ALTERATIONS OF THE ELECTROPHORETIC MOBILITY DISTRIBUTION OF RAT MAST CELLS AFTER IMMUNOLOGIC ACTIVATION

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ABSTRACT Changes in the electrophoretic mobility distributions of rat serosal mast cells after immunologic activation have been measured using the laser Doppler technique of electrophoretic light scattering. Rat serosal mast cells of 98% purity isolated by isopycnic and velocity gradient sedimentation had a highly negative electrophoretic mobility which was unaffected by incubation with normal rabbit serum or, at 4°C or in the absence of Ca^{+2}, with rabbit anti-rat $F(ab')_2$ antiserum. Immunologic activation of the cells with this antiserum in the presence of Ca^{+2} at 37°C resulted in a dose- and time-dependent increase in the electrophoretic mobility. Thus at a 1:25 dilution of anti-$F(ab')_2$ the mean and mode electrophoretic mobilities of the mast cell population increased 25 and 21%, respectively. The width of the electrophoretic mobility distribution also increased with activation, indicating a heterogeneous response of the mast cells in the population. The increase in electrophoretic mobility after immunologic activation is not diminished by treatment of the cells with 1 M NaCl to solubilize adsorbed mast cell granules or heparin.

INTRODUCTION

The mast cell is an immunologically activated secretory cell capable of releasing a diverse group of active pharmacologic materials which together possess all the necessary functions for a fully expressed but controlled local inflammatory response. The membrane-bound granules within the mast cell are known to contain vasoactive amines, chemotactic peptides, structural proteoglycans, and lysosomal hydrolases. The surface of the mast cell possesses specific receptors for the Fc portion of IgE; bridging of two IgE molecules by antigen or by antibody directed against the F(ab) portion of cell-surface-bound IgE leads to a noncytolytic secretion of the contents of these unique granules (1). An early event in the release reaction is a rapid influx of Ca^{+2} into the cell (2), which is followed by alterations in phospholipid metabolism including generation of phosphatidic acid and phosphatydyl inositol, increases in cyclic adenosine 3′,5′ monophosphate, phosphorylation of cytosol proteins, and fusion of the

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perigranular membrane with the plasmalemma (3–6). Morphological changes in the mast cell surface which have been shown to accompany immunologic activation include a lateral redistribution of surface receptors and intramembranous particles (7, 8), fusion of the perigranular membrane with the plasmalemma, and formation of pores between the granule interior and the external milieu, resulting in secretory release (6–10). The increase in the number of surface folds and ruffles in the mast cell plasmalemma after secretion is generally interpreted as the result of an increased surface area caused by addition of perigranular membrane to plasmalemma (10). These morphologic and biochemical alterations could be expressed at the cell surface as a change in the surface charge density.

Using the laser Doppler technique of electrophoretic light scattering (ELS),[1] we have measured the electrophoretic mobility distributions of rat serosal mast cells before and after immunologic activation. The activation process results in a substantial increase in the electrophoretic mobility of the mast cells which, like the secretion of mediators, is Ca^{+2}-, dose-, and temperature-dependent.

MATERIALS AND METHODS

Metrizamide, analytical grade (Accurate Chemical and Scientific Corp., Hicksville, N. Y.), histamine acid phosphate (Mann Research Labs, N. Y.), calcium ionophore A23187 (Eli Lilly and Co., Indianapolis, Ind.), heparin (Sigma Chemical Co., St. Louis, Mo.), and serotonin (Calbiochem, San Diego, Calif.) were obtained as indicated.

