

A Study of the Role of Hemolysis in the Hemostatic Defect of Transfusion Reactions

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The hemoclastic crisis following an incompatible blood transfusion is in many ways similar to the reaction that occurs following the infusion of tissue thromboplastin (1). The systemic manifestations associated with hemolytic transfusion reactions could result from the liberation of an active thromboplastic material following hemolysis. This is an attractive hypothesis since erythrocytes have been reported to contain thromboplastic activity (2, 3). If the hemostatic defect associated with the hemolytic transfusion reaction is the result of the liberation of an active thromboplastic substance by hemolysis, an identical response should result from quantitatively similar cell lysis from other causes. In order to test this hypothesis, erythrocyte lysis was produced in dogs by several methods, including incompatible blood transfusions. Under the conditions of these experiments, the systemic effects of incompatible blood transfusions do not occur with equivalent hemoglobinemia produced by other means.

Materials and Methods

Adult mongrel dogs of either sex were used in these experiments. Blood samples were obtained from the external jugular vein. Platelet counts were done by phase microscopy (4). Fibrinogen was determined by measuring the tyrosine content of clottable protein colorimetrically (5). Hemoglobin was determined by the cyanmethemoglobin method (6) and the benzidine method (7). Leukocyte counts were done by the direct method.

Hemoglobinemia was produced by four methods: (a) Hemoglobin solutions were infused in amounts of 2 ml per kg body weight. These solutions were prepared by repeated freezing and thawing of citrated whole blood (one part 0.11 M sodium citrate solution to 9 parts whole blood). After the final thawing, the material was centrifuged and the cellular debris removed.

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The resulting solution contained approximately 200 mg hemoglobin per ml. (b) Sterile distilled water was infused intravenously in amounts of 8 ml per kg body weight. (c) Blood stored in the presence of high glucose concentration was infused intravenously in amounts of 6 ml per kg body weight. It has been shown previously that erythrocytes stored under these conditions appear to be well preserved but have an increased osmotic fragility and hemolyze when injected (8). Blood was collected in ACD solution (N. I. H. formula A) in a ratio of one part ACD solution to 5 parts whole blood. To this, one part by volume of sterile 50% glucose solution was added. The blood was stored under sterile conditions at 4° C for 7 days and then infused intravenously into the original donor animal. (d) Incompatible plasma and erythrocyte transfusions were given in amount of 3 ml per kg body weight.

Several agglutinins have been demonstrated in dogs, of which canine anti-A is the most apt to cause reactions in dogs transfused indiscriminately (9). Canine erythrocytes were typed by an indirect Coombs technique using a potent canine anti-A serum and anticanine serum prepared by injecting rabbits with pooled dog serum. Under these circumstances, canine erythrocytes are of two types: (a) those which agglutinate (A-positive) and (b) those which do not agglutinate (A-negative). Anti-A isoantibody formation was stimulated by a series of injections of A-positive erythrocytes into dogs of A-negative type. Prior to infusion, bloods were tested for incompatibility using an indirect Coombs crossmatch technique.

Results

The results of 5 representative experiments in which similar plasma hemoglobin levels were produced by different methods are given in Table. All animals developed moderate hemoglobinemia and later hematuria.

Hemoglobinemia following infusion of hemoglobin solution. Dogs tolerated their own hemoglobin well. Following infusion, plasma hemoglobin rose to levels of 300—400 mg%. Except for this, there was no evidence of intolerance. The hemoglobinemia cleared rapidly; only a trace of plasma hemoglobin was detected 6 hours after infusion.

Hemoglobinemia following infusion of distilled water. Dogs tolerated the administration of distilled water well and showed no subjective effects. Plasma hemoglobin rose to 200—300 mg% within 10 minutes after the infusion. No appreciable change in fibrinogen concentration, platelet or leukocyte numbers occurred in the circulating blood.

Hemoglobinemia following infusion of glucose-damaged erythrocytes. The blood after storage with increased glucose concentration showed no evidence of hemolysis prior to infusion. However, following infusion, plasma hemoglobin rose to levels of 250—300 mg% during the first 30 minutes. The hemoglobinemia cleared rapidly so that only a trace could be detected 6 hours after infusion. No evidence of intolerance of this material could be detected except for the hemoglobinemia and hematuria. There was no significant change in fibrinogen concentration or numbers of leukocytes and platelets in the circulation.

Hemoglobinemia following incompatible transfusions. Transfusion reactions were produced by the injection of both incompatible erythrocytes and plasma.

In the incompatible erythrocyte experiments, blood from a dog of A-positive type was collected in sodium citrate and infused into a dog of A-negative type. The recipient animal had been previously stimulated to have a high titer of anti-A antibody. This resulted in hemolysis of the donor's cells as evidenced by the hemoglobinemia which occurred. This was associated with a precipitous fall in the number of circulating platelets and leukocytes. There was a less dramatic decrease in plasma fibrinogen concentration. The dogs developed respiratory irregularities, hyporeflexia, and spontaneous defecation and micturition. A transitory phase of peripheral vascular collapse was also a prominent feature.

The marked leukopenia and thrombocytopenia were of short duration. Leukocytosis occurred after the first hour reaching 30 000—40 000/mm³ during the first 24 hours. The platelet count returned toward normal, but remained below the pre-infusion level during the first 24 hours. All animals were able to effectively clear the hemoglobinemia with no detectable evidence of renal impairment. All recovered spontaneously.

The same type of reaction occurred following the infusion of incompatible plasma. In these experiments dogs of A-positive type were given plasma obtained from dogs of A-negative type previously stimulated to have a high titer of anti-A antibody. This resulted in hemolysis of the recipient's erythrocytes. The sequence of events following such an infusion was identical to that occurring with hemolysis of the donor's cells.

