Staff Meeting Bulletin
Hospitals of the » » »
University of Minnesota

Transfusion Studies

Volume XV | Friday, May 12, 1944 | Number 25

STAFF MEETING BULLETIN
HOSPITALS OF THE . . .
UNIVERSITY OF MINNESOTA

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William A. O'Brien, M.D.

I. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS
No. 25 May 15 - May 20

Visitors Welcome

Monday, May 15

9:00 - 10:00 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff, Todd Amphitheater, U. H.

9:00 - 11:00 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff, Interns Quarters, U. H.

12:30 - 1:30 Pediatrics Seminar; The Possible Specificity of Cutaneous and Mucus Membrane Reactions Due to Sulfonamides, Dr. Sher, W-205 U. H.

12:30 - 1:30 Pathology Seminar; The Pathology and Bacteriology of Gas Gangrene; R. M. Marwin, 104 I. A.

4:00 - Preventive Medicine and Public Health Seminar; Vitamins as Taught and Used; Ancel Keys, 6th Floor, H. S. Lounge.

Tuesday, May 16

8:00 - 9:00 Surgery Journal Club; O. H. Wangensteen and Staff, Main 515 U. H.

9:00 - 10:00 Roentgenology-Pediatrics Conference; L. G. Rigler, I. McQuarrie and Staff, Eustis Amphitheater, U. H.

11:00 - 12:00 Urology Conference; C. D. Creevy and Staff, Main 515, U. H.

12:30 - 1:30 Pathology Conference; Autopsies, Pathology Staff, 104 I. A.

12:30 - 1:30 Physiology-Pharmacology Seminar; The Thyroid--Pituitary Relationship with Special Reference to Iodine Metabolism; J. T. King; 214 M. H.

4:30 - 5:30 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff, Station 54, U. H.

4:00 - 5:00 Pediatric Grand Rounds; I. McQuarrie and Staff, W-205 U. H.

5:00 - 6:00 Roentgen Diagnosis Conference; A. T. Stenstrom, M-515 U. H.

Wednesday, May 17

9:00 - 11:00 Neuropsychiatry Seminar; J. C. McKinley and Staff, Station 60 Lounge, U. H.

10:30 - 12:30 Otolaryngology Case Studies; Out-Patient Ear, Nose and Throat Department; L. R. Boies and Staff.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Carcinoma of Head of Pancreas, E. T. Bell, C. J. Watson, O. H. Wangensteen, and Staff, Todd Amphitheater, U. H.

12:30 - 1:30 Pharmacology Seminar; Pharmacology of Acridine Compounds, A. Neva, 105 M. H.

12:30 - 1:20 Physiological Chemistry Journal Club; Current Literature Reviews; Staff, 116 M. H.

4:30 - 5:30 Neurophysiology Seminar; The Effect of Hypoglycemia on the Brain; J. F. Bosma; 113 M. S.

Thursday, May 18

9:00 - 10:00 Medicine Case Presentation; C. J. Watson and Staff, Todd Amphitheater, U. H.

10:00 - 12:00 Medicine Rounds; C. J. Watson and Staff, East 214 U. H.

12:30 - 1:30 Physiology Chemistry Seminar; The Use of Heavy Isotopes as Tracers; H. G. Wood, 116 M. H.

4:30 - 5:30 Bacteriology Seminar; Report of New York Meetings, "Antibiotics," 113 M. S.

5:00 - 6:00 Roentgenology Seminar; Pyelolymphatic Reflux; G. M. Kelby; M-515, U.H.

Friday, May 19

9:00 - 10:00 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

8:30 - 10:00 Pediatrics Grand Rounds; I. McQuarrie and Staff.

10:00 - 12:00 Medicine Ward Rounds; C. J. Watson and Staff; East 214 U. H.

11:45 - 1:15 University of Minnesota Hospital General Staff Meeting; Surgical Management of Ulcerative Colitis, Clarence Dennis, Powell Hall Recreation Room.

1:30 - 2:30 Medicine Case Presentation; C. J. Watson and Staff, Eustis Amphitheater.

1:00 - 2:50 Dermatology and Syphilology: Presentation of selected cases of the week; Henry E. Michelson and Staff; W-306 U. H.

1:30 - 3:00 Roentgenology-Neurosurgery Conference; H. O. Peterson, W. T. Peyton and Staff, Todd Amphitheater, U. H.

Saturday, May 20

9:00 - 10:00 Medicine Case Presentation, C. J. Watson and Staff, Main 515 U. H.

9:15 - 11:30 Surgery-Roentgenology Conference; O. H. Wangensteen, L. G. Rigler, and Staff, Todd Amphitheater, U. H.

10:00 - 12:00 Medicine Ward Rounds; C. J. Watson and Staff, E-214 U. H.

11:30 - 12:30 Anatomy Seminar; Hematopoiesis in the Rat Embryo and Fetus; Arthur Kirschbaum; Histochemistry of Cytoplasmic Granules; Richard N. Winger, I.A. 226.

II. TRANSFUSION STUDIES

E. B. Flink
K. B. Skubi

1. Survival of Transfused Red Blood Cells

The demands of the present war have stimulated a new impetus to the investigation of blood and blood substitutes. As a result the last few years have seen numerous contributions to our knowledge in this field.

This study will devote itself chiefly to the fate of the erythrocyte in the human body under varying circumstances. With the increasing use of the blood transfusion as a therapeutic measure it is necessary to take into account the limitations and dangers of this procedure.

Numerous studies have been devoted to the fate of the transfused erythrocyte, the effect of storage on its in vitro properties and the effectiveness of various preservative solutions. It is now well accepted that the most accurate criterion for assessing the value of the transfused erythrocyte is its in vivo survival time. Such commonly measured in vitro properties as spontaneous hemolysis, osmotic and mechanical fragility have not been found by Mollison and Young[1] to be related in any constant way to in vivo survival and therefore conclusions based on such tests may be entirely fallacious.

Methods of Study

The in vivo fate of the red cell can be studied by three methods. Namely, by (a) the differential agglutination method of Ashby[2] and Wiener[3]; (b) by the use of radioactive iron; and (c) by the study of urobilinogen excretion following transfusion.

