–50%) redistribution of plasma-membrane enzymes (phosphodiesterase in L cells, 5'-nucleotidase in macrophages) into latex phagolysosomes. Estimates of the proportion of plasma membrane interiorized during these experiments gave a similar 30–50% value (260).

POLYPEPTIDES Hubbard & Cohn (261) found that L cells contain at least 15–20 polypeptides that are accessible to iodination with lacto-peroxidase and ^{125}I. After phagocytosis (207), all of these radioiodinated polypeptides were identified in phagolysosomes, and apparently in the same relative proportions to those found in the plasma membrane. Again, a similar

percentage of radioiodine and plasma-membrane area was interiorized (260).

LIPIDS Ulsamer et al (262) found that the lipid composition of *Acanthamoeba* phagolysosome membrane was identical to that of the plasma membrane from which it was derived. Comparable experiments have not been done in mammalian cells.

Fate of the Interiorized Plasma Membrane—Biochemical Studies

It is clear that endocytosed membrane components can be degraded. Plasma-membrane marker enzymes [L-cell phosphodiesterase (207) and macrophage 5'-nucleotidase (208)] are rapidly ($t_{1/2}$ of 2 hr) inactivated following inclusion in latex phagolysosomes. More impressively, some 70% of interiorized, radioiodinated L-cell–membrane proteins are rapidly ($t_{1/2}$ of 2 hr) and completely degraded after phagocytosis (labeled monoiodotyrosine was recovered in the culture medium). It is not known whether all plasma-membrane proteins and enzymes, or just the ones that are exteriorly exposed and therefore end up facing the interior of the phagolysosome, are degraded. However, it is evident that despite the presence of lysosomal phospholipases, the membrane lipids of the phagosome are conserved (204, 205).

Endocytosis and Plasma-Membrane Turnover

It has long been suspected that endocytosis contributes to the turnover of plasma membrane, since it brings this membrane into a degradative organelle—the lysosome. The evidence is hardly clear, however. Some workers have studied individual markers like 5'-nucleotidase in macrophages (263) and acetylcholine receptors in myotubes (258). Both enzymes appear to enter the cell in endocytic vacuoles, but it has not been demonstrated that this is responsible for, or correlates with, the observed turnover. Most workers have radiolabeled the cell surface—by iodination (207, 214), acetylation (265, 266), or after biosynthetic incorporation of sugars and amino acids (264, 267–271)—and have then made two sorts of measurements:

TURNOVER OF BULK RADIOLABEL For radioiodinated plasma membrane proteins, degradation follows first-order kinetics with a rapid ($t_{1/2}$ of a few hours or less) and a slow ($t_{1/2}$ of 20–100 hr) component. Other labels appear to yield only a single slow component. The rapid component accounts for 0–50% of the total and has been attributed by Kaplan (272) to be the result of pinocytosis. Other explanations seem possible: elution of adsorbed labeled materials, extracellular degradation, or shedding. It is also

possible that this rapid turnover is not physiological but is a result of the iodination technique. The slow component has an extremely long half-life when compared to the rate at which plasma membrane is likely interiorized by pinocytosis, e.g. half of the surface of mouse L cells is endocytosed hourly (246). The wide difference between the average rate of interiorization of membrane and protein half-lives of 20–100 hr can be reconciled if (*a*) plasma-membrane components are excluded from the pinocytic vesicles; (*b*) labeled components are efficiently recycled without degradation; or (*c*) the rate of pinocytosis of the surface is not the rate-limiting process in turnover.

TURNOVER OF INDIVIDUAL COMPONENTS In some studies, many individual membrane proteins turn over at similar rates (207, 264, 266), while in others, different protein species turn over at dissimilar rates (268–271). It is not clear if these differences reflect physiological processes or are merely technical in origin. The finding of homogeneous turnover prompts the conclusion that degradation occurs in bulk, as would occur when endocytosis delivers a large segment of plasma membrane to the lysosome. Again, one must determine the rate-limiting process(es), for one might expect that the susceptibility of different membrane components to lysosomal digestion varies considerably, e.g. Hubbard & Cohn (207) noted that certain components of plasma membrane included in latex phagolysosomes were degraded more slowly than the bulk of labeled proteins. Also the rate at which pinocytosed contents are degraded varies considerably. For instance, lysosomal release of [^{125}I]monoiodotyrosine from [^{125}I]HRP has a $t_{1/2}$ of 20–30 hr, whereas enzymatic activity is lost with a $t_{1/2}$ of 7–9 hr (21). In contrast, the $t_{1/2}$ of degradation of [^{3}H]leucine-labeled outer-coat proteins of reovirus is about 1 hr, while the RNA transcriptase activity of reovirus core proteins is unaffected by lysosomal enzymes (178).

ENDOCYTOSIS AND THE PENETRATION OF INTRACELLULAR PARASITES

Ingestion and intracellular killing of microbial parasites is generally recognized as a central mechanism in host defense. It is less widely appreciated that many obligate intracellular parasites utilize endocytic pathways to penetrate and replicate within their host cells. Many of these microorganisms gain entry into their host cells by virtue of specific receptor molecules on the cell's surface. For instance, human but not mouse cells bear a genetically defined surface receptor for poliovirus (273). While only human cells can be infected with the intact virus, both human and mouse cells can be infected with poliovirus RNA (274, 275). Thus it is the presence or

absence of this receptor that determines the effect of poliovirus on these cells. Similarly, the presence of the Duffy surface antigen (276, 277) is essential for the penetration of malarial merozoites into human red blood cells (278) and a protease-sensitive surface component of mouse macrophages mediates the binding and interiorization of *Trypanosoma cruzi* (279). In all of these cases, possession of surface receptors appears to place the host in a disadvantageous position for survival. The continued expression of cellular receptors for potentially lethal disease-causing organisms suggests that these receptors may mediate as yet unrecognized normal physiological functions and that viruses and protozoa have merely adapted these receptors for their own ends.

A variety of infectious agents enter their host cells within endocytic vacuoles [cf (278, 280, 281) for reviews], but have different ultimate intracellular destinations.

Agents That Enter the Cytoplasmic Matrix

The interaction of vaccinia virus with the membrane of the endocytic vacuole causes dissolution of both the outer lipoprotein coat of the virus and the vacuolar membrane, and results in the release of the DNA-containing viral core into the cytoplasmic matrix (78, 280, 282) where viral replication occurs. The surface properties of the virus are critical determinants of its capacity to enter the cytoplasmic matrix; treatment of vaccinia virus with heat or antibodies prevents its escape from the endocytic vacuole and results in its sequestration in lysosomes, where it is destroyed (282). Like vaccinia, the trypomastigotes of *T. cruzi* (279) must also escape from the phagocytic vacuole and enter the cytoplasmic matrix to initiate replication.

