is that peptide sequences analogous to human α and β chains or parts of these are being synthesized at different rates. Possible reasons for globin peptides being better utilized than equivalent amounts of free amino acids in new hemoglobin formation are discussed.

Distribution and Radioactivity of Glycine and Serine in Globin Peptides

Peptide No.	μM. Serine and Glycine* (S.D.†=±0.1μM.)	Counts for 10 Minutes (S.D.=±24)
2	0.3	232
4	0.8	129
5	0.15	82
7	0.4	182
9	0.6	266
13	2.0	1859
14	0.1	382
15	0.4	162
16	0.4	201

* Determined by ninhydrin method. Molar ratios were found to be approximately same using fluorodinitrobenzene method with another sample of globin.
† Standard deviation.

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Supported by grants from the National Heart Institute, U.S. Public Health Service, the Wisconsin Alumni Research Foundation, and the Committee on Research of the American Medical Association.

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Problems in Hemostasis During and After Open-Heart Surgery

VI—Over-All Changes in Blood Coagulation Mechanism

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THE HEMORRHAGIC SYNDROME in patients operated upon for correction of cardiac defects with the aid of a pump oxygenator constitutes a significant problem. Study of this problem in over 60 patients has furnished information indicating that, as a rule, more than one factor is responsible for postoperative bleeding tendencies occasionally noted in these patients.

In a number of instances, preexisting defects can be demonstrated in the cellular and plasmatic components of blood. These abnormalities may disturb the normal coagulation mechanism. During and after cardiac bypass these changes may become magnified and severe enough to result in hemorrhagic diathesis.

Methods

In 60 patients the fibrinogen concentration, plasminogen concentration, platelet count and thrombin time, preoperative hemoglobin, Lee White clotting time, one-stage prothrombin time (Quick), and platelet count were determined as described elsewhere.[2]

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Preexisting Defects

Prolongation of Lee White clotting time in a number of patients appeared to occur more frequently in cyanotic than in acyanotic patients. In the patients studied only 8% had prolonged Lee White clotting time (Table 1).

The prothrombin time was found to be elevated in 28% of the patients. Occasionally, the abnormality noted was considerable. Profound prothrombin deficiencies were again observed in cyanotic patients. The finding of antithrombin activity in the blood was noted. The antithrombin activity, like that of heparin, was found to be neutralized in the test tube by small amounts of polybrene.

Platelet counts at times were low. All other factors studied were normal. There was no evidence of free plasmin activity. No significant plasminogen

activator activity could be demonstrated in these patients preoperatively. The fibrinogen and plasminogen concentrations were found to be normal.

In summary, the alterations observed in the preoperative period were deficiency in the prothrombin complex, at times considerable antithrombin activity, occasionally prolonged clotting time, and, less frequently, a decreased number of circulating platelets.

Table 1.—Extent of Preexisting Clotting Defects

Parameters Studied (Their Normal Range)	No. Cases Studied: Total	Cases With Normal Values	Cases With Abnormal Values	Range of Abnormal Values
Platelet count (200,000-400,000)	71	62(88%)	9(12%)	90,000-150,000
Lee White clotting time (12-22 min.)	49	45(92%)	4(8%)	25-35 min.
Prothrombin time (13/13-16/13 sec.)	73	54(74%)	19(28%)	16/13-35/13 sec.
Thrombin time (20-30 sec.)	42	29(67%)	14(33%)	50-180 sec.

Induced Defects

During cardiac bypass, the blood is exposed to considerable trauma. The effect of the trauma on the cellular and plasmatic components of blood was experimentally determined by recirculating freshly drawn human blood through a pump oxygenator. Destruction of significant numbers of platelets and red blood cells was observed. The first alteration could be determined directly. The second was inferred from the finding of a rise in plasma hemoglobin concentration.

As a result of the above cellular injuries, significant shortening of recalcification and thrombin generation time became apparent during these experiments. The extent of the changes indicated the development of a state of marked hypercoagulability (Table 2). When platelets and hemoglobin-free plasma was used for recirculation instead of whole blood, no changes in recalcification time and trombin generation time were present. It was concluded that the alteration of blood cells was related to the hypercoagulability changes observed in blood.

Table 2.—Effect of Perfusion on Blood during in Vitro Studies

Findings	Remarks
Platelet count .....Decreased	The longer the perfusion, the more marked the decline in platelet number
Fibrinogen concentration .....Unaltered	
Plasma hemoglobin concentration .....Increased	The longer the perfusion, the greater the rise in plasma hemoglobin concentration
Recalcification time ...Shortened	Marked shortening occurs after 30-45 min. of perfusion
Thrombin generation time .....Shortened	Marked shortening occurs after 30-45 min. of perfusion

These in vitro experiments find their counterpart in patients undergoing open-heart surgery. In vivo in patients, platelet count declines, and the plasma hemoglobin concentration becomes elevated. The extent of these changes increases as the time of bypass becomes longer. These changes are similar to the ones observed in the in vitro experiments. The implication is that the changes result from trauma to the blood.

