

## Limitation of Hemolysis in Experimental Transfusion Reactions Related to Depletion of Complement and Isoantibody in the Recipient

### Observations on Dogs Given Successive Transfusions of Incompatible Red Cells Tagged with Radioactive Iron

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**T**HE RECOVERY of some patients after transfusion of large volumes of incompatible erythrocytes suggests that the effects of such unfortunate accidents do not depend solely on the volume of blood given.<sup>1, 2, 3</sup> In experimental transfusion reactions produced by the injection of incompatible whole blood into isoimmunized dogs<sup>4, 5</sup> it was noted that the degree of hemoglobinemia induced was not directly related to the amount of blood transfused, which varied from 0.5 to 2.5 Gm. of hemoglobin per Kg. weight of the recipient. This indicated some limitation of the rate at which the body could destroy incompatible transfused cells. It was also noted that the complement and antibody titers declined following transfusion of incompatible blood.

The experiments reported here were designed to assess the importance of depletion of complement and antibody in limiting the rate of destruction of transfused incompatible erythrocytes and the related degree of hemoglobinemia. The plan of the experiments was to give a sufficiently large transfusion of labeled red cells to cause the disappearance of antibody or complement and then follow this within ninety to one hundred and twenty minutes by another transfusion of similar size. Survival of the transfused cells, antibody titers, complement activity and plasma hemoglobin levels were then determined at appropriate intervals.

In animals with a high antibody titer, available complement appeared to limit the rate of destruction of donated incompatible erythrocytes. On the other hand, in the presence of adequate complement the rate of hemolysis was limited by the disappearance of antibody when the initial titer of antibody was low.

#### METHODS

The recipient dogs were healthy adult mongrels vaccinated against distemper. No attempt was made to control the volume or pH of the urine of the transfused dogs. Recipients were immunized by multiple small injections of whole blood as previously described.<sup>4</sup> The cells of each recipient lacked the canine A agglutinin (previously referred to as the Do-

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factor)<sup>4</sup> while the cells used for immunization and transfusion contained the A factor. Incompatibility with respect to the A factor was thus the only immunologic factor involved in the transfusion reactions. The characteristics of the canine anti-A antibody are described in detail elsewhere.<sup>6</sup> Briefly, it is a warm agglutinin and hemolysin, it fixes complement and under proper conditions it may become attached to cells without producing agglutination, but its presence on the cell is detectable by the Coombs anti-globulin test.

The donor dogs were prepared by the intravenous administration of adequate amounts of radioactive iron to insure a high level of activity in the donated red cells. Blood for transfusion was collected in sterilized bottles containing a saturated solution of sodium citrate as an anticoagulant. The transfusions were begun within thirty minutes after the blood was drawn and were given through the external jugular veins of the unanesthetized recipients.

Methods of determining blood volume and the ashing and electroplating procedures used in determinations of radioactive iron have been described.<sup>7</sup> Red cells for radioiron determination taken after a transfusion were washed twice with an isotonic solution of sodium chloride, and the volume of packed red cells was then noted after which aliquots were taken for radioiron determination.

Estimation of the percentage of donated cells remaining in circulation was based on the dilution of isotopically labeled red cells in samples of the recipient's blood. Correction was made for baseline radioactivity if any in the recipient, removal of radioiron by sampling and destruction of donated cells.

Methods for measuring isoantibody,<sup>4</sup> complement<sup>4</sup> and plasma hemoglobin<sup>8</sup> (as hemochromogen) have been previously described. Antibody titers are expressed in terms of the final dilution of serum causing microscopically detectable agglutination of the dog erythrocytes, using saline or normal dog serum as diluents. In experiments 1 and 3 the indirect antiglobulin titer of the recipient's serum refers to the dilution of serum which was capable of sensitizing the donor's red cells to such an extent that they were agglutinated by a 1 to 10 dilution of anti-dog-serum rabbit serum.<sup>6</sup> Complement titers refer to the highest dilution of the recipient's serum which completely hemolyzed sheep cells sensitized with anti-sheep-cell rabbit serum.