Agents That Replicate Within Lysosomes

Several obligate intracellular bacterial pathogens (e.g. *Brucella,* tubercle bacilli, *Listeria monocytogenes*) are sequestered within host-cell lysosomes where they replicate, eventually destroying the cell (283). Tubercle bacilli slow the fusion of bacillus-containing phagosomes with lysosomes (284). In contrast, antibody-coated tubercle bacilli are rapidly sequestered within lysosomes (285). Under both conditions, however, the tubercle bacilli grow equally well intracellularly.

The capacity of reovirus to survive the lysosomal environment is unique among animal viruses. Infecting reovirus particles are concentrated within host-cell lysosomes where they are uncoated and thereby converted to a biosynthetically active infectious form (178).

Agents That Replicate Within Phagosomes

Chlamydia and toxoplasma utilize the vacuolar apparatus in yet another way. Chlamydia (286) and toxoplasma (287) enter cells within endocytic

vacuoles, but prevent the fusion of these vacuoles with host-cell lysosomes. Presumably their capacity to inhibit lysosome-phagosome fusion requires continued production of metabolite(s) or biosynthetic product(s) by these organisms, since dead Chlamydia and toxoplasma are sequestered in lysosomes.

It is evident that each of the organisms described above has utilized one or more steps in the endocytic pathway in its replicative cycle. Hence these agents should provide excellent tools for the dissection of this pathway and for the identification of the effector molecules that control them.

ENDOCYTOSIS AND HOMEOSTASIS

Although the ingestion of infectious microorganisms is one of the most dramatic illustrations of the role of endocytosis in the physiology of multicellular organisms, it is almost certainly of secondary importance in quantitative terms. Quantitatively, senescent and dying cells, cell fragments, and a variety of small particles and soluble macromolecules probably represent the bulk of materials endocytized daily by the cells of multicellular organisms. This is most easily illustrated by the turnover of red blood cells in man.

A 60–70 kg adult has about 5×10^{13} red blood cells. Each day 1/120th of these or 3×10^{11} cells are removed from the circulation by mononuclear phagocytes. In the course of a year the mononuclear phagocytes ingest and digest over 2.7 kg of hemoglobin alone. Loss of sialic acid from the surface of red blood cells during aging may regulate their removal from the circulation (288). Winchester et al (289) have shown that desialated red blood cells express antigen(s) that react with antibodies present in all normal sera, and Kay (174) has found that phagocytosis of aged red blood cells by mononuclear phagocytes is regulated by an IgG species present in homologous normal serum. Thus, the turnover of red blood cells, like the clearance of infectious agents, may be regulated by Fc-receptor–mediated phagocytosis.

Phagocytic leucocytes also play essential roles in wound healing (290) and in the repair and/or involution of organs and tissues (158).

The importance of endocytic activity in facultative phagocytes is clearly illustrated by the role of pigment epithelial cells in the neural retina. These cells selectively ingest senescent fragments of rod outer segments (291–295). Failure of the pigment epithelial cells to remove these fragments results in retinitis pigmentosa, a disease that leads to blindness (295).

The regulation of cholesterol metabolism also may be under endocytic control. The work of Goldstein & Brown (reviewed in 66a) indicates that cholesterol biosynthesis is controlled by the uptake of cholesterol containing low-density lipoproteins. Endocytosis of low-density lipoproteins is regulated by a genetically defined receptor in the surface of fibroblasts. Absence

of this receptor, as occurs in familial hypercholesterolemia, leads to markedly reduced lipoprotein uptake, overproduction of cholesterol, premature atherosclerosis, and death.

Endocytosis also plays a central role in regulating the levels of at least one essential hormone, thyroxine. Thyroid-stimulating hormone stimulates the endocytosis of thyroglobin by thyroid epithelial cells. The endocytized thyroglobin is digested by the lysosomes of these cells and thyroxine is released as a result (296–298).

SPECULATIONS

Lewis (42), in his initial description of pinocytosis in 1931, predicted: "The importance of this phenomenon in cellular metabolism and in the economy of intercellular fluids seems almost self-evident." The importance of endocytosis in the physiology of cells and organisms is becoming more and more evident, and we suggest that the processes outlined below may also be governed by endocytosis.

Nutrition

The terms *phagocytosis* and *pinocytosis* imply a nutritional function, but this has not been fully explored. In vivo, the uptake of effete and damaged cells by professional phagocytes, asialoglycoproteins by hepatocytes, and proteins by renal proximal-tubular cells, results in the intracellular digestion of these materials and allows for reutilization of their components. But at the individual cell level, there is no evidence in vivo that uptake and digestion of macromolecules is an important source of metabolic substrates. Eagle & Piez (299) proved that labeled amino acids contained in exogenous proteins were not themselves reutilized for protein biosynthesis during HeLa-cell growth in vitro. Direct transport of substrates across the cell surface likely provides most metabolic substrates. However, it is probable that a variety of essential small molecules, especially those complexed to macromolecular carriers, may gain access to cells following an initial endocytic step, e.g. vitamins like B-12 (300), cholesterol (66a), and even metals.

Control of Metabolism

Biologically active molecules such as protein hormones are generally thought to act by binding to specific cell-surface receptors, thereby initiating chemical signals that alter cell functions. Many biologically active macromolecules—hormones (301), growth factors (302), antigens (303), neurotransmitters (258), and even toxins (304, 305)—are endocytosed following adsorption to the cell surface. The significance of endocytosis in the function

of these agents is not established. It may assist delivery of the ligand, or of its biologically active subunit, such as a toxin, to the cytoplasmic matrix, or in the initiation of some other signal such as cyclic-nucleotide formation. Endocytosis may also function to terminate signals generated by ligand binding at the cell surface, either by direct removal of ligand and/or receptor from the surface, such as occurs on antigen binding to lymphocytes (303), or by altering the formation of new receptors, such as occurs in the decrease of low-density lipoprotein receptors following lipoprotein uptake (66a), and as may occur in the "down regulation" of human growth hormone (306) and insulin receptors (307). Moreover, several situations are known where the levels of intracellular enzymes and proteins are altered following endocytic uptake of particles and macromolecules [e.g. heme oxygenase (314), apoferritin (315), and lysosomal hydrolases (215)].

The Cell Surface

It is likely that the influx of plasma membrane during endocytosis does more than just enclose soluble and particulate materials. Membrane interiorization may allow cells to remove altered portions of the cell surface, reorganize its composition and topography, and acquire information initiated by ligand binding.

Therapy

Endocytosis may provide a means for effecting a variety of therapeutic maneuvers, especially the selective delivery of drugs into cells (308). There are two approaches to selective delivery. One is to complex the therapeutic agent to some carrier so that it can only enter cells by bulk uptake. Only cells that are active in endocytosis will take up the complex, digest the carrier, and release the drug locally. De Duve et al (308) have termed such complexes "lysosomotropic" drugs. Another approach is to attach the drug to a specific ligand, which is then selectively recognized by a restricted class of cells. Binding then *may* be followed by uptake, digestion, and local release. Some of the vehicles (reviewed in 309a) that have been considered are listed below.

