Fc-Receptor-mediated Phagocytosis Occurs in Macrophages without an Increase in Average [Ca^{++}]$_i$

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Abstract. The calcium ion has been implicated as a cytosolic signal or regulator in phagocytosis. Using the Ca^{++}-sensitive photoprotein aequorin we have measured intracellular free Ca^{++} ion concentration ([Ca^{++}]$_i$) in thioglycolate-elicited mouse peritoneal macrophages during phagocytosis and IgG-induced spreading. Macrophages plated on glass were loaded with aequorin and [Ca^{++}]$_i$ was then measured from cell populations, both as previously described (McNeil, P. L., and D. L. Taylor, 1985, *Cell Calcium,* 6:83–92). Aequorin indicated a resting [Ca^{++}]$_i$ in adherent macrophages of 84 nM and was responsive to changes in [Ca^{++}]$_i$ induced by the addition of Mg-ATP (0.1 mM) or serum to medium. However, during the 15 min required for phagocytosis of seven or eight IgG-coated erythrocytes per macrophage loaded with aequorin, we measured no change in [Ca^{++}]$_i$. Similarly, the ligation of Fc-receptors that occurs when

macrophages spread on immune complex–coated coverslips did not change macrophage [Ca^{++}]$_i$. In contrast, a rise in [Ca^{++}]$_i$ of macrophages was measured during phagocytosis occurring in a serum-free saline of pH 7.85, and as a consequence of incubation with quin2 A/M. We estimate that had a change in [Ca^{++}]$_i$ occurred during phagocytosis, aequorin would have detected a rise from 0.1 to 1.0 μM taking place in as little as 2% of the macrophage's cytoplasmic volume. We therefore suggest that either Ca^{++} is not involved as a cytoplasmic signal for phagocytosis or that increases in [Ca^{++}]$_i$ during phagocytosis are confined to such small regions of cytoplasm as to be below the limits of detection by our cellular averaging method. Our data emphasizes, moreover, the need for well-defined, nonperturbing conditions in such measurements of [Ca^{++}]$_i$.

PHAGOCYTOSIS is initiated when Fc-receptors on the macrophage's plasma membrane engage IgG molecules coating the surface of a particle (17, 18). Movement of enveloping pseudopods around the particle's circumference and fusion of those pseudopods with one another complete particle engulfment (11). Importantly, ingestion of one surface-bound particle by a macrophage is not accompanied by indiscriminate ingestion of other particles (3). The requirement for specific receptor-ligand interactions and the segmental nature of the phagocytic response led Silverstein et al. (17, 18) to suggest that signals regulating particle engulfment are generated within and remain restricted to the segment of cytoplasm immediately adjacent to plasma membrane containing ligated Fc-receptors. It will therefore be of fundamental importance to establish the chemical identity of the cytoplasmic signal for phagocytosis, as well as the location and kinetics of its generation.

The calcium ion has been proposed as a cytoplasmic signal for phagocytosis (4, 20), and recent quin2 measurements suggest that a considerable change in total cytoplasmic free Ca^{++} occurs upon phagocyte challenge with opsonized parti-

cles (7, 26). In this paper we have used the Ca^{++}-sensitive photoprotein aequorin to measure the intracellular free calcium ion concentration ([Ca^{++}]$_i$)[1] of thioglycolate-elicited mouse peritoneal macrophages during Fc-receptor-mediated phagocytosis and spreading. Both events occurred without detectable changes in [Ca^{++}]$_i$ when assayed in culture medium of pH 7.4. A striking rise in [Ca^{++}]$_i$ of macrophages was, however, measured during phagocytosis in a saline of pH 7.8. We therefore suggest either that a rise in [Ca^{++}]$_i$ is not necessary for phagocytosis or that rises in [Ca^{++}]$_i$ are confined to such highly localized regions of cytoplasm as to be below the limits of detection by our methods.

Materials and Methods

Cell Preparation and Loading with Aequorin

Thioglycolate-elicited mouse peritoneal macrophages, obtained as described (22), were plated on 60-mm glass petri dishes (which had been washed in alkaline detergent) at confluent densities (1–3 × 10^7 cells/dish) in Dulbecco's

1. *Abbreviations used in this paper:* [Ca^{++}]$_i$, intracellular free Ca^{++} ion concentration; DNP, dinitrophenol; EA, IgG-coated erythrocyte(s).

modified Eagle's medium with 10% heat-inactivated fetal bovine serum (M10F). 1 h later, cultures were washed three times with Ca-Mg-free PBS (containing 10 μM EDTA), and aequorin was introduced into the macrophage cytoplasm by scrape-loading (8, 10). Cells (10^5) were then placed in Sykes-Moore chambers and allowed to recover for 5–18 h at 37°C in M10F in a CO_2 (5%) incubator before use in experiments, unless stated otherwise.