Isolation of Cells

Cells from 20 to 50 male or female Sprague-Dawley rats, each weighing 200–300 g, were collected by lavage of the peritoneal cavity of each rat with 20 ml of Tyrode's buffer lacking Ca^{+2} and Mg^{+2} and containing 0.1% gelatin and 40 mg/ml of commercial heparin. The cells were sedimented at 400 *g* for 15 min at room temperature, pooled, and washed twice with Ca^{+2}- and Mg^{+2}-free Tyrode's buffer containing 0.1% gelatin and 10 mg/liter of DNA (TGD^-).[2] 1-ml volumes of from 3 to 8 $\times$ 10^7 cells in the above buffer were layered on each of 20 to 40 2-ml cushions of 22.5% wt/vol metrizamide in the same buffer (density, 1.125 g/ml) and centrifuged at room temperature for 15 min at 400 *g* at the buffer-metrizamide interface. The cells remaining at this interface, principally eosinophils and mononuclear leukocytes, were retained. The cell pellets, rich in mast cells, were pooled, washed, and resuspended in 10 ml of TGD^- and applied to each of two 30-ml continuous 3–9% metrizamide gradients. The gradients were centrifuged at room temperature for 12 min at 35 *g*. The mast cells were present in a pellet at the bottom of the gradient, while contaminating nucleated cells and erythrocytes were found in a band sedimenting approximately one-third through the gradient. The mast cell purity was 95–98% by differential count of the nucleated cells in smears stained with toluidine blue. The yield was $\sim 10^6$ mast cells per rat; erythrocytes were absent.

Samples enriched in either eosinophils or mononuclear leukocytes were prepared according to the method of Boyum (10). Cells collected from the upper interface of the 22.5% metrizamide band (1.5 $\times$ 10^7 cells in 3 ml) were separated with LSM (leukocyte separation medium, Bionetic Inc., N. Y.) by centrifugation at 400 *g* for 30 min. These preparations were separately washed twice and employed in the electrophoresis experiments.

Rat peripheral blood erythrocytes were obtained from cardiac puncture and washed twice in TGD^-.

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[1] *Abbreviations used in this paper:* ELS, electrophoretic light scattering; anti-$F(ab')_2$, rabbit anti-rat $F(ab')_2$; TGD^-, Tyrode's buffer + 0.1% gelatin + 10 mg/liter deoxyribonuclease; TGD, Complete Tyrode's buffer + 0.1% gelatin + 10 mg/liter deoxyribonuclease.

Isolation of Mast Cell Granules

Rat mast cells of 95% purity were washed twice with 0.9% NaCl, centrifuged at 400 *g* for 10 min at room temperature, and resuspended in distilled water at a concentration of 0.5×10^6 mast cells/ml. The pH of the mast cell suspension was adjusted to 7.1 with 0.01 M NaOH and the cells were agitated for 5 min at room temperature; the sequence of pH adjustment and agitation was repeated twice. The resulting suspension was centrifuged at 100 *g* for 10 min at room temperature, and the supernatant suspension containing the granules was decanted from the sediment of large cellular debris. The granule suspension was sedimented by centrifugation at 3,000 *g* for 20 min at 4°C. The mast cell granules were resuspended and washed with the electrophoresis buffer before electrophoresis.

Noncytolytic Activation of Mast Cells

Purified rat mast cells at a concentration of ~ 1–2×10^6 cells/ml in complete Tyrode's buffer containing 0.1% gelatin and 10 mg/l DNase (TGD)[2] were prewarmed to 37°C for 15 min and mixed with buffer or various dilutions of heat-inactivated rabbit anti-rat $F(ab')_2$ antiserum. After incubation at 37°C for various times, the cells were sedimented by centrifugation at 400 *g* for 5 min at room temperature. The supernates were retained, the cells were washed with 1 ml of TGD, and the wash was pooled with the original supernates. In some experiments a final wash with TGD^- made 1.0 M in NaCl was performed to remove adsorbed mast cell granules. This procedure has been described in other reports (12), and the effectiveness of granule removal was verified in this work by light microscope observation.

Supernates and cell pellets from challenged and unchallenged mast cells were disrupted by freezing and thawing six times and the resulting extracts assessed for histamine by bioassay (13).

The net percentage of histamine released was determined by subtracting the spontaneous release from the release obtained with challenge and dividing by the amount of the constituent in the starting cell pool, corrected for that amount released spontaneously. Therefore, net percentage of release =

$$\frac{\text{stimulated release} - \text{unstimulated release}}{\text{stimulated release} + \text{residual} - \text{unstimulated release}} \times 100.$$

Electrophoresis Buffer

The electrophoresis buffer employed in these studies was composed of 0.01 M HEPES, 0.01 M NaCl, 4.5% sorbitol, and NaOH to a final pH of 7.5. The conductivity of this iso-osmotic, low ionic strength buffer is 1.21 ± 0.02 mmho/cm at 20.0°C. The mobilities measured with this solution were corrected to those of pure water since its viscosity is significantly greater than that of water.