Ashby's agglutination method consists of transfusing a group A or B recipient with group O blood. Periodically thereafter small samples of blood are taken and the recipient's cells are selectively agglutinated with an appropriate serum, leaving the donor's cells free to be counted. Wiener's[3] method is similar in principle but makes use of the M and N blood types.

Recently Ross and Chapin[4] have utilized a very ingenious way of measuring the survival of transfused red cells by the use of a radioactive isotope of iron. When fed or injected into persons with hypochromic anemia of iron deficiency, this isotope is incorporated into the hemoglobin of newly formed erythrocytes. Blood was drawn from these donors into citrated flasks and stored for periods varying from one to fourteen days. 40 to 50 c.c. of this labeled blood was injected intravenously into healthy adults all of whom had normal hemoglobin levels. At varying intervals of time after injection of the blood, samples were withdrawn for blood volume determinations and for counts of the radioactive cells which were done with the Geiger-Muller apparatus. It is possible to detect quantitatively the iron of such labeled cells in the recipient's blood stream even though they may be mixed with thousands of cells containing no radioactive substance.

The method incorporating the study of pigment metabolism before and after transfusion was used in the present study and will be discussed in more detail later.

The Survival Rate of the Transfused Erythrocyte

It is generally agreed by most investigators[5,1,6,7,8] that the average in vivo survival time of the transfused erythrocyte is 80 days and that some survive as long as 120 days. Fresh blood is composed of cells of varying ages and their potential life is inversely proportional to their age even under the most favorable circumstances. Data collected by Mollison and Young[9] shows that about 85% of the fresh transfused red cells are still present at the end of two weeks, 70% in four weeks, and about 45% in eight weeks. (See chart 1)

As the storage time of transfused citrated blood increases the red cell survival rate decreases. The data of various investigators[4,6,7,8,9] on the survival rate of stored citrated blood

of varying ages is not in complete agreement and does not lend itself too well for comparison. However, from the figures available it appears that citrated blood stored three days or less matches fresh blood in respect to red cell survival time.

According to the data of Mollison and Young[11] citrated blood 5 to 9 days of age has a fairly good survival rate, with about 50% of the cells still viable at three weeks and 27% at the end of two months. (See chart 1 and Table I). In this respect Belk and Barnes[7] state that with blood stored seven days or more the post transfusion survival was in no case longer than 24 to 48 hours. (See Table 2)

Blood between 4 and 6 days of age occupies an intermediate position and according to present day standards probably should be considered only "moderately" efficient.

Chapin and Ross[4] in studying the 24 hour red cell survival rate by means of radioactive iron found that only 60% of 6 day old cells survived for 24 hours and about 5% survived for 24 hours when storage was prolonged for 10 days. (See chart 2).

Although many blood banks continue to use citrated blood stored as long as ten and even fourteen days, most investigators in this field recommend the use of citrated blood no older than 5 days and others advocate that storage should not exceed 3 days.[10,11,12,13]

From the above statement it should not be assumed that blood older than four days is of no value. These red cells are less efficient, but even after destruction, can be of considerable benefit to the recipient. Chapin and Ross[4] have data to suggest that iron liberated from destroyed erythrocytes appears to be used for the resynthesis of hemoglobin in preference to and more rapidly than iron present in the blood plasma, the tissue reserves, and food stuffs. They raise the question whether or not some of the products of hemoglobin breakdown can be reused for synthesis of hemoglobin while still in a fairly complex state and without being completely broken down.

Denstedt et al[10] using the agglutination technique have noted a distinct rise in the circulating donor cells between the 15th and 25th days and a second less marked rise at about the 60th day. Others have observed a similar effect. Denstedt feels that some transfused cells are probably stored in the spleen. The increase is not accompanied by a rise in total cell count or in the hemoglobin level.

It is obvious that the fate of the transfused red cell remains far from solved. However, even though destroyed its degradation products may still be of considerable use to the host in some instances, especially in iron deficiency anemias.

Effect of Preservatives

As early as 1916 Rous and Turner[14] found that the addition of glucose to citrated blood enhanced its keeping qualities. However, the large volumes of citrate and glucose used made this an impractical procedure. Since then several investigators have modified this solution and now a satisfactory small volume glucose-citrate mixture is available.[15] It has the following composition: 100 cc. 2% disodium citrate (monohydric), 20 c.c. 15% glucose (or 10 c.c. of 30% glucose). The amount of blood mixed with this is usually 420 c.c. (See Table 1). This solution can be autoclaved after the glucose and citrate are mixed. Furthermore, cells stored in the mixture as long as three weeks have a survival time equivalent to that of fresh blood. (See chart 1 and Table 1).

The cell survival time in these mixtures depends somewhat upon the concentration of glucose and citrate, but the optimal range is quite wide. It has been shown[11] that when approximately one volume of glucose-citrate mixture is added to four volumes of blood, variation of the concentration of glucose in the final mixture from 0.6 to 2.2 makes little difference to subsequent survival. The amount of citrate generally used varies between 1.25 grams and 3.0 grams per 500 c.c. of blood.

Caramelization of glucose solutions upon autoclaving has been offered as an objection to their use. As little as a 1:10,000 dilution of caramel discolors a solution. The reactions encountered coincident with the use of caramelized solutions are no different qualitatively and quantitatively from those seen in uncaramelized solutions. So far as is known, no evidence has been produced to show that intravenous injections of caramel is in any way injurious to man.[16]

Other Factors Influencing Survival Time

(a) Temperature: Immediate storage is usually carried out at temperatures between 4-10 degrees C. At higher temperatures the rate of hemolysis is markedly increased. Freezing causes considerable hemolysis of erythrocytes.

(b) The recipient: i.e., increased tendency to destroy blood due to acquired hemolytic tendencies. Dacie and Mollison[28] found that transfused blood was usually completely destroyed within 20 days in such individuals.

