J. Cell Sci. 11, 569–579 (1972)
Printed in Great Britain

TURNOVER OF PLASMA MEMBRANE DURING PHAGOCYTOSIS

R. J. GOODALL, Y. F. LAI AND J. E. THOMPSON
Department of Biology, University of Waterloo, Waterloo, Ontario, Canada

SUMMARY

Levels of radioactive glycine and glycerol incorporated into the plasma membrane of *Acanthamoeba castellanii* during phagocytosis were determined in order to elucidate how surface membrane expended during this process is replaced. The amoebae were allowed to ingest latex beads in the presence of the labelled membrane precursors and plasma membrane was then isolated and analysed for the presence of radioactivity. The isolated membrane fragments were found to be quite highly labelled. In order to ascertain whether this represented preferential synthesis of plasma membrane in response to phagocytosis, the specific radioactivities of the isolated membrane fractions were compared with those of corresponding particulate homogenates, which were composites of all cellular membranes. Enrichment values calculated in this manner proved to be essentially similar for both phagocytosing and non-phagocytosing amoebae. This indicates that *de novo* synthesis of plasma membrane is not essential for phagocytosis and in turn suggests that pre-existing cytoplasmic membrane is used to replace surface membrane consumed during ingestion. Presumably the incorporation of membrane precursors that was observed represents molecular turnover that occurs irrespective of phagocytosis.

INTRODUCTION

Expenditure of plasma membrane is a fundamental feature of phagocytosis. Transmission electron microscopy has revealed that during ingestion of latex beads by *Acanthamoeba castellanii*, the beads are encapsulated in surface membrane-forming vesicles which are ultimately internalized (Korn & Wiesman, 1967). Consequently, large amounts of plasma membrane are consumed and presumably must ultimately be replaced. Scanning electron microscopy of *Acanthamoeba* sp. has revealed that there is a surplus of plasma membrane in the form of numerous folds and filopodia over the cell surface (Pasternak, Thompson, Schultz & Zachariah, 1970; Goodall & Thompson, 1971). Some of this reserve appears to be used during phagocytosis (Goodall & Thompson, 1971), meaning that replacement of expended plasmalemma need not necessarily occur during actual ingestion.

Weisman & Korn (1967) have suggested that replenishment could occur either by intussusception of pre-existing cytoplasmic membrane into the plasmalemma or by direct *de novo* synthesis. Biosynthesis of membrane comprises both synthesis of components and their assembly into a functional membrane. Dallner, Siekevitz & Palade (1966*b*) have pointed out that this could be achieved through a single step assembly, contingent upon prior synthesis of all components, or a multi-step process involving the sequential incorporation of components. There is some evidence indicating that

chloroplast lamellae membranes are synthesized in a manner consistent with a single step assembly (Ohad, Siekevitz & Palade, 1967; Goldberg & Ohad, 1970*a*), but other membrane types such as plasma membrane and endoplasmic reticulum appear to be synthesized through a multi-step process. For example, from labelled precursor experiments with developing rat hepatocytes, it would appear that membrane fragments destined to become smooth endoplasmic reticulum are initially synthesized on rough endoplasmic reticulum and subsequently undergo transformation into smooth endoplasmic reticulum (Dallner, Siekevitz & Palade, 1966*a*, *b*). Similarly there are indications for a number of different types of cells that a precursor of plasma membrane is synthesized on the endoplasmic reticulum and subsequently modified and incorporated into the plasmalemma (Warren & Glick, 1968; Bosmann, Hagopian & Eylar, 1969; Chlapowski & Band, 1971*b*). There is also evidence suggesting that constituent molecules can be added on to pre-existing membrane during its growth or as replacements for degenerate portions (Omura, Siekevitz & Palade, 1967; Warren & Glick, 1968; Bosmann *et al.* 1969; Goldberg & Ohad, 1970*b*).

