Journal of Supramolecular Structure 2:517–528 (1974)

ENDOCYTOSIS AND EXOCYTOSIS: ROLE OF MICROFILAMENTS AND INVOLVEMENT OF PHOSPHOLIPIDS IN MEMBRANE FUSION

Edward D. Korn, Blair Bowers, Shmuel Batzri, Susan R. Simmons, and Edward J. Victoria

National Heart and Lung Institute, Laboratory of Biochemistry, Section on Cellular Biochemistry and Ultrastructure, Bethesda, Maryland 20014

A brief description of endocytosis and exocytosis is followed by a discussion of the experimental approaches to the study of the initial events of endocytosis, the possible involvement of microfilaments, and in particular the possible role of membrane lipids in the events of membrane fusion. Recently developed model systems are also discussed.

INTRODUCTION

Endocytosis includes all uptake processes in which extracellular material is internalized within a vesicle whose membrane originated in the plasma membrane of the cell; that is, pinocytosis of solutes and phagocytosis of particulate material. Exocytosis is the secretory process by which the contents of cytoplasmic vesicles are excreted as the vesicle membrane joins the plasma membrane. At least superficially exocytosis is the morphological reciprocal of endocytosis. We do not mean to imply, however, that the two processes are necessarily indistinguishable ultrastructurally or biochemically or, as one of us has discussed at greater length elsewhere (1) (other recent reviews of membrane fusion (2) and endocytosis (3) are available), even that pinocytosis and phagocytosis are necessarily identical processes differing only in the size of the endocytic vesicle. One should also keep in mind the possibility that significant differences may exist among

*This paper served as the introduction to the session on Endocytosis, Exocytosis, and Fusion. In addition to presenting data from our own laboratory an effort was made to establish briefly the general framework within which such research is conducted and to speculate on the directions in which future research might turn.

endocytic (and exocytic) processes as they occur in different cells. Endocytosis and exocytosis are examples of processes that probably evolved in unicellular organisms and then were adapted to quite different purposes in metazoa.

Some cells – small amoebas such as Acanthamoeba castellanii, for example – are continuously pinocytic under conditions of growth on soluble media and are phagocytically competent whenever contact is made with appropriate particulate material. In such cells pinocytosis is probably the sole nutritive mechanism. In large amoebas, phagocytosis is the only feeding process known to be sufficient to maintain life, and pinocytosis may be largely an experimentally induced, perhaps abnormal, situation. In mammalian macrophages and polymorphonuclear leukocytes phagocytosis serves a protective function for the organism but is probably of no specific advantage to the endocytic cell. Similarly, endocytosis by thymocytes is apparently of great importance in the mammalian immune response but is not known to be specifically beneficial to the thymocyte. One significant consequence of the different physiological roles of endocytosis in different cells is that only in the protozoa, where endocytosis is the sole nutritional process, is there equal exocytosis, which serves, in addition to its excretory function, to maintain the cell surface which would otherwise be rapidly depleted by endocytosis. In mammalian cells the endocytic vesicles ultimately accumulate as intracellular residual bodies, and if the cell surface is to be replaced, it is more likely to be by synthesis de novo. Exocytosis does, of course, occur in many metazoan cells, serving essential purposes other than excretion (for example, secretion of enzymes, plasma proteins, hormones, and neurotransmitters), and in highly specialized exocytic cells an endocytic mechanism may be necessary to maintain an adequate intracellular membrane pool.

Endocytosis and exocytosis are complex, integrated biochemical and morphological processes that can be divided arbitrarily into several stages for the purposes of discussion and experimentation. Initiation of the process, in many cases, is triggered by unknown cellular events. [A basic tetrapeptide moiety of γ-globulin will increase the rate of phagocytosis in polymorphonuclear leukocytes (4). There is disputed evidence for more rapid pinocytosis of charged molecules (1), and there certainly are specific receptor sites on endocytic mammalian cells for lectins (5), antibodies (6), and asialoglycoproteins (7).]

The mechanisms of vesicle formation in endocytosis and of movement of vesicles to and from the plasma membrane in endocytosis and exocytosis are not clear but may well involve cytoplasmic microtubules and/or microfilaments. Certainly close association of at least microfilaments with the plasma membrane has been observed (8) (for a review of cytoplasmic actin and myosin see ref. 9). Experiments with inhibitors of microtubules and microfilaments (colchicine and cytochalasin B, respectively) bear on this problem (1, 2) although their effects in some instances may finally turn out to be due to interaction with membrane, rather than with cytoplasmic, proteins.

