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**Hypertonic Saline Dextran: Immunological and
Hematological Effects Following Single and Multiple
Doses in Dogs and Pigs**

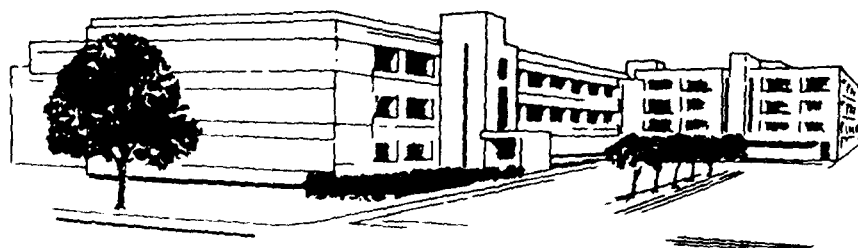
A.F. Kilani,
J.J. Summary
and M.A. Dubick

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Hypertonic Saline Dextran: Immunological and Hematological Effects Following Single and Multiple Dose in Dogs and Pigs, A.F. Kilani et.

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At the doses used, there were no adverse effects on the immune or coagulation systems. Differences in the past studies may be due to different doses of dextrans used, high molecular weight contaminants and higher degrees of branching.

HYPERTONIC SALINE DEXTRAN:
IMMUNOLOGICAL AND HEMATOLOGICAL EFFECTS FOLLOWING
SINGLE AND MULTIPLE DOSES IN DOGS AND PIGS

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PREFACE

The research effort reported here served as a thesis submitted by the senior author to the faculty of the Center for Advanced Medical Technology at San Francisco State University in partial fulfillment of the requirements for a Master of Science degree in Clinical Science. This paper adheres to the format requirements established by San Francisco State University.

ABSTRACT

Dextran has been used in resuscitation from hypovolemia and trauma for the past 40 years. This project examined the effects of multiple doses of 20 ml/kg body weight Hypertonic Saline Dextran-70 on the immune system in dogs and the effects of a single dose of 4 ml/kg body weight on blood aggregation and clot formation in pigs.

The project focused on the use of a new dextran formula, Hypertonic Saline Dextran, HSD (6% Dextran-70 in 7.5% NaCl). Immunoprecipitation experiments using specimens from 24 Beagle dogs revealed no significant changes in the levels of IgG, IgM, and C3 protein following daily infusions of HSD at doses up to five times the recommended dose.

Following a single HSD infusion of the recommended dose of 4 ml/kg body weight, specimens from 21 pigs revealed no significant changes in prothrombin time (PT) and activated partial thromboplastin time (APTT) throughout the testing period of 168 hours. Following HSD infusion in 7 of these pigs, platelet aggregation was tested using the agonists ADP, collagen, epinephrine and ristocetin. ADP was the only agonist

that caused a slight increase in the percent platelet aggregation. The platelet aggregation increased 17% in the hemorrhaged pigs between 0 and 168 hours. Bleeding times analyzed for 4 of the pigs revealed no significant effects following HSD infusion.

At the doses used, there were no adverse effects on the immune or coagulation systems. Differences in the past studies may be due to different doses of dextrans used, high molecular weight contaminants and higher degrees of branching.

Hypertonic Saline Dextran: Immunological and
Hematological Effects Following Singles and Multiple
Doses -- Kilani et al.

INTRODUCTION

Acute hemorrhage counts for almost 90% of deaths in military and civilian trauma (35). While several resuscitative fluids have been used to manage hemorrhagic shock (3,9,14,15,24,35,40,42), there has always been a need for a formula that can be used in emergency situations in non-hospital settings to resuscitate from hypovolemic shock. A new formulation, Hypertonic Saline Dextran-HSD (6% Dextran-70/7.5% NaCl), is the focus of this project.

Dextrans have been used in resuscitation for more than 40 years (9,40,42). These macromolecules have been suspended in electrolyte solutions to achieve the longest duration in circulation and the maximum efficacy in volume replacement (15,24,40).

There are reports of hemorrhagic complications as a result of dextran administration (4). Large doses of dextran (>1.5 g/kg body weight) with average molecular weights of >60,000 daltons were reported to prolong bleeding and clotting times following infusion (4,27).

Prolonged bleeding was confirmed in about 5% of normal dextran recipients (2). About 46% of individuals infused with a liter of dextran developed increased bleeding time (6).

Dextran may interfere with several coagulation mechanisms. Fibrinogen and factor VIII levels decrease more than could be attributable to simple hemodilution by the resuscitation fluid; the dextran is reported to accelerate the rate of the action of thrombin in the conversion of fibrinogen to fibrin (40).

Another potential risk in using dextrans is the immune reaction to the dextran moiety. Dextran antigenicity is dependent on its molecular weight (43). Low molecular weight clinical dextrans, e.g. D-70 and D-40, do not induce immune reactions (43). There have been a few reports of severe anaphylactoid reactions (18,20-22,32) to high molecular weight dextrans (>100,000 daltons) or those with higher degrees of branching (22,29).

In phase III clinical trials, it was determined that the therapeutic dose for HSD is 4 ml/kg body weight (31). This project addresses some potentially adverse effects such as disturbances in bleeding time, platelet aggregation, prothrombin time, activated

partial thromboplastin time,as well as anaphylactoid
reactions following administration of HSD.

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Literature Review

Resuscitative Fluids:

Acute blood loss is a major cause of shock and probably death in freeway accidents, on battle fields, and in other acute bleeding cases. Ninety percent of deaths in warfare are due to hemorrhage and cardiac arrest (23,35). The pre-hospital use of resuscitative fluids can save lives. Although the use of conventional isotonic fluids can be helpful in the severely injured, it might not be applicable in emergency situations because of the difficulty of insertion of large-bore catheters in the patient's vein (24). There is an urgent need for a resuscitative fluid that can be used in smaller amounts and sustain its effect of volume replacement until the patient can be moved to a fixed clinic (24).

