# Desensitization of the Neutrophil Aggregation Response to Chemotatic Factors

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**In the presence of $Ca^{2+}$ and $Mg^{2+}$, the chemotactic fragment of C5, the synthetic chemotactic oligopeptide formyl-methionly-leucyl-phenyl-alanine, and the ionophore A23187 aggregated human neutrophils. Aggregation induced by the two chemotactic factors was transient and reversed within 2 to 4 minutes after exposure; aggregation induced by A23187 was sustained and continued to increase over 15 minutes. In the absence of the bivalent cations, none of these three agents aggregated the cells. If bivalent cations were added after cell contact with a chemotactic factor, aggregation was detected after, but not before, addition of the cations. Under these conditions, the magnitude of the aggregation response was sharply reduced: cells preincubated with a chemotactic factor for longer than 2 to 4 minutes aggregated minimally after addition of bivalent cations. Moreover, cells preincubated with a chemotactic factor for 4 minutes, exposed to bivalent cations, and then rechallenged with the same chemotactic factor also showed a minimal aggregation response, ie, the cells were "desensitized" to the original stimulus. However, cells desensitized to one of the chemotactic factors still aggregated prominently when exposed to the other chemotactic factor or to A23187. Cells could not be desensitized to the ionophore A23187. Desensitization of the neutrophil aggregation response closely resembles desensitization of mast cell and leukocyte degranulation. Degranulation and aggregation appear to be closely related cellular responses to immunologic stimuli. Both responses may reflect alterations in surface membrane permeability to bivalent cations and/or changes in surface membrane adhesiveness to other biologic membranes. (Am J Pathol 93:693–706, 1978)**

BIVALENT CATIONS play an important role in mast cell and leukocyte degranulation. In the absence of $Ca^{2+}$ and $Mg^{2+}$, mast cells [1-9] and basophils [10-17] release little or no histamine when exposed to antigenic or anaphylatoxic stimuli; under similar conditions, polymorphonuclear neutrophils (PMNs) release submaximal amounts of their lysosomal constituents when exposed to chemotactic factors.[18-21] Moreover, agents such as the ionophore A23187, which are known to transport bivalent cations across biologic membranes,[22,23] induce mast cell [2,5,7,8,24,25] and PMN [19,21,26-28] degranulation. Finally, specific antigen or A23187 stimulates mast cells to take up $Ca^{2+}$ [2,5,7] and chemotactic factors or A23187 stimulates PMNs to do the same.[19,29-33] It has been postulated, therefore, that changes in cytosolic $Ca^{2+}$ modulate cellular degranulation.[1-9,13,19-21,24-28,32,34]

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Further evidence bearing on the role of bivalent cations in the degranulation response has come from studies on mast cell and basophil desensitization. Mast cells incubated with specific antigen *before* exposure to bivalent cations release less histamine than cells incubated with antigen after exposure to bivalent cations.[4,5,7,8] The longer the preincubation period of cells with antigen before exposure to bivalent cations, the less histamine is released. This desensitization was found even when cells were restimulated with the antigen after exposure to bivalent cations. However, these antigen-desensitized cells released nearly normally in response to A23187 or dextran.[5,8] Basophils respond similarly: cells can be desensitized to specific antigen or to anaphylatoxin and then can be made to degranulate when exposed to the opposite releasing agent.[10-17] These results have suggested to some investigators that certain immunologic stimuli induce responding cells to open $Ca^{2+}$ gates in their surface membrane.[5,7,8] These gates close spontaneously and are not reopened by repeated stimulation with the same agent. Apparently, releasing agents do not require extracellular bivalent cations to stimulate these changes. Thus, in the presence of extracellular $Ca^{2+}$, releasing agents stimulate $Ca^{2+}$ influx and, therefore, cellular degranulation; in the absence of extracellular $Ca^{2+}$, releasing agents desensitize the cells.

We have studied the aggregation of PMNs induced by chemotactic factors [35-42] and have found this PMN response to have many similarities to chemotactic-factor–induced PMN degranulation. Both PMN responses require extracellular $Ca^{2+}$[35,37-40] and are enhanced by cytochalsin B,[36,40] high concentrations of extracellular $Ca^{2+}$,[37,40] and extracellular phosphates.[40] Lanthanum,[40] cellular antimetabolites,[36] and antagonists [41] or inactivators [42] of chemotactic factors inhibit both responses. Because of these similarities and the recent finding that chemotactic-factor–induced PMN degranulation can be desensitized in a manner somewhat similar to mast cell and basophil desensitization,[43,44] we studied the desensitization of the aggregation response of human PMNs.