(c) The character of the red cell: Dacie and Mollison[28] showed that blood taken from a patient with familial hemolytic anemia both before and after splenectomy disappeared rapidly when transfused into a normal recipient. On the other hand, normal blood transfused into six patients with familial hemolytic anemia survived normally in five. In one case which was Rh negative survival was somewhat diminished.

Pigment Studies Following Fresh and Old Blood Transfusions.

In order to study the relative efficiency for in vivo survival of the blood in the University Hospital Bank, the values of the serum bilirubin, plasma hemoglobin, and feces urobilinogen were observed before and after fresh and old blood transfusions. Though not as accurate quantitatively, as the actual counting of surviving donor cells, this method gives a rough estimate of the degree of cellular destruction after transfusion.

Method: After preliminary feces urobilinogen, serum bilirubin, and plasma hemoglobin determinations were done, fresh blood (less than 24 hours of age) was given over a period of one to one and a half hours. Within an hour after completion of the transfusion blood was drawn for plasma hemoglobin level, five hours later the serum bilirubin level was determined. Quantitative feces urobilinogen studies were carried out for the next eight days. After an interval of eight days or longer these same studies were then repeated following an old blood transfusion (stored 6 to 8 days). The amount of blood was 450 c.c. except in case II where two fresh blood transfusions were given, and only one old blood transfusion. The urobilinogen was determined in each instance by the method of Watson,[20] the plasma hemoglobin by the method of Flink and Watson.[21]

Results: The values for plasma hemoglobin showed no appreciable change and thus it could be stated that no significant intravascular hemolysis took place following either fresh or old blood transfusions.

In each of the four cases the serum bilirubin values showed a much greater rise with old blood than with fresh blood. The average rise in serym bilirubin following fresh blood was 0.23 mg% and following old blood it was 1.87 mg%.

Feces urobilinogen exeretion followed the same pattern in two cases. In a third case there was an increase in feces urobilinogen after old blood, however, data was incomplete for fresh blood. A fourth case showed slightly higher excretion following fresh blood as compared with old. The average increase in feces urobilinogen following fresh blood was 34 mg per day and after old blood was 96 mg per day. (See chart 3).

Wasserman, Volterno and Rosenthal[22] in a similar study on three patients with apastic anemia found an increase in feces urobilinogen excretion proportional to the length of storage of the transfused blood. In case I following 5 day old

blood there was a rise of 31 mg per day. There was no rise with fresh blood. In case II following 5 day old blood there was a rise of 74 mg per day, after 12 day old blood 118 mg per day. In case III after fresh blood the values were three times normal--after blood stored 17 days the values were 20 times normal.

Others[8,6] have observed the marked frequency of bilirubinemia following the use of blood stored for more than five days as compared to that stored for a shorter period.

From the above data it is evident that the increased destruction of old blood in the reticuloendothelial system, as reflected by a rise in serum bilirubin and feces urobilinogen, makes it definitely less efficaceous for in vivo survival than fresh blood.

University Hospitals Blood Bank

Blood at the University Hospitals is drawn into sterile flasks containing 50 c.c. of 2.5% sodium citrate solution. This is stored at 4 degrees C. for no longer than ten days, after which plasma is withdrawn and the cells discarded. (An average of ten bottles of cells are discarded each day and a large surplus of plasma is accumulating. Storage facilities for this are becoming quite a problem.)

The storage age of 300 consecutive blood transfusions given over a period of 45 days at the University Hospitals were recorded. (See Table 3) It was found that 52% of the blood was stored three days or less, whereas, 33% was stored six days or longer. On the basis of the data previously presented it can be seen that about one third of the blood used must be considered relatively inefficient.

Effect of Storage on Other Factors

Most authors agree that marked deterioration of both leucocytes and platelets occurs within 24 hours. Immune globulin substances appear to be quite stable but complement and perhaps other antibacterial substances rapidly degenerate in stored blood.[8,6,23]

Quick[49] has shown that prothrombin is probably made up of two components, "A" and "B". Component "A" is stable when the plasma is in its native and unmodified state, whereas in oxilated or citrated plasma it is easily destroyed, supposedly due to oxidation. The "B" component, which is the only one reduced in clinical prothrombin deficit and following the use of Dicoumarol is not affected by storage. Thus stored plasma or whole blood are just as effective as fresh plasma in Dicoumarol poisoning or hypoprothrombinemia.

Red Cell Transfusions

In the past few years demands for plasma have created a large surplus of red cells at donor centers. For some time efforts have been directed toward salvaging these cells and numerous reports are now available in this regard.[11,17,18,19] Although survival rates are not yet reported it appears that cells resuspended in normal saline or dextrose-citrate solutions are a satisfactory substitute for whole blood, at least as far as the erythrocytes are concerned. Thalhimer[13] has recommended that the cells must be used within five days of the date of bleeding.

Miscellaneous

Tolerance to Sodium Citrate: Sodium citrate is relatively non toxic since 6 to 8 grams may be injected intravenously during a ten minute period without producing symptoms. Larger amounts can be given over longer periods of time without untoward effect. It is rapidly oxidized and excreted, 90% being removed from the blood within 10 minutes.[25]

Rate of Transfusion: The rate of blood injection depends upon the integrity of the cardiovascular system. In normal individuals as much as 650 c.c. can be given in three minutes without adverse reactions. However, the recommended rate is 250 c.c. per hour.[26] In discussing the use of blood in wartime emergencies Vaughan[29] states that it is safe and often essential to give one liter of blood or blood derivative in the first half hour. It is often necessary to give this initial

volume with the help of pressure, since circulatory failure may have led to complete collapse of the veins and gravity alone is insufficient to cause a free flow of fluid. This author also states that no case should be regarded as hopeless until at least 3 liters have been given without any rise in pressure.

The belief that transfusion of blood in gastrointestinal bleeding may raise the blood pressure and "blow off" a clot from the site is probably erroneous. The rapid infusion of 500 c.c. of blood in 5 to 10 minutes produces only a 10 mm. of mercury rise in blood pressure. This is inadequate to dislodge recent thrombi.[27] Rise in blood pressure may not, however, be the only factor of importance.

