The Assessment of Drug-Dependent and Isoimmune Antiplatelet Antibodies by the Use of Platelet Aggregometry

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ABSTRACT The technique of platelet aggregometry provides a simple, quantitative, and specific method for the detection of drug-dependent and isoimmune antiplatelet antibodies. In the presence of antiquinidine antibody, quinidine causes lysis of normal platelets in platelet-rich plasma. The resulting changes in optical density are readily detected in the aggregometer. The initial rate of lysis is a function of the antibody titer, but is relatively independent of the platelet count. In vitro, quinidine produces platelet swelling and inhibits aggregation of platelets by adenosine diphosphate, epinephrine, and collagen. Isoimmune antibodies cause aggregation of platelets in platelet-rich plasma. In studies of a single family the rate of aggregation is proportional to the number of HL-A antigens present on the normal platelets against which the antibody is directed. The simple technique of platelet aggregometry may be a useful adjunct in the selection of compatible donors for platelet transfusion. Serum derived from patients with idiopathic thromboytopenic purpura did not cause platelet aggregation.

INTRODUCTION

Drug-induced immune reactions are one of the most common causes of acquired thrombocytopenia. Further, the widespread use of platelet transfusions has emphasized the importance of isoimmune antiplatelet antibodies in determining the survival of infused platelets (1). Not surprisingly, a variety of techniques have been utilized to detect drug-induced and isoimmune antiplatelet antibodies (2–16). These include direct visual assays such as observation of platelet lysis (2–5), platelet agglutination, (6–10), and inhibition of clot retraction (3, 11, 12). Unfortunately, these tests, while simple to perform, are relatively insensitive. More sensitive tests—complement fixation (4, 7, 13), antiglobulin consumption (4, 7), platelet factor 3 activation (10,14), and inhibition of serotonin uptake or release of serotonin and ^{51}Cr from platelets (15, 16)—are highly specialized, and are not readily available in most clinical laboratories.

The purpose of this paper is to describe a new technique for measuring drug-induced and isoimmune antiplatelet antibodies. The assay is an optical one, in which changes in optical density (or light transmission) of platelet-rich plasma are recorded as antibody-induced changes occur. The test is simple, but quantitative and highly sensitive. It has been used to test for the presence of antiplatelet antibodies in 30 normal subjects and in 29 patients with various forms of thrombocytopenia.

METHODS

Platelet-rich plasma (PRP).[1] Blood was collected by a two-syringe technique with plastic disposable syringes (Roehr Products, Philadelphia, Pa.) and uncoated, thin-wall, 19-gauge needles ("Minicath," Deseret Pharm. Co., Sandy, Utah) from normal subjects and from patients. The whole blood was transferred to plastic centrifuge tubes (Falcon Plastics, No. 2027, Oxnard, Calif.) containing either: 0.5 ml 3.8% sodium citrate (w/v); 0.005 ml or 0.05 ml heparin (Upjohn Company, Kalamazoo, Mich., 1,000 U/ml) or 0.15 ml 5% disodium EDTA (w/v). In each instance sufficient blood was added to reach a final volume of 5 ml. The anticoagulated blood was centrifuged at room temperature (21–23°C) at 180 *g* for 10 min, and the PRP was removed with silicone-coated Pasteur pipettes. The platelet count of the PRP from normal subjects ranged from 150

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[1] *Abbreviations used in this paper:* PPP, platelet-poor plasma; PRP, platelet-rich plasma.

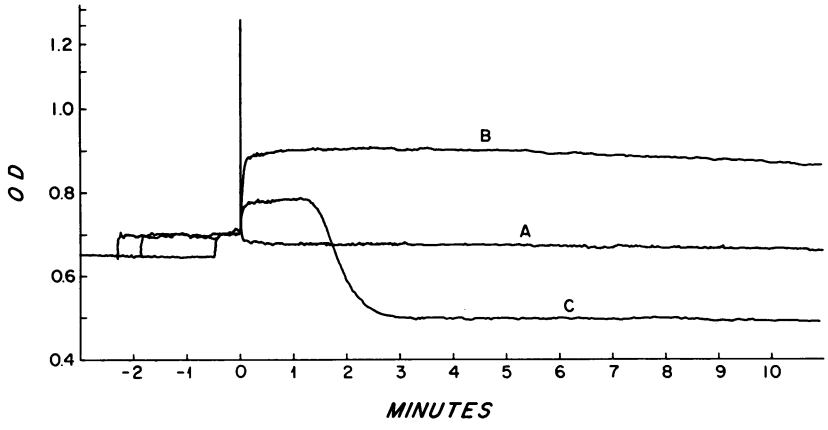


FIGURE 1 Representative tracing of the response to quinidine. Curve A: 0.3 ml normal PRP (3.8% Na citrate 1:10 v/v), 0.2 ml normal serum. Curve B: 0.3 ml normal PRP, 0.2 ml normal serum. Quinidine (1.5×10^{-3} M final concentration) added at zero time. Curve C: (displaced downward by 0.1 optical density [OD] unit for clarity of presentation) 0.3 ml normal PRP, 0.2 ml serum from patient with quinidine-induced thrombocytopenia. Quinidine (1.5×10^{-3} M final concentration) added at zero time.

to 500,000 cells/mm³. The PRP was maintained in plastic tubes at room temperature.

Platelet-poor plasma (PPP). PPP was prepared in a similar fashion except that whole blood was centrifuged at 2,400 *g* for 20 min. The platelet count of the PPP ranged from 4 to 10,000 cells/mm³. In experiments in which the platelet count of the PRP was varied, dilutions were made in PPP derived from the same subject.

Serum. Serum was prepared by transferring whole blood from normal donors or from patients into new, uncoated glass tubes. The blood was allowed to clot for 2 hr at 37°C. The serum was separated by centrifugation at 2,400 *g* for 20 min. It was then heated at 56°C for 30 min to inactivate traces of thrombin. Serum samples were stored at −20°C and were warmed to room temperature before use. Sera from seven patients with idiopathic thrombocytopenic purpura and from one normal patient were the gift of Drs. William Maloney and Robert Handin, Peter Bent Brigham Hospital, Boston, Mass.