In order to distinguish between *de novo* synthesis and utilization of pre-existing membrane as means of replacing plasma membrane expended during phagocytosis, we followed the incorporation of labelled membrane precursors into specific types of membrane of *A. castellanii* during phagocytosis of latex beads. In a previous study with the same organism Ulsamer, Smith & Korn (1969) measured the incorporation of radioactive precursors into total cell lipid during phagocytosis. They found no evidence for increased turnover of phospholipid components as a result of ingestion. However, this type of analysis would not necessarily detect a preferential synthesis of plasma membrane and thus, as indicated by the authors, their observations do not unequivocally preclude *de novo* synthesis of the plasmalemma.

MATERIALS AND METHODS

Culturing and induction of phagocytosis

Amoeboid cells of *Acanthamoeba castellanii* (Neff strain) were cultured as previously described (Schultz & Thompson, 1969). At a population density of $3–5 \times 10^5$ cells/ml, the amoebæ from 4 l. of culture were harvested by centrifugation at 800 *g* for 10 min at 4 °C and resuspended in 190 ml of the endocytosis medium described by Weisman & Korn (1967). This medium consists of 1·5 % proteose-peptone in 0·015 M KH_2PO_4, pH 6·7. The suspension of cells was incubated for 40 min in a shaking water bath adjusted to 30 °C and rotating at 80 rev/min. At the end of this period phagocytosis was initiated by adding polyvinyl toluene latex beads (2·02 ± 0·0135 μm diameter, Dow Chemical Co.) at a concentration of 2 mg/ml. Incubation was continued for an additional 90 min. The cells were then harvested as before and used for fractionation. Any uningested latex beads were removed as previously described (Goodall & Thompson, 1971).

For experiments in which the incorporation of radioactive precursors (glycine-2-C^{14}, glycine-2-H^3 or glycerol-2-H^3, Amersham Searle Inc.) into membranes during phagocytosis was being determined, the labelled compound was added to the incubation mixture 30 min before the addition of the latex beads at concentrations of 0·15 mCi for ^{14}C and 0·4 mCi for ^3H. In each case the labelled compounds were administered at a specific activity of 53 mCi/mmol. This was followed by the addition of 100-fold molar excess of cold chase after 60 min.

In some experiments the procedure for endocytosis was altered from the routinely used scheme described above. In order to determine the influence of proteose-peptone on phagocy-

tosis, the cells were resuspended in 0·015 M KH_2PO_4, pH 6·7 rather than in the enriched endocytosis medium. Also, the effects of a higher concentration of latex (6 mg/ml of incubation medium rather than 2 mg/ml) were determined. In a few experiments, label was added to the incubation medium at the same time as the latex beads rather than 30 min before. Experiments in which these altered conditions were used are clearly designated in the tables. For all other experiments described in the tables, the routine procedure for phagocytosis described above was used.

The effects of cycloheximide on the incorporation of labelled glycine into membranes during phagocytosis were also determined. In these experiments cycloheximide was added at a concentration of 5 mg/ml 5 min after the cells were suspended in the endocytosis medium. Labelled glycine, latex beads and cold glycine were added at 30-min intervals respectively thereafter. The cells were harvested 30 min after the last addition.

The amount of latex internalized by the amoebae during the incubation period was determined by measuring the absorbancy at 267 nm of a dioxane extract of the cells as described by Weisman & Korn (1967).

Isolation of membrane fractions

Purified plasma membrane was isolated as previously described (Schultz & Thompson, 1969). The layer from the sucrose gradient constituting the purified membrane was removed with a syringe, diluted with approximately 5 vol. of 1 mM $NaHCO_3$ (pH 7·5), pelleted by centrifugation, resuspended in a small volume of $NaHCO_3$ solution and stored at −4 °C.

A microsomal fraction was obtained from the same fractionation procedure. This involved centrifuging the 1500-*g* supernatant derived from the plasma membrane preparation, initially at 10000 *g* for 10 min at 4 °C in order to sediment non-microsomal particulate material, and finally at 133000 *g* for 2 h at 4 °C. The microsomal pellet so obtained was resuspended in a small volume of 1 mM $NaHCO_3$ (pH 7·5) and retained at −4 °C for subsequent analyses.

