Modulation of the Random Migration of Human Platelets

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A B S T R A C T Random migration of human platelets has been recognized as a parameter of platelet function which can be assessed in a reproducible manner by modification of the Boyden micropore filter technique for evaluating this function in other cells (Boyden, S. 1962. *J. Exp. Med.* **115**: 453–466). Because platelets are extremely susceptible to aggregation, the conditions for collecting and isolating platelets and the migration buffer (Ca^{++} and Mg^{++}-free phosphate buffered saline, pH 6.8, with glucose and gelatin) were selected to minimize such a possibility. The random movement of platelets into the micropore filter was maximal at 30–37°C and was contingent upon the metabolic integrity of the cell; thus, it can be attributed to active spontaneous migration. While the initiating and enhancing effects of epinephrine on the platelet aggregation-release reaction are mediated by an α-adrenergic receptor, the inhibition of random migration involved a β-receptor. Equimolar propranolol but not phentolamine prevented epinephrine inhibition of random migration, and isoproterenol had activity comparable to epinephrine while phenylephrine was inactive. The capacity of the cholinomimetic agent, carbachol, to increase platelet migration is reminiscent of the recent findings in several cell systems in which β-adrenergic and cholinergic stimuli have opposite effects. The prostaglandins E_1 and E_2 augmented spontaneous migration in contrast to their well established inhibitory action on platelet aggregation at the concentrations employed. The suppression by indomethacin of prostaglandin enhancement and of spontaneous migration implies a requirement for the prostaglandin biosynthetic pathway during the migration process. Thus, the spontaneous migration of human platelets, an additional parameter of platelet function for in vitro investigations, disclosed not only a β-adrenergic receptor for epinephrine, but also a capacity for cholinergic augmentation and an apparent requirement for prostaglandin biosynthesis.

INTRODUCTION

The methods for in vitro assessment of human platelet function have included platelet adherence to synthetic surfaces, aggregation as measured by changes in the optical density of platelet suspensions, and release of specific platelet constituents induced by aggregating agents (1, 2) but have not included directed or random migration. The coincidental observation that platelets as well as polymorphonuclear and mononuclear leukocytes moved through a micropore filter during studies of random migration of mixed leukocyte populations prompted a specific investigation of platelet migration. The Boyden chamber method (3) for studying spontaneous and directed migration of various classes of leukocytes was adapted for such platelet studies by appropriate selection of pore size, buffer, and other conditions. This in turn permitted analysis of the effects of adrenergic and cholinergic stimuli and prostaglandins on spontaneous migration of human platelets.

METHODS

Polystyrene disposable chemotactic chambers (Adaps Inc., Dedham, Mass.) and micropore filters (Millipore Corp., Bedford, Mass.) were assembled as previously described (4). L-epinephrine (Sigma Chemical Co., St. Louis, Mo.), phentolamine (Regitine, Ciba Pharmaceutical Company, Summit, N. J.), propranolol (Inderal, Ayerst Laboratories, New York), gelatin (Difco Laboratories, Detroit, Mich.), and prostaglandins PGE_1, PGE_2, and $PGF_{2\alpha}$ (The Upjohn Co., Kalamazoo, Mich.) were obtained as noted. Siliconized (Dricote, Fisher Scientific Co., Fair Lawn, N. J.) glassware and needles or plastic materials were used for all platelet suspensions.

Platelet isolation. Fresh human blood was collected from normal volunteers who had taken no drugs known to affect platelet function for at least 5 days before venipuncture. 5-ml portions of blood were mixed with 1 ml of sodium citrate anticoagulant (citric acid 0.076 M, sodium citrate 0.15 M, pH 5.2) in 14-ml plastic tubes and were immediately centrifuged at 200 g for 15 min at room temperature. The platelet-rich plasma was removed from the buffy coat

and red cell infranate and was centrifuged at 900 g for 20 min. The platelet-poor supernate was discarded, and the platelet pellet was resuspended by repeated gentle aspiration with a pipette in the standard buffered medium (pH 6.8, 0.005 M KH_2PO_4, 0.005 M Na_2HPO_4, 0.1 M NaCl, 0.2 g/100 ml glucose, and 0.5 g/100 ml gelatin). Platelet counts were performed by phase-contrast microscopy.