#### EXPERIMENTAL OBSERVATIONS

The sequelae of transfusing immunized "A-negative" (or "Do-negative") dogs with "A-positive" (or "Do-positive") red cells have been described in detail in an earlier report.<sup>4, 5</sup> In this paper attention will be directed only to the concentration of hemoglobin, complement and antibody in the plasma or serum of the recipient dogs and to the rate of disappearance of the donated cells from the recipients' circulation.

The results of transfusing dogs having relatively low isoantibody titers are shown in figures 1 and 2. In experiment 1 (fig. 1) the antibody titer fell from an initial value of 1:8 (using serum as a diluent) to 0 within ten minutes after the first transfusion, and antibody was not again detected until seventy-two hours later. Antibody measurable by the indirect antiglobulin test was still present in a titer of 1:4 two hours after the first transfusion but was not present ten minutes after the second transfusion. Following the first transfusion the donated cells disappeared rapidly and only 2 per cent remained at ten minutes, while after the second transfusion the donated cells disappeared more slowly. At ten minutes, 68 per cent of the donated cells remained in the circulation and 16 per cent of the cells were still present after twenty-four hours. The plasma hemochromogen rose rapidly to 520 mg. per 100 mg. ten minutes after the first transfusion

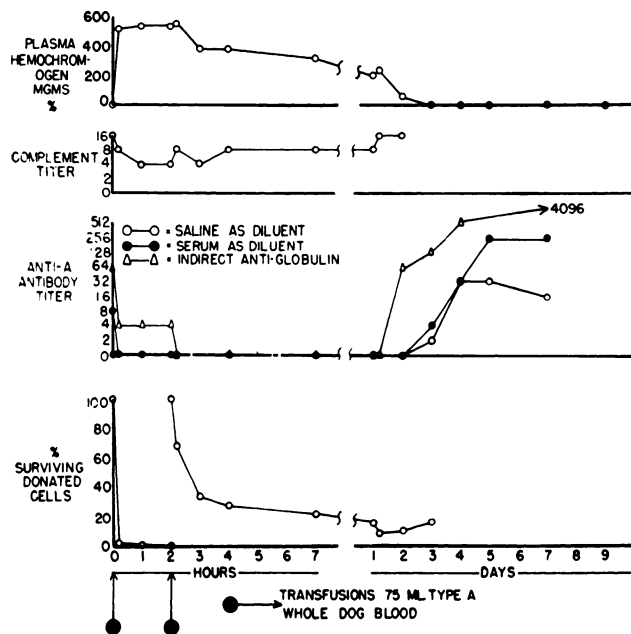


FIG. 1.- Plasma hemochromogen concentration, complement and isoantibody titers and proportion of donated red cells surviving after successive transfusions of incompatible blood into a dog having low anti-A titer. Donated blood contained 79,000 counts per minute per 75 ml. Dog 49E. Wt. 20 Kg.

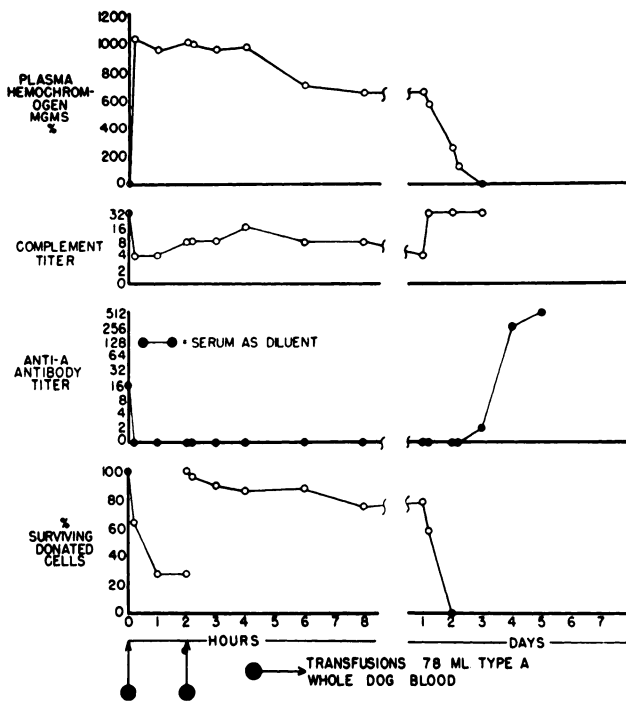


FIG. 2.- Plasma hemochromogen concentration, complement and isoantibody titers and proportion of donated red cells surviving after successive transfusions of incompatible blood into a dog having low anti-A titer. Donated blood contained 45,000 counts per minute per 78 ml. Dog 43-380. Wt. 13.5 Kg.

and did not increase significantly following the second transfusion. Hemoglobinemia persisted for at least forty-eight hours.