## Materials and Methods

### Chemotactic Factors

The chemotactic fragment of C5 (C5fr) and the chemotactic tripeptide formyl-methionyl-leucyl-phenylalanine (FMLP) were obtained and used as previously described.[35] The C5fr was employed in a final protein concentration of 1 mg/ml of PMN suspension. The majority of this protein consisted of albumin which separates poorly from the C5fr.

### Compounds and Buffers

The buffer used throughout these studies was a modified Hanks' balanced salt solution containing (mM): NaCl, 130; KCl, 5.5; $Na_2HPO_4$, 0.6; $NaH_2PO_4$, 0.6; glucose, 10; and tris,

25. Where indicated, $Ca^{2+}$ and $Mg^{2+}$ were added to the buffer in the form of chloride salts. The ionophore A23187 was a gift of Dr. Robert Hammill (Eli Lilly Company, Indianapolis, Ind). The A23197 and FMLP were dissolved in dimethylsulfoxide which, in the concentrations used here (less than 0.1%), did not influence PMN aggregation. All chemicals were of reagent grade or better and the chemotactic factors, bivalent cations, and buffers were made pH 7.4 before use.

### Isolation of Neutrophils

Normal human whole blood was centrifuged over Ficoll-Hypaque discontinuous gradients to obtain leukocyte populations containing greater than 96% PMNs, as previously described.[38]

### Aggregation Assay

The isolated PMNs were freed of contaminating erythrocytes by hypotonic lysis and then were washed and suspended (4600 cells/$\mu$l) in the Hanks' buffer. All subsequent studies were performed in a 37 C room and all reagents were made 37 C before use. The assay was performed as previously described.[38,48] One milliliter of the PMN suspension was placed in a plastic vial and stirred continuously with a magnetic bar. After 1 minute of equilibration, $Ca^{2+}$ and $Mg^{2+}$, where indicated, were added to the suspension. Following another 2 minutes of equilibration, a small volume (ie, less than 60 $\mu$l) of an aggregating substance was added to the suspension and, at ¼, ½, 1, 2, 4, 8, and 15 minutes after this addition, 25-$\mu$l samples were taken from the suspension and analyzed in the Coulter Counter system. In other experments, cells were equilibrated without bivalent cations, exposed to an aggregating substance, and then, at various times thereafter, treated with $Ca^{2+}$ and $Mg^{2+}$. Samples were taken from the suspension at the above specified times after addition of the bivalent cations. Finally, in some experiments, cells were equilibrated without bivalent cations for 3 minutes, exposed to an aggregating substance for 4 minutes, and then treated with bivalent cations. One minute after addition of the cations, a second aggregating substance was added to the PMN suspension and samples were taken at the times specified above.

As soon as they were obtained, samples were immediately diluted in 10 ml of Isoton solution (Coulter Electronics, Hialeah, Fla) and analyzed in the Coulter Counter system. The Coulter Counter was a Model ZBI (Coulter Electronics, Hialeah, Fla). The counter was set at the following values for amplification, aperture, and upper threshold: ¼, 4, and infinity, respectively. For each sample, the particle concentration enumerated at a lower threshold of 10 (called the "T particle concentration") and the particle concentration enumerated at a lower threshold of 80 (called the "A particle concentration") were obtained. Since at a lower threshold of 10 all PMNs in a sample are enumerated, whereas at a lower threshold of 80 only particles larger than 1.8 times the size of an unaggregated PMN are enumerated,[38] T is the total particle concentration and A is the large or aggregated particle concentration. Results for a given sample of the PMN suspension are reported as the large particle percentage (100 × A/T).