Platelet migration. 0.5 ml of platelet suspension containing 4–6×10^7 platelets and less than 0.1% leukocytes was added to the cell compartment of a modified Boyden chamber which was separated from the medium compartment below by an 8-μm micropore filter. After a 3-h incubation at 37°C, the filters were washed twice in 0.15 M NaCl, fixed in 10% (vol/vol) formalin (5) containing 0.15 M sodium acetate (pH 7.2) for 1 h, stained with Harris' hematoxylin (5) and mounted on glass slides. All experiments were performed in duplicate or triplicate. Migration into each filter was quantitated by counting the number of platelets in 10 high-power fields (HPF)[1] at a fixed level for each experiment of 40–70 μm from the source of the platelets at the top of the filter. The microscope slides containing the mounted micropore filters were coded and counted in blind fashion by two investigators. The variation in interpretation was less than 20% of the mean of the separate readings. Modifications of the medium composition or agents tested for their effect on platelet migration were present on both the platelet and medium sides of the filter with no concentration gradient. Statistical analysis of data was performed with a standard paired t test.

RESULTS

Determination of the optimal conditions for migration

The influence of buffer composition, filter pore size, and conditions of incubation on the rate of migration of platelets was assessed by counting triplicate filters at hourly intervals up to 5 h for each set of conditions. The time-course of migration for platelets suspended in the standard buffered medium and incubated at 37°C always revealed a continuous increase of platelet counts as the duration of incubation increased. After 3 h of incubation, platelet counts per HPF 50 μm from the platelet layer at the top of the micropore filter in a typical experiment were 3–5 for 3 μm, 9–13 for 5 μm, and 19–25 for 8 μm pore size. In all subsequent experiments platelets were incubated for 3 h on 8-μm millipore filters and counted at a fixed depth from 40 to 70 μm for each experiment.

Platelet migration was maximal at temperatures between 30 and 37°C and diminished at either lower or higher temperatures (Fig. 1). 37°C was used as the standard temperature for examining all other variables.

The concentration of gelatin in the standard buffered medium was varied between 0 and 3.0 g/100 ml. Maximal migration (Fig. 2) occurred between gelatin concentrations of 0.5 and 1.0 g/100 ml. All subsequent studies utilized 0.5 g/100 ml gelatin.

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[1] *Abbreviations used in this paper:* HPF, high-power fields; ID_{50}, dose required for 50% inhibition of migration.
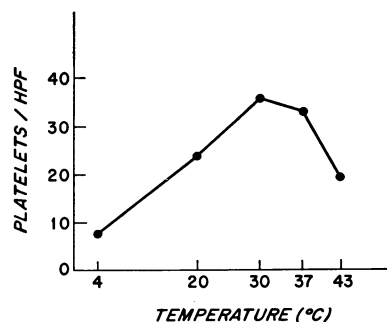

FIGURE 1 Temperature dependence of the random migration of purified human platelets. Each point represents the mean number of platelets per high-power field (HPF) in 8-μm pore filters at 50 μm from the platelet source for triplicate chambers incubated at specified temperatures for 3 h.

pH. In order to provide a broader buffering range Tris(hydroxymethyl)aminomethane (0.005 M) and acetic acid (0.005 M) were added to standard medium without NaCl. The pH was adjusted to levels between 4.0 and 11.0 with 0.1 M NaOH or 0.1 M HCl, followed by adjustment of the conductivity with 3.0 M NaCl to that of the standard buffer (12.6 mS at 22°C). pH and conductivity were recorded after the platelets were suspended in the buffers. A peak of maximal migration was observed between pH 5.0 and 6.5 with a plateau being noted between 6.5 and 8.5; migration was not detected at pH less than 4.5 or greater than 10.0 (Fig. 3).