Essentially similar results were obtained in experiment 2 (fig. 2) in which the initial antibody titer (using serum as a diluent) was 1:16. Antibody had disappeared by ten minutes after the first transfusion and was not again detected for three days. The donated cells disappeared much less rapidly following the second transfusion, but the values given are less reliable than those in experiment 1 because of high baseline radioactivity in the recipient's red cells remaining from

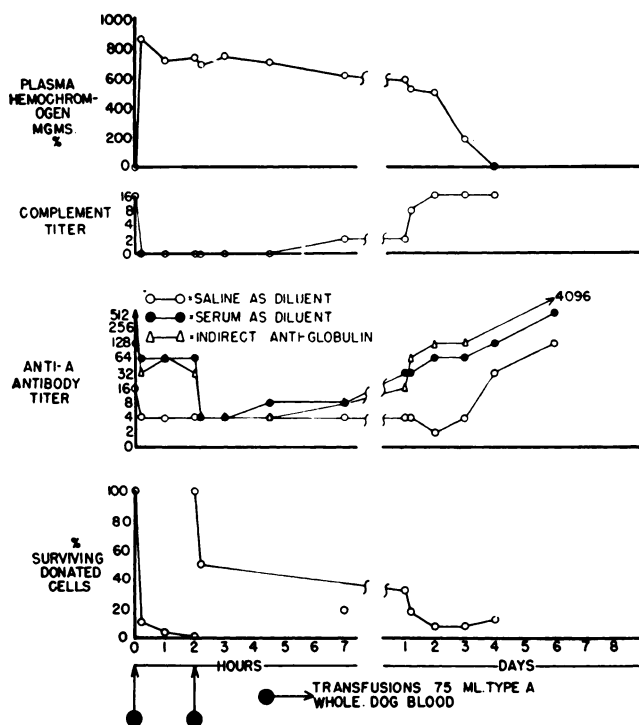


FIG. 3.—Plasma hemochromogen concentration, complement and isoantibody titers and proportion of donated red cells surviving after successive transfusions of incompatible blood into a dog having high anti-A titer. Donated blood contained 76,000 counts per minute per 75 ml. Dog 1298. Wt. 11 Kg.

a previous transfusion. The plasma hemochromogen reached a maximum of 1,032 mg. per 100 ml. ten minutes after the first transfusion and did not rise following the second transfusion. Hemoglobinemia persisted for fifty-four hours.

In both of these experiments complement activity decreased only after the first transfusion and remained low for several hours, but was always present in a titer of at least 1:4.

The results of experiments with high antibody titers (1:128 using serum as a diluent) are shown in figures 3 and 4. In experiment 3 (fig. 3) the initial complement titer was 1:16. Complement was not present in measurable amounts ten minutes after the first transfusion and was not detected until seven hours later.

After the first transfusion the donated cells disappeared rapidly, 11 per cent remaining at ten minutes and 1 per cent at two hours. After the second transfusion the donated cells disappeared more slowly, 50 per cent remaining at ten minutes and 32 per cent at twenty-four hours. The maximal plasma hemochromogen concentration of 860 mg. per 100 ml. was attained ten minutes after the first transfusion and did not rise after the second transfusion. Hemoglobinemia persisted for at least three days. Similar results were obtained in experiment 4

