

Detection of Platelet Isoantibodies by [³H]Serotonin Platelet Release and Its Clinical Application to the Problem of Platelet Matching

JON P. GOCKERMAN, ROBERT P. BOWMAN, and MARCEL E. CONRAD
with the technical assistance of SANDRA L. ECKLEY

*From the Department of Hematology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D. C. 20012*

ABSTRACT The detection of platelet isoantibodies by the release of [³H]serotonin from platelets has been evaluated. The conditions for optimal release of [³H]serotonin with platelet isoantibodies using a microtechnique have been defined. A group of cardiac surgery patients were followed pre- and post-transfusions, with 48% developing a positive serotonin release assay. Of these patients, 16% also had a platelet complement-fixing and/or lymphocytotoxic isoantibody. There was variation in the degree of correlation between [³H]serotonin release and lymphocytotoxicity using individual National Institutes of Health typing serum. The matching obtained between family members by both techniques showed a close correlation when each technique was evaluated separately using the same NIH typing serum. The detection of isoantibodies in patients with hematological malignancies correlated with the unresponsiveness to unmatched platelet transfusions in 15 out of 17 cases. The use of the patient's isoantibody to matched platelets of family members by [³H]serotonin release correlated well with the clinical response to transfusion with these platelets. The data suggest that (a) platelet isoantibodies can be

detected with increased frequency by [³H]serotonin release; (b) [³H]serotonin release is a specific reaction depending on the surface antigen of the platelet; and (c) the method can be used to match compatible family members for platelet transfusions.

INTRODUCTION

The use of platelet transfusions to support patients with thrombocytopenia has become widespread in the last 5 yr and has resulted in a marked decrease in hemorrhagic complications associated with thrombocytopenic states (1, 2). The aggressive use of chemotherapeutic agents in all types of neoplasia has increased the need for longer and more frequent periods of platelet support. Repeated transfusions of platelets from unmatched donors frequently results in the development of refractory states characterized by a markedly shortened lifespan of the infused platelets (3, 4). In an attempt to overcome this problem, Yankee and his co-workers have evaluated the use of HL-A lymphocyte-matched platelet donors in patients with aplastic anemia (5-7). HL-A lymphocyte-matched siblings as platelet donors have worked exceedingly well in their studies, and the use of unrelated HL-A-matched donors has been successful (5-7). These findings have been extended recently to patients with hematological malignancies with mean increases in platelet counts between 5,000 and 13,000/mm³ × m³ per unit of platelet transfused at 20 h after HL-A-matched platelet transfusion (8).

Several problems arise with widespread use of HL-A lymphocyte platelet matching. There is the technical problem of obtaining accurate HL-A typing sera which is in limited supply. The general use of HL-A lymphocyte matching requires a large and expensive computer-

This work was presented in part at Annual Meeting of the American Society of Hematology, Hollywood, Fla. 1972 (*Blood*. 40: 972).

Dr. Gockerman's present address is the Division of Hematology-Oncology, Department of Medicine, University of Kentucky, Lexington, Ky. 40506; Dr. Bowman's present address is Division of Hematology-Oncology, Department of Medicine, Brook Army Medical Center, Ft. Sam Houston, Tex. 78234; Dr. Conrad's present address is Division of Hematology-Oncology, Department of Medicine, University of Alabama, Birmingham, Ala. 35294.

Received for publication 22 August 1973 and in revised form 6 September 1974.

based operation. Most patients receiving platelets have acute hematological malignancies and possibly an altered immunological status both due to the disease and its treatment with immunosuppressive drugs (9). Platelets possess isoantigens along with antigens of the HL-A system (10, 11). Platelet-specific antigens, which in certain clinical situations are of prime importance, are not detected by HL-A lymphocyte typing (12). Further, certain lymphocyte HL-A antigens may not be present on platelets and thus need not be matched (8).

The use of serum from an isoimmunized patient as a source of antibodies to help in excluding ineffective platelet transfusions has rarely been used as the primary basis to select donors (13). The problem has been that the sensitivity of the techniques for detecting platelet isoantibodies have been relatively poor. Complement-fixing platelet isoantibodies have been identified in 10–60% of multitransfused patients (3, 14, 15). Isoantibodies as detected by lymphocytotoxicity or other currently available techniques are identified in about 30% of this group of patients (15–17).

In 1963, Bridges, Baldini, Fichera, and Dameshek evaluated the inhibition of [^{14}C]serotonin uptake by human platelets as a means of detecting platelet isoantibodies (18). All sera from four multitransfused patients showed suppression of serotonin uptake into unmatched donor platelets but not into their own platelets. [^{14}C]Serotonin uptake was not influenced by sera from six patients with ITP. In 1973, Hirschman and Shulman described the release of [^{14}C]serotonin from prelabeled platelets as a sensitive method of detecting ITP factor and platelet isoantibodies (19).

We have modified the serotonin platelet release assay and have evaluated its specificity and use as a means of detecting platelet isoantibodies. The assay was used with HL-A lymphocytotoxic typing sera to permit comparison between [^3H]serotonin platelet release and lymphocytotoxicity. We have used the reaction of the patient's isoantibody in the [^3H]serotonin release test as a means of predicting to which family member's platelets the patient will respond.