Calcium, magnesium, and potassium. Because the platelet suspensions in the standard buffered medium contain no added Mg^{++} or Ca^{++}, the concentration re-
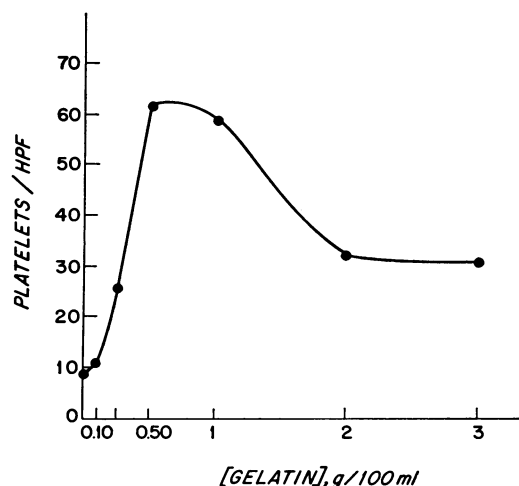

FIGURE 2 Influence of gelatin concentration on the random migration of human platelets. Each point represents the mean number of platelets per HPF at 40 μm from the platelet source for triplicate chambers containing buffer constituted to the specified gelatin concentration.

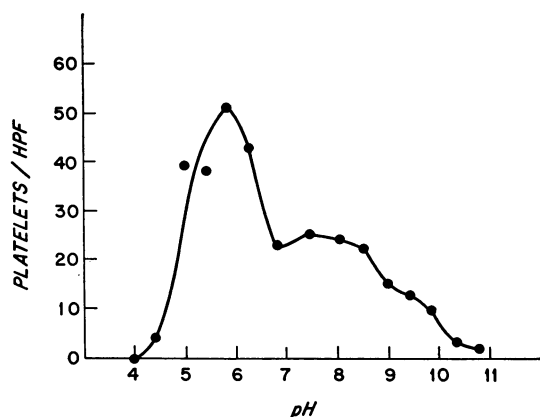*Modulation of the Random Migration of Human Platelets* 1101

FIGURE 3 Influence of pH on the random migration of human platelets. The pH of the standard buffer containing added 0.005 M Tris and 0.005 M acetic acid and no NaCl was adjusted to the specified values, and the conductivity was then adjusted with 3.0 M NaCl to that of the standard buffer. Each point represents the mean number of platelets at 60 μm from the platelet source in duplicate chambers.

corded (Fig. 4) is that added to the buffer, namely 10^{-2}–10^{-6} M. Both divalent cations inhibited migration in a dose-related manner at concentrations greater than 10^{-4} M. Platelet aggregates were observed on the top of the filters at 10^{-2} M. No concentration of these cations increased migration above that seen in the standard Mg^{++} and Ca^{++}-free medium. Washing platelets three times also did not decrease random migration.

Varying amounts of KCl were substituted for NaCl in standard medium made with NaH_2PO_4 (0.005 M)
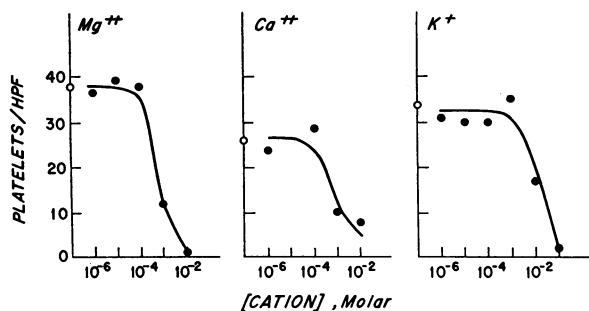
instead of KH_2PO_4. K^+ concentrations greater than 10^{-2} M were found to inhibit migration in a dose-related manner. The migration observed in standard buffer with a potassium concentration of 5×10^{-3} M was not exceeded by raising or lowering potassium concentration.

As the optimal conditions for platelet movement appeared to reflect an active rather than passive process and as platelets fixed in 10% formalin before study in standard platelet buffer failed to migrate into the filters, platelet movement was attributed to active migration and studies of its biochemical and pharmacologic modulation were undertaken.

Modulation of platelet migration

Metabolic inhibitors. Migration in glucose-free buffer was 25% of that observed in the standard buffer containing glucose (200 mg/100 ml), suggesting the requirement of glucose metabolism for migration to occur. 2-Iodoacetamide at concentrations between 10^{-3} and 10^{-5} M inhibited migration in a dose-related manner (Fig. 5) with the dose required for 50% inhibition (ID_{50}) being approximately 10^{-3} M. As the 2-iodoacetamide ID_{50} may also have been sufficient to influence not only glycolysis but also the hexose monophosphate shunt, the effect of 6-aminonicotinamide on migration was examined. 6-Aminonicotinamide functions as an analog of NADP to inhibit selectively the hexose monophosphate shunt (6). At a concentration of 10^{-5} M, 6-aminonicotinamide suppressed migration by 45 and 75% in two experiments.