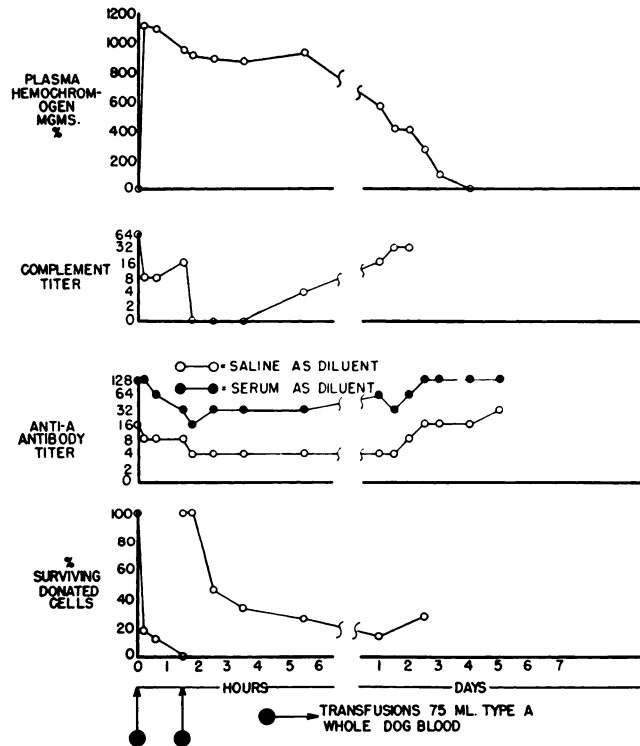


FIG. 4.—Plasma hemochromogen concentration, complement and isoantibody titers and proportion of donated red cells surviving after successive transfusions of incompatible blood into a dog having high anti-A titer. Donated blood contained 35,000 counts per minute per 75 ml. Dog 43-380. Wt. 14.3 Kg.

(fig. 4) except that complement persisted until ten minutes after the second transfusion.

Although there was a fall in antibody titer in both experiments 3 and 4, antibody was detectable, even with saline as a diluent throughout the period of observation. Toward the end of the first week after the transfusion the antibody titer rose in each of the four experiments to figures higher than those observed immediately prior to transfusion.

In each of the three experiments in which radioiron determinations were made more than two days after transfusion there was a rise in the radioiron in the cir-

culating red cells. This is presumably a reflection of the incorporation into the recipient's newly formed cells of radioiron released from the destroyed donated erythrocytes.<sup>9</sup>

Renal failure did not develop in any of the transfused dogs nor was there significant nitrogen retention. The hematologic findings following the transfusion were similar to those previously reported.<sup>4</sup> In no instance could the transfused cells be detected in samples of the recipient's blood by the use of either canine anti-A serum or anti-dog-serum rabbit serum. The curves expressing osmotic fragility of erythrocytes drawn from the recipients after transfusion were normal. Any increase in fragility of the donated corpuscles present in the recipient's circulation at the time of the tests was insufficient to produce "tailed" curves, that is, curves revealing increased fragility of a small portion of the cell population.

#### DISCUSSION

Renal failure associated with hemoglobinuric or "lower nephron" nephrosis is the major complication following hemolytic transfusion reactions in human beings. The frequency of uremia following hemolytic transfusion reactions is difficult to determine because hemolytic reactions are frequently overlooked especially in anesthetized patients.<sup>2, 10</sup> The mortality of hemoglobinuric nephrosis of this variety has been estimated to be about 50 per cent but modern management has considerably reduced this figure.<sup>3, 11</sup> Some recipients have recovered after large quantities of incompatible blood have been given, while in others a relatively small amount of blood has produced fatal renal damage.<sup>3, 12, 13, 14, 15</sup>

Attempts to produce hemoglobinuric nephrosis experimentally have been successful for the most part only (1) with the production of very high levels of hemoglobinemia,<sup>16, 17, 18</sup> or (2) by the intravenous injection of hemoglobin or laked erythrocytes to animals suffering from shock, dehydration or previously damaged kidneys.<sup>19, 20, 21</sup> Thirty-four experimental transfusion reactions have been produced in dogs in this laboratory under various conditions and only one death from renal failure has occurred, although mild nitrogen retention and oliguria have been produced at times. The concentration of hemochromogen in the dogs' plasma never exceeded 1640 mg. per 100 ml. even though recipients with isoantibody titers as high as 1:256 received 10 ml. of incompatible cells per Kg. The mild effect of these transfusions on the kidneys of the dogs can be understood in the light of reported observations that plasma hemoglobin concentrations as high as 3700 to 8000 mg.<sup>16, 17, 18</sup> per 100 ml. were required in order to produce renal damage in normal animals.

