Calcium Transport by Macrophage Plasma Membranes*

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Phagocytic vesicles, inside-out plasma membrane vesicles purified from rabbit lung macrophages (Stossel, T. P., Mason, R. J., Pollard, T. D., and Vaughan, M. (1972) J. Clin. Invest. 51, 604–614), have a calcium transport system that pumps calcium from the cytoplasmic to the external membrane surface. The activity was dependent on the presence of Mg** and ATP, although ADP promoted 50% of the uptake observed in the presence of ATP; AMP, GTP, CTP, and UTP were inactive. The calcium transport reaction required oxalate for sustained uptake >10 min, and the ionophore A23187 (3.3 μM) released sequestered calcium, indicating uptake against an electrochemical gradient. Calcium uptake was ouabain- and azide-insensitive and temperature-dependent. The \( V_{\text{max}} \) of calcium uptake under optimal conditions was 2.25 nmol/mg of protein/min at 37°C; the apparent \( K_a \) for MgATP was 0.5 mM and for free Ca** was 0.48 μM. Trypsinization of the vesicles rapidly inactivated calcium uptake. Washing of vesicles with ethylene glycol bis(β-aminoethy ether)N,N,N',N'-tetraacetic acid (EGTA) decreased their calcium uptake by 50%, suggesting removal of endogenous calmodulin. Bovine brain calmodulin activated the calcium uptake activity of washed phagocytic vesicles by 2- to 3-fold. Trifluoroperazine, an agent that inhibits calmodulin-stimulated reactions in the presence of calcium, inhibited calcium uptake by 50%. A peripheral plasma membrane vesicle preparation prepared by gentle sonication of macrophages (Davies, W. A., and Stossel, T. P. (1977) J. Cell Biol. 75, 941–955) had a calcium uptake system with properties similar to those of phagocytic vesicles. The findings demonstrate the presence of a calcium pump that could be involved in the control of cytoplasmic movement.

Pulmonary macrophages are motile cells that respond to contact with suitable surfaces or micron-sized objects by undergoing movements that lead to spreading and phagocytosis. There is evidence that interactions of actin and other proteins in the cortical cytoplasm of macrophages provide the motor power for these movements and that variations in free calcium concentrations in the cortical cytoplasm of macrophages might control their directionality (1). If the plasma membrane maintains a large electrochemical gradient of calcium between the extracellular environment and the cytoplasm, a slight alteration of its activity could result in a considerable variation of cytoplasmic free calcium concentrations. If contact of the external surface of the plasma membrane with certain surfaces were somehow coupled to the calcium gradient-maintaining activity of the membrane, the interaction could lead to changes in peripheral cytoplasmic calcium levels.

We have studied calcium transport across the macrophage plasma membrane, using phagocytic vesicles. Phagocytic vesicles arise from the internalization of plasma membrane and thus constitute a system to study easily its inner surface. Furthermore, when prepared from macrophages that have ingested oil droplets, they can be purified rapidly by flotation with good yield and in an intact state (2). Using this approach, we have characterized a high affinity MgATP-dependent calcium pump located in the inner side of the plasma membrane.

MATERIALS AND METHODS

Macrophages—Cells were obtained from the lungs of New Zealand white rabbits by the procedure of Myrvik et al. (3) as described previously (4), 14 to 21 days after the animals received an intravenous injection of 1 ml of Freund's complete adjuvant (5) to increase cell yields. The cells suspended in 0.15 M NaCl were washed three times in the cold by centrifugation (250 × g, 10 min) and finally resuspended in a modified Krebs-Ringer phosphate medium (130 mM NaCl, 4 mM KCl, 1.3 mM MgCl2, 1 mM CaCl2, 10 mM sodium phosphate buffer, pH 7.4) (hereafter designated as "medium") and kept ice cold until use. Yields ranged from 3 × 10⁸ to 2.4 × 10⁹ cells/rabbit, of which more than 80% were macrophages.

Sera—Rabbit serum was obtained from freshly clotted blood derived from anesthetized animals by cardiac puncture. Sera were used on the same day or stored at −70°C until use.