METHODS

[^3H]Serotonin platelet release assay. 30 ml of blood was collected in plastic tubes containing 0.45 ml of 10% disodium EDTA and centrifuged at 4°C in a Sorvall RC2B centrifuge (Ivan Sorvall, Inc., Newtown, Conn.) at 1,085 *g* for 2.5 min. The platelet-rich plasma (PRP)¹ was measured in a siliconized graduate cylinder (Siliclad, Clay-Adams, Inc., Parsippany, N. J.) using a siliconized Pasteur pipette. The appropriate dose of [^3H]serotonin (calculated as described below) was added to the PRP in a

plastic tube. This was incubated in a shaking 37°C water bath for 40 min, at which time maximal uptake of [^3H]serotonin had occurred. The platelets were then centrifuged at 4°C in a Sorvall RC2B at 1,085 *g* for 10 min, and the supernate was decanted. With the aid of a siliconized Pasteur pipette, the platelets were resuspended in a solution which contained 140 mM NaCl, 5 mM KCl, 5 mM glucose, and human albumin at a concentration of 5 mg/ml (Pentex Fraction II, Miles Laboratories, Inc., Kankakee, Ill.) and 0.1 M Tris hydrochloride brought to pH 7.4 with 0.1 M NaOH as described by Rossi (20). This solution decreased nonspecific [^3H]serotonin platelet release. Minimal differences occurred in platelet antibody-induced [^3H]serotonin release over the pH range of 5.0–7.5. The resuspended platelets were spun at 1,085 *g* for 10 min in a 4°C Sorvall RC2B centrifuge. The supernate was discarded, and the platelets were resuspended in Rossi's solution at 4°C to a platelet count of 300,000/mm³ which gave optimal release in this system. The platelets were kept at 4°C until used.

The desired amount of test sera, 1–10 μl (as described below), was added to a V-shaped microtiter plate (Cooke Engineering Co., Alexandria, Va.) followed by the addition of 10 μl of [^3H]serotonin-labeled platelets. All pipetting was done using an Eppendorf pipetter with disposable tips (Fisher Scientific Co., Pittsburgh, Pa.) or a Hamilton syringe with a repetitive dispensing attachment (Hamilton Co., Reno, Nev.). The plate was covered with sealing tape (Cooke Engineering Co.) and incubated 40 min at 37°C. The maximal release of [^3H]serotonin in the presence of isoantibody occurred at 40 min. The plate was then placed in a 4°C ice bath, and 150 μl of Rossi's solution at 4°C was added. It was incubated at 4°C for 5 min and then spun in a PR-6 IEC centrifuge (Damon/IEC, Needham Heights, Mass.) at 4°C for 10 min at 1,500 *g*. Afterwards the plate was placed in a 4°C ice bath, and 100- μl samples were removed using an Eppendorf pipette with care taken not to disturb the platelet button. The plate was kept at 4°C so that nonspecific release would not occur. The sample was placed into a counting vial containing 10 ml of a scintillation fluid consisting of 667 ml/liter scintillation grade toluene, 333 ml/liter of scintillation grade Triton X-100, 5 g/liter of 2,5-diphenyloxazole and 0.125 g/liter of 1,4-bis[2-(5-phenyloxazolyl)]-benzene. The samples were counted for 10 min in a Beckman scintillation counter LS-345 (Beckman Instruments, Inc., Fullerton, Calif.).

Use of pre-poured microtiter plates. The wells of V-shaped microtiter plates were covered with paraffin oil (Fisher Scientific Co.), and the excess oil was shaken out. The desired amount of test sera (1–10 μl) was added to each well beneath the oil with a Hamilton syringe modified with a repetitive dispenser. The plate was then covered with sealing tape and frozen at –70°C. It was thawed before the addition of 10 μl of [^3H]serotonin-labeled platelets beneath the oil layer using a Hamilton syringe. The remainder of the procedure was done as detailed previously. Serum samples prepared in this manner gave the same results as when they were prepared fresh.

Labeling of platelets with serotonin. [^3H]5-Hydroxytryptamine creatinine sulphate was obtained as a freeze-dried powder (Amersham/Searle Corp., Arlington Heights, Ill.). It was stored at –70°C until diluted with 0.15 M NaCl to final concentration of 40×10^{-6} M. 130 μl of this standard dilution resulted in a concentration of 8.6×10^{-15} μmol of [^3H]5-hydroxytryptamine creatinine sulphate per platelet when added to 15 ml of PRP with a platelet count of 400,000/mm³. Using the following formula, the appropri-

¹ Abbreviation used in this paper: PRP, platelet-rich plasma.

ate dose for any PRP was obtained:

$$\mu\text{l of standard dilution to add} = \frac{\text{volume PRP in ml}}{15 \text{ ml}} \times \frac{\text{Platelet count/mm}^3}{400,000/\text{mm}^3} \times 130 \mu\text{l}.$$

This was the optimal dose of [³H]serotonin for detection of isoantibodies by [³H]serotonin platelet release; at higher and lower doses, the degree of isoantibody-mediated release was decreased. The dose used (8.6×10^{-15} $\mu\text{mol/platelet}$) maximized the release of [³H]serotonin, decreased scintillation counting time, and allowed use of microtiter volumes of platelets and test serum.

Serum samples. Whole blood was allowed to clot in glass tubes, the serum was separated by centrifugation, heated at 56°C for 30 min, and then stored at -20°C. Preliminary studies have shown that heating did not decrease the releasing capacity of a positive serum but did eliminate false positive serum samples. Repetitive freeze-thawings had no effect on the releasing capacity of a serum. It was noted that samples stored for 9 mo showed some decrease in releasing capacity. Preliminary studies showed no difference in results between either heated citrated plasma samples or heated sera.