For phagocytosis in Acanthamoeba the morphological evidence is clear. Fibrillar actin is locally concentrated where the phagocytic vesicle is formed (Fig. 1), but actin filaments are apparently not associated with the internalized vesicles (Fig. 2). This observation is supported by the absence of a detectable actin band on SDS-polyacrylamide electrophoretic gels of isolated phagosomes (9a). Actin is a major component of isolated Acanthamoeba plasma membranes (8, 10). Lipophosphonoglycan, a major component of the amoeba plasma membrane (10–13), is present in the isolated phagosomes (9). It is

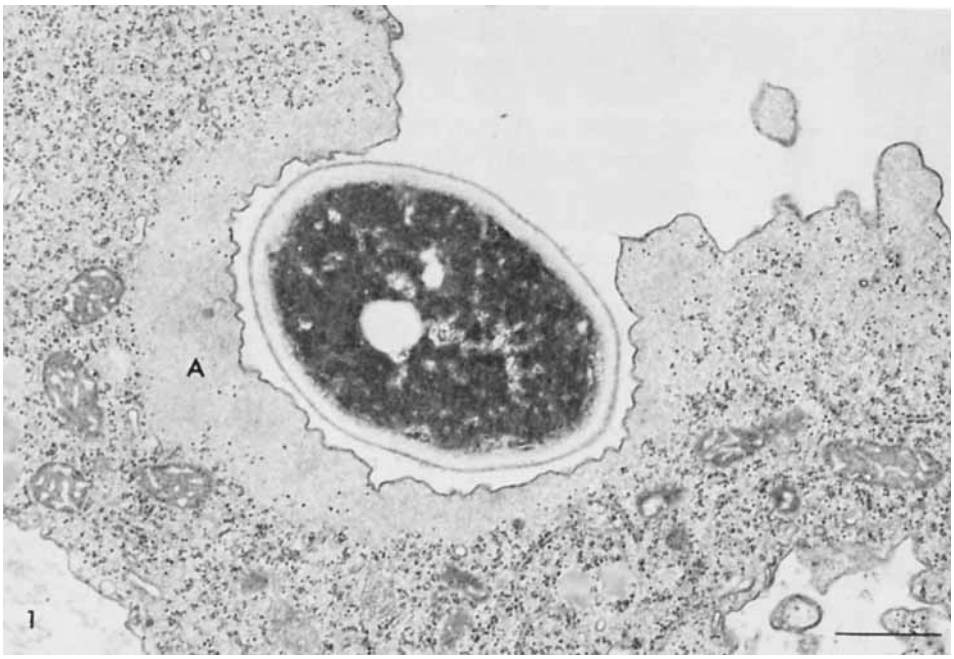

Fig. 1. Early stage in the ingestion of a yeast cell by Acanthamoeba. The cell cortex at the site of ingestion shows a thick layer of actin filaments (A). Bar is 1 μm.

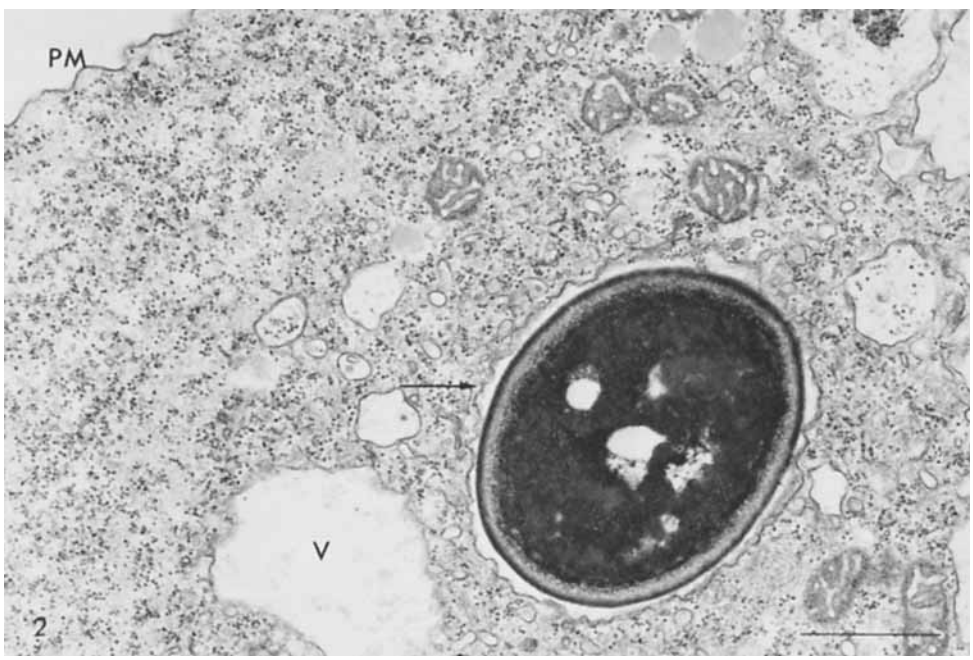

Fig. 2. After ingestion of a yeast cell the phagosome membrane (arrow) no longer shows an association of actin. PM, plasma membrane; V, vacuole. Bar is 1 μm.