The fibrinogen concentrations dropped considerably in 2 periods: The first decline in fibrinogen concentration was observed in the first 5 minutes of cardiac bypass. These changes in fibrinogen concentration greatly exceeded the hemodilution, resulting from the use of plasma expanders (low molecular dextran and albumin). The second drop in fibrinogen concentration occurred after polybrene administration. This decline has been ascribed to the immediate loss of heparin anticoagulation. Therefore, a permissive defibrination as in clotting may occur.

Similar changes in the fibrinogen concentration were observed in patients pretreated with epsilon amino caproic acid. The above noted decline in fibrinogen concentration is probably not the result of fibrinolytic activity.

Table 3.—Changes in Blood Constituents During Extracorporeal Circulation in Patients

	Cardiac Bypass: Before	After
Platelet number	100%	55%
Plasma hemoglobin concentration	36 mg.%	156 mg.%
Fibrinogen concentration	100%	57%
Prothrombin time (control 13 sec.)	15 sec.	19 sec.

A decrease in prothrombin complex concentration as measured by the one-stage prothrombin time determination before and after surgery, was noted (Table 3). The changes in fibrinogen and prothrombin concentration are assumed to be the result of intravascular clotting. The opportunity for intravascular clotting is present if less than excess amounts of heparin are used during cardiac bypass. Under normal conditions, excess amounts of heparin are necessary to prevent clotting of blood which has been exposed to foreign surfaces.[7] Since the products of cellular breakdown have a heparin-neutralizing effect (Conley et al.[1] and Rapaport et al.[9]) it is readily apparent that the balance in the organism preventing intravascular clotting is delicate and easily upset.

As a result of hypercoagulability and intravascular clotting, the body responds to attempt to lyse the formed clots. A similar effect is noted in various other hypercoagulability states (i.e., after injection of trypsin, venoms, thrombin, endotoxin,[3, 4] or thromboplastin in animals or after severe hemorrhage, incompatible blood transfusion, or various obstetrical conditions in humans [5, 6]). Intravascular clotting has, directly or indirectly, been demonstrated and associated with increased fibrinolytic activity in these various pathological conditions.

A similar sequence of events seems to occur in patients undergoing open-heart surgery (figure). As a result of the intravascular clotting, considerable

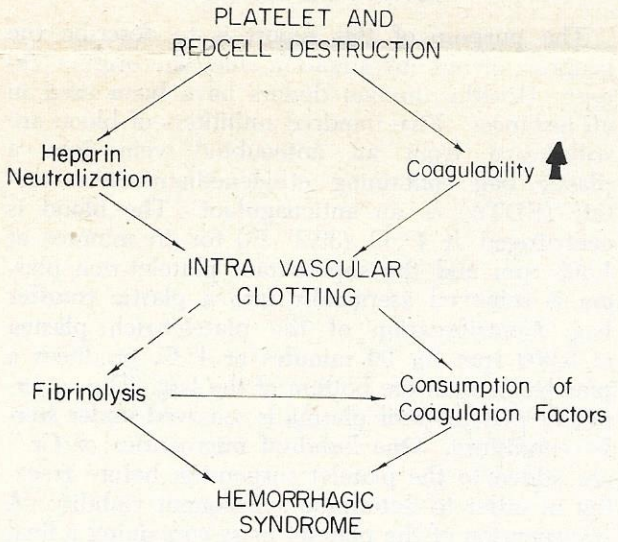
plasminogen activator activity is apparent shortly after the initiation of cardiac bypass. The degree of plasminogen activator activity could be related to the duration of cardiac bypass.[2] Exaggerated release of plasminogen activator was observed after operation for correction of acquired (mitral and aortic valvular) heart disease. Changes in blood pH, defibrillation, or duration of pulmonary hypoxia did not appear to affect the release of plasminogen activator.[2]

Maximum plasminogen activator activity occurred at the time of termination of cardiac bypass or shortly thereafter. The quantity of plasminogen activator released during cardiac bypass was, at times, considerable.

Therapy

Administration of epsilon amino caproic acid, an inhibitor of plasminogen activator activity, resulted in marked amelioration and, in a few instances, in dramatic cessation of bleeding. This observation would indicate that persistent hemorrhage due to the plasminogen activator activity must be considered a clinical problem.

Therefore, the effect of epsilon amino caproic acid was studied in a group of 20 patients. In alternating manner, 10 patients were pretreated with the drug, and another 10 were not pretreated. Both groups of patients were found to be comparable in respect to the type of cardiac defect, age, and preoperative status. As further evidence of the similarity of the control and pretreated group is the fact



Mechanism of hemorrhagic syndrome in patients undergoing open-heart surgery.

that the average plasminogen activator activity for each group was the same. Yet, fibrinolytic activity, as measured by the plasma clot lysis time—which was markedly increased in the nonpretreated group of patients—appeared to be absent in the group of patients pretreated with epsilon amino caproic acid (Table 4). Amelioration of bleeding was noted at times after administration of the drug. Evidence of toxicity associated with the use of the drug is lacking so far.