The amount of test serum used in the assay varied. When the ratio of test serum to final reaction volume was altered, three patterns of variation in serotonin release were seen (Fig. 1). There was no change in a negative sera (J. V.); the degree of positivity decreased (E. L., V. W.), or there was a prozone-like effect in which the degree of positivity of a serum, already positive, increased (R. B., D. J.). The increased release of serotonin with dilution of R. B. and D. J. sera could have been, alternatively, due to the dilution of a serotonin release inhibitor. In the clinical studies where an antibody was suspected, a final serum-plus-platelet volume ratio of 1:2, 1:4.3, and 1:11 was run. In the studies with NIH HL-A typing sera, a ratio of 1:11 was used because of the limited supply of typing sera. All samples were assayed in duplicate.

Controls for the [³H]serotonin platelet release assay. These consisted of a pooled serum control obtained from combining sera from 300 normal blood donors. The pooled serum was heated at 56°C for 30 min. 10 samples of the pooled serum were run for every test. The standard deviation was calculated from the counts per minute of these 10 samples. A serum sample was said to release serotonin (give a positive serotonin release assay) if the specimen contained radioactivity in excess of the mean ± 3 SD of that observed in the control samples (i.e., cpm of the sample divided by the mean cpm ± 3 SD of the control was greater than 1).

In terms of percent release the average of the control ± 3 SD represented not more than 20% release (usually 13–18%) of the total [³H]serotonin in the platelet. Positive sera samples released 20–40% of the total [³H]serotonin in the platelet with a few strongly positive sera releasing up to 90% of the [³H]serotonin.

The SD obtained from the pool of 300 normal blood donors was the same as assaying each sample separately and then calculating the standard deviation. If instead of the mean ± 3 SD, the mean ± 2 SD was taken as the lower limit of positivity, 10% false positives occurred. These false positives were in a group of young individuals who had

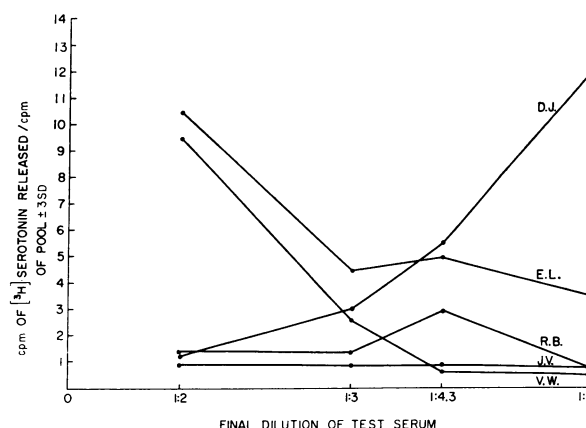


FIGURE 1 [³H]Serotonin platelet release as a function of the final dilution of test serum used in the release assay. Each line represents an isoantibody from a different patient and was obtained with the same platelets in the same experiment.

never received blood products and had never been pregnant. In no cases have we found a positive release with heated serum and platelets from the same donor. A 100% control was a pooled serum specimen that was sampled for radioactivity before the final centrifugation of the platelets in the microtiter plates.

Characterization of the platelet antibody. Platelet elution studies using the pooled serum and positive [³H]serotonin-releasing sera were done by the method of Shulman, Marder, Hiller, and Collier (21). These eluates were concentrated and dialyzed against 0.15 M NaCl, and the protein concentration was adjusted to 1.6 mg/ml. Sample sizes of 20 μl were used in the [³H]serotonin platelet release system. Serum samples were fractionated on DEAE giving a 7S peak and then eluting the remainder of the material off the column as peak II (22). The peaks were concentrated and dialyzed against 0.15 M NaCl and adjusted to protein concentration of 18 mg/ml. Sample sizes of 10 and 20 μl were used in the [³H]serotonin platelet release system.

Platelet complement fixation studies. Quantitative platelet complement fixation studies were performed by the method of Shulman and co-workers using serum samples of 0.1 ml and platelets at $10^6/\text{mm}^3$ for a screening assay (21).

Lymphocytotoxicity studies. Lymphocytotoxicity studies were done by the modified Amos method (23). Glass wool

TABLE I
Detection of Isoantibodies from Cardiac Surgery Patients

Total number of cardiac patients	31
Total number of complement-fixing platelet antibodies	2 (6.45%)*
Total number of lymphocytotoxic antibodies	3 (9.7%)
Total number of [³ H]serotonin platelet-releasing antibodies	15 (48.4%)
Number of sera with complement-fixing antibodies and [³ H]serotonin platelet release	2 out of 2
Number of sera with lymphocytotoxic antibodies and [³ H]serotonin platelet release	3 out of 3

* Number in parenthesis is percent of total patients studied.

TABLE II
Reaction Pattern of Isoantibodies from Cardiac Surgery Patients*

Patient	Procedure	Panel of donors						
		J. G.	B. B.	S. E.	G. S.	G. H.	L. M.	C. B.
A. W.	Platelet complement fixation	—	—	+	—	+	—	—
	[³ H]Serotonin platelet release	—	—	+	—	+	—	—
	Lymphocytotoxicity	—	—	+	+	+	—	—
I. M.	C fixation	—	—	—	—	—	—	—
	[³ H]Serotonin platelet release	+	—	—	—	+	—	—
	Lymphocytotoxicity	—	—	—	—	+	—	—
L. J.	C fixation	+	+	ND	ND	—	ND	+
	[³ H]Serotonin platelet release	+	+	+	+	+	+	+
	Lymphocytotoxicity	+	+	+	+	ND	+	+
L. S.	C fixation	—	—	—	—	—	—	—
	[³ H]Serotonin platelet release	+	+	—	—	+	—	—
	Lymphocytotoxicity	—	—	—	—	—	—	—

* ND, not done; +, positive assay; —, negative assay.

instead of nylon wool was used to decrease granulocyte and platelet contamination. This resulted in less than 1% granulocyte contamination of the mononuclear cells. All typing was graded according to the NIH system. A serum was said to show a positive reaction if the score was 4 NIH U or greater. HL-A antiserum was obtained from the NIH typing bank through the kindness of Dr. Donald Kayhoe; this antiserum was not monospecific.