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2. Transfusion Reactions

The importance of trying to classify reactions to transfusions is not generally appreciated. Reactions naturally fall into the following groups: (1) simple febrile reactions due to pyrogenic substances usually undetermined, (2) allergic reactions, both anaphylactic and urticarial, (3) circulatory reactions from too rapid administration, and (4) febrile reactions following intra-vascular hemolysis.

The incidence of reactions of all kinds varies with different reports but usually ranges from three to five per cent of all infusions of blood given. Erf and Jones[12] report an incidence of 3.2 per cent in a series of 2,889 transfusions of blood. Only two transfusions resulting in non-fatal reactions were found to be due to incompatible blood. Urticaria was found in 0.3 per cent. Levine[30] reported the incidence of transfusion reactions at this hospital in 1942, as 4.2 per cent of 522 blood transfusions and 2.0 per cent of 149 plasma infusions. Of these none were clearly hemolytic. Wiener et al[31] report an incidence of 2 cases of incompatibility in 3000 transfusions. Carlson[32] reported 6 per cent of 3,388 transfusions of banked blood resulted in reactions. Of these 11 cases or .32 per cent were serious. These included 3 hemolytic reactions, 3 cases of jaundice without other evidence of hemolytic reaction, 2 anaphylactic reactions and 3 cases in which cardiovascular embarrassment was caused by a transfusion.

Wiener and Schaeffer[6] state that blood older than 10 days caused chills and fever quite regularly, but most investigators agree that the age of blood when not older than 8 or 9 days does not influence the incidence of febrile reactions. DeGowin et al[33] have demonstrated that the potassium content of the plasma of old stored blood does not cause any untoward symptoms or signs. Furthermore they have warned against the warming of donors' blood and found no greater number of febrile reactions when the blood was given at room temperature.

It is generally conceded that a hemolytic reaction is the most serious complication of transfusions of whole blood. Most of the case reports depend on the appearance of hemoglobin in the urine, on oliguria or anuria, or on transient jaundice for recognition of the hemolytic component. In 1667 Jean Baptiste Denys first described a severe hemolytic transfusion reaction with hemoglobinuria - "urine of a color as black as if it had been mixed with the soot of chimneys." Undoubtedly a large number of hemolytic reactions go unrecognized. There have been a large number of reports of anuria and death from transfusion reactions since 1900. Baker and Dodds,[34] DeGowin et al,[35] Lindau,[36], Goldring and Graef,[37] Daniels et al,[38], Bordley,[39] and Ayer and Gould[40] have contributed the most to the study of cases of transfusion complicated by hemolytic reactions. Very little attention has been paid to the presence of hemoglobin free in the plasma, and surprisingly enough, little mention of the actual amount of hemoglobinuria.

Daniels et al[38] reported 7 deaths out of 13 cases of hemolytic reactions - 54%. Hesse[41] in a review of the literature reported 20 deaths out of 46 hemolytic reactions - 42%. Bordley[39] reported 10 deaths out of 17 hemolytic reactions - 59%. These data indicate

the seriousness of hemolytic reactions and emphasize the importance of correctly defining the type of transfusion reactions as soon as they occur.

Hemolytic reactions are due to group incompatibility, the occurrence of isoagglutinins such as anti-Rh agglutinins, and occasionally to overheated blood. Hesse[41] stresses the importance of the use of uncross-matched group O blood as a cause of fatal reactions. Aubert et al[42] demonstrated that in 40 per cent of donors of group O anti-A titers of over 1:512 were found and noted that transfusions of O blood of such high anti-A titer or higher rarely produced an elevation of the red blood cell levels of recipients of group A. Neither did they produce severe or fatal hemolytic reactions.

One of the purposes of this presentation is to call attention to the value of the determination of the plasma hemoglobin content after any suspected transfusion reaction in order to classify the reaction correctly with regard to intravascular hemolysis. The highest pre- or post-transfusion plasma hemoglobin concentration in 60 transfusions given during the past 2 years at this hospital regardless of the age of blood (up to 10 days) was 10 mg. per cent and most of the levels were below 5 mg. per cent. The post-transfusion specimens were obtained during the first hour after the transfusion had stopped. When two specimens were obtained in the hour after transfusion, no appreciable difference was found in the value immediately after and one hour after transfusion. Of course, it is quite important that no artificial hemolysis be introduced. In order to avoid hemolysis in withdrawing blood it is necessary to do a clean-cut venapuncture, use a clean dry or a clean saline-rinsed syringe and needle, and very gently agitate the blood with one volume of 3 per cent sodium citrate for each nine volumes of blood. Hemolysis becomes grossly visible when the plasma hemoglobin concentration amounts to 20 mg. per cent.

Ottenberg and Fox[43] have clearly demonstrated in human beings that when the plasma hemoglobin level does not exceed some rather variable limit there is a fairly uniform and gradual removal of hemoglobin from the plasma. They found an average level of about 150 mg. per cent at which hemoglobin appeared in the urine but that there was a rather wide range of variations. As much as 50 grams of hemoglobin in solution has been injected into human beings,[44] without visible reactions. It is apparent that a small amount of hemoglobin dissolved in plasma can produce no damage.

During the past several years it has been our privilege to study sixteen patients with febrile non-hemolytic reactions to infusions of blood or blood substitutes (two of plasma). As an illustration of the severity of the reactions seven of these patients had temperatures of 103° to 106° F and all patients had chills and at least some rise in temperature. Clinically it was impossible to differentiate the reactions from hemolytic reactions. The post-transfusion plasma hemoglobin concentrations ranged from traces to 8.4 mg. per cent and there was no hemoglobinuria. The blood was drawn within an hour of the reaction in each instance.

In at least some of the above instances it was of very crucial importance to be assured immediately that no hemolysis had occurred because of the critical conditions of the patients.

Five cases of hemolytic transfusion reactions have been studied during the past two years. Undoubtedly others have occurred for several patients have developed jaundice following transfusion and at least one case of anuria has occurred but our attention was called to them too late to permit a study of the above type.

, female age 36, received 200 c.c. of blood and developed a chill, pain in the back and fever of 104° so the transfusion was discontinued. Thirty minutes and two hours after the chill, the plasma hemoglobin levels were 36 and 38 mg. per cent respectively. No hemoglobin appeared in the urine. Jaundice became apparent the following day.

