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Research Article

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Increased Vascular Permeability Produced

by Human Platelet Granule Cationic Extract

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A B S T R A C T   A cationic protein extract obtained from isolated human platelet granules increased vascular permeability in mouse and rabbit skin. The permeability-enhancing effect was not inhibited by soybean trypsin and pancreatic trypsin inhibitor, methylsergide maleate, carboxypeptidase B, and CĪ inactivator. Permeability-enhancing activity was blocked by prior treatment of challenged animals with antihistamine. The nondializable relatively heat-stable cationic granule protein extract possessed potent mastocytolytic activity. The experiments described suggest that human platelets exert a permeabil:ty-enhancing effect by lysosomal release of cationic proteins which cause histamine release from adjacent tissue mast cells.

INTRODUCTION

Early inflammatory changes in injured tissues involve the release of various biologically active materials from the lysosomes of polymorphonuclear leukocytes, including the cationic proteins (1–5). This family of prote:ns possesses a spectrum of activities including antibacterial action (6), enhancement of vascular permeability (3), anticoagulant activity (7), and fever production (8). The cationic lysosomal proteins therefore appear to play important roles as mediators of the inflammatory response.

Human platelets contain intracellular granules similar to the classical lysosomes of other cells (9). Platelets degranulate during the formation of the hemostatic plug (10) and during interaction with antigen-antibody complexes, bacteria, and viruses (11). Recent studies of Packham, Nishizawa, and Mustard (11) indicate that platelets may contribute to the inflammatory response accompanying tissue injury by the release during such interactions of intracellular constituents which increase vascular permeability.

In order to investigate the role of human platelets as early mediators of the inflammatory response we have examined the biological activities of cationic proteins extracted from isolated platelet granules. This cationic protein extract increased vascular permeability and released histamine from mast cells. By these mechanisms human platelets may contribute to the development of the acute inflammatory reaction.

METHODS

Preparation of platelet fractions. Human platelet concentrates prepared from 10-20 U of freshly collected whole blood in acid citrate dextrose anticoagulant were generously supplied by the New York Blood Center. The concentrates were usually processed within 24 hr of the day of collection. Platelets were separated by means of the "oil bottle" centrifugation technique (12). Platelet counts were performed by phase microscopy. Contamination of platelets by leukocytes in multiple samples was less than one cell per 10,000 platelets. The isolated platelets were washed four times in Alsever's solution (13) and four more times in Gaintner buffer (14). The platelets were homogenized in 0.44 M sucrose containing 0.001 M ethylenediaminetetraacetate (EDTA) and subcellular fractions were prepared by sucrose density gradient ultracentrifugation as described by Marcus, Zucker-Franklin, Safier, and Ullman (9). The granule fraction was washed twice with Gaintner buffer, dialyzed extensively against water, and lyophilized. Platelet protein was obtained from intact washed platelets by sonication and prepared as previously described (12).

Extraction of cationic proteins from platelets. Cationic protein fractions were obtained from lyophilized granules by extraction three times with 2-ml volumes of ice cold 0.2 N H_2SO_4. The combined acid extracts were cleared by centrifugation at 12,000 g. Similar acid extracts were prepared from platelet protein preparations and from whole washed platelets. Before experimental use, the acid extracts were dialyzed for 4 hr at 10°C against three changes of buffered saline pH 7.4 (0.15 M NaCl, 0.0175 M phosphate). The 20% ethanol fraction was prepared from the acid platelet granule extract as described by Seegers and Janoff (3). All fractions were adjusted to physiologic pH and salt concentration before use. It was noted that biologic activity diminished with storage at 4°C. Thus in the experiments described below studies were performed generally within 1-2 wk of extraction.

Vascular permeability tests. Swiss Webster mice, weighing 20–25 g, were injected intravenously with 0.1 ml of 0.5% solution of Evans Blue (Warner Lambert Pharmaceutical Co., Morris Plains, N. J.). Beginning 5 min later unless otherwise noted, 0.1 ml of test material was injected intracutaneously into the back of the mice using No. 26 gauge disposable needles. Between 60 and 90 min later, the animals were killed, the dorsal skin reflected, and the injected site excised, weighed, and placed in 1.5 ml formamide solution. After dye elution for 72–96 hr the color intensity was quantitated by spectrophotometric analysis at 620 mμ. The permeability activity in this system was defined as the increase in OD units in the formamide solution per 100 mg of excised mouse skin. Vascular permeability tests were performed in rabbits which had received an intravenous injection of Evans Blue (1.0 ml/kg of a 0.5% solution). The permeability activity was expressed as the average diameter of bluing of duplicate lesions 60 min after the test challenge.