⁵¹Cr platelet survivals. Platelet chromium-51 survival studies were performed by the techniques of Aster with modification in the counting procedure so as to obtain higher counts above background in our severely thrombocytopenic patients (24). At appropriate intervals, 10-ml samples of blood were collected in 17 × 100-mm polypropylene tubes (Falcon Plastics, Oxnard, Calif.) containing 0.15 ml of 10% Na₂ EDTA. The radioactivity was quantified in a small body animal counter (Packard Instrument

Co., Inc., Downers Grove, Ill.). Duplicate studies were done by the platelet separation technique and whole blood counting technique on four normal donors with similar normal results. Results were calculated on linear coordinates by the method of Aster (24).

TABLE IV
Comparison between Lymphocytotoxicity and [³H]Serotonin Release*

HL-A sera†	Panel of normal donors						
	B. B.	J. G.	S. E.	L. M.	G. H.	G. S.	C. B.
1		○	○			○	○
2					○		
3	⊕				○		
7							
9	⊕	+			+	⊕	+
13				+			
4a	○			○		○	
4b	○	○	○	⊕	○	⊕	○
w5	○						
17	+	+	+	+	+	+	+
Isoantibodies‡	B. B.	J. G.	S. E.	L. M.			
E. P.		⊕	+	+			
D. K.		+					
H. C.	+	+	+	ND			
K. B.		+					
V. W.		+					
E. L.	⊕	⊕		⊕			
L. J.	⊕	⊕	⊕	⊕			
D. J.	⊕		○	⊕			
G. C.	⊕	⊕	⊕	ND			
S. O.	⊕	⊕	⊕	⊕			

* +, positive [³H]serotonin platelet release; ○, positive lymphocytotoxicity assay; ⊕, positive in both assays; ND, not done.

† HL-A designation as given by NIH typing bank.

‡ Obtained from multitransfused patients.

TABLE III
Platelet Elution and DEAE Eluate Studies with Platelet Isoantibodies

Serum sample	Untreated serum	Platelet eluate	DEAE 7S peak	DEAE peak II
E. L.	6.8*	3.3	1.9	0.6
V. W.	6.2	1.4	1.1	0.5
D. K.	1.3	1.5	1.0	0.7
E. P.	1.8	1.2	1.2	0.4
D. J.	1.2	1.1	1.7	1.2
H. C.	1.7	ND‡	2.0	0.6
C. C.	2.2	ND	1.2	0.5
P. W.	1.5	ND	1.0	0.7

* Controls consisted of pool serum untreated or treated in the same manner as the platelet isoantibody serum. A value greater than 1.0 is considered positive [³H]serotonin platelet release assay.

‡ ND, not done.

TABLE V
Comparison between [³H]Serotonin Platelet Release and Lymphocytotoxicity in a Family

HL-A sera*	5+	5	1	2	2	9	5,4A	7	7,12	Multi	9	4C,W18
Haplotype†												
(1) BC§	+		+			+	+					
(2) BC	+		+			+	+					
(3) BC	+	+	+	+					+			+
(4) BD							+					+
(5) BD			+			+	+				+	+
(6) AD								+		+		
(7) AD						+		+		+		+
(8) CD	+											
(9) AB	+		+				+			+		
(10) AC	+		+		+	+	+	+		+	+	+

* HL-A sera designation from NIH Typing Bank. These sera are not monospecific.

† Haplotypes determined using NIH Lymphocytotoxic Tissue Typing Trays: A, HL-A10, 7; B, Te 9; C, HL-A2, Te 18; D, HL-A2, 5.

§ Patient (1) was transplanted with a kidney from patient (2) with an excellent take; patient (8) is the mother and patient (9) is the father.

Positive [³H]serotonin platelet release assay; each family member evaluated with all sera, and only positive results indicated.

Patient studies. Samples were obtained from hospitalized patients either receiving or expected to receive transfusions. A group of cardiac patients had no hematological problems but were receiving platelets and whole blood at the time of open heart surgery. Serum samples were drawn before, 1 wk after, and 2 wk after surgery. A panel of lymphocytes and platelets from seven normal laboratory workers was used to evaluate these sera for isoantibodies by the techniques of platelet complement fixation, lymphocytotoxicity, and [³H]serotonin platelet release.

A second group of patients had hematological malignancies, and serum samples were drawn at intervals during their hospital course. These samples were evaluated for antibodies against lymphocytes and platelets of blood donors by the techniques of platelet complement fixation, lymphocytotoxicity, and [³H]serotonin platelet release.

Platelet transfusion studies. Unmatched platelets refer to platelets obtained from the general blood-donating population by standard techniques (25) and administered to the patient within 72 h. A single unit of freshly drawn unmatched platelets was tagged with ⁵¹Cr and immediately given to the patient. Compatible platelets were obtained from family members whose platelets did not release serotonin when reacted with the prospective recipient's serum. Their platelets were administered immediately after separating or ⁵¹Cr tagging.

Platelet counts were done a few hours before platelet transfusion and 24 h after transfusion with a Technicon platelet counter (Technicon Instrument Corp., Tarrytown, N. Y.). No response to platelet transfusion was said to occur when there was no increase in platelet count 24 h after platelet transfusion in a patient who had no fever, bleeding, infection, hypersplenism, or evidence of disseminated intravascular coagulation.