### Studies on A23187 Fluorescence

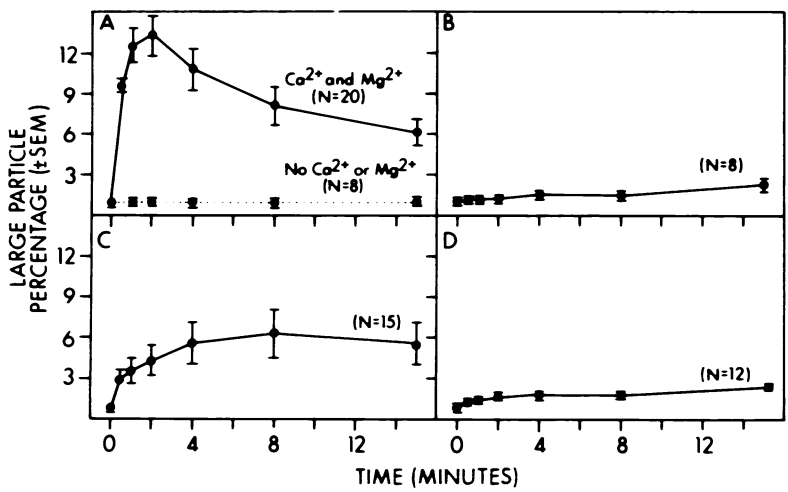
The effect of albumin or a chemotactic factor on the fluorescent emission of A23187 was measured with a Perkin-Elmer fluorescence spectrophotometer Model 4A (Wilton, Conn) set at a sensitivity range of 3 and a gain of 3. The A23187 was dissolved at a concentration of $3.3 \times 10^{-7}$ M in the modified Hanks' buffer containing 0.0008% dimethylsulfoxide, 1.4 mM $Ca^{2+}$, and 0.7 mM $Mg^{2+}$. Excitation and emission were performed and recorded at 380 mM and 440 mM, respectively. The solvent alone did not fluoresce under these conditions. The influence of salt-poor human albumin (National Red Cross product) or chemotactic factor on the fluorescence of the A23187 was found by adding the albumin (1

mg/cc, final concentration), C5fr (1 mg protein/cc, final concentration), or FMLP ($5 \times 10^{-8}$ M, final concentration) directly to the A23187 solution. The albumin and FMLP did not fluoresce by themselves. The C5fr did fluoresce slightly under these conditions, and this fluorescence was considered when analyzing its influence on A23187 fluorescence.

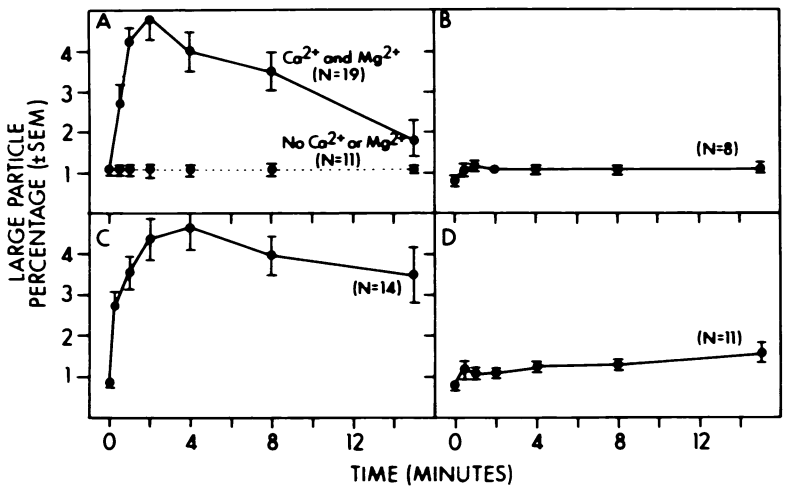
## Results

The PMNs suspended in medium containing $Ca^{2+}$ and $Mg^{2+}$ transiently aggregated after exposure to FMLP or C5fr: the large particle percentage of these suspensions rose to a maximum within 2 to 4 minutes of exposure to the chemotactic factor and fell thereafter (Text-figures 1A and 2A, *solid lines*). When bivalent cations were omitted from the suspending medium, these changes did not occur (Text-figures 1A and 2A, *interrupted lines*). Several nonchemotactic proteins and peptides, when added to PMNs suspended in media containing both bivalent cations, did not aggregate the cells.[38,40] Thus, $Ca^{2+}$ and $Mg^{2+}$, as well as a chemotactic factor, are required for the aggregation response of PMNs.