A modified homogenate designated 'particulate homogenate' was prepared by centrifuging the true homogenate at 133000 *g* for 2 h at 4 °C. The pellet was simply resuspended in 1 mM bicarbonate solution and stored at −4 °C.

Biochemical, chemical and radioactivity determinations

Succinate dehydrogenase (EC 1.3.99.1) was assayed according to the procedure of Pennington (1961). Glucose-6-phosphatase (EC 3.1.3.9) and 5′-nucleotidase (EC 3.1.3.5) activities were determined as described by Hubscher & West (1965) and Michell & Hawthorne (1965) respectively, with the following modification. In addition to enzyme and substrate blanks, a further blank containing trichloroacetic acid (TCA) was run routinely. This value was added to compensate for the level of phosphorus in the TCA which in the unmodified procedure is subtracted twice.

Protein determinations were routinely performed by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as a standard.

Levels of phospholipid in the microsomal fraction were determined by measuring the amount of P released by acid digestion of lipid extracts. Lipids were extracted by a technique essentially similar to that described by Dallner *et al.* (1966*a*). A known volume of suspended microsomes was treated with 10% TCA (2·5 ml/ml of fraction) and a pellet obtained by centrifugation at 1700 *g* for 10 min. This pellet was suspended in 12 ml of chloroform–ethanol solution (1:1 by volume), containing 10 mg DL-α-tocopherol per ml, and incubated with stirring in an atmosphere of N_2 for at least 12 h at 4 °C. The suspension was filtered, and the filtrate transferred to a digestion flask and evaporated to dryness. Digestion and determination of P were carried out as described by King (1932) with the modification that the samples were predigested in concentrated nitric acid. P levels of undigested samples were subtracted.

For radioactivity determinations, 0·3 ml of fraction were suspended in 20 ml of a Cab-o-sil scintillation fluid mixture (30 g of Cab-o-sil per l. of fluid) and the samples counted in a Packard TriCarb Scintillation Counter.

Table 1. *Enzyme data for fractions isolated from A. castellanii after phagocytosis of latex beads*

Enzyme	Expt.	Specific activity: Homogenate	Specific activity: Plasma membrane	Relative enrichment
5′-Nucleotidase	A	92·4	462	5·0
	B	75·6	424	5·6
	C	66·4	408	6·1
Glucose-6-phosphatase	A	38·3	31·0	0·8
	B	24·2	n.d.	—
	C	47·1	n.d.	—
Succinate dehydrogenase	A	0·031	0·034	1·1
	B	0·032	n.d.	—
	C	0·025	0·038	1·6

5′-Nucleotidase and glucose-6-phosphatase activities are expressed as μg P per mg protein per h and succinate dehydrogenase as μmol reduced iodonitrotetrazolium violet per mg protein per h. n.d. means not detectable. Relative enrichment is the ratio of the specific activity in the plasma membrane to that of the homogenate.

RESULTS

Enzymic properties of the plasma membrane fraction

Enzyme data for preparations of purified plasma membrane isolated from *Acanthamoeba* cells which had ingested latex beads, are presented in Table 1. Consistently the membrane fragments were enriched in 5′-nucleotidase by 5- to 6-fold with respect to homogenate on a specific activity basis. In contrast, levels of glucose-6-phosphatase and succinate dehydrogenase were very low and in some cases not detectable (Table 1), indicating that the fraction was essentially plasma membrane.

