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Clinical Transfusion Medicine

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I. Pretransfusion Considerations

A. Typing and Cross Matching

The ability of veterinarians to obtain blood types of potential donors and recipients is limited by the scarcity of reagents and laboratories that perform typing of animal blood. At this time, canine typing reagents are avaiable only through the laboratory of Dr. Robert Bull at Michigan State University. In addition, several veterinary schools and at least one commercial laboratory (Stormont Laboratories, Vacaville, California) perform typing of canine blood using these reagents. In most cases canine donors are typed only for the two alleles at the DEA-1 locus with antibodies combined and termed DEA-1.1,2. (For procedures for typing and cross matching, see Smith, this volume ["Erythrocytes"].) Dogs that react with this reagent are commonly called A positive. Red cells that are A positive are most likely to sensitize recipients that lack these antigens. Because A-positive dogs represent 60% of the population, they can be utilized as donors for recipients that are also positive. This requires that typing be available for recipients. Blood group A_2 dogs may develop antibodies when given A_1 red cells, but this is rare (Swisher and Young, 1961). These antibodies would be detectable if a major cross match were done before subsequent transfusions. Although a single transfusion of A-positive blood could be given to an A-negative dog never previously transfused, antibodies may be formed that increase the risk for reaction on subsequent transfusion, for premature destruction of transfused cells 7–14 days after the first transfusion, and for neonatal hemolytic disease in pups of transfused bitches (see Paradis, this volume). If typing is not immediately available, A-negative donors can be used for all transfusions. Testing for blood group antigen DEA-7, present in 45% of dogs, is not routinely done, but this antigen may also elicit an antibody response in DEA-7 negative dogs (Swisher and Young, 1961). Some DEA-7-negative dogs have naturally occurring anti-7 antibody. Although this has generally been thought not to be of clinical significance, R. W. Bull (personal communication, 1990) has observed shortened survival of transfused DEA-7-positive cells into these dogs. The remaining antigens are either weakly reactive, or else very prevalent or rare so that donor and recipient are likely to match.

Because typing is not done for all known (or unknown) antigens, dogs that have received transfusions more than 4 days previously should be cross matched. A major cross match, testing donor red cells against recipient plasma, will detect clinically significant antibodies that have formed after a previous transfusion. The cross match does not prevent

sensitization; it only detects sensitization already present. An autocontrol with recipient red cells and plasma is included because some recipients have immune-mediated hemolysis with autoagglutination or direct antiglobulin test (DAT) positivity interfering with both typing and cross matching. If the control is only weakly positive and the test sample is strong, results may be valid. If both are equal, no conclusions as to compatibility can be drawn. A minor cross match testing donor plasma with recipient red cells is not helpful in dogs.

Feline typing reagents are not readily available, although potentially incompatible transfusions are detectable by a major cross match. The frequency of blood groups in cats varies geographically and by breed (Auer and Bell, 1981; Bird *et al.*, 1988; Giger, 1989, 1990). Greater than 99% of cats in the United States, over 90% in Europe and Japan, and approximately 70% in Australia are blood group A. Certain breeds, especially the Persian, Devon rex, British shorthair, Himalayan, Abyssinian, Birman, Scottish fold, and Somali, have a high prevalence of group B, up to 50% in some breeds (Giger, 1990). The oriental breeds such as Siamese and Burmese tend to be group A. Any feline transfusion across blood groups results in rapid destruction of the transfused cells, even if the recipient has never been previously transfused. The most severe clinical reactions have been seen in the group B cats given group A cells. These cats have high naturally occurring titers of anti-A antibody. Less severe reactions are seen if group B blood is given to a group A cat, but survival of transfused cells is short. A practical approach to feline transfusions is to have group A donors available. Typing is available at the same locations that type canine blood. When a transfusion is needed, a major cross match is done. If the recipient is a group B cat, strong agglutination occurs even without use of Coombs reagent. If group B blood is needed, a group B donor may be identified form one of the high-prevalence breeds. If the need is not urgent, blood may be obtained from Dr. Urs Giger at the University of Pennsylvania.

In horses, compatibility tests including direct agglutination, antiglobulin, and hemolytic testing were not effective in predicting survival of transfused Fe^{59}-labeled cells (Kallfelz *et al.*, 1978). Despite lack of evidence of incompatibility on testing, transfusions were only of value for periods of 2–4 days. Second transfusions in the same group of six horses resulted in two severe anaphylactic reactions. Similar short red cell survival was noted when labeled, cross match-compatible red cells were given to normal cattle (Kallfelz and Whitlock, 1973). From days 3 to 4 posttransfusion, the surviving fraction dropped from 60 to 24%. In goats and sheep the half-life of transfused autologous red cells was 8 days (Gulliani *et al.*, 1975; Giles *et al.*, 1975). When unmatched homol-

ogous goat red cells were transfused, the survival times were 3–4 days with no cells remaining in the circulation by the seventh day. The most likely explanation for poor survival of transfused red cells in horses and ruminants is the presence of naturally occurring isoantibodies and numerous blood group antigens. Any transfusions in these species must be given with caution because cross matching may not predict incompatibility. Also, because survival of cells is so poor, maximum use of alternatives to transfusions should be made.

B. Pretransfusion Testing

In most animals needing a transfusion, the cause of the blood loss or anemia is known. In cases of anemia of unknown cause, blood should be drawn for any tests anticipated before the transfusion is given. The testing should include a complete blood count (CBC) with reticulocyte count (except in horses) and a platelet count. Red cell indices and morphology can be helpful in finding the cause. A DAT is run if there is any suspicion of immune-mediated disease, because a positive DAT posttransfusion could be caused by development of alloantibodies. If iron deficiency is present in an adult animal, one should search especially diligently for a bleeding gastrointestinal lesion such as ulcer or neoplasia. If the anemia is nonregenerative, renal function should be evaluated. Human recombinant erythropoietin has been shown to be an effective treatment for dogs and cats with anemia associated with renal disease (Cowgill *et al.*, 1990). Examination of the marrow is indicated in cases of nonregenerative anemia of unknown cause. Anemia of chronic disease is associated with a mild decrease in the hematocrit (Hct), and occurs secondary to many chronic or debilitating diseases. If the Hct drops below 20–25%, an additional problem must be sought. Additional information on the clinical workup of the anemic patient is provided elsewhere (Cotter, 1989).

C. Assessment of the Need for Transfusion

Red blood cells are needed whenever the red cell number becomes so depleted that oxygen-carrying capacity is insufficient to maintain adequate performance. The definition of adequate may vary. For example, an otherwise healthy animal with mild to moderate weakness after acute blood loss may be allowed to recover spontaneously. A severely ill patient with the same degree of anemia caused by surgical blood loss may recover more quickly with fewer complications if transfused. One

must avoid the temptation of arbitrary transfusion at a given Hct. Several factors are considered, including the cause and severity of anemia, expectation of further blood loss, alternative treatment options, and the time of onset of anemia. Typically, anemia caused by lack of red cell production is slow in onset and compensatory mechanisms are functioning by the time the anemia is severe. These mechanisms include increased cardiac output and pulmonary ventilation, and in some species, increased red cell 2,3-diphosphoglycerate (2,3-DPG). Thus, oxygen delivery is maximized and sedentary patients may show only mild clinical signs despite a Hct of less than 10%. Conversely, the acutely anemic patient may require red cell support at a higher Hct. In fact a patient with acute hemorrhage may die with a normal Hct. The decision to transfuse also depends upon the overall evaluation of the patient, especially cardiac, pulmonary, and renal function. The availability of alternative treatment such as iron replacement after chronic blood loss may avert the need for blood. Ventricular function is depressed in animals when the Hct is less than 30%, however, oxygen extraction and central venous P_{O_2} remain normal until the Hct reaches 20% (Messmer, 1975).

In a chronically anemic patient, one must consider the benefit of transfusion in raising arterial oxygen content against the risk of overloading the already hyperkinetic circulation.

D. Red Cell Options

Red cells may be available as fresh or stored whole blood, packed red cells (PRBCs), or less commonly, as washed red cells. Because in most anemias the need is for red cells only, PRBCs are the treatment of choice. This minimizes exposure to donor histocompatibility antigens and to citrate. The volume of the transfusion can be adjusted to the individual patient's needs. The Hct of PRBCs is approximately 80%, so saline is usually added to facilitate administration, using small volumes in congestive heart disease and larger volumes in dehydration or hypovolemia.