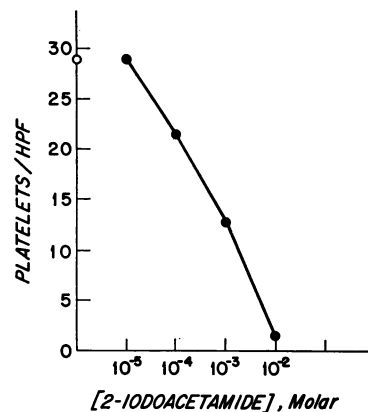*Adrenergic and cholinergic agents.* At concentrations of 10^{-8} M or greater, epinephrine caused a dose-

FIGURE 4 Influence of cation concentration on the random migration of human platelets in duplicate chambers (●). Mg^{++}: the standard Mg^{++} and Ca^{++}-free buffer was constituted with $MgCl_2$ at the specified concentrations. Mean migration in Mg^{++}-free buffer (○) was 38 platelets per HPF at 50 μm from the platelet source. Ca^{++}: the standard Mg^{++} and Ca^{++}-free buffer was constituted with $CaCl_2$ at the specified concentrations. Mean migration in Ca^{++}-free buffer (○) was 25 platelets per HPF at 60 μm from the platelet source. K^+: the specified amount of KCl was substituted for NaCl in Mg^{++} and Ca^{++}-free buffer in which 0.005 M NaH_2PO_4 was substituted for KH_2PO_4 to make a K^+-free buffer. Mean migration in K^+-free buffer (○) was 34 platelets per HPF at 50 μm from the platelet source.

FIGURE 5 Influence of 2-iodoacetamide on the random migration of human platelets (●). Platelets were preincubated at 37°C for 15 min in buffer containing the specified concentrations of 2-iodoacetamide before the platelet suspensions were added to the Boyden chambers. Each point represents the mean number of platelets per HPF at 50 μm from the platelet source in duplicate chambers. Control migration in buffer not containing iodoacetamide was 28 platelets per HPF (○).
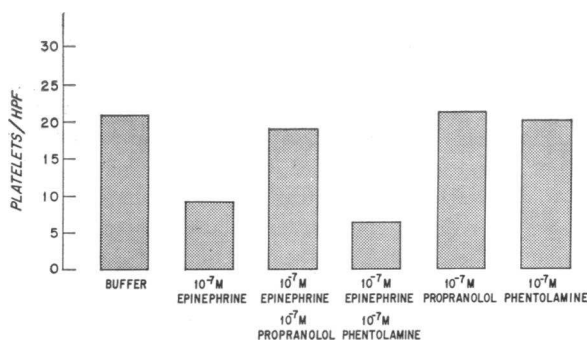
FIGURE 6 Adrenergic receptor specificity of epinephrine-induced inhibition of migration. Platelets were preincubated with propranolol, phentolamine, or buffer for 15 min at 37°C before epinephrine was added. The suspensions were immediately added to the Boyden chambers, and migration was assessed in duplicate filters at 50 μm from the platelet source.

related reduction in migration with 59±12% (mean± SD) inhibition at 10^{-7} M in 10 experiments ($P < 0.01$). Platelets were not visibly aggregated on the upper surface of the filters at concentrations of epinephrine as high as 10^{-5} M. To determine whether the action of epinephrine was mediated by the α or β receptor, the ability of equimolar amounts of phentolamine, an α-blocker, or propranolol, a β-blocker, to interfere with this effect was studied. The inhibition of migration by 10^{-7} M epinephrine was reversed by equimolar propranolol but not by phentolamine (Fig. 6). Propranolol and phentolamine alone had no effect on migration. Isoproterenol, a pure β-agonist, was nearly as active on a molar basis ($ID_{50} \cong 10^{-7}$ M) as epinephrine in terms of inhibiting migration; while phenylephrine, a pure α-agonist, had no effect at concentrations up to 10^{-5} M.