The effects of the antihistamine, chlorpheniramine maleate, and the serotonin antagonist, methylsergide maleate, upon the permeability-enhancing properties of platelet granule extract were tested in mice by injecting 1 mg chlorpheniramine or 0.01 mg methylsergide intraperitoneally ½ hr before intravenous Evans Blue. Control animals were pretreated with intraperitoneal injection of buffer. The antihistamine effect was tested in rabbits by the intradermal injection of chlorpheniramine maleate 30 min before intravenous Evans Blue injection. The test agents were then injected into the pretreated intradermal sites. The effect of carboxypeptidase B, an inhibitor of bradykinin (15), on the permeability-enhancing property was tested by prior incubation of platelet granule extract with 15 U of carboxypeptidase B for 1 hr at room temperature before intracutaneous challenge. The effect of trypsin was determined by incubating platelet granule extract with the enzyme at an extract to enzyme protein ratio of 10:1. The reaction mixture, containing 0.002 M Ca^{++}, was adjusted to pH 8.2 with sodium bicarbonate and incubated for 19 hr at 37°C. Control incubations included granule extract in the absence of trypsin and buffer plus trypsin. At the completion of the incubation period, the pH was adjusted to 7.4 with 0.1 N HCl. The effect of pancreatic trypsin inhibitor (final concentration 50 μg/ml) and soybean trypsin inhibitor (final concentration 125 μg/ml) was determined by incubation of the enzyme inhibitors with the extract at room temperature for 30 min. Partially purified C$\bar{1}$ inactivator [1] was kindly provided by Dr. Peter Harpel, Cornell University Medical College. The effect of this inhibitor was determined after incubation of the platelet extract with 50 inhibitor U/ml for 30 min.

Assay of mastocytolytic activity in vitro. Adult Sprague Dawley rats, exsanguinated by cardiac puncture, were injected intraperitoneally with 20 cc of cold Tyrode's solution containing 50 U heparin/ml. Peritoneal cells were obtained by aspiration, washed by centrifugation, and resuspended in Tyrode's solution. The suspensions contained 5–10% mast cells. Cells were collected similarly from adult Swiss Webster mice, weighing 20-25 g, after injection of 5 ml of Tyrode's solution. Aliquots of cells were pipetted onto Millipore filters (Millipore Corp., Bedford, Mass.) in modified Lucite Boyden chambers. Buffer, platelet cationic extract, and (or) inhibitors were added by micropipette and mixed gently. Chambers were incubated at 37°C for 30 min, after

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[1] C$\bar{1}$ inactivator is World Health Organization current term for the former C′1 esterase-inhibitor.

which the reaction was stopped by adding fixative directly to the chambers. The fixative solution contained 10% formalin and 0.1% toluidine blue, which stained the mast cells selectively. After dehydration in serial alcohols and clearing in xylene, the filters were examined under the microscope at 400 × magnification. 500 mast cells were counted per filter; cells with smooth borders were classed as intact and those with granules adherent to the surface or scattered in a halo around the cells were considered degranulated. Control samples, containing cells and buffer only, were run simultaneously in every experiment. All samples were tested in duplicate and all counts were performed by the same observer.

Analytical polyacrylamide-gel electrophoresis. This was carried out as described by Reisfield (16) using β-alanine buffer pH 4.5 at 5 ma current per tube for 60 min. The gels were stained with Coomassie Blue.

Enzyme assays. Ribonuclease and desoxyribonuclease were assayed by the method of Schneider and Hogeboom (17). Lysozyme was measured by the method of Prockop and Davidson (18) using the lysozyme assay kit (Worthington Biochemical Corp., Freehold, N. J.). Cathepsin activity was measured as previously described (19).

The platelet aggregating property of the platelet granule extract was studied after the addition of 0.1 ml buffered extract (230 μg/ml) to 1.2 ml citrated human platelet-rich plasma by the method of Born (20) using a platelet aggregometer (Chronolog Corp., Broomall, Pa.).