If bivalent cations were added to the PMN suspensions after a chemotactic factor, aggregation was detected after, but not before, addition of




TEXT-FIGURE 1—Aggregation and desensitization of PMNs induced by FMLP. A—Large particle percentage found after adding $5 \times 10^{-8}$ M FMLP (final concentration) to PMNs suspended in media with (*solid line*) or without (*interrupted line*) $Ca^{2+}$ and $Mg^{2+}$. B—Large particle percentage found after adding $Ca^{2+}$ and $Mg^{2+}$ to PMNs preincubated with $5 \times 10^{-8}$ M FMLP for 4 minutes in the absence of bivalent cations. C—Large particle percentage found after adding 50 $\mu$l/ml C5fr to PMNs which had been preincubated with $5 \times 10^{-8}$ M FMLP for 4 minutes and then exposed to bivalent cations for 1 minute. D—Large particle percentage found after adding $5 \times 10^{-8}$ M FMLP (final concentration) to PMNs which had been preincubated with $5 \times 10^{-8}$ M FMLP for 4 minutes and then exposed to bivalent cations for 1 minute. For all studies the final concentrations of the bivalent cations were 1.4 mM $Ca^{2+}$ and 0.7 mM $Mg^{2+}$.




TEXT-FIGURE 2—Aggregation and desensitization of PMNs induced by C5fr. A—Large particle percentage found after adding 50 μl/ml C5fr to PMNs suspended in media with (*solid line*) or without (*interrupted line*) $Ca^{2+}$ and $Mg^{2+}$. B—Large particle percentage found after adding $Ca^{2+}$ and $Mg^{2+}$ to PMNs preincubated with 50 μl/ml C5fr for 4 minutes in the absence of bivalent cations. C—Large particle percentage found after adding $5 \times 10^{-8}$ M FMLP (final concentration) to PMNs which had been preincubated with 50 μl/ml C5fr for 4 minutes and then exposed to bivalent cations for 1 minute. D—Large particle percentage found after adding 50 μl/ml C5fr to PMNs which had been preincubated with 50 μl/ml C5fr for 4 minutes and then exposed to bivalent cations for 1 minute. For all studies the final concentrations of the bivalent cations were 1.4 mM $Ca^{2+}$ and 0.7 mM $Mg^{2+}$.

the cations. Table 1 shows the large particle percentages found after adding $Ca^{2+}$ and $Mg^{2+}$ to PMN suspensions preincubated with FMLP for varying periods: the changes in the large particle percentages found after adding bivalent cations diminished as the duration the cells were preincubated with FMLP was lengthened. Studies using C5fr instead of FMLP produced nearly identical results (not shown). Cells preincubated with either chemotactic factor for longer than 2 to 4 minutes aggregated minimally after exposure to bivalent cations (Text-figures 1B and 2B). Thus, in the presence or absence of bivalent cations, PMNs appear to lose their reactivity 2 to 4 minutes after exposure to a chemotactic factor, ie, cells pretreated with bivalent cations before exposure to a chemotactic factor began to disaggregate 2 to 4 minutes after the exposure and cells preincubated with a chemotactic factor before treatment with bivalent cations aggregated minimally when the preincubation period exceeded 2 to 4 minutes (compare Text-figures 1A and 2A with Table 1 and Text-figures 1B and 2B).

The PMNs also aggregated in response to A23187 (Text-figure 3A, *solid lines*), and, like the aggregation induced by chemotactic factors, this PMN response also required bivalent cations (Text-figure 3A, *interrupted line*).

Table 1—Influence of the Sequence and Time of Addition of FMLP and Bivalent Cations on the Aggregation of Neutrophils*

| Time after starting aggregation (min) | Time (min) $Ca^{2+}$ and $Mg^{2+}$ added after FMLP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | −2† | 0‡ | ¼ | ½ | 1 | 2 | 4 | 8 |
| 0 | 1.0 ± 0.1§ | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.6 ± 0.1 |
| ¼ | 1.5 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 |
| ½ | 9.3 ± 1.0 | 6.3 ± 0.9 | 4.7 ± 0.6 | 3.7 ± 0.3 | 2.6 ± 0.3 | 1.8 ± 0.1 | 1.2 ± 0.1 | 0.8 ± 0.1 |
| 1 | 12.6 ± 1.3 | 8.7 ± 1.3 | 6.3 ± 0.8 | 5.2 ± 0.5 | 3.2 ± 0.4 | 1.4 ± 0.1 | 1.5 ± 0.1 | 1.3 ± 0.1 |
| 2 | 14.3 ± 1.4 | 10.9 ± 1.4 | 7.8 ± 2.3 | 6.8 ± 0.6 | 3.9 ± 0.5 | 1.5 ± 0.2 | 1.2 ± 0.1 | 1.4 ± 0.3 |
| 4 | 10.8 ± 1.4 | 10.8 ± 1.6 | 8.0 ± 1.5 | 6.8 ± 1.0 | 4.2 ± 0.8 | 1.6 ± 0.2 | 1.6 ± 0.2 | 1.4 ± 0.1 |
| 8 | 8.1 ± 1.3 | 7.3 ± 1.2 | 5.6 ± 1.4 | 5.0 ± 1.0 | 3.5 ± 0.5 | 1.7 ± 0.1 | 1.5 ± 0.1 | 1.2 ± 0.1 |
| 15 | 6.2 ± 1.1 | 3.8 ± 0.4 | 4.0 ± 0.6 | 4.2 ± 0.9 | 3.2 ± 0.6 | 2.5 ± 0.3 | 2.5 ± 0.4 | 1.5 ± 0.1 |
| N‖ | 30 | 15 | 12 | 10 | 7 | 6 | 8 | 3 |