A number of factors probably limit the degree of hemoglobinemia following *in vivo* hemolysis by immune mechanisms. Of importance are the rate of destruction of the incompatible red cells and the rate of clearance of hemoglobin from the plasma. Previous studies have shown that hemoglobin is cleared from the plasma in part by the kidneys after the renal threshold is reached and that from 10 to 40 per cent of the hemoglobin from incompatible cells may be so excreted.<sup>5</sup> The remaining 60 to 90 per cent of the liberated hemoglobin is presumably removed by phagocytosis or other mechanisms.

In the experimental transfusion reactions reported here it is clear that the rate of destruction of transfused incompatible erythrocytes was influenced by the availability of both antibody and complement. Following depletion of either factor from the recipient's plasma, which depended in large part upon the initial antibody titer, the originally rapid rate of destruction of the donated cells was slowed and no further rise in the level of hemoglobinemia was noted after a second transfusion of incompatible erythrocytes. The persistence of slowly declining hemoglobinemia during the periods when either complement or antibody was undetectable in the recipient's plasma suggests that one or the other of these factors is utilized as rapidly as it reappears, as long as incompatible cells are present. The successive transfusions at intervals of ninety to one hundred and twenty minutes were more or less equivalent in this respect to single large transfusions, but allowed separation of the effects produced. In interpreting the results of these experiments it should be appreciated that the rate of disappearance of incompatible cells may be influenced by other factors in addition to the quantity of complement and isoantibody present in the circulation of the recipient.

Stavitsky, Stavitsky and Ecker<sup>22</sup> have reported the disappearance of complement in immunized rabbits following the injection of a variety of antigenic substances. These investigators demonstrated that complement activity did not diminish if the circulating antibody was depleted by prior injection of antigen—a state comparable to that produced in the experiments described in this report.

#### SUMMARY

Each of 4 dogs was given two successive transfusions of incompatible dog erythrocytes at intervals of ninety or one hundred and twenty minutes. Measurements were made of the concentration of hemoglobin, complement and isoantibody in the plasma or serum of the recipients and of the rate of disappearance of the donated cells labeled with radioactive iron.

In each experiment the donated red corpuscles were destroyed much more slowly after the second transfusion than after the first, and the hemoglobinemia produced by the first transfusion was not appreciably augmented by the second transfusion. The successive transfusions at short intervals were considered nearly equivalent to single large transfusions but permitted separate study of the effects produced.

The rate of hemolysis was influenced by the initial titers of both antibody and complement. In dogs with high antibody titer, available complement appeared to limit the rate of destruction of incompatible donated cells. In the presence of adequate complement the rate of hemolysis was limited by the disappearance of antibody when the initial titer of antibody was low.

Limitation of the degree of hemoglobinemia due to limitation of the rate of destruction of transfused incompatible erythrocytes and the rather efficient clearance of plasma hemoglobin explains in part the failure of some hemolytic transfusion reactions to produce severe or fatal renal damage.

It is emphasized that most investigations of hemoglobinuric nephrosis in animals have dealt with the injection of hemoglobin solutions which resulted in hemoglobinemia of much greater intensity than it has been possible to produce by transfusion of large volumes of incompatible red corpuscles.