LIPOSOMES Liposomes are artificial lipid membranes that have an aqueous content and can be coated with specific ligands (309), e.g. Weissmann et al delivered liposomes containing HRP to leucocytes by coating them with immunoglobulins (310).

CONJUGATES OF INTERCALATING DRUGS WITH DNA The tumoricidal drug, duanorubicin, exhibits reduced cardiac toxicity following complexing with DNA. The complex, when endocytosed by tumor cells, is digested, liberating the drug locally (311).

POLYCATIONIZATION Basu et al polycationized low-density lipoproteins so that they were bound electrostatically to, and were interiorized by, mutant fibroblasts lacking the physiological lipoprotein receptor (312). This approach is unlikely to be useful therapeutically since polycations will bind nonspecifically to most serum proteins and cells.

ALTERATION OF OLIGOSACCHARIDES Rogers & Kornfeld (313) linked oligosaccharides containing terminal galactose residues to albumin. Albumin modified in this way was bound to the asialoglycoprotein receptors on the hepatocyte's membrane, and was endocytosed by these cells.

ENTRY OF MACROMOLECULES INTO THE CYTOPLASMIC MATRIX Infectious agents and toxins gain access to the cytoplasm, and in some instances this follows an initial endocytic step. Conceivably these same routes could be used for the delivery of nucleic acids and other macromolecules to the cytoplasm.

Whether or not these specific approaches prove of therapeutic value, it is clear that endocytic uptake holds great promise as a means of delivering biologically active macromolecules to the cytoplasmic matrix and from this site to the nucleus.

ACKNOWLEDGMENT

We are grateful to Drs. William Arend, Alex Novikoff and Thomas Stossel for helpful suggestions.

This work was supported by grants AI08697, AI13013, and AI07012 from the National Institute of Allergy and Infectious Diseases.

Literature Cited

1. Holter, H. 1959. *Int. Rev. Cytol.* 8:481–504
2. Chapman-Andresen, C. 1962–1963. *C. R. Trav. Lab. Carlsberg* 33:73–264
3. Gosselin, R. E. 1967. *Fed. Proc.* 26: 987–93
4. Ryser, H. J.-P. 1968. *Science* 159: 390–96
5. Jacques, P. J. 1969. In *Lysosomes in Biology & Pathology,* ed. J. J. Dingle, H. B. Fell, 2:395–420. Amsterdam: North-Holland
6. Roth, T. F., Porter, K. R. 1964. *J. Cell Biol.* 20:313–32
7. Fawcett, D. W. 1965. *J. Histochem. Cytochem.* 13:75–91
8. Anderson, R. G., Goldstein, J. L., Brown, M. S. 1976. *Proc. Natl. Acad. Sci. USA* 73:2434–38
9. Roth, T. F., Cutting, J. A., Atlas, S. B. 1976. *J. Supramol. Struct.* 4:527–48
10. Friend, D. S., Farquhar, M. G. 1967. *J. Cell Biol.* 35:357–76
11. Novikoff, A. B. 1976. *Proc. Natl. Acad. Sci. USA* 73:2781–87
12. Franke, W. W., Luder, M. R., Kartenbeck, J., Zerban, H., Keenan, T. W. 1976. *J. Cell Biol.* 69:173–95
13. Franke, W. W., Herth, W. 1974. *Exp. Cell Res.* 89:447–51
14. Franke, W. W., Kartenbeck, J., Spring, H. 1976. *J. Cell Biol.* 71:196–206
15. Rees, R. P., Bunge, M. B., Bunge, R. P. 1976. *J. Cell Biol.* 68:240–63
16. Rodewald, R. 1973. *J. Cell Biol.* 58:189–211
17. Kanaseki, T., Kadota, K. 1969. *J. Cell Biol.* 42:202–20