Anticoagulant activity of the platelet granule extract. This was studied as described by Saba, Roberts, and Herion (7). The following tests were performed in duplicate with and without platelet granule extract at a protein concentration of 100 μg/ml: one-stage prothrombin time (21), recalcification time (22), thrombin time (23), and partial thromboplastin time (24). Before use the platelet granule extract was dialyzed for 4 hr into barbital buffer pH 7.35 ionic strength 0.154. Buffer replaced platelet granule extract in control clotting mixtures.

Chemical assay for vasoactive amines. Serotonin in the platelet granule extract was assayed by the fluorometric method of Bertler (25). This assay was performed by Dr. Donald Reis, Cornell University Medical College. Histamine in the platelet granule extract was assayed fluorometrically by the method of Shore, Burkhalter, and Cohn (26). The limit of sensitivity of the assay was 0.005 μg histamine. Intradermal histamine at this level did not produce increased vascular permeability in mice or rabbits.

Protein determinations were carried out by the Folin method (27).

Special chemicals and pharmacologic agents. Chlorpheniramine maleate (Schering) supplied 100 mg/ml was diluted in buffered saline to desired concentration. Methylsergide maleate, an antiserotonin agent, was the gift of Sandoz Pharmaceuticals, Hanover, N. J. Carboxypeptidase B (COBC 8JA) supplied as 176 U/mg, soybean trypsin inhibitor 3 x crystallized, and pancreatic trypsin inhibitor (salt free) were obtained from Worthington Biochemical Corp., Freehold, N. J. Bradykinin triacetate was obtained from Calbiochem, Los Angeles, Calif. Diisopropyl fluorophosphate (DFP) was reconstituted to a 1 M solution in isopropyl alcohol, and diluted to desired concentration in buffer. Sodium fluoride was obtained from Fisher Scientific Co., Union, N. J. Iodoacetamide and *para*-chloromercuribenzoate (*p*-CMB), recrystallized before use, was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Vascular Permeability Produced by Platelet Granule Cationic Extract   275

TABLE I
Permeability-Enhancing Activity of Platelet Fractions

Mixture tested	Animals	Permeability* activity
Buffered saline	10	0.095 ±0.00
Platelet granule extract, 100 µg/ml	10	0.440 ±0.09
Platelet granule extract–20% ethanol fraction, 140 µg/ml	5	0.405 ±0.14
Whole platelet extract, 680 µg/ml	8	0.360 ±0.09
Platelet protein extract, 1.4 mg/ml	5	0.260 ±0.06
Histamine, 1 mg/ml	4	0.750 ±0.07

* Mean ±1 SD.

RESULTS

Permeability-enhancing activity of platelet cationic protein. The cationic protein extract from the isolated platelet granules significantly increased vascular permeability in mouse skin (Table I). The 20% ethanol fraction prepared from the granule acid extract and acid extracts derived from whole platelets as well as from solubilized platelet protein were less active than the starting material. In view of the apparent greater specific biologic activity of the granule cationic extract, these preparations primarily were used for subsequent experimental studies.

The permeability changes induced in the mouse skin by single injections of granule cationic extract appeared to be relatively acute, disappearing over a short period of time. The maximum enhancement of vascular permeability was observed when animals were in-
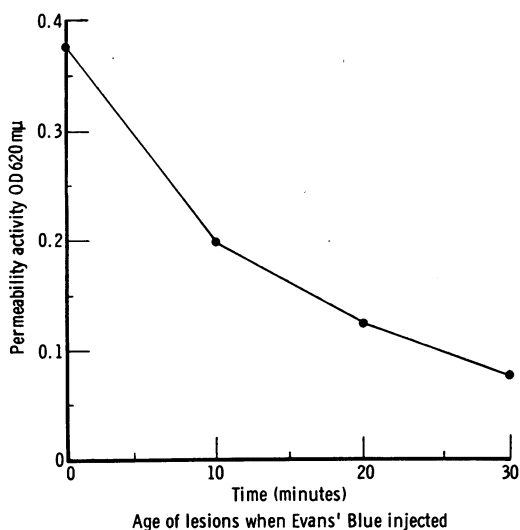

FIGURE 1 The duration of the permeability-enhancing effect after the injection of platelet granule extract (100 µg/ml). Each point represents the average of four animals.

jected with Evans Blue immediately before or after intracutaneous challenge (Fig. 1). When the dye was injected 10, 20 or 30 min after intracutaneous challenge, permeability changes were diminished.

Effect of various agents on the permeability-enhancing property. Pretreatment of mice with intraperitoneal chlorpheniramine maleate completely abolished the permeability-enhancing property of the platelet granule extract (Table II).