Effects of Intravenous Infusion. Samples Obtained 30 Minutes after Infusion

Material Infused	Hemoglobin mg %	Thrombocytes/cmm		Leukocytes/cmm		Fibrinogen mg%	
		before	after	before	after	before	after
Autologous Hemoglobin	350	285,000	260,000	14,800	16,420	315	330
Distilled Water	275	240,000	210,000	15,000	14,500	276	270
Glucose-damaged Erythrocytes	290	250,000	235,000	14,200	11,520	280	270
Incompatible erythrocytes	325	350,000	10,000	14,700	3,000	290	175
Incompatible plasma	300	397,000	47,000	16,000	5,000	320	240

Discussion

The infusion of incompatible plasma or erythrocytes results in a hemoclastic crisis characterized by hemoglobinemia, thrombocytopenia, leukopenia and a moderate decrease in plasma fibrinogen. This is accompanied by a pronounced somatic disturbance. Such a response was not detected following the other forms

of hemolysis under study indicating that the hemoglobinemia of the degree produced does not appear responsible for the reaction. No detectable change occurred with similar plasma hemoglobin levels resulting from the infusion of autologous hemoglobin. No attempt was made to determine the amount of erythrocyte stroma present in circulation following in-vivo hemolysis, but the amount of cell debris should be proportional to the amount of hemoglobin liberated. The hemoglobinemia resulting from the infusion of distilled water and glucose damaged erythrocytes was of the same order of magnitude as produced by the incompatible blood transfusions.

This group of experiments was designed to compare the effect of moderate hemolysis produced by several mechanisms. It is realized that it is possible to produce severe systemic reactions by infusing sufficient amounts of any solution used in these studies. Toxic manifestations on a hemodynamic basis result if sufficient fluid is infused rapidly (10). A transitory relative decrease in all blood elements can be produced on a volumetric dilution basis if a sufficient volume of fluid is infused. By injecting large amounts of any hemolytic substance it would be possible to produce a sudden massive decrease in circulating erythrocytes resulting in anemic anoxia. None of these mechanisms were a part of these studies.

Quick has postulated that erythrocytin, a clotting factor liberated when erythrocytes disintegrate, may be responsible for the toxic manifestations observed in the various conditions in which hemolysis occurs (2). This would not appear to be a major cause of the sequence of events associated with incompatible blood transfusions since with the other forms of hemolysis studied, no toxic manifestations occurred. In addition, erythrocytin is reported to require thromboplastinogen (AHF) for its activity (2). Prior to routinely cross-matching canine bloods for transfusion purposes, transfusion reactions were inadvertently produced in our hemophilic dogs (11). This should not occur if erythrocytin activated by AHF is a major cause of the systemic manifestations associated with incompatible blood transfusions.

It is generally recognized that incompatible blood transfusions are the result of antibody-antigen interaction. Thus, certain aspects of this can be said to be similar to anaphylactic shock. It has been known for many years that associated with peptone or anaphylactic shock a disturbance in coagulability occurs in addition to the other systemic reactions (12, 13). Except for hemolysis, there appears to be little difference between transfusion reactions and other forms of anaphylactic shock. The associated hemolysis may be contributory but does not appear to be the primary precipitating event in the syndrome of incompatible transfusion reactions.

Summary

Hemoglobinemia of approximately 300 mg⁰/₀ was produced in dogs by five separate methods. Resulting from the infusions of incompatible plasma or erythrocytes there was a hemostatic defect characterized by severe thrombocytopenia and moderate fibrinogenopenia. Associated with this were leukopenia, neuromuscular and cardio-respiratory disturbances. Except for hemoglobinemia resulting from infusion of autologous hemoglobin solution, distilled water, or glucose-damaged autologous erythrocytes, no systemic reaction was detected. These findings indicate that the liberation of thromboplastic erythrocyte components by hemolysis is not the mechanism of the hemostatic defect in hemolytic transfusion reactions.

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Résumé

Les auteurs ont provoqué avec 5 méthodes différentes une hémoglobinémie expérimentale de 300 mg⁰/₀ chez des chiens. Suite à la transfusion de plasma ou d'érythrocytes incompatibles, l'hémostase était perturbée par une thrombocytopenie et une fibrinogénémie modérée. Une leucopénie et des troubles cardio-respiratoires et neuromusculaires étaient également présents. A l'exception de l'hémoglobinémie causée par la transfusion d'une solution d'hémoglobine autologue, d'eau distillée et d'érythrocytes autologues endommagés par le glucose aucune réaction de système ne fut observée. Ces observations démontrent que la libération par l'hémolyse de facteurs érythrocytaires thromboplastiques ne sont pas la cause des troubles de l'hémostase dans les transfusions avec réaction hémolytique.

Zusammenfassung

Bei Hunden wurde eine Hämoglobinämie von ungefähr 300 mg⁰/₀ unter Verwendung von 5 verschiedenen Methoden hervorgerufen. Durch Infusionen von unverträglichem Plasma oder Erythrozyten wurde eine Gerinnungsstörung erzeugt, die durch eine schwere Thrombopenie und eine mäßig starke Fibrinogenopenie charakterisiert war. Mit dieser waren Leukopenie, neuromuskuläre und kardio-respiratorische Störungen verbunden. Nach der Infusion von autologen Hämoglobinlösungen, destilliertem Wasser oder glukosegeschädigten auto-

logen Erythrozyten entstand eine Hämoglobinämie, aber keine Allgemeinreaktion. Diese Befunde zeigen, daß die Freisetzung thromboplastischer Substanzen aus den Erythrozyten durch die Hämolyse nicht Ursache der Gerinnungsstörung bei hämolytischen Transfusionsreaktionen sein kann.

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