Fresh whole blood may be indicated in actively bleeding, anemic animals with thrombocytopenia. For convenience, fresh whole blood is also used in cats and small dogs less than 5–6 kg. In ambulatory large animal practice, blood may be drawn from one animal in a herd and given immediately to another. Although fresh whole blood may supply some platelets, stored whole blood does not contain viable platelets after 12–24 hours. If equipment and personnel are available to make components, there is no value in storing whole blood. An exception

would be in the cat, for which there is a limit to the amount of blood (approximately 50 ml) that can be drawn from a donor. This volume can be collected in a standard human blood bag after citrate is removed by expelling it into a satellite bag. Enough citrate is left in the collection line for 50 ml, but the volume is too small for plasma removal by standard plasma extractors. Presently, the only way that feline PRBCs can be collected is in an open system. Occasionally for treatment of neonatal hemolytic disease, washed maternal red cells may be given. Most of the antibody can be removed with the plasma, allowing for survival of the remaining neonatal red cells along with the transfused maternal cells.

Products rarely used in animals but used in humans are leukocyte-poor red cells and frozen red cells. Leukocytes can be removed by centrifugation, washing, and filtration techniques to minimize febrile reactions that may occur in multiply transfused patients that have developed antibodies to histocompatibility antigens on donor white cells. Freezing of red cells is practiced for long-term storage of rare types, of autologous units, and for further removal of unwanted histocompatibility antigens. The process for freezing and preserving dog red cells has been described, but is somewhat expensive and time consuming, so is not practical for routine veterinary use (Contreras *et al.*, 1979). In addition, dog red cells are more prone to hemolysis during processing than are human cells. Freezing of red cells may be used for long-term storage from rare species of valuable animals where a donor may not be available when a transfusion is needed.

E. Administration of Packed Red Cells and Whole Blood

Various formulas have been derived to estimate the Hct increment expected in the recipient after red cell transfusion. This is assuming that transfused cells will survive normally and that the recipient has a normal blood volume. For example, if whole blood is given to a dehydrated animal over a 2- to 4-hour period and the Hct measured immediately afterward and 24 hours later, one will observe an initial rise in Hct followed by a further rise after 24 hours as fluid shifts from vasculature to interstitium. A secondary drop in Hct might occur in an animal given PRBCs as fluid is drawn into the vasculature by the effects of the red cells.

A simple rule of thumb is that a transfusion of 20 ml/kg of whole blood or 10 ml/kg of PRBCs will raise the Hct of the recipient by 10 points. For practical reasons, canine blood is drawn into human "unit" bags holding 450 ml of blood and stored as approximately 200-ml

"units" of PRBCs and 250-ml "units" of plasma. Because of this, most transfusions, except for dogs weighing less than 20 pounds are given in unit volumes, and the Hct is raised to a clinically safe range. For cats, the unit is often defined as 50 ml, because this is the maximum amount safely drawn from a 5- to 6-kg cat.

The intravenous route should be used for all transfusions except for those in tiny pups or kittens. This is true even if a cutdown is required for access. An indwelling catheter should be placed into the vein so the transfusion can be given slowly without the need to restrain the recipient. The needle size should be at least 20–22 gauge even in cats. Absorption from the intraperitoneal route is slow and inconsistent, whereas intramedullary transfusion allows 95% of the cells to enter the circulation within 5 minutes (Clark and Woodley, 1959).

The rate that blood is given depends upon several factors. In general, the transfusion should be completed within 4 hours according to standards set by the American Association of Blood Banks (Widmann, 1985) to prevent growth of bacteria in the event of contamination. In cases of acute massive hemorrhage, blood may be pumped in within minutes. The full 4 hours is used in cases of cardiac insufficiency, either primary or secondary to chronic anemia.

For routine transfusion in treatment of anemia, it is not necessary to warm the blood after taking it from the refrigerator. An unopened bag of saline may be stored in a 37°C waterbath and used to dilute PRBCs when a transfusion is needed. Warming of blood is indicated for neonatal transfusion and for administration to hypothermic patients. Hypothermia causes vasoconstriction and also interferes with platelet function (Valeri *et al.*, 1987). Warming should be done only with a waterbath with a thermostat or a standard blood warmer. If blood is heated to a temperature greater than 50°C, hemolysis will occur.

A blood administration set with a 170-μm filter is used to prevent clots from entering the recipient. The 40-μm microaggregate filters are not indicated for routine transfusion, and are only used in massive transfusions of stored blood where microaggregates of white cells or platelets can accumulate and contribute to pulmonary insufficiency.

F. Survival of Transfused Cells

Transfused compatible cells stored in standard citrate anticoagulants have a normal survival when transfused. Autologous or homologous transfused cells can be labeled, with ^{51}Cr or other isotopes, and persistence in the circulation studied (see Smith, this volume ["Erythrocytes"]). Compatible red cells are removed steadily over a period of

time up to the normal red cell life span for each species. This is because transfused blood contains cells of all ages. Transfused cells survive and undergo aging changes in the same manner as nontransfused cells. As they become senescent, IgG can be detected on the cell membrane (Garratty, 1987). The autologous antibody is directed against membrane antigens normally hidden by sialic acid on the surface of the red cell. Antibody-covered red cells are removed by macrophages predominantly in the spleen. It is uncertain whether splenectomy prolongs red cell life span in normal individuals. Two studies, one in rabbits and one in rats, provided conflicting results (Miescher, 1956; Belcher and Harriss, 1959).

II. Transfusion in Hemorrhagic Shock

Trauma with subsequent internal or external hemorrhage and anticipated or unanticipated hemorrhage in surgery are common emergencies. In many cases the amount of blood lost is unknown. Animals can withstand the loss of 25–30% of their total blood volume without replacement. Following a sudden loss of 20% of the blood volume, it takes 20–60 hours to restore lost volume through endogenous plasma replacement (Sohmer, 1979). No alterations in plasma albumin levels occur. After severe hemorrhage, levels of albumin and other serum proteins are decreased. Increased synthesis of albumin begins in approximately 48 hours, so initially preformed albumin moves to circulation from the interstitial space (Malt *et al.*, 1969). Erythropoietin levels begin to rise within about 6 hours. The Hct falls gradually over 2–3 days after acute blood loss. A linear fall in the Hct causes a logarithmic rise in erythropoietin levels (Adamson and Hillman, 1968).

After internal hemorrhage, the plasma and about 50% of the red cells will reenter the circulation after several hours. Blood in a body cavity initially clots, and then is defibrinated, leaving most of the red cells intact. Defibrinated blood has been transfused, both as autotransfusion from a body cavity and historically as cadaver blood (see Cotter, this volume ["History of Transfusion Medicine"], and Dodds, this volume ["Autologous Transfusion"]).

A. Clinical and Physiological Changes

Clinical manifestations of hemorrhagic shock become evident in dogs when about 30% of the blood volume is lost, especially if the dog is exercised. Initial signs are tachycardia, tachypnea, weak pulses, pale

mucous membranes, and hypotension. Compensatory changes that occur are increased rate and force of cardiac contraction, and increased oxygen extraction in the tissues. In dogs, sudden loss of 40% of the blood volume was fatal 50% of the time if untreated, whereas, all survived 50% blood loss if blood volume was maintained with bovine albumin (Rawson *et al.*, 1959; Moores *et al.*, 1983). Similarly, baboons survived an exchange transfusion with hetastarch replacement to a Hct of 15% (Levine *et al.*, 1990). These studies indicate that a significant percentage of blood volume can be lost so long as circulating volume is maintained.

When bleeding occurs into tissues one may underestimate actual losses. In human patients, 1.5 liters of blood are commonly lost with a closed femur fracture and 1.5–2 liters are lost with a fractured pelvis. Large amounts of blood may also be lost in gastrointestinal hemorrhage. Surgical blood loss may be greater than commonly expected. For example, in a series of human patients undergoing meniscectomy, the red cell volume decreased by an average of 9% in the first week after surgery (Davies and Fisher, 1958).

After acute hemorrhage, arterial pressure is maintained longer than is cardiac output. Release of catacholamines occurs quickly, after stimulation by baroreceptors in the aortic arch and the carotid sinus. α-Adrenergic receptors in arterioles of the skin, and receptors in splanchnic tissues respond to norepinephrine by causing vasoconstriction. Renal blood flow is maintained by reflex relaxation of afferent arterioles. Eventually, urine production decreases to maintain circulating volume. Prolonged hypovolemia and hypotension lead to decreased glomerular filtration and eventually to tubular necrosis. At the same time most of the circulation is diverted to vital organs such as the heart, adrenal glands, and the brain, which are low in adrenergic receptors. With acute hemorrhage, epinephrine causes a rapid increase in the number of neutrophils and platelets. The neutrophilia occurs because of mobilization of the marginated pool.