18. Pearse, B. M. F. 1975. *J. Mol. Biol.* 97:93–98
19. Pearse, B. M. F. 1976. *Proc. Natl. Acad. Sci. USA* 73:1255–59
20. Ehrenreich, B. A., Cohn, Z. A. 1967. *J. Exp. Med.* 126:941–58
21. Steinman, R. M., Cohn, Z. A. 1972. *J. Cell Biol.* 55:186–204
22. Williams, K. E., Kidston, E. M., Beck, F., Lloyd, J. B. 1975. *J. Cell Biol.* 64:123–34
23. Cohn, Z. A., Benson, B. 1965. *J. Exp. Med.* 122:445–66
24. Steinman, R. M., Silver, J. M., Cohn, Z. A. 1974. *J. Cell Biol.* 63:949–69
25. Neufeld, E. F., Lim, T. W., Shapiro, L. J. 1975. *Ann. Rev. Biochem.* 44:357–76
26. Bruns, R. R., Palade, G. E. 1968. *J. Cell Biol.* 37:277–99
27. Simionescu, N., Simionescu, M., Palade, G. E. 1975. *J. Cell Biol.* 64:586–607
28. Walker, W. A., Cornell, R., Davenport, L. M., Isselbacher, K. J. 1972. *J. Cell Biol.* 54:195–205
29. Orlic, D., Lev, R. 1973. *J. Cell Biol.* 56:106–19
30. Hemmings, W. A. 1976. *Maternofetal Transmission of Immunoglobulins.* Cambridge, Engl: Cambridge Univ. Press
31. Brambell, F. W. R. 1970. *The Transmission of Passive Immunity from Mother to Young.* New York: Elsevier
32. Tomasi, T. B. 1976. *The Immune System of Secretions.* Englewood Cliffs, NJ: Prentice Hall
33. Silverstein, S. C., Astell, C., Levin, D. H., Schonberg, M., Acs, G. 1972. *Virology* 47:797–806
34. Mantovani, B., Rabinovitch, M., Nussenzweig, V. 1972. *J. Exp. Med.* 135: 780–92
35. Michl, J., Ohlbaum, D. J., Silverstein, S. C. 1976. *J. Exp. Med.* 144:1465–83
36. Michell, R. H., Pancake, S. J., Noseworthy, J., Karnovsky, M. L. 1969. *J. Cell Biol.* 40:216–24
37. Weisman, R. A., Korn, E. D. 1967. *Biochemistry* 6:485–97
38. Githens, S., Karnovsky, M. L. 1973. *J. Cell Biol.* 58:536–48
39. Wetzel, M. G., Korn, E. D. 1969. *J. Cell Biol.* 53:90–104
40. Stossel, T. P., Mason, R. J., Hartwig, J., Vaughan, M. 1972. *J. Clin. Invest.* 51:615–24
40a. Cox, J. M., Stossel, T. P. 1976. In *In Vitro Methods in Cell Mediated and Tumor Immunity,* ed. B. R. Bloom, J. R. David, pp. 363–68. New York: Academic
41. Cohn, Z. A., Morse, S. I. 1959. *J. Exp. Med.* 110:419–43
41a. Rabinovitch, M., Manejias, R. E., Nussenzweig, V. 1975. *J. Exp. Med.* 142:827–38
42. Lewis, W. H. 1931. *Bull. Johns Hopkins Hosp.* 49:17–36
43. Cohn, Z. A. 1966. *J. Exp. Med.* 124:557–71
44. Palade, G. E. 1956. *J. Biophys. Biochem. Cytol. Suppl.* 2:85–98
45. Bennett, H. S. 1956. *J. Biophys. Biochem. Cytol. Suppl.* 2:99–103
46. Fedorko, M. E., Hirsch, J. G., Cohn, Z. A. 1968. *J. Cell Biol.* 38:392–402
47. Cohn, Z. A., Fedorko, M. E., Hirsch, J. G. 1966. *J. Exp. Med.* 123:757–66
48. Gordon, G. B., Miller, L. R., Bensch, K. G. 1965. *J. Cell Biol.* 25:Pt. 2, pp. 41–55
49. Odor, D. L. 1956. *J. Biophys. Biochem. Cytol. Suppl.* 2:105–8
50. Farquhar, M. G., Palade, G. E. 1960. *J. Biophys. Biochem. Cytol.* 7:297–304
51. Graham, R. C. Jr., Karnovsky, M. J. 1966. *J. Histochem. Cytochem.* 14:291–302
52. Straus, W. 1964. *J. Cell Biol.* 20:497–507
53. Silverstein, S. C., Marcus, P. I. 1964. *Virology* 23:370–80
54. Goldstein, J. L., Brown, M. S. 1974. *J. Biol. Chem.* 249:5153–62
55. Wagner, R., Rosenberg, M., Estensen, R. 1971. *J. Cell Biol.* 50:804–17
56. Bowers, B., Olszewski, T. E. 1972. *J. Cell Biol.* 53:681–94
57. Williams, K. E., Kidston, E. M., Beck, F., Lloyd, J. B. 1975. *J. Cell Biol.* 64:113–22
58. Steinman, R. M. 1976. In *In Vitro Methods in Cell Mediated and Tumor Immunity,* ed. B. R. Bloom, J. R. David, pp. 379–86. New York: Academic
59. Schumaker, V. N. 1958. *Exp. Cell Res.* 15:314–31
60. Bach, G., Friedman, R., Weissmann, B., Neufeld, E. F. 1972. *Proc. Natl. Acad. Sci. USA* 69:2048–51
61. Von Figura, K., Kresse, H. 1974. *J. Clin. Invest.* 53:85–90
62. Gosselin, R. E. 1956. *J. Gen. Physiol.* 39:625–49
63. Seljelid, R., Silverstein, S. C., Cohn, Z. A. 1973. *J. Cell Biol.* 57:484–98
64. Edelson, P. J., Cohn, Z. A. 1974. *J. Exp. Med.* 140:1364–86
65. Silverstein, S. C., Dales, S. 1968. *J. Cell Biol.* 36:197–230

66. Simpson, R. W., Hauser, R. E., Dales, S. 1969. *Virology* 37:285–90
66a. Goldstein, J. L., Brown, M. S. 1977. *Ann. Rev. Biochem.* 46:897–930
67. Goldstein, J. L., Basu, S. K., Brunschede, G. Y., Brown, M. S. 1976. *Cell* 7:85–96
68. Ashwell, G., Morell, A. G. 1974. *Adv. Enzymol.* 41:99–128
69. Rabinovitch, M. 1968. *Semin. Hematol.* 5:134–55
70. Hoff, S. F., Huang, Y. O., Wisnieski, B., Fox, C. F. 1976. *J. Cell Biol.* 70:A127
71. Rabinovitch, M. 1967. *Exp. Cell Res.* 46:19–28
72. Karnovsky, M. L. 1962. *Physiol. Rev.* 42:143–68
73. Karnovsky, M. L., Simmons, S., Glass, E. A., Shale, A. W., Hart, P. D. 1970. In *Mononuclear Phagocytes,* ed. R. VanFurth, pp. 103–20. Oxford, Engl: Blackwell
74. Karnovsky, M. L., Lazdins, J., Simmons, S. R. 1975. In *Mononuclear Phagocytes in Immunity, Infection and Pathology,* ed. R. VanFurth, pp. 423–39. Oxford, Engl: Blackwell
75. Sbarra, A. J., Shirley, W. 1963. *J. Bacteriol.* 86:259–65
76. Griffin, F. M. Jr., Griffin, J. A., Leider, J. E., Silverstein, S. C. 1975. *J. Exp. Med.* 142:1263–82
77. Roberts, J., Quastel, J. H. 1963. *Biochem. J.* 89:150–56
78. Dales, S., Kajioka, R. 1964. *Virology* 24:278–94
79. Sbarra, A. J., Karnovsky, M. L. 1959. *J. Biol. Chem.* 234:1355–62
80. Oren, R., Farnham, A. E., Saito, K., Milofsky, E., Karnovsky, M. L. 1963. *J. Cell Biol.* 17:487–501
81. Michl, J., Ohlbaum, D. J., Silverstein, S. C. 1976. *J. Exp. Med.* 144:1484–93
82. Cohn, Z. A., Benson, B. 1965. *J. Exp. Med.* 121:279–88
83. Stossel, T. P., Murad, F., Mason, R. J., Vaughan, M. 1970. *J. Biol. Chem.* 245:6228–34
84. Atkinson, D. E. 1970. In *The Enzymes,* ed. P. D. Boyer, Vol. 1, pp. 461–89. New York: Academic. 3rd ed.
85. Malawista, S. E., Bodel, P. T. 1967. *J. Clin. Invest.* 46:786–96
86. DeChatelet, L. R., Cooper, M. R., McCall, C. E. 1971. *Infect. Immun.* 3:66–72
87. Mudd, E. B. H., Mudd, S. 1933. *J. Gen. Physiol.* 16:625–36
88. van Oss, C. J., Gillman, C. F., Neumann, A. W. 1975. *Phagocytic Engulfment and Cell Adhesiveness as Cellular Surface Phenomena.* New York: Dekker
89. Elsbach, P., Farrow, S. 1969. *Biochim. Biophys. Acta* 176:438–41
90. Elsbach, P. 1974. In *The Inflammatory Process,* ed. B. W. Zweifach, L. Grant, R. T. McCluskey, pp. 363–408. New York: Academic
91. Jamieson, J. D., Palade, G. E. 1968. *J. Cell Biol.* 39:589–603
92. Stroobant, P., Kaback, H. R. 1975. *Proc. Natl. Acad. Sci. USA* 72:3970–74
93. Korn, E. D. 1975. In *Biochemistry of Cell Walls and Membranes,* ed. C. F. Fox, pp. 1–26. Baltimore: Univ. Park Press
94. Pesanti, E. L., Axline, S. G. 1975. *J. Exp. Med.* 142:903–13
95. Bhisey, A. N., Freed, J. J. 1971. *Exp. Cell Res.* 64:430–38
96. Pesanti, E. L., Axline, S. G. 1975. *J. Exp. Med.* 141:1030–46
97. Ukena, T. E., Berlin, R. D. 1972. *J. Exp. Med.* 136:1–7
98. Vassalli, J. D., Silverstein, S. C. 1977. *Exp. Cell Res.* In press
99. Pollard, T. D., Weihing, R. R. 1974. *Crit. Rev. Biochem.* 2:1–65
100. Symposia on Quantitative Biology, Vol. XL. 1975. Cold Spring Harbor, Cold Spring Harbor Lab.
101. Altman, P. L., Katz, D. D., eds. In *Cell Biology I,* pp. 341–56. Bethesda, Md: Fed. Am. Soc. Exp. Biol.
102. Stossel, T. P., Pollard, T. D. 1973. *J. Biol. Chem.* 248:8288–94
103. Hartwig, J. H., Stossel, T. P. 1975. *J. Biol. Chem.* 250:5696–5705
104. Reaven, E. P., Axline, S. G. 1973. *J. Cell Biol.* 59:12–27
105. Allison, A. C., Davies, P., De Petris, S. 1971. *Nature New Biol.* 232:153–55
106. Spudich, J. A., Lin, S. 1972. *Proc. Natl. Acad. Sci. USA* 69:442–46
107. Hartwig, J. H., Stossel, T. P. 1976. *J. Cell Biol.* 71:295–303
108. Weihing, R. R. 1976. *J. Cell Biol.* 71:303–7
109. Allison, A. C., Davies, P. 1974. *Symp. Soc. Exp. Biol.* 28:419–46
110. Malawista, S. E., Gee, J. B. L., Bensch, K. G. 1971. *Yale J. Biol. Med.* 44:286–300
111. Axline, S. G., Reaven, E. P. 1974. *J. Cell Biol.* 62:647–59
112. Boxer, L. A., Hedley-Whyte, E. T., Stossel, T. P. 1974. *New Eng. J. Med.* 291:1093–99
113. Stossel, T. P., Hartwig, J. H. 1975. *J. Biol. Chem.* 250:5706–12