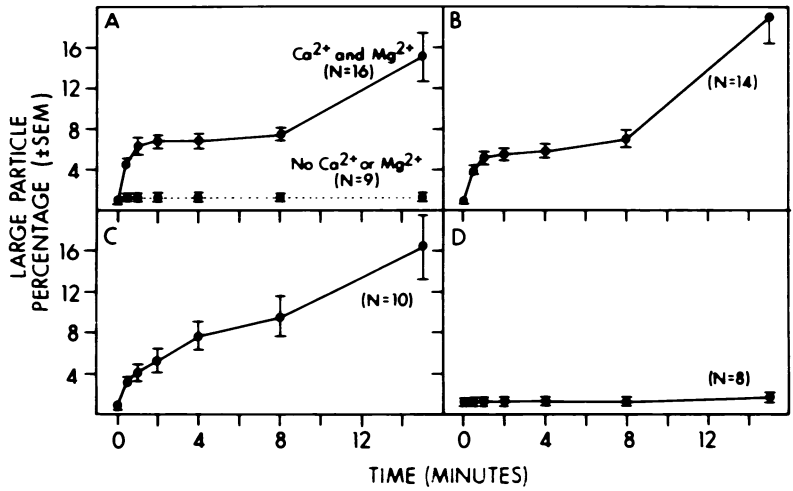
* Cells were preincubated with FMLP in the absence of bivalent cations for the indicated time and were then exposed to $Ca^{2+}$ and $Mg^{2+}$. The large particle percentage was obtained at the indicated time after adding the cations.

† Cells were preincubated with bivalent cations for 2 minutes and then exposed to FMLP. The large particle percentage was obtained at the indicated time after adding FMLP.

‡ Bivalent cations and FMLP were added simultaneously and the large particle percentage was obtained at the indicated time thereafter. For all studies the final concentrations of bivalent cations and chemotactic factor were 1.4 mM $Ca^{2+}$, 0.7 mM $Mg^{2+}$, and $5 \times 10^{-8}$ M FMLP.

§ Large particle percentage ± SEM

‖ Number of separate experiments performed




TEXT-FIGURE 3—Influence of A23187 on PMN aggregation. A—Large particle percentage found after adding $3.3 \times 10^{-7}$ M A23197 (final concentration) to PMNs suspended in media with (*solid line*) or without (*interrupted line*) $Ca^{2+}$ and $Mg^{2+}$. B—Large particle percentage found after adding $Ca^{2+}$ and $Mg^{2+}$ to PMNs preincubated with $3.3 \times 10^{-7}$ M A23187 (final concentration) for 4 minutes in the absence of bivalent cations. C—Large particle percentage found after adding $3.3 \times 10^{-7}$ M A23187 (final concentration) to PMNs which had been preincubated with $5 \times 10^{-8}$ M FMLP for 4 minutes and then exposed to bivalent cations for 1 minute. D—Large particle percentage found after adding $3.3 \times 10^{-7}$ M A23187 (final concentration) to PMNs which had been preincubated with 50 μl/ml C5fr for 4 minutes and then exposed to bivalent cations for 1 minute. For all studies the final concentrations of the bivalent cations were 1.4 mM $Ca^{2+}$ and 0.7 mM $Mg^{2+}$.

In striking contrast to aggregation induced by chemotactic factors, however, aggregation induced by A23187 was sustained and continued to increase over 15 minutes. Moreover, cells preincubated with A23187 in the absence of bivalent cations for 4 minutes aggregated normally when exposed to $Ca^{2+}$ and $Mg^{2+}$ (Text-figure 3B). Thus, in the presence or absence of bivalent cations, PMNs showed no decay in their reactivity to A23187.