The serotonin antagonist, methylsergide maleate, had no inhibitory effect on the vascular permeability-enhancing property of the granule cationic extract (Table II). An intracutaneous challenge with serotonin was effectively blocked by prior treatment with the inhibitor.

Carboxypeptidase B, a known inhibitor of the inflammatory promoting activity of bradykinin (16), was incubated with the platelet granule extract before intracutaneous challenge (Table III). No impairment of biologic activity was observed.

Incubation of the platelet cationic extract with trypsin for 24 hr at 37°C significantly inhibited the permeability enhancing activity (Table IV).

Soybean trypsin inhibitor and pancreatic trypsin inhibitor, known blockers of the plasmin system (28), did not interfere with the permeability-enhancing property of the platelet acid extract (Table V). The in vivo permeability experiments reported in this table were performed using two separate batches of platelet granule extract. The platelet granule extract used with the pancreatic trypsin inhibitor was more potent than the preparation used with the soybean trypsin inhibitor. Both granule extract preparations produced significant enhancement of vascular permeability which was not blocked by either enzyme inhibitor. Pancreatic trypsin

TABLE II
Effect of Histamine and of Serotonin Antagonists on the Permeability-Enhancing Property of Platelet Granule Extract

Test materials	No. of animals	Permeability activity after	
		Inhibitor	Buffer*
		Chlorpheniramine‡	
Buffer	6	0.09 ±0.02	0.10 ±0.01
PGE§, 100 µg/ml	6	0.08 ±0.02	0.36 ±0.07
Histamine, 0.1 mg/ml	3	0.12 ±0.02	0.52 ±0.10
		Methylsergide‖	
Buffer	3	0.09 ±0.01	0.08 ±0.01
PGE§, 108/µg per ml	3	0.29 ±0.07	0.31 ±0.04
Serotonin, 1 mg/ml	3	0.08 ±0.01	0.22 ±0.04

* 0.1 ml injected intraperitoneally 1 hr before challenge.
‡ 1.0 mg injected intraperitoneally 1 hr before challenge.
§ PGE = platelet granule extract.
‖ 0.01 mg injected intraperitoneally 1 hr before challenge

inhibitor and soybean trypsin inhibitor alone were inactive.

CĪ inactivator has been shown to abolish permeability enhancement by several different protein systems including: CĪ, PF/dil, and kallikrein (29). Preincubation of the platelet granule extract with this inhibitor did not interfere with the permeability-enhancing property (Table V).

Vascular permeability studies in rabbits. The permeability-enhancing activity of the platelet granule extract in rabbits closely paralleled the observations made in mice. The platelet granule extract produced a significant increase in permeability which was effectively blocked by prior local treatment with the antihistamine, chlorpheniramine maleate (Table VI). Chlorpheniramine and other antihistamines also have an anti-bradykinin action in rabbits (30). Carboxypeptidase B did not inhibit the permeability-enhancing property of the platelet granule extract. This enzyme alone was inactive. Soybean trypsin inhibitor was similarly inactive in blocking the biologic activity of the cationic extract.

Mastocytolytic activity of platelet granule extract in vitro. The platelet granule extract was shown to possess a potent mastocytolytic effect using both mouse and rat peritoneal cells. Fig. 2 shows the concentration dependence of the degranulation effect using a potent preparation of cationic extract. In these studies the rat peritoneal cell suspension was incubated with the platelet extract for 30 min at 37°C. At high concentrations of extract, almost complete degranulation of the mast cells was observed.

Effect of metabolic inhibitors on the mastocytolytic effect of the platelet cationic extract. Incubation of rat peritoneal mast cells with platelet granule cationic extract at 4°C resulted in no degranulation whereas degranulation by platelet granule extract was consistently observed at 37°C. The effect of various inhibitors on the mastocytolytic activity of platelet cationic extract at 37°C is shown in Table VII. Significant inhibition of degranulation occurred in the presence of sodium fluoride, an inhibitor of glycolysis, as well as with iodoacetamide and *p*-CMB, inhibitors of thiol enzymes.

TABLE III

Effect of Carboxypeptidase B on Permeability-Enhancing Property of Platelet Granule Extract

Mixture tested	Animals	Permeability activity
Carboxypeptidase B, 15 U	3	0.065 ±0.02
PGE* diluted 1:1 with buffer	3	0.23 ±0.06
PGE* diluted 1:1 with carboxy peptidase B 15 U	3	0.21 ±0.03

* PGE = platelet granule extract 108 μg/ml.

