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INTRODUCTION

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This conference is designed to evaluate the use of low molecular weight dextran in the management of injured man. Our aim is to summarize the current status of this agent and to suggest potentially fertile fields for future study.

The participants in this conference are sophisticated students in this field of research and all realize:

1) That "shock" is a term that encompasses many clinical and physiologic states. The participants are therefore asked to define the type of "shock" to which they are referring in all presentations.

2) That responses to various types of injury differ markedly in various species. Participants are therefore asked to define both the species employed and the experimental conditions when describing results of investigation.

Although our primary concern is with injured man, certain investigations require animal experimentation. It will be understood that experimental findings in animals may not be applicable in man, but we will not belabor this obvious point.

In order to clarify terminology in regards to dextrans, let us define the three types as follows:

Low Molecular Weight Dextran (LMWD) or Low Viscous Dextran (LVD) has a mean molecular weight of approximately 45,000. It is marketed under the name of Rheomacrodex(R). It is of this substance which we will primarily speak today.

Ordinary dextran as used clinically in the United States and in Sweden has a molecular weight ranging between 50,000 and 200,000. This is the material that has been stockpiled in such large quantities and which is marketed under the name of Macrodex(R).

Finally, there are the dextrans with molecular weights over 200,000 which are used only under experimental conditions in this country. In Great Britain, however, various high molecular weight dextrans have been given extensive clinical trial.

THE CHEMISTRY OF DEXTRAN AND PROPERTIES OF LOW MOLECULAR WEIGHT DEXTRAN AND OTHER DEXTRAN PREPARATIONS

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Dextrans are polysaccharides produced by certain bacteria, such as Leuconostoc mesenteroides. These bacteria convert sucrose into dextran. All dextrans are polyglucoses in which the majority of bonds linking the glucose units are of the alpha 1:6 type.

The detailed structure of the molecules is, however, dependent on which strain of bacteria is used for dextran production. Besides 1:6 glucosidic linkages, the dextrans have a varying content of 1:3 and 1:4 glucosidic linkages, and the molecules are more or less branched. Figure 1 shows a part of a dextran molecule. As the properties of dextrans depend on the detailed structure of the molecules, it is of importance to remember that investigations performed with different dextrans from different strains of bacteria are not always exactly equivalent.

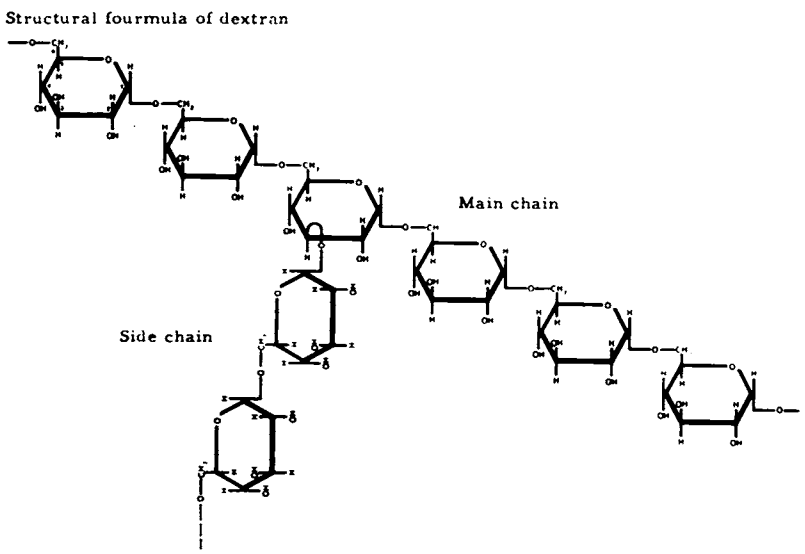

Figure 1. Structural Formula - Dextran

It has been found that dextrans suitable for clinical use have a low degree of branching with more than 90 per cent α - 1:6 glucosidic linkages. I will not go into more details regarding this question, because today the same structural type of dextran with a low degree of branching is used both in the United States and in most European countries.

Native dextran consists of a mixture of molecules of different chain lengths and molecular weights. This occurs because of the great variation in the number of anhydroglucose units within the molecules. The dextran molecules produced by the bacteria can be of extremely high molecular weight. It should be mentioned also that in solution the dextran molecules are hydrated and that the dextran chains are highly flexible. In accordance with their physical-chemical properties, the molecular chains should be assumed to be randomly coiled in solution. From native dextran of very high molecular weight, dextran fractions of lower molecular weights and with rather narrow molecular weight distributions can be obtained by partial acid hydrolysis and fractionations.

I will now briefly compare the physical-chemical properties of low molecular weight dextran with other dextran preparations.

All dextran preparations consist of mixtures of molecules of varying size even if the product has been well fractionated in order to obtain a narrow molecular weight distribution. For heterogeneous mixtures average molecular weights are determined. However, it is of importance to remember that different methods for determining molecular weights give different kinds of molecular weight averages. These averages may differ appreciably for a polymolecular sample. This sometimes leads to confusion if one does not state how the molecular weights have been measured or what kind of average is used.

Today the average molecular weights of dextran samples are largely determined by light scattering, which gives a value known as weight average molecular weight. The symbol $\overline{M}_w$ is used in chemical literature to denote this average. The molecular weights which I refer to in the following are such values.

The average molecular weight of the low molecular weight dextran preparation, known as Rheomacrodex^R in Sweden, is about 40,000. The ordinary dextran plasma expander used in the United States and in Sweden and many other European countries has an average molecular weight of about 75,000. Perhaps one could call this type medium molecular weight dextran. In England, dextran plasma expanders having average molecular weights of about 150,000 are used.

It should be mentioned that so-called high molecular weight dextrans, which have been used in animal experiments to produce intravascular aggregation, are dextran fractions that have molecular weights of some hundred thousands or even over a million. I wish to stress that such high molecular weight dextran fractions are not equivalent with the ordinary clinical dextran preparations used in the United States or Sweden. The properties of dextran are to a high degree dependent upon the molecular weight.

Not only is the average molecular weight of a clinical dextran preparation important, but also the whole molecular weight distribution as well. In a somewhat simplified manner, the molecular weight distribution of the low molecular weight dextran can be roughly described by saying that more than 90 per cent of the preparation has molecular weights within the range 10,000 to 80,000 (when determined according to the procedure given in the American specifications for clinical dextran). The corresponding range for ordinary clinical dextran of average molecular weight

75,000 is, according to American specifications, 25,000 to 200,000. The same limits have been applied in Sweden for many years but we have lately decreased the upper limit of the range to 150,000.

Figure 2 shows the molecular weight distributions of the three types of clinical dextran preparations in more detail. In this figure molecular weights have been plotted on the X-axis and cumulative weight per cent of the dextran preparation on the Y-axis. From the curves it is possible to calculate the percentage of the preparation within different molecular weight ranges. Curve A is the integral molecular

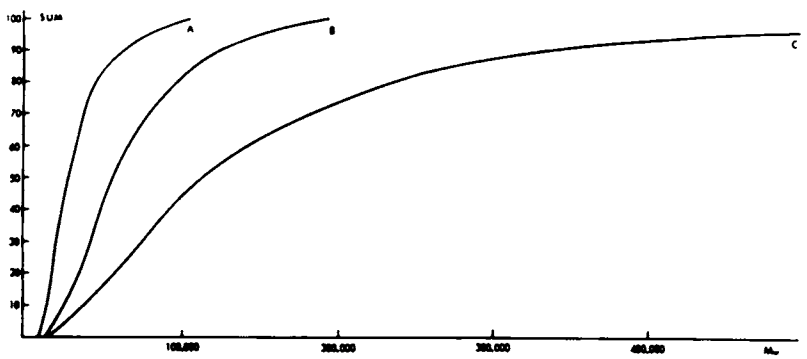

Figure 2. Molecular weight distribution of Rheomacrodex^{R} (A), Swedish/American dextran (B), as a plasma expander and British dextran (C).

weight distribution curve for the low molecular weight dextran of average molecular weight 40,000. Curve B is the corresponding curve for ordinary clinical dextran of average molecular weight 75,000. Curve C is the result of a corresponding determination on a British dextran plasma expander which, according to our opinion, contains molecules too large for clinical application. (Preparations of such high molecular weights may cause certain untoward side effects such as aggregation of erythrocytes, interference with coagulation factors, and an increased tendency to allergic reactions.)

Figure 3 shows that the viscosity of the new low molecular weight dextran solution is lower than the viscosity of the ordinary dextran plasma expander when compared at the same concentrations.

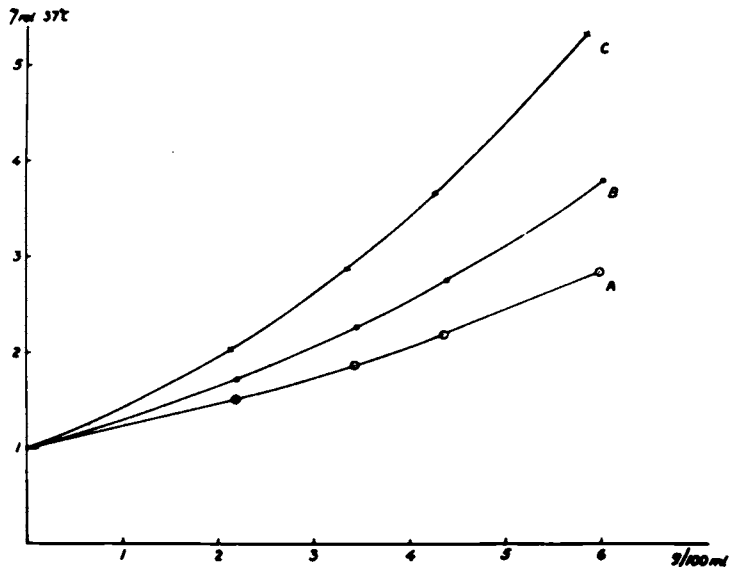

Figure 3. Relative viscosity η_{rel} at 37 degrees Centigrade as a function of dextran concentration in g/100 ml. A, Low molecular weight dextran (M_w = about 40,000). B, Ordinary dextran plasma expander (M_w about 75,000). C, British type of dextran plasma expander.

HEMATO-RHEOLOGICAL PROPERTIES OF LOW MOLECULAR WEIGHT DEXTRAN AND OTHER DEXTRANS

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Introduction

In surgery very little interest has been given to rheological disturbances. This depends mainly on the fact that flow, according to the Poiseuilles law, is proportional to the pressure head and the fourth power of the diameter of the vessels and inversely proportional to the viscosity. As long as the fluid is a Newtonian fluid like water or oil, the flow will vary only with the variations in pressure head and diameter. Blood is, however, a tissue comprised of a suspension of corpuscles and an emulsion of fat in a vehicle—the plasma.

While the pressure and volume flow of water and other Newtonian fluids vary in proportion to one another, this is not the case with pseudo-plastic fluids unless and until a certain critical pressure and velocity of flow has been established. Below this critical pressure and velocity the increase in flow is much less than one would expect from an increase in pressure. This signifies that the viscosity of blood increases—or the fluidity of blood decreases—at low flow rates. In rheological terms this means that blood behaves like a pseudo-plastic fluid.

Tissue injury is followed by alterations in the physical properties of blood with aggregation of cells and of chylomicra. This alteration influences its fluidity. Knowledge about the circulatory disturbances due to such alterations has accumulated from investigations on the microcirculatory flow pattern of blood in the living body, especially those of Knisely and his co-workers, and in artificial capillary systems. The flow characteristics of blood in arterioles, true capillaries and venules are of importance for the distribution of red cells and thereby the supply of oxygen to the tissue and removal of carbon dioxide from the tissue.

The term rheological disturbances is used for such changes in the physical state of blood which influence flow. The increased knowledge of rheological disturbances of blood justifies its application on pathogenetic and therapeutic problems in the field of surgery.

In most studies on shock an increasing amount of evidence has underlined the importance of reduced blood volume, lowered blood pressure, arteriolar constriction resulting in a diminishing cardiac output and cessation of capillary flow. However, deaths still occur as the result of shock, or the consequences of shock, which are not related to the above-mentioned factors. This brings us back to the question of how to define the term "shock." I have not been able to accept the definitions of

shock generally used; the definition I would propose is the following: "Shock is any acute haemodynamic disturbance which causes such a degree of reduced capillary flow that tissue hypoxia of a degree leading to functional and/or morphological changes occurs."