, female age 17, received 500 cc. of apparently compatible blood and developed a mild chill and fever of 103.6°F. Plasma obtained 3 and 5 hours after transfusion was red and the hemoglobin concentrations were 124 and 75 mg. per cent respectively. The next day the serum bilirubin concentration was 2.7 mg. per cent and she was visibly jaundiced. A urine specimen for the whole twenty-four hour period contained no hemoglobin.

, female age 14, received 500 cc. of group O blood (four days old), 540 cc. of group O plasma and 250 cc. of group A plasma during an operation for the ligation of a patent ductus arteriosis. No visible reaction occurred, but she was anesthetized during the administration of blood. Red urine and jaundice were noticed twenty-four hours after the operation. The plasma was red and the plasma hemoglobin level was 54 mg. per cent at that time; the total bilirubin was 4.6 mg. per cent and most of it was indirect reacting (van den Bergh). Thirty hours after the operation the plasma hemoglobin was 43 mg. per cent and 40 hours after the operation the level was 12 mg. per cent. There was a small amount of hemoglobin in the urine specimen obtained just before the first blood specimen (24 hours after transfusion) but none afterwards.

male age 45, has an intracranial meningioma and during craniotomy severe bleeding and shock were encountered repeatedly. He received 16 bottles of group O bank blood, some of which was uncross-matched, and 2000 cc. of plasma during an eight hour operation. His urine output was 25 c.c. during the first sixteen hours postoperatively and he became intensely jaundiced. A specimen of blood obtained twenty hours after the beginning of the operation revealed a bright red plasma and a hemoglobin level of 254 mg. per cent. Forty-four hours after the operation the plasma was definitely brownish-red, and the level of hemoglobin was 160 mg. per cent. Direct spectroscopic examination of the plasma revealed only oxy-hemoglobin bands in the twenty-hour specimen, but the forty-four hour and sixty-eight hour specimens revealed in addition a distinct band at 623 - 624 mu. This band was compared with a methemoglobin spectrum and the band was distinctly different from the first band of methemoglobin, which is at 630 mu. The above described band is characteristic of methemalbumin, a combination of hematin and serum albumin recently described by Fairley.[45] The postoperative course was very stormy with a fever as high as 105°F. The patient died of uremia (B.U.N. 103 mg. per cent) seventy-six hours after operation. Unfortunately, permission for autopsy could not be obtained.

., male age 32, had a lobectomy for removal of bronchial adenoma. He had 1800 cc. of plasma and 2000 cc. of blood (all of it eight or nine days old) during the operation. Hemoglobinuria was noted the morning after operation. Blood specimens were not obtained until forty-two hours after the last transfusion. The plasma hemoglobin was 38 mg. per cent at that time, and was 33 mg. per cent at forty-eight hours and 16 mg. per cent at sixty-seven hours. The bilirubin reached a peak at forty-eight hours of 6.1 mg. per cent and returned to 0.47 mg. per cent at ninety hours. A urine specimen from the thirty-sixth to the forty-eighth hours had 1.9 gm. of hemoglobin but subsequent specimens were free of hemoglobin. No methemalbumin was found in the serum. He recovered completely from the effects of the reactions and the operation.

From the description of the cases of hemolytic reaction it will be seen that two of the cases would have been completely unrecognized if the plasma had not been examined for hemoglobin. One patient died of uremia secondary to an hemolytic reaction to multiple transfusions. The three cases with hemoglobinuria were transfused during operation and consequently had no clinically recognizable reaction such as rigor or fever. Undoubtedly there are cases of mild hemolysis such as the first two cases and a certain number of cases even with hemoglobinuria which go unrecognized.

The following procedures should be carried out whenever any kind of a reaction occurs after a transfusion of whole

blood:

1. Notify the blood bank immediately and bring back the transfusion set intact if the reaction occurs before completion of the transfusion.

2. Ask for re-crossmatching of the donor's and patient's blood.

3. Have all the urine collected from the patient for a 24-hour period and send it to the main laboratory for test for hemoglobin.

4. Collect 4.5 c.c. of blood in a tube containing 0.5 c.c. of 3% sodium citrate solution, invert the centrifuge tube just once, and then have it centrifuged immediately. If a serious hemolytic reaction has occurred the plasma will be highly colored with hemoglobin, but if the reaction is merely a febrile one, no visible hemoglobin will appear in the plasma. A plasma hemoglobin determination should be obtained.

5. Whenever a large amount (even one unit) of group O blood is used in an emergency where time does not permit cross-matching a specimen of plasma should be obtained as in (4) at completion of the transfusion.

As to the efficacy of alkalinization of the urine prophylactically or therapeutically to prevent renal complications no definite statement can be made. There is quite a controversy in the literature about the use of alkali. Their use continues to have a vogue in blackwater fever and transfusion reactions. The basis of the treatment is the experimental evidence obtained by De Gowin et al,[35] Baker and Dodds,[34] and Bywaters[46] that acidifying the urine in animals receiving hemoglobin solutions resulted in death whereas similar doses of hemoglobin in animals with alkaline urine produced no renal insufficiency. Foy et al[47] state that in blackwater fever there is no evidence that oliguria is less frequent in patients with alkaline urine than those with acid urine. Richards and Walker[48] have demonstrated that the glomerular filtrate first becomes acid normally in the region of Henle's loop. This corresponds to the location of the beginning of damage to tubular epithelium and the appearance of pigment casts in the kidneys of individuals dying of blackwater fever and transfusion reactions. Because of the experimental evidence for the benefits of alkalinization it seems wise to recommend it routinely in patients receiving transfusions in spite of some objections by various recent writers. The alkalinization should be carried out by giving sodium bicarbonate in doses of 10 to 12 grams just before the transfusion orally in all patients who can take it orally.