Preparation of Phagocytic Vesicles—This technique, described in detail elsewhere (2), is outlined briefly below. Disododecylphosphate (1 ml) (practical grade, Matheson, Coleman and Bell, E. Rutherford, N. J.) was added to 3 ml of medium containing 20 mg/ml of bovine serum albumin, and the suspension was sonicated for 90 s to form oil droplets coated with albumin (albumin particles). In some experiments, Escherichia coli lipopolysaccharide 0.2686 (Difco) at a final concentration of 10 mg/ml was used instead of albumin (lipopolysaccharide particles).

Albumin or lipopolysaccharide particles were incubated with fresh or freshly thawed rabbit serum for 20 min at 37°C, during which time an opsonic fragment of the third component of complement deposited on them (6) (opsonized particles). Cells were suspended in medium and warmed to 37°C. Suspensions of opsonized particles (20%, v/v) and cells (5%, v/v) were mixed and agitated gently in a shaking bath at 37°C for 20 min. The number of particles ingested was routinely much greater when the lipopolysaccharide-coated droplets were employed, which made them more suitable for obtaining sufficient quantities of phagocytic vesicles. The cells which ingested opsonized particles were washed once in cold 0.15 M NaCl, suspended in deionized ice-cold water, and immediately centrifuged at 4°C. Exposure to water caused the cells to swell but not break, and made subsequent homogenization easier.

The cell pellets were suspended in an equal volume of ice-cold 0.34 M sucrose containing 5 mM EGTA, 5 mM dithiothreitol, 20 mM DFP, 5 mM ethylene glycol bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid; DFP, diisopropyl fluorophosphate.

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1 The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid; DFP, diisopropyl fluorophosphate.
imidazole-HCl buffer, pH 7.4, and several protease inhibitors, including 1 mM phenylmethylsulfonyl fluoride, 0.25 mM of a-1-antitrypsin, and 0.25 mM of soybean trypsin inhibitor. The cells were homogenized in this medium in a 40-ml Dounce homogenizer with a tight-fitting pestle. The progress of cell disruption was monitored as the percentage of free nuclei visible by phase contrast microscopy. About 25 strokes were required to break 90% of the cells. Ca" (CaCl2) was then added to a final concentration of 3 mM. The homogenates were then transferred to a 30-ml Sorvall centrifuge tube and carefully overlaid by means of a peristaltic pump (Pharmacia) with 2 volumes of a solution containing 100 mM KCl, 30 mM imidazole-HCl, pH 6.8, and centrifuged for 60 min at 150,000 x g. The floating white layer, representing phagocytic vesicles, was then carefully removed with a pipette and kept at ice temperature until it was further diluted for use. The protein concentration of phagocytic vesicles was determined by the Folin procedure (7). In some experiments, the cells were incubated with 5 mM disopropyl fluorophosphate (DFP) (Sigma) for 5 min at 0°C to inhibit serine proteases. Prior to homogenization, the cells were washed twice in cold 0.15 M NaCl.

Preparation of Peripheral Hyaline Vesicles of Macrophages—This preparation containing a mixture of right-side-out and inside-out blebs of plasma membrane, and virtually free of organelles, was prepared by gentle sonication of a warm suspension of cells according to the method published by techniques (12). The vesicles were then isolated from residual cell bodies by sedimenting the latter (250 g for 10 min) and pelleting the resultant supernatant. The pellet was washed in a solution containing 100 mM KCl, 30 mM imidazole-HCl, pH 6.8, and resuspended in the same solution and kept on ice until use. The calcium uptake by phagocytic vesicles or peripheral vesicles of macrophages was measured in the following medium: 100 mM KCl, 1 mg/ml of albumin, 30 mM imidazole-HCl buffer, pH 6.8, and various concentrations of MgCl2, ATP (pH adjusted to 6.8), and CaCl2, in a total volume of 1.5 ml. Phagocytic or peripheral vesicles, at concentrations of approximately 0.2 to 0.3 mg of protein/ml of medium, were warmed at 37°C in the above medium and, after 3 min, ammonium oxalate was added to a final concentration of 5 mM. The suspension was mixed and the reaction was started by the addition of CaCl2 (usually 25 mM containing 1 to 10 mM Ca" and the mixture was quickly agitated. Samples (150 ml) were taken from the mixture at defined intervals and added to 2 ml of solution containing 100 mM KCl, 30 mM imidazole-HCl, pH 6.8. Vesicles were trapped by vacuum filtration with a manifold (Millipore) on 0.45-µm pore filters (Millipore) which were washed before addition of test samples with 0.25 M KCl (2 ml), followed by water (11 ml). The filters containing vesicles were washed once with 2 ml of the same KCl/imidazole-HCl buffer and were dissolved in Aquasol (New England Nuclear) and counted for Ca" radioactivity in a Packard scintillation spectrometer. Calcium uptake rates were expressed as nanomoles/mg of protein/min after the values bound in the absence of ATP were subtracted. The free Ca" concentration of solutions was calculated using a model of the computer program of Perrin and Marcoux (9) as described by Potter and Gergely (10).