114. Stossel, T. P., Hartwig, J. H. 1976. *J. Cell Biol.* 68:602–19
115. Wang, K., Ash, J. F., Singer, S. J. 1975. *Proc. Natl. Acad. Sci. USA* 72:4483–86
116. Boxer, L. A., Stossel, T. P. 1976. *J. Clin. Invest.* 57:964–76
117. Lehninger, A. L. 1971. In *Biochemistry.* New York: Worth
118. Tilney, L. G., Hatano, S., Ishikawa, H., Mooseker, M. S. 1973. *J. Cell Biol.* 59:109–76
119. Tilney, L. G. 1975. *J. Cell Biol.* 64:289–310
120. Storti, R. V., Rich, A. 1976. *Proc. Natl. Acad. Sci. USA* 73:2346–50
121. Pollack, R., Rifkin, D. 1975. *Cell* 6:495–506
122. Lazarides, E. 1975. *J. Cell Biol.* 65: 549–61
123. Stossel, T. P. 1973. *J. Cell Biol.* 58: 346–56
124. Wills, E. J., Davies, P., Allison, A. C., Haswell, A. D. 1972. *Nature New Biol.* 240:58–60
125. Stossel, T. P., Pollard, T. D., Mason, R. J., Vaughan, M. 1971. *J. Clin. Invest.* 50:1745–57
126. Stossel, T. P., Mason, R. J., Pollard, T. D., Vaughan, M. 1972. *J. Clin. Invest.* 51:604–14
127. Rabinovitch, M. 1969. *Exp. Cell Res.* 56:326–32
128. North, R. J. 1966. *J. Ultrastructure Res.* 16:96–108
129. Griffin, F. M. Jr., Silverstein, S. C. 1974. *J. Exp. Med.* 139:323–36
130. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., Wood, W. B., McCarty, M. 1973. In *Microbiology,* pp. 633–34. New York: Harper & Row
131. Griffin, F. M. Jr. 1977. In *Biological Amplification Systems in Immunity,* ed. N. K. Day, R. A. Good. New York: Plenum. In press
132. Uhr, J. W. 1965. *Proc. Natl. Acad. Sci. USA* 54:1599–1606
133. Inchley, C., Grey, H., Uhr, J. W. 1970. *J. Immunol.* 105:362–69
134. Berken, A., Benacerraf, B. 1966. *J. Exp. Med.* 123:119–44
135. Okafor, G. O., Turner, M. W., Hay, F. C. 1974. *Nature* 248:228–30
136. Yasmeen, D., Elerson, J. R., Dorrington, K. J., Painter, R. H. 1973. *J. Immunol.* 110:1706–9
137. Ciccimarra, F., Rosen, F. S., Merler, E. 1975. *Proc. Natl. Acad. Sci. USA* 72:2081–83
138. Edelman, G. M., Cunningham, B. A., Gall, W. E., Gottlieb, P. D., Rutishauser, U., Waxdal, M. J. 1969. *Proc. Natl. Acad. Sci. USA* 63:78–85
138a. Alexander, M. D., Leslie, R. G. Q., Cohen, S. 1976. *Eur. J. Immunol.* 6:101–7
138b. Ovary, Z., Saluk, P. H., Quijada, L., Lamm, M. E. 1976. *J. Immunol.* 116:1265–71
139. Arend, W. P., Mannik, M. 1972. *J. Exp. Med.* 136:514–31
140. Arend, W. P., Mannik, M. 1975. See Ref. 74, pp. 303–14
141. Rhodes, J. 1973. *Nature* 243:527–28
142. Müller-Eberhard, H. J. 1975. *Ann. Rev. Biochem.* 44:697–724
143. Stossel, T. P., Field, R. J., Gitlin, J. D., Alper, C. A., Rosen, F. S. 1975. *J. Exp. Med.* 141:1329–47
144. Griffin, F. M. Jr., Bianco, C., Silverstein, S. C. 1975. *J. Exp. Med.* 141:1269–77
145. Bokisch, V. A., Dierich, M. P., Müller-Eberhard, H. J. 1975. *Proc. Natl. Acad. Sci. USA* 12:1989–93
146. Wilkinson, P. C. 1974. In *Chemotaxis and Inflammation,* pp. 78–98. Edinburgh: Churchill Livingston
146a. Unkeless, J. C. 1977. *J. Exp. Med.* In press
147. Askenase, P. W., Hayden, B. J. 1974. *Immunology* 27:563–76
148. Lay, W. H., Nussenzweig, V. 1968. *J. Exp. Med.* 128:991–1009
149. Messner, R. P., Jelinek, J. 1970. *J. Clin. Invest.* 49:2165–71
150. Abramson, N., Gelfand, E. W., Jandl, J. H., Rosen, F. S. 1970. *J. Exp. Med.* 132:1207–15
151. Edelman, G. M., Gall, W. E. 1969. *Ann. Rev. Biochem.* 38:415–66
152. Unkeless, J. C., Eisen, H. N. 1975. *J. Exp. Med.* 142:1520–33
153. Parish, W. E. 1965. *Nature* 208:594–95
154. Boyden, S. V., Sorkin, E. 1960. *Immunology* 3:272–83
155. Boyden, S. V., Sorkin, E. 1961. *Immunology* 4:244–52
156. Phillips-Quagliata, J. M., Levine, B. B., Quagliata, F., Uhr, J. W. 1971. *J. Exp. Med.* 133:589–601
157. Arend, W. P., Mannik, M. 1973. *J. Immunol.* 110:1455–63
158. Holtzman, E. 1976. *Lysosomes: A Survey.* New York: Springer
159. Mannik, M., Arend, W. P., Hall, A. P., Gilliland, B. C. 1971. *J. Exp. Med.* 133:713–39
160. Arend, W. P., Mannik, M. 1971. *J. Immunol.* 107:63–75
161. Steinman, R. M., Cohn, Z. A. 1972. *J. Cell Biol.* 55:616–34