possible that depolymerization of actin (the F to G transition) is involved in release of the actin filaments from the phagosome membrane. On this evidence, filaments would be involved in the formation of the phagocytic vesicle but perhaps not in the movements of cytoplasmic phagosomes. Similar morphological evidence for the possible involvement of actin filaments in the formation of pinocytic vesicles is much more difficult to obtain because of the small size of the pinosome.

In any case, membranes eventually get very close to one another and fuse, either to form two membranes where one existed (endocytosis) or to join two membranes into one (exocytosis). Many questions arise concerning the role and disposition of membrane proteins during these events; some of these problems are discussed in papers by Berlin (14) and Satir (15). But what of the role of membrane lipids?

MEMBRANE LIPIDS AND MEMBRANE FUSION

At least two hypotheses have been offered. The first states that membrane fusion is essentially a passive event with respect to the lipid bilayer – that is, whenever membrane lipid bilayers are brought into close proximity they will fuse. Specificity in the fusion process then is reserved entirely for those processes which bring membranes together. Because of the stress invoked by the very small radius of curvature created during endocytosis [and possibly by micropseudopods that may form on vesicles undergoing exocytosis (16, 17)] bilayers will fuse, according to this hypothesis, and realign to form membranes with greater radii of curvature. It has also been speculated that the very small surface area at the tip of a micropsuedopod may reduce the replusive forces at the sites of fusion between two similar membrane surfaces. It has been further suggested that changes in charge may arise in fusing membranes, facilitating their interaction. This reduction in charge might be indirectly accomplished by bridging negatively charged groups – for example, with Ca^{++} (2). None of these hypotheses has yet generated convincing experimental support.

One major proposal (18) requires a more active role for the membrane lipids in the fusion process. The suggestion is made that the local conversion of phospholipids to lysophospholipids at fusion sites would lead to bilayer instability and micellization with consequent mixing of membrane components Subsequent reacylation of the lysophospholipids might then allow separation of newly stable bilayer membranes. Lateral diffusion of lysophospholipids away from the fusion sites might also allow reformation of a stable bilayer, in which case reacylation could occur spatially and temporally out of phase with membrane fusion. Clearly, even if these events do occur the fusion process must be more complex than just described, but is there any evidence for such a phenomenon?

LYSOLECITHIN AND FUSION

Erythrocytes can be caused to fuse into large syncytia by addition of rather high concentrations of lysolecithin (19) and similar detergents referred to as fusogenic agents (20). There is only minimal evidence, however, for the formation of lysolecithin during "normal" membrane fusion processes. For example, we find (21) phagocytic vesicles

TABLE I. Phospholipid Composition of Plasma Membranes and Phagosome Membranes Isolated from Acanthamoeba Castellanii[a]

	Plasma membrane	Phagosome membrane
Acidic phosphatides	5.0	2.1
Diphosphatidylgylcerol	2.6	1.2
Phosphatidylethanolamine	47.2	43.1
Phosphatidylserine	26.9	25.0
Phosphoinositide	0.2	0.6
Phosphatidylcholine	18.6	23.3
Lysophosphatidylcholine	0	4.5

[a]Data are from Ulsamer et al. (21).

isolated from Acanthamoeba to have a lipid composition almost identical to that of plasma membranes except for the presence of as much as 5% lysophosphatidylcholine (Table I); as much as 10% of the phosphatidylcholine and phosphatidylethanolamine of chicken erythrocytes may be converted to lysophospholipids following Ca^{++}-induced fusion at pH 10.5 (22) but not during viral-induced fusion. These elevated levels of lysophospholipids might be artifacts of the high pH in the one case or of phospholipase activity present in the phagocytic vesicles in the other. Still, they may be real and, therefore, significant in the fusion process.