Electrophoretic Light Scattering

The principles involved in electrophoretic light scattering have been described previously (14). Briefly, the frequency of laser light scattered by a cell moving under the influence of an electric field is Doppler shifted. The velocity of the cell can be determined by the magnitude of the Doppler shift. The electrophoretic mobility is defined as the velocity divided by the electric field. Hence, the frequency spectrum observed may be interpreted as an electrophoretic mobility distribution.

The methods and apparatus employed in ELS have been described in detail (15). All experiments were performed with a He-Ne (Spectra-Physics, Mountain View, Calif.) laser operating at 632.8 nm with a scattering angle of 57.7°. Electric field pulses of constant magnitude between 25 and 30 V/cm were provided by a timed switching circuit. Spectra were calculated by a Saicor model 51B real time spectrum analyzer (Honeywell, Denver, Colo.) operating on a frequency range of 200 Hz.

RESULTS

The electrophoretic mobilities of rat mast cells, erythrocytes, and peritoneal eosinophils and macrophages were determined in a low-ionic-strength iso-osmotic electrophoresis buffer (0.01 M; 20 Å Debye-Huckel screening length). The mode and mean electrophoretic mobilities of

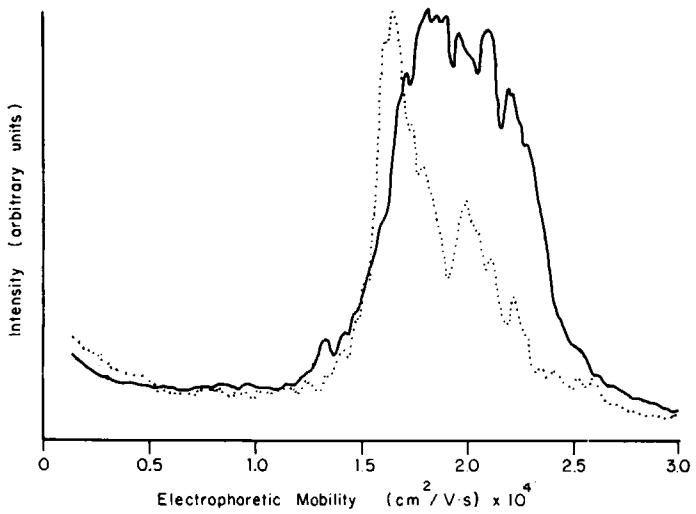

FIGURE 1. Electrophoretic mobility distribution of resting rat serosal mast cells (· · · ·) and of mast cells incubated with a 1:40 dilution of heat-activated rabbit anti-rat $F(ab')_2$ antiserum (—).

resting mast cells were 1.86 ± 0.03 and 1.98 ± 0.03 × 10^{-4} $cm^2/V \cdot s$, respectively, whereas the mode electrophoretic mobilities of the symmetric distributions of eosinophils, macrophages, and erythrocytes were 1.57 ± 0.03, 1.56 ± 0.03, and 2.36 ± 0.03 × 10^{-4} $cm^2/V \cdot s$, respectively. Initial observations of the effect of immunologic activation upon the electrophoretic mobility distribution of rat mast cells were determined after exposure of 2 × 10^6 cells/ml to buffer or to a 1:40 dilution of heat-inactivated rabbit anti-rat $F(ab')_2$ antiserum for 10 min at 37°C (Fig. 1). Spontaneous release of histamine was 4% and after activation 24%. In this particular experiment, the mean and mode electrophoretic mobilities of mast cells increased