## REFERENCES

- <sup>1</sup> WIENER, A. S. AND SCHAEFER, G.: Limitation in the use of preserved blood for transfusion; study of fate of transfused erythrocytes in recipient's circulation. *M. Clin. North America* 24: 705, 1940.
- <sup>2</sup> YOUNG, L. E., PLATZER, R. F., YUILE, C. L. AND WOODRUFF, R. L.: Recovery of group O recipient after transfusion of two liters of group A blood. Report of cases with autopsy following ultimate death from carcinoma of lung and pyothorax. *Am. J. Clin. Path.* 17: 777, 1947.
- <sup>3</sup> MUIRHEAD, E. E., HALEY, A. E., HABERMAN, S. AND HILL, J. M.: Acute renal insufficiency due to incompatible transfusion and other causes, with particular reference to management. *Blood* 3: 101, Supp. 2, 1948.
- <sup>4</sup> YOUNG, L. E., ERVIN, D. M. AND YUILE, C. L.: Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. I. Serological and hematological aspects. *Blood* 4: 1219, 1949.
- <sup>5</sup> YUILE, C. L., VANZANDT, T. F., ERVIN, D. M. AND YOUNG, L. E.: Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. II. Renal aspect following whole blood transfusions. *Blood*: 4: 1232, 1949.
- <sup>6</sup> CHRISTIAN, R. M., ERVIN, D. M. AND YOUNG, L. E.: Characteristics of dog isohemagglutinins. Submitted for publication.
- <sup>7</sup> YUILE, C. L., BLY, C. G., STEWART, M. B., IZZO, A. J., WELLS, J. C. AND WHIPPLE, G. H.: Plasma and red cell radioiron following intravenous injection. Turpentine abscesses in normal and anemic dogs. *J. Exper. Med.* 90: 273, 1949.
- <sup>8</sup> FLINK, E. B. AND WATSON, C. J.: A method for the quantitative determination of hemoglobin and related heme pigments in feces, urine and blood plasma. *J. Biol. Chem.* 146: 171, 1942.
- <sup>9</sup> GIBSON, J. G., II, PEACOCK, W. C., EVANS, R. D., SACK, T. AND AUB, J. C.: The rate of post-transfusion loss of non-viable stored human erythrocytes and the re-utilization of hemoglobin-derived radioactive iron. *J. Clin. Investigation* 26: 739, 1947.
- <sup>10</sup> DRUMMOND, R.: Symptomless incompatible transfusion and resultant changes in isoagglutinin titre. *Brit. M. J.* 1: 483, 1944.
- <sup>11</sup> MERRILL, J. P., SMITH, S., III, CALLAHAN, E. J., III AND THORN, G. W.: The use of an artificial kidney. II. Clinical experience. *J. Clin. Investigation* 29: 425, 1950.
- <sup>12</sup> BORDLEY, J., III: Reactions following transfusion of blood with urinary suppression and uremia. *Arch. Int. Med.* 47: 288, 1931.
- <sup>13</sup> DEGOWIN, E. L.: Grave sequelae of blood transfusions; a clinical study of 13 cases occurring in 3500 blood transfusions. *Ann. Int. Med.* 11: 1777, 1938.
- <sup>14</sup> DANIELS, W. B., LEONARD, B. W. AND HOLTZMAN, S.: Renal insufficiency following transfusion. Report of thirteen cases. *J. A. M. A.* 116: 1208, 1941.
- <sup>15</sup> STOCK, R. J.: Acute urinary suppression: Observations in twenty-two patients. *Am. J. Med.* 7: 45, 1949.
- <sup>16</sup> FLINK, E. B.: Blood transfusion studies. III. The relationship of hemoglobinemia and of the pH of the urine to renal damage produced by injection of hemoglobin solutions into dogs. *J. Lab. & Clin. Med.* 33: 223, 1947.
- <sup>17</sup> HARRISON, H. E., BUNTING, H., ORDWAY, N. K. AND ALBRINK, W. S.: The pathogenesis of the renal injury produced in the dog by hemoglobin or methemoglobin. *J. Exper. Med.* 86: 339, 1947.
- <sup>18</sup> OLSON, W. H. AND NECHELES, H.: Kidney excretion during and after hemoglobinemia; a method for production of hemoglobinemia by high sonic vibration. *J. Clin. Lab. Med.* 34: 1733, 1949.



- <sup>19</sup> YUILE, C. L., GOLD, M. A. AND HINDS, E. G.: Hemoglobin precipitation in renal tubules. A study of its causes and effects. *J. Exper. Med.* *82*: 361, 1945.
- <sup>20</sup> LALICH, J. J.: The influence of available fluid on the production of experimental hemoglobinuric nephrosis in rabbits. *J. Exper. Med.* *87*: 157, 1948.
- <sup>21</sup> MALUF, N. S. R.: Factors inducing renal shutdown from lysed erythrocytes: an experimental study. *Ann. Surg.* *130*: 49, 1949.
- <sup>22</sup> STAVITSKY, A. B., STAVITSKY, R. AND ECKER, E. E.: Loss of hemolytic-complement activity and granulocytes following reinjection of an antigen into the rabbit. *J. Immunol.* *63*: 389, 1949.