If one considers the proposed lytic cycle it is apparent that at least two enzymes are necessary, phospholipase A (perhaps A_2) and acyl-CoA-lysophospholipid acyl transferase, both of which should then be present in the plasma membrane of endocytic cells. We have found (23) both enzymes with high specific activities in highly purified plasma membranes isolated from Acanthamoeba (Table II), an amoeba in which endocytic activity is particularly pronounced (24, 25). Unfortunately, plasma membranes from active endocytic cells other than Acanthamoeba have not been isolated and characterized. Plasma membranes from liver parenchymal cells, which certainly are exocytic, contain phospholipase (26) but no acyl-CoA transferase (27). In contrast, erythrocytes (which are not normally thought to be endocytic or exocytic, although the isolated membranes

TABLE II. Subcellular Distribution of Enzymes of Phospholipid Metabolism in Acanthamoeba Castellanii

Enzyme	Plasma membrane	Total homogenate	Mitochondria	Microsomes	Soluble phase
	(nmoles/min/mg protein)				
Phospholipase A_2	9.9	8.0	13.5	2.7	6.9
Phospholipase A_1	1.6	2.6	11.4	4.8	1.9
Oleoyl-CoA lysolecithin acyltransferase	2.8	3.4	0.1	10.4	0.4
Palmitoyl-CoA synthetase	8.2	4.4	1.5	19.8	1.0

Fractions were isolated as described (21) and initial reaction rates were determined (23) under conditions in which the rates of reaction were proportional to enzyme concentration as determined with the plasma membrane fraction.

can be induced to vesiculate) contain an acyl-CoA transferase but no phospholipase (28).

If the lytic cycle functions in endocytic cells it might be expected to stimulate a turnover of membrane phospholipids that could be detected by suitable tracer studies. Endocytosis and exocytosis do stimulate the turnover of phosphatidylinositol in some cells (29, 30) but phosphatidylinositol is not a major membrane phospholipid and, in any case, the increased turnover probably occurs in internal membranes and not in the plasma membrane (31). Endocytosis and exocytosis have never been shown to affect the rate of incorporation of radioactive precursors into phospholipids of the plasma membrane. Even in the case of Acanthamoeba, where the normal pinocytic process is so rapid (24) that the cell surface is internalized as rapidly as 5–50 times an hour (generation time = 12 hr), we have been unable to find any concomitant turnover of plasma membrane phospholipids (9a, 23). We calculate for Acanthamoeba that, even if all of the nutrients taken in by pinocytosis were used only for membrane synthesis, less than 10% of the plasma membrane used to form endocytic vesicles could be synthesized de novo. The plasma membrane internalized by endocytosis must be replaced by exocytosis of an equivalent mass of membrane. Nonetheless, one might have expected to find incorporation of fatty acids and lysophospholipids into membrane phospholipids if the lytic cycle functioned at the sites of membrane fusion. Perhaps, as suggested before, the acylation step occurs subsequent to the fusion events when the membrane is no longer plasma membrane – for example, after its internalization as endocytic vesicles. Elsbach has found increased acylation of lysolecithin-^{32}P in polymorphonuclear leukocytes during phagocytosis (32), and the labeled lecithin does, in fact, seem to be in the phagosome membrane (33). It is not yet established whether the enhanced acylation of lysophospholipid in those experiments occurs during membrane fusion or subsequent to it, but this observation is certainly of great relevance to the hypothesis of the lytic cycle.

DIRECTIONS OF FUTURE RESEARCH

Several model systems show promise for elucidating the role of phospholipids in membrane fusion. These are being exploited in a number of laboratories, but what follows is a preliminary description of research underway in this laboratory.

Fusion of Liposomes with Liposomes

We have recently initiated experiments in which we attempt to follow the possible fusion of liposomes by measuring the mixing of their internal contents (34). Liposomes are prepared, with either 0.5 M potassium ferricyanide or 0.5 M potassium ascorbate in their internal aqueous space, by a novel method (35) not involving sonication. Such liposomes are fully equivalent to the single-bilayer vesicles obtained by high-energy sonication (Fig. 3). It is useful to recall that these liposomes, with an outside diameter of about 25 nm and an inside diameter of 15 nm, will have an internal volume of about 1.86×10^{-18} ml. When that aqueous space contains solute at a concentration of 0.5 M, as in these experiments, only about 600 molecules of solute will be inside each vesicle. Each vesicle will consist of about 3,600 molecules of phospholipid of which about 70% will be on the outer half of the bilayer and 30% on the inner half.