Shock is not merely a problem of blood volume, blood pressure and anemia, but essentially a problem of flow. Hence, though blood volume and blood pressure must be maintained, this in itself affords no guarantee that the tissues will be adequately perfused with blood, i.e., that the red cells will pass in single file through true capillaries. This definition, however, puts other requirements than the usual ones on a substitute for treatment of shock, i.e., rheological properties.

Rheologic Disturbances from Injury

I should like first to present some data and experiments illustrating what we consider to be main blood changes resulting from injury, changes which have an important bearing on the problem of substitution treatment for traumatic shock.

Figure 1 shows changes in the haematocrit, erythrocyte sedimentation rate (ESR) and plasma protein patterns of two patients with compound fractures of the lower leg. They were treated only for their local injuries and received no intravenous therapy. In both cases there was a decrease in the haematocrit, an increase in the sedimentation rate, a decrease of albumin, an increase in globulins, including especially the α_2 fraction and an increase in fibrinogen. This accumulation of large protein molecules at the expense of albumin leads to an increase in the viscosity of the plasma and a decrease in the suspension stability of the blood, with consequent aggregation of cells. These changes vary in degree depending upon the severity of the injury.

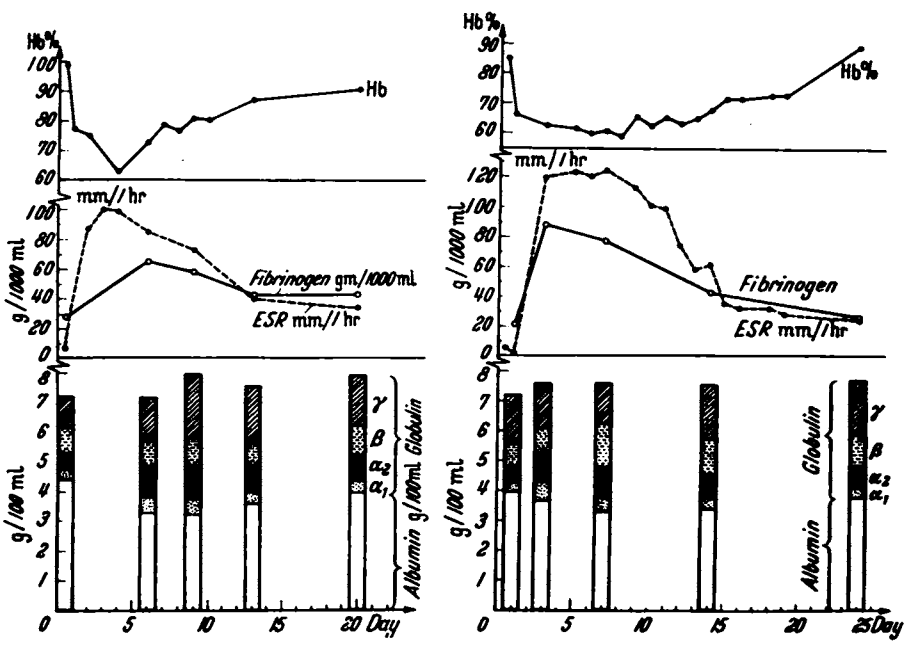

Figure 1. Changes in haemoglobin, suspension stability and plasma protein pattern in two patients with compound fractures of the lower leg.

Figure 2 shows the capillary flow in the bulbar conjunctiva of one of these patients. Aggregation and stasis of cells in the venules is visible, and blood is being shunted through arteriolo-venular anastomoses 18 hours after the injury, thus before any elevation of ESR is apparent. In the second picture occlusive aggregates are seen in post-capillary venules. Later the stasis is less marked and the flow improves despite ESR which is now increased.

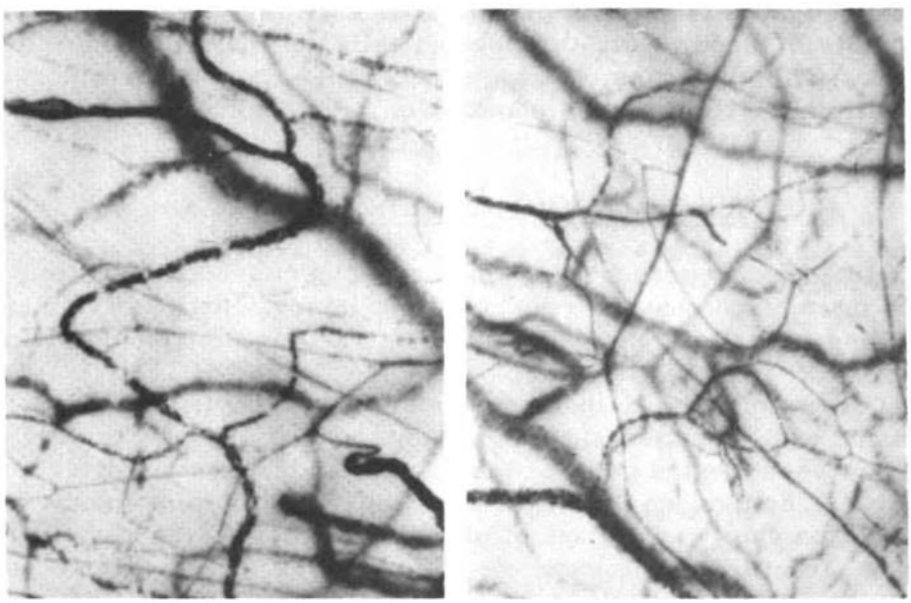

Figure 2. Capillary flow in the bulbar conjunctiva 18 hours and 2 days after the injury (from the first patient in Fig. 1).

Figure 3 shows the capillary flow in the bulbar conjunctiva of a rabbit with an untreated ten per cent third-degree burn injury of the back. In the top picture aggregated cells are visible in the smallest capillary venules four hours after burning. In the middle picture, we see larger aggregates occluding the venules 12 hours after burning. The bottom picture illustrates the flow characteristics three days after the injury. Marked changes in the blood flow are apparent, with obstructing aggregates in larger collecting venules as well as arteriolovenular shunting of blood.

Figure 4 lists the changes in the haematocrit, ESR, urinary output and blood volume of the same burned animal. After primary haemoconcentration, a progressive decrease is observed in the haematocrit, together with oliguria and a diminution in the circulating red cell volume, but a spontaneous restoration of blood volume occurs. ESR is rather reduced early after the injury. Thus aggregation of blood cells in vivo is not comparable to ESR in vitro.

The in vivo aggregation of cells which follows tissue injury may be differentiated in four phases:

1) The early phase of "white emboli," which consists of aggregated platelets and chylomicra appearing within minutes of injury and reproducible with intravenous injection of thromboplastin.

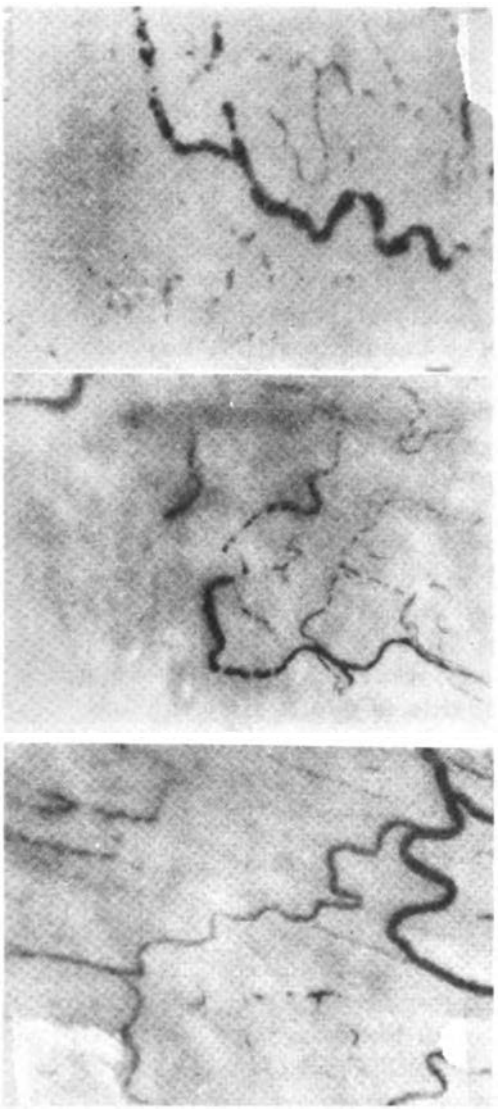

Figure 3. Capillary flow in the bulbar conjunctiva of a rabbit with an untreated burn injury.

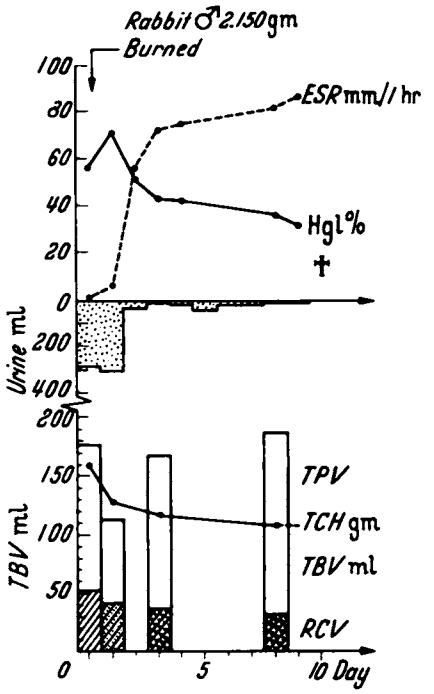

Figure 4. Blood volume and blood changes in the same rabbit as in figure 3.

2) After some few hours an early red cell aggregation occurring together with a reduced ESR and reproducible with purified thrombin injections.

3) Red cell aggregation running parallel with increasing ESR and reproducible with injections of large macromolecules.

4) High ESR, which is not parallel to any marked changes in the microcirculation at all, despite decreasing intravascular aggregation of red cells.

The variations in the viscosity of whole blood and plasma occurring at different time intervals following a contusion injury in a dog are given in the following figure 5.

The curves show that the viscosity of whole blood and the haematocrit have clearly increased two hours after the contusion (curve B compared with curve A).

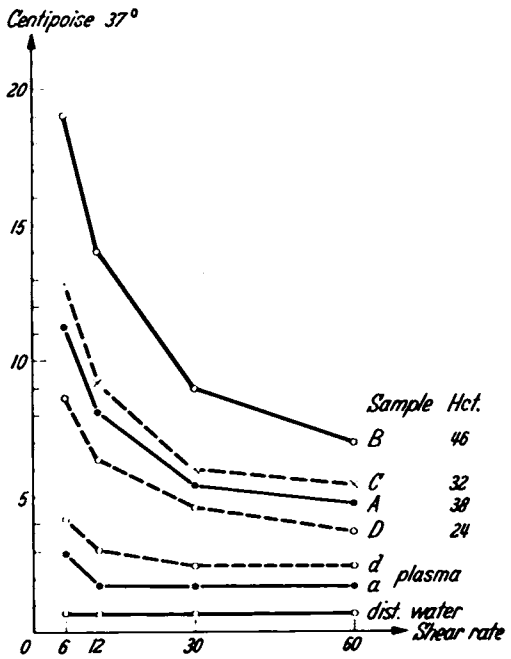

Figure 5. Variations in the viscosity of blood and plasma at different time intervals after a contusion injury in a dog.

This increased viscosity is most marked at low rates of shear, which must be of special importance for the flow of blood in venules and sinusoids where the flow rate may be very slow, especially under pathological conditions.

The viscosity curve 16 hours after the contusion shows a drop in whole blood viscosity to about pre-experimental values (curve C). The haematocrit is, however, lower than pre-experimentally. Since a lowering of the haematocrit should result in a decreased viscosity of the whole blood, the unchanged viscosity despite a lowered haematocrit indicates an alteration in the viscous properties of the blood.

The viscosity curve 96 hours after the contusion shows a decreased viscosity of whole blood compared to the pre-experimental value; this is due to the very low haematocrit.

The viscosity of the plasma is slightly increased 2 and 16 hours after the contusion; 96 hours after the contusion the viscosity of plasma is significantly increased at any rate of shear. These changes indicate an altered distribution of polymers in the plasma (compare curves a and d).