Summary

From the above data one must conclude that where simple citrated blood is used to treat anemias, in which new blood formation is impaired or in which an acquired hemolytic tendency prevails, the storage should be limited to 4 days and preferably not longer than 2 days. In iron deficiency anemias and instances of acute blood loss where the functional capacity of erythrocyte regeneration can be assumed to be normal, citrated blood up to 6 days of age can be used.

On the other hand a satisfactory glucose-citrate mixture is now available in which blood can be stored safely for much longer periods of time. Even when stored for three weeks such blood has a post transfusion survival rate equivalent to fresh blood.

According to the present day standards a considerable number of transfusions at the University Hospitals are poor from the standpoint of erythrocyte survival. In view of this the use of the above mentioned citrate-glucose mixture should be considered.

It is important to classify febrile transfusion reactions with special reference to the occurrence of or absence of hemolysis.

Five cases of hemolytic reactions are reported.

Sixteen cases of febrile reactions were proven to be non-hemolytic.

The usefulness of determining the plasma hemoglobin content after reactions is stressed.

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We wish to express our appreciation to Dr. C. J. Watson, Mr. G. Needham, Miss Betty Bertie, and Miss Violet Hawkinson for their cooperation in this study.

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Table 1

Erythrocyte Survival Rate of Blood Stored in Various Preservatives

Preservative	c.c.	Length of storage in days	Per cent Survival in Vivo			
			24 hours	1 week	3 weeks	2 months
I. Sodium citrate 3%	100	0	100	95	85	45
Blood	440	5-9	72	68	51	27
		11-17	25	11		
II. Defibrinated blood	450	6-9	89	76	63	20
III. Disodium citrate 2%	100	20	92	87	83	50
Glucose 15%	20	28	81	74	59	25
Blood	420					

Table 2

In Vivo Red Cell Destruction of Citrated Blood
(Belk and Barnes)

Age of blood	
4 days - - - -	no donor cells detected after 35 days
6 days - - - -	" " " " " 20 days
7 days - - - -	" " " " " 48 hours

Table 3

University Hospitals Blood Bank

300 Consecutive Transfusions
over a period of 45 days

Age of blood in days	Number of Transfusions	% of Total
1-3	156	52
4	28	
5	17	15
6	36	
7	25	
8	14	33
9	20	
10	4	
	300	100