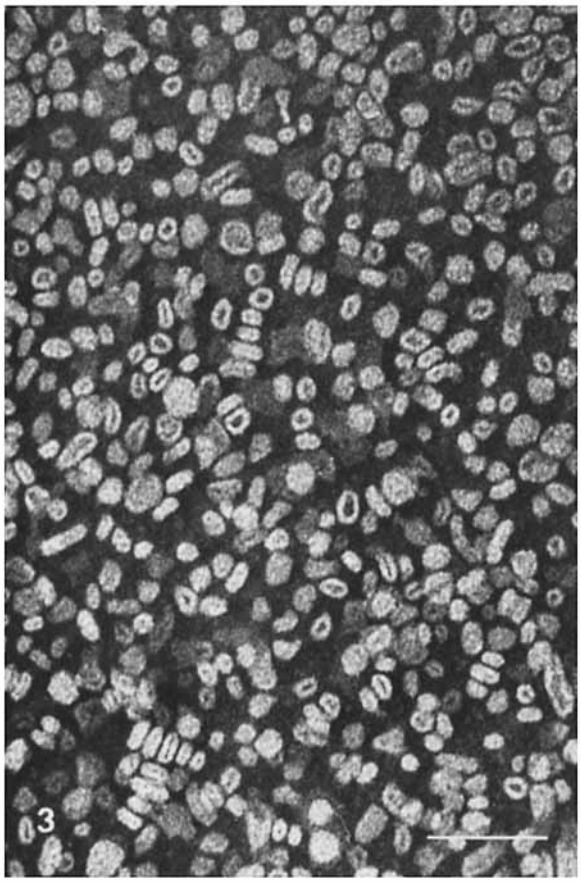

Fig. 3. Liposomes negatively stained with 1% sodium phosphotungstate. A representative field of unfractionated liposomes prepared as described (35). Bar is 200 nm.

When mixed in solution 1 mole of ascorbate reduces 2 moles of ferricyanide and reduces the absorbance at 420 nm to zero (the millimolar extinction coefficient of ferricyanide is approximately 1). When dipalmitoyl lecithin (DPL) liposomes containing either ferricyanide or ascorbate are mixed in a spectrophotometer cell at room temperature with excess external ascorbate or ferricyanide, respectively, no reduction occurs. In fact, these preparations can be kept for days with no detectable leak. Addition of deoxycholate in the presence of 0.1 M KCl results in immediate and total reduction of the ferricyanide. When DPL-liposomes containing ascorbate and DPL-liposomes containing ferricyanide are mixed at room temperature again no reduction of the ferricyanide occurs. This is true for neutral liposomes, for liposomes with opposite charges (introduced by addition of 5% dicetylphosphate or 5% cetyltrimethylammonium bromide), and for negative liposomes plus Ca^{++}. In the two latter experiments the liposomes aggregate immediately upon mixing in distilled water, but addition of 0.1 M KCl dissociates them and allows the spectrophotometric measurement to be made. Aggregation does not result

in mixing of the internal spaces of the liposomes. Similarly, no evidence for mixing of internal spaces was found when DPL-liposomes were mixed with egg lecithin-liposomes or with DPL-liposomes which contained 10% phosphatidylserine.

Phospholipase A_2 has been added to liposomes to test directly the lytic cycle hypothesis for membrane fusion. Addition of enzyme to DPL-ferricyanide-liposomes in the presence of excess external ascorbate caused a rapid, Ca^{++}-dependent hydrolysis of 40–50% of the lecithin to lysolecithin and palmitic acid as measured by radioactivity and phosphorus distribution on thin-layer chromatograms. There was a concomitant rapid reduction of a maximum of 20% of the ferricyanide by ascorbate. This reduction seems to result from a temporary "leak" since the remaining 80% of the internal ferricyanide was not reduced even after prolonged incubation. Similar results have been obtained upon the addition of phospholipase to a mixture of DPL-ferricyanide-liposomes and DPL-ascorbate-liposomes and with liposomes prepared from egg lecithin; no evidence for enzyme-induced fusion of liposomes has thus far been obtained in these experiments. It is remarkable that, after a transitory interval, lecithin-liposomes appear to be stable and impermeable in the presence of approximately equimolar concentrations of lysolecithin and fatty acid. Do these prelimary results indicate that single-bilayer liposomes may be more stable in the presence of lytic agents than generally thought?

Fusion of Liposomes with Cells

Another experimental approach to ascertaining the possible role of lipids in membrane fusion is to study the interaction of cells with liposomes. Papahadjopoulos (36), for example, has demonstrated that liposomes can induce the fusion of cultured mammalian cells. The most effective liposomes were negatively charged and in the liquid-crystalline state; the presence of lysolecithin was not essential. Although it seems likely that the cells were fused through a bridge created by the fusion of one liposome with the plasma membrane of two cells, the time scale of this experiment (24 hr) was much different from that of normal membrane fusions.