Preparation of Surfaces for Phagocytosis

Sheep erythrocytes were opsonized with rabbit anti-sheep erythrocyte IgG (Cappel Laboratories, Cochranville, PA). Coverglasses suitable for Sykes-Moore chambers were coated with dinitrophenol (DNP) as described (25), coated with immune complexes by incubation for 45 min in a 1/30 dilution of rabbit antisera against DNP, and washed three times with saline.

Measurement of [Ca⁺⁺]ᵢ using Aequorin

Aequorin luminescence was measured from 10^5 macrophages adherent to the lower glass coverslip of a Sykes-Moore chamber. The luminometer was similar to a previous design (8) but capable of photon counting. In experiments with erythrocytes, a mirror normally (8) placed over the Sykes-Moore chamber to reflect light downwards toward the photocathode was removed so that absorption of luminescence by erythrocytes in suspension and/or landing from above did not diminish measured resting or stimulated luminescence from aequorin in macrophage cytoplasm. The interior temperature of the luminometer housing surrounding the cells was maintained at 37°C by circulating warm air. Particles for phagocytosis or other compounds were placed in M10F (pH 7.4) at 37°C and gassed with CO_2 (5%), and then rapidly perfused (chamber volume was replaced three times in ~15 s) into cell chambers. Cell lysis (for determination of maximal luminescence rates) and the in vitro calibration of aequorin fractional luminescence as a function of [Ca⁺⁺], both necessary for estimating [Ca⁺⁺]ᵢ, were performed as previously described (8). Sykes-Moore chambers containing macrophages were removed from the luminometer for microscopic photography or for determination of phagocytic indices at various times during the measurements of [Ca⁺⁺]ᵢ.

Cell Treatment with Quin2 A/M

Quin2 A/M was purchased from Sigma Chemical Co. (St. Louis, MO) or Lancaster Labs (Windham, NH). Both products gave identical results. Quin2 A/M was stored as a 50 mM stock solution in dry dimethylsulfoxide at −30°C in the dark and desiccated until use, usually within 4 wk of receipt. Macrophages or J774 cells (10^5) in Sykes-Moore chambers were incubated for 90 min at 37°C in a 20-μM solution of quin2 A/M, which had been dissolved in M10F (pH 7.4) by dilution of the dimethylsulfoxide stock during vigorous vortexing. After quin2 A/M treatment, cells were washed with 4 vol fresh M10F. Any solution of quin2 A/M noticed microscopically to contain particulates was discarded.

Results

Resting [Ca⁺⁺]ᵢ of Macrophages

The resting intracellular free calcium ion concentration ([Ca⁺⁺]ᵢ) of adherent, thioglycolate-elicited mouse peritoneal macrophages was 84 nM (±38 SD; $n = 9$) as estimated using aequorin. The [Ca⁺⁺]ᵢ of suspended macrophage-like J774 cells was previously estimated using quin2 to be 87 nM (26). These are typical values for the [Ca⁺⁺]ᵢ of unstimulated mammalian cells.

[Ca⁺⁺]ᵢ of Macrophages during Phagocytosis of Erythrocytes

During 15 min of phagocytosis of IgG-coated erythrocytes (EA), macrophage [Ca⁺⁺]ᵢ measured using aequorin did not change (Fig. 1). Average counts per second from aequorin luminescence (over a background dark count of 28 ± 11) were 75 (±52 SD; $n = 6$) when measured between 1 and 2 min after control perfusions of culture medium to macrophages loaded with aequorin, and were subsequently 77 (±47) when measured from the same populations between 1 and 2 min after perfusion of EA. After making such measure-
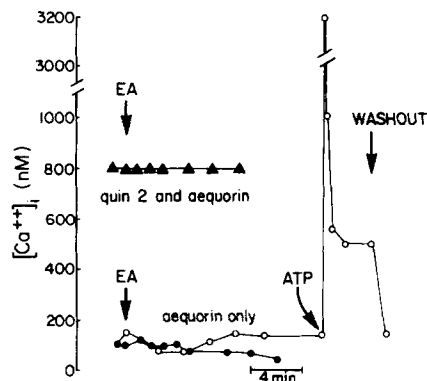

Figure 1. Intracellular free Ca⁺⁺ ion concentration ([Ca⁺⁺]ᵢ) of adherent mouse macrophages during phagocytic challenge. IgG opsonized erythrocytes (EA) (~10^8/ml) in M10F were perfused to untreated macrophages loaded with aequorin (● and ○) or macrophages previously incubated in 20 μM quin2 A/M for 90 min (▲). One population of macrophages was perfused with ATP (0.1 mM) in M10F after 15 min of phagocytosis (○).