Incorporation of radioactive precursors into plasma membrane during phagocytosis

Estimations of the extent to which labelled glycine was incorporated into the amoeboid plasma membrane during active ingestion of latex beads were obtained by calculating the specific radioactivity (cpm per mg protein) of purified plasma membrane fragments isolated from cells which had undergone phagocytosis. Preparations of membrane which served as controls were isolated from cells treated in exactly the same manner except that the latex beads were not added to the endocytosis medium. Finally, in order to get an indication of whether the presence of label in these isolated fragments represented preferential synthesis of plasma membrane in response to phagocytosis, the specific radioactivities of the isolated membrane fractions were compared with those of corresponding particulate homogenates. The results of these experiments are shown in Table 2. Both [^{14}C]glycine and [^{3}H]glycine were used and for each isotope considerable label was present in the isolated plasma membrane fragments irrespective of the presence of latex beads. Enrichments in radioactivity relative

Table 2. *Incorporation of radioactive glycine into plasma membrane of A. castellanii during phagocytosis of latex beads*

Expt.	Latex beads, mg/ml	Specific radioactivity of isolated plasma membrane	Enrichment relative to particulate homogenate
A	0	1637	1·44
B	0	2905	1·82
C	0	2230	2·09
D*	0	9735	1·30
E	2	1906	2·35
F	6	2002	1·72
G*	6	3222	1·34
H†	6	5034	1·80

Specific activities are quoted as cpm per mg protein. Enrichment values are ratios of the specific radioactivity in the plasma membrane to that of the corresponding particulate homogenate. For enrichments in the presence and absence of latex beads $t_6 = 0{\cdot}51$. * The cells were incubated in 0·015 M KH_2PO_4, pH 6·7, rather than in the proteose-peptone medium. † Latex beads and radioactive glycine were added to the incubation mixture simultaneously.

to corresponding particulate homogenates, expressed as a ratio of the specific radioactivity of the purified membrane fraction to that of the particulate homogenate, were essentially similar both in the presence and absence of ingestion. For experiments in which latex beads were present, a mean relative enrichment of 1·80 was obtained as compared to 1·66 for the controls. Statistical analysis (Student's *t* test) of these enrichment figures showed no significant difference between results obtained under the 2 conditions.

Increasing the amount of latex in the incubation system from 2 to 6 mg/ml had no effect on the enrichment of label in the isolated plasma membranes (Table 2). Moreover, enrichments were not significantly different for experiments in which the incubation was carried out in simple buffer rather than in the enriched medium containing proteose-peptone (Table 2). It is also apparent from Table 2 that the addition of latex beads and label simultaneously to the incubation medium rather than in sequence with a 30-min period between each addition did not significantly change the enrichment of radioactivity.

In order to be certain that the presence of radioactivity in the isolated plasma membrane fragments represented *de novo* protein synthesis, the effects of cycloheximide on the incorporation were determined. From Table 3, it is apparent that in the presence of this compound, the incorporation of [^{3}H]glycine was decreased to about 5–10% of the normal level. Observations by phase-contrast microscopy showed that cycloheximide does not prevent uptake of the latex beads.

In a series of experiments similar to those involving the use of glycine, the incorporation of [^{3}H]glycerol into plasma membrane was investigated as a function of phagocytosis. The results are illustrated in Table 4. A mean relative enrichment of 1·47 was obtained for experiments in which latex beads were present in the incubation

Table 3. *Effect of cycloheximide on the incorporation of [^3H]glycine into plasma membrane of A. castellanii during phagocytosis of latex beads*

Expt.	Cycloheximide	Specific radioactivity of isolated plasma membrane
A	Absent	1524
B	Absent	2591
C	Present	123
D	Present	125

Specific radioactivities are quoted as cpm per mg protein.

Table 4. *Incorporation of [^3H]glycerol into plasma membrane of A. castellanii during phagocytosis of latex beads*

Expt.	Latex beads	Specific radioactivity of isolated plasma membrane	Enrichment relative to particulate homogenate
A	Absent	2295	0·91
B	Absent	14365	1·42
C	Absent	1972	1·84
D	Present	4115	1·33
E	Present	4310	1·68
F	Present	2534	1·40

Specific radioactivities are quoted as cpm per mg protein. Enrichment values are ratios of the specific radioactivity in the plasma membrane to that of the corresponding particulate homogenate. For enrichments in the presence and absence of latex beads $t_4 = 0{\cdot}28$.

mixture. This is to be compared with a corresponding figure of 1·39 for incubations in which the beads were absent. Statistical analysis (Student's *t* test) of the individual enrichment figures confirmed that there is no significant difference for the incorporation of glycerol between a phagocytosing and non-phagocytosing system.