Platelet aggregometry. The changes in optical density of stirred PRP induced by platelet aggregation or lysis were measured in a platelet aggregometer (Chrono-Log Corp, Broomall, Pa.) with a 609 nm filter. A measured volume of normal PRP (usually 0.3 ml) was added to a 0.312 inch diameter, silicone-coated cuvette containing a 1 × 4 mm stirring bar (cut segment of a paper clip). A measured volume of heated test serum (usually 0.2 ml) was added, and the cuvette was then placed in the light path of the instrument. The contents of the cuvette were maintained at 37°C; the rate of stirring was 1,100 rpm. Changes in optical density (or light transmission) were continuously recorded on a strip chart recorder. We have used a Varicord Model 43 (Photovolt Corp., New York), but any potentiometric 10 mv full scale recorder is suitable. In some experiments aggregation of platelets was initiated with ADP (Sigma Chemical Company, St. Louis, Mo.) or by epinephrine.

Electron microscopy. Samples of PRP-serum-drug mixtures were fixed in a solution containing 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. After fixation the samples were washed and stained in 1% osmium. They were then dehydrated through graded alcohols, imbedded in Epon, and sections were viewed in a Philips Model 200 electron microscope (Philips Electronic Instruments, Mount Vernon, N. Y.).

RESULTS

The detection of drug-induced antibodies. When normal, heat-treated serum (0.2 ml) was added to normal PRP (0.3 ml) prepared with 3.8% Na citrate (1:10 v/v) in the absence of quinidine, no changes in optical density of the stirred mixture occurred over a period of observation as long as 60 min (Fig. 1, curve A). When quinidine gluconate (final concentration 1.5×10^{-3} M) was added to such a mixture of normal PRP and normal heated serum, an increase in optical density promptly occurred, but there was no subsequent change in the optical density of the mixture (Fig. 1, curve B). When serum containing antibodies against quinidine was added to normal PRP, no change in optical density occurred. However, when quinidine was added, there was a biphasic change in optical density (Fig. 1, curve C). Initially, the optical density increased as it did when quinidine alone was added. Shortly thereafter there was a progressive decline in the optical density of the mixture.

The morphologic counterpart of the changes in optical density of PRP that occurred when quinidine was added in the presence or absence of antibodies are shown in Fig. 2. Quinidine (final concentration 1.5×10^{-3} M) was added to a mixture of PRP (citrate anticoagulant) from a normal subject and heated serum from a patient with quinidine-induced thrombocytopenia. At intervals before and after the addition of quinidine, samples were removed and fixed for electron microscopy. Quinidine was similarly added to an incubation mixture con-