The fluoroperoxidase (Ste-lazine, kindly provided by Smith Kline and French) was preincubated with vesicles in the presence of 50 µM calcium at room temperature for 20 min. Calcium uptake was measured as previously described at 37°C, but the reaction was started with the addition of ATP. Ionomophore A23187 (Calbiochem) was dissolved in dimethylsulfoxide and then diluted with the incubation buffer to a final concentration of 3.3 mM. Control experiments were performed in order to ensure that dimethylsulfoxide at similar concentrations did not alter calcium uptake by the vesicles. When calculating the specific activity of calcium uptake, the values obtained were not corrected for the albumin coating the lipid incorporated into the phagocytic vesicles, estimated to represent 20% of the total protein (2). In some experiments using the same technique, 45Ca uptake by whole macrophages was also measured in a medium similar to that described above.

Trypsinization Procedure—Bovine pancreatic trypsin, type IX, treated with diphenyl carbamyl chloride to inactivate chymotrypsin, and soybean trypsin inhibitor, type T-S, were obtained from Sigma. Solutions of 10 mM Ca"/ml of each were made in 100 mM KCl, 30 mM imidazole-histidine buffer, pH 6.8. Vesicles were performed at 37°C. Prewarmed trypsin stock solution was added to make a final concentration of 1 mg/ml. After 5 or 10 min of incubation, soybean trypsin inhibitor was added to a final concentration of 1 mg/ml. The suspension was immediately placed on ice after mixing.

ATPase Activity—Vesicles were suspended as described for Ca" uptake in medium containing 100 mM KCl, 30 mM imidazole-HCl buffer, pH 6.8, and various concentrations of MgCl2 and [32P]ATP (New England Nuclear; 5 µCi/ml). For each condition, two sets of tubes were prepared, the first containing 50 µM CaCl2, and the second containing 1 mM EGTA. (Ca" + Mg")-ATPase (calcium-stimulated Mg"ATPase) was estimated as the difference between the tubes containing calcium and the tube containing EGTA. 3P was determined by the method of Martin and Doty (11) as described by Shamoo et al. (12).

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Results

Uptake of Calcium by Phagocytic Vesicles—Fig. 1 shows a time course of calcium uptake by phagocytic vesicles from alveolar macrophages. In the absence of ATP, approximately 1.5 nmol of Ca"/mg of protein became associated with the vesicles within the 1st min of incubation and thereafter did not increase further. The uptake of calcium by phagocytic vesicles was measured at 37°C in a medium containing 100 mM KCl, 30 mM imidazole-HCl buffer, pH 6.8, and 0.25 mg/ml of soybean trypsin inhibitor. The cells were treated with diphenyl carbamyl chloride to inactivate chymotrypsin, and pelleting the resultant supernatant. The pellet was washed in a solution containing 100 mM KCl, 30 mM imidazole-HCl buffer, 2.5 mM MgCl2, pH 6.8. The collected phagocytic vesicles were further diluted in 4 volumes of the above, still containing EGTA, and kept at ice temperature with occasional agitation for 30 min. Sucrose and CaCl2 were then added to final concentrations of 100 and 1 mM, respectively, and the phagocytic vesicles were overlaid by means of a peristaltic pump with a solution containing 100 mM KCl, 30 mM imidazole-HCl buffer, 2.5 mM MgCl2, 100 mM CaCl2, pH 6.8, in Sorvall centrifuge tubes and spun at 40,000 x g for 10 min. The phagocytic vesicle fraction was suspended in the same calcium-containing buffer to which 10 µCi/mg of Ca was added for measurement of the effect of calmodulin on Ca uptake. The phagocytic vesicles were incubated with various concentrations of bovine brain calmodulin (13) for 1 h at ice-cold temperature, with occasional agitation. The overlay solution used for separation of the phagocytic vesicles was prepared by gentle sonication of a warm suspension of cells according to the method published by techniques (12) and then further diluted in 4 volumes of the above, still containing EGTA, and kept at ice temperature with occasional agitation for 30 min. The overlay solution was then removed with a pipette and the overlay solution was washed twice in cold 0.15 M NaCl.