An estimate of the amount of plasma membrane consumed as a result of phagocytosis was obtained by measuring the level of latex taken up by the cells. When the latex beads were present in the incubation medium at a concentration of 2 mg/ml, at the cell concentration used each cell ingested an average of 48 beads. Korn & Weisman (1967) have shown that for beads of this size, each bead is encapsulated separately and is thus surrounded by membrane. Assuming an average cell diameter of 20 μm this means that an amount of plasma membrane equivalent to approximately 48 % of the surface area of the cell is consumed. Corresponding figures for the higher concentration of beads (6 mg/ml of incubation medium) were an average of 120 beads ingested per cell which accounts for an expenditure of membrane equivalent to about 120 % of the cell surface as a result of phagocytosis. It is possible to have an amount of membrane accounting for more than 100 % of the cell surface consumed because the surface of these cells is convoluted (Goodall & Thompson, 1971).

Table 5. *Incorporation of [^{3}H]glycerol into microsomes isolated from A. castellanii after phagocytosis of latex beads*

Expt.	Latex beads	Specific radioactivity of isolated microsomes	Enrichment relative to particulate homogenate
A	Absent	327	0·11
B	Absent	229	0·09
C	Absent	299	0·17
D	Present	379	0·15
E	Present	1213	0·12
F	Present	142	0·13

Specific radioactivities of isolated microsomes are quoted as cpm per μg P. Enrichment values are ratios of the specific radioactivity in microsomes to that of the corresponding particulate homogenate. For enrichments in the presence and absence of latex beads $t_4 = 0{\cdot}39$.

Analyses of radioactivity in a microsomal fraction

An indication of the extent to which [^{3}H]glycerol was incorporated into endoplasmic reticulum during phagocytosis was obtained by determining the specific radioactivities of microsomal fractions. Ribosomal protein, either in the form of rough endoplasmic reticulum or free ribosomes, is a source of non-membranous protein present in microsomal fractions to a variable extent among experiments and this renders calculations of specific activity on a protein basis somewhat unreliable. This complication was obviated by calculating specific radioactivities relative to phospholipid. On this basis, the mean relative enrichments are 0·13 and 0·12 in the presence and absence of beads respectively and are clearly not different (Table 5). Statistical analysis of the individual enrichment figures confirms this (Student's t test).

DISCUSSION

Plasma membrane fragments were isolated according to the procedure previously described by Schultz & Thompson (1969) for the same organism. This procedure has since been shown to compare favourably with an alternative method developed by Ulsamer, Wright, Wetzel & Korn (1971) for *Acanthamoeba*. An assessment of the enzymic properties of the isolated membranes indicated that the purity of the preparation was not seriously affected by the presence of internalized beads. The membrane fragments were enriched in 5′-nucleotidase, an enzyme which serves as a marker for plasma membrane of these cells as well as various mammalian tissues (Coleman & Finean, 1966; Schultz & Thompson, 1969). In addition, levels of glucose-6-phosphatase and succinate dehydrogenase, markers for microsomes and mitochondrial membrane fragments respectively for this cell (Schultz & Thompson, 1969) were minimal. This means that the purified fraction was relatively free of the usual contaminating cytoplasmic membranes.

In this context, vacuolar membranes are also a possible source of contamination, but these membranes, although certainly modified in this state, are for the most part

derived from plasma membrane in this organism (Korn & Weisman, 1967; Weisman & Korn, 1967).