Table I. Phagocytosis by Macrophages Loaded with Aequorin or Fluoresceinated Dextran

Experiment	Percent macrophages phagocytosing EA	Phagocytic index
Macrophages scrape-loaded in fluoresceinated dextran*		
Fluorescent	86	650
Not Fluorescent	83	750
Macrophages scrape-loaded in aequorin‡		
Untreated	100	660
Quin 2	14	2

EA (3 ml at 10^8 per ml) in M10F (37°C, 5% CO_2) were presented for phagocytosis in Sykes-Moore chambers for 15 min (during which time [Ca⁺⁺]ᵢ was monitored in those cells loaded with aequorin; see Fig. 1A); uningested EA were lysed with NH₄Cl₂ (150 mM, pH 7.0); cultures were rinsed with 3 vol M10F; and EA ingested per macrophage ($n = 50$) were counted using phase-contrast microscopy. Macrophages loaded with fluoresceinated dextran were scored as fluorescent (or not) using a 40× Zeiss phase-contrast lens (0.6 numerical aperture). The phagocytic index [(No. EA ingested/No. macrophages scored) × percentage macrophages phagocytosing] for erythrocytes not opsonized with IgG was <0.1.
* See Fig. 2, A and B.
‡ See Fig. 1A.

ments of macrophage [Ca⁺⁺]ᵢ for 15 min in the presence of EA, we determined from microscopic counts of the same cells that an average of 6.5 EA had been ingested per macrophage, and that >99% of the macrophage had ingested at least one EA (Table I). Thus, phagocytosis was clearly occurring during our measurements of [Ca⁺⁺]ᵢ.

The efficiency of loading aequorin into the macrophages by the scrape-loading method has been estimated to be ~67%. When macrophages were loaded by scraping with a 10-kD fluoresceinated dextran (approximately the same radius of gyration of aequorin, 20 kD) and examined by fluorescence microscopy, 33% of these cells were not visibly fluorescent. However, such nonfluorescent macrophages phagocytosed to an extent comparable to those fluorescent members of the same population subject to scrape-loading (Table I). Fluoresceinated dextran loaded by scraping was present throughout the macrophage cytoplasm (Fig. 2), including the cytoplasm
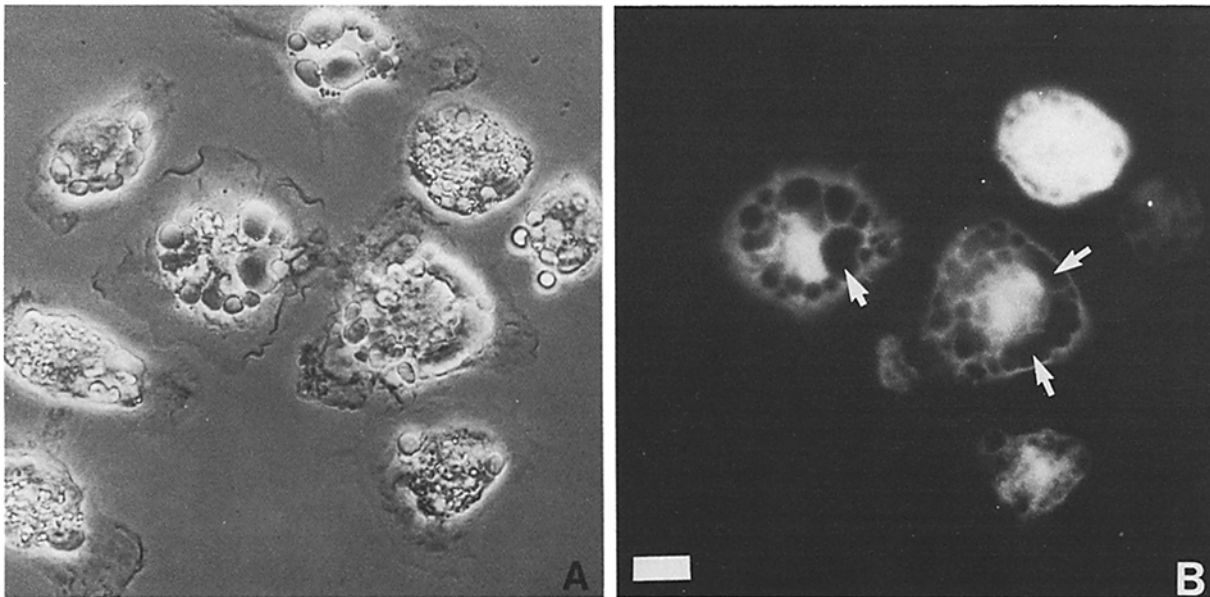
Figure 2. Photomicrographs of living macrophages loaded by scraping with fluoresceinated dextran. (*A* and *B*) Macrophages were scrape-loaded (glass substratum) in 10 kD fluorescein-dextran (10 mg/ml dissolved in 150 mM KCl, 5 mM Hepes, pH 7.0; see reference 10 for general methodology). 5 h later these fluorescent cells were challenged with EA for 15 min, and their phase (*A*) or fluorescence (*B*) images were recorded photographically. Note that the fluoresceinated dextran is present throughout macrophage cytoplasm but excluded from phagolysosomes (arrows) containing ingested EA. Bar, 10 μm.