Because adrenergic and cholinergic agents often have opposing actions on biologic systems, the effect of the cholinomimetic agent carbachol on migration was determined. Carbachol caused an increase in platelet migration over a broad concentration range (10^{-7} M–10^{-13} M) with a maximum enhancement of 55±27% (mean± SD) increase at 10^{-9} M in four experiments ($P < 0.05$).

Prostaglandins and inhibitors of prostaglandin synthesis. Indomethacin, an agent known to inhibit platelet prostaglandin synthetase (7), produced a dose-related inhibition of migration at concentrations greater than 10^{-8} M (Fig. 7) with 53±17% (mean±SD) inhibition at 10^{-5} M in seven experiments ($P < 0.01$). On the presumption that the effect of indomethacin was mediated by changes in the availability of endogenous prostaglandins, the effect of exogenous prostaglandins PGE₁, PGE₂, and PGF₂α was investigated (Fig. 8). PGE₁ caused a dose-related enhancement of migration which was maximal by 2.8 × 10^{-7} M and fell to a lesser value at concentrations of 1.4 × 10^{-5}–2.8 × 10^{-5} M. The
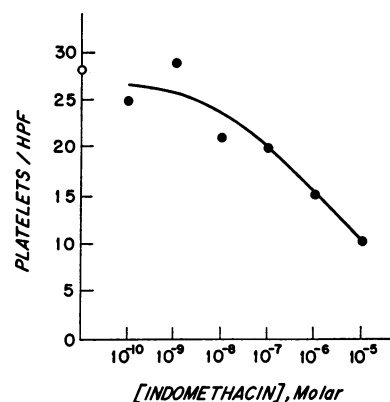

FIGURE 7 Influence of indomethacin on the random migration of human platelets. Platelets were preincubated at 37°C for 15 min in buffer containing the specified concentrations of indomethacin before the platelet suspensions were added to duplicate Boyden chambers. Each point (●) represents the mean number of platelets per HPF at 50 μm from the platelet source in duplicate chambers. Control migration in buffer not containing indomethacin was 28 platelets per HPF (○).

enhancement observed at 2.8 × 10^{-7} M in six experiments was 133±86% (mean±SD) ($P < 0.01$). The concentration of PGE₂ required to achieve enhancement comparable to PGE₁ was 10- to 100-fold greater. The maximal enhancement by PGE₂ occurring at concentrations of 1.4 × 10^{-5} M to 2.8 × 10^{-5} M was 121±76% (mean± SD) in four experiments ($P < 0.05$). PGF₂α had no effect over the range in which the enhancing action of PGE₁ and PGE₂ was recognized.

As exogenous prostaglandins increased platelet migration while an inhibitor of prostaglandin synthetase,
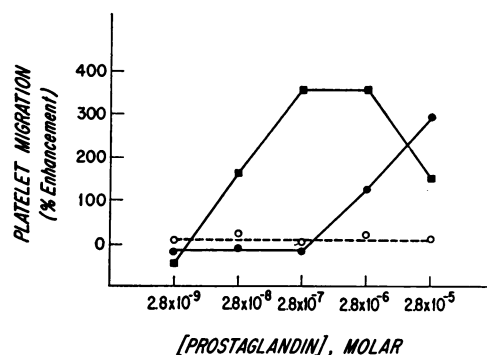
FIGURE 8 Enhancement of the random migration of human platelets by exogenous prostaglandins. Platelets were preincubated for 15 min at 37°C with either PGE₁ (■), PGE₂ (●), or PGF₂α (○) at the specified concentrations before being added to the Boyden chambers. Control migration in buffer alone was 10 platelets per HPF at 70 μm in the study of PGE₁ and PGE₂ and 19 platelets per HPF at 50 μm from the platelet source in the study of PGF₂α; triplicate chambers were used.

indomethacin, suppressed migration, their opposing actions were examined by their simultaneous presence in the Boyden chambers. A PGE_1 concentration of 1.4×10^{-6} M, a multiple of the lowest concentration causing maximal enhancement, yielded full enhancement despite the presence of 10^{-5} M indomethacin, while the action of lesser concentrations of PGE_1 ranged from partial reversal of indomethacin-induced inhibition to restoration of enhancement.