A positive response was considered present if there was an increase in the platelet count of 5,000/mm³/m² body surface area at 24 h for each unit of platelets given. In the neoplastic patients the white cell counts and bone marrows were followed so as to be certain a platelet response was not due to recovery from drug-induced hypoplasia.

Results of platelet transfusions are presented by one of two graphic representations. The method of indicating platelet count rise and duration on a linear time scale allows an estimate of platelet survival. The other method used by Yankee, Grumet, and Rogentine takes into account body surface area and the number of units of platelets administered (5).

RESULTS

Serum samples from 375 random blood donors were examined for isoantibodies by the technique of [³H]serotonin platelet release. 16 (4.3%) of these samples caused [³H]serotonin platelet release. Of the 16 positive samples, none showed lymphocytotoxicity; however, 3 samples did show a complement-fixing platelet antibody.

31 cardiac patients were followed during their hospital stay for open heart surgery. At least three serum samples from each patient were obtained at intervals during their hospital course and were evaluated for isoantibodies by platelet complement fixation, [³H]serotonin platelet release, and lymphocytotoxicity against a panel of lymphocytes and platelets from seven laboratory volunteers. 48% of these patients developed a [³H]serotonin platelet-releasing isoantibody (Table I). Two of the patients developed a complement-fixing isoantibody, and [³H]serotonin platelet release. Three of the patients developed lymphocytotoxic antibody, and these three showed [³H]serotonin platelet release.

A positive serum did not release with all unmatched platelet samples but did release repeatedly with the same platelet samples. This specificity always coincided with the complement-fixing antibody when present and in general with the specificity of the lymphocytotoxic antibody (Table II). Whenever there was complement-fix-

TABLE VI
Clinical Correlation of [³H]Serotonin Platelet Release Assay

Patients	Disease*	[³ H]Serotonin platelet release†	Platelet complement fixation†	Lymphocyto-toxicity†	Response platelet transfusions†	⁵¹ Cr t _{1/2} platelet survivals§
(1) H. B.	AML	+	—	—	—	1 day
(2) L. B.	AML	+	—	+	—	
(3) M. B.	AA	+	—	+	—	
(4) U. B.	AML	+	—	—	—	
(5) Y. B.	AML	+	—	—	—	1.2 days
(6) R. B.	AML	+	—	—	—	18 h
(7) I. B.	AML	+	—	—	—	10.5 h
(8) G. C.	AML	+	—	+	—	18 h
(9) T. H.	AML	+	—	+	—	
(10) E. L.	AML	+	—	+	—	
(11) D. L.	AA	—	—	—	—	
(12) S. O.	AA	+	—	+	—	20 h
(13) E. S.	MM	+	—	—	—	1.8 days
(14) J. S.	AML	+	+	+	—	
(15) K. S.	MP	+	—	—	—	
(16) J. V.	AML	—	—	+	—	
(17) P. W.	AML	+	—	+	—	

* AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; AA, aplastic anemia; MP, myeloproliferative syndrome; MM, multiple myeloma.

† —, Negative; +, Positive.

§ Platelet survivals were done with single units of random platelets at the time when the [³H]serotonin release assay was positive using these platelets and the serum of the patient.

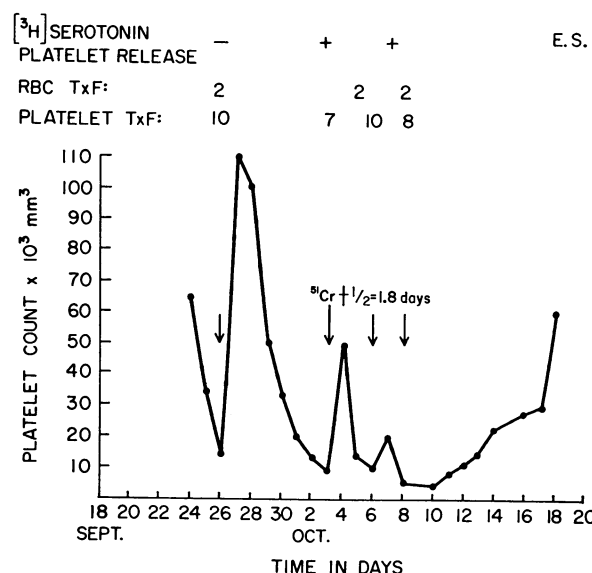


FIGURE 2 Relation between the response of patient to platelet transfusions and [³H]serotonin platelet release assay. The arrows indicate times at which unmatched platelets were given; the numbers above indicate the number of units given. The negative (—) and positive (+) signs indicate results of [³H]serotonin release assay with unmatched platelets. The ⁵¹Cr t_{1/2} platelet survival was done with platelets positive in the [³H]serotonin release assay.

ing antibody, [³H]serotonin platelet release also occurred. Four patients who had isoantibodies that resulted in [³H]serotonin platelet release with random platelets showed no release of serotonin when reacted with their own platelets. Their platelets did release when reacted with nonautologous isoantibodies.

Table III indicates the results showing that the [³H]-serotonin platelet-releasing material can be eluted from platelets and is in the 7S peak on DEAE columns. One sample (D. J.) has some activity in the second peak off the DEAE column.

Comparison between [³H]serotonin platelet release and lymphocytotoxicity. A panel of sera obtained from the NIH typing bank was used to test [³H]serotonin release and lymphocytotoxicity on a group of normal individuals. No correlation was obtained between serotonin release and lymphocytotoxicity using the same sera in both tests (upper part, Table IV).