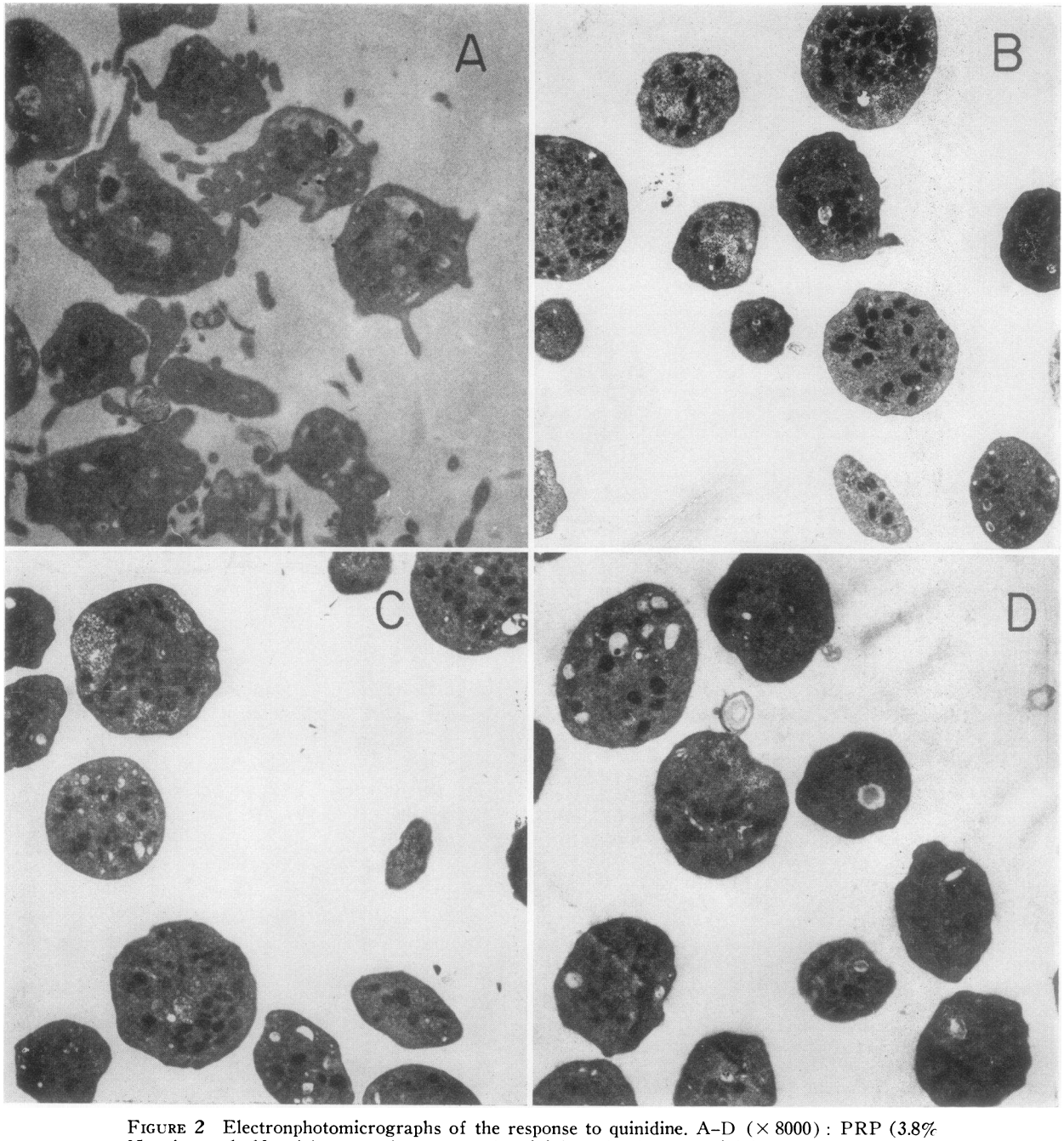
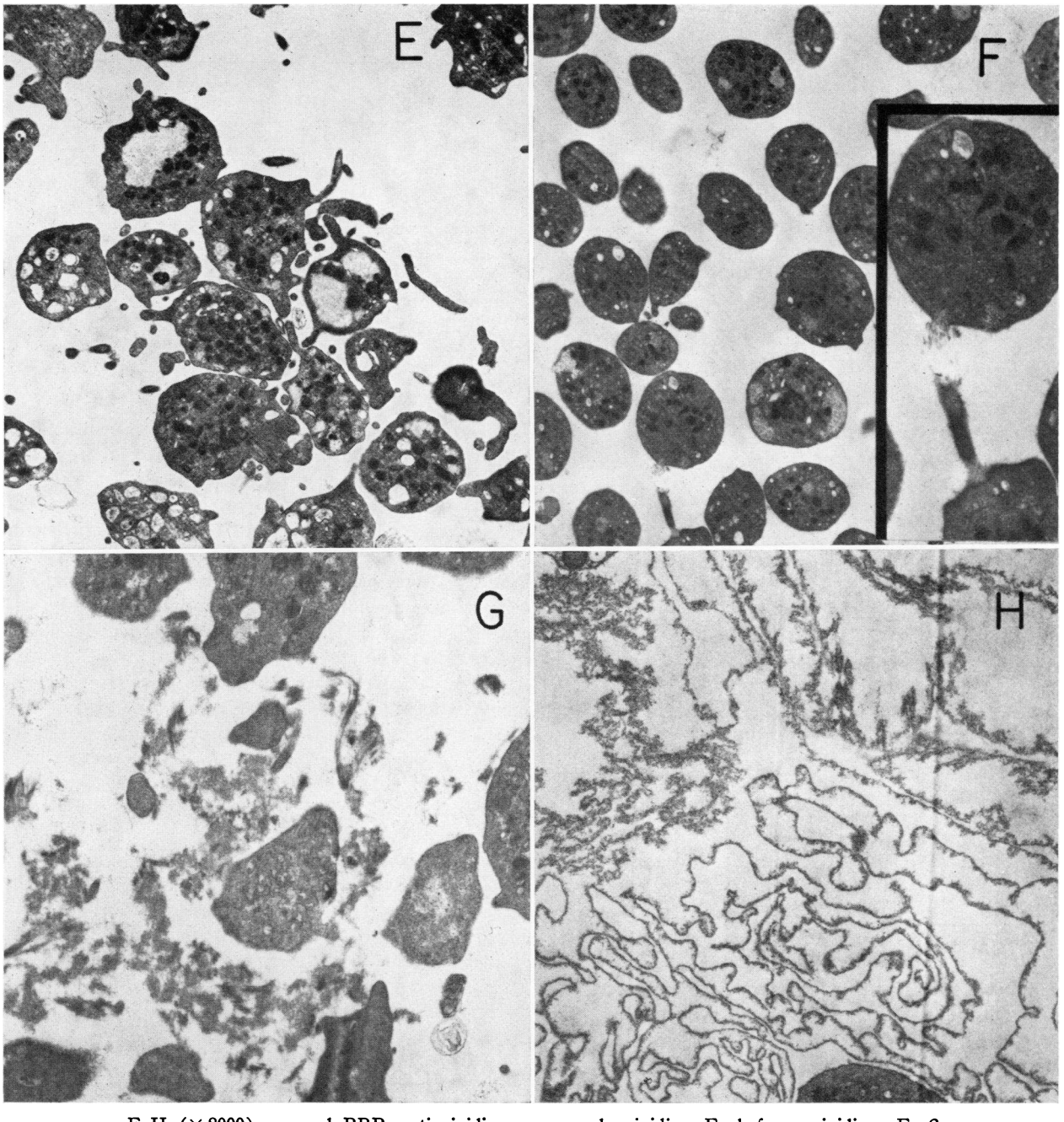


FIGURE 2 Electronphotomicrographs of the response to quinidine. A–D (× 8000): PRP (3.8% Na citrate 1:10 v/v), normal serum and quinidine. A: before quinidine; B: 2 min after quinidine; C: 6 min after quinidine; D: 12 min after quinidine.

taining normal PRP and normal serum. In the control incubation before the addition of drug (Fig. 2 A) platelets showed the effect of stirring in that some vacuoles were present and pseudopods had formed. The addition of quinidine to the mixture of normal serum and normal PRP (Fig. 2 B) caused a prompt change in the appearance of the platelets. They became spherical and swollen and the marginal band of tubules was no longer present. The granules and plasma membranes, however, remained intact throughout the remainder of the 12 min incubation (Figs. 2 C and D). In the mixture of normal PRP and antibody-containing serum, before the addition of quinidine (Fig. 2 E), the platelets again showed only the effect of stirring. The addition of quinidine



E–H (× 8000): normal PRP, antiquinidine serum and quinidine. E: before quinidine; F: 2 min after quinidine (insert: magnification showing membrane rupture [× 13,500]); G: 6 min after quinidine; H: 12 min after quinidine.

caused the initial changes seen in the control incubation, but continued incubation resulted in progressive disruption of the platelet. Within 2 min (Fig. 2 F) discrete areas of membrane rupture were evident. At longer time intervals, progressive lysis of the cell architecture was apparent (Fig. 2 G), culminating in total disruption of the platelets at 12 min (Fig. 2 H).