surrounding the forming phagosome (not shown). These experiments and the low resting $[Ca^{++}]_i$ measured with aequorin suggest that the luminescent signal we measured came from healthy, functionally competent macrophages, and that lack of a measurable increase in $[Ca^{++}]_i$ during phagocytosis cannot be explained by a subpopulation of macrophages that were loaded with aequorin but that remained phagocytically inactive.

$[Ca^{++}]_i$ of Macrophages during Frustrated Phagocytosis

As a second means of stimulating Fc-receptors, we provided macrophages with an opsonized surface too large for ingestion, namely, a coverglass coated with IgG-containing immune complexes. A rapid exaggerated spreading response and tight circumferential adherence of the macrophage's ventral surface to the IgG-coated substratum are two consequences of such stimulation, termed frustrated phagocytosis (25). An advantage of this system is that measurement of $[Ca^{++}]_i$ during frustrated phagocytosis can be carried out in the absence of light-absorbing particles such as red blood cells.

To induce frustrated phagocytosis, we plated macrophages, 30 or 60 min after loading with aequorin, on DNP-coated coverglasses coated with anti-DNP IgG (25). As controls, macrophages were plated on DNP-coated coverglasses that had not been treated with anti-DNP IgG. During 30 min on these two substrata, dramatic spreading was observed on the DNP/anti-DNP IgG-coated coverglass but not on the coverglass coated with DNP alone (Fig. 3, *A* and *B*). As further expected of frustrated phagocytosis (25), macrophages containing aequorin that had spread upon the DNP/anti-DNP IgG-coated coverglass excluded fluoresceinated anti-substrate antibodies from the zone of contact with the IgG-coated surface (Fig. 3, *C* and *D*).

$[Ca^{++}]_i$ of both frustrated phagocytes and control cells differed by no more than 50 nM during a 150-min interval of spreading, over which $[Ca^{++}]_i$ dropped in both populations (Fig. 4*A*). This drop in $[Ca^{++}]_i$ may be the result of cell attachment since $[Ca^{++}]_i$ of cells in suspension was ~300–400 nM whether cells were given 30, 60, or 90 min to recover in suspension after scrape-loading. $[Ca^{++}]_i$ dropped to 100–200 nM within 60 min in cells plated immediately after scrape-loading. The membrane potential of suspended J774 cells is depolarized relative to that of attached J774 cells (21), suggesting, in conjunction with our measurements of $[Ca^{++}]_i$, that several aspects of cytoplasmic ion physiology are dependent on attachment to a substratum. It is also possible that attachment facilitates recovery of macrophage $[Ca^{++}]_i$ after the scrape-loading procedure.

As a second means of inducing frustrated phagocytosis, macrophages plated 5–18 h previously on DNP-coated coverglasses were subsequently perfused with anti-DNP. This method of inducing frustrated phagocytosis also failed to produce any detectable rise in $[Ca^{++}]_i$ (Fig. 4*B*).

$[Ca^{++}]_i$ of Macrophages during Phagocytosis of Latex Beads

In addition to erythrocyte and glass surfaces opsonized with IgG, we also challenged macrophages with latex spheres coated with bovine serum albumin (BSA) and spheres coated with BSA/anti-BSA. Both types of spheres were phagocytosed by the cells, but neither caused any change in macrophage $[Ca^{++}]_i$ (data not shown).