TABLE I
MAST CELL ELECTROPHORETIC MOBILITIES

Treatment	Electrophoretic mobility		Width	% Histamine release
	Mode	Mean		
	(± 0.03 × 10^{-4} $cm^2/V \cdot s$)			
Untreated cells	1.86	1.98	0.18	4
Cells + salt wash	1.92	1.95	0.17	3
Normal rabbit serum	1.99	2.00	0.16	5
Antiserum, 1:25, 4°C	1.81	1.91	0.17	4
Antiserum, 1:25, no Ca^{+2}, Mg^{+2}	1.83	1.97	0.16	3
Antiserum, 1:100	1.84	2.04	0.21	14
Antiserum, 1:50	1.90	2.27	0.32	26
Antiserum, 1:25	2.40	2.36	0.27	42
Antiserum*, 1:25, 1 min.	2.04	2.22	0.25	45
Antiserum*, 1:25, 30 min.	2.11	2.20	0.24	42
Cells + histamine, 10 μg/ml	1.84	1.92	0.18	
Cells + serotonin, 1 μg/ml	1.83	1.96	0.17	
Cells + heparin, 50 μg/ml	1.85	1.95	0.16	

by 14 and 24%, respectively, after immunologic activation. The width of the distribution increased significantly, indicating a heterogeneous electrokinetic response of the cell surface. Removal of adsorbed mast cell granules by washing the cells with TGD$^-$ made 1 M in NaCl did not release histamine or create a significant alteration in the electrophoretic mobility distribution of resting or activated mast cells with TGD$^-$ made 1 M in NaCl did not release histamine or create a significant alteration in the electrophoretic mobility distribution of resting or activated mast cells (Table I).

Incubation of rat mast cells under conditions which did not promote histamine release also did not alter the electrophoretic mobility distribution. For example, incubation of mast cells

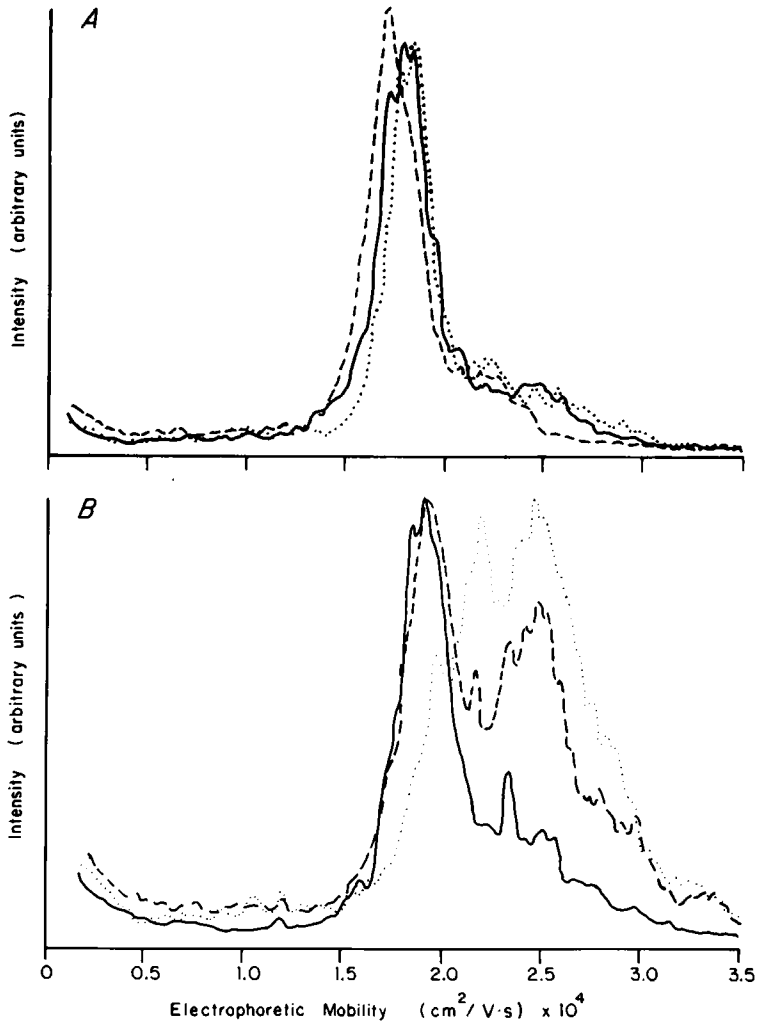

FIGURE 2. Effect of immunologic activation upon rat mast cell electrophoretic mobility distribution. Panel A: resting rat mast cells (—), mast cells plus 1:25 dilution of rabbit anti-rat $F(ab')_2$ at 4°C (----) and mast cells plus 1:25 dilution of rabbit anti-rat $F(ab')_2$ at 37°C but in the absence of Ca^{+2} and Mg^{+2} (· · ·). Panel B: dependence of electrophoretic mobility distribution change upon amount of rabbit anti-rat $F(ab')_2$ antiserum. Mast cells plus 1:100 (—), 1:50 (----), and 1:25 (· · · ·) dilution of antiserum.