DISCUSSION

Random migration of human platelets represents a parameter of platelet function which can be assessed in a reproducible manner by modification of the Boyden micropore filter technique for evaluating this function in leukocytes. Random movement of platelets was attributed to active migration because it was maximal at 30–37°C (Fig. 1), required metabolic integrity of the platelets (Fig. 5), and was not observed if the platelets had been prefixed in formalin. The suppression of migration by an adrenergic agent (Fig. 6) acting through a β receptor and the enhancement achieved with a cholinomimetic agent or certain prostaglandins (Fig. 8) support the view that movement is an active process modulated by specific pharmacologic agents. Platelet movement as assessed by egress from capillary tubes has also been considered an active process based upon recognition of a temperature optimum of 30–37°C, inhibition by cytochalasin B, and chemotactic responsiveness to stimuli (8–10). Studies of leukocyte migration have also demonstrated that movement from capillary tubes is an active migration but is more dependent on surface adherence and more resistant to metabolic inhibitors than migration in Boyden chambers (11).

Because the platelet is extremely susceptible to aggregation the conditions for collection and handling as well as migration were selected to minimize such a possibility. Calcium ions, which are essential for the aggregation phenomenon, were not added to the migration buffer and were chelated during platelet harvesting by the addition to blood of sufficient sodium citrate anticoagulant to achieve final concentration of 3×10^{-2} M and pH 6.8. In addition, aggregation occurs most readily at pH above 7.2 (12) while the migration was most striking at pH 5.0–6.5 (Fig. 3). That aggregation was in fact difficult to achieve under the experimental conditions for migration was supported by the failure to recognize aggregates on the filters at the highest concentration studied of the aggregating agent epinephrine (Fig. 6). On the other hand, the platelets did have the capacity to aggregate, as evidenced by the presence of aggregates on the upper surface of the stained micropore filter when the calcium-free buffer was constituted with 10^{-2} M calcium or Mg^{++} (Fig. 4);

aggregation was possibly due to residual thrombin in the platelet suspension.

Compared to previous studies utilizing purified suspensions of human neutrophilic or eosinophilic polymorphonuclear leukocytes and mononuclear leukocytes, random migration of purified human platelets required a 25-fold greater cell concentration and enumeration halfway through the filter in order to achieve counts comparable to those found on the underside of the filter in leukocyte random migration. The pore size of 8 μm required for platelet migration is also utilized in studies of mononuclear leukocytes and basophils (13, 14) while the retarding effect of a 3 μm pore size is essential to the quantitation of eosinophilic and neutrophilic polymorphonuclear leukocytes. The finding of an optimal protein concentration (Fig. 2) for platelet migration is reminiscent of the protein requirements for migration of various leukocyte populations (15) and is consistent with the interpretation that low protein concentration impeded the process by allowing excessive surface adherence, while a high concentration prevented the degree of adherence required for progressive movement. Random migration of platelets, like that of polymorphonuclear leukocytes (16), is suppressed by 2-iodoacetamide and 6-aminonicotinamide (Fig. 5), agents which suppress glycolysis and the hexose monophosphate shunt, respectively. One apparent difference between the inherent requirements for spontaneous migration of human platelets as compared to rabbit neutrophilic leukocytes is that platelet migration is optimal in the absence of added magnesium and calcium (Fig. 4), while leukocyte migration is reduced by low divalent cation concentration (17). The inhibition of platelet migration by divalent cations can be attributed, however, to the aggregation of platelets by thrombin contaminating the suspension of platelets. It should be noted in considering any of the differences in the migration characteristics for platelets as compared to other cell types that the standard buffer is phosphate-buffered saline, pH 6.8, containing only added glucose and gelatin, whereas leukocyte migration is assessed in enriched buffers, e.g., Medium 199 (13, 14). The basis for selecting a simple buffer was to minimize platelet aggregation and to facilitate determination of medium requirements.