If shock is untreated, vasomotor failure ensues followed by acidosis and formation of microthrombosis from slowed blood flow. Factors contributing to decompensation are prostaglandins, endogenous opioids, and capillary obstruction by neutrophils and fibrin. Capillary permeability increases and endotoxins are released form damaged tissues. When this happens the osmotic gradient is reversed and blood volume cannot be effectively restored. In the lung, interstitial edema interferes with alveolar elasticity and impaired oxygenation may be unresponsive to oxygen therapy. Other changes that occur are centrilobular hepatic necrosis and erosions of the small intestinal mucosa from hypoxia.

Intestinal lesions allow further loss of fluid and protein into the lumen as well of entry of Gram-negative bacteria and endotoxins into the circulation.

B. Fluid Support

Survival from hypovolemic shock is dependent on the restoration of oxygen transport, blood volume, and blood flow. It has been said that the controversy over the use of crystalloids or colloids in critically ill patients has an emotional tenor that is inversely proportional to the supporting physiologic data (Shoemaker, 1982). All agree that the first approach to the acutely bleeding patient is to stop bleeding and restore circulating volume. Most clinicians prefer crystalloid replacement at least initially. Several studies of experimentally induced hemorrhagic shock have reported improved survival when crystalloids were compared to colloids (Collins *et al.*, 1973; Gaisford *et al.*, 1972). Although smaller volumes of colloids are required they were more likely to cause pulmonary edema because of leakage into the pulmonary tissues (Vergilio *et al.*, 1979). In a randomized human study and an experimental primate study, colloids were associated with a lower incidence of pulmonary edema (Rackow *et al.*, 1983; Gaisford *et al.*, 1972). In two additional studies, hemodynamic and oxygen transport responses were greater and more prolonged after infusion of colloids (Nees *et al.*, 1978; Dawidson *et al.*, 1979). Colloids improved hemodynamic and oxygen transport whereas crystalloids improved arterial pressure and peripheral resistance, but not flow and oxygen transport.

In most clinical situations outcomes have been comparable. In one randomized human study, resuscitation times were shorter and complications were fewer when approximately 25% of the fluid given was colloid, and the rest was crystalloid (Shoemaker *et al.*, 1981). It may be that in previously healthy patients, either solution is effective, but in elderly patients, or those with underlying heart failure or hepatic or renal insufficiency, it may be prudent to avoid large doses of crystalloids (Rackow *et al.*, 1983).

Albumin is used in human patients as a source of colloid, but species-specific albumin is not available for animals. Hetastarch performs as well as albumin, is less antigenic, and although expensive could be used in dogs and cats. Because crystalloids are readily available, inexpensive, and free of allergic reactions, they have remained the mainstay for initial resuscitation.

Recently the use of hypertonic saline (7%) has been evaluated (Rocha *et al.*, 1987). The advantage over isotonic crystalloids is that a small

volume of hypertonic saline more quickly draws fluid from the interstitial space to restore circulating volume. This can then be followed with additional support if needed.

Replacement of a volume of crystalloids equal to the volme of blood removed does not restore blood volume. For canine and feline patients, a commonly used initial dose of Ringer's lactate for the dog is 90 ml/kg over the first 1–2 hours, and 50 ml/kg for the cat. This represents replacement of one blood volume (Breznock and Strack, 1982). Adequacy of treatment is determined by overall clinical appearance of the patient, heart and respiratory rates, mucous membrane color, capillary refill time, pulse quality, Hct, and urine output. These are sometimes supplemented by serial measurements of arterial and central venous pressure, and in specialized centers, pulmonary wedge pressure. These parameters do not always correlate well as predictors of survival (Shoemaker *et al.*, 1979). An additional evaluation may be made of the ability of the circulatory system to accept a large rapid infusion of fluid without producing pulmonary edema. This assumes that hypovolemia is associated with normal venous compliance. Complete descriptions of treatment and monitoring techniques for humans, dogs, and cats are provided by Shoemaker (1982) and Hartsfield (1985).

The most valuable indicators of response are restoration of oxygen transport and tissue perfusion. Therapeutic goals are to raise mean arterial pressure, maintain central venous pressure below 12 cm H_2O to avoid pulmonary edema, and to maintain normal urine output, blood gases, and heart rate. The Hct is monitored as fluid is replaced, but is imprecise because massive hemorrhage can cause death with a minimal drop, and overhydration can lower the Hct leading to a false sense of urgency for red cell replacement. The decision as to whether red cells are needed is based upon a combination of known and estimated blood loss, response to initial fluid resuscitation, Hct, and total serum protein level.

C. Blood Transfusion

Assuming that initial attempts at resuscitation with crystalloid or colloid replacement have been unsuccessful, red cells must then be replaced. Historically, physicians and veterinarians have relied upon fresh or stored whole blood to replace losses. On first thought it seems logical that blood loss should be replaced with whole blood, but initial blood replacement should consist of PRBCs. The movement of albumin from the interstitial space to capillaries will replace up to 50% of lost blood volume without the need for supplementary plasma. In addition,

PRBCs have less adenine, citrate, sodium, ammonia, histocompatibility antigens, and antibodies than whole blood. Packed red cells are also active in increasing plasma volume. Blood was removed from dogs and equal-volume replacement using PRBCs was given to one group, and plasma was given to the other group. The red cells caused movement of interstitial plasma into the circulation, resulting in an equal increase in plasma volume in both groups (Valeri *et al.*, 1986a). Valeri and Altschule (1976) described a "missing blood syndrome" in soldiers resuscitated with crystalloid, colloid, and blood products. These patients had chronic hypovolemia not detectable by measurement of Hct or blood pressure. Large volumes of transfused red cells were required to raise the plasma volume and reexpand the peripheral circulation.

Rapid transfusion is necessary in life-threatening hemorrhagic shock. A large-bore needle is used with positive pressure achieved by wrapping a blood pressure cuff around the bag and blowing up the cuff. Because the Hct of PRBCs is approximately 80%, saline is added to achieve a more rapid flow. No fluid other than saline should be mixed with blood. The amount of blood to be given depends upon the amount of blood lost and patient's response. An initial dose of 10 ml/kg PRBCs can be given and the patient is then reassessed. If large volumes of fluids and PRBCs are given, one must watch for signs of volume overload such as distended or pulsating jugular veins, increased central venous pressure, or pulmonary edema.

In cases requiring massive transfusion—defined as administration of a total estimated blood volume of red cells within 24 hours—loss of clotting factors and platelets and effects of red cell storage lesions on oxygen delivery must be considered. Large reserves of platelets are present in the spleen of normal individuals. These can be released in times of increased need. It is common for platelet counts to be increased after moderate hemorrhage. In humans the platelet count generally remains over 100,000/μl until after transfusion of 18 units of stored blood, because platelets are not present in either stored whole blood or PRBCs (Counts *et al.*, 1979). Some of the drop in platelet count that occurs after large volumes of fluids or PRBCs is dilutional. Platelet replacement is indicated only if abnormal bleeding is occurring, not just because the count is low. As fluid shifts reestablish equilibrium, an increase in platelet count and serum proteins occurs. Decreased platelet counts after only moderate hemorrhage and transfusion might indicate the presence of diffuse intravascular coagulation (DIC) or some other underlying disease.

Clotting factor depletion may occur after massive transfusion with PRBCs and may require replacement either by fresh-frozen plasma

(FFP) or fresh whole blood. There is no advantage of stored whole blood over PRBCs as treatment for acute hemorrhage. A patient who has received a transfusion equivalent of one blood volume will retain approximately 25–30% of his original blood elements. Even without the additional consumption of clotting factors that occurs in hemorrhage, this begins to approach a level at which coagulation becomes impaired (Sohmer and Scott, 1982). Patients who receive a large volume of PRBCs may develop decreased levels of clotting factors, but the likelihood of severe depletion is low if coagulation was normal before the hemorrhage. In normal individuals, circulating levels of clotting factors are in excess of those needed, and additional extravascular distribution of most factors is present. Except for factors V, VIII, and fibrinogen, most of the factors are roughly the size of albumin and share its 40% intravascular and 60% extravascular distribution (Counts, 1984). A pool of factor VIII is bound to vascular endothelium and is released with stress or β-adrenergic stimulation (Wall *et al.*, 1980). It is difficult to predict whether replacement of clotting factors will be needed after massive transfusion. Monitoring of prothrombin time (PT) and activated partial thromboplastin time (APTT) or at least activated clotting time (ACT) is useful to determine need for, and efficacy of, replacement.