Some correlation was obtained when sera obtained from multitransfused patients was compared in the lymphocytotoxicity and [³H]serotonin platelet release assays (lower part, Table IV). Chi-square analysis of this data gave a *P* value > 0.001.

Table V shows a family of known HL-A type in which there is some correlation between matches by [³H]serotonin platelet release and lymphocytotoxicity. As with Table IV, comparisons between the techniques for indi-

vidual serum specimens is poor, but the overall pattern of [³H]serotonin platelet release results in the same matching pattern as does lymphocytotoxicity testing.

Use of [³H]serotonin platelet release to detect isoantibodies. We have studied 23 patients with hematological disorders during their hospital course. Four of these patients were never transfused during their hospitalization for chemotherapy and had negative [³H]serotonin release assays with random platelets. Two patients required multiple platelet and packed red cell transfusions during their hospitalizations for chemotherapy. They remained responsive to random platelet transfusions and had negative [³H]serotonin release assays. The remaining 17 patients developed unresponsiveness to random platelet transfusions after a variable number of platelet and red cell transfusions. At the time of unresponsiveness they were evaluated as to the presence of detectable platelet and lymphocyte antibodies (Table VI). 15 of these patients had a positive [³H]serotonin release assay, 9 had a positive lymphocytotoxicity assay, and 1 a positive platelet complement fixation assay.

Two patients with a negative serotonin release had no response to platelet transfusion. One of these (J. V.) had lymphocytotoxic antibodies when first studied, and later in his course the serotonin release test became positive. D. L. had Fanconi's aplastic anemia and had become refractory to platelet transfusions. We were unable to detect an antibody to platelets or lymphocytes by any of the methods we used.

The presence of a lymphocytotoxic antibody correlated less well with the patient's clinical response to platelets than did [³H]serotonin platelet release. In one

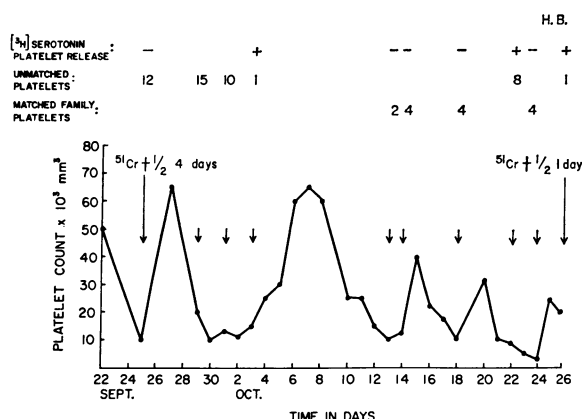


FIGURE 3 Clinical course of patient transfused with unmatched platelets and platelets of family members negative by the [³H]serotonin platelet release assay. The arrows indicate when platelets were given. The number above the arrow indicates the number and type of platelets given. The negative (-) and the positive (+) signs indicate the results of [³H]serotonin release assay with the platelets given on that day.

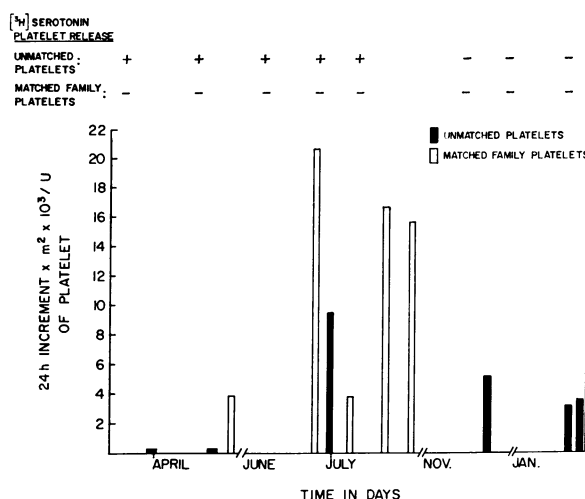


FIGURE 4 Clinical course of patient transfused with unmatched platelets and platelets of family members negative by [³H]serotonin platelet release assay. The negative (-) and the positive (+) signs indicate the result of [³H]serotonin release assay with unmatched platelets and platelets from sister and father.

patient (J. V.) lymphocytotoxicity antibodies were present when the patient showed no platelet response and the [³H]serotonin release was negative.

The development of [³H]serotonin platelet-releasing isoantibodies correlated with the onset of platelet unresponsiveness, as seen in Figs. 2 and 3. The patient in Fig. 2 had not been previously transfused. 7 days after transfusion with 10 U of platelets and 2 U of red blood cells, he showed a decreased platelet response (⁵¹Cr t_{1/2} 1.8 days). At this time his serum caused serotonin release from platelets. The patient in Fig. 3 had been transfused on numerous occasions in the past. His first transfusion in several months had been with 3 U of packed red blood cells on 13 September. He responded to platelets when first given on 25 September. 4 days later he became unresponsive to platelet transfusion, and his serum caused serotonin release. The rapid appearance of antibody probably represented an anamnestic response.