The initial rate of platelet lysis was a function of the potency and concentration of the antibody. The final degree of lysis, however, was relatively independent of antibody concentration, for with prolonged incubation the lytic reaction proceeded to complete lysis. Therefore, to compare the potency of different sera or to titer a single serum, we have utilized an arbitrary quantitation

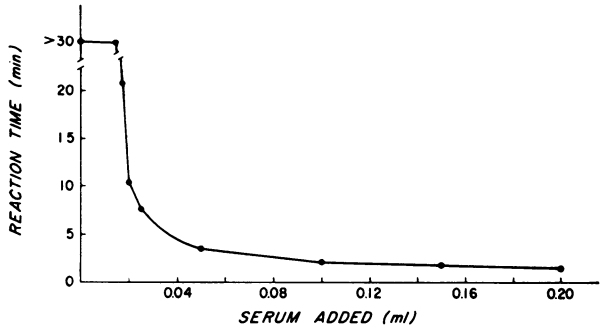


FIGURE 3 The relationship between the rate of lysis of normal platelets and the amount of antiquinidine antibody added in the presence of a constant concentration of quinidine (1.5×10^{-3} M). PRP 0.3 ml (3.8% Na citrate 1:10 v/v). Varying amounts of antiquinidine antibody serum added to normal serum to reach a final volume of 0.2 ml.

of the rate of reaction. We have defined the "reaction time" as the interval between the addition of a drug to a mixture of PRP and serum to be tested and the decrease of optical density by 0.1 optical density units. In the special case of quinidine, which causes swelling, we have measured the change in optical density from the peak of the swelling reaction. The reaction time as a function of the time-course of antibody production after quinidine ingestion by a sensitized subject is shown in Table I.

The patient was a 65-yr old lady admitted for the evaluation of the sudden onset of generalized purpura and bleeding. 4 yr before admission the patient had received quinidine sulfate for the treatment of leg cramps. She took the medication sporadically. 2 yr before admission, after the ingestion of quinidine sulfate she developed a purpuric eruption. Several days later, as the purpura was fading she was noted to have a platelet count of 93,000 which rose to 630,000 within a week. 3 days before admission, because of recurring leg cramps she again took quinidine, despite repeated advice to avoid the drug. She noted the rapid onset of sweating, joint pains, and nausea. 2 days before admission, generalized purpura developed and on the day before admission vaginal bleeding, gingival oozing, and melena occurred and the patient was admitted. The patient was otherwise remarkably free of other diseases and except for moderate hypertension her physical examination was unremarkable.

TABLE I
Time Course of Antibody Production after Quinidine Ingestion

Days after ingestion	Reaction time*	Platelet count
	min	*cells/mm³*
3	2.8	6,600
4	2.5	8,800
5	2.1	8,000
6	1.6	8,900
7	1.3	20,500
8	1.8	63,000
14	1.5	158,460
163	3.7	400,000
391	6.2	Not done

* Time after addition of quinidine (1.5×10^{-3} M) until decrease of 0.1 optical density unit. In each instance, test system contained 0.2 ml heated serum and 0.3 ml PRP (3.8% Na citrate 1:10 v/v). All samples tested on a single day, 392 days after ingestion. In the absence of quinidine, the reaction time for each sample exceeded 30 min.

TABLE II
Complement Fixation vs. Reaction Time

Titer	(Serum added)	Complement fixed*	Reaction time‡
	μl	*U*	*min*
1:12.5	(40 μl)	4.6	4.9
1:25	(20 μl)	4.2	11.4
1:50	(10 μl)	1.4	24.5
1:100	(5 μl)	0.6	>30

* Study performed by Dr. N. Raphael Shulman. 10 U of complement added to each dilution.
‡ PRP (heparin, 2 U/ml) 0.3 ml; sufficient normal heated serum was added to the patient's serum to reach a final total volume of 0.2 ml. Patient serum from day 7, Table I.

The peak antibody level was found on the 8th day after quinidine ingestion, but antibody was readily detectable in serum drawn on the 391st day after ingestion of a single quinidine tablet (Table I).

The relationship between the rate of lysis and antibody concentration was determined by diluting a single serum of high potency (Fig. 3). The reaction time progressively increased as the antibody-containing serum was diluted in normal heated serum. With the serum employed in this experiment, a reaction was detected with 20 μl of serum, but lesser amounts did not produce visible platelet lysis. The sensitivity of the optical method was compared to that of complement fixation (Table II). In general, the sensitivity of both techniques was similar, but the complement fixation assay detected one dilution greater than the optical method. The reaction between normal platelets, antibody, and drug is specific (Table III). There was no reaction between quinidine, PRP, and quinidine antibody; nor was there a reaction between quinidine, PRP, and quinine antibody. In the presence of the appropriate drug and antibody there was brisk lysis.

The relationship between the rate of platelet lysis and the platelet count was examined by performing serial dilutions of PRP and PPP. In these experiments PRP

TABLE III
Specificity of the Reaction

Antibody	Drug*	Reaction time‡
		min
Antiquinidine	Quinidine	2.4
Antiquinidine	Quinine	30
Antiquinine	Quinine	13.5
Antiquinine	Quinidine	>30
Normal serum	Quinidine	>30
Normal serum	Quinine	>30

* Quinidine: 1.5×10^{-3} M; quinine: 5 μl of saturated solution in saline, prepared by suspending quinine sulfate powder, U. S. P., in saline at a final concentration of 1.5×10^{-3} M.
‡ 0.3 ml PRP (3.8% Na citrate 1:10v/v); heated serum 0.2 ml.

and PPP were prepared from both citrate—or heparin—anticoagulated plasma. With both preparations, over a broad range from 100,000 to 500,000 cells/mm³, there was no significant change in the reaction time in the presence of quinidine and quinidine antibody. Therefore, the exact quantitation of platelets in the PRP was not critical.

The relationship between the concentration of drug added to the test system and the onset of lysis was examined in detail with quinidine (Fig. 4). At high concentrations of quinidine the swelling phenomenon was prominent. It decreased rapidly at lower concentrations. Nevertheless the reaction time remained constant over a 10-fold concentration range from 1.5 to 15×10^{-4} M. At final concentrations of quinidine less than 0.6×10^{-4} M there was a sharp rise in the reaction time. At concentrations of drug between 3×10^{-3} and 10^{-2} M the swelling reaction was so prominent that it interfered with the accurate determination of the rate of reaction, and at higher concentrations the platelet ruptured.

A cation requirement for lysis was striking, in that PRP collected using EDTA as an anticoagulant did not react in the presence of quinidine and antiquinidine antibody (Table IV). Addition of $MgCl_2$ restored reactivity. The optimal concentration was 5×10^{-3} M, but at this concentration, the rate of reaction was slower than with platelets collected using citrate as an anticoagulant. Heparin could also be used as an anticoagulant. PRP prepared from blood containing heparin at a final concentration of 2 U/ml of whole blood was consistently more reactive than PRP prepared from citrated blood. At higher concentrations of heparin (10 U/ml) the platelets were less reactive than platelets derived from citrated blood.