The following experiments have been performed to analyze the effect of vehicle viscosity on the red cell suspension viscosity at different haematocrits and at different rates of shear. Red cells which were washed twice have been added to different vehicles in concentrations to cause a progressive increase of the haematocrit of the suspension. As apparent from figure 6 the viscosity of red cells suspended in saline, low viscous dextran, Macrodex and high viscous dextran shows an increase in viscosity with increasing haematocrit and decreasing rate of shear. This increased viscosity is, however, unproportionally greater than expected from only the haematocrit, when the viscosity of the vehicle is increasing. This is also valid for plasma, albumin, and fibrinogen used as vehicles as evident from figure 7. Figure 8 demonstrates the unexpected greater increase of red cell suspension viscosities in different vehicles and at different haematocrits at the shear rate of 6 which equals 1 inverse second. We have called this unexpected increase in viscosity a "viscosity plus" factor. This "viscosity plus" factor increases with increasing vehicle viscosity, increasing haematocrit and decreasing rate of shear.

Thus tissue injury is followed by significant alterations in the viscous properties of blood. These alterations must influence the flow of blood, especially at low flow rates, i.e., in venules and sinusoids, and thus influence the venous return

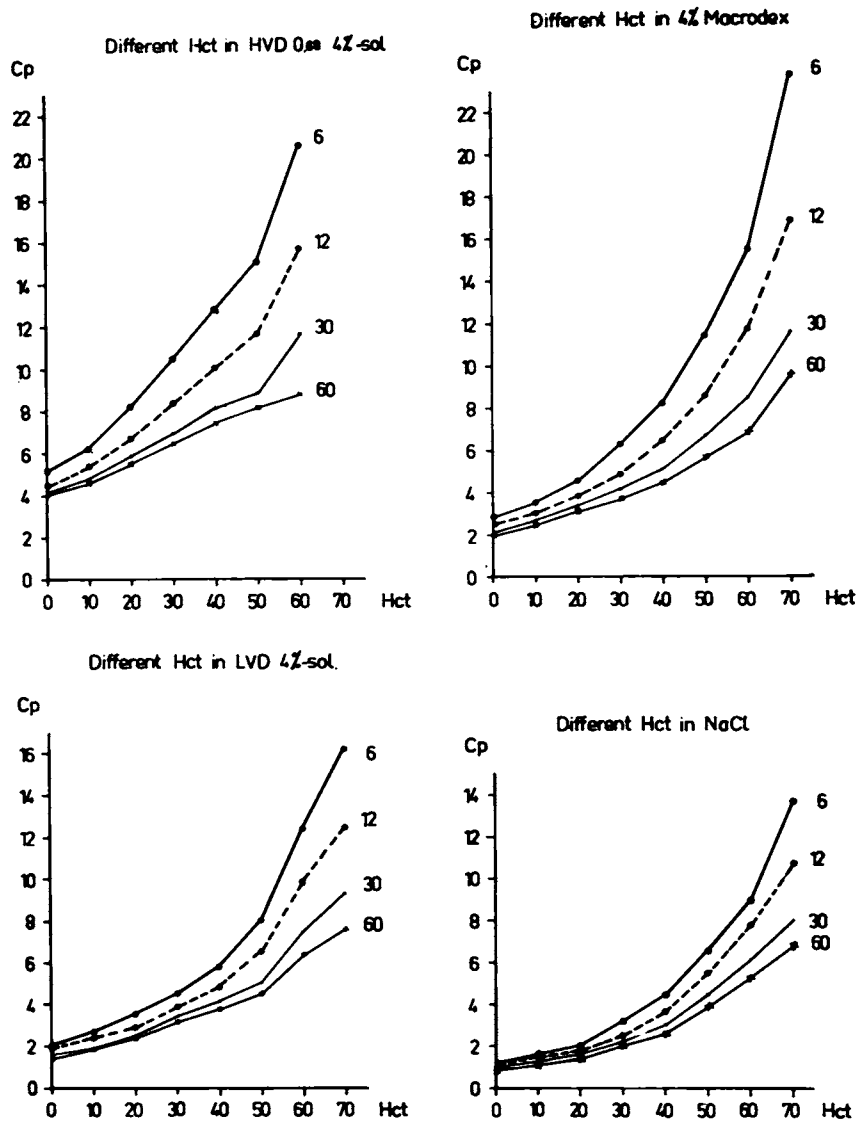

Figure 6. Viscosity values on red cell suspensions at different haematocrits in different 4 per cent dextran solutions.

of blood. Determinations of the viscosity of blood, however, can only give information on the viscous properties of the blood as such, and not on the behavior of blood in vessels of varying diameters.

Flow of Blood in Capillary Tubes

From studies on the flow of blood in tubes with diameters less than 300μ it is known that the viscosity of whole blood decreases with decreasing width of the tubes. This drop in viscosity is explained by the corpuscles being more and more concentrated to the faster axial stream, with the result that the haematocrit decreases and the viscosity of whole blood is then determined more and more by the viscosity of plasma.

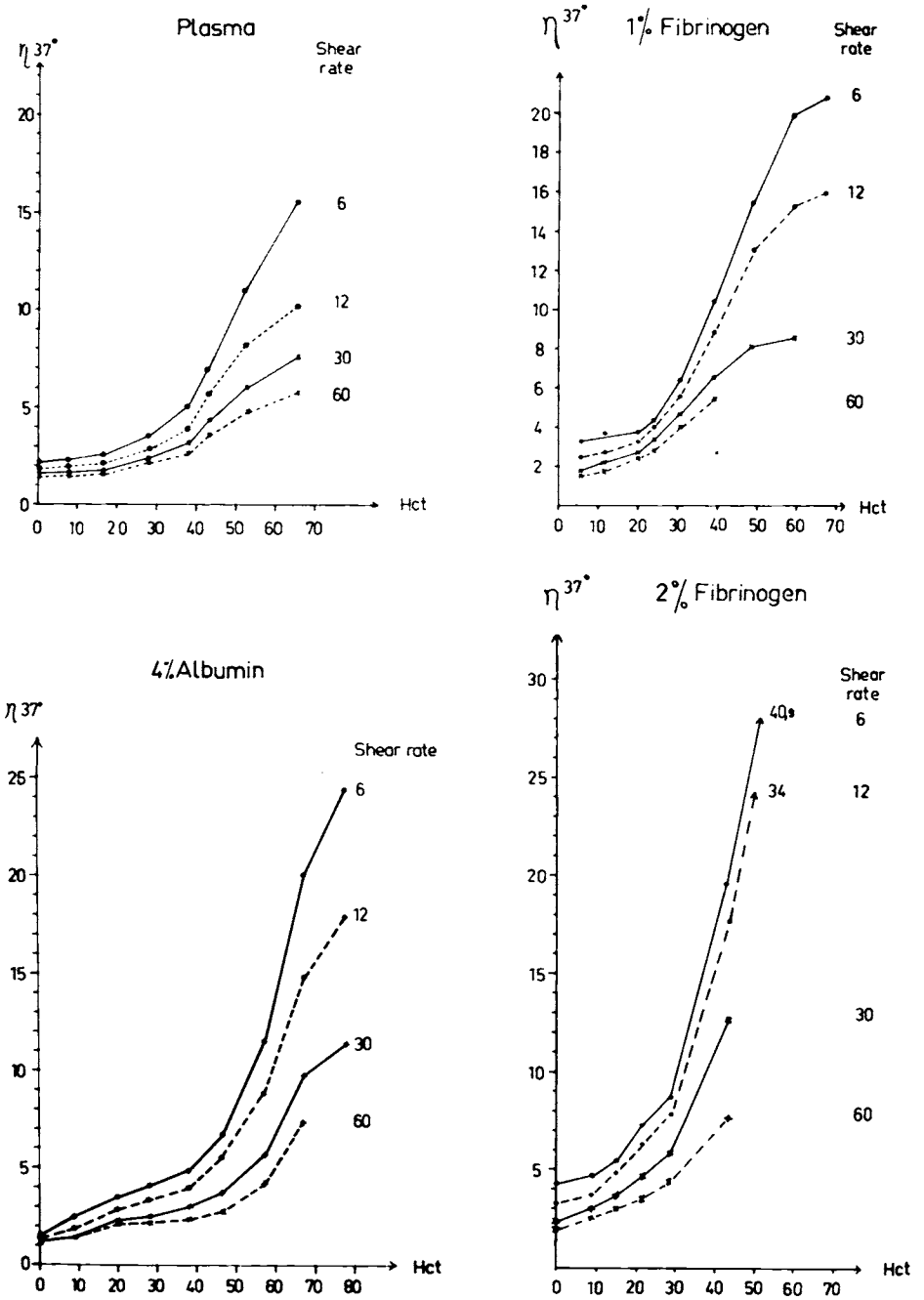

Figure 7. Viscosity values on red cell suspensions at different haematocrits in plasma, 4 per cent albumin, and 1 per cent and 2 per cent fibrinogen solutions.

These flow properties have been clarified for blood streaming in linear, rigid tubes. Such model studies, however, imitate arteriolar but not venular flow. Since erythrostasis and occlusion by aggregated cells in the venules are such dominant findings in the microcirculatory flow pattern of blood after tissue injury, it has become essential to study these flow properties of blood in capillary models with branching and postcapillary tubes.

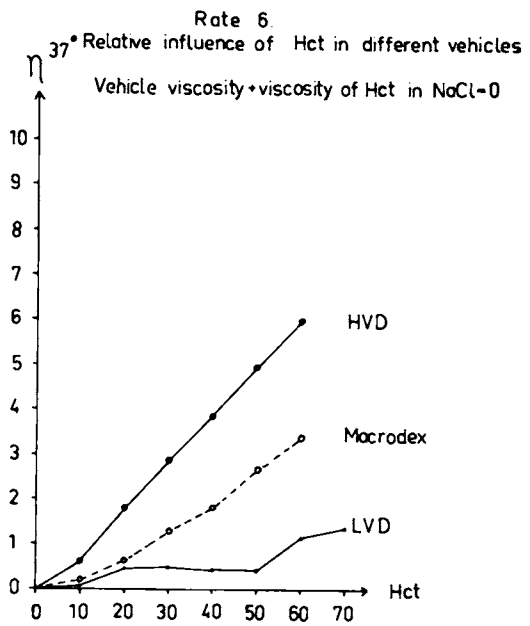

Figure 8. "Viscosity plus" factor at the shear rate of 6.

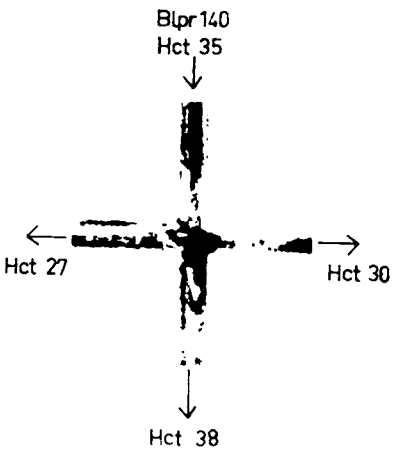

Figure 9. Flow pattern of blood with decreased suspension stability when perfused in a branched capillary device with a diameter of 110μ.

When blood is perfused through a branched capillary device according to figure 9, one consequence of the orientation of the cells becomes apparent, namely the skimming of plasma through the lateral outlets and erythrostasis in the central outlet. The figure is taken from an experiment where blood from the femoral artery of a heparinized dog with ESR 130 mm/hour perfuses the branched capillary device. A marked axial orientation of the cells is apparent in the inflow capillary tube (110μ in diameter). In the cross area there is turbulence. On introduction, the haematocrit of the blood was 35; this had dropped to 27-30 in the lateral outlets and risen to 38 in the central outlet.

The postcapillary flow, seen in prolonged tubes as in figure 10, shows stasis of cells relative to plasma. (This erythrostasis is apparent at clinical examination in so-called "red shock," when hypostasis of cells and persistent pale pressure marks are seen, signifying a very poor flow of blood.)

Thus, the perfusion of blood with decreased suspension stability through branched, narrow capillary tubes demonstrates a separation of plasma flow and cell flow. This separation results in skimming of plasma and in postcapillary erythrostasis; this separation tendency increases with decreasing suspension stability and decreasing flow rate.

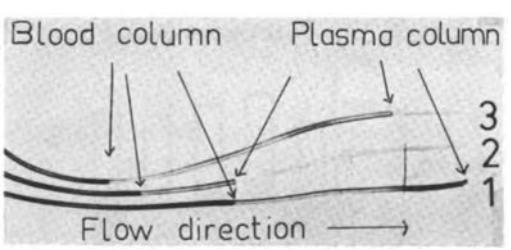

Figure 10. Flow of blood in postcapillary tubes from the outlet capillaries of the model in figure 9.