with normal rabbit serum or with rabbit anti-rat $F(ab')_2$ antiserum at 37°C in the absence of divalent cations or at 4°C in the presence of divalent cations produced little histamine release (3 ± 2%) and had no perceptible effect on the electrophoretic mobility distribution, as is illustrated in Fig. 2 and documented in Table I.

Activation by anti-$F(ab')_2$ incubated with mast cells at 37°C in the presence of Ca^{+2} and Mg^{+2} produced both histamine release and alterations of the electrophoretic mobility which were directly proportional to the dose of the antiserum. Activation by 1:25, 1:50, and 1:100 dilution of anti-$F(ab')_2$ antiserum in the presence of Ca^{+2} and Mg^{+2} resulted in 38, 22, and 10% net histamine release, respectively. Mean and mode electrophoretic mobilities of rat mast cells after activation by anti-$F(ab')_2$ antiserum at 1:100 dilution were 1.84 ± 0.03 and 2.04 ± 0.03 × 10^{-4}, whereas a 1:50 dilution resulted in a bimodal response with overall mean and mode values of 1.90 ± 0.03 and 2.27 ± 0.03 × 10^{-4} $cm^2/V \cdot s$, respectively. Activation of mast cells with a 1:25 dilution of antiserum caused a greater negative shift in mean and mode electrophoretic mobilities to 2.40 ± 0.03 and 2.36 ± 0.03 × 10^{-4} $cm^2/V \cdot s$, respectively (Fig. 2, Table I). The spectra in Fig. 2 illustrate that the immunologic activation of rat mast cells produces a substantial increase in the width of the electrophoretic mobility distribution, indicating a heterogeneous response of the mast cells to activation as expressed in changes in the surface charge density.

To determine the period of time necessary for the completion of the electrokinetic alterations, we incubated 2 × 10^6 rat mast cells/ml with a 1:25 dilution of rabbit anti-rat $F(ab')_2$ antiserum for 0, 1, 35, and 120 min. Activation was then terminated by adding 3 vol of ice-cold TGD^- and rapidly sedimenting the cells. Histamine release (46 ± 4%) was complete within 1 min. The effects of activation for different time periods upon the electrophoretic mobility distribution are illustrated in Fig. 3 and summarized in Table I. The resting mast cells had mean and mode electrophoretic mobilities of 1.92 and 1.95 ± 0.03 × 10^{-4}, respectively, which were 2.04 ± 0.03 and 2.22 ± 0.03 × 10^{-4} $cm^2/V \cdot s$ 1.0 min after chal-

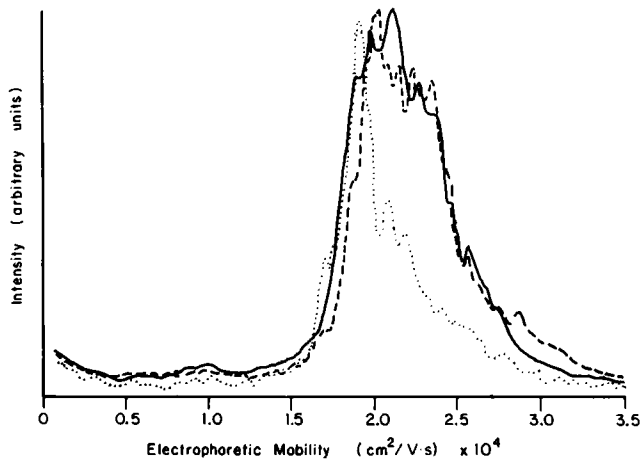

FIGURE 3. Kinetics of alteration in electrophoretic mobility distribution of rat mast cells after interaction with 1:25 dilution of rabbit anti-rat $F(ab')_2$ antiserum. Resting mast cells (· · · ·), mast cells 1 min (—) and 30 min after activation (----).

lenge. No further significant alteration of cell surface charge density could be detected at 35 or 120 min of incubation. Thus the electrokinetic alteration which accompanies activation is, like histamine release, complete within 1 min.