The final determinants, then, of the rate of reaction include the amount of antibody added, its potency, and the anticoagulant employed. The rate of reaction is relatively independent of the platelet count in the PRP or of the concentration of quinidine.

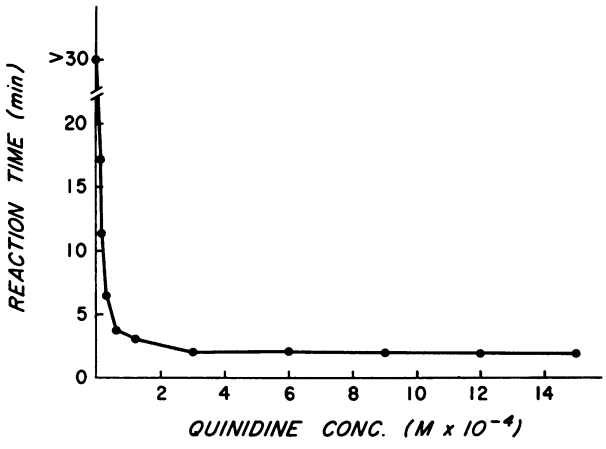


FIGURE 4 The relationship between the rate of lysis of normal platelets and the amount of quinidine added in the presence of a constant amount of antibody (0.2 ml).

The exposure of platelets to quinidine impaired the ability of platelets to respond to a variety of aggregating agents. At a quinidine concentration of 1.5×10^{-3} M there was neither a primary nor a secondary wave of aggregation in response to the addition of 5×10^{-6} M adenosine diphosphate (ADP). At lower concentrations of quinidine, there was rapid disaggregation of platelets in response to 10^{-6} M ADP. Similarly, in the presence of 1.5×10^{-3} M quinidine, no aggregation occurred in response to the addition of 40 μl of a stock collagen solution which produced vigorous aggregation in the absence of quinidine. Quinidine completely blocked the response to epinephrine at a quinidine concentration of 6×10^{-5} M and its effect was detectable at 3×10^{-5} M. Representative experiments are shown in Fig. 5.

TABLE IV
Influence of Anticoagulants on the Reaction Time

Anticoagulant*	Reaction time
	min
Citrate (3.8%, 1:10 v/v)	2.8
EDTA‡	>30.0
EDTA + $MgCl_2$ (2.5×10^{-3} M)	4.3
EDTA + $MgCl_2$ (5×10^{-3} M)	3.4
EDTA + $MgCl_2$ (1×10^{-2} M)	4.1
EDTA + $MgCl_2$ (2×10^{-2} M)	5.8
Heparin (2 U/ml)	1.9

* Incubation mixture: 0.4 ml normal PRP, 0.1 ml serum from patient with quinidine-dependent antibody, quinidine gluconate, final concentration 1.5×10^{-3} M.
‡ 0.15 ml 5% disodium EDTA, 0.15:5 (v/v).

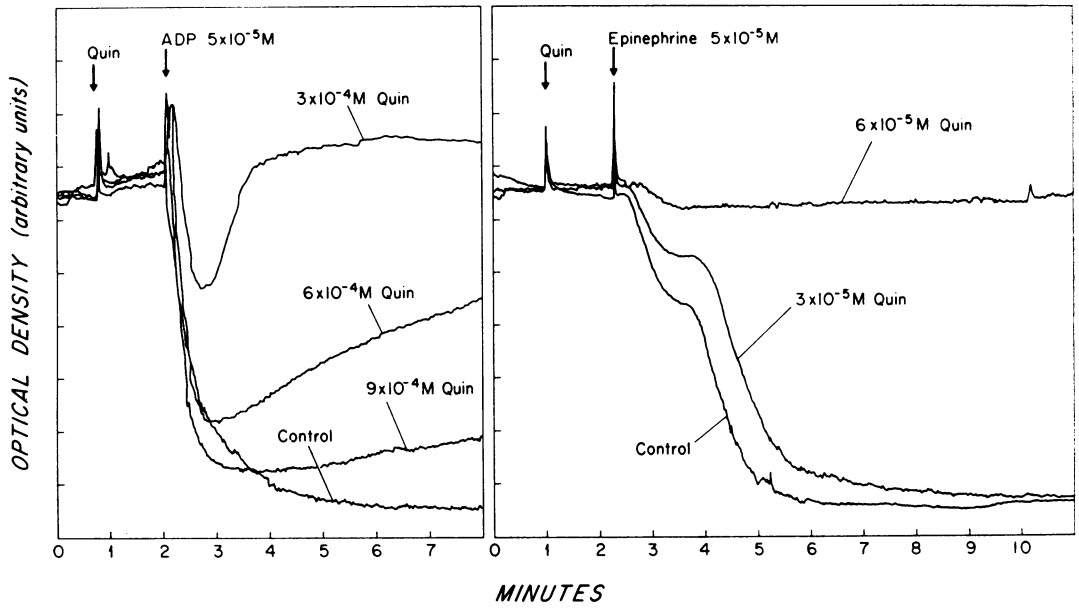


FIGURE 5 The inhibitory effect of quinidine on platelet aggregation initiated by adenosine diphosphate (ADP) and epinephrine.

The detection of isoimmune antibodies. During the course of our studies of drug-induced antibodies, we noted that the serum from one patient with quinidine antiplatelet antibodies produced a pronounced decrease in optical density when added to PRP in the absence of quinidine. The patient had received multiple blood and platelet concentrate transfusions and we postulated that the decrease in optical density that occurred in the absence of quinidine reflected the presence of an isoantibody the patient had acquired in addition to the quinidine antibody.

The mechanism of the decrease in optical density was examined with the electron microscope (Fig. 6). In contrast to the lytic reaction seen with quinidine, the decrease in optical density caused by the isoantibody reflected increasing platelet aggregation, apparent at 2 min (Fig. 6 B) and culminating in the formation of massive platelet aggregates (Fig. 6 D).