Plasma has been used widely as a blood volume substitute. It has provided hypovolemic recipients with volume, coagulation factors, and albumin; however, it is not as commonly used anymore because of the risk of transmission of blood-borne diseases (42).

Over the past 60 years, many polymers, e.g., hydroxyethyl starch (HES), acacia, pectin, dextran, plastic, and gelatin, suspended in electrolyte solutions, have been tested as acellular resuscitative fluids for the management of hypovolemia and trauma (40). Gum Arabic, a polysaccharide, was used during World War I (14). Dextran was used extensively during the Korean War but was not without problems. There were military autopsy reports of anaphylactoid complications (33) and precipitates of high molecular weight dextrans in the reticuloendothelial organs (15).

These synthetic resuscitative fluids were favored over isotonic crystalloid solutions, e.g. normal saline, Ringer's lactate, etc., which must be administered in 2-4 times the volume of lost plasma and consequently cause tissue edema (16). Velasco et al. (53) reported 100% survival of anesthetized dogs infused with hypertonic 7.5% NaCl in a volume equal to 10% of the lost blood volume compared to 0% survival in a group of dogs infused with normal saline. Electrolyte solutions had the advantage of being non-reactive and of having relatively long shelf lives;

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but, compared to other resuscitative fluids, they have the shortest duration in circulation (42). Electrolyte solutions were beneficial when used with synthetic plasma volume expanders which are considered more effective in long term blood component therapy (15).

Preparation of Dextrans:

Dextrans are made up of glucose residues linked in 1,6 linkages with some 1,2,1,3, and 1,4 branching linkages (18,51). Dextrans are considered a storage polysaccharide in yeasts and bacteria (51). During the growth of the Streptococcus strain Leuconostoc mesenteroides B512 NRRL in a sucrose-containing medium, the enzyme dextran sucrose manufactures native dextrans of several million daltons molecular weight (4,14,40,43). The preparation of clinical dextrans involves the acid hydrolysis of the native large dextran into smaller molecules (4,43), or alteration of the bacterial strain to narrow the range of molecular weights produced (9). Earlier allergic reactions to dextrans may have been due to the molecular weights and different molecular structures of the dextrans and the different bacterial strains used (15). Now, the most commonly used clinical dextrans are Dextran-70 (D-70)

and Dextran-40 (D-40), which have average molecular weights of 70,000 and 40,000 daltons, respectively (2,49). Pharmacia preparations of D-70 have more than 90% of the molecules within the range of 25,000 to 125,000 daltons (7); similarly, D-40 preparations have 90% or more of the molecules between 10,000 and 80,000 daltons (7).

Clinical Aspects of the Use of Resuscitative Fluids:

For safety, it is required that all plasma volume expanders in resuscitative fluids meet high purity standards and when metabolized they should completely break down or be excreted by the body (42). Dextran is an expander of choice because molecules smaller than 50,000 daltons are filtered passively through the kidneys (7) and what remains in the body is metabolized to CO_2 and H_2O (15). The spleen can remove dextrans from circulation and parenchymal cells can metabolize the polymers (42). Temporary tissue storage of dextran causes reversible histological alterations while the uptake of some other expanders, e.g., polyvinylpyrrolidone can render some organs dysfunctional (40). Hydroxyethyl starch (HES) causes storage problems in the liver and kidneys and is

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present in plasma months after infusion. Multiple infusions of HES preparations which contain traces of ethylene glycol led to nephrotoxicity and hepatic toxicity (33). Ringer's Lactate might cause immunological and metabolic complications in burned patients due to the high concentration of lactate and the lactate's slow clearance rate (11). Gelatins, the least tolerated among expanders, cause cell aggregation and anaphylactoid reactions (33). The use of dextrans is not risk-free. D-70 and D-40 are highly oncotic solutions. When D-40 is infused in dehydrated patients, it must be accompanied by water and electrolytes; otherwise, the situation will be aggravated (15).

Rheological Effects of Dextran:

Dextran affects blood viscosity (12), platelet function, coagulation factors and hemostasis. Because dextrans prolong bleeding, they have been used to prevent deep venous thrombosis following surgery (4,10,42). Alexander et al. (2) reported that dextrans tend to increase erythrocyte sedimentation rate, to coat erythrocytes, platelets and vascular endothelium, to change platelet electromobility and factor 3 release

and to alter renal tubular function. Adsorption of dextran onto red cell surfaces causes aggregation by bridging forces (30,34) which overcome the repulsion between red cells due to the negative charges exerted by sialic acids (28). D-70 has been reported to aggregate platelets and erythrocytes (2,26,37) while D-40 is reported to disaggregate erythrocytes and decrease platelet spreading (49).

Lim et al. (32) considered the dilution effects of the infused fluids in the improvement of blood flow. They measured the blood viscosity of subjects before and after infusion with Macrodex (6% Dextran-70 in saline) and Rheomacrodex (10% Dextran-40 in saline). Viscosities increased following infusion with D-70 and decreased following infusion with D-40 (8,32). In general, dextran tends to adsorb to the surfaces of large proteins like factor VIII (4) resulting in symptoms that resemble von Willebrand's disease (1,40). Testing the levels of fibrinogen and -macroglobulin, large plasma molecules, Aberg et al. (1) found that the concentration promptly decreased immediately after dextran infusion. They also found that injection of a concentrate of factor VIII could reverse the

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prolongation in bleeding time (1,42). In vitro, when human blood is incubated with HSD, dextran increases platelet aggregability and leads to formation of small platelet aggregates which disperse to yield platelets that are partially refractory to aggregating stimuli (10). It also prolongs prothrombin time (PT) while the activated partial thromboplastin time (APTT) does not change (23). The dextran may modify the action of thrombin to convert fibrinogen to fibrin and the resulting weak fibrin structure may cause prolonged bleeding (4,54). A decrease in platelet counts was observed after infusion of more than a liter of clinical dextrans (55).