The possibility that mast cell granules or their contents might adhere to or otherwise alter the electrokinetic properties of mast cell surfaces was explored by directly adding purified granules or mediators to rat mast cells and assessing the effect with ELS. The ELS spectra of mast cells which had been incubated at 37°C for 10 min with histamine, serotonin, and commercial porcine heparin at concentrations of 10, 1, and 50 μg/ml, respectively, were

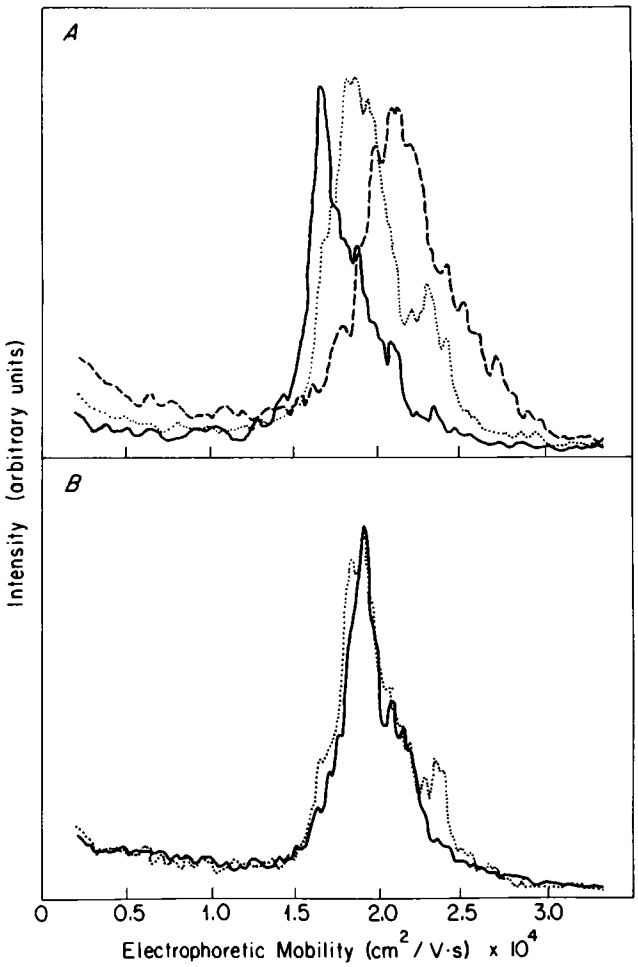

FIGURE 4. Effect of membrane-free rat mast cell granules upon mast cell electrophoretic mobility distribution. Panel A: electrophoretic mobility distributions of 1×10^6 resting mast cells (—), membrane-free mast cell granules from 5×10^5 rat mast cells (----), and of a mixture of 1×10^6 rat mast cells and membrane-free granules from 5×10^5 rat mast cells (· · · ·) in complete Tyrode's buffer. Panel B: electrophoretic mobility distribution of 1×10^6 resting mast cells (—) and a mixture of 1×10^6 mast cells and membrane-free granules from 5×10^5 rat mast cells (· · · ·) in Tyrode's buffer made 1 M with NaCl.