Grant and McConnell (37) have reported the fusion of liposomes with the membrane of Acholeplasma laidlawii. The primary evidence for fusion in these experiments was the movement of an electron spin reporter molecule from the environment of the dipalmityl phosphatidylcholine (DPL) of the liposome into the more fluid lipid phase of the mycoplasma membrane. It is of interest that the apparent mixing of the phospholipids of the liposome and the bacterium was accompanied by the loss of the aqueous content of the liposome into the surrounding medium. These bacterial cells are not known normally to undergo endocytic or cell–cell fusions.

We have been carrying out similar experiments with liposomes and Acanthamoeba. When single-bilayer liposomes prepared from egg phosphatidylcholine or DPL are incubated with amoebas there is a linear uptake of phospholipid at more than 15 times the rate of endocytosis (34). The aqueous contents of the liposomes are taken in at the same rate as the phospholipid bilayer, as can be shown by labeling the internal aqueous space with ^{3}H-inulin (Table III). The process is partially inhibited, however, by dinitrophenol (Table III) and at 12°C. Both these conditions inhibit endocytosis more extensively (24, 25, 38), and it seemed possible that at least the inhibition-resistant uptake of liposomes might be by a process other than pinocytosis. Attempts to resolve this question by auto-

TABLE III. Interaction of Liposomes and Acanthamoeba

Experiment	Substrate	"Uptake" (nmoles/2 × 10^6 cells)
I	^{3}H-egg-liposomes (+)	33
	^{3}H-egg-liposomes (+) DNP	5
	^{3}H-DPL-liposomes (+)	43
	^{3}H-DPL-liposomes (+) DNP	17
	^{3}H-DPL-liposomes (−)	37
	^{3}H-DPL-liposomes (−) DNP	15
II	^{3}H-egg-liposomes (+)	4.7%
	^{3}H-inulin	0.1%
	^{3}H-inulin inside egg liposomes (+)	4.7%

Expt. I. Amoebas (2 × 10^6/ml) were incubated for 1 hr at 28°C with liposomes (3 μmoles of phospholipid/ml) prepared (35) from either egg lecithin or synthetic dipalmitoyl lecithin. The liposomes contained either 5% stearylamine (+) or 5% dicetylphosphate (−) and a trace of ^{3}H-lecithin isolated from amoebas grown in the presence of ^{3}H-oleate. "Uptake" was determined by measuring the radioactivity of washed cells.

Expt. II. The three substrates were ^{3}H-egg-lecithin-liposomes prepared as in Expt. I, ^{3}H-inulin, and nonradioactive egg lecithin in liposomes containing ^{3}H-inulin in the internal aqueous space. ^{3}H-inulin uptake is a measure of nonconcentrative pinocytosis. The uptake of ^{3}H-inulin inside liposomes demonstrates that the internal aqueous space is taken up by the cells at the same rate as the phospholipid bilayer.

radiography with ^{3}H-phospholipid-liposomes have provided ambiguous results (34). At early times the label is associated primarily with the cell surface and at later times (Fig. 4) with excess "membranes" associated with cytoplasmic vacuoles. These proliferating internal "membranes" could be the result of fusion of liposomes with each other after their endocytic uptake, but they might derive from the internalization of excess cell surface formed by fusion of the liposomes with the plasma membrane. The uptake of liposomes doubles the phospholipid content of the cell which, if fusion with the plasma membrane were the mechanism, would cause perhaps a 20-fold increase in the surface area of plasma membrane. This excess plasma membrane would then have to be removed from the surface. It might be this secondary process of membrane internalization, and not pinocytosis, that is inhibited by low temperature and dinitrophenol.

To test further whether at least the noninhibited uptake of liposome phospholipid might represent fusion of liposomes with the cell membrane we have prepared single-bilayer DPL-liposomes with α-amylase in the internal space and incubated the liposomes with Acanthamoeba at 12°C to inhibit pinocytosis. Approximately 85% of the amylase activity expected to be incorporated into the cell (calculated from the uptake of phospholipid) was recovered in the cells, but all of this activity could be sedimented at 100,000 × g. We had anticipated that if the mechanism of liposome uptake were fusion all of the amylase would have been soluble in the cell cytoplasm. These results would suggest that the amylase, in fact, was in cytoplasmic vacuoles, as might have been expected following pinocytosis of the liposomes. However, when soluble amylase is pinocytosed by Acanthamoeba at 28°C the enzyme is rapidly inactivated and no enzymatic activity can be recovered from the cells. It is, of course, possible that the liposome bilayer would