Measuring both PT and APTT in the current study allowed the monitoring of both intrinsic and extrinsic coagulation pathways (see Appendices B & C). Although these two pathways act independently, they interact through factor X which when activated to Xa acts together with calcium ions, factor V and platelet factor 3 to convert prothrombin into thrombin. Evaluating both systems gives a more complete picture of the overall coagulation response (23,39,47).

No effects on blood typing and cross matching were observed following in vitro incubation of human blood with HSD (41). No phlebitis or blood typing and cross-matching problems were reported following HSD infusion (24). Hemoglobin loss from red blood cells was reduced by as much as 80% in the presence of dextran. A resealing process in which dextrans of at least 2,000 dalton molecular weight reseal the inner membrane of the red blood cells after the loss of 20% or more of cellular hemoglobin was proposed as the mechanism (56).

Anaphylactoid Reactions Caused by Dextran:

Dextran use has been limited due to concerns of anaphylactoid reactions similar to those experienced in the combat casualties in the Korean war (33). Like many other synthetic plasma volume expanders, it may cause adverse reactions, e.g., skin rashes, urticaria, and severe circulatory shock (18,20,33) which mimic the clinical picture of anaphylaxis (20,21,42,45). In anaphylaxis, IgE antibodies are responsible for the clinical symptoms (42,46); in anaphylactoid reactions, IgG antibodies (especially IgG₂) and some IgA antibodies are the mediators (42) while IgM antibodies levels are low and IgD antibodies are absent (43).

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The immune response induced by dextran was described by Hedin and Richter (20) to be an immune complex-mediated (Type III) allergy. This implies that IgG, IgM and the complement system are involved. In type III allergy, the insoluble antigen-antibody complexes lead to complement fixation and the release of anaphylatoxins as split products of C3 and C5 (46). The anaphylatoxins cause the release of cell mediators that attract polymorphonuclear cells which phagocytize the immune complexes and release enzymes and mediators that inflame the surrounding tissues (46). Antibodies of the IgE class were not demonstrable in patients who developed anaphylactic shock due to dextran infusion (42,43). C3 was analyzed in this project because it is the most important indicator of the complement cascade activation and it increases during the acute phase immune response (21). Dextran can also activate the alternative pathway of complement in vitro (43).

In guinea pigs no antibodies are produced following injection of native dextran (15); but it is a B-cell mitogen in mice (18). It appears that the immunogenicity of dextran is molecular weight-dependent in man (15,29) and in mice (25). Clinical dextrans,

D-70 and D-40, do not induce immune responses (13,15,18,33).

Although there are several reports of dextran-induced anaphylactoid reactions (DIAR), IgE class antibody concentrations as measured by passive cutaneous anaphylaxis, the radioallergosorbent technique, and the modified radioactive red cell-linked antigen-antiglobulin reaction in monkeys were normal after using clinical dextran (19,20,22,42,43).

Anti-dextran antibodies present in the blood are probably due to previous exposures to serologically active dextrans in food, and on bacteria in the gastrointestinal tract (18,40). Cross reactivity was reported between dextran and polysaccharides from some microorganisms such as Micrococci, Salmonella typhi, Klebsiella, Lactobacilli and other Streptococci (18,20,21).

To minimize the risk of anaphylactoid reactions, it is important to supervise the infusion of the first 50 ml of clinical dextran because this is when anaphylactoid reactions are most likely to occur (42,50). An effective preventive measure against anaphylactoid reactions has been the injection of

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hypovolemic patients with 20 mL of a hapten dextran, Dextran I, (Promit 15%, Pharmacia AB) (20,42) two minutes before the clinical dextran injection (43). The average molecular weight of this hapten is 1,000 daltons (21,38,40). These monovalent haptens in Dextran I work by binding to the dextran specific antibody reactive sites, which blocks the potentially reactive dextran molecules from binding to specific antibodies (42,43).

The incidence of DIAR varies from 0.03% to 4.7% depending on the molecular weight of the dextran given (18,20,21,33). The incidence of these reactions has dropped recently due to the improved purity of dextran preparations (less branched and high molecular weight contaminants). Summary et al. (52) stated that there have been very few reports of immunologic responses to dextrans with an average molecular weight of less than 100,000 daltons.

Proposed New Resuscitative Fluid:

HSD has an advantage over many other resuscitation formulas because the volumes needed for infusion are reduced. Three volumes of Ringer's lactate (57) or one half to one volume of Macrodex or Rheomacrodex are

needed to replace each lost volume of blood (41) while only one fourth volume of HSD can replace each volume of lost blood (24,36). The effect of HSD is sustained for a duration longer than that of any other formula and the side effects are minimal if doses do not exceed the maximal suggested dose of 1.5 g/day (42).

Anaphylactoid reactions and prolonged bleeding and hemostatic abnormalities reported earlier are probably due to the higher molecular weights and chemical structures of contaminants of the dextrans used (15,40,42,43). The present dextran preparations meet higher purity standards and have a narrower range of molecular weights (40,000-70,000 daltons).

Dogs were used in this project because they have been well established for use in resuscitative fluid toxicology studies (57). Swine gained popularity after being used for the first time in 1919 for hemorrhage studies at the University of Wisconsin and proved to be excellent models (17).

Materials and Methods

The Immunology Study:

Frozen serum specimens previously drawn from 24 euvoletic (normal blood volume) male and female Beagle dogs employed in a subacute toxicity study (57), were tested to evaluate changes in immunoglobulins and C3 protein concentrations following multiple HSD infusions. Selection of these components to test for dextran-induced reactions was based on their importance in the immune response and commercial availability of the antiserum.