TABLE IV

Effect of Trypsin on Permeability-Enhancing Ability of Platelet Granule Extract

Mixture tested	Animals	Permeability activity
Buffer	3	0.09 ±0.02
PGE*, 140 μg/ml	3	0.35 ±0.08
PGE*, 140 μg/ml plus trypsin 15 μg/ml	3	0.11 ±0.01
Buffer plus trypsin 15 μg/ml	3	0.07 ±0.01

* PGE = platelet granule extract.

Profound inhibition of degranulation occurred in the presence of the esterase inhibitor, diisopropyl fluorophosphate (DFP). Incubation of the mast cell suspension with the inhibitors alone resulted in no significant degranulation.

Additional characterization of the platelet granule extract. Acrylamide-gel electrophoresis of the platelet granule acid extract revealed at least three distinct cationic protein bands (Fig. 3). This electrophoretic pattern was observed on repeated analyses of multiple

TABLE V

Effect of Pancreatic Trypsin Inhibitor, Soybean Trypsin Inhibitor, and CĪ Inactivator on Permeability-Enhancing Activity of Platelet Granule Extract

Mixture tested	No. of mice	Permeability activity
Buffer	4	0.11 ±0.02
PGE*	6	0.41 ±0.05
PGE* + pancreatic trypsin inhibitor, 50 μg/ml	6	0.43 ±0.10
Buffer + pancreatic trypsin inhibitor, 50 μg/ml	6	0.16 ±0.07
PGE‡	4	0.34 ±0.05
PGE‡ + soybean trypsin inhibitor, 50 μg/ml	4	0.36 ±0.08
Buffer + soybean trypsin inhibitor, 50 μg/ml	4	0.07 ±0.03
PGE§	4	0.52 ±0.11
PGE§ + CĪ-inactivator, 50 U/ml	4	0.50 ±0.02
Buffer + CĪ-inactivator, 50 U/ml	4	0.08 ±0.02

* PGE = platelet granule extract 100 μg/ml.
‡ PGE = a different preparation of platlet granule extract 230 μg/ml.
§ PGE = third preparation of platelet granule extract 130 μg/ml.

separate platelet granule preparations. No deoxyribonuclease, ribonuclease, lysozyme, or cathepsin enzyme activity was detected in the platelet cationic extract using protein concentrations up to 1 mg/ml. Similarly, no serotonin or histamine was detected in the platelet cationic protein solution using spectrofluorometric methods. Extensive dialysis as well as heating the platelet granule solution to 80°C for 15 min did not abolish the biologic activity. The preparation at protein concentrations of 100 μg/ml did not cause aggregation of normal platelets in citrated plasma differentiating this activity from platelet factor 2 (31). No anticoagulant activity of the platelet granule cationic extract was detected using four separate clotting systems: prothrombin time, recalcification time, thrombin time, and partial thromboplastin time.

DISCUSSION

We have demonstrated that a cationic protein fraction extracted from the intracellular granules of human platelets increases vascular permeability in mouse and rabbit skin. This platelet granule fraction has many characteristics similar to the mastocytolytic, lysosomal
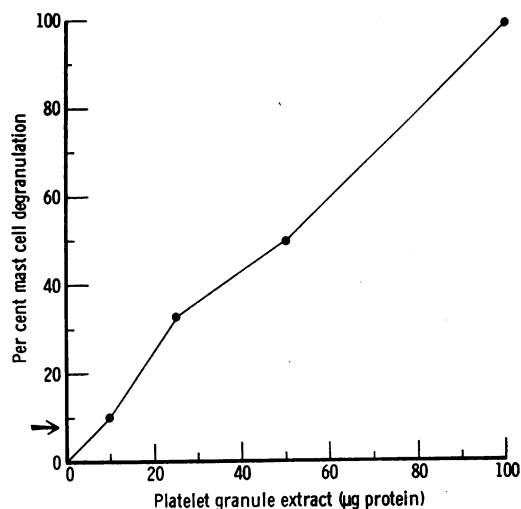

FIGURE 2 Rat mast cell degranulating effect of increasing amounts of cationic platelet granule extract. The arrow indicates the upper limit of degranulation observed in the presence of buffer. Each point represents average of four preparations.