The transfusion of large volumes of cold blood extracts energy from the patient for heat production. Hypothermia also interferes with platelet function by inhibiting thromboxane B_2 production, decreases oxygen release in tissues, and interferes with metabolism of citrate (Valeri *et al.*, 1987). A reduction in bleeding time correlated well with warming of the patient, indicating that the platelet function defect is reversible. In situations of massive transfusions with stored PRBCs or whole blood, storage lesions such as decreased pH, 2,3-DPG, and ATP may interfere with oxygen delivery and red cell survival. The metabolism of citrate to bicarbonate tends to neutralize the drop in pH. Although 2,3-DPG can be regenerated and other by-products of transfusion cleared, this places an increased burden on the patient, who is not in an ideal position for such demands. The patient undergoing massive transfusion is at greatest risk of suffering adverse effects of a transfusion. A practical outline for management of human patients in hemorrhagic shock has been outlined with crystalloid and colloid infusions followed by PRBCs (Sohmer and Scott, 1982). Depending upon the PT, APTT, and platelet count, FFP or platelets are added. Occasionally cryoprecipitate may be needed if bleeding continues and fibrinogen levels remain low despite FFP administration. Significant bleeding is not likely unless the PT and APTT are at least 1.5 times that of the control.

Veterinarians have the advantage of being able to give fresh whole

blood when it is needed. Because of the requirement to test human blood for disease-causing organisms, fresh whole blood is not readily available. The major indication for fresh whole blood is in patients who need red cells, clotting factors, and platelets, such as patients with massive hemorrhage or with bleeding from severe liver disease, DIC, or thrombocytopenia.

III. Transfusion in Surgery

As discussed in Section II, PRBCs are indicated first when additional oxygen-carrying capacity is needed.

Historically, anesthesiologists and surgeons have felt that a Hct of 30% (hemoglobin of 10 gm/dl) was required for humans to undergo surgical procedures requiring general anesthesia (Levine *et al.*, 1990). At this level compensatory mechanisms are in place if the anemia has been present for more than a few days. Coronary vessels are maximally dilated and cardiac output is increased. If pulmonary and cardiac function are normal, a Hct of 20% or even less might be acceptable in patients under anesthesia when activity requirements are not of concern (Moore, 1974, Mollison *et al.*, 1987). If transfusion is needed for a surgical patient, and if it is not an emergency, it is better given near the end of the procedure. This allows for maximum conservation of transfused cells. (see Dodds, this volume ["Autologous Transfusion"], for discussion of intraoperative salvage). All of these methods minimize the need for homologous blood and make use of an immediately available and safe supply of autologous blood.

IV. Treatment of Hemolytic Anemia

Hemolysis, or abnormal shortening of the red cell life span beyond the ability of the marrow to compensate, may be caused by intrinsic red cell defects or by exogenous effects from substances in the plasma. Examples of intrinsic defects include inherited red cell enzyme abnormalities, such as pyruvate kinase deficiency in Basenji and beagle dogs and phosphofructokinase deficiency in English springer spaniels. Hemoglobinopathies such as sickle cell anemia and thalassemia are common causes of chronic hemolytic anemia in man. The only hemoglobinopathy reported in animals is congenital erythropoietic porphyria occurring rarely in cattle, cats, and pigs, usually not associated with significant anemia. If a compatible red cell transfusion is given to a dog

with an intrinsic red cell disorder, the survival of the transfused cells is normal. Hemolysis of exogenous cause is much more common in both man and animals than that caused by intrinsic red cell defects. Exogenous causes include infectious agents, antibodies, and toxins. Transfused cells are destroyed as readily as the recipient's own cells in cases of hemolytic anemia of exogenous cause, provided the cause is still present. The oldest cells in donor blood will be most susceptible to hemolysis, whereas reticulocytes are most resistant. The rate of destruction of transfused cells is also increased in the presence of alloantibodies.

A. Immune-Mediated Hemolytic Anemia

A common problem encountered by veterinary clinicians is acute fulminating immune-mediated hemolytic anemia (IMHA). This is one of the most common causes of anemia in dogs. It occurs equally in both sexes, with males more at risk under 2 years of age, and females more so after that (Switzer and Jain, 1981). Poodles, cocker spaniels, and old English sheep dogs may have a higher prevalence. The overall death rate is approximately 40%.

Complications of IMHA include cardiac, hepatic, and renal failure; hemoglobinemia; hemoglobinuria; icterus; pulmonary thromboembolism; and DIC (Klein *et al.*, 1989). Most are DAT positive, and some have spontaneous autoagglutination that is noted immediately when blood is drawn. Most dogs with autoagglutination and intravascular hemolysis have IgG plus complement on their red cells (Slappendel, 1979). The mortality in this form is quite high, 75–80%. Most dogs with IMHA, and almost all with the severe form, require one or more transfusions in addition to immunosuppressant therapy. Those that are strongly DAT positive or that have autoagglutination are difficult or impossible to type or cross match. These patients may be serologically incompatible with their own red cells and with those of most if not all donors. If they have been previously transfused, they may also have alloantibodies that may be impossible to detect. Sometimes difficulties in typing human patients with positive controls are overcome by incubating the patient's red cells at 45°C for 5–10 minutes and washing at the same temperature. This may elute the antibody and allow typing to proceed, but must be done cautiously to prevent hemolysis (Petz, 1982). This procedure has not been evaluated in dogs. Destruction of transfused cells may also be increased in the presence of fever or splenomegaly, possibly from increased activity of mononuclear phagocytes.

Although it is true that rapid hemolysis of transfused red cells adds to

morbidity, hypoxia from severe anemia may contribute even more to damage to heart, liver, and kidneys. Thus, transfusion should not be withheld in life-threatening IMHA. The decision as to when to transfuse must be made on an individual basis using more clinical parameters than simply the Hct to decide. Serial determinations of the Hct are helpful in determining the rate of progression or stabilization of the disease.

If a transfusion is needed, most human and canine patients will tolerate seemingly incompatible red cells with only infrequent reactions. Survival of the transfused cells is about equal to that of the patient's own cells and the net result is that the transfusion provides at least temporary benefit. Rarely in very severe IMHA, the transfused cells are destroyed rapidly (Petz, 1982). In humans patients with IMHA, the specificity of the autoantibody can sometimes be determined by elution techniques and testing the antibody against red cells of known phenotypes. In dogs this is not practical, so the identity of canine autoantibodies remains unknown.

About 50% of human patients, and a slightly smaller percentage of dogs with warm-antibody IMHA, will respond to immunosuppressive doses of corticosteroids during the first few days, although the Hct may not reach a normal level until weeks later. Dogs that fail to respond to corticosteroids may respond when stronger immunosuppressive drugs such as cyclophosphamide or azathiaprine are added.

B. Other Causes of Hemolytic Anemia

Table I lists causes of hemolytic anemia in animals. The general approach to treatment is to remove the cause when possible. Any drug currently being given is considered suspect. Those known to cause hemolysis must be discontinued along with any others that are not essential. Semimoist cat foods containing propylene glycol shorten red cell life span and should not be fed to any anemic cat. If transfusion is required in treatment of hemolytic anemia of any cause, the approach is the same as for IMHA. Discussion of specific therapy for the underlying cause is beyond the scope of this article.