In that study, the dogs were infused daily with 20 ml/kg body weight which is the maximum dose tolerated with no side effects (57). This dose is five times the recommended therapeutic dose of 4 ml/kg and was chosen because it was previously shown that at a single dose of 20 ml/kg, no effects on immunoglobulins or C3 protein were observed (52). Specimens were drawn before infusion and at days 1, 2, 3, 7, & 14 following infusion. Control animals were infused with RL as a volume control. To test the HSD components and decide which caused the immunoglobulin and C3 changes, other dogs were infused with D-70 and a fourth group was infused with 7.5% Saline.

Double diffusion plates--DD (Ouchterlony) with pH=7.2 phosphate buffered 1% agarose were used to determine the titers of IgG, IgM and C3 protein. Results were recorded after 24 hours and 48 hours but only the 48 hour values were used according to standard protocol in clinical laboratories. To determine the titers of IgG, IgM and C3 protein, dilutions of 1/64-1/2084 were used for IgG, 1/2-1/64 for IgM and 1/4-1/128 for C3 protein. Normal saline was used as a diluent. The plates were inoculated with 10 ul of diluted serum aliquots in each well and 10 ul of the antiserum in the central well. Rabbit anti-dog IgG was obtained from Sigma Chemical Company (Saint Louis, MO), while Cappel goat anti-dog IgM and C3 were obtained from Organon Teknika Corporation (West Chester, PA). The plates were incubated at room temperature (25°) in a humid environment (a wetted gauze was inserted inside a zip lock bag containing two plates). Each plate contained two 7 well patterns. (Supplier: Kallestad; Austin, TX). Six DD plates were used for every animal subject to test the serum at days 0, 3, 7, & 14 for each parameter. Readings were taken over a dark

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background to obtain the optimal resolution of precipitation lines.

The Hematology Study:

The PT and APTT study:

Twenty four female Yorkshire pigs were used for the coagulation study. To eliminate interferences from the stored blood and platelets pool in the spleen, these pigs underwent splenectomy prior to this study. The design of the pig experiments was meant to mimic the clinical use of HSD in the resuscitation from hypovolemia. An important event occurred two hours after infusion. This experimental structure represents a patient admitted to a hospital 2 hours after injury for full resuscitation and replacement of blood. To monitor the changes in PT and APTT following hemorrhage and/or HSD administration, the tested pigs were divided into three groups:

- a. Hemorrhaged pigs: 27 ml blood/kg body weight (n=4). Hemorrhage was done over a sixty minute period with no subsequent infusions.
- b. Hemorrhaged pigs infused with a single therapeutic dose of 4 ml HSD/kg body weight (n=9).

c. Non-hemorrhaged pigs infused with a single dose of 4 ml HSD/kg body weight (n=11).

Blood specimens (20 ml) were drawn at times: 0 (before infusion), 60 minutes post hemorrhage (in the hemorrhaged animals), and at 0.5, 1, 2, 3, 4, 24, 48, 72 hours and 7 days following HSD infusion. Specimens were stored at -20 C° until tested. Total storage time was 6-12 weeks.

Testing was done on BBL Fibrosystem with Ortho Diagnostic Systems kits for PT and APTT. (Supplier: Sigma Diagnostics, Saint Louis, MO). General Diagnostics Simplastin kits were used in PT and APTT experiments. (Supplier: Organon Teknika Corporation; Durham, NC). Controls for both PT and APTT were provided by the same manufacturers. Prior to starting each test, samples and reagents were equilibrated to 37° C in heating blocks.

When measuring PT, 0.2 ml of Ortho Brain Thromboplastin (made up of rabbit brain and lung tissues) was added to 0.1 ml of the tested serum. The timer was started at the same time the reagent was added. All PT tests were done in duplicate. Mean values and ranges were recorded.

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When performing the APTT, 0.1 ml of serum was incubated with 0.1 ml of platelet factor 3 reagent plus activator for APTT (contains phospholipids extracted from rabbit brain tissue and micronized silica for surface activation of plasma samples) for five minutes before addition of 0.1 ml of 0.025 M calcium chloride and starting the timer. This test was also run in duplicate, and the means and ranges reported. Plasma controls with the same lot number of reagents were used since control results vary with different lot numbers.

Bleeding Times Study:

Bleeding time determinations were performed by LAIR personnel using the ear incision technique described by Bowie et al. Due to technical limitations in this technique, only three measurements were available on 4 euvoletic pigs infused with HSD. Bleeding times were determined at baseline (time 0), and one and two hours after HSD infusion.

Platelet Aggregation Study:

The platelet aggregometry study was done on a Chrono-Log Aggregometer (Model 540, Chrono-Log Corporation; Havertown, PA). Citrated plasma specimens were collected at specific time intervals (see below). The collected blood was centrifuged at 800 g for 6 minutes to separate the platelet-rich plasma (PRP) fraction. The PRP was aspirated into siliconized tubes (since platelets have a tendency to adhere to regular glass surfaces), and the remaining blood was centrifuged at 2500 g for 10 minutes to obtain the platelet-poor plasma (PPP) which served as a blank in this study. The PPP was also aspirated into siliconized tubes. The PRP was tested for aggregability using each of the following substances: collagen, ADP, epinephrine, and ristocetin. Percent aggregation was determined using the following equation: $[90 - CR] / [90 - 10] * 100$ (48). CR=Chart Recorder Reading.

To 450 ul of plasma was added 50 ul of collagen, ADP, or epinephrine or 10 ul of ristocetin to 490 ul of plasma (44,48). Collagen, ADP, and epinephrine were

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obtained from Sigma Diagnostics (Sigma Chemical Company; Saint Louis, MO). Ristocetin was provided by Chrono-Log; Havertown, PA.