The compounds chosen as precursors for membrane were the most elementary components of lipid and protein; glycerol has been shown to be a specific marker for membrane phospholipids (Chlapowski & Band, 1971*a*) and glycine is an absolute growth requirement for this organism (Adam, 1959). In order to ensure that the nature of plasma membrane replenishment was monitored both during and after the period of most active phagocytosis, the incubation interval in all experiments exceeded by 20 min the length of time required for the amoeba to return to its prephagocytotic morphology, as estimated by scanning electron microscopy (Goodall & Thompson, 1971). For experiments involving each of the radioactive precursors, there was some variation in the absolute specific radioactivities for both plasma membrane and microsomal fractions (Tables 2–5). However, the degree of incorporation is dependent upon the amount of labelled compound taken up by the intact cells in the incubation medium and this uptake does vary from experiment to experiment. Moreover, a microsomal fraction by its very nature has a variable composition. Accordingly, some variation in the absolute specific radioactivities for the isolated fractions is to be expected. This inherent difficulty was circumvented, however, by comparing enrichments in radioactivity relative to corresponding particulate homogenates.

Estimations of the proportions of plasma membrane interiorized during phagocytosis indicated that an amount of membrane equivalent to about 48% of the cell surface area was consumed when latex beads were present at a concentration of 2 mg/ml of incubation medium. At the higher concentration of latex beads (6 mg/ml of incubation medium), a corresponding figure of 120% was obtained. In this light, it seems reasonable that phagocytosis might stimulate *de novo* synthesis of the plasmalemma. However, during ingestion of latex beads by *A. castellanii* there was no indication of a corresponding stimulated incorporation of precursors into either the lipid or protein components of the surface membrane. Had there been preferential synthesis of plasma membrane in response to ingestion, the specific radioactivities in the isolated fraction should have been significantly higher than those of corresponding particulate homogenates, which were composites of all the cellular membranes. Enrichment values calculated in this manner proved to be essentially similar for both phagocytosing and non-phagocytosing cells. Comparisons were made against modified homogenates rather than true homogenates in order to eliminate any contribution to the radioactivity count made by unincorporated glycine remaining in the cytoplasm. It is apparent, therefore, that ingestion of latex beads does not induce *de novo* synthesis involving a single-step assembly of precursors at the plasmalemma itself. An effect attributable to ingestion of latex beads could theoretically have been masked by phagocytosis of nutrients in the proteose-peptone endocytosis medium. However, this appears not to be the case, for enrichments of radioactivity in the isolated plasma membrane obtained when proteose-peptone was absent from the incubation medium were quite comparable in magnitude to those obtained in its presence.

An alternative possibility is that surface membrane expended during phagocytosis

is replaced by *de novo* synthesis involving a multi-step process. This is a particularly attractive hypothesis for *Acanthamoeba* in the light of a recent study on the biosynthesis of plasma membrane in this organism by Chlapowski & Band (1971*b*). They were able to detect collapsed vesicles, derived from rough endoplasmic reticulum, which become inserted into the plasma membrane during logarithmic growth. These observations were interpreted as indicating that plasma membrane biosynthesis involves the formation of a primary phospholipid membrane on the rough endoplasmic reticulum and its ultimate intussusception into the plasmalemma. If plasma membrane expended during phagocytosis were replaced in this manner, there should have been a corresponding preferential incorporation of label into the endoplasmic reticulum. However, this also appears not to be the case, for there was no enrichment of radioactivity in microsomal fractions for phagocytosing as opposed to non-phagocytosing organisms.

Clearly, however, labelled precursors of both phospholipid and protein are incorporated into the plasma membrane during this period, as indicated by the relatively high specific radioactivities of the isolated membrane fraction. In the absence of detectable preferential membrane synthesis, this can be interpreted as reflecting routine molecular turnover occurring independently of phagocytosis. The inhibiting action of cycloheximide on the incorporation of glycine indicates that such turnover involves proteins.