Pathophysiology of Induced Flow Disturbances

In order to study the effect of the above-mentioned flow disturbances occurring under pathophysiological conditions, we have induced them experimentally by a variety of means: injury of various kinds, hypothermia, intravenous infusion of thrombin, thromboplastin, fat, and large macromolecules in solution. Critical experiments designed to demonstrate the significance of these disturbances must, however, also meet the requirement of reversibility. For this reason, we have used dextran of very high molecular weight to produce the desired changes and dextran of low molecular weight to reverse them. Thus, one and the same colloid was employed both to produce and to reverse aggregation by varying its molecular properties. In this way changes in the flow characteristics of blood in capillaries can be graded by varying the amounts and properties of the macromolecules infused.

Figure 11 shows an experiment on a rabbit which had been given highly viscous dextran (HVD) having a molecular weight of about one million and intrinsic viscosity 0.7 in order to produce increased plasma viscosity and red cell aggregation, i.e., to imitate the accumulation of large and viscidizing protein molecules such as takes place after injury. Following administration of high viscosity dextran, a drop in haematocrit occurs together with an increase in the ESR, oliguria or anuria, an increase in blood volume, but a decrease in the red cell volume. These changes are thus quite similar to those following untreated burn injuries, even though there is no trauma and despite a slight increase in blood volume.

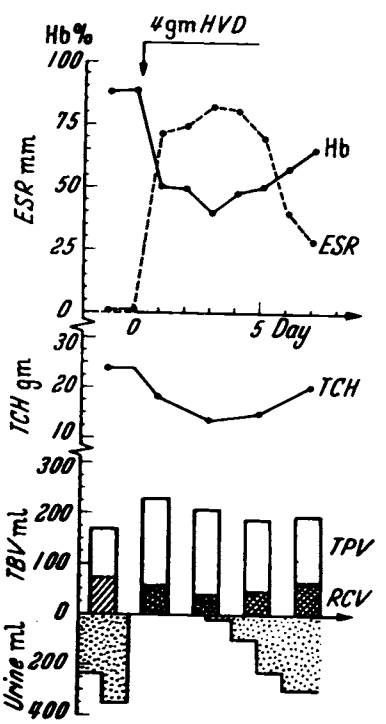

Figure 11. Plasma volume, red-cell volume, and urinary output in a rabbit given high-viscosity dextran.

Figure 12 shows pictures of the capillary flow in the bulbar conjunctiva of the rabbit, as well as of blood smears. On the left can be seen aggregation and stasis of cells in the venules and pronounced aggregation of cells in smears following administration of highly viscous dextran. On the right are pictures from the same rabbit after subsequent administration of low viscous dextran (LVD) showing disaggregation of aggregated cells previously accumulated in the venules as well as more even suspension of the cells in the smears. The low molecular weight dextran used had an average molecular weight of about 40,000 and intrinsic viscosity 0.19. The influence of dextran-induced intravascular aggregation on cardiac output, peripheral resistance, hind-limb blood flow, renal and hepatic blood flow has been investigated before and after administration of HVD and subsequent treatment with LVD.

Figure 13 shows changes in cardiac output, determined with the cardio green method, in control and experimental dogs.

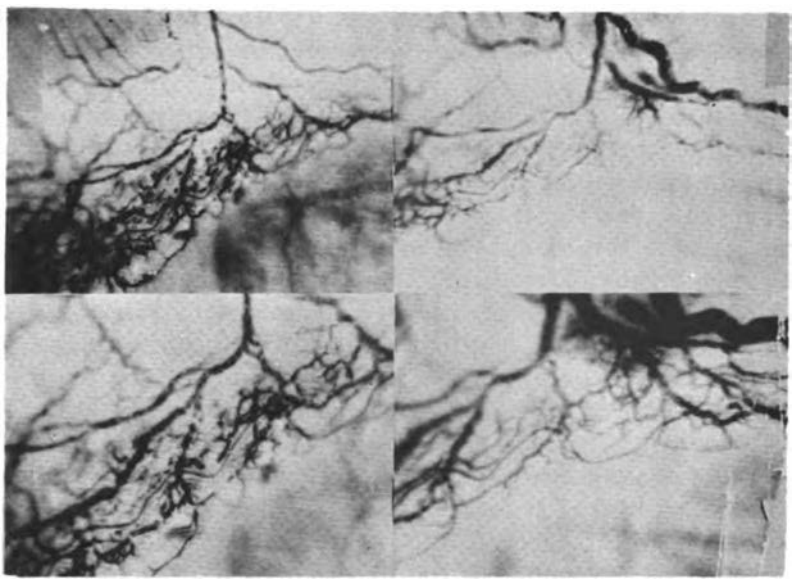

Figure 12. Capillary flow in the bulbar conjunctiva of a rabbit given first high-viscosity dextran (left) and subsequently low-viscosity dextran (right).

The experimental dogs were given 1 g HVD to produce aggregation and subsequently 2 g LVD per kg body weight to obtain disaggregation. During aggregation, cardiac output decreased progressively but returned to control values after disaggregation with LVD.

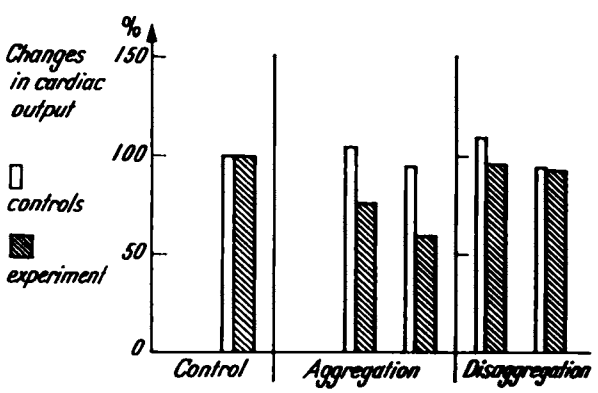

Figure 13. Changes in cardiac output during a period with intravascular aggregation and during disaggregation in control and experimental dogs.

In a study on the acid-base balance in dogs in which intravascular aggregation and disaggregation were induced with HVD and LVD, we have found a normal pH, a slight decrease in pCO_2, an increase in lactic acid, and a marked decrease in oxygen consumption and carbon dioxide elimination during the period of aggregation. After reversal of the aggregation in response to infusion of LVD, the dogs show a transient increase in acidosis with a lowering of the pH, and increase in pCO_2 and lactic acid which points to an accumulation in the tissue of acid material which is released first when the flow is improved.

Microscopic examination of organs from animals subjected to standardized contusion, or cold injury, or to administration of HVD, and sacrificed during a period when capillary flow was markedly changed owing to intravascular aggregation of cells revealed morphological damages in the liver, kidneys and heart, the intravascular aggregation being due either to injury or infusion of HVD. Animals

subjected to the same standardized injury but treated with LVD to counteract the flow changes did not present signs of damage to the organs.

The influence of traumata and induced intravascular aggregation on wound healing as a parameter on effective nutritive blood flow has been studied by Dr. Zederfeldt using a method based on the determination of the tensile strength in healing wounds. Figure 14 gives the main results of this study. In the figure are seven groups of animals, with ten rabbits in each group, subjected to three different experimental procedures: femoral fracture, induced intravascular aggregation with HVD, and withdrawal of blood. Femoral fracture, administration of HVD, and withdrawal of blood were all followed by a diminution in the rate of healing. This decreased rate of healing could not be explained by the accompanying anemia since withdrawal of blood, if substituted with ordinary commercial clinical dextran having an average molecular weight of 75,000, did not cause any reduction of the rate of healing. Nor could the decreased rate of healing be ascribed to the reduction of blood volume in the case of femoral fracture, since in this instance substitution with commercial dextran did not significantly improve the rate of healing. The decreased rate of healing can, however, be counteracted by reversing intravascular aggregation with the aid of LVD infusions; the intravascular aggregation being due either to injury, such as a femoral fracture, or to the presence of large and viscidizing molecules as after the infusion of HVD.

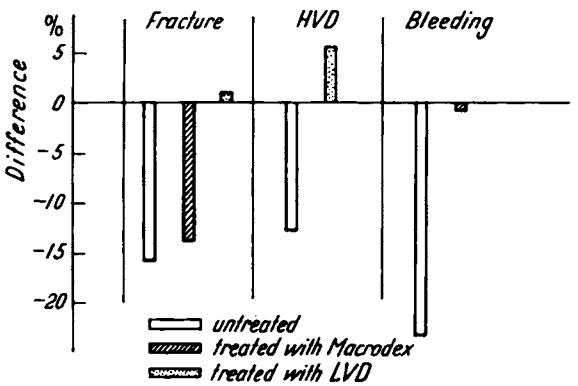

Figure 14. Difference in rate of healing of pre- and post-experimental wounds in seven groups of rabbits under the following experimental conditions: femoral fracture without treatment, after treatment with commercial dextran (Macrodex^{R}), and after treatment with LVD; administration of HVD without and with treatment with LVD; withdrawal of blood without and with treatment with commercial dextran (Macrodex^{R}).

We, therefore, conclude that volume replacement is not sufficient to restore capillary flow and that the disordered capillary flow after tissue injury is due to disturbances in the flow properties of the blood.

Effects of Rheomacrodex^{R} in Humans

Infusion of this low viscosity dextran solution (Rheomacrodex^{R}) to patients with intravascular aggregation of blood cells and impaired capillary flow reverses or reduces the aggregation, increases the fluidity of blood and improves the capillary flow. Figure 15 shows the influence of this dextran solution on the viscosity of whole blood and plasma in a patient with bile peritonitis. In this case the infusion clearly diminished the viscosity of the blood. This increased fluidity is most pronounced at low rates of shear, which is of importance for the flow of blood in the sinusoids and venules where the flow rate is low, especially in a patient with disease. In this case the increasing fluidity of blood after the infusion of low viscous dextran rapidly improved the general condition of the patient.

Figure 16 shows the capillary flow pattern of blood in the bulbar conjunctiva from a man with a fractured lower leg, before, during, and after infusion of 500 ml of this dextran solution. Prior to the infusion, the venules showed aggregation and stasis of cells and marked axial orientation of cells in the arterioles. During and after the infusion the red cells are much more evenly distributed and the stasis of cells disappears.

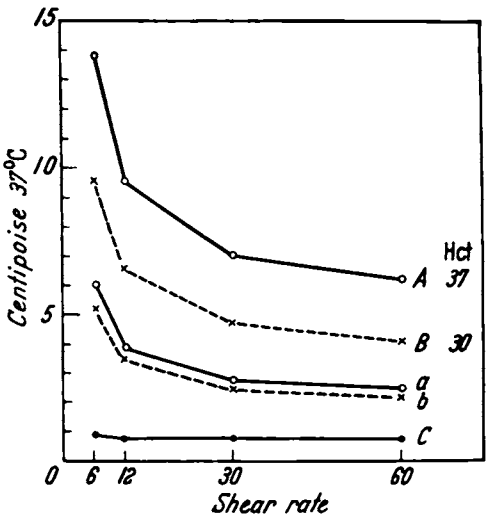

Figure 15. Viscosity of whole blood (A) and plasma (a) before and after (B and b) the infusion of 500 ml 15 per cent LVD in a patient with bile peritonitis. C: viscosity of water.

Figure 17 illustrates the effect of this dextran solution on the viscosity of blood, peripheral resistance and blood flow in a patient suffering from a 40 per cent burn injury. It shows that prior to the infusion the viscosity of whole blood was high at all rates of shear (curve A), that the haematocrit was high, that the perfusion pressure was 110 mm Hg, and that the volume flow was about 10 ml per 100 ml tissue per minute. After infusion of 1000 ml low viscous dextran the haematocrit dropped and the whole blood viscosity decreased at all rates of shear, especially at lower rates (curve B). The perfusion pressure was unchanged, but the blood flow increased to about 14 ml per 100 ml tissue per minute. This means that the peripheral resistance decreased. Curve C gives the viscosity of whole blood 24 hours later at continuous infusion of low viscous dextran. Despite an increased haematocrit, the viscosity remained low.

These cases thus demonstrate that this dextran preparation increases the fluidity of the blood, and increases the perfusion of tissues with blood.

The value of this dextran fraction when employed in patients suffering from so-called "irreversible shock," despite adequate volume replacement of blood, is illustrated by the following case.