Chart 1

In Vivo Red Cell Survival

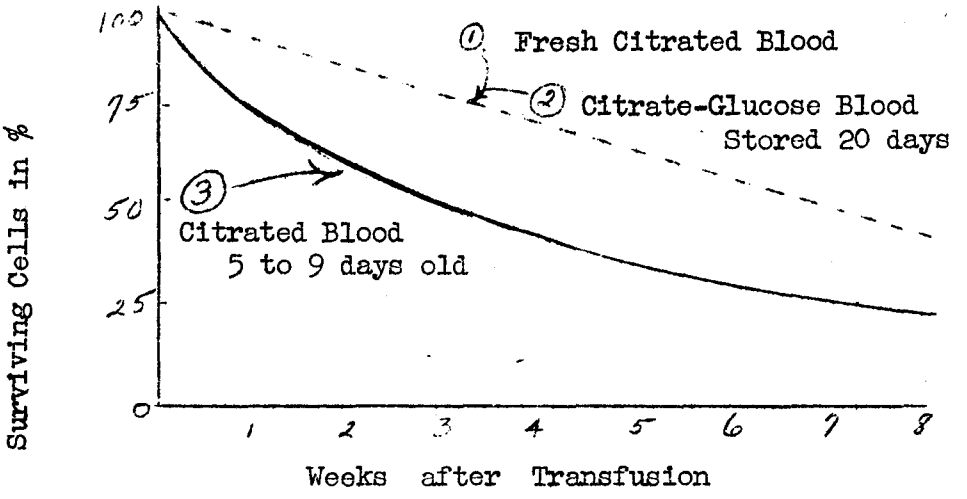


Chart 2

24 Hr. Survival Time of

Citrated Blood as Measured

by Tagged Iron

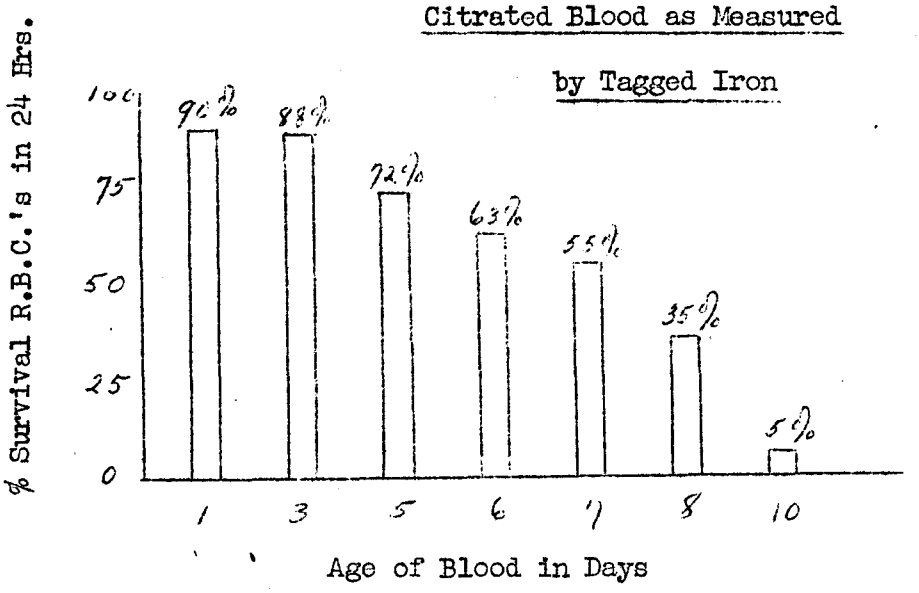
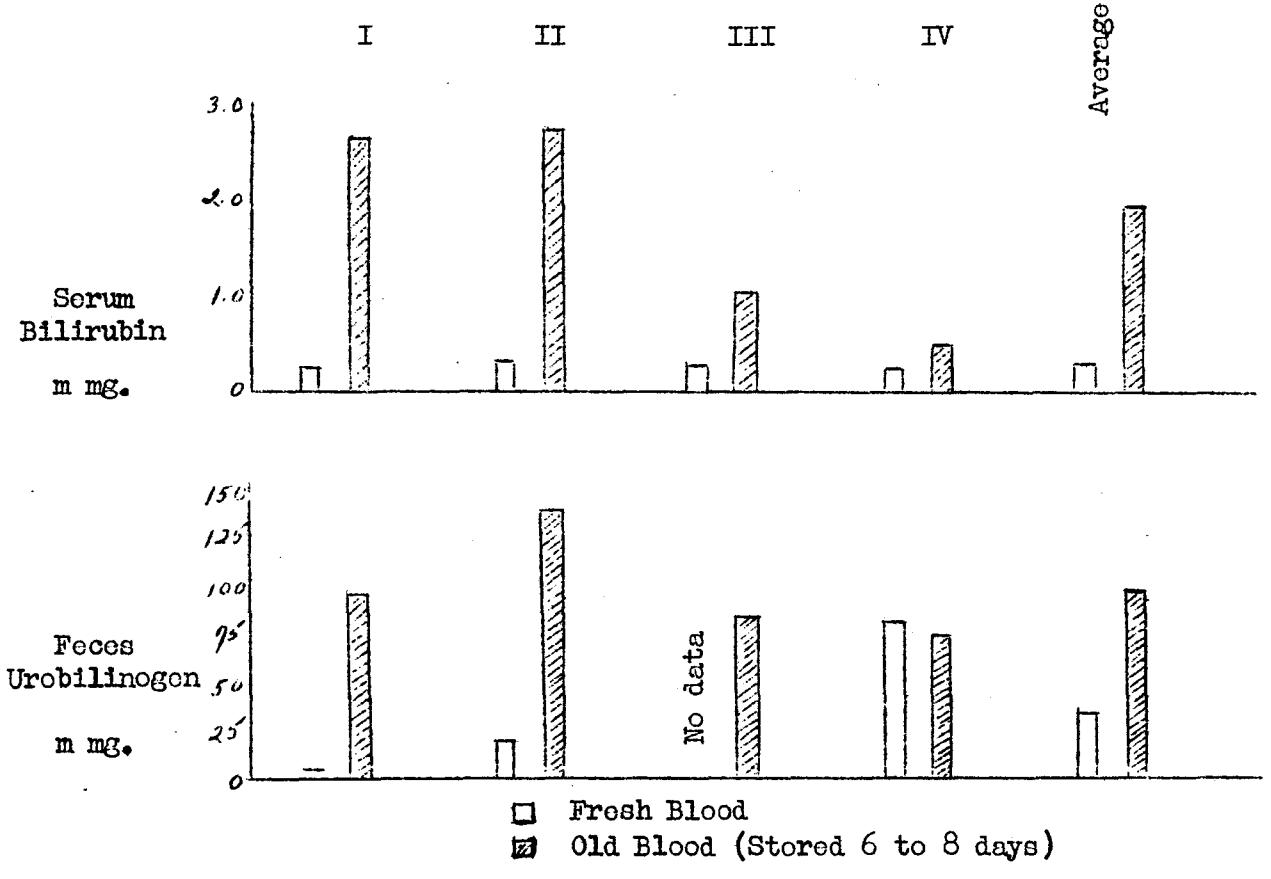


Chart 3



Rise in Serum Bilirubin and Feces Urobilinogen following Fresh and Old Blood Transfusions

III. SPECIAL REPORT

Physicians who desire to employ the new microbiotic chemotherapeutic agent, the sodium salt of penicillin, in the treatment of conditions hitherto not amenable to or resistant to therapy with sulfonamide drugs or other agents, should be guided by the following summary.

This report has been prepared by Dr. Chester S. Keefer, Chairman of the Committee on Chemotherapy of the National Research Council and Consultant to the Office of Scientific Research and Development.

The recommendations presented in this report have been based upon experience gained by clinical teams in a number of institutions, treating over three thousand cases of presumably amenable diseases representing a wide range of conditions. The long series of carefully controlled and reported cases, under supervision of the Office of Scientific Research and Development, has resulted in authoritative data upon which to base recommendations as to indications, contra-indications, mode of administration and dosage for penicillin.

In releasing penicillin for controlled use in the civilian practice of medicine, the War Production Board through the Office of Civilian Penicillin Distribution desires to make penicillin available to the greatest number of patients to whom its administration is justified without wasting precious material. Therefore, it recommends Dr. Keefer's summary as a guide for treatment with penicillin in hospitals and institutions at this time.

The text of the report prepared by Dr. Keefer, representing the recommendation of the Office of Scientific Research and Development and the National Research Council as follows:

"Based upon the experience in the past year with penicillin therapy, it has been found that penicillin is the best therapeutic agent available for the treatment of certain conditions, as follows:

Group I Indications

1. All staphylococcic infections with and without bacteremia:
   - Acute osteomyelitis
   - Carbuncles--soft tissue abscesse
   - Meningitis
   - Cavernous or lateral sinus thrombosis
   - Pneumonia--empyema
   - Carbuncle of kidney
   - Wound infections

2. All cases of clostridis infections:
   - Gas gangrene
   - Malignant edema

3. All hemolytic streptococcic infections with bacteremia and all serious local infections:
   - Cellulitis
   - Mastoiditis with intra-cranial complications, i.e., meningitis, sinus thrombosis, etc.
   - Pneumonia and empyema
   - Puerperal sepsis
   - Peritonitis

4. All anaerobic streptoccic infections:
   - Puerperal sepsis

5. All pneumococcic infections of
   - Meninges
   - Pleura
   - Endocardium

   All cases of sulfonamide-resistant pneumococcic pneumonia

6. All gonococcic infections complicated by
   - Arthritis
   - Ophthalmia
   - Endocarditis
   - Peritonitis
   - Epididymitis

   Also all cases of sulfonamide-resistant gonorrhoea

Indications in Group II

Penicillin has also been found to be an effective agent in the following diseases but its position has not been definitely defined:

1. Syphilis
2. Actinomycosis
3. Bacterial endocarditis

Conditions in Group III of Questionable Value

Penicillin is of questionable value in mixed infections of the peritoneum and liver in which the predominating organism is of the gram negative flora--i.e.,

1. Ruptured appendix
2. Liver abscesses
3. Urinary tract infections
4. It is also of questionable value in rat bite fever due to streptobacillus moniliformis

Group IV Conditions Contra-indicated

Penicillin is contra-indicated in the following cases because it is ineffective:

1. All gram negative bacillary infections:
   Typhoid--Para-typhoid
   Dysentery — B. Pyocyaneous
   E. Coli — Br. melitensis (undulant fever)
   H. influenza — Tularemia
   B. Proteus — B. Friedlanders
2. Tuberculosis
3. Toxoplasmosis
4. Histoplasmosis
5. Acute rheumatic fever
6. Lupus erythematosus diffuse
7. Infectious mononucleosis
8. Pemphigus
9. Hodgkin's disease
10. Acute and chronic leukemia
11. Ulcerative colitis
12. Coccidioidomycosis
13. Malaria
14. Poliomyelitis
15. Blastomycosis
16. Non-specific iritis and uveitis
17. Moniliasis

Treatment of Infections with Penicillin

The recommendations put forth in Dr. Keefer's report, based on the wide experience gained under varied conditions of use and purpose, follow:

Method of Preparing Penicillin for Treatment

Penicillin is supplied in ampoules of different sizes--25,000 units and 100,000 units each. Inasmuch as penicillin is extremely soluble, it may be dissolved in small amounts of sterile, distilled pyrogen-free water, or in sterile, normal saline solution. When large unit sizes are being used in hospitals, the contents of the ampoule should be dissolved in water or saline so that the final concentration is 5,000 units per cubic centimeter. This solution should be stored under aseptic precautions in the ice box, and made up freshly every day. Solutions for local or parenteral use may be diluted further, depending upon the concentration desired.

A. For intravenous injection

1. The dry powder may be dissolved in sterile physiological salt solution in concentrations of 1,000-5,000 units per cc. for direct injection through a syringe.

2. The dry powder may be dissolved in sterile saline or 5 per cent glucose solution in lower dilution (25-50 units per cc.) for constant intravenous therapy.

B. For intramuscular injection

1. The total volume of individual injections should be small, i.e., 5,000 units per cc. of physiological saline.

C. For topical application

1. The powdered form of the sodium salt is irritating to wound surfaces and should not be used.

2. Solutions in physiological salt solution with a concentration of 250 units per cc. are satisfactory. For resistant or more intense infections this concentration may be increased to 500 units per cc.

Methods of Administration of Penicillin

There are three common methods of administering penicillin--intravenous, intramuscular and topical. Subcutaneous injections are likely to be painful and should be avoided.

Repeated intramuscular injections may be tolerated less well than repeated or constant intravenous injections. In many cases, however, the intramuscular route

may be the one of choice.

In the treatment of meningitis, empyema, and surface burns of limited extent, penicillin should be used topically, that is, injected directly into the subarachnoid space, into the pleural cavity, or applied locally in solution containing 250 units per cc.

Dosage

The dosage of penicillin will vary from one patient to another depending on the type and severity of infection. In our experience recovery has followed in many serious infections following 40,000 to 50,000 Oxford units a day, in others 100,000 to 120,000 or even more is necessary. The objective in every case is to bring the infection under control as quickly as possible. The following recommendations are made at the present time with a full realization that revisions may be necessary as experience accumulates.

It is well to remember that penicillin is excreted rapidly in the urine so that following a single injection it is often impossible to detect it in the blood for a period longer than 2 to 4 hours. It is well, therefore, to use repeated intramuscular or intravenous injections every 3 or 4 hours, or to administer it as a continuous infusion.

A. In serious infections with or without bacteremia an initial dose of 15,000 or 20,000 Oxford units with continuing dosage as

1. Constant intravenous injection of normal saline solution containing penicillin so that 2,000 to 5,000 Oxford units are delivered every hour, making a total of 48,000 to 120,000 units in a 24-hour period. One-half the total daily dose may be dissolved in a liter of normal saline solution and allowed to drip at the rate of 30 to 40 drops per minute.

2. If continuous intravenous drip is undesirable, then 10,000 to 20,000 units may be injected intramuscularly every 3 or 4 hours.

3. After the temperature has returned to normal the penicillin may be stopped and the course of the disease followed carefully.

B. In chronically infected compound injuries, osteomyelitis, etc., the dosage schedule should be 5,000 units every two hours or 10,000 units every four hours parenterally with local treatment as indicated. This dosage schedule may have to be increased, depending upon the seriousness of the infection, and response to treatment.

C. Sulfonamide-resistant gonorrhea

1. 10,000 units every 3 hours intramuscularly or intravenously for 10 doses. It is not likely that the same effect may be obtained with 20,000 units every 3 hours for 5 doses. The minimum dosage has not been worked out completely. The results of treatment should be controlled by culture of exudate.

D. Empyema

1. Penicillin in normal physiological saline solution should be injected directly into the empyema cavity after aspiration of pus or fluid. This should be done once or twice daily, using 30,000 or 40,000 units depending upon the size of the cavity, type of infection and number of organisms. Penicillin solutions should not be used for irrigation. It requires at least 6 to 8 hours for a maximum effect of penicillin.

E. Meningitis

1. Penicillin does not penetrate the subarachnoid space in appreciable amounts, so that it is necessary to inject penicillin into the subarachnoid space or intracisternally in order to produce the desired effect. Ten thousand units diluted in physiological saline solution in a concentration of 1,000 units per cc. should be injected once or twice daily, depending upon the clinical course and the presence of organisms."

* * * * *

The above dosage schedules may require revision as increased experience is obtained. In many cases studied by accredited investigators, the above schedule has proved to be adequate.

Conclusion

The Office of Civilian Penicillin Distribution, War Production Board requests medical practitioners employing penicillin to carefully observe the recommendations stated above as to indications, contra-indications, mode of administration and dosage in order to gain the maximum value and advantage from this new medicinal agent.

F May 1, 1944