measured (Table I). None of these mediators resulted in any detectable change in the electrophoretic mobility distribution of the mast cells. The effect of binding of mast cell granules to the exterior surface of mast cells was studied by preparing granules by distilled-water lysis of mast cells and performing ELS measurements of suspensions of isolated mast cells, mast cell granules, and co-incubations of mast cells and granules (Fig. 4 *a*). The mean and mode electrophoretic mobilities of the granules are 2.40 and 2.42 $\times$ 10^{-4} $cm^2/V \cdot s$, respectively. Because the granules are much smaller than cells, their light scattering spectrum is diffusion broadened, and the direct correspondence of Doppler width and width of the electrophoretic mobility distribution does not apply. From studies of the electric field strength dependence of the linewidths, we conclude that the granules have a radius of 0.6–0.8 μm and a charge density which is sufficiently uniform that the electrophoretic heterogeneity is a minor contributor to the ELS linewidth. When membrane-free granules from 5 $\times$ 10^5 mast cells are incubated with 1 $\times$ 10^6 intact mast cells, a spectrum is obtained which has a mean electrophoretic mobility intermediate between the respective values for the resting cells and the isolated granules. This indicates that granules adhere to mast cells in physiologic buffer and adherence in turn alters mast cell electrophoretic mobility in a net negative direction. However, exposure of mast cells bearing adsorbed granules to TGD^- buffer made 1.0 M in NaCl (Fig. 4 *b*) results in an electrophoretic mobility distribution which is indistinguishable from that of the resting mast cells. The effect of 1 M NaCl is attributed to the solubilization of the adsorbed heparin-chymase granule complexes. Therefore, the electrokinetic alterations observed upon immunologic activation of mast cells, which were resistant to washing with the high-salt buffer, cannot be simply attributed to adsorption of granules released from activated cells.

DISCUSSION

The principal results of the present study are the demonstration that resting rat serosal mast cells possess a high net negative surface charge when compared to other resident peritoneal nucleated cell populations and that an increase in the net negative charge density at the rat mast cell surface occurs after their activation with anti-$F(ab')_2$ at 37°C in the presence of divalent cations (Fig. 1). This increase in surface charge density appears to be directly dependent upon and related to the extent of immunologic activation. Thus, interaction of mast cells with normal rabbit serum or with rabbit anti-rat $F(ab')_2$ antiserum at 4°C or at 37°C in the absence of divalent cations, conditions which do not support histamine release, also are not associated with electrokinetic changes. In contrast, a dose-dependent release of histamine and a proportional increase in mean and mode electrophoretic mobilities occur after stimulation of mast cells with rabbit anti-rat $F(ab')_2$ in the presence of Ca^{+2} and Mg^{+2} (Fig. 2, Table I).

The alteration in cell surface charge density occurred rapidly and was completely expressed within the first minute after mast cell activation (Fig. 3). The changes noted on brief interaction with anti-$F(ab')_2$ were maintained for up to 2 h.

The biochemical origin of the alterations in cell surface charge density remains unexplained. Our control experiments have shown that these alterations can not be attributed to the binding of released histamine, serotonin, or heparin, or to the binding of free mast cell granules. Certainly rabbit serum alone or the binding of anti-$F(ab')_2$ to surface immunoglobulin at a temperature below that sufficient to permit mediator release is not sufficient to induce

changes in the electrophoretic mobility distribution. The remaining possibilities include the effect of other mast cell mediators upon cell surface charge. The release of lysosomal proteases, such as chymase, and the B-exoglycosidases could be reflected in alterations of membrane elements; however, the rapidity of cell surface changes and the persistence of maximal charge change for periods of 2 h argue against this explanation. More likely is that the net negative cell surface charge is altered by the addition or exposure of negatively charged lipid moieties upon the cell surface. Such charged molecules could be present on the inner surface of the perigranular membrane and would be exposed after their fusion with the plasma membrane. Alternatively, or in addition, the rapid increase in phosphatidic acid and phosphatydyl inositol noted in the mast cell after immunologic activation (4, 5) could provide the necessary negatively charged groups.

We do not believe that the induced electrophoretic heterogeneity can be attributed to disruption of the stimulated mast cells. The technique of cell activation employed in our experiments has been demonstrated to be noncytotoxic (16). Moreover, we monitored cell viability by the criterion of dye exclusion and always observed a viability of >95% before and after stimulation.

Whatever the molecular explanation, the increase in negative surface charge density after exocytic granule release is in direct contrast to the decrease of net negative surface charge density observed in macrophages (17)[2] and polymorphonuclear leukocytes (18) after stimulation of endocytosis. It may be that a high negative surface charge density on the noncytoplasmic side of these vesicles is important for the formation or stability of the vesicle or for maintaining a transvesicular gradient of H^+ or other ions. In the case of the mast cell, the presence of a highly negatively charged surface might affect the state of activation of Hageman factor (19) and thereby provide the substrate for local activation of Hageman factor-dependent inflammatory pathways.