Concentrations were:

- a. Collagen: 2 mg/ml
- b. ADP: 0.0002 mol/l
- c. Epinephrine: 0.0001 mol/l
- d. Ristocetin: 15 mg/ml

Seven pigs were used in this study. Four pigs were infused with 4 ml/kg body weight HSD and the other three were hemorrhaged and then infused with a single dose 4 ml/kg body weight HSD. Mean values of percent aggregation were reported \pm Standard Error.

In preliminary studies of platelet aggregation in pigs using all four reagents, it was determined that pig platelet aggregation responded best to ADP. Therefore, only ADP was used in subsequent studies on effects of HSD on platelet aggregation.

Statistical Analysis:

Data were analyzed by 2-way analysis of variance with treatment and time as variables. The Newman-Keuls test was used to compare differences in the means of

the variables. Probabilities less than 0.05 indicated statistically significant differences.

Results

Coagulation Parameters:

For PT and APTT, there were very slight, although not clinically significant changes (Tables I and II). APTT decreased about 3-5 seconds and the PT decreased 1.2-2.6 seconds between time 0 and 168 hrs in 4 hemorrhaged, 11 nonhemorrhaged pigs which were infused with a single 4 ml/kg body weight dose of HSD, and the 9 hemorrhaged pigs treated with HSD. In the 4 hemorrhaged pigs, the change might be considered a physiological response to hypovolemia or hemodilution induced by HSD.

The percent aggregation of pig platelets with agonists used at concentrations used for human studies, revealed that ADP was the agonist that resulted in a desired response (data not shown). ADP was used for the studies presented here. Platelet aggregation studies done on 7 pigs revealed that platelet aggregation increased by 17% in hemorrhaged and HSD

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infused pigs and by 7% in nonhemorrhaged but HSD infused pigs between 0 and 24 hours (Table III). Although platelet aggregation seemed to increase slightly, bleeding times responded differently. Bleeding times were not significantly prolonged 2 hours after infusion of HSD (Table IV).

Immunology Study Results:

The 24 dogs used in this study were divided into 4 groups of 6 each. These four groups were infused with the following four solutions, respectively: HSD, D-70, Hypertonic Saline, and Ringer's Lactate. To consider the change a significant immune response, the minimum change in titers should be two dilution factors or more. In the four groups results showed no significant changes in the titers of IgG, IgM or C3 protein over the 14 day experimental period. There were no discrepancies among the four groups (Tables V-VIII).

Discussion and Conclusion:

The hematology findings are in agreement with results reported in a similar study by Hess et al. (23). They concluded that HSD in vitro had very slight effects on PT and APTT and the changes would be toward

faster clotting rather than longer coagulation times. They also concluded that these effects might be due to the hypertonic saline fraction and not dextran (23).

It is not clear yet why HSD might cause slight decreases in PT and APTT. Prolongation of bleeding times, however, was found to be due to the decrease in factor VIII levels soon after dextran infusion (1,42). This phenomenon is used clinically. Dextran is used to improve microcirculation and prevent deep venous thrombosis following surgeries (4,42). Prolonged bleeding was also reported to be due to the formation of a weak fibrin structure following dextran resuscitation (24,41). Increased platelet aggregability is one characteristic of D-70 (2). Not only does D-70 increase platelet aggregation but it also increases erythrocyte sedimentation rate due to its tendency to stick to surfaces (2).

The results in the immunology study agree with a previous study on the immunological effects of a single 20 ml/kg HSD infusion in dogs (52). Since immunogenicity of dextran is molecular weight-dependent (15,25,29), and dextrans with molecular weights less than 100,000 daltons do not evoke an immune response

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(52), clinical dextrans were not expected to induce an increase in the levels of IgG, IgM, or C3 protein (13,15,18,33). The IgG, IgM and C3 titers in the tested samples were not increased.

The efficiency of HSD in restoring the mean arterial pressure and improving renal function has been proven in hemorrhaged animals (9,35,42). Dextran, a major constituent of HSD was proven to improve blood flow, and prevent deep venous thrombosis through its effects on the coagulation system (42).

At the recommended doses of HSD, there were no effects on the pig coagulation system. At a dose five times the recommended therapeutic dose, there were no adverse effects on the immune systems of dogs. HSD shows a great potential for clinical use in the management of hypovolemia and trauma. There have been a number of reports on HSD effects in animal models. To assure the safety of HSD, it must be tested in humans. The experimental design of this present project simulates a human trauma case. A more complete picture would be obtained in future studies using a larger number of tested animals, and a more comprehensive study analyzing individual coagulation

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factors and plasma proteins levels, then comparing the results with similar data reported in human trials.

REFERENCES

1. Aberg, M.; Hedner, U.; Bergentz, S.E. Effect of dextran on factor VIII (antihemophilic factor) and platelet function. *Ann. Surg.* 189:243-247; 1979.
2. Alexander, B.; Odake, K.; Lawlor, D.; Swanger, M. Coagulation, hemostasis, and plasma expanders: a quarter century enigma. *Fed. Proc.* 34(6):1429-1440; 1975.
3. Bergman, A.; Andreen, M.; Blomback, M. Plasma substitution with 3% Dextran-60 in orthopedic surgery: influence on plasma colloid osmotic pressure, coagulation parameters, immunoglobulins and other plasma constituents. *Acta Anaesthesiol. Scand.* 34:21-29; 1990.
4. Berqvist, D. Dextran and hemostasis. *Acta Chir. Scand.* 148:633-640; 1982.
5. Bowei, E.J.W.; Owen, C.A. Jr.; Zollman, P.E.; et al.: Tests on hemostasis in swine: Normal values and values in pigs affected with von Willebrand's disease. *Am. J. Vet. Res.* 34: 1405-1407; 1973.
6. Bronwell, A.W.; Artz, C.P.; Sakoff, Y. Evaluation of blood loss from a standardized wound after dextran. *Surg. Forum* 5:809; 1954.
7. Buchanan, E.C. Blood and blood substitutes for treating hemorrhagic shock. *Am. J. Hosp. Pharm.* 34: 631-636; 1977.
8. Bygdeman, S.; Wells, R. Comparison of rheological effects of dextran 40 and upon erythrocyte aggregation and cell viscosity. *Bibl. Anat.* 10: 16-19; 1968.
9. Dubick, M.A.; Summary, J.J.; Ryan, B.A.; Wade, C.E. Dextran concentrations in plasma and urine following administration of 6% Dextran-70/7.5% NaCl to hemorrhaged and euvoletic conscious swine. *Circ. Shock* 29:301-310; 1989.