The capacity of epinephrine and the prostaglandins to initiate and/or modulate platelet function has been noted in the platelet aggregation and release reactions (18, 19) while an effect of a cholinomimetic agent has not received comparable attention. Although the effect of epinephrine on the aggregation-release reaction is to initiate or facilitate through its α potential (18), its action on random migration is inhibitory and dependent on interaction with a β receptor. This conclusion is

based on the capacity of equimolar propranolol but not phentolamine to prevent epinephrine inhibition of random migration (Fig. 6) and on the finding that isoproterenol is nearly as active on a molar basis as epinephrine while phenylephrine is without effect. This recognition of a platelet function modulated by a β receptor is consistent with the report of β-receptor-mediated platelet disaggregation (20). Whether β-receptor-modulated platelet functions will prove a characteristic of other in vitro functions under comparable buffer conditions is not established. In this regard, Ahlquist observed that the relative predominance of α vs. β potential in a model system is influenced by the buffer characteristics (21).

The opposing effect of adrenergic and cholinergic stimuli on biologic systems has more recently been appreciated in in vitro studies of effector cells of the inflammatory response such as tissue mast cells (22), thymus-derived lymphocytes (23), and polymorphonuclear leukocytes (24, 25). β-Adrenergic stimuli suppressed the antigen-induced release of chemical mediators from lung fragments sensitized with IgE, the thymus lymphocyte-mediated destruction of allo-incompatible target cells, and the release of lysosomal enzymes by neutrophilic leukocytes during phagocytosis, while cholinergic stimuli enhanced each of these phenomena. The concentration of cholinomimetic agents active in each of these systems was several orders of magnitude less than those of the adrenergic stimuli. Hence, the demonstration of cholinergic enhancement of spontaneous platelet migration with a peak effect in the nanomolar range or less is entirely consistent with the findings involving other cells engaged in different functions. By analogy to the other systems (26), the opposing action of β-adrenergic and cholinergic stimuli on platelet migration could relate to their influence on the intracellular levels of the cyclic nucleotides, cyclic AMP, and cyclic GMP, respectively.

The findings that PGE$_1$ as well as high concentrations of PGE$_2$ enhanced platelet migration (Fig. 8) are in contradistinction to their inhibitory action on the platelet aggregation-release reaction in a comparable dose (19). The additional finding that indomethacin, an inhibitor of platelet prostaglandin synthetase (7), suppressed random migration (Fig. 7) suggests that a certain level of endogenous prostaglandin formation is essential to the migration process. This is supported by the capacity of indomethacin to prevent PGE$_1$-mediated enhancement at the usual optimal dose, although a several fold increase in this dose will overcome the indomethacin effect. The influence of indomethacin on the expression of the prostaglandin enhancing action may mean that intermediates in prostaglandin biosynthesis act in concert with the end product. The capacity of indomethacin to diminish the effect of exogenous prosta-

glandins has also been observed in an in vitro system concerned with prostaglandin inhibition of antigen-induced mitogenesis (27). Irrespective of whether intermediates in prostaglandin biosynthesis or the end products themselves are essential to migration and its augmentation by exogenous prostaglandins is the additional consideration of whether these effects involve a change in the ratio of cyclic nucleotides. A precise interpretation of the indomethacin or PGE$_1$ effects on platelet function will require determination of the prostaglandins formed and the changes in the level of cyclic nucleotides in relation to pharmacologic and surface stimuli.