A 52-year-old woman weighing 55 kg was admitted to the hospital in poor condition with severe shock. She suffered from advanced peritonitis from a perforated intestinal tumor. Her blood pressure was 70 mm Hg, and her pulse rate was 140. Pre-operative treatment consisted of the administration of Macrodex^{R} and blood. Massive hemorrhage occurred during the operation. Operative and postoperative shock was combatted with whole blood and with Aramine^{R} as a pressor agent. Despite copious transfusions of whole blood, increasing doses of Aramine^{R} were given to maintain blood pressure. Blood volume was determined during this period and showed normal values: P.V. 2670 ml and RCV 1670 ml, haematocrit 44 per cent. Microscopy revealed a very poor capillary flow in the bulbar conjunctiva, standstill of aggregated blood cells in the venules, arteriolo-venular shunting, and marked constriction of the arterioles. There were persistent pale pressure-marks on the skin. The patient was considered to be in "irreversible shock."

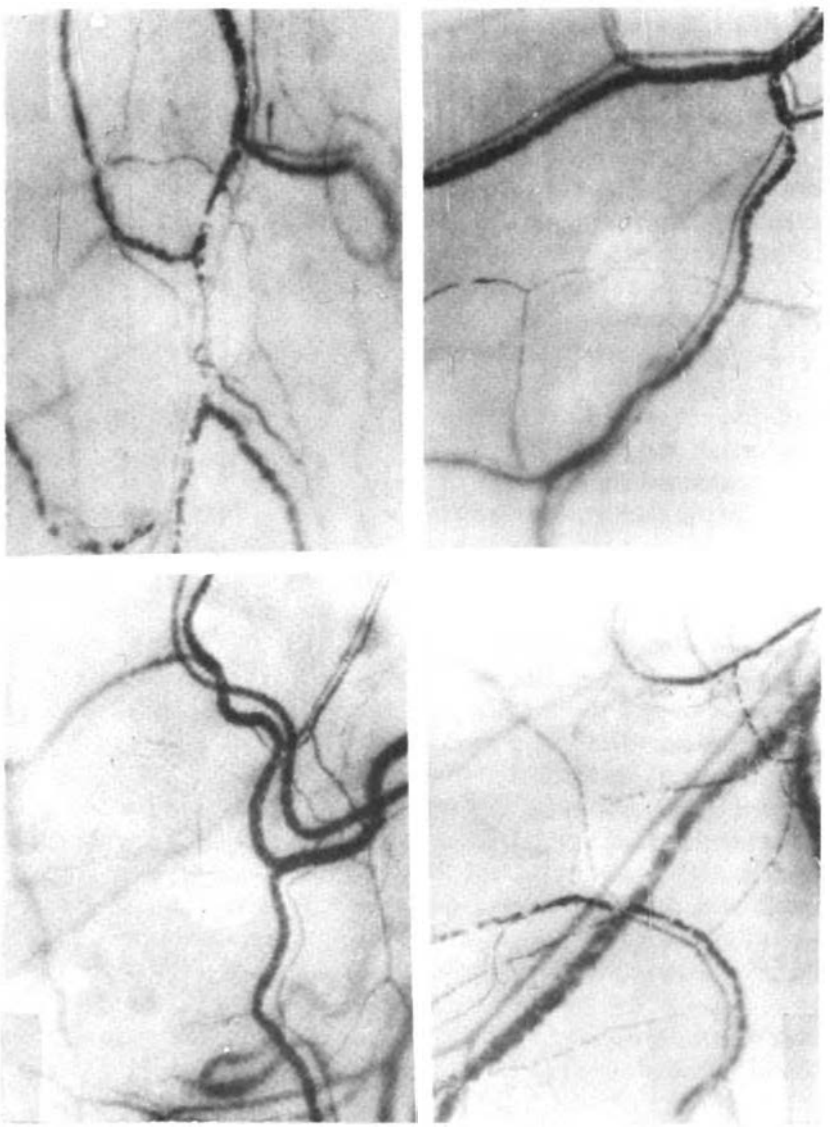

Figure 16. Capillary flow of blood in the bulbar conjunctiva in a patient with a fractured leg: upper left before, upper right during, lower left immediately after, and lower right 24 hours after a single infusion of 500 ml Rheomacrodex^{R}. Note the aggregation and stasis of cells in venules with marked axial orientation in the arterioles. Occlusive aggregates appear in the postcapillary venules. During and after the infusion this stasis and aggregation disappears.

An infusion of 500 ml of low viscous dextran in dextrose was rapidly administered; this was followed by continuous infusion of 2000 ml low viscous dextran for two days. Within one hour after the start of the low viscous dextran infusion, administration of Aramine^{R} could be stopped. The response of the blood pressure, pulse rate, and urine flow is recorded in figure 18. The patient's general condition

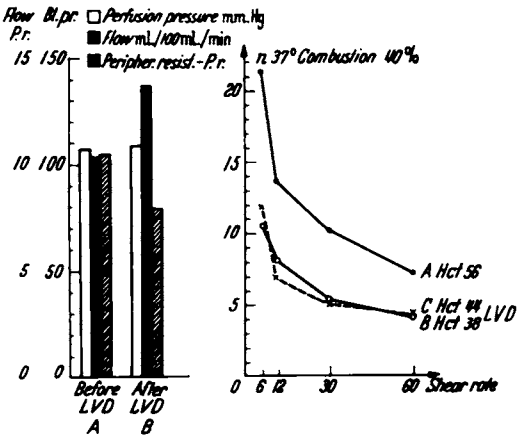

Figure 17. Flow of blood (after 5 minutes arterial occlusion) recorded with a forearm pletysmograph and whole blood viscosity in a case with 40 per cent burned area before and after infusion of 1000 ml 15 per cent LVD. A: before, B: 1 hour after the infusion, C: 24 hours later during continued infusion. The infusion was followed by unchanged perfusion pressure (open columns), increased flow (black columns), decreased peripheral resistance (shaded columns), and decreased viscosity of whole blood.

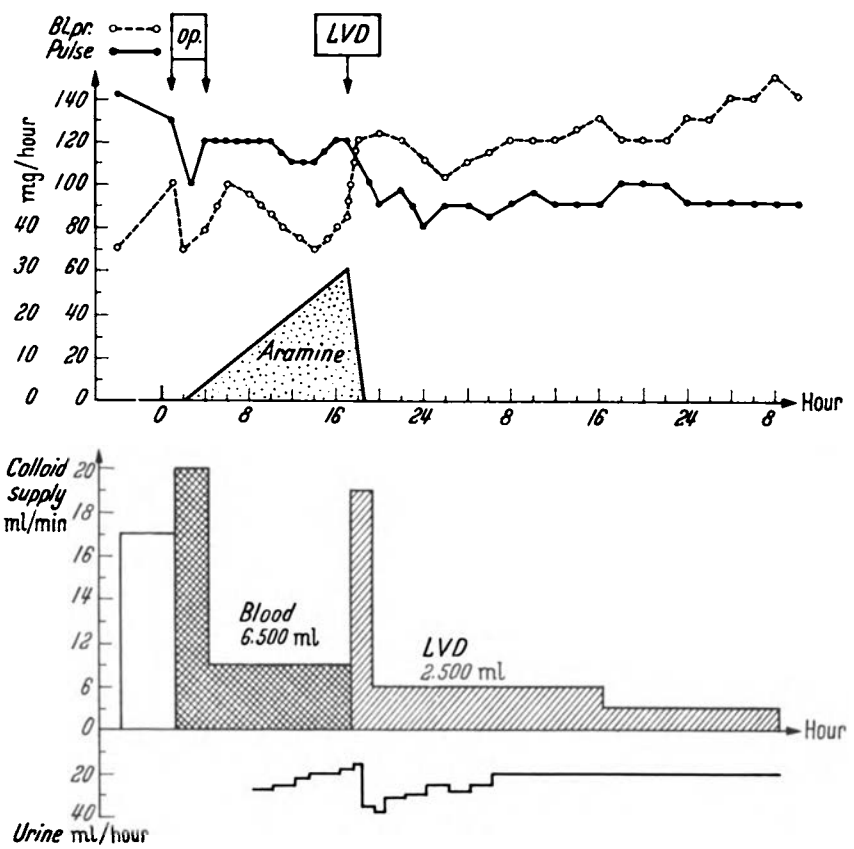

Figure 18. Case report of so-called "irreversible shock."

improved rapidly; the cold, cyanotic skin became warm and red. The capillary flow in the bulbar conjunctiva accelerated and cell stagnation disappeared. The blood pressure and pulse rate were maintained at adequate levels without the need for any extra drugs. The urine flow increased. In short, the "irreversible shock" had been reversed.

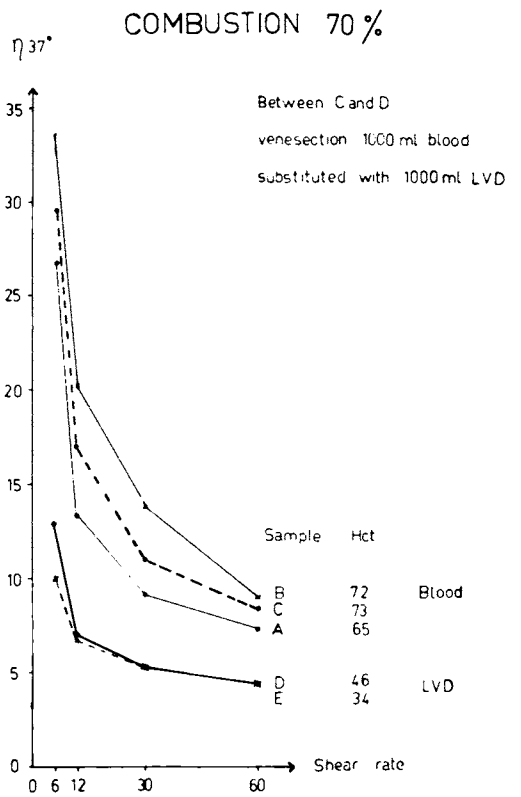

Figure 19. Viscosity values (A, B, C) from a severely burned patient (70 per cent burned area) primarily treated according to Evans schema and later (D, E) after venesection of 1000 ml of blood substitute with 1000 ml of Rheomacrodex[R].

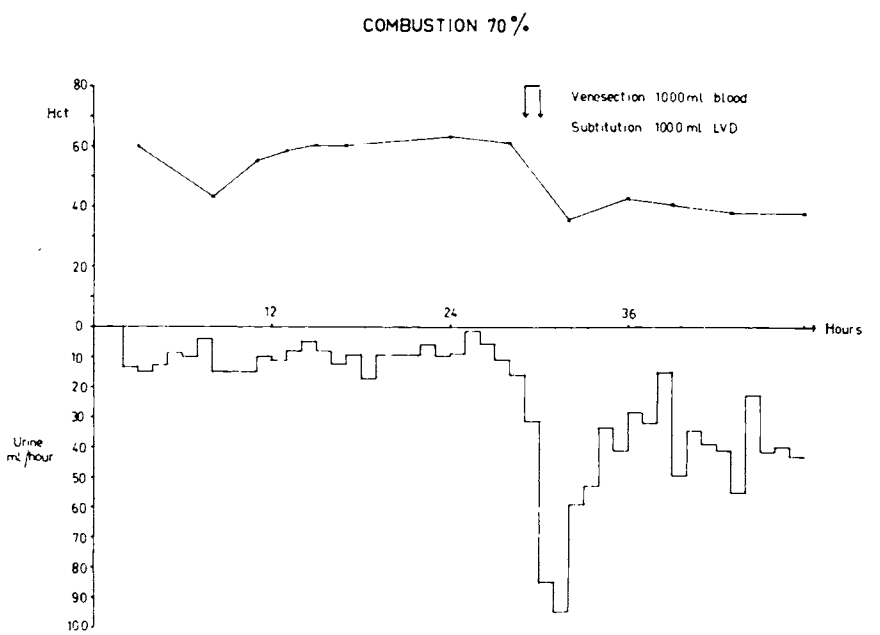

Figure 20. Hourly urinary outputs before and after venesection in case reported in figure 19.

In another case of "irreversible shock" from a severe burn injury of 70 per cent body area the following viscosity data were obtained. During treatment, according to Evans rules, with whole blood, plasma and electrolytes the viscosity of blood increased to very high values 30 centipoise, especially at low rates of shear. When the patient developed clinical signs of irreversibility of shock with oliguria, 1000 ml of whole blood was withdrawn and replaced with low viscous dextran. The viscosity of the blood then became normal. The clinical picture changed favorably and a high diuresis started. The "irreversible shock" was reversed. The patient, however, died from sepsis on the seventh day of injury. (See figures 19 and 20.)