10. Evans, R.J.; Jordon, J.L. Mechanism of the antithrombotic action of dextran. *N. Engl. J. Med.* 28:748; 1974. (Letter)
11. Fowler, C.L.; Miller, H.I.; Nance, F.C. Immunological and physiological effects of different resuscitation fluids in the guinea pig model. *Med. Sci. Res.* 16:795-796; 1988.
12. Gregersen, M.I.; Peric, B.; Usami, S.; Chien, S. Relation of molecular weight of dextran to its effects on viscosity and sedimentation rate of blood. *Bibl. Anat.* 4:58-61; 1964.
13. Gronwall, A. Antigenicity of Swedish clinical dextran (Macrodex). *Acta Soc. Med. Upsal.* 64: 244; 1959.
14. Gronwall, A.; Ingelman, B. The introduction of dextran as a plasma substitute. *Vox Sang.* 47:96-99; 1984.
15. Gruber, U.F. Blood replacement. 70-82; 1969. New York: Springer-Verlag.
16. Halonen, P.; Linko, K.; Myllyla, G. A study of hemostasis following the use of hydroxyethyl starch 120 and dextran in major laparotomies. *Acta Anaesth. Scand.* 31:320-324; 1987.
17. Hannon, J.P. Hemorrhage and hemorrhagic shock in swine: A review. Letterman Army Institute of Research; 1989. Institute Report No. 449. Available from: Letterman Army Institute of Research; Presidio of San Francisco, CA: U.S.A.
18. Hedin, H. Dextran-induced anaphylactoid reactions in immunological in vitro and in vivo studies; 1977. Available from: Institute of Zoophysiology, University of Uppsala; Uppsala: Sweden. (Dissertation)

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19. Hedin, H.; Kraft, D.; Richter, W.; Scheiner, O.; Devey, M.I. Dextran reactive antibodies in patients with anaphylactoid reactions to dextran. *Immunobiology* 156:289; 1979.
20. Hedin, H.; Richter, W. Pathomechanisms of dextran-induced anaphylactoid reactions in man. *Int. Archs. Allergy Appl. Immunol.* 68:122-126; 1982.
21. Hedin, H.; Richter, W.; Messmer, K.; Renck, H.; Ljungstrom, K-G.; Laubenthal, H. Incidence, pathomechanism and prevention of dextran-induced anaphylactoid/anaphylactic reactions. *Dev. Biol. Stand.* 48:179-189; 1981.
22. Hedin, H.; Richter, W.; Ring, J. Dextran-induced anaphylactoid reactions in man. *Int. Archs Allergy Appl. Immunol.* 52:145-159; 1976.
23. Hess, J.R.; Dubick, M.A.; Summary, J.J.; Bengal, N.R.; Ryan, B.A.; Wade, C.E. The effects of 7.5% NaCl/6% Dextran-70 on coagulation and platelet function in humans and rabbits. Letterman Army Institute of Research; 1989. Institute Report No.428. Available from: Letterman Army Institute of Research; Presidio of San Francisco, CA: U.S.A.
24. Holcroft, J.W.; Vassar, M.J.; Turner, J.E.; Derlet, R.W.; Kramer, G.C. 3% NaCl and 7.5% NaCl/Dextran-70 in the resuscitation of severely injured patients. *Ann. Surg.* 206:279-287; 1987.
25. Howard, J.G.; Vicari, G.; Courtenay, B.M. Influence of molecular structure on the tolerogenicity of bacterial dextrans. 1. The alpha 1-6 linked epitope of dextran B 512. *Immunology* 29:585; 1975.
26. Hummel, K. The influence of water-soluble colloids on the mechanism of erythrocyte aggregation. *Bibl. Hemat.* 33:322-329; 1969.

27. Jacobaeus, U. The effect of dextran on the coagulation of blood. *Acta Med. Scand.* 151:505-507; 1955.
28. Jan, K-m. Red cell interactions in macromolecular suspension. Third international congress in biorheology: Symposium on recent advances in hemorheology I. *Biorheology* 16:137-148; 1979.
29. Kabat, E.A.; Bezer, A.E. The effect of variation in molecular weight on the antigenicity of dextran in man. *Archs. Biochem. Biophys.* 78:306; 1958.
30. Knox, R.G.; Nordt, F.J.; Seaman, G.V.F.; Brooks, D.E. Rheology of erythrocyte suspensions: Dextran-mediated aggregation of deformable and nondeformable erythrocytes. *Biorheology* 14:75-84; 1977.
31. Kramer, G.C.; Perron, P.R.; Lindsey, D.C.; Sy Ho, H.; Gunther, R.A.; Boyle, W.A.; Holcroft, J.W. Small volume resuscitation with hypertonic saline dextran solution. *Surgery* 100:239-247; 1986.
32. Lim, Jr., C.; Kostrzewska, E.; Bergentz, S.; Gelin, L.E. Rheology of human blood following treatment with dextran-40 and dextran-70. *Bibl. Anat.* 10:9-15; 1969.
33. Litwin, M.S. To which extent is the clinical use of dextran, gelatin and hydroxyethyl starch influenced by the incidence and severity of anaphylactoid reactions? *Vox Sang.* 36:39-49; 1979.
34. Maeda, N.; Seike, M.; Shiga, T. Effect of temperature on the velocity of erythrocyte aggregation. *Biochem. Biophys. Acta* 904:319-329; 1987.