Correction of prothrombin and fibrinogen deficits at times is therapeutically indicated. Various observations indicate that these patients may benefit from administration of fibrinogen, either purified or in the form of whole blood and/or plasma.

Table 4.—Changes in Plasminogen-Plasmin System at End of Cardiopulmonary Bypass

	Plasminogen Activator Activity Measured by		Fibrinolytic Activity (Measured by Plasma Clot Lysis)
	Euglobulin Clot Lysis Time, Min.	Astrup Plate Lysis In Mm.[2]	
10 patients pretreated with E.A.C.A.*	18	180	15 hr.
10 unpretreated patients	18	180	50 min.

* E.A.C.A., epsilon amino caproic acid.

A complex therapeutic problem remains in that antithrombin activity was greatly increased postoperatively in a number of patients. Whether this finding represents a heparin rebound, interference with fibrinogen conversion, or release of other heparinoid material is not known. Marked antithrombin activity, however, was found frequently prior to surgery before the patients received any heparin.

Changes in thrombin activity were observed in patients adequately pretreated with epsilon amino caproic acid, thus suggesting that the antithrombin is not a product of fibrinogen breakdown.[8] The changes in the antithrombin activity were at times quite severe. Consequently, the abnormality may well have contributed to hemorrhagic diathesis in some of our patients.

Other abnormalities noted included prolonged bleeding time and poor clot retraction. This occurred mainly in patients whose platelet counts were low. Some of the above described abnormalities persisted for several days. Prothrombin times remained prolonged for varying periods of time. The possibility that this abnormality denotes liver damage is open for further investigation.

During and after cardiac surgery, the need for massive blood replacements is imperative. Blood replacement of this magnitude may itself result in hemorrhagic tendencies, thrombocytopenia, and citrate intoxication; and, occasionally, transfusion reaction itself can precipitate hemorrhagic states.

From these studies it may be concluded that several defects in the coagulation mechanism can be delineated in patients undergoing cardio-pulmonary bypass procedures. These defects—if severe enough—may, individually or in combination, be implicated as the cause of uncontrollable hemorrhage.

Prepared with the technical assistance of Audrey Runyan, B.S., Marion MacAulay, B.S., and Mary Ann Gans, R.N., B.S.

This study was supported by a grant from the U.S. Public Health Service.

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In Vivo and In Vitro Survival of Glycerolized Frozen Platelets

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IN RECENT YEARS it has been recognized that thrombocytopenic hemorrhage may occur from massive transfusion with stored whole blood, from toxic or immunologic causes, or from the depression of platelet production following radiation or chemotherapy. Restoration of the coagulation mechanism by replacement of viable platelets will effectively control the bleeding. In the event of nuclear warfare, the fatality rate from thrombocytopenia would be high. It is obvious that a method to store blood platelets for long periods of time should be developed in order that coagulation deficiencies secondary to thrombocytopenia may be treated rapidly and effectively.

These needs prompted us to report a series of experiments in 1958 in which canine platelets were frozen in the presence of glycerol and stored for periods up to 4 months at −79° C. (−110.2° F.).[1] The preservation of clot retracting ability following thawing indicated that at least a portion of the platelets retained viability. Since this report, Cohen and Gardner[2] extended these studies upon canine platelets and demonstrated viability of stored thrombocytes following transfusion. Similar studies performed upon human platelets resulted in a shortened life span of the frozen and thawed platelets. Other platelet preparations have been produced in which the platelets themselves were nonviable, but in which it was hoped that the hemostatic effect would be preserved. These have included platelets "preserved" in gelatin, lyophilized and frozen platelets, and disintegrated platelet material. Furthermore, phosphatides derived from cephalin and soybean have been shown to reproduce certain effects of platelets in vitro. In all of these examples, it has been possible to achieve at least a partial, transient correction of certain coagulation defects. However, it remains to be seen whether a true hemostatic effect in vivo will be achieved.

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Method and Materials

The purpose of this report is to describe the progress of our investigation since the original report. Healthy, human donors have been used in all instances. Five hundred milliliters of blood are withdrawn from an antecubital vein into a plastic bag containing ethylenediaminetetra-acetate (EDTA) as an anticoagulant. The blood is centrifuged at 4° C. (39.2° F.) for 15 minutes at 1,300 rpm and the supernatant platelet-rich plasma is removed aseptically into a plastic transfer bag. Centrifugation of the platelet-rich plasma at 3,000 rpm for 30 minutes at 4° C. produces a platelet mass at the bottom of the bag. The supernatant platelet-poor plasma is removed under sterile conditions. One hundred microcuries of Cr^{51} are added to the platelet suspension before freezing in order to determine subsequent viability. A resuspension of the platelet mass containing a final concentration of 8 per cent glycerol is made, and the entire suspension is cooled at 1° C. (33.8° F.) per minute to −20° C. (−4° F.) and then at 10° C. (50° F.) per minute to −79° C. (−110.2° F.). Although we have maintained canine platelets at −79° C. for as long as 4 months, the experiments reported here were not stored for more than a few hours. The platelets are rapidly thawed by immersion of the bag in a water bath which is maintained at 40° C. (104° F.).