Responsiveness of Aequorin as a Calcium Indicator in the Macrophage

Aequorin in the macrophage cytoplasm was clearly responsive to agents that mobilize cytosolic calcium. For example, ATP
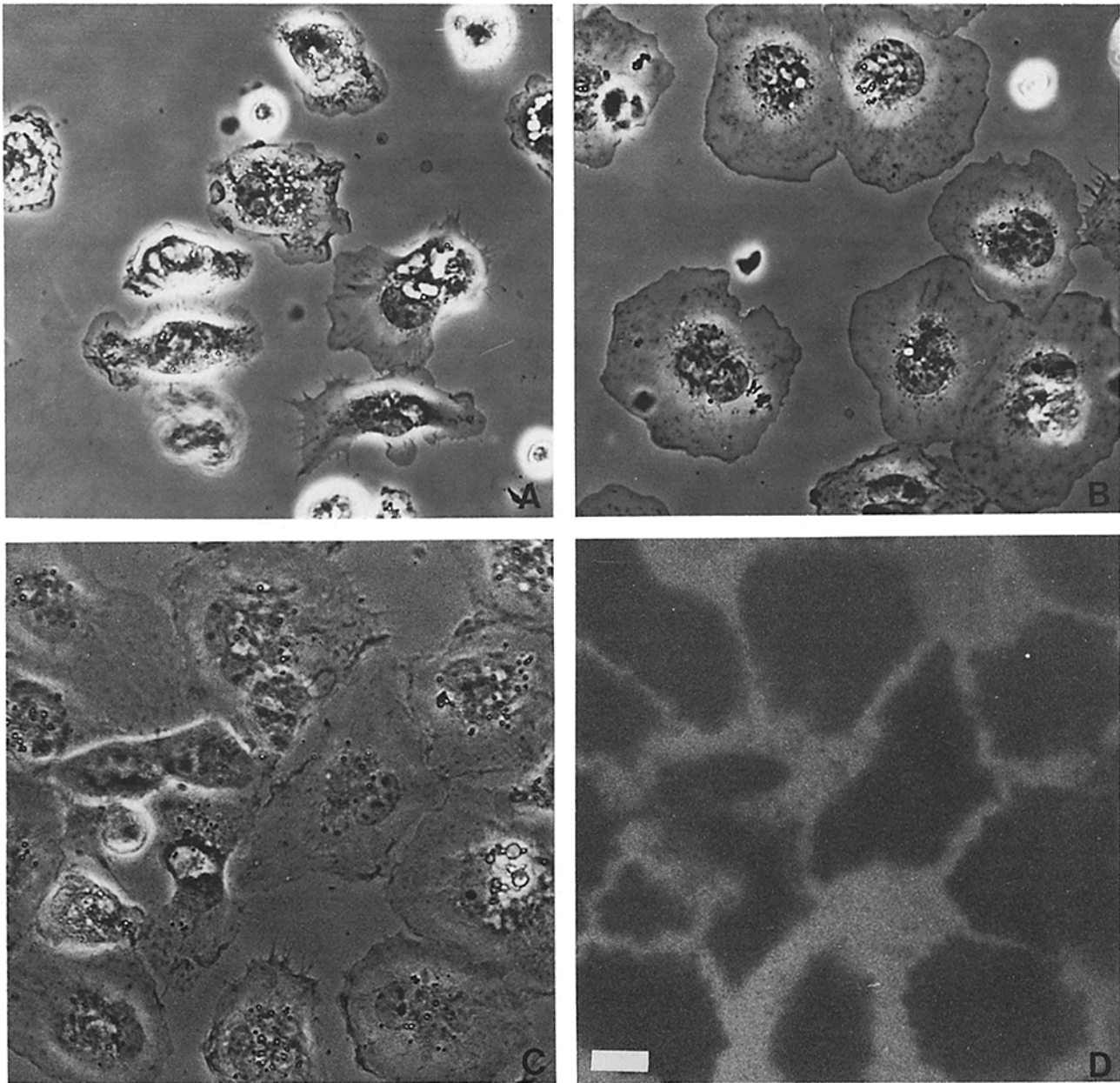
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Figure 3. Photomicrographs of macrophages whose $[Ca^{++}]_i$ was measured during frustrated phagocytosis. (*A* and *B*) Macrophages were scrape-loaded in aequorin, plated 30 min later on either a DNP-coated (*A*) or DNP/anti-DNP IgG-coated (*B*) coverglass, and then photographed after $[Ca^{++}]_i$ (Fig. 4*A*) was recorded for 30 min more. Note the well-spread appearance of macrophages on the DNP/anti-DNP IgG but not on the DNP-only coverglass. (*C* and *D*) Macrophages were scrape-loaded in aequorin and plated on a DNP/anti-DNP-coated coverglass for 30 min as in *B*, and then stained for 1 h at 37°C with a 1/20 dilution of fluoresceinated goat anti-rabbit IgG and washed with 3 vol M10F. Note that fluoresceinated antibody stain for anti-DNP IgG has been excluded from beneath the macrophages (*D*), as is characteristic of frustrated phagocytosis. Bar, 10 μM.