Decay in PMN reactivity was also found when the cells were exposed to the original chemotactic factor a second time. Cells were preincubated with FMLP for 4 minutes in the absence of bivalent cations, exposed to $Ca^{2+}$ and $Mg^{2+}$, and, 1 minute thereafter, treated with FMLP. Figure 1D shows that these PMNs were unresponsive. Identical results were found when cells were preincubated with C5fr, exposed to $Ca^{2+}$ and $Mg^{2+}$, and then again treated with C5fr (Text-figure 2D). These results suggest that decay in PMN reactivity is not simply the result of a loss of chemotactic factor activity during the incubation period. When PMNs were preincubated with either FMLP or C5fr, exposed to $Ca^{2+}$ and $Mg^{2+}$, and then treated with the opposite chemotactic factor, aggregation did occur (Text-figures 1C and 2C). Although the magnitude of these aggregation re-

sponses was blunted, these results suggest that the loss of PMN reactivity is not due to cell refractoriness to all stimuli. This suggestion is further supported by studies with A23187. Cells were preincubated with FMLP for 4 minutes in bivalent-cation–free medium, exposed to $Ca^{2+}$ and $Mg^{2+}$ for 1 minute, and then treated with A23187. These cells aggregated normally in response to the ionophore (Text-figure 3C). Cells preincubated with C5fr did not respond to A23187 (Text-figure 3D). Although the cause for this latter inhibition was not found, human albumin, in a protein concentration equivalent to the protein concentration of the C5fr preparation (1 mg/ml), also inhibited A23187-induced aggregation although FMLP-induced aggregation was not affected (Table 2). In separate studies it was found that either C5fr or human albumin approximately doubled the fluorescence of A23187 (see *Materials and Methods*). The FMLP did not influence the A23187 fluorescence. These results suggest that the A23187 may interact with the two protein preparations. This interaction may render A23187 inactive in the aggregation assay. Alternatively, the two protein preparations may render PMNs unresponsive to A23187 without influencing the PMN response to chemotactic factors. In either case, the inhibition of A23187-induced aggregation by C5fr seems related to the protein content and not the aggregative activity of the C5fr preparation. Taken together, these results suggest that PMN desensitization is selective for the agent which induces it.

Table 2—Influence of Albumin on the Large Particle Percentage Induced by FMLP or A23187*

| | A23187 | | FMLP | |
|---|---|---|---|---|
| Time (min) | No albumin | Albumin (1 mg/cc) | No albumin | Albumin (1 mg/cc) |
| 0 | 0.8 ± 0.1† | 0.8 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| ½ | 4.7 ± 0.6 | 0.9 ± 0.2 | 9.3 ± 1.0 | 9.6 ± 1.6 |
| 1 | 6.3 ± 0.8 | 1.0 ± 0.2 | 12.6 ± 1.3 | 10.7 ± 1.6 |
| 2 | 6.9 ± 0.9 | 0.9 ± 0.1 | 14.3 ± 1.4 | 11.6 ± 2.0 |
| 4 | 6.9 ± 0.9 | 0.9 ± 0.2 | 10.8 ± 1.4 | 8.5 ± 1.5 |
| 8 | 7.4 ± 0.8 | 0.9 ± 0.1 | 8.1 ± 1.3 | 5.4 ± 1.5 |
| 15 | 15.1 ± 2.5 | 0.9 ± 0.1 | 6.2 ± 1.1 | 2.0 ± 0.2 |
| N‡ | 16 | 8 | 30 | 5 |

* Human PMNs were suspended in media with or without human albumin and then exposed to either $3.3 \times 10^{-7}$ M A23187 (final concentration) or $5 \times 10^{-6}$ M FMLP (final concentration). The media contained 1.4 mM $Ca^{2+}$ and 0.7 mM $Mg^{2+}$.

† ± SEM

‡ N is the number of separate experiments performed.