Increasing amounts of antibody accelerated the initial rate of aggregation, and we, therefore, utilized the reaction time—now defined as the interval between the addition of serum until a decrease of 0.1 optical density unit had occurred—to titer the potency of an individual serum (Fig. 7) or to compare the potency of different sera.

In testing the patient's serum with a panel of 50 samples of PRP (heparin, 2 U/ml) derived from different donors, we observed aggregation in 23 instances. The antibody in the patient's serum was also tested by Dr. Richard Aster who found it to be monospecific for

TABLE V

Lymphocyte Cytotoxicity Cross-matching of Patient Compared with Aggregometry Cross-matching*

Patient	Blood type	Lymphocyte antigens	HL-A cross match post-transfusion	Aggregometry‡ reaction time pre-transfusion	Aggregometry‡ reaction time post-transfusion
				min	*min*
Recipient	O	HLA-2, HLA-3, TE-50			
Donor M	O	HLA-3, *HLA-9*, Te-50	Negative	>30	25.5
Donor A	O	HLA-2, *HLA-5*, *HLA-10* Te-50	Negative	>30	7.4
Donor H	A	HLA-2, Te-50	Negative	>30	>30

* Kindly performed by Dr. Ramon Patel.

‡ Test system: 0.4 ml donor PRP (anticoagulant: heparin, 2 U/ml) and 0.1 ml recipient serum.

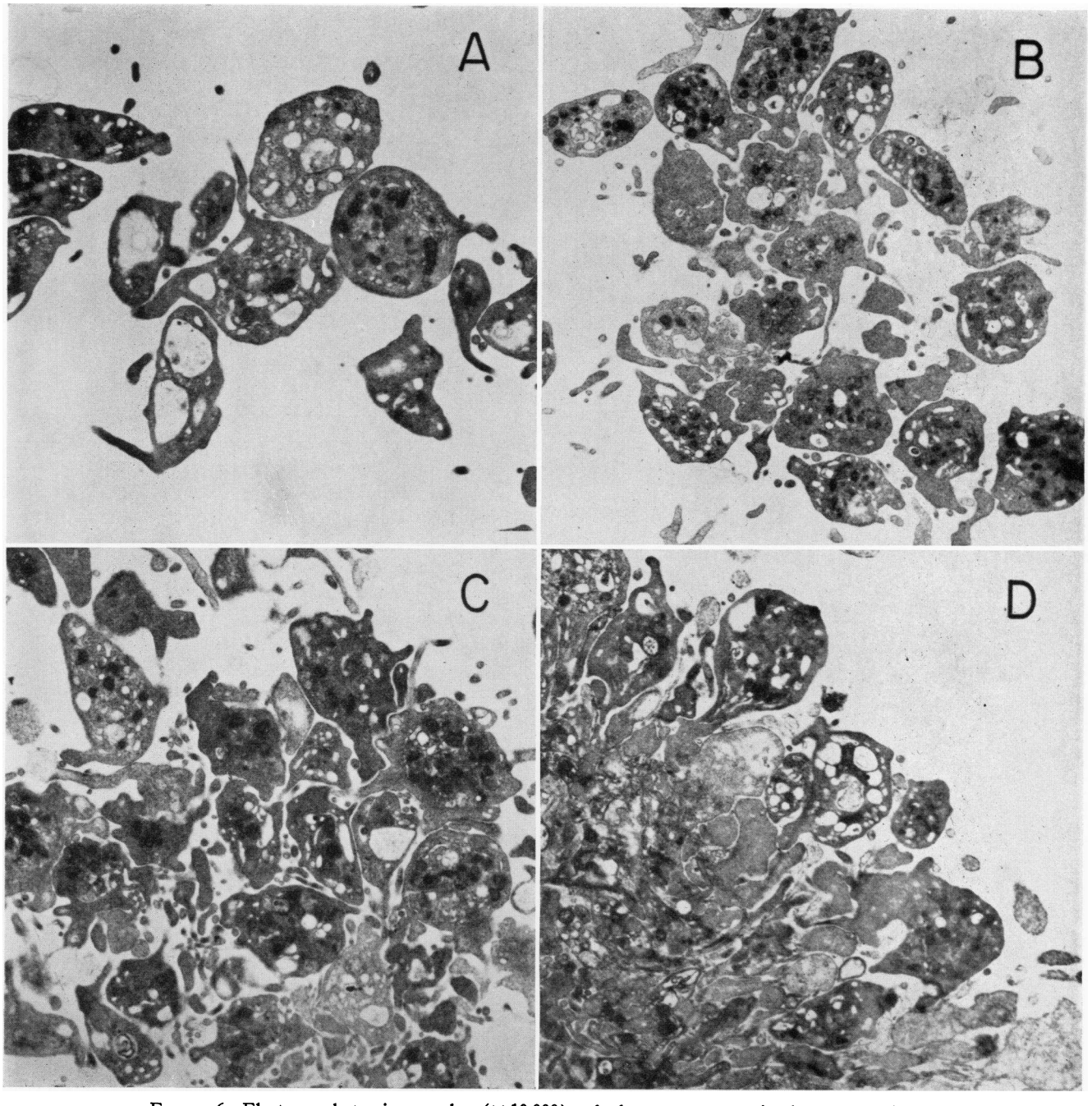


FIGURE 6 Electron photomicrographs (× 10,000) of the response to issoimmune antibody. A: before serum; B: 2 min after the addition of serum containing isoimmune antibody; C: 6 min after addition of serum; D: 12 min after the addition of serum.

HL-A-2. It reacted with the lymphocytes and platelets of 50% of his donor panel.