V. Transfusion in Chronic Blood Loss

The limiting factor for continued erythropoiesis is iron. As small amounts of blood are lost over a period of time, the marrow responds initially with reticulocytosis and the Hct remains normal. Depending

TABLE I

CAUSES OF ACQUIRED HEMOLYTIC ANEMIA

Cause	Species[a]
Immune mediated	
Primary IMHA	Human, dog
Neonatal isoimmune hemolysis	Horse, human
Transfusion reactions	Human
Drugs and toxins	
Oxidants (Heinz body anemia/methemoglobinemia)	
Acetaminophen	Cat
Plants	
Red maple	Horse
Onions	Cattle, dog
Rape, kale	Cattle
Nitrates	Cattle
Propylene glycol	Cat
Snake venom	Dog
Zinc from foreign body (e.g., pennies, metal nuts)	Dog
Copper	Ruminants
Propylthiouracil	Cat
Heparin	Horse
Fresh water intoxication (or drowning)	Cattle
Infectious agents	
Leptospirosis	Ruminants
Bacillary hemoglobinuria	Cattle
Retroviruses	Cats, horse
Parasites	
Anaplasmosis	Cattle
Hemobartonellosis	Cat
Babesiosis	Dog, horse, cattle
Eperythrozoonosis	Pig
Malaria	Human
Malignancies	
Leukemia	Cat, human
Hemangiosarcoma	Dog
Hypophosphatemia	Cattle
Microangiopathic	
Thrombotic thromobcytopenic purpura	Human
Splenic torsion	Dog
Dirofilaria vena caval syndrome	Dog
Hypersplenism	Cat
Hypophosphatemia	Cattle

[a] Species most commonly affected.

on the quantity of iron stores, microcytic and hypochromic anemia will eventually develop. Neonatal animals with low iron stores become deficient if the diet is inadequate even in the absence of hemorrhage. Older animals with adequate stores are more resistant, and iron deficiency usually indicates that chronic blood loss has occurred. Occult blood loss occurs most frequently via the gastrointestinal tract from blood-sucking parasites, ulcers, or tumors. If the cause of the blood loss can be identified and removed, and if the patient is stable, iron replacement may prevent the need for transfusion. If the anemia is severe, especially if further losses are anticipated, transfusion with PRBC is indicated.

One source of chronic blood loss in both human and animal patients is iatrogenic from repeated blood sampling. In hospitalized human patients, it was reported that 50% of patients transfused had more than one unit of blood drawn for diagnostic testing (Smoller and Kruskall, 1986). This can also be a problem in small dogs and cats. Those drawing blood on a repeated basis should keep the Hct in mind and remember that less blood needs to be drawn from an anemic patient to obtain a needed volume of plasma or serum.

VI. Transfusion in Anemia from Lack of Red Cell Production

Anemia secondary to decreased production is most commonly associated with a primary bone marrow disease such as pure red cell aplasia, aplastic anemia, myelodysplasia, hematopoietic malignancy, or retroviral infection. Other causes include decreased erythropoietin from chronic renal disease, iron deficiency, immune-mediated destruction of red cell precursors, and anemia of chronic disease.

Anemia of chronic disease may be the most common form of anemia seen in hospitalized human or animal patients. Because the anemia is generally mild, it is often not commented upon. Pathogenesis includes shortened red cell life span by a hyperactive reticuloendothelial system. In addition, mobilization of iron from stores is decreased and the marrow fails to increase production to compensate for the loss. Cats are especially prone to anemia of chronic disease because of their normally short red cell life span and small blood volume to body weight ratio compared to other species (Breznock and Strack, 1982). In addition, many cats with various illnesses carry retroviruses such as feline leukemia or immunodeficiency viruses, which further suppress the marrow. Anemia of chronic disease usually requires no specific therapy and resolves if the underlying disease is corrected. Severe nonregenerative

anemia should not be attributed to anemia of chronic disease, but probably has another cause.

With decreased red cell production, anemia is slowly progressive and may be quite severe before clinical signs become evident. Although compensatory mechanisms allow these patients to function at a low Hct, periodic transfusion of PRBCs may be required for survival. In contrast to anemia caused by hemolysis or blood loss, anemia from lack of production generally has a poorer prognosis for recovery. Evaluation of bone marrow may provide clues to diagnosis as well as prognosis. If clinical signs of anemia such as tachycardia, tachypnea, and weakness are present at rest, transfusion is indicated. If the patient is asymptomatic, transfusion may be withheld. However, if the Hct drops below 10%, the animal is at significant risk for acute collapse should it become stressed. Because the Hct in animals is rarely raised into the normal range by transfusion, one need not be concerned that the transfusion will further suppress hematopoiesis. Also, because hemosiderosis from iron overload becomes a possibility after multiple transfusions, additional iron supplementation should be avoided. Cross matching becomes progressively more important in animals receiving multiple transfusions because the risk of sensitization is greater. Advances in production and use of genetically engineered hematopoietic growth factors is discussed by Donahue (this volume). Dogs and cats with anemia secondary to chronic renal failure often respond to treatment with recombinant human erythropoietin (Cowgill *et al.*, 1990).

VII. Transfusion of Plasma

A. Uses for Fresh-Frozen Plasma

Military personnel were treated with plasma during World War II for treatment of hemorrhagic shock. The packed red cells obtained as a by-product were not recognized as a valuable resource until some time later. As more was learned about the value of crystalloids and colloids, and the risks associated with plasma, its use as a volume expander declined. A conference at the National Institutes of Health in 1985 concluded that there was no justification for the use of plasma as a volume expander or nutritional supplement, although approximately 50% of fresh-frozen plasma transfusions in human hospitals were given for such reasons. As defined in American Association of Blood Banks (AABB) Standards (Widmann, 1985), FFP is separated from fresh whole blood by centrifugation and frozen to −30°C within 6 hours of

collection (see Authement, this volume). The major use for FFP both in animals and man is as a source of all clotting factors.

When reasons for canine FFP transfusions were categorized at Tufts University School of Veterinary Medicine in 1987 and 1988, the most common use was for treatment of DIC (Stone *et al.*, 1991). Dogs normally have short clotting times compared to man, and seem especially prone to develop DIC secondary to many serious illnesses. The most common causes of DIC include hemangiosarcoma, other malignancies, immune-mediated hemolytic anemia, gastric torsion, pancreatitis, heat stroke, liver disease, and infection, and were similar to those reported previously (Feldman *et al.*, 1981).

The laboratory diagnosis of DIC has been overemphasized and may be confusing (Westphal, 1984). Tests may change rapidly, and the classic picture of low platelets and fibrinogen, prolonged PT and PTT, and elevated fibrinogen degradation product (FDP) levels may not be present. Whenever the fibrinogen level is decreased below 80–100 mg/dl, the PT and PTT will be prolonged because fibrinogen is needed for fibrin formation in both tests. In one study in dogs, factor V was depleted but factor VIII was not (Feldman *et al.*, 1981). Although this differs from reports in humans and from experimental studies in dogs, it does not change the approach to treatment (Rabiner and Friedman, 1968). Thrombocytopenia is so consistent that a diagnosis of DIC cannot be certain without it. The platelet count returns to normal slowly over several days as DIC resolves. The fibrinogen level, however, rapidly returns to normal. FDPs are cleared primarily in the Kupffer cells of the liver and some are excreted in the urine. Elevated FDPs may sometimes be seen in hepatic or renal failure (Westphal, 1984). A discussion of treatment of DIC generates much of the same emotion as does the discussion of crystalloids versus colloids for treatment of shock.

By the time DIC is clinically evident, clotting factors are severely depleted. Once a patient is bleeding and fibrinogen is low, and the PT and PTT are prolonged, replacement of clotting factors with FFP is needed in addition to supportive care (Burns, 1987). Withholding FFP in the presence of significant ongoing hemorrhage is much like the old argument that nutritional support should be withheld from cancer patients because it "feeds" the tumor cells. In addition to coagulation factors, FFP contains antithrombin III (AT III), a potent inhibitor of thrombin formation. Replacement of AT III will help prevent further thrombosis. The endogenous production of AT III in dogs was shown to increase rapidly beginning within 6 hours of induction of DIC by endotoxin injection (Tanaka *et al.*, 1986). Production was able to surpass consumption after 1–2 days despite ongoing DIC. If one can eliminate

the underlying cause of DIC and if bleeding is not evident, it is not necessary to "treat" the laboratory abnormalities; they can simply be monitored along with the patient. In the most severe cases of DIC with excessive blood loss, replacement of red cells, clotting factors, and platelets may be needed. Here fresh whole blood is indicated initially with individual components added later as needed. For example, severe depletion of fibrinogen might best be treated with supplementary cryoprecipitate. Heparin is sometimes used in conjunction with FFP and controversy exists as to when and how it should be used. There is general agreement that heparin used alone in the bleeding patient is risky and unlikely to be successful. Heparin is a specific activator of AT III and requires its presence for its anticoagulant effect. If a patient continues to bleed or shows evidence of thrombosis 4 hours after therapy is begun for the underlying disease and the clotting factors are replaced, then heparin should be added. An exception to this rule would apply in cases in which the risk of development of DIC is very high. For example, dogs with severe heat stroke frequently develop DIC and might benefit from prophylactic heparin when thrombosis is beginning and coagulation defects are not yet measurable.