TABLE VI

Permeability-Enhancing Activity of Platelet Granule Extract in the Rabbit

Mixture tested	No. of experiments	Bluing diameter*
		mm
Buffer	8	1.0 ±0.9
PGE‡	4	11.0 ±1.6
PGE‡ 30 min after intradermal chlorpheniramine maleate, 0.1 ml (0.1 mg)	3	3.0 ±0.0
Buffer 30 minutes following intradermal chlorpheniramine maleate, 0.1 ml	3	3.0 ±0.0
PGE‡ diluted 1:1 with buffer	4	8.0 ±1.6
PGE‡ diluted 1:1 with carboxypeptidase B, 15 U	3	9.0 ±1.0
Buffer with carboxypeptidase 5, 15 U	3	2.0 ±1.0
Bradykinin, 4.8 μg/ml	3	12.0 ±1.0
Bradykinin, 4.8 μg/ml with carboxypeptidase B, 15 U	3	3.0 ±2.0
PGE‡ plus soybean trypsin inhibitor, 125 μg/ml	3	10.0 ±2.0
Buffer plus soybean trypsin inhibitor, 125 μg/ml	3	1.0 ±1.0

* Mean ±1 SD.
‡ PGE = platelet granule extract, 180 μg/ml.

cationic protein obtained from rabbit exudative polymorphonuclear leukocytes (3). Thus, the active principle of platelet granules is extracted in weak mineral acid from whole cell homogenates as well as from isolated granules and is retained in the cationic fraction precipitated by 20% ethanol. The active principle extracted from whole platelets was completely localized to the cationic fraction as all other anionic fractions of the human platelets obtained chromatographically on DEAE-cellulose including those containing cathepsin A failed to induce increased vascular permeability upon intradermal injection.[2] The platelet cationic extract, which was inactivated by prior incubation with the proteolytic enzyme trypsin, revealed up to three discrete bands on cationic polyacrylamide gel electrophoresis. Lysozyme, ribonuclease, deoxyribonuclease, well defined cationic enzyme constituents in leukocyte lysosomes, were not detected in the platelet granule cationic extract.

It is well known that the platelet actively adsorbs onto its plasma membrane a population of plasma proteins including a majority of the coagulation factors (32). The platelet may retain some of these activities even after extensive washing (33). For this reason, it was necessary to be certain that the permeability-enhancing activity observed utilizing the platelet cationic fraction was not due to the participation of one or more contaminating plasma protein systems.

TABLE VII
Effect of Metabolic Inhibitors on Mast Cell Degranulation by Platelet Granule Extract

Mast cells incubated with:	10^{-3} M iodoacetamide	10^{-5} M p-CMB	10^{-4} M NaF	10^{-10} M DFP	None
PGE*	—	—	—	—	41.3
Buffer plus	6.6	8.7	5.1	2.1	6.7
PGE*	12.2	6.1	10.6	0.9	—

p-CMB = parachloromercuribenzoate; NaF = sodium fluoride; DFP = diisopropyl fluorophosphate.
* PGE = platelet granule extract 87 μg/ml.

Human platelets contain plasminogen (12), and plasmin has been shown to produce increased vascular permeability (34). Soybean trypsin inhibitor and pancreatic trypsin inhibitor, which block the action of plasmin, did not appreciably inhibit the permeability-enhancing activity of the platelet cationic granule extract.

Carboxypeptidase B, a specific inhibitor of bradykinin (15), did not inhibit the vascular permeability-enhancing activity of the platelet granule extract in mouse and rabbit skin. C1-inactivator, which blocks the action of C1, PF/dil, kallikrein, and plasmin (29), had no inhibitory effect on the permeability-enhancing property of the platelet granule extract. It thus seems unlikely that these well defined plasma protein systems contributed significantly to the vascular permeability effect of the platelet extract.

Platelets contain appreciable quantities of granule-bound serotonin (35); however, in contrast to rabbit platelets, human platelets contain little or no histamine (36). The specific serotonin antagonist, methylsergide maleate, did not inhibit the permeability-enhancing property of the platelet granule extract. Extensive dialysis of the platelet granule extract did not abolish the biologic activity. In addition, spectrofluorometric analysis of active aliquots of the granule extract revealed no measurable serotonin or histamine. It would therefore seem likely that these intrinsic platelet vasoactive amines were not directly responsible for the permeability-enhancing properties of the cationic extract.