162. Bianco, C., Griffin, F. M. Jr., Silverstein, S. C. 1975. *J. Exp. Med.* 141:1278–90
163. Bianco, C. 1977. See Ref. 131. In press
164. Reynolds, H. Y., Atkinson, J. P., Newball, H. H., Frank, M. M. 1975. *J. Immunol.* 114:1813–19
165. Wellek, B., Hahn, H. H., Opferkuch, W. 1975. *J. Immunol.* 114:1643–45
166. Wellek, B., Hahn, H. H., Opferkuch, W. 1975. *Eur. J. Immunol.* 5:378–81
167. Ehlenberger, A. G., Nussenzweig, V. 1977. *J. Exp. Med.* 145:357–71
168. Huber, H., Polley, M. J., Linscott, W. D., Fudenberg, H. H., Müller-Eberhard, H. J. 1968. *Science* 162:1281–83
169. Scribner, D. J., Fahrney, D. 1976. *J. Immunol.* 116:892–97
170. Mantovani, B. 1975. *J. Immunol.* 115: 15–17
171. Yusko, S. C., Roth, T. F. 1976. *J. Supramol. Struct.* 4:89–97
172. Allison, A. C., Harington, J. S., Birbeck, M. 1966. *J. Exp. Med.* 124: 141–54
173. Wigler, M., Weinstein, I. B. 1976. *Nature* 259:232–33
174. Kay, M. M. B. 1975. *Proc. Natl. Acad. Sci. USA* 72:3521–25
175. Rabinovitch, M. 1968. *Proc. Soc. Exp. Biol. Med.* 127:351–55
176. Kerbel, R. S. 1976. *Nature* 263:196
177. Boxer, L. A., Davis, J. Bachner, R. L. 1977. *J. Cell. Physiol.* In press
178. Silverstein, S. C., Christman, J. K., Acs, G. 1976. *Ann. Rev. Biochem.* 45:375–408
179. Michl, J., Silverstein, S. C. 1976. *J. Cell Biol.* 70:286a
180. Jones, T. C., Hirsch, J. G. 1971. *J. Exp. Med.* 133:231–59
181. Griffin, F. M. Jr., Griffin, J. A., Silverstein, S. C. 1976. *J. Exp. Med.* 144:788–809
182. Taylor, R. B., Duffus, W. P. H., Raff, M. C., De Petris, S. 1971. *Nature New Biol.* 233:225–29
183. Polliack, A., Gordon, S. 1975. *Lab. Invest.* 33:469–77
184. Kaplan, G., Gaudernack, G., Seljelid, R. 1975. *Exp. Cell Res.* 95:365–75
185. Orenstein, J. M., Shelton, E. 1977. *Lab. Invest.* In press
186. Palade, G. E., Bruns, R. 1968. *J. Cell Biol.* 37:633–49
187. Okada, Y. 1969. *Curr. Top. Microbiol.* 48:102–28
188. Parkes, J. G., Fox, C. F. 1975. *Biochemistry* 14:3725–29
189. Poole, A., Howell, J., Lucy, J. 1970. *Nature* 227:810–13
190. Oates, P. J., Touster, O. 1976. *J. Cell Biol.* 68:319–38
191. Scheid, A., Choppin, P. W. 1974. *Virology* 57:475–90
192. Poste, G., Papahadjopoulos, D. 1976. *Proc. Natl. Acad. Sci. USA* 73:1603–7
193. Papahadjopoulos, D., Poste, G., Schaeffer, B. E. 1973. *Biochim. Biophys. Acta* 323:23–42
194. Martin, F. J., MacDonald, R. C. 1976. *J. Cell Biol.* 70:506–14
195. Pagano, R. E., Huang, L. 1975. *J. Cell Biol.* 67:49–60
196. Graham, R. C. Jr., Karnovsky, M. J., Shafer, A. W., Glass, E. A., Karnovsky, M. L. 1967. *J. Cell Biol.* 32:629–47
197. Johnston, R. B., Lehmeyer, J. E., Guthrie, L. A. 1976. *J. Exp. Med.* 143:1551–56
198. Johnston, R. B., Lehmeyer, J. E. 1977. In *Superoxide and Superoxide Dismutase,* ed. A. M. Michelson, J. M. McCord, I. Fridovich. London: Academic. In press
199. Briggs, R. T., Drath, D. B., Karnovsky, M. L., Karnovsky, M. J. 1975. *J. Cell Biol.* 67:566–86
200. Rossi, F., Zabucchi, E., Romeo, G. 1975. See Ref. 74, pp. 441–62
201. Klebanoff, S. J. 1975. *Semin. Hematol.* 12:117–42
202. Root, R. K. 1975. In *The Phagocytic Cell in Host Resistance,* ed. J. A. Bellanti, D. H. Dayton, pp. 201–26. New York: Raven
203. Baehner, R. L. 1975. See Ref. 202, pp. 173–200
204. Elsbach, P. 1968. *J. Clin. Invest.* 47:2217–29
205. Elsbach, P., Patriarca, P., Pettis, P., Stossel, T. P., Mason, R. J., Vaughan, M. 1972. *J. Clin. Invest.* 51:1910–14
206. Sastry, P. S., Hokin, L. E. 1966. *J. Biol. Chem.* 241:3354–61
207. Hubbard, A. L., Cohn, Z. A. 1975. *J. Cell Biol.* 64:461–79
208. Werb, Z., Cohn, Z. A. 1972. *J. Biol. Chem.* 247:2439–46
209. Ignarro, L. J., Lint, T. F., George, W. J. 1974. *J. Exp. Med.* 139:1395–1414
210. Ignarro, L. J., George, W. J. 1974. *J. Exp. Med.* 140:225–38
211. Zurier, R. B., Weissmann, G., Hoffstein, S., Kammerman, S., Tai, H.-H. 1974. *J. Clin. Invest.* 53:297–309
212. Bainton, D. F. 1973. *J. Cell Biol.* 58:249–64
213. Werb, Z., Dingle, J. T. 1976. In *Lysosomes in Biology and Pathology,* ed. J. T. Dingle, R. T. Dean. 5:127–56. New York: Elsevier