A further comparison of the platelet aggregation assay for isoantibodies with HL-A typing was afforded by a study of a 34-yr old patient with acute granulocytic leukemia. Serum was obtained before antitumor therapy. After chemotherapy, the patient became thrombocytopenic and, over the course of 44 days received transfusions of 141 platelet concentrates derived from random donors. During this interval his platelet count did not rise above 55,000/mm^3. In an effort to raise his peripheral platelet count 30 platelet concentrates were

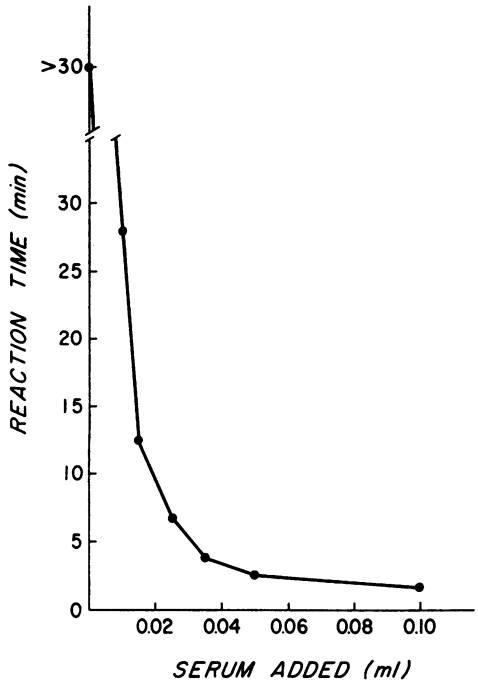


FIGURE 7 The relationship between the rate of aggregation of normal platelets and the amount of isoimmune antibody added. PRP (heparin, 2 U/ml) 0.4 ml; varying amounts of isoimmune antibody serum added to normal serum to achieve a final volume of 0.1 ml.

given over a period of 36 hr, but the platelet count remained below 8,000/mm³. Serum obtained at this time produced direct aggregation of PRP (heparin, 2 U/ml) from all random donors tested. In contrast, serum drawn before therapy was unreactive with the PRP from these same donors.

The patient had three healthy siblings who were potential platelet donors. HL-A typing of the patient and of his siblings was performed using the lymphocyte cytotoxicity technique (17), as were studies of aggregation reactions between the patient's serum (before and after transfusion) and of his siblings' PRP (Table V). One sibling, donor M, had one incompatible antigen; one (donor A) had two; and one (donor H) had none. With at least the three siblings tested there appeared to be a direct correlation between the number of HL-A incompatible antigens present in the donor serum and the initial rate of platelet aggregation of the patient's PRP.

The detection of the antibody associated with the syndrome of idiopathic thrombocytopenic purpura. Sera from seven patients, who had been diagnosed as having idiopathic thrombocytopenic purpura by Dr. William Maloney, and of one normal subject were tested by an observer who was unaware of the diagnoses. Of the seven patients, six were thrombocytopenic at the time the blood was drawn; the seventh patient had a normal count, after splenectomy. All eight sera failed to induce direct aggregation of normal PRP (citrate anticoagulant).

The clinical use of the optical technique for the detection of antiplatelet antibodies. The results of our clinical experience to date with the optical technique for the detection of antiplatelet antibodies are summarized in Table VI. We detected drug-induced antibodies in 11 patients (9 quinidine, 1 quinine, 1 sulfisoxazole) and isoimmune antibodies in 4 patients. We found no reaction in seven patients with idiopathic thrombocytopenic purpura nor in two patients with systemic lupus erythematosus.

DISCUSSION

Since quinidine is the most common of the drugs that elicit antiplatelet antibodies, we have utilized it as the model for the detection of drug-induced antiplatelet antibodies. The data shown in Figs. 1–3 and in Table I indicate that in the presence of quinidine and antiquinidine antibodies, platelets lyse. The rate of lysis as recorded optically is proportional to the concentration and potency of the antibody. These observations permitted the quantitation of the time-course of antibody production and also allowed a comparative titration of the potency of individual serum samples. Within broad limits the concentration of platelets in the PRP used in the test system did not alter the rate of lysis. Therefore, precise enumeration of the platelets was not critical. Boullin and O'Brien (18) have shown that the platelet uptake of quinidine from plasma reaches a peak value at quinidine concentrations between 10^{-4} and 2×10^{-4} M. Below 10^{-4} M quinidine uptake by platelets falls off sharply. These data correlate well with the effects of changing quinidine concentration in our system (Fig. 4). At concentrations of quinidine above 1.5×10^{-4} M the reaction time was constant, as would be predicted from the binding of quinidine by platelets. At concentrations below 0.6×10^{-4} M there was a pronounced rise in reaction time, again consonant with the data of Boullin and O'Brien.

The sensitivity of the optical method approached that of complement fixation (Table II). In contrast to the platelet aggregation technique, complement fixation is a much more elaborate procedure which requires special reagents and a high order of technical skill. Furthermore, in addition to being sensitive, the platelet aggregation method is specific in that there was no cross-reactivity between antibodies elicited by quinine and those that formed in response to quinidine (Table III). We have not directly examined the relative sensitivity of the optical method to that of platelet factor 3 immunoinjury which is reported to be as sensitive as the

complement fixation technique (10, 14). In our hands, we find that platelet aggregation reactions require less technical sophistication than does the PF-3 assay.

In their study of quinidine binding by platelets, Boullin and O'Brien (18) also report the morphologic changes in platelets caused by quinidine. They studied these effects at three concentrations of quinidine: 10^{-5} M, 10^{-4} M, and 2×10^{-3} M. At the two lower concentrations they found no clear-cut changes in platelet ultrastructure, but they found gross swelling and partial platelet disruption at the high concentration. Our data are similar in that we observed minimal swelling at 10^{-4} M quinidine, with progressively prominent swelling at higher concentrations. At 1.5×10^{-3} M quinidine we found marked swelling, but no gross internal disruption of the platelets (Fig. 2). At higher concentrations there was progressively severe damage to the platelets.

The swelling of normal platelets produced by quinidine is strikingly similar to the effects on platelets of other plant alkaloids such as colchicine, vincristine, and Velban (19). In each instance, incubation of platelets with the alkaloid disrupts the marginal band of tubules, producing spherical, swollen platelets. Despite the loss of marginal tubules, clot retraction is not inhibited by colchicine, and the Vinca alkaloids (19). Similarly, the exposure of platelets to quinidine in the concentration we have employed does not impair clot retraction. Although several alkaloids produce the platelet swelling reaction, there is a high degree of specificity, for we have found no swelling when platelets were incubated with quinine. The mechanism by which plant alkaloids cause dissolution of platelet microtubules has not been established, but the phenomenon is presumed to be related to the binding of the drugs to specific microtubular proteins (20). Indeed, a specific colchicine-binding protein has been isolated from human platelets (21). This protein shares in common with other colchicine-binding proteins the ability to control the viscosity of the cytosol.