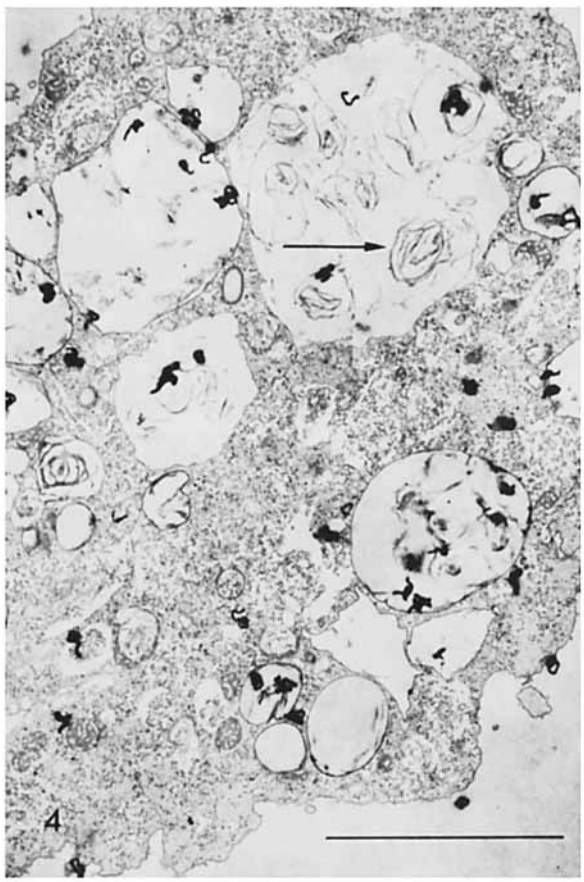

Fig. 4. Autoradiogram of an Acanthamoeba incubated for 150 min with ^{3}H-egg-lecthin-liposomes. Note the proliferation of membranes within the vacuoles (arrow). Such structures are normally not seen in the cytoplasmic vacuoles (see, for example, Figs. 1 and 2). The silver grains lie over the plasma membrane, the vacuole membrane, and the intravacuole membranes. Bar is 5 μm.

protect its internal amylase from degradative enzymes in the endocytic vacuoles. The paradox exists, however, that pinocytosis does not normally occur under the conditions (12°C) used in the experiments with liposomes.

SUMMARY

The hypothesis for a controlled enzymatic interconversion of phospholipids to lysophospholipids accompanying the membrane fusion event remains an attractive one. It is supported by the fusogenic ability of lysolecithin and other detergents when added to cell suspensions, albeit at rather high concentrations. There is some evidence for a net increase in membrane lysophospholipids during membrane fusion, although a detectable

increase need not occur if the lysophospholipids are rapidly reacylated. But only one laboratory has detected an increased rate of acylation of lysophospholipids associated with the endocytic event, and that acylation has not been demonstrated to be involved specifically in the fusion process. It is possible that the lytic cycle functions in two stages. Phospholipase action might generate lysophospholipids which could promote fusion at a locus and then rapidly diffuse within the membrane lipid bilayer, thus reducing the local concentration of lysophospholipid below the fusogenic level. Reacylation could occur at a later stage and perhaps not specifically in the plasma membrane. This would allow an increase in concentration of lysophospholipid that would not necessarily be accompanied by an increased rate of turnover of phospholipids in the plasma membrane.

Preliminary evidence suggests that liposome-liposome fusion might serve as a useful model system. Techniques are available to follow fusion by the effects on reporter molecules included in the phospholipid bilayer (39, 40) and to measure the rate of mixing of internal aqueous spaces of liposomes undergoing fusion.

Liposome-cell fusion is also a viable experimental model. Moreover, should it prove possible to induce the fusion of liposomes in quantity with cell membranes a mechanism will have been provided for the direct introduction of otherwise impermeable molecules (enzymes, nucleic acids, etc.) into the cell cytoplasm.* The experimental and potential clinical applications of such a procedure are obvious. Even the rapid pinocytosis of liposomes allows the introduction of large amounts of otherwise impermeable solute molecules into the cell – in this case into the phagosomes (lysosomes) where they might serve a useful purpose and from which they might be able to enter the cell cytoplasm.