The overlay solution used for separation of the phagocytic vesicles was then carefully removed with a pipette and diluted in 4 volumes of the above, still containing EGTA, and kept at ice temperature with occasional agitation for 30 min. The overlay solution was then removed with a pipette and the overlay solution was washed twice in cold 0.15 M NaCl.

The overlay solution used for separation of the phagocytic vesicles was then carefully removed with a pipette and washed twice in cold 0.15 M NaCl. The overlay solution used for separation of the phagocytic vesicles was then carefully removed with a pipette and washed twice in cold 0.15 M NaCl.
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not increase above this basal level. The presence of both Mg
and ATP was essential for calcium uptake, which was continuous for about 10 min. Addition of the calcium ionophore A23187 (3.3 µM) to the vesicles after 10 min of incubation with magnesium and ATP caused a rapid release of calcium from the loaded vesicles to basal levels, demonstrating that accumulation occurred against an electrochemical gradient (14).

The presence of oxalate as a trapping agent for calcium was not increase above this basal level. The presence of both Mg
and ATP was essential for sustained calcium uptake that persisted for about 30 min.

U7P, CTP, or GTP did not support Ca2+ uptake, and ADP was considerably less effective than was ATP (Table I). Calcium uptake by the phagocytic vesicles was temperature-dependent, being reduced at 25°C to 48% of the activity at 37°C, and was not detectable at 4°C.

Ouabain did not inhibit calcium uptake. Sodium azide (Table II), at concentrations that almost totally inhibit calcium uptake by mitochondria from several tissues (15, 16), had no effect on the uptake of calcium by phagocytic vesicles. The rate of calcium uptake by phagocytic vesicles was not very different between pH 6.6 and 7.2, but declined markedly when the pH was beyond these limits (not shown). Fig. 2 shows the effect of free Ca2+ concentrations on uptake by phagocytic vesicles. Using the values up to 1.5 × 10^{-7}M, the calculated K_a for free calcium was 0.48 ± 0.01 µM with a V_max of 2.25 nmol/mg of protein/min. At higher free Ca2+ concentrations, an increase of calcium associated with the phagocytic vesicles was observed in the absence of ATP, suggesting leakage or nonspecific calcium oxalate precipitation. If these non-ATP-dependent values are subtracted from the values in the presence of ATP, it appears that the active uptake system is saturable with respect to free calcium concentration.

Fig. 3 shows the calcium uptake rate by phagocytic vesicles as a function of the MgATP concentration. Calcium uptake

![FIG. 2. Ca2+ uptake by phagocytic vesicles of rabbit alveolar macrophages in the presence of oxalate as a function of free Ca2+ concentration.](image1)

![FIG. 3. MgATP-dependent calcium uptake by phagocytic vesicles as a function of MgATP concentration.](image2)

reached maximum values at about 2.5 mM MgATP, and the apparent K_a for MgATP was about 0.5 mM.

If the phagocytic vesicles were kept at ice temperature for several hours, a decrease in their calcium uptake activity was observed (about 40% decrease after 4 h). This decline in activity could be prevented if the macrophages were treated with DFP before homogenization.