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35. Maningas, P.A.; DeGuzman, L.R.; Tillman, F.J.; Hinson, C.S.; Priegnitz, K.J.; Volk, K.A.; Bellamy, R.F. Small volume infusion of NaCl in 6% Dextran-70 for the treatment of severe hemorrhagic shock in swine. *Ann. Emer. Med.* 15:1131-1137; 1986.
36. Mattox, K.L.; Maningas, P.A.; Moore, E.E.; Mateer, J.R.; Marx, J.A.; Aprahamian, C.; Burch, J.M.; Pepe, P.E. Prehospital hypertonic saline dextran infusion for post-traumatic hypotension. *Ann. Surg.* 213(5):482-491; 1991.
37. Meiselman, H.J. The influence of dextran on the sedimentation behavior of human red cells: macro and micro studies. *Bibl. Anat.* 10:20-31; 1968.
38. Messmer, K.; Ljungstrom, K-G; Gruber, U.; Richter, W.; Hedin, H. Prevention of dextran-induced anaphylactoid reactions by hapten inhibition. *Lancet* i: 975; 1980.
39. Miale, J.B. *Laboratory medicine hematology*, 6th edition: 821-823; 1982. Saint Louis: The C.V. Mosby Company.
40. Mishler IV, J.M. Synthetic plasma volume expanders- Their pharmacology, safety and clinical efficacy. *Clin. Hemat.* 13:75-92; 1984.
41. Moore, G.L.; Summary, J.J.; Dubick, M.A.; Ledford, M.E.; Ryan, B.A.; Gonzales, A.; Wade, C.E. The effects of hypertonic saline (7.5%)/dextran-70 (HSD) on human red cell typing, lysis, and metabolism in vitro. Letterman Army Institute of Research; 1989. Institute Report No.401. Available from: Letterman Army Institute of Research; Presidio of San Francisco, CA: U.S.A.
42. Perkins, F.T. Plasma volume expanders: Report of a WHO meeting. *Dev. Biol. Stand.* 48:155-189; 1981.

43. Richter, A.W.; Hedin, H.I. Dextran hypersensitivity. *Immunol. Today.* 3(5):132-138; 1982.
44. Riess, H.; Braun, G.; Brehm, G.; Hiller, E. Critical evaluation of platelet aggregation in whole human blood. *Amer. J. Clin. Path.* 85:50-56; 1986.
45. Ring, J.; Messmer, K. Incidence and severity of anaphylactoid reactions to common volume substitutes. *Lancet i.* 466-469; 1977.
46. Roitt, I. *Essential Immunology*, 6th edition: 193-195; 1988. Palo Alto: Blackwell Scientific Publications.
47. Seiverd, C.E. *Hematology for medical technologists*, 5th edition: 387; 1983. Philadelphia: Lea and Febiger.
48. Sigma Diagnostics platelet aggregation reagents: Collagen, ADP, Epinephrine. Procedure No. 885. Available from: Sigma Diagnostics; St. Louis, MO: U.S.A. (Package insert-see Appendix A)
49. Sobel, M.; Adelman, B.; Greenfield, L.J. Dextran-40 reduces heparin-mediated platelet aggregation. *J. Surg. Res.* 40:382-387; 1986.
50. Stetz, J.J.; Jelinek, G.N.; Wolfram, R.M.; Antell, H.; Fries, C.C.; Stuckey, J.H. Blood flow in the intact extremity. *Arch. Surg.* 99:989; 1969.
51. Stryer, L. *Biochemistry*, 2nd edition: 376; 1981. San Francisco: W.H. Freeman and Company Press.
52. Summary, J.J.; Ryan, B.A.; Zaucha, G.M.; Dubick, M.A.; Wade, C.E.; Frost, D.F.; Korte, Jr., D.W. Serum immunoglobulin and complement responses to 6% Dextran-70/7.5% Hypertonic Saline solution in dogs and pigs. Letterman Army Institute of Research; 1989. Institute Report No. 431. Available from: Letterman Army Institute of Research; Presidio of San Francisco, CA: U.S.A.

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53. Velasco, I.T.; Pontieri, V.; Roca E Silva, M. Hyperosmotic NaCl and severe hemorrhagic shock. Amer. J. Physiol. 239:664-673; 1980.
54. Weislander, J.B.; Dougan, P.; Stjernquist, U.; Aberg, M.; Bergentz, S.E.; Sweden, M. The influence of dextran and saline solution upon platelet behavior after microarterial anastomosis. Surg. Gynec. Obstet. 163:256-262; 1986.
55. Weiss, H.J. The effect of clinical dextran on platelet aggregation, adhesion, and ADP release in man: In vivo and in vitro studies. J. Lab. Clin. Med. 69(1):37-46; 1967.
56. Zade-Oppen, A.M.M.; Ostling, S.G.; Marsden, N.V.B. On how macromolecules reduce hemoglobin loss in hypotonic hemolysis. Uppsala J. Med. Sci. 84:155-161; 1979.
57. Zaucha, G.M.; Frost, D.F.; Omaye, S.T.; Clifford, C.B.; Korte, Jr., D.W. Fourteen-day subacute intravenous toxicity study of Hypertonic Saline/Dextran-70 and its constituents in beagle dogs. Letterman Army Institute of Research; 1989. Institute Report No. 404. Available from: Letterman Army Institute of Research; Presidio of San Francisco, CA: U.S.A.