We would emphasize that the swelling phenomenon observed in vitro occurs at concentrations grossly in excess of those in the blood stream after quinidine ingestion. However, both Davis and Wilson (22) and Boullin and O'Brien (18) have shown that at concentrations from 4×10^{-6} M to 6.4×10^{-4} M, quinidine both inhibits the uptake of serotonin into platelets and causes release of serotonin from platelets. This observation, therefore, raises serious questions about the applicability of techniques which depend on either the inhibition of uptake or the release of serotonin from platelets to the detection of quinidine-dependent antibodies.

The effect of quinidine on the platelet response to aggregating agents (Fig. 5) may well reflect its binding to the platelet membrane. It is of interest that the response to epinephrine was completely blocked by a quinidine concentration (6×10^{-5} M) that did not impair the response to ADP.

TABLE VI

Clinical Experience with the Optical Technique for the Detection of Antiplatelet Antibodies

	No. tested	No. positive*
Drug-dependent antibodies		
Quinidine	9	9
Quinine	1	1
Sulfisoxazole	1	1
Isoimmune antibodies		
Post-transfusion	3	3
Neonatal purpura	1	1
Idiopathic thrombocytopenic purpura	7	0
Systemic lupus erythematosus	2	0
Controls		
Normal subjects	30	0
Thrombocytopenic patients (nonimmune)	5	0

* A positive reaction is defined as a decrease in optical density of a stirred mixture of normal PRP and test serum of >0.1 U occurring in less than 30 min. In negative reactions either no change or a change <0.1 U occurred during a similar period of observation.

Platelets share with lymphocytes the HL-A system of antigens involved in tissue histocompatibility. In addition there are other antigens such as P^{A1} which are recognized only on platelets. These may also cause alloimmunization (23). However, these antigens are found so frequently in the normal population, in contrast to the great diversity of the HL-A system, that the chances of sensitization by these antigens is of much less importance. Indeed, it is now clear that multiple transfusions of platelets derived from randomly selected donors will inevitably produce HL-A incompatibility in the recipient if sufficient transfusions are given. The resulting antiplatelet antibodies drastically shortened the survival of these randomly selected concentrates. In contrast, infusion of HL-A compatible platelets into refractory patients results in prolonged platelet survival and heightened hemostatic response (1, 24). Our observation that, in the single family that we studied, there was a positive correlation between the number of incompatible HL-A antigens and the rate of platelet agglutination observed in vitro suggests that the direct optical assay system may be a useful adjunct to the HL-A tissue typing technique. It was of interest to note (Table V) that the direct cross-matching test between potential donors' serum and lymphocytes did

not produce evidence of cytotoxic incompatibility which would have been predicted by both HL-A typing and by the platelet aggregometry reaction. Yankee (24) has shown, however, that there may be an initial discrepancy between the direct cross-matching technique and HL-A typing and, furthermore, that there may be a transient response to cross-match-negative donors. Inevitably, however, continued infusion of such platelets produces the expected accelerated disappearance of the circulating platelets, reflecting the HL-A incompatibility.

Henson (25) has divided the action of heterologous antiplatelet antibody on rabbit platelets into two components. In the absence of complement, the antibody released vasoactive amines from platelets, but did not produce cytolysis. Antibody in the presence of complement caused lysis. In further studies (26) he suggested that the reaction sequence involves first the initial aggregation of platelets by antigen-antibody-complexes mediated by the process of C3 immune adherence. Subsequent activation of the terminal complement sequence then would produce lysis. As predicted by his hypothesis, when C6-deficient plasma was used as the source of complement, aggregation occurred but lysis did not. Henson also described a complement-dependent, nonlytic immune injury to platelets that followed adherence of platelets to particulate antigen. This process presumably involved the by-pass activiation of C3 and did not involve complement components beyond C5.

Our findings with human platelets differ from those of Henson. We have demonstrated immune lysis of platelets in the absence of aggregation (Fig. 2). Indeed, at the quinidine concentrations that we used, aggregation reactions were blocked. Therefore, our data do not support the concept that immune lysis must first be preceded by aggregation. Conversely, the isoimmune antibody produced massive platelet aggregation (Fig. 6), but cell lysis did not occur. Since there was an abundant source of complement in the PRP and since the concentration of heparin used as the anticoagulant (2 U/ml) does not block complement-mediated lysis, absence of lysis is not consonant with the concept that soluble antigen-antibody complexes will necessarily produce lysis of platelets if complement is present. The pattern of activation of the complement sequence in our test system remains to be clarified.

The data present in Table VI summarize our clinical experience with the optical technique for the detection of antiplatelet antibodies. In contrast to earlier visual methods, the present assay system is sensitive and quantitative. Drug-dependent antiplatelet antibodies are readily detected as are the isoimmune antibodies induced by multiple transfusions. Furthermore, the lack of reaction with control sera and with sera drawn from patients who have thrombocytopenia on a nonimmune basis adds specificity to the assay system.

Although the PF-3 assay system, complement fixation, and the optical system are approximately equal in sensitivity, there is a marked divergence in their ability to detect the abnormality present in the sera of patients with idiopathic thrombocytopenic purpura. Karpatkin, Strick, Karpatkin, and Siskind (27) have recently summarized their experience with the PF-3 assay system. They find an incidence of 65% of patients with the diagnosis who demonstrate a positive PF-3 test. In contrast, our system does not detect an antiplatelet antibody, nor do complement-fixing techniques (28). The reason for the discrepancy is not yet established.

Previous attempts to utilize direct visual assays to measure the agglutination or lysis of platelets by antiplatelet antibodies have been limited by the relative imprecision of these tests compared to quantitative, but cumbersome assays. The quantitative direct optical assay system that we have described offers the advantages of sensitivity, and specificity. Furthermore, the utilization of normal platelets as the target issue and of heated serum as the source of antibody makes the collection, processing, and testing of samples relatively simple and rapid. The assay is sensitive to both drug-dependent and isoimmune antibodies and can detect both in the same serum but it does not detect the abnormality in patients with idiopathic thrombocytopenic purpura. Our preliminary observations indicate that the simple technique of platelet aggregometry may be a useful adjunct in the selection of compatible donors for platelet transfusion.

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