(0.1 mM) presented after 15 min of phagocytosis of EA produced an immediate and transient rise in macrophage $[Ca^{++}]_i$ to a peak value of 3.3 μM (Fig. 1). Higher doses of ATP (1–5 mM) produced even larger, prolonged (minutes) rises, but they could not be fully reversed by washing the ATP out of the chambers (data not shown). A similar but smaller rise in $[Ca^{++}]_i$ has been obtained with J774 cells using quin2 as the indicator (21). Perfusion of serum to macrophages deprived of serum for 12 h also raised the $[Ca^{++}]_i$ of these cells (data not shown).

The Effect of Quin2 and Medium Composition on Macrophage $[Ca^{++}]_i$

Both of the published quin2 studies (7, 26) differed from ours in three respects: quin2 had been loaded by cytoplasmic esterase activity into cells stirred during phagocytosis in a Hepes-buffered, serum-free saline solution. When we incubated macrophages loaded with aequorin for 90 min in 20 μM quin2 A/M (as described in reference 26), we recorded no change in aequorin luminescence upon challenge with EA
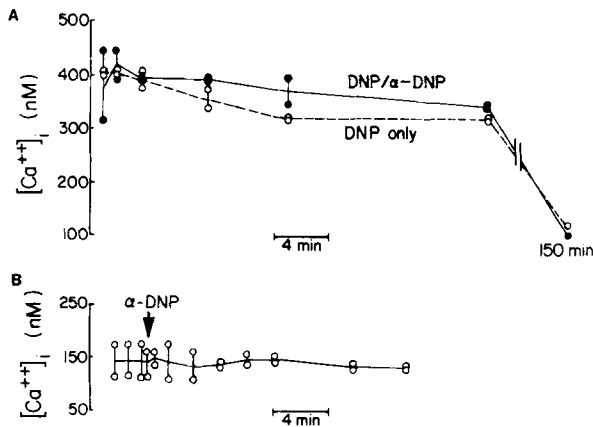
Figure 4. [Ca^{++}]$_i$ of macrophages during frustrated phagocytosis. (*A*) Macrophages (in replicate experiments) were plated on coverglasses coated with DNP only (O) or with DNP/anti-DNP IgG (●) 30 or 60 min after loading of aequorin. The [Ca^{++}]$_i$ of both populations is initially elevated, probably because [Ca^{++}] of suspended cells does not recover to levels typical of macrophages allowed to adhere to the substrate before measurement (as in *B*). (*B*) Anti-DNP IgG (diluted 1:30 in M10F) was perfused (in replicate experiments) to macrophages previously adherent for 18 h to a coverglass coated with DNP.

(Fig. 1). However, such quin2 loading did raise the resting [Ca^{++}]$_i$ of these cells, monitored with aequorin, almost 10-fold, to ~1 μM. We also observed that such adherent quin2-loaded cells could not phagocytose normally (Table I) and had assumed rounded morphologies. Inhibition of phagocytosis by quin2 in Ca^{++}-free medium has previously been reported (7, 26) and was interpreted to result from the Ca^{++}-buffering action of this dye. When we incubated J774 cells with 20 μM quin2 A/M (under conditions identical to those used for the macrophages), phagocytosis by these cells was unaffected as compared with that of control J774 cells not treated with quin2 A/M (data not shown). Thus, the macrophage appears to be peculiarly susceptible to quin2 toxicity.

When macrophages were challenged with EA in Hepes-buffered saline, pH 7.4, rather than culture medium, we failed similarly to record any change in [Ca^{++}]$_i$ monitored with aequorin. However, if the pH of this NaHCO$_3$-containing medium was raised to 7.85 by exposure to air, a small rise in resting and a much larger rise in phagocytosing [Ca^{++}]$_i$ were detected (Fig. 5).