Use of [³H]serotonin platelet release to match platelet donors. Patients with hematological malignancies were followed during their hospital course. Their [³H]serotonin platelet release assay was correlated with their clinical response to platelets by following post-transfusion platelet counts and in selected cases by doing ⁵¹Cr platelet survival studies. Fig. 3 shows an example of a patient who early in his course had a normal platelet response to unmatched platelets with a platelet ⁵¹Cr t_{1/2} survival of 4 days. Later, with the development of a positive [³H]serotonin platelet release, his platelet response to unmatched donors was decreased with a platelet survival ⁵¹Cr t_{1/2} of 1 day. His response to platelets

from members of his family, negative by the [^3H]serotonin platelet release assay, was excellent. The rise and the fall in the platelet count from 6–10 October was a bone marrow response to stopping and starting chemotherapy. Fig. 4 is an example of another patient who showed a poor response to unmatched platelets, but platelets from family members, negative by the [^3H]serotonin platelet release assay, provided a good platelet response. Of the patient's family members studied (father, mother, brother, sister, three children, and two nephews), only the father and sister were negative by the [^3H]serotonin platelet release assay. When the patient had not received platelet transfusions for long periods of time, an antibody could not be detected by [^3H]serotonin release, and she responded to unmatched platelet transfusions once again. The patient had a good response to a transfusion with random platelets in July despite her serum causing [^3H]serotonin release from random platelets.

DISCUSSION

The human platelet obtains its stores of serotonin from the blood by an active transport mechanism (26). Serotonin is stored in physiological concentrations in the dense granules of the platelet (27). The role serotonin plays in normal platelet function is unclear. The original work of Bridges et al. showed inhibition of [^{14}C]serotonin uptake by platelet antibodies made in rabbits and those induced by blood transfusions (18). Hirschman and Schulman devised a test that measures release of [^{14}C]serotonin from platelets in the presence of platelet antibodies (19). The test used in our studies is a modification of their original assay. The optimal conditions for uptake and release of radioactive serotonin were redetermined and are similar to those reported in their original studies. The modification we have introduced decreases spontaneous [^3H]serotonin release and allows the use of microtiter quantities of reagents to evaluate HL-A typing sera which are in limited supply.

The [^3H]serotonin platelet release assay appears to be measuring antibody as indicated by its heat stability, absorption, and elution from platelets and its appearance in the 7S fraction off a DEAE column (Table III). This confirms the earlier work of Hirschman and Schulman (19).

The [^3H]serotonin platelet release assay gives 4.3% false-positive results as determined by looking at 375 random blood donors. The percent of falsepositives are slightly lower if the multiparous females are excluded.

The frequency with which platelet isoantibodies are detected by [^3H]serotonin platelet release assay is greater than that seen with the techniques of complement fixation and lymphocytotoxicity. This was observed with two groups of patients. A prospective series of 31 cardiac surgery patients who had no hematological problems

developed a positive serotonin release in 48% of the patients (Table I). This high percentage would not appear inconsistent in light of the 88% incidence of lymphocyte stimulation induced in a similar group of patients as reported by Schechter, Soehnlen, and McFarland (28). A second group of 23 patients with hematological malignancies were followed prospectively; 17 developed no response to platelet transfusion and of these, 15 had a positive [^3H] serotonin release assay (Table VI). This would indicate a false negative result of 12%. The development of the positive [^3H]serotonin release correlates well with the patient's unresponsiveness to random platelet transfusions (Figs. 2, 3, and 4).

The release of [^3H]serotonin from platelets was not a nonspecific phenomenon. It appears to be dependent upon the antigenic characteristics of the platelet surface (Tables II and IV). The serum samples, especially, the NIH HL-A typing sera, showed a poor correlation between the [^3H]serotonin platelet release assay and lymphocytotoxicity. The reasons for this are not clear. These NIH HL-A typing sera were not monospecific. There are at least four specific platelet isoantigens. Some of the serotonin-releasing antibodies are probably directed against these isoantigens, especially those that fix complement on platelets but do not cause lymphocytotoxicity. There is much literature supporting the presence of HL-A antigens on the platelet (10, 29). However, it is extremely difficult to find good monospecific HL-A platelet complement-fixing isoantibodies. This suggests that either the amount, the spacial relationships, or the exposure of HL-A antigens on the platelet membrane are different than on the lymphocyte. However, it may reflect primarily a difference in sensitivity between the two tests. This may result in different levels of detection of HL-A antigens by [^3H]serotonin platelet release techniques. Despite this, our family study indicates that [^3H]serotonin platelet release can match compatible family members similar to matches obtained with lymphocytotoxicity. This agrees with previous workers who use platelet complement fixation tests as an adjunct to lymphocytotoxicity matching (29). Recently Hirschman, Yankee, Collier, and Garlnick have found good correlation between [^3H]serotonin release and lymphocytotoxicity using very selected monospecific HL-A antisera with our modification of the [^3H]serotonin platelet release assay (30).

The ability of [^3H]serotonin release to show antigenic specificity has allowed it to be used in platelet matching. The use of the patient's own serum to screen prospective family donors correlated well with the subsequent response to platelet transfusions (Figs. 3 and 4). We have used this method of platelet matching only within family lines, and thus its application to random donors needs further evaluation.

ACKNOWLEDGMENTS

We would like to thank Dr. Brack G. Hattler, Chief of Organ Transplantation Service, Major John H. Radcliffe, Chief of Blood Transfusion Service, and Miss F. Olivia Reeder, Donor Center Supervisor, without whose help these studies could not have been done; the housestaff and hematology fellows for their cooperation in our studies; and Colonel David C. Green, Chief of Thoracic-Cardiovascular Surgery, for allowing us to study his patients.