The recommended doses of heparin vary from 80 units/kg every 4–6 hours (Bick, 1985) to 5–10 units/kg intravenously or subcutaneously every 8 hours (Ruehl *et al.*, 1982). In general, smaller doses are preferred (Westphal, 1984). Despite the controversy, heparin has never been shown to increase survival in Gram-negative sepsis or other states in which DIC is encountered (Corrigan and Jordan, 1970). Harker (1974) argued that heparin aggravates bleeding and the resulting rise in fibrinogen level reflects defective fibrin stabilization, not improved hemostasis. Some have advocated incubating heparin with FFP before administering to activate antithrombin III to help prevent further thrombosis (Ruehl *et al.*, 1982). Other therapeutic agents such as inhibitors of fibrinolysis (ε-aminocaproic acid) and steroids are not likely to help and may be dangerous. Steroids slow the clearance of FDPs, bacteria, and immune complexes, but may be indicated in spetic shock from Gram-negative bacteria. Antithrombin III concentrates have recently been used with some success in human patients, but are not available for animals. In a thoughtful review of the topic, Feinstein (1982) stressed that beyond identification and removal of the underlying cause, treatment of DIC must be individualized and generalizations are difficult. Patients that are actively bleeding or require surgery should have factors replaced, and not receive heparin.

In hepatic failure, production of clotting factors, especially the vitamin K-dependent factors, is decreased. If ascites or edema is present,

the effectiveness of FFP is less because factors diffuse into the fluid. Vitamin K may be of benefit to these patients. FFP has been used in treatment of hemophilia A and von Willebrand's disease, although cryoprecipitate, if available, is more appropriate. In these patients, unless bleeding has been extensive, it is advantageous to avoid transfusing red cells. This will prevent or delay development of alloantibodies that could interfere with future transfusions.

B. Uses for Frozen Plasma

Frozen plasma (FP) is defined as that salvaged from whole blood stored in the refrigerator for longer than 6 hours before freezing, or FFP that has been stored from 1 to 5 years. Frozen plasma may be used as a source of stable clotting factors such as the vitamin K-dependent factors II, VII, IX, and X. Frozen plasma does not contain therapeutic levels of unstable factors such as VIII and V. Uses for FP include acute severe warfarin toxicity and hemophilia B. Plasma is sometimes used in animals as a source of albumin because species-specific albumin solutions are not available. When plasma was removed from normal dogs and replaced with crystalloid, hypoalbuminemic edema developed after 70% plasma dilution over a 3-hour period (Cervera and Moss, 1978). Albumin is difficult to supply in adequate amounts to reverse hypoalbuminemia. Approximately 60% of the total body albumin is located in the interstitial space and the concentration is in equilibrium with that in the plasma. If hypoalbuminemia develops, the calculated plasma albumin deficit represents only 40% of the whole-body deficit. If albumin is being lost via kidney or gastrointestinal tract, the albumin supplied by transfusion is quickly lost. Acute reversible hypoalbuminemia, such as might occur with burns, might respond well to plasma transfusion. Otherwise, hypoproteinemia is better treated by parenteral or enteral alimentation or other nutritional support. In catabolic states, infused albumin is metabolized as a calorie source. The additional sodium contained in plasma might also aggravate edema or ascites.

C. Cryoprecipitate

Cryoprecipitate is a concentrated solution of factor VIII, vWF, fibrinogen, and fibronectin, prepared from FFP by a slow thaw and separation of cryopoor plasma from the cryoprecipitate. It is used primarily to treat hemophilia A and von Willebrand's disease (vWD). Bleeding must be stopped as soon as possible in these animals, not

only to conserve red cells but also to minimize damage to joints. Other approaches to treatment of hemophilia A and vWD include deamino-8-D-arginine vasopressin (DDAVP) and danazol (a testosterone derivative), which have some efficacy of increasing levels of factor VIII and vWF. Thyroid hormone has also been helpful in decreasing bleeding episodes in some dogs with vWD. These approaches are described in more detail by Dodds (this volume ["Blood Substitutes"]). Obviously, drugs, inhibiting platelet function, especially aspirin, should be avoided in patients with coagulopathies. Although hypofibrinogenemia occurs in DIC, specific replacement of fibrinogen is usually not needed because adequate amounts are supplied in FFP. Cryoprecipitate has been used in human surgery as a source of tissue glue.

D. Administration of Plasma

In dogs, plasma can be given without concern for blood type because few red cells are present, and plasma donors are unlikely to have preformed antibodies if they have not been previously transfused. When plasma is separated from PRBCs, the majority of the citrate remains in the plasma so it is generally given over a 2 to 4 hour period. The dose is dependent on the degree of clotting factor deficit, so pre- and post-transfusion evaluations of the PT and APTT are indicated. An appropriate starting dose is 1 unit per 10–20 kg. If cryoprecipitate or FFP is to be given prior to surgery, the infusion should begin about 1 hour before anesthesia for maximum benefit. For example, factor VIII has a half-life of 6–8 hours so minimal benefit is obtained by giving it several hours before surgery.

VIII. Transfusion of Platelets

The major indication for platelet transfusion is to stop bleeding in patients with decreased platelet production or function. Platelet transfusions are given less frequently in animals than in man because of difficulties in preparing the needed volumes and because storage time is so short. Essentially no functional platelets are present in stored refrigerated whole blood or packed red cells. Even fresh whole blood will not contain adequate platelets in volumes commonly transfused to raise the platelet count of a thrombocytopenic animal. In humans patients, a standard platelet transfusion contains platelets concentrated from 6–8 units of blood. These are stored as platelet-rich plasma for up to 4–5 days at room temperature with constant rocking to prevent

aggregation. A platelet pack of 6 units is expected to raise the platelet count of the adult human recipient by approximately 60,000/μl immediately after the transfusion. Thus a 50-pound dog receiving 1 unit of fresh whole blood or platelet-rich plasma would be expected to have an increase of approximately 20,000/μl; 1 unit per 10 kg has been established as a reasonable starting dose. Further units can be given depending on the posttransfusion count and clinical status of the patient. A practical approach in bleeding thrombocytopenic animals is to supply at least some platelets as fresh whole blood. Red cell replacement has the added benefit of increasing platelet-to-platelet and platelet-to-endothelium interaction. Thus bleeding time may be improved in thrombocytopenic patients through red cell transfusion (Escolar *et al.*, 1988).

Most patients with a platelet count over 50,000/μl will tolerate surgery without the need for platelets (Tomasulo and Lenes, 1984). If unexpected bleeding occurs, platelets can be given, but the need is seldom urgent because bleeding from thrombocytopenia is not rapid. If bleeding is more severe it may indicate inadequate surgical hemostasis rather than effects of thrombocytopenia. Platelet transfusions are considered urgent only if bleeding is into the central nervous system or eye. Platelet transfusions may be ineffective in the presence of splenomegaly, sepsis, DIC, or hypothermia.

The adverse effects of local or systemic hypothermia on platelet function are often overlooked. Blood coagulation reactions are enzymatic and are dependent on temperature. Cooling of the skin causes reversible platelet dysfunction and prolongation of the bleeding time (Valeri *et al.*, 1987).

In the presence of idiopathic thrombocytopenic purpura (ITP) or alloimmunization from previous transfusions, platelet transfusions may be totally ineffective. In humans, HLA-matched platelets, obtained by apheresis, are used for patients that have developed alloantibodies. Platelet transfusions, are frequently ineffective even in nonimmunized hosts (Tomasulo and Lenes, 1984). The dog has been used as a model to study platelet function and survival. In one study, 18 of 21 (86%) dogs given up to eight platelet transfusions from a single, unrelated donor become immunized after an average of 2.3 transfusions (Slichter *et al.*, 1988). This alloimmunization resulted in a rapid destruction of transfused platelets that could not be prevented by pretreatment with prednisone, splenectomy, cyclophosphamide, or vincristine.

Those studies emphasized the highly immunogenic nature of platelet transfusions and the importance of posttransfusion monitoring.

In situations of platelet destruction such as ITP, the survival of

transfused platelets is a matter of minutes rather than 3–4 days as it may be in conditions of marrow failure. The spleen is where the major loss of platelets occurs. It is imprudent to assume that platelet transfusions, once administered, have benefited the patient. The platelet count should be run before and within 1 hour after the transfusion. If the count does not rise as expected, destruction of platelets may be occurring. Any platelet transfusion that does not result in an increase in platelet count immediately after the transfusion should be considered a failure (Tomasulo and Lenes, 1984). Animal studies have shown that an increment is necessary before the bleeding time is shortened (Roy and Djerassi, 1972).