Trypsinization of Phagocytic Vesicles—This experiment was performed to see whether a protein component of the calcium transport system was accessible to trypsin. Trypsinization rapidly decreased the Mg2+-ATP-dependent calcium uptake, with 83.6 and 87.4% inhibition after 5 and 10 min, respectively, of trypsin treatment.

Uptake of Calcium by Peripheral Vesicles—Fig. 4 shows a typical time course of calcium uptake by peripheral vesicles which was very similar to that of phagocytic vesicles, although of lower specific activity. The presence of both magnesium and ATP was essential for calcium uptake, which was contin-

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**TABLE I**
**Calcium uptake by phagocytic vesicles: temperature and nucleotide requirements**

The incubation medium was the complete system described in the legend to Fig. 1, except for the nucleotides which were those shown and which were present at a concentration of 5 mm. The procedure for measuring the calcium uptake is described in the legend to Fig. 1 and in the text.

| Nucleotide | Temperature °C | Calcium uptake nmol/mg protein/20 min |
|------------|---------------|-------------------------------------|
| MgATP      | 37            | 45                                  |
|            | 25            | 22                                  |
|            | 4             | 0.9                                 |
| MgADP      |               | 23                                  |
| MgUTP      |               | 1.0                                 |
| MgCTP      |               | 1.6                                 |
| MgGTP      |               | 1.7                                 |

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**TABLE II**
**ATP-dependent calcium uptake by phagocytic vesicles: effect of azide, ouabain, NaCl, and boiling**

The incubation medium was the complete system described in the legend to Fig. 1. The procedure for measuring the calcium uptake is described in the legend to Fig. 1 and in the text. Vesicles were boiled for 1 min.

| Additions to incubation medium | Calcium uptake nmol/mg protein/20 min |
|-------------------------------|-------------------------------------|
| Control (none)                | 45                                  |
| Ouabain (2 mM)                | 44                                  |
| Azide (5 mM)                  | 47                                  |
| KCl (50 mM) + NaCl (50 mM)    | 43                                  |
| Boiled vesicles               | 0.7                                 |
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Calcium uptake by peripheral plasma membrane vesicles prepared by gentle sonication of rabbit alveolar macrophages. The incubation medium and the experimental procedure are described in the legend to Fig. 1. Ionophore A23187 was added at 6 min of incubation of a sample without oxalate.

Addition of the calcium ionophore A23187 (3.3 μM) to vesicles after 6 min of incubation with magnesium and ATP caused a release of calcium from the loaded vesicles to basal levels, demonstrating accumulation against an electrochemical gradient. In the presence of oxalate, uptake was continuous for about 30 min. Trypsinization (1 mg/ml) rapidly decreased calcium uptake with more than 70% inhibition being evident after 5 min of trypsin treatment.

Experiments were also done to examine ATP dependence and trypsin sensitivity of calcium uptake by intact macrophages, as a homogeneous population of right-side-out membrane. Unlike phagocytic or peripheral vesicles, calcium uptake did not require the addition of ATP. 45Ca uptake in a similar medium and under the same experimental conditions at 25 μM total added calcium (10 μCi/ml) was 10,047 ± 2,923 cpm/mg of protein/20 min (mean ± S.E. of three experiments) and was not affected by previous trypsin treatment (1 mg/ml of trypsin for 10 min at 37°C) of the cells.

ATPase Activity of Phagocytic Vesicles—Under conditions that promoted maximal calcium uptake, phagocytic vesicles had a powerful Mg2+ATPase activity in the absence of added calcium, and calcium stimulation of ATP hydrolysis could not be detected with consistency.

Calmodulin Stimulation of Calcium Transport into Phagocytic Vesicles—Vesicles were washed in buffer containing EGTA in order to remove endogenous calmodulin. They were then preincubated in the presence of calcium and various amounts of calmodulin binding to the vesicles. After 3 min at 37°C, the calcium uptake reaction was started by the addition of ATP.