Discussion

Aequorin is extremely sensitive to, and unlikely to buffer, local changes in [Ca^{++}]$_i$ (8, 9). Between the physiologically relevant Ca^{++} concentrations of 0.1 and 10 μM, the measured fractional luminescence rate of aequorin increases by >1,000-fold. By contrast, the fluorescence of quin2 increases only twofold over this concentration range. Therefore, whereas quin2 might provide accurate estimates of resting [Ca^{++}]$_i$ and of uniform changes in [Ca^{++}]$_i$ occurring across the cytoplasmic space not exceeding micromolar levels, it would report transient, localized changes in [Ca^{++}]$_i$ poorly. Conversely, if the activation of cytoplasm during phagocytosis involves local, transient mobilization of Ca^{++}, aequorin should be of considerable advantage in detecting the change. Indeed, in a direct comparison of the two indicators, aequorin measured an order of magnitude greater thrombin-induced
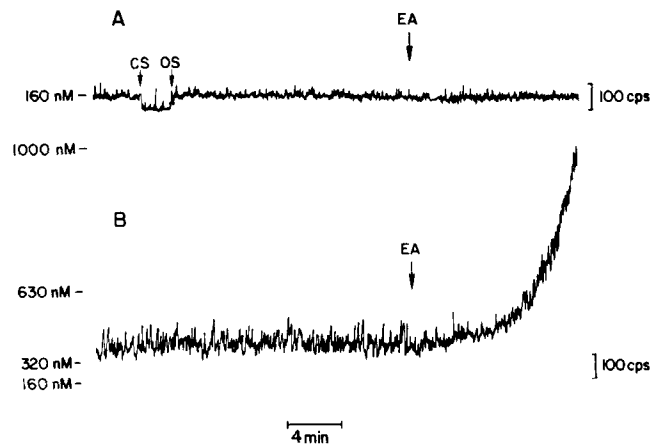

Figure 5. Effect of medium pH on macrophage [Ca^{++}]$_i$ during phagocytosis. Macrophage [Ca^{++}]$_i$ was measured for 30 min in (*A*) M10F or (*B*) a buffered, serum-free saline (20 mM Hepes, 8.5 mM Na$_2$CO$_3$, 118 mM NaCl, 5 mM KCl, 1.8 mM CaCl$_2$, 0.8 mM MgSO$_4$, and 5.5 mM glucose, initial pH 7.4) whose pH had risen to 7.85 upon prolonged exposure to air. EA suspended in M10F (*A*) or saline, pH 7.85 (*B*) were then added at the arrow. Resting [Ca^{++}]$_i$ was greater and less steady in macrophages kept in the buffered saline (*B*) than was the [Ca^{++}]$_i$ of cells in M10F (*A*), and a rise in macrophage [Ca^{++}]$_i$ was observed in saline, pH 7.85, during phagocytosis. No such rise was measured in the same saline, pH 7.4. *CS*, close shutter; *OS*, open shutter.

rise in platelet [Ca^{++}]$_i$ than did quin2 (5). Similarly, growth factor–induced rises in fibroblast [Ca^{++}]$_i$ were 5–50 times larger measured with aequorin than in independent measurements made with quin2 (9).

Increases in [Ca^{++}]$_i$ during phagocytosis have previously been measured using quin2 in both neutrophils (7) and J774 cells (26), but the characteristics of such increases were kinetically and quantitatively very different in these two phagocytes. Upon challenge of J774 cells with EA, the fluorescence of quin2 rose immediately and decayed slowly thereafter (26). These data are indicative of an immediate and sustained rise in [Ca^{++}]$_i$ to micromolar levels throughout J774 cytoplasm. By contrast, an increase in quin2 fluorescence was not detected until 2–3 min after presentation of IgG or C3 coated yeast to neutrophils, and [Ca^{++}]$_i$ did not peak until after 5–6 min (7). These measurements indicated a maximal, sustained rise of average [Ca^{++}]$_i$ to 300 nM (from a resting level of 120 nM).