Techniques have been developed for freezing human and canine platelets in dimethyl sulfoxide (Melaragno *et al.*, 1985; Valeri *et al.*, 1986b). The technology can be applied to other species, but is currently only done for research purposes.

IX. Transfusion of Granulocytes

Granulocyte transfusions were popular in the mid-1980s to treat human patients with sepsis and neutropenia. As new, improved-spectrum antibiotics have been developed, the use of granulocyte transfusions has declined. An additional reason for the decline was the difficulty in collecting adequate numbers of cells daily for each patient. Neutrophils are normally totally replaced at least twice daily, so large numbers (at least 10×10^{10}/day) must be given (Mollison *et al.*, 1987). Moreover, alloimmunization occurs rapidly even from previous whole blood transfusions, and pulmonary toxicity from sequestration of cells in the lungs is a problem (Westrick *et al.*, 1977). Some benefit from granulocyte transfusions occurs in septic newborn infants and transfusions are still used in this situation. In veterinary medicine, some use has been made in septic newborn foals, but otherwise use in animals has been restricted to research protocols. (For further discussion on white cell transfusions, see Weiss, this volume.)

X. Adverse Effects of Transfusions

Transfusion of blood or its components is generally a safe procedure in animals, particularly in the dog and cat. This discussion will primarily concern these species. The time of onset of side effects of transfusion varies from immediate, in a cat receiving as little of 2 ml of

incompatible blood, to delayed for years, as with transfusion-transmitted retroviral diseases. The severity of reactions varies from mild, transient pyrexia to death. Some of the reactions are preventable and some are not. The risks and benefits must be weighed in each situation in which transfusion is being considered. Because of an increasing concern about the dangers of human transfusions, especially concerning virus transmission, increasing emphasis is being placed on development of safe and effective alternatives to blood.

A. Hemolytic Reactions

When one thinks of a transfusion reaction, what usually comes to mind is an acute intravascular hemolytic catastrophe as might occur in an ABO-incompatible human transfusion. Fortunately in veterinary medicine, reactions are rare because of the low prevalence of naturally occurring isoantibodies. The group B cat is an exception because it is likely to have strong anti-A antibodies. If group A red cells are given to a group B cat, the cells are destroyed within minutes and the cat may also experience an acute complement-mediated shocklike reaction. In dogs, clinically significant naturally occurring isoantibodies are not a problem. Antibodies can develop and persist after an initial transfusion and become a problem if a second transfusion is given containing the same antigen.

Signs of an acute hemolytic reaction from incompatible red cells include acute fever, tachycardia, weakness, tremors, vomiting, and collapse. If signs occur the transfusion should be stopped and the line kept open with saline. A blood sample is drawn into EDTA and a portion is spun in a capillary tube to check for hemoglobinemia. A positive DAT can also be seen, but if all transfused cells have been destroyed, it could be negative. Both the recipient blood sample and any remaining donor blood in the bag should be saved for further testing for immunologic incompatibility, or for bacteriologic evaluation by gram stain and culture.

If intravascular hemolysis occurs, the potential for acute renal failure exists. Free hemoglobin was thought to cause renal damage, but antibody-coated red cell stroma is now known to cause renal vasoconstriction and ischemia. As little as 3 g of stroma from incompatible red cells can induce renal failure (Schmidt and Holland, 1967). In dogs, the transfusion of even autologous hemolyzed red cells leads to decreases in factors X, VIII, and fibrinogen and pulmonary artery thrombosis (Rabiner and Friedman, 1968). Presumably thromboplastic substances

in red cell membrane stroma are responsible (Shinowara, 1951). Antigen–antibody complexes and the decrease in blood pressure from complement, serotonin, and histamine predispose to development of DIC. Clearly, recognition of an acute hemolytic transfusion reaction calls for immediate action, not just repetition of lab tests or monitoring.

The major aims of treatment are to maintain blood pressure and renal blood flow and prevent DIC. Fluid therapy is supplemented with furosemide. Osmotic diuretics such as mannitol can also be used to increase urine output, but do not significantly increase renal blood flow (Peschel, 1986). If pressor agents, are needed, dopamine hydrochloride is most effective because it dilates renal vasculature while increasing cardiac output. Because the risk of DIC is high, prophylactic heparin should be given immediately as an initial loading dose and continued for approximately 6 hours. Heparin is contraindicated if the underlying reason for the transfusion pertained to trauma, surgery, or hemorrhage. A particularly difficult problem is an acute hemolytic reaction in a patient under anesthesia, wherein the only signs might be unexpected hypotension or increased capillary oozing of blood. If a reaction is suspected, the workup is the same as for the awake patient.

Although hemolytic reactions are most serious, they also are the most rare reactions. These should be preventable by pretransfusion testing, but the most common reasons for human acute hemolytic reactions are administrative errors, such as mixups in patient identification, rather than laboratory errors. There is no evidence that pretreating the recipient with antihistamines or steroids can prevent or suppress a hemolytic transfusion reaction (Schmidt, 1982).

When red cells are transfused to sensitized individuals or when sensitization occurs after the first transfusion, the most common reaction is delayed hemolysis. In a delayed reaction, hemolysis may occur from a few days to 2 weeks later as antibodies are produced. This occurs most frequently in previously transfused patients that have developed an antibody titer too low to be detected on cross match. The transfusion initiates a secondary immune response with a rapid rise in antibody. This may be subclinical and not noticeable unless transfused cells were labeled and survival measured. If destruction is more rapid, icterus may develop and the beneficial effect of the transfusion is shortened. The DAT should be checked whenever the drop in Hct is more rapid than expected because antibody-coated transfused cells will be positive. This effect is difficult to appreciate in patients transfused for treatment of immune-mediated hemolytic anemia. These patients are DAT-positive pretransfusion and often destroy transfused cells rapidly. The

antibody may not be detectable in the patient's serum because it is all absorbed onto the red cells. Typically, antibody becomes detectable at peak levels 10–15 days after the transfusions (Mollison *et al.*, 1987).

Hemolytic reactions can occur from nonimmunologic causes if blood is hemolyzed prior to transfusion. Causes include (1) overheating above 50°C, (2) freezing if refrigerator temperature drops too low, (3) mixing with hypotonic solutions such as 5% dextrose in water, (4) mechanical trauma to red cells during drawing or administration of blood, and (5) contamination with hemolytic bacteria (Peschel, 1986).

B. Fever and Allergic Reactions

The development of fever during transfusion could indicate bacterial contamination of the blood, however, this is rare. If blood is contaminated, the reaction of the recipient is usually immediate and severe. In addition to fever, trembling and acute collapse usually occur. If such a reaction occurs, the transfusion is stopped immediately and the remaining contents of the bag are Gram stained and cultured. Intravenous antibiotics and treatment for shock are instituted immediately.

Most febrile reactions are mild and not related to infection. Previously transfused animals become sensitized to histocompatibility antigens of donors and react with fever during or after the transfusion. Despite the fever, the patient usually is clinically better after the transfusion than before. Sometimes severely anemic cats are presented to the hospital with normal or subnormal temperatures and develop a fever 8–24 hours after transfusion. In some of these cats, the fever is caused by the underlying disease, and this becomes evident after the cat's condition is improved by transfusion. When a fever develops after transfusion but the patient appears stable or improved, it is often advantageous to monitor without treatment for 24 hours because nonspecific transfusion-induced fevers often spontaneously resolve within that time.

Allergic reactions are rare and have been seen more in canine and human patients than in other species. The most common manifestations are urticaria and angioneurotic edema. The cause is probably donor plasma antigens to which the recipient is sensitive. This is one reason why donors should not be receiving medications at the time of donation. In a series of atopic human patients known to be sensitive to such common allergens as pollen, dust, milk, and eggs, the transfusion of pooled serum was almost always followed by moderate to severe urticaria, whereas normal subjects rarely had any reaction (Maunsell, 1944). If an allergic reaction occurs, the transfusion is stopped and

intravenous antihistamines such as diphenhydramine given. If the reaction is severe with any sign of laryngeal or bronchopulmonary involvement, corticosteroids or even epinephrine (0.1 ml of 1:1000 solution intravenously to dog or cat) can be given as well. Most typically, the reaction is mild and subsides quickly, in which case the transfusion may be continued. It can be argued that it is dangerous to continue to deliver a proved allergen, but there are no clinical reports to support that contention.