Weisman & Korn (1967) have shown that in spite of a considerable expenditure of plasma membrane when *Acanthamoeba* cells ingest latex beads, there is essentially no change in the size of the organism. An examination of the same process by scanning electron microscopy indicated that although the filopodia and surface convolutions become less distinct during active phagocytosis, the organism ultimately re-attains its pre-phagocytotic morphology (Goodall & Thompson, 1971). Both of these observations imply that there is replenishment of that surface membrane consumed. This new membrane is apparently not furnished by *de novo* synthesis. There is no evidence for a single-step synthetic process in which all constituents of the membrane would be simultaneously assembled in a single operation. Neither is there evidence for synthesis by a multi-step process in which membrane fragments serving as precursors of plasma membrane would be synthesized elsewhere in the cell and subsequently acquire the characteristic properties of plasma membrane either before or after incorporation into the plasmalemma as new membrane. It seems reasonable to conclude, therefore, that the source of replenishment is pre-existing membrane which becomes inserted into the plasmalemma. Indeed there may be a cytoplasmic pool of such membrane which in the event of extensive phagocytosis is, as suggested by Chlapowski & Band (1971*b*), to a large extent derived from a fragmentation of vacuolar membranes. The fact that cycloheximide does not prevent uptake of latex beads is consistent with the conclusion that phagocytosis does not require *de novo* synthesis of membrane. It has also been previously reported that phagocytosis by macrophages and granulocytes is not affected by the presence of inhibitors of protein synthesis (Cline, 1966; Cohn, 1966). On the other hand, pinocytosis in *Amoeba proteus* is impaired by puromycin, implying dependence upon membrane synthesis (Sanders &

Bell, 1970). This indicates that in spite of many apparent similarities, the 2 processes are fundamentally different.

It would appear, therefore, that phagocytosis of latex beads by *Acanthamoeba* involves turnover of plasma membrane at 2 levels; cycling of membrane which enables replacement of expended surface membrane without *de novo* synthesis, and turnover at the molecular level involving input of newly synthesized material. It has been suggested by Warren & Glick (1968) that molecular turnover of membranes may provide a basis for their modification. Thus, in the case of phagocytosis, such turnover may very well be the means whereby newly incorporated membrane acquires those characteristics that distinguish plasma membrane from the various cytoplasmic membranes.

We are grateful to the National Research Council of Canada for a grant-in-aid of this research and for a Scholarship to one of us (R. J. G.).

REFERENCES

ADAM, K. M. G. (1959). The growth of *Acanthamoeba* sp. in a chemically defined medium. *J. gen. Microbiol.* **21**, 519–529.

BOSMANN, H. B., HAGOPIAN, A. & EYLAR, E. H. (1969). Cellular membranes: the biosynthesis of glycolipid and glycoprotein in HeLa cell membranes. *Archs Biochem. Biophys.* **130**, 573–583.

CHLAPOWSKI, F. J. & BAND, R. N. (1971*a*). Assembly of lipids into membranes in *Acanthamoeba palestinensis*. I. Observations on the specificity and stability of choline-^{14}C and glycerol-^{3}H as labels for membrane phospholipids. *J. Cell Biol.* **50**, 625–633.

CHLAPOWSKI, F. J. & BAND, R. N. (1971*b*). Assembly of lipids into membranes in *Acanthamoeba palestinensis*. II. The origin and fate of glycerol-^{3}H-labelled phospholipids of cellular membranes. *J. Cell Biol.* **50**, 634–651.

CLINE, M. J. (1966). Phagocytosis and synthesis of ribonucleic acid in human granulocytes. *Nature, Lond.* **212**, 1431–1433.

COHN, Z. A. (1966). The regulation of pinocytosis in mouse macrophages. I. Metabolic requirements as defined by the use of inhibitors. *J. exp. Med.* **124**, 557–571.

COLEMAN, R. & FINEAN, J. B. (1966). Preparation and properties of isolated plasma membranes from guinea pig tissues. *Biochim. biophys. Acta* **125**, 197–206.

DALLNER, G., SIEKEVITZ, P. & PALADE, G. E. (1966*a*). Biogenesis of endoplasmic reticulum membranes. I. Structural and chemical differentiation in developing rat hepatocyte. *J. Cell Biol.* **30**, 73–96.