ACKNOWLEDGMENTS

REFERENCES

1. Hellem, A. J. 1968. Platelet adhesiveness. Ser. Haematol. 1: 99–145.
2. Hampton, J. R. 1967. The study of platelet behavior and its relevance to thrombosis. J. Atheroscler. Res. 7: 729–746.
3. Boyden, S. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J. Exp. Med. 115: 453–466.
4. Lachmann, P. J., A. B. Kay, and R. A. Thompson. 1970. The chemotactic activity for neutrophil and eosinophil leukocytes of the trimolecular complex of the fifth, sixth, and seventh components of human complement (C567) prepared in free solution by the "reactive lysis" procedure. Immunology. 19: 895–899.
5. Luna, L. G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, New York. 3rd edition. 3.
6. Ammon, H. P. T., and J. Steinke. 1972. Effect of 6-aminonicotinamide on insulin release and C-14 glucose oxidation by isolated pancreatic rat islets: difference between glucose, tolbutamide and aminophylline. Endocrinology. 91: 33–48.
7. Smith, J. B., and A. L. Willis. 1971. Aspirin selectively inhibits prostaglandin production in human platelets. Nat. New Biol. 231: 235.
8. Nathan, P. 1973. The migration of human platelets in vitro. Thromb. Diath. Haemorrh. 30: 173–177.
9. Lowenhaupt, R. W., M. A. Miller, and H. I. Glueck. 1973. Platelet migration and chemotaxis demonstrated in vitro. Thromb. Res. 3: 477–486.
10. Lowenhaupt, R. W., and H. I. Glueck. 1974. The effect of cytochalasin B on platelet migration. Fed. Proc. 33: 243. (Abstr.)
11. Miller, M. E. 1974. Chemotaxis and random mobility. Their clinical and biologic differentiation. In Chemotaxis: Biology and Biochemistry. E. Sorkin, editor. S. Karger, Basel. In press.
12. Aster, R. H., and J. H. Jandl. 1964. Platelet sequestration in man. I. Methods. J. Clin. Invest. 43: 843–855.
13. Goetzl, E. J., I. Gigli, S. I. Wasserman, and K. F. Austen. 1973. A neutrophil immobilizing factor derived from human leukocytes. II. Specificity of action on polymorphonuclear leukocyte mobility. J. Immunol. 111: 938–945.

14. Kay, A. B., and K. F. Austen. 1972. Chemotaxis of human basophil leukocytes. *Clin. Exp. Immunol.* **11**: 557–563.

15. Zigmond, S. 1972. Studies on polymorphonuclear leukocyte locomotion and chemotaxis. Ph.D. Thesis at the Rockefeller University, Division of Cellular Immunology. New York.

16. Goetzl, E. J., and K. F. Austen. 1974. Stimulation of human neutrophil leukocyte aerobic glucose metabolism by purified chemotactic factors. *J. Clin. Invest.* **53**: 591–599.

17. Becker, E. L., and H. L. Showell. 1972. The effect of Ca^{2+} and Mg^{2+} on the chemotactic responsiveness and spontaneous motility of rabbit polymorphonuclear leukocytes. *Z. Immun. Forsch. Bd.* **143**: S466–476.

18. Mills, D. C. B., and G. C. K. Roberts. 1967. Effects of adrenaline on human blood platelets. *J. Physiol. (Lond.).* **193**: 443–453.

19. Kloeze, J. 1969. Relationship between chemical structure and platelet-aggregation activity of prostaglandins. *Biochim. Biophys. Acta.* **187**: 285–292.

20. Abdulla, Y. H. 1969. β-Adrenergic receptors in human platelets. *J. Atheroscler. Res.* **9**: 171–177.

21. Ahlquist, R. P. 1966. The adrenergic receptor. *J. Pharm. Sci.* **55**: 359–367.

22. Kaliner, M., R. P. Orange, and K. F. Austen. 1972. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV. Enhancement by cholinergic and alpha adrenergic stimulation. *J. Exp. Med.* **136**: 556–567.

23. Strom, T. B., C. B. Carpenter, M. R. Garovoy, K. F. Austen, J. P. Merrill, and M. Kaliner. 1973. The modulating influence of cyclic nucleotides upon lymphocyte-mediated cytotoxicity. *J. Exp. Med.* **138**: 381–393.

24. Ignarro, L. J., and C. Colombo. 1973. Enzyme release from polymorphonuclear leukocyte lysosomes: regulation by autonomic drugs and cyclic nucleotides. *Science (Wash. D. C.),* **180**: 1181–1185.

25. Ignarro, L. J. 1974. Stimulation of phagocytic release of neutral protease from human neutrophils by cholinergic amines and cyclic 3′,5′-guanosine monophosphate. *J. Immunol.* **112**: 210–214.

26. Ignarro, L. J., and W. J. George. 1974. Mediation of immunologic discharge of lysosomal enzymes from human neutrophils by guanosine 3′,5′-monophosphate. *J. Exp. Med.* **140**: 225–238.

27. Shenton, B. K., E. J. Field, A. F. Rogers, J. A. Carney, J. A. C. Sykes, and J. Mertin. 1973. Prostaglandins and cellular immunity: a regulatory mechanism. *Int. Res. Commun. Sys.* Dec. 8-10-1: 21.