About 2 nmol of calcium/mg of protein were associated with the phagocytic vesicles at zero time in all the incubations. In the absence of ATP and various amounts of calmodulin, the values thereafter did not increase. In the presence of ATP and no added calmodulin, calcium uptake values were approximately 50% lower than those of vesicle preparations not washed in EGTA. Under these conditions, the calcium uptake was constant with time for about 3 min. In the presence of calmodulin, a stimulation of calcium transport rates in the vesicles was observed.

Fig. 5 shows stimulation of calcium uptake by various calmodulin concentrations. Two- to three-fold increases in calcium uptake rates were detected at added calmodulin con-

Fig. 4. Calcium uptake by peripheral plasma membrane vesicles prepared by gentle sonication of rabbit alveolar macrophages. The incubation medium and the experimental procedure are described in the legend to Fig. 1. Ionophore A23187 was added at 6 min of incubation of a sample without oxalate.

Fig. 5. Effect of calmodulin on the initial rate of calcium uptake by phagocytic vesicles. For these experiments, vesicles from macrophages that had been treated with DFP before homogenization were first washed in EGTA as described under "Materials and Methods," and then preincubated with 100 μM calcium chloride and various amounts of calmodulin for 1 h at 0°C in the presence of 100 mM KCl, 30 mM imidazole-HCl buffer, pH 6.8, and 2.5 mM MgCl2. The vesicles were warmed for 3 min at 37°C and the reaction was started by the addition of 2.5 mM ATP. Samples (50 μl) were removed at zero time and filtered every minute thereafter, up to 6 min, and 45Ca was determined as described in the text.

Fig. 6. Effect of trifluoperazine on calcium uptake by phagocytic vesicles. The incubation medium is the complete system described in the legend to Fig. 1. For these experiments, vesicles were preincubated in medium in the presence of 50 μM calcium and various concentrations of trifluoperazine for 30 min at 25°C, followed by 3 min at 37°C. Then 5 mM oxalate was added and the reaction was started by the addition of ATP. After 12 min at 37°C, two 150-μl samples were withdrawn and filtered, and 45Ca was determined as described in the text.
centrations of 1 to 10 µg/ml, depending on the preparation used on different days. With the addition of ionophore A23187 (3.3 µM), a rapid release of calcium occurred to basal levels. As shown in Fig. 6, incubation of vesicles with calcium and trifluoroperazine inhibited calcium uptake. This inhibition was about 50% of control at trifluoroperazine concentrations of 50 µM. By contrast, no inhibition of calcium uptake was detected when vesicles that had been washed twice in an EGTA-containing buffer were incubated in the presence of trifluoroperazine (≤200 µM) and calcium.

**DISCUSSION**

We have characterized an ATP-dependent calcium transport system of phagocytic vesicles of alveolar macrophages. Compared to other techniques of plasma membrane purification in which sideness may be heterogeneous and purity often doubtful, phagocytic vesicles offer several advantages. First, they arise from the invagination of plasma membrane and thus constitute a homogeneous preparation of inside-out plasma membrane vesicles. Second, they can be obtained in high purity. Particularly, they are relatively free from other potent intracellular calcium transport systems, mitochondria and endoplasmic reticulum, as shown by enzyme markers and electron microscopy (12). A possible disadvantage of this vesicular preparation is its intravacuolar lysosomal content arising from fusion of lysosomes with the plasmalemma during phagocytosis. Since the calcium pump is destroyed rapidly by trypsin, its loss of activity after some hours, even at ice-bath temperature, which is preventable by use of DFP, is probably ascribable to leakage of lysosomal enzymes from a small fraction of ruptured vesicles. Another theoretical disadvantage is that phagocytic vesicles might not be representative of the composition of plasma membrane as a whole because of fusion of the vesicle membranes with lysosomal membranes or else because of lateral segregation of membrane molecules out of the vacuoles during phagocytosis (17). However, the significance of these possible disadvantages was diminished by the finding of an energy-dependent calcium transport system with the features of the phagocyte vesicle system in peripheral plasma membrane vesicles prepared by gentle sonication of macrophages. These vesicles are peripheral blebs of macrophage plasma membrane which are representative of the total cell plasma membrane with respect to the marker adenylate cyclase and externally labeled proteins (8). Electron micrographs of these vesicles contain some empty sacs which have filaments on their outer surfaces (8). Although the bulk of these blebs appear to be right-side-out vesicles, presumably these blebs appear to be right-side-out vesicles, presumably the measured calcium uptake took place in the subpopulation of inverted vesicles. The calcium uptake by peripheral vesicles was trypsin-sensitive, whereas calcium uptake by intact macrophages was not, suggesting that right-side-out vesicles do not have this transport system.