DALLNER, G., SIEKEVITZ, P. & PALADE, G. E. (1966*b*). Biogenesis of endoplasmic reticulum membranes. II. Synthesis of constitutive microsomal enzymes in developing rat hepatocyte. *J. Cell Biol.* **30**, 97–117.

GOLDBERG, I. & OHAD, I. (1970*a*). Biogenesis of chloroplast membranes. IV. Lipid and pigment changes during synthesis of chloroplast membranes in a mutant of *Chlamydomonas reinhardi* Y-1. *J. Cell Biol.* **44**, 563–571.

GOLDBERG, I. & OHAD, I. (1970*b*). Biogenesis of chloroplast membranes. V. A radioautographic study of membrane growth in a mutant of *Chlamydomonas reinhardi* Y-1. *J. Cell Biol.* **44**, 572–591.

GOODALL, R. J. & THOMPSON, J. E. (1971). A scanning electron microscopic study of phagocytosis. *Expl Cell Res.* **64**, 1–8.

HUBSCHER, G. & WEST, G. R. (1965). Specific assays of some phosphatases in subcellular fractions of small intestinal mucosa. *Nature, Lond.* **205**, 799–800.

KING, E. J. (1932). The colorimetric determination of phosphorus. *Biochem. J.* **26**, 292–297.

KORN, E. D. & WEISMAN, R. A. (1967). Phagocytosis of latex beads by *Acanthamoeba*. II. Electron microscopic study of the initial events. *J. Cell Biol.* **34**, 219–227.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MICHELL, R. H. & HAWTHORNE, J. N. (1965). The site of diphosphoinositide synthesis in rat liver. *Biochem. biophys. Res. Commun.* **21**, 333–338.

OHAD, I., SIEKEVITZ, P. & PALADE, G. E. (1967). Biogenesis of chloroplast membranes. II. Plastid differentiation during greening of a dark-grown algal mutant (*Chlamydomonas reinhardi*). *J. Cell Biol.* **35**, 553–584.

OMURA, T. P., SIEKEVITZ, P. & PALADE, G. E. (1967). Turnover of constituents of the endoplasmic reticulum membranes of rat hepatocytes. *J. biol. Chem.* **242**, 2389–2396.

PASTERNAK, J. J., THOMPSON, J. E., SCHULTZ, T. M. G. & ZACHARIAH, K. (1970). A scanning electron microscopic study of the encystment of *Acanthamoeba castellanii*. *Expl Cell Res.* **60**, 290–298.

PENNINGTON, R. J. (1961). Biochemistry of dystrophic muscle. *Biochem. J.* **80**, 649–654.

SANDERS, E. J. & BELL, L. G. E. (1970). Inhibition of pinocytosis in *Amoeba proteus* by puromycin. *Expl Cell Res.* **63**, 379–384.

SCHULTZ, T. M. G. & THOMPSON, J. E. (1969). Enrichment of 5′-nucleotidase in membrane fragments isolated from *Acanthamoeba* sp. *Biochim. biophys. Acta* **193**, 203–211.

ULSAMER, A. G., SMITH, F. R. & KORN, E. D. (1969). Lipids of *Acanthamoeba castellanii*. Composition and effects of phagocytosis on incorporation of radioactive precursors. *J. Cell Biol.* **43**, 105–114.

ULSAMER, A. G., WRIGHT, P. L., WETZEL, M. G. & KORN, E. D. (1971). Plasma and phagosome membranes of *Acanthamoeba castellanii*. *J. Cell Biol.* **51**, 193–215.

WARREN, L. & GLICK, M. C. (1968). Membranes of animal cells. II. The metabolism and turnover of the surface membrane. *J. Cell Biol.* **37**, 729–746.

WEISMAN, R. A. & KORN, E. D. (1967). Phagocytosis of latex beads by *Acanthamoeba*. I. Biochemical properties. *Biochemistry, N.Y.* **6**, 485–497.

(*Received 7 February* 1972)