Summary

In summary our indications for the clinical use of low viscous dextran in surgery are: To improve the capillary flow when this is impaired by disturbances in the flow properties of blood; that is, in different kinds of shock, especially from burn, crush, and toxins; in fat embolism and the so-called hepato-renal syndrome; in thrombosis, especially phlegmasia alba dolens and in incipient gangrene; in major surgery, artificial perfusions, vascular grafting procedures and in the use of large doses of X-ray contrast media intravascularly.

REFERENCES

Bayliss, L. E.: Rheology of blood and lymph. Frey-Wyssling, A: Deformation and Flow in Biological Systems, Amsterdam, North Holland Publishing Co., 1952, p. 355.

Bergentz, S.-E.: Studies on the Genesis of Post-Traumatic Fat Embolism, Acta Chir. Scand. Suppl., 282, 1961.

Bergentz, S.-E.; Carlsten, A.; Gelin, L.-E.; Litwin, M.; Rudenstam, C.-M.; Söderholm, B.; and Thulesius, O.: Metaboliska Effekter Av Intravaskular Blodkroppsaggregation Hos Hund, Nord Med., 66:1515, 1961.

Bergentz, S.-E.; Eiken, O.; and Nilsson, I.-M.: The Effect of Various Molecular Weight on the Coagulation in Dogs, Thromb. Diath. Haemorrh., 6:15, 1961.

Bergentz, S.-E.; Gelin, L.-E.; and Rudenstam, C.-M.: Experimental Studies on Fat Embolism, Bibl. Anat., 1:297, 1961.

Bergentz, S.-E.; Gelin, L.-E.; Rudenstam, C.-M.; and Zederfeldt, B.: Indications for the Use of Low Viscous Dextran in Surgery, Acta Chir. Scand., 122:343, 1961.

Bergentz, S.-E.; Gelin, L.-E.; Helander, C.-G.; Linder, E.; Nilsson, N. J.; and Rudenstam, C.-M.: Perifera Mosttåndet i Stora Och Lilla Kretsloppet Vid Inducerad Blodkroppsaggregation, Nord Med., 67:486, 1962.

Bingham, E. C., and Roepke, R. R.: The Rheology of the Blood, J. Gen. Physiol., 28:79, 1944.

Bingham, E. C., and Roepke, R. R.: The Fluidity of Whole Blood at 37 Degrees Centigrade, J. Gen. Physiol., 28:131, 1944.

Borgström, S.; Gelin, L.-E.; and Zederfeldt, B.: The Formation of Vein Thrombi Following Tissue Injury, Acta Chir. Scand., Suppl., 247, 1959.

Branemark, P. I.: Vital Microscopy of Bone Marrow in Rabbit, Scand. J. Clin. Lab. Invest., Suppl., 38, 1959.

Dahlback, O.; Delaney, L. T.; Gelin, L.-E.; Kugelberg, J.; Linder, E.; and Nilsson, E.: A Plasma Substitute in Extracorporeal Circulation: An Attempt to Minimize the Damage to the Blood, Acta Chir. Scand., Suppl., 245, p. 265, 1959.

Eiken, O.: Thrombotic Occlusion of Experimental Grafts as a Function of the Regional Blood Flow, Acta Chir. Scand., 121:410, 1961.

Fajers, C.-M., and Gelin, L.-E.: Kidney-Liver-and Heart Damages from Trauma and from Induced Intravascular Aggregation of Blood Cells, Acta Path. Microbiol. Scand., 46:97, 1959.

Fåhraeus, R.: The Suspension Stability of the Blood, Physiol. Rev., 9:241, 1929.

Fåhraeus, R.: The Erythrocytes-Plasma Interface and Consequences of Its Diminution, Lancet, 2:630, 1939.

Fåhraeus, R., and Lindquist, T.: The Viscosity of the Blood in Narrow Capillary Tubes, Amer. J. Physiol., 96:562, 1931.

Gelin, L.-E.: Studies in Anemia of Injury, Acta Chir. Scand., Suppl., 210, 1956.

Gelin, L.-E.: Disturbance of the Flow Properties of Blood and Its Counteraction in Surgery, Acta Chir. Scand., 122:287, 1961.

Gelin, L.-E.: Effect of Low Viscous Dextran in the Early Postoperative Period, Acta Chir. Scand., 122:333, 1961.

Gelin, L.-E.: A Method for Studies of Aggregation of Blood Cells, Erythrostasis and Plasma Skimming in Branching Capillary Tubes, To be published in Biorheology.

Gelin, L.-E., and Löfström, B.: A Preliminary Study on Peripheral Circulation During Deep Hypothermia, Acta Chir. Scand., 108:402, 1954.

Gelin, L.-E., and Ingelman, B.: Rheomacrodex - A New Dextran Solution for Rheological Treatment of Impaired Capillary Flow, Acta Chir. Scand., 122:294, 1961.

Gelin, L.-E.; Korsan-Bengtsen, K.; Ygge, J.; and Zederfeldt, B.: Influence of Low Viscous Dextran on the Hemostatic Mechanism, Acta Chir. Scand., 122:324, 1961.

Gelin, L.-E.; Persson, E.; and Zederfeldt, B.: Influence of Low Viscous Dextran on the Electrolyte Balance in Healthy Subjects, Acta Chir. Scand., 122:329, 1961.

Gelin, L.-E., and Shoemaker, W. C.: Hepatic Blood Flow and Microcirculatory Alterations Induced by Dextran of High and Low Viscosity, Surgery, 49:713, 1961.

Gelin, L.-E.; Sölvell, L.; and Zederfeldt, B.: The Plasma Volume Expanding Effect of Low Viscous Dextran and Macrodex, Acta Chir. Scand., 122:309, 1961.

Gelin, L.-E., and Thorén, O.K.A.: Influence of Low Viscous Dextran on Peripheral Circulation in Man, A Pletysmographic Study, Acta Chir. Scand., 122:303, 1961.

Gelin, L.-E., and Zederfeldt, B.: Experimental Evidence of the Significance of Disturbances in the Flow Properties of Blood, Acta Chir. Scand., 122:336, 1961.

Knisely, M. H.; Eliot, T. S.; and Bloch, E. H.: Sludged Blood In Traumatic Shock, AMA Arch. Surg., 51:220, 1945.

Knisely, M. H.: The Settling of Sludge During Life, Acta Anatomica, Suppl., 41, 1961.

Long, D. M.; Sanchez, L.; Varco, R. L.; and Lillehei, C. W.: The Use of Low Molecular Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation, Surgery, 50:12, 1961.

Löfström, B.: Intravascular Aggregation and Oxygen Consumption: Aggregation of Red Blood Cells Produced by High Molecular Weight Dextran or by Hypothermia, Acta Anaesth. Scand., 3:41, 1959.

Swank, R. L.: Suspension Stability of the Blood After Injections of Dextran, J. Appl. Physiol., 12:125, 1958.

Thorsén, G., and Hint, H.: Aggregation, Sedimentation, and Intravascular Sludging of Erythrocytes, Acta Chir. Scand., Suppl., 154, 1950.

Zederfeldt, B.: Studies on Wound Healing and Trauma, Acta Chir. Scand., Suppl., 224, 1957.

DISCUSSION

Dr. Gregerson: Blood is, of course, a non-Newtonian fluid, and its viscosity depends upon the shear rate. The evidence which Dr. Gelin has presented to you has been obtained with a so-called Brookfield viscometer, an instrument we have utilized but which we believe has important limitations.

Construction of the GDM viscometer at MIT has provided us with an instrument that measures viscosity at very low shear rates far below those measured by capillary viscometers or even by the Brookfield viscoelastic or comb plate viscometer. The Polarad viscometer measures even lower shear rates.

We measured the viscosity of dog blood replacing varying amounts of the plasma with dextrans of varying molecular weight. The haematocrit was kept constant. At higher shear rates the Brookfield viscometer was used while the Polarad instrument was used at very low shears going down to 0.2 reciprocal seconds. Finally we used the GDM viscometer for measure at extremely low rates of shear (.05 reciprocal seconds) which was about 1/100 that measured by Dr. Gelin.

We thought that low molecular dextran might lower the viscosity of blood but it does not, even at the very low shear rates of .05 reciprocal seconds. In all cases the higher the molecular weight of dextran employed, the greater the viscosity.

I want to emphasize the difference between the shear rates used in Dr. Gelin's experiments and in ours. The minimum that can be measured in his instrument is about 6. Measurement of viscosity at very low shear rates is difficult but is being undertaken with the help of the Bell Telephone Company.

As Dr. Gelin indicated, dextran affects viscosity by altering the cells, not primarily the plasma. There was scarcely any viscosity change with addition of LMWD to plasma, to saline, or to Ringer's solution. However, when we added large molecules to the blood, the changes in viscosity at low shear rates were of the order of 300 to 1000 times.

Dr. Gelin also pointed out the effect of haematocrit upon viscosity. We discovered that if we increased the haematocrit, when dealing with shear rates of about 10, relatively small changes occurred, as Dr. Gelin has indicated. If, however, we utilized very low shear rates of .2 or .05 reciprocal seconds, viscosity increased remarkably even at haematocrits of 50. If we increased the haematocrit still further, viscosity alterations were even more profound at these low shear rates.

PHARMACOLOGY OF LOW MOLECULAR WEIGHT DEXTRAN AND ITS EFFECT ON CLOTTING AND CROSSMATCHING*

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Definition

Low molecular weight dextran as we think of it today is a dextran fraction with a weight average molecular weight (M_w) of approximately 40,000 and a number average molecular weight (M_n) of 22,000.[1] This particular fraction has a narrow M_w distribution with a range of about 15,000 in the lower 10 per cent fraction to about 60,000 in the upper 10 per cent fraction. The descriptive term low molecular weight dextran contributes some confusion in discussions since it is not specific. There are available dextran fractions of lower M_w and even fractions with higher M_w which by comparison could be called low molecular weight dextran. All of these fractions have slightly different physical and pharmacologic properties. Therefore, for the purpose of clarity in this discussion, the above described fraction will be referred to by its generic name, Rheomacrodex^R.

History

The drug Rheomacrodex^R evolved from the continuous studies of a group of Swedish investigators who were seeking the perfect plasma expander. Dr. Bjorn Ingelman can be credited with initiating the concept that dextran solutions could be used for plasma expanders. His original studies were provoked by the urgent need for a plasma expander to serve the Swedish Armed Forces during World War II. He continued these studies after the war in collaboration with his colleagues at Uppsala and at other Swedish medical centers. The first dextran fractions caused serious side effects primarily due to their high average molecular weight, wide molecular weight distribution and high frequency of side chains or branching.[2] Studies continued to the present date have helped to elaborate on the basic toxicity of the original dextrans.

*The opinions and assertions expressed herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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While attempting to explain the complications due to dextran infusions, Gronwall and Ingelman[3] noted that high molecular weight dextran caused intravascular aggregation and that this tendency decreased with lower molecular weight fractions. Thorsen and Hint[4] then performed detailed quantitative studies on the effects of dextran on erythrocyte sedimentation and intravascular aggregation. They found the lowest M_w causing aggregation of red cells was 59,000 for dextran and 18,000 for gelatin. They also found that dextrans with lower M_w would reverse aggregation induced by dextrans with higher M_w. Gelin[5] hypothesized that intravascular aggregation was the principal cause of the anemia of injury. He and his collaborators elaborated on this hypothesis by using low molecular weight dextran fractions to prevent circulating red cell loss[5] and intravascular thrombosis[6] following experimental trauma. By demonstrating a cause and effect relationship between intravascular aggregation and diffuse necrotic lesions in parenchymatous organs[7], Gelin supplied the counterproof to Knisely's[8] contributions on the pathophysiologic significance of blood sludging.