REFERENCES

- Freireich, E. J. 1966. Effectiveness of platelet transfusion in leukemia and aplastic anemia. *Transfusion (Phila.)*. 6: 50-54.
- Henderson, E. S. 1969. Treatment of acute leukemia. *Semin. Hematol.* 6: 271-319.
- Shulman, N. R. 1966. Immunological considerations attending platelet transfusions. *Transfusion (Phila.)*. 6: 39-49.
- Stefanini, M., W. Dameshek, and E. Adelson. 1952. Platelets. VII. Shortened "platelet survival time" and development of platelet agglutinins following multiple platelet transfusions. *Proc. Soc. Exp. Biol. Med.* 80: 230-235.
- Yankee, R. A., F. C. Grumet, and G. N. Rogentine. 1969. Platelet transfusion therapy. The selection of compatible platelet donors for refractory patients by lymphocyte HL-A typing. *N. Engl. J. Med.* 281: 1208-1212.
- Yankee, R. A. 1971. Importance of histocompatibility in platelet transfusion therapy. *Vox Sang.* 20: 419-426.
- Yankee, R. A., K. S. Graff, R. Dowling, and E. S. Henderson. 1973. Selection of unrelated compatible platelet donors by lymphocyte HL-A matching. *N. Engl. J. Med.* 288: 760-764.
- Lohrmann, H. P., M. I. Bull, J. A. Decter, R. A. Yankee, and R. G. Graw, Jr. 1974. Platelet transfusions from HL-A compatible unrelated donors to alloimmunized patients. *Ann. Intern. Med.* 80: 9-14.
- Schwartz, R. S. 1965. Immunosuppressive drugs. *Prog. Allergy*. 9: 246-303.
- Svejgaard, A. 1969. Iso-antigenic system of human blood platelets: a survey. *Ser. Haematol.* 2(Pt. 3): 5-87.
- Dausset, J., and F. T. Rapaport. 1966. Transplantation antigen activity of human blood platelets. *Transplantation (Baltimore)*. 4: 182-193.
- Adner, M. M., G. R. Fisch, S. G. Starobin, and R. H. Aster. 1969. Use of "compatible" platelet transfusions in treatment of congenital isoimmune thrombocytopenic purpura. *N. Engl. J. Med.* 280: 244-247.
- Aster, R. H., R. H. Levin, H. Cooper, and E. J. Freireich. 1964. Complement-fixing platelet iso-antibodies in serum of transfused persons. Correlation of antibodies with platelet survival in thrombocytopenic patients. *Transfusion (Phila.)*. 4: 428-440.
- Aster, R. H., H. E. Cooper, and D. L. Singer. 1964. Simplified complement fixation test for detection of platelet antibodies in human serum. *J. Lab. Clin. Med.* 63: 161-172.
- Heinrich, D., C. Müller-Eckhardt, and W. Stier. 1973. The specificity of leukocyte and platelet alloantibodies in sera of patients with nonhemolytic transfusion reactions. *Vox Sang.* 25: 442-456.
- Shaw, J. F. 1973. Preliminary screening and tentative identification of HL-A lymphocytotoxic antibodies in a hospital blood bank. *Transfusion (Phila.)*. 13: 44-49.
- Salmon, C., and D. Schwartz. 1960. Statistical analysis of a series of 639 polytransfused patients: attempt to interpret the conditions of iso-immunization. *Rev. Hematol.* 15: 162-173.
- Bridges, J. M., M. Baldini, C. Fichera, and W. Dameshek. 1963. Effect of anti-platelet antibodies on the *in vitro* uptake of 5-hydroxytryptamine by blood platelets. *Nature (Lond.)*. 197: 364-366.
- Hirschman, R. J., and N. R. Shulman. 1973. The use of platelet serotonin release as a sensitive method for detecting anti-platelet antibodies and a plasma anti-platelet factor in patients with idiopathic thrombocytopenic purpura. *Br. J. Haematol.* 24: 793-802.
- Rossi, E. C. 1972. The effect of albumin upon the loss of enzymes from washed platelets. *J. Lab. Clin. Med.* 79: 240-246.
- Shulman, N. R., V. J. Marder, M. C. Hiller, and E. M. Collier. 1964. Platelet and leukocyte isoantigens and their antibodies: serologic, physiologic and clinical studies. *Prog. Hematol.* 4: 222-304.
- Sober, H. A., and E. A. Peterson. 1958. Protein chromatography on ion exchange cellulose. *Fed. Proc.* 17: 1116-1126.
- Amos, B. D. 1972. Cytotoxicity testing. In *Manual of Tissue Typing Techniques*. J. G. Ray, Jr., R. C. Scott, D. B. Hare, C. E. Harris, and D. E. Kayhoe, editors. Transplantation and Immunology Branch Collaborative Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. 14-17.
- Aster, R. H. 1971. The study of platelet kinetics with ⁵¹Cr-labeled platelets. In *Platelet Kinetics*. J. M. Paulus, editor. American Elsevier Publishing Co., Inc., New York. 317-323.
- Chappell, W. S. 1966. Platelet concentrates from acidified plasma: a method of preparation without the use of additives. *Transfusion (Phila.)*. 6: 308-309.
- Zucker, M. B. 1962. Serotonin (5-hydroxytryptamine): hematologic aspects. *Prog. Hematol.* 2: 206-224.
- Mustard, J. F., and M. A. Packham. 1970. Factors influencing platelet function: adhesion, release and aggregation. *Pharmacol. Rev.* 22: 97-187.
- Schechter, G. P., F. Soehnen, and W. McFarland. 1972. Lymphocyte response to blood transfusion in man. *N. Engl. J. Med.* 287: 1169-1173.
- Colombani, J. 1971. Blood platelets in HL-A serology. *Transplant. Proc.* 3: 1078-1087.
- Hirschman, R. J., R. A. Yankee, B. S. Collier, and H. R. Garlick. 1973. Sensitive methods for the detection and characterization of platelet isoantibodies. *Thromb. Diath. Haemorrh.* 29: 408-415.