Had a rise from 0.1 to 0.3 or 3.0 μM [Ca^{++}]$_i$ occurred throughout the cytoplasm of phagocytosing macrophages, as implied by previous studies of neutrophils (7) and J774 cells (26), respectively, then the expected rise in aequorin luminescence rate would have been 4.0 (neutrophils) or 254.0-fold (J774 cells). However, using aequorin loaded into adherent, actively phagocytic cells maintained under well-defined culture conditions, we could not confirm the results obtained in the above studies with quin2. We conclude that a generalized or total cytoplasmic rise in [Ca^{++}]$_i$ above resting levels does not occur in phagocytosing macrophages under buffer conditions accepted as physiological, and is therefore not necessary for macrophage phagocytosis. We emphasize, moreover, the need for well-defined nonperturbing conditions in such measurements of [Ca^{++}]$_i$.

There are many potential reasons for the differences in the

calcium data presented here and those described in neutrophils (8) and J774 cells (7). First, the cell types were different. Second, the environment of the cells could have been different. Finally, the calcium indicator in our studies was aequorin, whereas the other two investigations used quin2. These issues must be addressed in future studies.

In the neutrophil, a significant generalized rise in $[Ca^{++}]_i$ may not be involved in phagocytosis. When changes in $[Ca^{++}]_i$ detected with quin2 were visualized in chemotaxing/phagocytosing neutrophils, $[Ca^{++}]_i$ was slightly elevated (up 29 nM) in that segment of cytoplasm nearest the source of chemoattractant (15). Importantly, in the absence of exogenous Ca^{++} no changes in $[Ca^{++}]_i$ were detected in any region of the neutrophil despite the fact that both of these motile events still occurred (15). Unfortunately, we have two concerns with this latter study that make it difficult for us to evaluate the significance. First, quin2 is not an optimal calcium indicator (23). Second, the use of ratio imaging to make spectroscopic measurements in living cells is exciting, but there are important parameters of imaging technology such as temporal and spatial signal to noise ratio and probe distribution and mobility that were not addressed (1).

The question remains whether highly localized changes in $[Ca^{++}]_i$ might be involved in phagocytosis by macrophages. Working from approximations of the volume of cytoplasm activated during phagocytosis and the duration of such activation, we can estimate roughly the minimum rise in $[Ca^{++}]_i$ that would have been detected by aequorin. On average the macrophage ate one EA every 2–3 min (from our measurements of phagocytic index). If each such event took 2–5 min, then on average, each macrophage was continuously ingesting one EA. Phagocytic envelopment proceeds by repeated local generation of cytoplasmic signals (16). From electron microscopic and immunofluorescence micrographs it appears that reorganization of the cytoskeleton and organelle exclusion occur below the point of particle attachment to a depth ~0.5 μm (2, 12, 14, 19). If extended to this depth for the diameter of an EA (~8 μm), a cylinder of activated cytoplasm would occupy a volume of ~25 μm^3. Each macrophage (~15 μm diam) has a volume of ~1,800 μm^3. Thus, ingestion of a single EA would engage ~1.4% of the total macrophage volume. If $[Ca^{++}]_i$ had risen from 0.1 to 3 μM in a region of cytoplasm of this volume, the luminescence rate would have risen 254-fold there, producing an easily detectable increase in whole cell signal (photon counts) of 356%. If, however, the rise in $[Ca^{++}]_i$ had been only to 0.3 μM in this localized region, then the luminescence rate there would have risen only fourfold, giving a measured increase of only 5.6%. Our methods are not sensitive enough to detect a 5.6% increase averaged over the whole cell.

There is considerable circumstantial evidence that calcium may be involved in regulating cytoplasmic structure and contractility in phagocytic cells (20, 24). The activities of gelsolin, nonmuscle alpha-actinin, and calmodulin are all regulated in vitro by $[Ca^{++}]$ between 0.1 and 1.0 μM. However, a variety of Ca^{++}-independent mechanisms may also exist. For example, dissociation of the profilin/actin complex is caused in vitro by phosphatidylinositol 4,5-biphosphate, and results in actin polymerization, which in vivo would be expected to occur adjacent to regions of ligand activated membranes (6). Our data cannot rule out calcium as a regulator of phagocytosis, if such regulation is confined to <1.4% of the cytoplasmic volume, and involves a rise in $[Ca^{++}]_i$ of <0.5 μm in that domain. A final assessment of the role of Ca^{++} in phagocytosis by the macrophage must therefore await measurements of $[Ca^{++}]_i$ made in cytoplasm immediately surrounding the forming phagosome, and this will require a sensitive, nonperturbing microscopic assay. Direct imaging of aequorin luminescence from single cells may answer the question. Alternatively, Fura-2, a new fluorescent calcium indicator (13), could yield important results when it has been characterized in more detail.

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