Although earlier work on low molecular weight dextrans had been carried out by members of the United States Army[9], the present interest in this country stems from the observations on the use of Rheomacrodex^R during periods of extracorporeal circulation at the University of Minnesota.[10-14]

Pharmacologic Effects

Intravascular Aggregation

Probably the principal pharmacologic importance of Rheomacrodex^R is related to its ability to prevent or reverse aggregation of corpuscles *in vivo* or *in vitro*. When intravascular aggregation occurs, several phenomena can be observed in the microcirculation.[11] Initially, there is an increase in plasma skimming and axial accumulation of the corpuscles. Then there is occlusion of small venules where shear rates are low. When the intravascular aggregation is prolonged and severe, the adhesive forces between corpuscles increase, and occlusions occur in vessels where shear rates are high, such as the arterioles. Some of the occlusive phenomena are sufficiently permanent to cause thrombosis and microinfarcts. The administration of Rheomacrodex^R prevents or minimizes intravascular aggregation and microinfarction.[11,14]

The cause of intravascular aggregation and the method of prevention are still disputed. With phase microscopy[4] and with electron microscopy[15] a film has been observed on the surface of aggregated blood cells. This surface film probably is composed of the gel phase of macromolecules, such as dextran, gelatin or plasma proteins. The addition of Rheomacrodex^R to blood containing aggregated corpuscles results in the disappearance of the surface film and the dispersion of aggregates. The explanation presented by Thorsen and Hint[4] seems to apply best to the accumulated observations at this time. These authors used the following formula to explain their observations:

$$\ln \frac{C_{gel}}{C_{sol}} = KM$$

where C_{gel} equals the concentration of macromolecules in the gel phase, C_{sol} equals the concentration in the dissolved phase and M equals the number average molecular weight. The constant K is dependent on the nature of the macromolecules or mixture of macromolecules present in the system. The blood corpuscles and possibly also the vascular endothelium provide the phase boundary on which gelation of macromolecules occurs. Using this formula, it can be seen that addition of high molecular weight substances or partial polymerization of plasma proteins could result in an increase in C_{gel}. The addition of abnormal or denatured proteins increases the asymmetry of the macromolecules and increases the value of K. Any change increasing C_{gel} would increase the thixotropism of blood. Addition of Rheomacrodex^R would result in a decrease in M or average number molecular weight of a given system with a decrease in C_{gel} below a critical value.

Why the presence of a film on the corpuscles results in a change in their suspension stability could be explained on the basis of adherent properties of the gel or on the decrease of the normal, mutually repelling negative charges on the corpuscles. On phase microscopy it has been observed that this film does have stringy, adhesive properties when cells are partially separated by forceful action.[4]

Plasma Expansion

Rheomacrodex^R also serves as an effective plasma expander, although the duration of effect is not as prolonged as with expanders with larger molecules. After administration of Rheomacrodex^R in normal subjects, Gelin[16] found that the plasma volumes returned to preinfusion values approximately 90 minutes after the end of infusion. Renal excretion and decrease in plasma concentration occurred rapidly during the first hour after the infusion. When 500 ml of 10 per cent Rheomacrodex^R in saline was administered in one hour, approximately one-half of the dextran was excreted in the urine in the first three to five hours. Rheomacrodex^R also had a greater initial diuretic effect than Macrodex^R.[16] However, part of the rapid excretory rate and the shortness of plasma expanding effect reported by Gelin[16] may have been due to the rapid overloading of the blood volume in normovolemic subjects. When Rheomacrodex^R was given during extracorporeal circulation, plasma dextran values were elevated for longer periods of time than Gelin reported and excretion of approximately one-half of the dextran administered occurred within six to ten hours.[11,13]

Colloid Osmotic Pressure

The addition of Rheomacrodex^R to blood results in an increase in the number of colloid particles and therefore an increase in colloid osmotic pressure of the blood. The colloid osmotic pressure due to a given concentration of Rheomacrodex^R may be estimated from the following formula:[17]

$$P_{mm\ H_2O} = \frac{RT}{M_n} \cdot c + Ac^2$$

where

R = 8494 (100 cm^3 - mm H_2O/mole degrees)

T = 310 degrees K

M_n = 22,000

c = concentration in gm/100 cm^3

A = 14, the experimentally determined virial (?) coefficient for dextran.

Further calculations result in this simplified formula for easy reference in physiologic studies:

$$P_{mm\ H_2O} = 120\ C + 14\ C^2$$

From this formula it can be seen that a plasma concentration of Rheomacrodex^R of approximately 1 gm/100 cm^3 will result in an increase in colloid osmotic pressure of 10 mm Hg, and plasma concentrations of 1.5 gm/100 cm^3 and 2.0 gm/100 cm^3 will result in increments of about 15 and 22 mm Hg colloid osmotic pressure, respectively. Probably the principal errors introduced in this calculation result from the more rapid excretion of the smallest molecules and the dilution of colloid by fluid drawn from the interstitial space into the intravascular space. Hence, the time between cessation of administration and sampling would be of some importance. The significant increase in colloid osmotic pressure following Rheomacrodex^R infusions was shown in the studies by Gelin[16] in which volumes of 500 ml of 15 per cent Rheomacrodex^R to two normal individuals increased plasma volumes by 880 and 910 ml. A practical application of this principle is the prevention or therapy of pulmonary edema with Rheomacrodex^R.[11]

In estimating the total osmotic or colloid osmotic pressure effects of dextran and other colloids, one must use the number average molecular weight (M_n) and not the weight average molecular weight (M_w). The M_n is a measure of the number of molecules per unit weight of a substance and is determined by end group analysis or measurements of osmotic pressure across a membrane of a known quantity of substance. The M_w is measured by light dispersion and is a measure of the distribution of molecules according to size. The physical chemical principles of this important distinction have been given in detail by Wallenius[18] and by Thorsen and Hint.[4]

Diuresis

The clearance of different dextran fractions by the kidney has been studied by Wallenius.[18] In humans with no detectable proteinuria, the largest dextran molecules detected in the urine varied between molecular weights of 42,500 and 49,000. Larger molecules probably pass the glomerular membrane slowly. Thus, most if not all of the Rheomacrodex^R fraction can be filtered through the kidney. In extracorporeal circulation, the prevention of intravascular aggregation with Rheomacrodex^R was found to improve the renal cortical distribution of blood flow.[19] Under such

circumstances, the rheological properties of Rheomacrodex^R may contribute a relative diuretic effect. Large colloid molecules such as dextran exert only a small total osmotic diuretic effect when compared with small molecular weight substances such as mannitol. However, the physiologic effect may be out of proportion to the obvious expected effect when considering a colloid diuretic in the perspective of the counter-current theory for urine formation. The concentration of dextran in the urine may be several times that of the blood, depending on the state of hydration of the patient, the dose of Rheomacrodex^R and the time period after administration. Under such circumstances, the dextran molecules exert a colloid osmotic pressure effect across the capillary and tubule loops.

Viscosity

The addition of Rheomacrodex^R to whole blood or plasma results in an increase in viscosity when measured by the capillary tube method at high shear rates. This phenomenon corresponds with the increase in viscosity obtained by increasing the concentration of macromolecules such as albumin and dextran. Dintenfass[20] used a rotational viscometer and found that in patients with intravascular aggregation the viscosities were five to ten times greater at low shear rates than in normal patients. These observations tend to corroborate those of Gelin[1] in which he found a decrease in whole blood and plasma viscosity when Rheomacrodex^R was given to subjects with intravascular aggregation.

Therapeutic Dose and Evaluation of Effectiveness

The blood level of dextran effective in preventing intravascular aggregation was determined to be approximately 1.2 to 1.6 gm per cent.[11] This effective blood level was determined by administering Rheomacrodex^R to subjects undergoing extracorporeal circulation[11,14] or dogs subjected to hypercapnic acidosis.[12] The microcirculation of the ocular conjunctiva or the mesentery was observed for the appearance of intravascular aggregation as well as its prevention. Blood samples were drawn to determine the concentration of dextran at any given time and the blood levels were correlated with the microcirculatory observations. Dextran in blood was determined by the anthrone method.[18]

After determining the effective dosage for experimental extracorporeal circulation, a comparable therapy scale was used in patient studies. The microcirculation of the ocular conjunctiva of patients was studied postoperatively for the onset of intravascular aggregation. Depending on the cardiotomy blood loss after extracorporeal circulation and on the volume of postoperative blood replacement, intravascular aggregation was observed 2 to 12 hours after extracorporeal circulation. Dextran blood levels at the latter time averaged 0.8 gm per cent. Rheomacrodex^R was also given postoperatively to patients with intravascular aggregation and to two patients with idiopathic hemolytic anemia. Blood samples for dextran levels were drawn during and after the effective inhibition of intravascular aggregation. The lowest blood level at which intravascular aggregation was inhibited was 1.0 gm per cent.

The basic dosage scale suggested by Gelin[5] in his initial report was found to be accurate and was adapted with appropriate modifications. In patients undergoing extracorporeal circulation, doses of 2.0 to 3.0 gm/Kg have been used when the pump oxygenator circuit required priming volumes approximately equal to that of the patient's own blood volume. When the priming volume of the circuit is smaller, the dosage may be decreased accordingly. In postoperative patients with normal urine output and other patients requiring therapy, 2.0 to 3.0 gm/Kg should be given over a 24 hour period as part of the normal fluid requirements. The initial priming dose of 1.0 gm/Kg of Rheomacrodex^R may be given in 10 to 15 minutes. Bergentz[21] has presented an outline for the Rheomacrodex^R therapy of patients with burns, trauma, and surgery for vascular insufficiency. Bernstein[22] has given the method of therapy for the prevention of complications due to injections of radio-opaque media.

Methods for evaluating the effectiveness of Rheomacrodex^R therapy include direct observation and photographic recording of the microcirculation of the ocular conjunctiva, erythrocyte sedimentation rate, erythrophoresis, and viscosity measurements with rotational viscometers at low shear rates. Microcirculatory studies have the advantage of evaluating the primary point of interest. In experienced hands, microcirculatory studies can be precise and accurate. However, the method is inherently qualitative, and patient cooperation is required. Bjork[23] has used this method routinely and successfully in postoperative patients. Sedimentation rates are effective methods of evaluation when elevated, but false negative results have been recorded, particularly immediately after injury. Erythrophoresis, or measurements of red cell charge, have been used but require dilution of whole blood. Viscosity measurements at low shear rates, as mentioned above,[1,20] have not been used widely but may prove to be the most effective and quantitative method for evaluating the adequacy of Rheomacrodex^R therapy.

Toxicity and Side Effects

Rheomacrodex^R has been administered to mice to determine a lethal dose of 42 gm/Kg.[1] This dose is much higher than that obtained in dogs. The discrepancy probably is due to differences in species, differences in the rate of administration, or differences in the endpoint used to determine toxicity. Tables 1, 2, and 3 illustrate the results of tolerance studies with Rheomacrodex^R in this laboratory using healthy dogs under pentobarbital sodium anesthesia. Human serum albumin, 10 per cent solution in saline, was used for comparison (Table 4). Serum albumin rather than homologous plasma was selected because it is an established plasma expander commercially available in sterile solutions. Dogs do not manifest hypersensitivity to human serum albumin on initial intravenous injection although hypersensitivity reactions have been recorded with homologous dog plasma.[24] When Rheomacrodex^R, 10 per cent solution in saline, was administered intravenously in a dosage of 10 to 15 gm/Kg over an average time of 48.2 minutes, 50 per cent of the dogs died.[14] The results were similar in human serum albumin studies in which five surviving dogs received 11.7 gm/Kg and five non-survivors received 11.0 gm/Kg. The average duration of administration was 48.1 minutes. Pulmonary hemorrhage, intestinal hemorrhage, mesenteric hemorrhage, or combinations of these phenomena were thought to be the immediate cause of death in all dogs except one.

TABLE 1

Tolerance to Rheomacrodex^{R} 10 per cent in Saline 10 to 15 gm/Kg

Dog	Weight Kg	Dose gm/Kg	Time given min.	Results
1	13.0	11.5	48	Died after 60 min. Bleeding from mouth. Diffuse pulmonary hemorrhage.
2	9.0	15.0	65	Died after 10 min. Bleeding from mouth. Diffuse pulmonary hemorrhage.
3	9.0	12.8	43	Died after 4 min. Hemorrhage into mesentery. Hemoperitoneum of 300 ml.
4	9.1	10.9	40	Survived. Bleeding from nasal orifices stopped spontaneously.
5	13.	11.5	31	Survived.
6	10.	10.	40	Died after 15 min. Bleeding from mouth. Diffuse patchy pulmonary hemorrhage.
7	11.5	12.6	30	Died after 30 min. Bleeding from nose. Diffuse patchy pulmonary hemorrhage.
8	9.	11.5	55	Survived. Sacrificed two days later. Normal gross findings.
9	9.	11.	65	Survived. Sacrificed two days later. Normal gross findings.
10	9.	12.	65	Survived. Bleeding from mouth stopped spontaneously. Two days later patchy pulmonary hemorrhage.