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REFERENCES

1. METZGER, H. 1977. The cellular receptor for IgE. *In* Receptors and Recognition, Series A. P. Cuatrecasas and M. F. Greaves, editors. John Wiley & Sons, New York. 73.
2. UVNÄS, B. 1971. Quantitative correlation between degranulation and histamine release in mast cells. *In* Biochemistry of Acute Allergic Reactions. K. F. Austen and E. L. Becker, editors. Blackwell, Oxford. 175.
3. SULLIVAN, T. J., and C. W. PARKER. 1979. Possible role of arachidonic acid and its metabolites in mediator release from rat mast cells. *J. Immunol.* **122**:431.
4. KENNERLY, D. A., T. J. SULLIVAN, and C. W. PARKER. 1979. Activation of phospholipid metabolism during mediator release from stimulated rat mast cells. *J. Immunol.* **122**:152.
5. COCKCROFT, S., and B. D. GOMPERTS. 1979. Evidence for a role of phosphatidylinositol turnover in stimulus-secretion coupling. Studies with rat peritoneal mast cells. *Biochem. J.* **178**:681.
6. LAGUNOFF, D. 1973. Membrane fusion during mast cell secretion. *J. Cell Biol.* **57**:252.
7. HENSON, P. M., M. H. GINSBERG, and D. C. MORRISON. 1978. Mechanism of mediator release by inflammatory cells. *In* Membrane Fusion. G. Poste and G. L. Nicolson, editors. Elsevier-North Holland, New York. 407.

[2]Petty, H. R., manuscript submitted for publication.

8. LAWSON, D., M. C. RAFF, B. GOMPERTS, C. FEWTRELL, and N. B. GILVA. 1977. Molecular events during membrane fusion. A study of exocytosis in rat peritoneal mast cells. *J. Cell Biol.* **72**:242.
9. CHI, E. Y., D. LAGUNOFF, and J. K. KOEHLER. 1976. Freeze-fracture study of mast cell secretion. *Proc. Natl. Acad. Sci. U.S.A.* **73**:2823.
10. BURWEN, S. J., and B. H. SATIR. 1977. A freeze-fracture study of early membrane events during mast cell secretion. *J. Cell Biol.* **73**:660.
11. BOYUM, A. 1976. Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol.* **5**:9. (Suppl. 5).
12. YURT, R. W., R. W. LEID, J. SPRAGG, and K. F. AUSTEN. 1977. Immunologic release of heparin from purified rat peritoneal mast cells. *J. Immunol.* **118**:1201.
13. STECHSCHULTE, D. J., K. F. AUSTEN, and K. J. BLOCH. 1963. Antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea-pig and rat. *J. Exp. Med.* **125**:127.
14. WARE, B. R. 1974. Electrophoretic light scattering. *Adv. Colloid Interface Sci.* **4**:1.
15. SMITH, B. A., and B. R. WARE. 1978. Apparatus and methods for laser Doppler electrophoresis. *In* Contemporary Topics in Analytical and Clinical Chemistry. Vol. 2. D. M. Hercules et al, editors. Plenum Publishing Corp., New York. 29.
16. LYNCH, S. M., K. F. AUSTEN, and S. I. WASSERMAN. 1978. Release of arylsulfatase A but not B from rat mast cells by noncytolytic secretory stimuli. *J. Immunol.* **121**:1394.
17. PETTY, H. R., and B. R. WARE. 1979. Macrophage response to concanavalin A: effect of surface crosslinking on the electrophoretic mobility distribution. *Proc. Natl. Acad. Sci. U.S.A.* **76**:2278.
18. PETTY, H. R. 1979. Biophysical studies of immunological reactions at the cell surface. Ph. D. Thesis, Biophysics, Harvard University, Cambridge, Massachusetts.
19. SPRAGG, J., and K. F. AUSTEN. 1977. Plasma factors: the Hageman-factor-dependent pathways and the complement sequence. *In* Immunopharmacology. J. W. Hadden, R. G. Coffey, and F. Spreafico, editors. Plenum Publishing, New York. 125.