C. Circulatory Overload

Circulatory overload is most likely to occur in cats or small dogs if large volumes of blood are given to a normovolemic patient, or if a standard volume is given to an animal with underlying cardiac insufficiency. Anemic animals susceptible to circulatory overload should always receive slowly infused PRBCs, not whole blood. Chronically anemic animals are more at risk because cardiac hypertrophy or dilatation is often present from long-term increased cardiac output. By the time the animal requires a transfusion, the cardiac compensatory mechanisms are already stressed maximally. The additional blood volume may produce pulmonary edema. Signs include, cough, cyanosis, dyspnea, and moist lung sounds. If overload is suspected, the transfusion is stopped and furosemide and oxygen are administered.

D. Electrolyte and Acid–Base Abnormalities

Citrate is an ideal anticoagulant from the standpoint of safety, because it is metabolized in the liver within minutes of administration. Experimentally, citrate toxicity is hard to produce. The equivalent dose of citrate to that in 7 liters of citrated blood was given without reaction to an adult man in 90 minutes (Nakasone *et al.*, 1954). When toxic changes were produced in dogs, signs included EKG changes: prolongation of QT, pulsus alternans, and depression of P and T waves. Muscle tremors occurred as well. If additional doses were given, ventricular fibrillation occurred. Diagnosis cannot be made by measuring serum calcium because only the ionized fraction is decreased. Animals with portosystemic shunts, severe liver disease, or hypothermia will metabolize citrate more slowly than normal and are more prone to toxicity. Components with the most citrate are FFP, PRP, and whole blood. Packed red cells are low in citrate and unlikely to cause toxicity. In most cases, citrate toxicity is reversible if the transfusion is stopped for 5–10 minutes and restarted at a lower rate. Injection of calcium gluco-

nate reverses the effect but is rarely needed. The alkaline by-product of citrate metabolism offsets the acidosis induced by stored blood (Collins *et al.*, 1971). Because of this, acidosis is rare after transfusion of stored blood.

Potassium toxicity is primarily a concern in humans and horses receiving stored blood. Animals other than horses have potassium concentrations in red cells equal to that in plasma, so hyperkalemia is not a concern. Even in man, clinically significant hyperkalemia is rare and occurs less frequently than hypokalemia. Increased potassium predisposes to citrate toxicity because potassium and citrate both adversely affect cardiac rhythm and function. Ammonia levels rise in stored blood and may cause a problem in patients with liver failure.

E. Miscellaneous Reactions

Much has been written about the dangers to pulmonary function caused by microaggregates after massive transfusions. These consist of platelet, white cell, and red cell stromal debris, which increase after the first week of storage. These can be removed by the use of a 40-μm filter. Recent clinical studies indicate that the problem of microaggregates has been overemphasized (Seldon, 1979). Routine use of millipore filters is not only unnecessary, but may be detrimental because rapid transfusion cannot be delivered through such a small filter.

Hemosiderosis is a rare complication that can occur in a patient that has received numerous transfusions and is not bleeding. Packed red cells contain 1 mg Fe/ml, and the normal daily excretion of iron from the body is only about 1 mg. Initially, excess iron is stored in reticuloendothelial cells and does not cause clinical signs. Once transferrin is saturated, additional iron stored in hepatic parenchymal cells may damage the liver (Marcus and Huehns, 1985). Few animals receive multiple transfusions, but hematinic therapy should be avoided in animals that are being transfused, unless iron deficiency is present.

Immunological effects of transfusions are not limited to alloimmunization of recipients. The first suspicion that transfusion might be immunosuppressive was related to the observation of improved renal allograft survival in transfused patients (Opelz and Terasaki, 1978). More recently, human cancer patients that were transfused at the time of tumor resection had shorter survival times than patients of the same stage of disease that were not transfused (Blumberg and Heal, 1987). In addition, those patients that received whole blood did worse than those receiving packed red cells. It has been suggested that plasma contains histocompatibility antigens, antiidiotypic antibodies, or other immunoregulatory substances that could cause immunosuppression. In an

animal model of blood transfusion and tumor recurrence, plasma was the component associated with the highest rate of tumor growth (Horimi *et al.*, 1983). In addition to immune suppression, transfusion, especially with stored red cells, has been thought to cause reticuloendothelial blockade. The fixed macrophages in the spleen and liver engulf red cells and then are unable to phagocytize organisms. This phenomenon is not likely to be clinically significant.

Most human deaths caused by transfusions result from transmission of infectious diseases. Examples of transfusion-transmitted infections include AIDS and HTLV-I infection, hepatitis, malaria, babesiosis, Chagas' disease, cytomegalovirus and Epstein–Barr virus infections, syphilis and, rarely, other infections such as brucellosis, salmonellosis, toxoplasmosis, trypanosomiasis, and filariasis. These agents share common properties of prolonged persistence in the blood of healthy carriers, long incubation periods, and stability in stored blood. Ideally, testing should be done for all agents, but accurate tests are only available for a few, and some carriers are not detectable.

The same or related organisms can be expected to be transmitted from animal to animal by transfusion. Retroviruses are of major importance in cats, cattle, and horses. The cat is most at risk, with up to 5 % of random cats carrying either feline leukemia virus or feline immunodeficiency virus. Although horses and cattle are less frequently transfused than are dogs and cats, bovine leukemia virus and equine anemia virus are relatively prevalent. Depending on geographic prevalence of disease, dogs are at risk for transfusion transmitted babesiosis, ehrlichiosis, and the microfilarial stage of dirofilariasis. In addition to retroviruses, cats may carry the coronavirus that causes feline infectious peritonitis, although transmission by blood has not been proved. Hemobartonellosis is another disease that increases the risk of transfusion of cats.

XI. Therapeutic Apheresis

The use of apheresis to obtain single-donor platelets or plasma for administration to a patient has been described by Authement (this volume). The procedure has also been used to remove abnormal components from the blood, either cells or plasma. Examples of clinical indications are removal of very large numbers of blasts in acute leukemia (leukopheresis); excess red cells in polycythemia vera; abnormal antibodies, lipids, or proteins (plasmapheresis); and excess platelets in thrombocythemia (plateletpheresis).

Several machines are available that collect the desired component by

continuous-flow separation and centrifugation or filtration. These are most efficient for rapid processing of large volumes of blood. Because the extracorporial volume is relatively large, these machines are not readily adaptable for animals under 10–15 kg. If equipment is unavailable or if the patient is very small, apheresis may be done manually by removing a volume of blood, separating the component by centrifugation, and returning the rest to the animal. Because the volume exchanged in a standard protocol is usually one plasma volume, manual apheresis requires removal of approximately four samples of 20 ml/kg each. Each exchange results in a progressive decrease in removal of the abnormal substance, because of dilution of plasma by replacement fluid.

Plasmapheresis has been shown to be of definite value in treatment of hyperviscosity syndrome both in human and canine patients (Nose *et al.*, 1983; Klausner *et al.*, 1986). Because the paraprotein is confined primarily to the vascular space and the rate of synthesis is slow, removal every few weeks may be the only treatment required early in the disease. In certain immune-mediated diseases in which antibody production is more rapid, removal results in temporary improvement, but rapid rebound of antibody systhesis requires follow-up immunosuppressive therapy. Immune-mediated diseases most likely to respond to plasmapheresis are myasthenia gravis, Guillian–Barré syndrome, immune-mediated hemolytic anemia, and thrombocytopenia. Plasmapheresis is the treatment of choice for thrombotic thrombocytopenic purpura in human patients; this condition has not been reported in animals. Therapeutic apheresis has been used clinically by this author and others in small numbers of dogs (Matus *et al.*, 1985; Klausner *et al.*, 1986).

The amount of the specific pathologic substance to be removed depends upon the volume of plasma exchanged and the frequency of the procedure. If a large volume of plasma is removed, clotting factors and antithrombin III are removed as well. Despite this, very few thrombotic or hemorrhagic complications have been reported (Mollison *et al.*, 1987). Plasma levels of immunoglobulins and albumin are decreased, and result in immunosuppression and decreased colloid osmotic pressure. Because species-specific albumin is not available for animals, the replacement fluid should contain some colloid or homologous plasma.

Clinical applications for apheresis in humans and animals are continuing to expand into diseases that are difficult to treat by other means. As equipment becomes more available in academic veterinary centers, the procedure should become more available for veterinary patients as well.

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