214. Gordon, S., Unkeless, J. C., Cohn, Z. A. 1974. *J. Exp. Med.* 140:995–1010
215. Axline, S. G., Cohn, Z. A. 1970. *J. Exp. Med.* 131:1239–60
216. Manganiello, V., Evans, W. H., Stossel, T. P., Mason, R. J., Vaughan, M. 1971. *J. Clin. Invest.* 50:2741–44
217. Seyberth, H. W., Schmidt-Gayk, H., Jakobs, K. H., Hackenthal, E. 1973. *J. Cell Biol.* 57:567–71
218. Muschel, R. J., Rosen, N., Bloom, B. R. 1976. *J. Exp. Med.* 145:175–86
219. Brandt, P. W. 1958. *Exp. Cell Res.* 15:300–13
220. Marshall, J. M., Nachmias, V. T. 1965. *J. Histochem. Cytochem.* 13:92–104
220a. Schekman, R., Singer, S. J. 1976. *Proc. Natl. Acad. Sci. USA* 73:4075–79
221. Unanue, E. R., Perkins, W. D., Karnovsky, M. J. 1972. *J. Exp. Med.* 136:885–906
222. Gonatas, N. K., Gonatas, J. O., Stieber, A., Antoine, J. C., Avrameas, S. 1976. *J. Cell Biol.* 70:477–93
223. Cohn, Z. A., Parks, E. 1967. *J. Exp. Med.* 125:1091–1104
224. Masur, S. K., Holtzman, E., Schwartz, I. L., Walter, R. 1971. *J. Cell Biol.* 49:582–94
225. Teichberg, S., Holtzman, E., Crain, S. M., Peterson, E. R. 1975. *J. Cell Biol.* 67:215–30
226. Ceccarelli, B., Hurlbut, W. P., Mauro, A. 1973. *J. Cell Biol.* 57:499–524
227. Heuser, J. E., Reese, T. S. 1973. *J. Cell Biol.* 57:315–44
228. Schacher, S., Holtzman, E., Hood, D. C. 1976. *J. Cell Biol.* 70:179–92
229. Holtzman, E., Teichberg, S., Abrahams, S. J., Citkowitz, E., Crain, S. M., Kawai, N., Peterson, E. R. 1973. *J. Histochem. Cytochem.* 4:349–85
230. Geuze, J. J., Kramer, M. F. 1974. *Cell Tissue Res.* 156:1–20
231. Orci, L., Malaisse-Lagae, F., Ravazzola, M., Amherdt, M., Renold, A. E. 1973. *Science* 181:561–62
232. Amsterdam, A., Ohad, I., Schramm, M. 1969. *J. Cell Biol.* 41:753–73
233. Farquhar, M. G., Skutelsky, E. H., Hopkins, C. R. 1975. In *The Anterior Pituitary,* ed. A. Tixier-Vidal, M. G. Farquhar, pp. 83–135. New York: Academic
234. Pelletier, G. 1973. *J. Ultrastruct. Res.* 43:445–59
235. Nagasawa, J., Douglas, W. W., Schulz, R. A. 1971. *Nature* 232:341–42
236. Baker, P. F., Ravazzola, M., Malaisse-Lagae, F., Orci, L. 1974. *Nature* 250: 155–57
237. Theodosius, D. T., Dreifuss, J. J., Harris, M. C., Orci, L. 1976. *J. Cell Biol.* 70:294–303
238. Palade, G. E. 1959. In *Subcellular Particles,* ed. T. Hayashi, pp. 64–80. New York: Ronald
239. De Camilli, P., Peluchetti, D., Meldolesi, J. 1976. *J. Cell Biol.* 70:59–74
240. Hausmann, K., Allen, R. D. 1976. *J. Cell Biol.* 69:313–26
241. Edelson, P. J., Zwiebel, R., Cohn, Z. A. 1975. *J. Exp. Med.* 142:1150–64
242. Kaplan, J. 1976. *Nature* 263:596–97
243. Quintart, J., Bartholeyns, J., Baudhuin, P. 1976. *J. Cell Biol.* 70:134a
244. Weibel, E. R., Bolender, R. P. 1973. In *Principles and Techniques of Electron Microscopy,* ed. M. A. Hayat, 3:237–96. New York: Van Nostrand-Reinhold
245. Elias, H., Hennig, A., Schwartz, D. E. 1971. *Physiol. Rev.* 51:158–200
246. Steinman, R. M., Brodie, S. E., Cohn, Z. A. 1972. *J. Cell Biol.* 68:665–87
247. Nadler, V., Goldfischer, S. 1970. *J. Histochem. Cytochem.* 18:368–71
248. Gonatas, N. K., Steiber, A., Kim, S. U., Graham, D. I., Avrameas, S. 1975. *Exp. Cell Res.* 94:426–31
249. Jamieson, J. D. 1972. *Curr. Top. Membr. Transp.* 3:273–338
250. Bowers, B. 1976. *J. Cell Biol.* 70:109a
251. Allen, R. D. 1976. *J. Cell Biol.* 70:386a
252. Allen, R. D. 1974. *J. Cell Biol.* 63: 904–22
253. Tsan, M. F., Berlin, R. D. 1971. *J. Exp. Med.* 134:1016–35
254. Widnell, C. C. 1972. *J. Cell Biol.* 52: 542–58
255. Farquhar, M. G., Bergeron, J. J. M., Palade, G. E. 1974. *J. Cell Biol.* 60:8–25
256. Edelson, P. J., Cohn, Z. A. 1976. *J. Exp. Med.* 144:1596–608
257. Bergeron, J. J. M., Evans, W. H., Geschwind, I. I. 1973. *J. Cell Biol.* 59:771–76
258. Devreotes, P. N., Fambrough, D. M. 1975. *Cold Spring Harbor Symp. Quant. Biol.* 40:237–52
259. Schneider, Y. G., Tulkens, P., Trouet, A. 1976. *J. Cell Biol.* 70:323a
260. Steinman, R. M., Cohn, Z. A. 1976. In *Biogenesis and Turnover of Membrane Macromolecules,* ed. J. S. Cook, pp. 1–14. New York: Raven
261. Hubbard, A. L., Cohn, Z. A. 1975. *J. Cell Biol.* 64:438–60
262. Ulsamer, A. G., Wright, P. L., Wetzel, M. G., Korn, E. D. 1971. *J. Cell Biol.* 51:193–215
263. Edelson, P. J., Cohn, Z. A. 1976. *J. Exp. Med.* 144:1581–95