TABLES

Effect of Hemorrhage and/or HSD infusion on Coagulation Parameters in Pigs¹

TABLE I
Activated Partial Thromboplastin Time (sec)

Time (hr)	Hem (n=4)	HSD (n=11)	Hem +HSD (n=9)
BL ²	25.1±4.1	26.7±0.8	28.3±1.1
Hem ³	22.3±3.5	—	23.8±0.7
0.5	20.5±3.2	24.1±0.9	23.0±0.7
1	21.0±3.0	24.6±0.9	24.6±1.1
2	22.5±2.9	24.3±0.7	25.1±1.0
3	22.7±3.0	24.3±1.0	25.2±1.0
4	23.4±2.3	25.9±0.8	25.7±1.0
24	22.5±2.3	26.1±1.0	25.5±1.2
48	23.0±1.8	24.6±1.7	24.2±2.0
72	22.8±1.3	22.1±1.3	21.2±1.4
168	19.6±0.7	21.9±1.5	25.6±0.9*

TABLE II
Prothrombin Time (sec)

Time (hr)	Hem (n=4)	HSD (n=11)	Hem +HSD (n=9)
BL ²	15.8±0.6	16.3±0.3	16.2±0.5
Hem ³	14.8±0.6	—	15.2±0.5
0.5	15.0±1.4	15.8±0.3	15.4±0.5
1	15.0±1.0	15.0±0.5	15.5±0.5
2	15.4±1.1	14.9±0.5	15.8±0.4
3	15.1±0.8	14.7±0.5	15.4±0.4
4	16.1±0.3	15.2±0.4	15.6±0.5
24	16.3±0.6	15.5±0.4	14.9±0.5
48	15.3±0.8	15.2±0.2	14.4±0.4
72	15.8±1.3	14.6±0.2	13.4±0.6
168	14.6±1.0	14.6±0.2	13.6±0.6

¹Data Expressed as mean ± S.E. (Standard Error)

²BL- Baseline values

³Hem- values following hemorrhage

For both PT and APTT two-way analysis of variance indicate no significant difference within each group from 0 to 168 hours

* One-way analysis between the groups indicated $P < 0.05$ at 168 hours, but student Newman-Keuls test (done by Dr. Virginia Gildengorin) failed to show significant differences among the groups.

Serum Immunoglobulins and C₃-Complement Concentrations in Dogs¹

ID #	DAYS
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0	3	7	14
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0	3	7	14
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0	3	7	14
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TABLE V
HSD

Animals	IgG				IgM				C ₃ Complement			
2	256	256	256	256	4	2	2	2	32	32	16	16
25	256	256	256	256	4	4	2	2	—	16	16	16
33	128	128	128	128	16	16	8	8	—	8	8	16
45	512	512	256	256	8	8	8	4	32	32	16	16
52	256	256	256	256	4	4	2	2	32	32	16	16
64	512	512	256	256	16	16	8	8	64	64	32	32

TABLE VI
D70

Animals	IgG				IgM				C ₃ Complement			
5	256	256	256	128	16	16	16	16	64	64	32	32
26	128	128	128	128	4	4	2	—	32	32	16	16
37	256	256	256	256	8	8	4	4	16	8	8	8
49	512	512	512	512	8	8	4	4	32	16	16	16
55	1024	512	512	512	8	8	8	8	32	16	—	—
60	512	512	256	256	8	8	8	4	32	16	16	16

TABLE VII
HS

Animals	IgG				IgM				C ₃ Complement			
7	256	256	256	256	4	4	4	4	32	32	32	32
29	1024	1024	1024	1024	8	8	8	8	64	64	64	64
41	512	512	512	512	8	8	8	8	32	32	32	32
50	256	256	256	256	4	4	4	4	32	64	64	64
51	256	256	256	256	8	8	8	8	32	32	16	16
61	1024	1024	1024	1024	8	8	8	8	16	16	16	16

TABLE VIII
RL

Animals	IgG				IgM				C ₃ Complement			
6	512	512	512	512	—	—	4	4	—	—	—	16
34	256	256	512	512	4	4	4	4	32	32	32	32
44	512	1024	512	512	32	32	32	32	64	64	32	32
57	256	256	512	512	8	8	8	8	64	32	32	32
59	512	512	512	256	8	8	8	8	64	64	64	64
67	512	512	512	512	8	8	4	4	64	64	64	64

¹Data expressed as dilution factor based on 1:n, where n is value represented

No significant changes were observed at any time with any treatment. A significant change is a two dilution factor change in titers between days 0 & 14

TABLE III
Platelet Aggregation² (%)

Time (hr)	HSD (n=4)	Hem +HSD (n=3)
BL ³	58.1±3.1	66.2±1.2
Hem ⁴	—	77.5±2.5
0.5	65.5±3.2	67.8±5.7
1	66.0±3.0	65 (1)
2	61.9±3.4	74.5±7.6
3	—	—
4	—	—
24	65.1±4.3	83.8±8.1

¹Data expressed as mean ± S.E. (Standard Error)

²Platelet aggregation using ADP as agonist

³BL- Baseline values

⁴Hem- values following hemorrhage

No statistically significant difference was observed between 0 and 24 hours (P>0.05)

TABLE IV
Bleeding times of Euvoletic Pigs Infused with HSD¹

Time	Bleeding time (min)	
Baseline	3.8±0.7	(4)
1 hr post HSD	3.4±0.7	(4)
2 hrs post HSD	4.2±1.8	(2)

¹Data expressed as mean ± S.E. (n)
 No statistically significant change between 0 and 2 hrs ($P > 0.05$).