REFERENCES

1. Korn, E. D., in "The Biochemistry of Cell Walls and Membranes," C. F. Fox, (Ed.). Medical and Technical Publishing Co. Ltd., London (in press).
2. Poste, C., and Allison, A. C., Biochim. Biophys. Acta 300:421–405 (1973).
3. Jacques, P. J., in "Lysosomes in Biology and Pathology," vol. 2, H. Fell and J. Dingle (Eds.). North Holland Press, Amsterdam (1969).
4. Nishioka, K., Constanopoulos, A., Satoh, P. S., and Nayjar, V. A., Biochim. Biophys. Res. Commun. 47:172–179 (1972).
5. Sharon, N., and Lis, H., Science 177:949–959 (1972).
6. Pierce, C. W., Asofsky, R., and Solliday, S. M., Federation Proc. 32:41–43 (1973).
7. Ashwell, G., and Morell, A., in "Glycoproteins of Blood Cells and Plasma," G. A. Jamieson and T. J. Greenwalt (Eds.). J. B. Lippincott, Philadelphia (1971) p. 173.
8. Pollard, T. D., and Korn, E. D., J. Biol. Chem. 248:448–450 (1973).
9. Pollard, T. D., and Weihing, R. R., CRC Crit. Rev. Biochem. 2:1–65 (1974).

9a. Simmons, S., and Korn, E. D., unpublished results.

10. Korn, E. D., and Wright, P. L., J. Biol. Chem. 248:439–447 (1973).
11. Korn, E. D., Dearborn, D., Sokoloski, E., and Fales, H. M., J. Biol. Chem. 248:2257–2259 (1973).
12. Korn, E. D., Dearborn, D., and Wright, P. L., J. Biol. Chem. (in press).
13. Dearborn, D., and Korn, E. D., J. Biol. Chem. (in press).
14. Berlin, R. D., J. Supramol. Struc. (in press).
15. Satir, B., J. Supramol. Struc. 2:529 (1974).
16. Schramm, M., Selinger, Z., Salomon, Y., Eytan, E., and Batzri, S., Nature New Biol. 240:203 (1973).
17. Raz, A., and Goldman, R., Nature 247:206–208 (1974).
18. Lucy, J. A., Nature 227:815–817 (1970).

*Recent data strongly support liposome-cell fusion as the mechanism of incorporating of dipalmitoyl lecithin liposomes into Acanthamoeba.

19. Poole, A. R., Kowell, J. I., and Lucy, J. A., Nature 227:810–814 (1970).
20. Ahkong, Q. F., Fisher, D., Tampion, W., and Lucy, J. A., Biochem. J. 136:147–155 (1973).
21. Ulsamer, A. G., Wright, P. L., Wetzel, M. G., and Korn, E. D., J. Cell Biol. 51:193–215 (1971).
22. Toister, Z., and Loyter, A., J. Biol. Chem. 248:422–432 (1973).
23. Victoria, E., and Korn, E. D., J. Lipid Res. (in press).
24. Bowers, B., and Olszewski, T. E., J. Cell Biol. 53:681–694 (1972).
25. Weisman, R. A., and Korn, E. D., Biochemistry 6:485–497 (1967).
26. Victoria, E. J., Golde, L. M. G. van, Hostetler, K. Y., Scherphof, G. L., and Deenen, L. L. M. van, Biochim. Biophys. Acta 239:443–457 (1971).
27. Eibl, H., Hill, E. E., and Lands, W. E. M., Eur. J. Biochem. 9:250–258 (1969).
28. Paysant, M., Bitran, M., Wald, R., and Polonovski, J., Bull. Soc. Chim. Biol. 52:1257–1269 (1970).
29. Berger, R. R., and Karnovsky, M. L., Federation Proc. 25:840 (1966).
30. Sastry, P. S., and Hokin, L. E., J. Biol. Chem. 241:3354–3361 (1966).
31. Gerber, D., Davies, M., and Hokin, L. E., J. Cell Biol. 50:736–745 (1973).
32. Elsbach, P., J. Clin. Invest. 47:2217 (1968).
33. Elsbach, P., Patriarca, P., Pettis, P., Stossel, T. P., Mason, R. J., and Vaughan, M., J. Clin. Invest. 51:1910 (1972).
34. Batzri, S., and Korn, E. D., unpublished results.
35. Batzri, S., and Korn, E. D., Biochim. Biophys. Acta 298:1015–1019 (1973).
36. Papahadjopoulos, D., Poste, G., and Schaeffer, B. E., Biochim. Biophys. Acta 323:23–42 (1973).
37. Grant, C. W. M., and McConnell, H. M., Proc. Nat. Acad. Sci. U.S. 70:1230–1240 (1973).
38. Bowers, B., and Korn, E. D., unpublished results.
39. Taupin, C., and McConnell, H. M., Fed. Eur. Biochem. Soc., 8th Meeting, vol. 28, (1972), p. 219.
40. Metcalfe, J. C., Birdsall, N. J. N., and Lee, A. G., Fed. Eur. Biochem. Soc., 8th Meeting, vol. 28 (1972), p. 197.