## Discussion

In the presence, but not in the absence, of $Ca^{2+}$ and $Mg^{2+}$, chemotactic factors transiently aggregate PMNs (Text-figures 1A and 2A). The effect abates 2 to 4 minutes after the cells are exposed to the chemotactic factor. If PMNs are preincubated with a chemotactic factor in the absence of bivalent cations for varying periods and then treated with bivalent cations, aggregation is detected after, but not before, the addition of the cations. The magnitude of this aggregation response decreases as the preincubation period lengthens (Table 1): when the duration of the preincubation period exceeds 2 to 4 minutes, aggregation is barely detectable (Text-figures 1B and 2B). Thus, in the presence or absence of bivalent cations, PMNs appear to lose their reactivity to chemotactic factors after 2 to 4 minutes of exposure. Mast cells and basophils react similarly: these cells show a decay in the magnitude of histamine release when preincubated with a releasing agent under conditions not supporting release.[4,5,7-17,45] Here also, the degree of decay increases as the preincubation period is lengthened, and the rate of this decay correlates with the rate of histamine release under control conditions.[45] Normally occurring histamine release, therefore, may be controlled and limited by cellular desensitization to the releasing substance.[45] A similar explanation may apply to the aggregation response of PMNs to chemotactic factors. Independently of extracellular bivalent cations, chemotactic factors may stimulate PMNs to form a state favoring aggregation. This state may be short-lived, and as it dissipates so may the tendency to form aggregates. Bivalent cations are necessary for aggregates to form. Hence, in the presence of bivalent cations, chemotactic factors stimulate self-limiting aggregation; in the absence of bivalent cations, chemotactic factors cannot stimulate this effect, although an underlying aggregation potential develops and then dissipates. When bivalent cations are added to PMNs after a chemotactic factor, the ensuing aggregation reflects the evolution of the underlying aggregation potential: aggregation is blunted.

In the presence but not the absence of bivalent cations, A23187 also aggregates PMNs (Text-figure 3A). Unlike the aggregation induced by chemotactic factors, however, A23187-induced aggregation continues to increase over a 15-minute period. Similarly, cells preincubated with the ionophore in bivalent-cation–free medium for 4 minutes respond normally after exposure to $Ca^{2+}$ and $Mg^{2+}$ (Text-figure 3B). Mast cells also cannot be desensitized to A23187.[5,7,8] The aggregation tendency of PMNs exposed to the ionophore, unlike the tendency induced by chemotactic factors, appears to be long-lived.

This chemotactic-factor–induced decay in PMN reactivity is also found

when cells are re-exposed to the same chemotactic factor to which they were first exposed (Text-figures 1D and 2D). Cells preincubated with FMLP or C5fr, however, did aggregate when exposed to the opposite chemotactic factor (Text-figures 1C and 2C). Similarly, PMNs preincubated with FMLP aggregated normally when exposed to A23187 (Text-figure 3C). The inhibition of A23187-induced aggregation found when PMNs were preincubated with C5fr (Text-figure 3C) may result from the protein content of the C5fr preparation (Table 2). We conclude, therefore, that decay in PMN responsiveness to one aggregating substance is not necessarily accompanied by decay in responsiveness to other aggregating substances. Since PMNs have different receptors for FMLP and C5fr,[41,46,47] desensitization may reflect interference with intercellular sequences at a level close to the chemotactic factor receptor. Mast cells and basophils show similar selectivity in desensitization,[5,8,10,11,13,15-17] and it has been postulated that this desensitization also reflects events occurring at or close to the receptors for releasing agents.[5,7,8,15-17]

In the mast cell, degranulation and desensitization may result from the opening and closing of transmembranous $Ca^{2+}$ gates.[5,7,8] Similar events may explain PMN degranulation [19-21,24-28,32] and aggregation [40] as well as the desensitization of these two responses. It is interesting to note that aggregation may reflect PMN surface membrane adherence to the membranes of other cells and degranulation may reflect PMN surface membrane adherence to the membranes of intracellular granules. Thus, aggregation and degranulation may reflect increases in surface membrane adhesiveness. Recently, it has been found that chemotactic factors increase PMN adhesiveness to various foreign surfaces.[39,40,48,49] We suggest, therefore, that chemotactic factors may also increase PMN surface membrane adhesiveness to other biologic membranes. Cytosolic $Ca^{2+}$ may modulate the adhesiveness of cellular membranes directly or indirectly: $Ca^{2+}$ may span intermembranous spaces, decrease intermembranous repulsive forces, or influence various biochemical events controlling membrane adhesiveness. Chemotactic factors may increase membrane adhesiveness by increasing membrane permeability to $Ca^{2+}$ and/or through other actions on the PMN. In any event, changes in membrane adhesiveness may be important events in cellular function.

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