The calcium transport system of macrophage vesicles shares several properties with intracellular calcium transport systems described previously, e.g. mitochondria (18) and sarcoplasmic reticulum (19), including those of red cell membranes (20). The macrophage system has a requirement for the presence of Mg\(^{2+}\)-ATPase activity, associated with the vesicles, a parallel hydrolysis of ATP coupled to transport (\(\text{Ca}^{2+} + \text{Mg}^{2+}\)-ATPase) could not be shown. Recently, an ATPase activity with high calcium affinity (24) has been described in a putative plasma membrane fraction from rabbit alveolar macrophages. However, the ATPase activity described was active in the absence of magnesium, an ion required in our system as well as in most mammalian subcellular calcium transport systems (25).

The component of the calcium transport system susceptible to tripticy inactivation is on the cytoplasmic face of peripheral vesicles. Since rabbit pulmonary macrophages contain lysosomal proteases that are presumably delivered into phagocytic vacuoles during phagocytosis (26), the existence of a protease-sensitive component on the external face of the membrane seems unlikely. DFP, a potent serine protease inhibitor, prevented the loss of calcium pump activity prior to storage. Therefore, the loss of activity is more likely due to leakage of lysosomal enzymes which attack the cytoplasmic component of the pump than to destruction of a component at the external membrane surface.

Calcium, a ubiquitous heat-stable 16,700-dalton calcium-binding protein that confers calcium sensitivity to several enzymes, stimulates active calcium transport into inside-out vesicles of red blood cell membranes (27). Several experiments showed that calmodulin has a similar effect on calcium transport of the macrophage plasma membrane vesicles. First, washing the vesicles in EGTA decreased calcium transport rates, suggesting that the washing removed endogenous calmodulin (28). Second, addition of purified calmodulin in the presence of calcium led to a dose-dependent activation of calcium transport into phagocytic vesicles. Maximal activation was obtained at lower calmodulin concentrations (0.05 to 0.52 µM) than those estimated to be present in cells in which it had been measured (1 to 2 µM) (29, 30). Finally, trifluoperazine, a drug that binds calmodulin in the presence of calcium, concomitantly inhibiting calmodulin-activated reactions (31, 32), inhibited calcium transport into macrophage phagocytic vesicles. In preliminary studies, we have also observed that boiled extracts of macrophages activated phosphodiesterase in a calcium-dependent manner which is consistent with the presence of calmodulin in these extracts. Calmodulin is a soluble protein rather than an intrinsic membrane protein. Thus, the possibility that certain soluble proteins may interact with the cytoplasmic face of the plasma membrane of the macrophage to modulate active calcium transport is also established.

Most studies to date of the relationship between calcium and leukocyte functions concerned effects of extracellular calcium on these functions (e.g. Ref. 33–35) or changes in concentration of membrane (36), tracer (37, 38), or total (39) calcium levels of leukocytes. These studies reported complex changes in the measured parameters following surface stimulation of leukocytes with phagocytosable particles or chemotactic peptides consistent with a possible involvement of calcium changes in some cellular compartment in the effects of stimulation. Phagocytosis by rabbit lung macrophages can take place in the absence of extracellular calcium (33), indicating that a calcium influx may not create the changes in cytosolic calcium concentrations presumably associated with the movement of phagocytosis. When compared with muscle functions coupled to changes in calcium concentrations, macrophage movement occurs at a much slower rate (seconds to tens of seconds) and is not an all-or-none phenomenon, and movements may be localized to certain regions of the plasma.
membrane. Therefore, a localized calcium efflux controlled by a plasma membrane calcium pump could have a function in the coupling of membrane stimulation to cytoplasmic movement.

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