264. Tweto, J., Doyle, D. 1976. *J. Biol. Chem.* 254:872–82
265. Roberts, R. M., Yuan, B. O.-C. 1975. *Biochem. J.* 13:4846–55
266. Roberts, R. M., Yuan, B. O.-C. 1975. *Arch. Biochem. Biophys.* 171:234–44
267. Warren, L., Glick, M. C. 1968. *J. Cell Biol.* 37:729–46
268. Dehlinger, P. J., Schimke, R. T. 1971. *J. Biol. Chem.* 246:2574–83
269. Alpers, D. H. 1972. *J. Clin. Invest.* 51:2621–30
270. Kaplan, J., Moskowitz, M. 1975. *Biochim. Biophys. Acta* 389:306–13
271. Simon, F. R., Blumenfeld, O. O., Arias, I. M. 1970. *Biochim. Biophys. Acta* 219: 349–60
272. Kaplan, J. 1977. Submitted for publication
273. Miller, D. A., Miller, O. J., Dev, V. G., Hashmi, S., Tantravahi, R., Medrano, L., Green, H. 1974. *Cell* 1:167–73
274. Holland, J. J., McLaren, L. C., Syverton, J. T. 1959. *J. Exp. Med.* 110:65–80
275. Holland, J. J. 1964. *Bacteriol. Rev.* 28:3–13
276. Miller, L. H., Mason, S. J., Dvorak, J. A., McGinniss, M. H., Rothman, I. K. 1975. *Science* 189:561–63
277. Miller, L. H., Mason, S. J., Clyde, D. F., McGinniss, M. H. 1976. *N. Engl. J. Med.* 295:302–4
278. Ladda, R., Aikawa, M., Sprinz, H. 1969. *J. Parasitol.* 55:633–44
279. Nogueira, N., Cohn, Z. A. 1976. *J. Exp. Med.* 143:1402–20
280. Dales, S. 1973. *Bacteriol. Rev.* 37: 103–35
281. Hirsch, J. G., Jones, J. E., Len, L. 1974. *Ciba Found. Symp. (NS)* 25:205–23
282. Silverstein, S. C. 1975. See Ref. 74, pp. 557–73
283. Mackaness, G. B., Blanden, R. V. 1967. *Prog. Allergy* 11:89–140
284. Armstrong, J. A., Hart, P. D. 1971. *J. Exp. Med.* 134:713–40
285. Armstrong, J. A., Hart, P. D. 1975. *J. Exp. Med.* 142:1–16
286. Friis, R. R. 1972. *J. Bacteriol.* 110: 706–21
287. Jones, T. C., Hirsch, J. G. 1972. *J. Exp. Med.* 136:1173–94
288. Durocher, J. R., Payne, R. C., Conrad, M. E. 1975. *Blood* 45:11–20
289. Winchester, R. J., Fu, S. M., Winfield, J. B., Kunkel, H. G. 1975. *J. Immunol.* 114:410–14
290. Leibovich, S. J., Ross, R. 1975. See Ref. 74, pp. 347–61
291. Bok, D., Hall, M. O. 1971. *J. Cell Biol.* 49:664–82
292. Hollyfield, J. G., Ward, A. 1974. *J. Ultrastruct. Res.* 46:327–38
293. Hollyfield, J. G., Ward, A. 1974. *J. Ultrastruct. Res.* 46:339–50
294. Hollyfield, J. G., Ward, A. 1974. *Invest. Ophthalmol.* 13:1016–23
295. Mullen, R. J., LaVail, M. W. 1976. *Science* 192:799–801
296. Wetzel, B. K., Spicer, S. S., Wollman, S. H. 1965. *J. Cell Biol.* 25:593–618
297. Seljelid, R., Nakken, K. F. 1968. *Scand. J. Clin. Lab. Invest. Suppl. 22* 106:125
298. Kowalski, K., Babiarz, D., Burke, G. 1972. *J. Lab. Clin. Med.* 79:258–66
299. Eagle, H., Piez, K. A. 1960. *J. Biol. Chem.* 235:1095–97
300. Wilson, T. H. 1964. *Medicine Baltimore* 43:669–77
301. Abel, J. H., McClellan, M. C., Chen, T., Sawyer, H. R., Niswender, G. D. 1976. *J. Cell Biol.* 70:367a
302. Carpenter, G., Cohen, S. 1976. *J. Cell Biol.* 71:159–71
303. Nossal, G. J. V., Layton, J. E. 1976. *J. Exp. Med.* 143:511–28
304. Nicolson, G. L. 1974. *Int. Rev. Cytol.* 39:89–190
305. Refsnes, K., Olsnes, S., Pihl, A. 1974. *J. Biol. Chem.* 249:3557–62
306. Lesniak, M. A., Roth, J. 1976. *J. Biol. Chem.* 251:3720–29
307. Gavin, J. R. III, Roth, J., Neville, D. M. Jr., De Meyts, P., Buell, D. N. 1974. *Proc. Natl. Acad. Sci. USA* 71:84–88
308. De Duve, C., De Barsy, T., Poole, B., Trouet, A., Tulkens, P., Van Hoof, F. 1974. *Biochem. Pharmacol.* 23:2495–2531
309. Gregoriadis, G. 1976. *N. Engl. J. Med.* 295:704–10, 765–69
309a. Gregoriadis, G. 1977. *Nature* 265: 407–11
310. Weissmann, G., Bloomgarden, D., Kaplan, R., Cohen, C., Hoffstein, S., Collins, T., Gotlieb, A., Nagle, D. 1975. *Proc. Natl. Acad. Sci. USA* 72:88–92
311. Trouet, A., Deprez-de Campeneere, D., De Duve, C. 1972. *Nature New Biol.* 239:110–12
312. Basu, S. K., Goldstein, J. L., Anderson, R. G. W., Brown, M. S. 1976. *Proc. Natl. Acad. Sci. USA* 73:3178–82
313. Rogers, J. C., Kornfeld, S. 1971. *Biochem. Biophys. Res. Commun.* 45: 622–29
314. Gemsa, D., Woo, C. H., Fudenberg, H. H., Schmid, R. 1973. *J. Clin. Invest.* 52: 812–22
315. Cross, N. L. 1974. *The fate of erythrocyte iron following phagocytosis by macrophages.* PhD thesis. Rockefeller Univ., New York, NY

CONTENTS