Prior treatment of the test animals with the antihistamine, chlorpheniramine maleate, consistently blocked the permeability-enhancing effect of the platelet granule extract. Since the increase in vascular permeability after the injection of platelet cationic extract appeared ultimately to be mediated by the challenged animals' histamine, we examined the effect of the extract on mast cells obtained from mouse and rat peritoneal washings. Cationic proteins from rabbit exudate neutrophilic leukocytes are known to contain a permeability factor which

releases histamine from mast cells (3). Such histamine release from mast cells is well correlated with the release of granules from the cells, providing a sensitive visual index of amine liberation. This granule release is an energy-requiring process which requires participation of cell-bound esterases in contrast to the toxic, nonspecific granule release caused by detergents and cytotoxic chemicals (37). The platelet granule cationic extract which produced striking changes in vascular permeability also caused marked degranulation of mouse and rat mast cells. The mastocytolytic effect of the cationic extract was inhibited by exposure of the mast cell suspension to diisopropyl fluorophosphate, a potent inhibitor of serine esterases. In these respects, the platelet cationic activity bears striking resemblances to at least one of the rabbit neutrophil cationic proteins (37).

The platelet cationic activity differed strikingly from one previously reported property associated with rabbit lysosomal cationic proteins. Saba, Roberts, and Herion (7) demonstrated that rabbit leukocyte lysosomal cationic preparations exert a strong anticoagulant effect on human blood. Using the identical test systems described by these authors, we have been unable to demonstrate a similar effect with the platelet cationic extract. This difference may be biologically important particularly in view of the crucial role that the platelet plays in the initiation of hemostasis.

These studies corroborate and extend earlier observations of Mustard and associates (11, 38) who first showed that incubation of human platelets with throm-
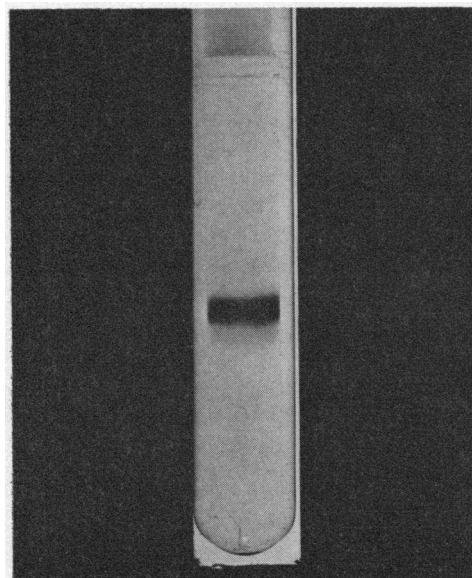

FIGURE 3   Acrylamide-gel electrophoresis of platelet granule extract (30 μg protein). Cathode toward the bottom.

bin, collagen or antigen antibody complexes was associated with the release of permeability factors from the aggregated platelets. These investigators postulated that the permeability effect resulted from the combined release of histamine, serotonin, and lysosomal enzymes. Subsequent studies by Packham, Nishizawa, and Mustard (11) showed that antihistamine pretreatment of test animals inhibited the permeability effect.

Our studies in addition to these earlier findings suggest that platelets may play an important role in the pathophysiologic mechanisms associated with the inflammatory response. It has been previously demonstrated that platelets accumulate in blood vessels adjacent to areas of tissue damage and inflammation (39). Various stimuli including circulating antigen-antibody complexes, endotoxin, and bacteria as well as endothelial cell disruption and damage may lead to platelet aggregation and degranulation with lysosomal discharge of the mastocytolytic cationic activity. We have previously demonstrated that human platelet granules contain potent proteases including cathepsin A (19). These proteolytic activities, although not apparently directly involved in the vascular permeability changes, may contribute to additional phases of the early cell-mediated inflammatory response.

The platelet cationic protein activity described in these studies obviously bears a close analogy to the well described system in rabbit neutrophil polymorphonuclear leukocytes. The techniques utilized in our study were primarily based on those developed in the rabbit leukocyte system. One important difference should be stressed when comparing the human platelet system to the rabbit leukocyte systems. To data no lysosomal cationic permeability activity has been detected in circulating rabbit leukocytes. All the previously described vascular permeability-enhancing activity has been obtained with fractions derived from *exudative* cells. Thus, the platelet in man (and probably in lower animals) may be a unique circulating cell with a precommitted exudative function which permits it to play an important role in mediating early blood vessel responses to focal inflammatory stimuli.

ACKNOWLEDGMENTS

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