TABLE 2

Tolerance to Rheomacrodex^{R} 10 per cent in Saline <10 gm/Kg

Dog	Weight Kg	Dose gm/Kg	Time given min.	Results
1	11.	4.	80	Survived.
2	15.	6.7	31	Survived. Bleeding from vagina 50 ml. Stopped spontaneously.
3	10.	7.	56	Survived. Three days later sacrificed. Normal gross findings.
4	9.5	8.	60	Survived. Three days later patchy pulmonary hemorrhage.
5	7.	7.	30	Died after 4 hours. Diffuse intestinal hemorrhages at tapeworm attachment.
6	10.	7.	30	Died after 4 hours. Diffuse hemorrhage in intestine, mesentery, peritoneum and lungs.
7	13.5	6.	30	Survived. Three days later sacrificed. Circumferential hemorrhage in 2nd part duodenum with gastric distention.
8	9.5	7.4	68	Died after 150 min. Bleeding from nose. Pulmonary edema and hemorrhage. Pericardial tamponade.
9	10.	7.5	75	Survived. Two days later patchy pulmonary hemorrhage.
10	9.	9.6	45	Survived. Two days later normal gross findings.

TABLE 3

Tolerance to Rheomacrodex^{R} in High Dose or High Concentration

Dog	Weight Kg	% Solution	Dose gm/Kg	Time given min.	Results
1	12.5	50	20.6	52	Died after 2 min. Bleeding from mouth. Diffuse pulmonary hemorrhage and intestinal and mesenteric hemorrhage.
2	12.2	31	10.	12	Survived. A few flecks of blood in vomitus.
3	12.2	30	12.3	30	Survived.
4	10.	20	16.5	20	Died after 5 min. Diffuse patchy pulmonary hemorrhage.
5	9.	20	9.6	180	Survived.
6	10.	20	8.	177	Survived.
7	10.9	15	20.	42	Died after 9 min. Pulmonary, intestinal, and mesenteric hemorrhage.
8	11.8	15	19.1	35	Survived.
9	10.	15	15.	23	Survived.
10	10.	15	13.1	35	Died after 5 min. Bleeding from mouth. Hemoperitoneum 50 ml.
11	10.	15	12.8	30	Survived.
12	10.	15	10.5	75	Sacrificed after 60 min. Normal gross findings.
13	8.	15	9.2	26	Died after 13 min. Patchy pulmonary hemorrhage and mesenteric hemorrhage.

TABLE 4

Tolerance to Human Serum Albumin 10 per cent in Saline 10 to 15 gm/Kg

Dog	Weight Kg	Dose gm/Kg	Time given min.	Results
1	11.5	11.7	45	Survived. Five days later sacrificed. Diffuse pulmonary hemorrhage. Hemorrhage in cardiac atria, gall bladder, cecum and appendix.
2	9.5	12.1	35	Died after 4 min. Diffuse patchy pulmonary hemorrhage. Intestinal congestion and hemorrhage. Hemorrhage into thymus and lumph nodes.
3	7.5	10.	40	Died after 5 min. Pulmonary edema and cardiac tamponade. Diffuse hemorrhage in intestines and gall bladder.
4	9.	13.9	35	Survived.
5	9.	12.8	42	Died after 10 min. Patchy pulmonary hemorrhage. Edema and hemorrhage of gall bladder.
6	9.5	10.5	45	Survived. Bleeding per rectum stopped spontaneously.
7	9.8	10.2	60	Survived. Two days later pleural effusion 400 ml. Hemorrhage in cardiac atria.
8	8.	10.	60	Died after 10 min. Pulmonary hemorrhage. Ascites 300 ml.
9	7.5	10.	50	Died after 5 min. Pulmonary congestion.
10	10.	12.	75	Survived. Two days later patchy pulmonary hemorrhage and hemorrhage in cardiac atria.

After determining the LD_{50} for Rheomacrodex^R to be approximately 10 to 15 gm/Kg, additional studies were performed to determine toxicity at sub-lethal dose levels. Persistent prolongation of bleeding time was selected as the most likely first sign of toxicity. The method for determining bleeding time was that of Jacobson[25] modified for use in dogs.[14] The results are given in Tables 5 and 6. Bleeding times were persistently prolonged after 4 to 6 gm/Kg of 10 per cent Rheomacrodex^R [13] and after 5 to 6 gm/Kg of 10 per cent albumin. When these dose levels were reached, intravascular aggregation was observed in the microcirculation of the ocular conjunctiva. The systemic venous pressure and portal venous pressures increased to levels of 40 and 50 cm of saline respectively.

TABLE 5

Toxicity of Rheomacrodex^R

Dog	Weight Kg	Per Cent Solution	Time given min.	Bleeding time persistently prolonged at dose in gm/Kg
1	11.	10	84	4.
2	8.5	10	140	6.
3	11.8	10	57	5.
4	9.	10	90	4.
5	10.	15	75	8.2
6	10.	20	83	6.
7	9.	20	190	9.6

TABLE 6

Toxicity of Human Serum Albumin

Dog	Weight Kg	Per Cent Solution	Time given min.	Bleeding time persistently prolonged at dose in gm/Kg
1	12.	10	46	5
2	7.5	10	102	6
3	10.5	10	113	5
4	14.5	10	70	5
5	10.	10	60	5

The etiology of the toxicity of large quantities of Rheomacrodex^R and albumin given in a short period of time may be the rapid overdistention of the vasculature with tissue injury, the production of intravascular aggregation or both. With these large doses the concentration of macromolecules may become sufficiently high to induce gelation of macromolecules such as fibrinogen on the surface of corpuscles according to the rules set forth by Thorsen and Hint.[4]

Bergentz, et al.,[26] compared the toxicity in dogs of therapeutic doses of Rheomacrodex^R with equal doses of high molecular weight dextran. They observed that Rheomacrodex^R did not produce any significant changes in the coagulation mechanism. High molecular weight dextran (M_W = 1,000,000) produced prolongation of the bleeding time and coagulation time, thrombocytopenia, pathologic prothrombin consumption, decrease in fibrinogen, prothrombin and factor VII, factor V and AHG. Heparin treatment prevented the decrease in fibrinogen, prothrombin, factor VII and factor V but not the thrombocytopenia. They concluded that the coagulation defects induced by dextran infusions were due to intravascular aggregation of platelets resulting in intravascular coagulation and consumption of coagulation factors. Long, et al.,[11] and Melrose[27] found that Rheomacrodex^R given during extracorporeal circulation in humans reduced the usual thrombocytopenia significantly and did not interfere with the coagulation mechanism or increase postoperative bleeding. Gelin, et al.,[28] administered 1000 ml of Rheomacrodex^R intravenously in two hours in ten healthy patients. They observed a transient thrombocytopenia, and a transient prolongation of bleeding time above control levels but within normal limits. Two patients had an increased capillary fragility, and in seven patients a pathologic thrombin generation was noted.

Antigenicity of various dextran moieties has been reviewed by Grönwall.[2] The studies reported indicated that antigenicity to dextran decreased with decreasing molecular weight and with decreased branching. In the clinical studies with Rheomacrodex^R reported thus far,[11,21] no suggestion of antigenicity of Rheomacrodex^R has been reported.

Contra-indications to the use of Rheomacrodex^R have been suggested by Gelin and his colleagues[22] to be pulmonary edema, thrombocytopenia, and localized septic processes. In the experience of Long, et al.,[11] Rheomacrodex^R was efficacious in the therapy of pulmonary edema. This concept is consistent with the physical principles regarding the mechanism of pulmonary edema secondary to cardiac failure. Increasing colloid osmotic pressure of blood should be and is effective in overcoming filtration due to increases in pulmonary capillary hydrostatic pressure. In the case of thrombocytopenia, further dilution of thrombocytes by intravenous infusions of dextran or platelet-poor bank blood has obvious dangers. However, some of the experimental[26] and clinical evidence[11,27] cited above suggests that a tendency to thrombocytopenia may be decreased by therapeutic doses of Rheomacrodex^R when the thrombocytopenia is due to intravascular aggregation. The evidence presented by Gelin[21] on septic processes is not convincing in that traumatized patients with septic processes frequently develop septicemia as demonstrated by Gelin's[21] data. Furthermore, there is no evidence to date to indicate that intravascular aggregation plays a role in isolating septic processes. Clinically a decrease in blood supply to an infected area is deleterious to the isolation and irradication of infection.

Effect on Crossmatching of Blood

Difficulties have been encountered in typing and crossmatching blood when high molecular weight dextran was present in the blood or when Macrodex^R was present and the typing was performed with whole blood and not 3 to 10 per cent suspensions of erythrocytes.[29] The difficulties were related to the production of

pseudoagglutination, aggregation, or rouleaux formation. Therapeutic doses of Rheomacrodex^{R} do not produce rouleaux formation or aggregation but will reverse this phenomenon when it is present. The question was raised whether Rheomacrodex^{R} would inhibit true agglutination and therefore interfere with typing or crossmatching of blood or even be effective in the treatment of incompatible blood transfusion reactions. Routine typing and crossmatching was performed with 2 per cent concentrations of Rheomacrodex^{R}. There was no interference with true agglutination and no false positive reactions were observed.[30]

Either systemic or selective cardiac hypothermia is frequently employed in cardiac surgery. Cold autoagglutinins have been found in a number of candidates for surgery. When Rheomacrodex^{R} was added to the blood of these patients in concentrations of 1 to 2 per cent, cold agglutination was inhibited.[13,31] This observation has added another indication for the use of Rheomacrodex^{R} in open heart surgery when hypothermia is employed.

REFERENCES

1. Gelin, L. E., and Ingelman, B.: Rheomacrodex^{R} - A New Dextran Solution for Rheological Treatment of Impaired Capillary Flow, Acta Chir. Scand., 122:294, 1961.

2. Grönwall, A.: Dextran and Its Use in Colloidal Infusion Solutions, Almquist and Wiksell, Uppsala, 1957.

3. Grönwall, A., and Ingelman, B.: Untersuchunger Über Dextran und sein Verhalten vei parenteraler Zufuhr II, Acta Phys. Scand., 9:1, 1945.

4. Thorsen, G., and Hint, H.: Aggregation, Sedimentation and Intravascular Sedimentation of Erythrocytes, Acta Chir. Scand., Suppl. 154, 1950.

5. Gelin, L. E.: Studies in Anemia of Injury, Acta Chir. Scand., Suppl. 210, 1956.

6. Zederfeldt, B.: Studies on Wound Healing and Trauma, With Special Reference to Intravascular Aggregation of Erythrocytes, Acta Chir. Scand., Suppl. 224, 1957.

7. Fajers, C. M., and Gelin, L. E.: Kidney, Liver and Heart Damage from Trauma and from Induced Intravascular Aggregation of Blood Cells: an experimental study, Acta Path. et Microbiol. Scand., 46:97, 1959.

8. Knisely, M. H.; Eliot, T. S.; and Bloch, E. H.: Sludged Blood in Traumatic Shock, Arch. Surg., 51:220, 1945.

9. Bromwell, A. W.; Artz, C. P.; and Sako, Y.: Evaluation of Blood Loss from a Standardized Wound after Dextran, Surg. Forum, 5:809, 1954.

10. Long, D. M., Jr.; Sellers, R. D.; Buri, P.; McKelvey, J.; and Lillehei, C. W.: Microcirculation and Splanchno-Hepatic and Renal Blood Flow Studies During Prolonged Total Cardiopulmonary Bypass, Read at 21st Annual Meeting, Society of University Surgeons, 1960.

11. Long, D. M., Jr.; Sanchez, L.; Varco, R. L.; and Lillehei, C. W.: The use of Low Molecular Weight Dextran and Serum Albumin As Plasma Expanders in Extracorporeal Circulation, Surg., 50:12, 1961.

12. Long, D. M., Jr.; Meyer, M. W.; Brown, E. B., Jr.; and Lillehei, C. W.: Myocardial Necrosis and Electrocardiographic Changes Related to Microcirculatory Abnormalities, Amer. J. Cardiol., 10:695, 1962.

13. Long, D. M., Jr.: The Status of Plasma Expanders in Open Heart Surgery, Dis. Chest, 41:578, 1962.

14. Long, D. M., Jr.; Folkman, M. J.; McClenathan, J. E.: The Use of Low Molecular Weight Dextran in Extracorporeal Circulation, Hypothermia, and Hypercapnea, Read at XI Congress of the European Society of Cardio-vascular Surgery, 1962. In press.

15. Lee, W. H., Jr.; Krumhaar, D.; Fonkalsrud, E. W.; Schjeide, O. A.; and Maloney, J. V., Jr.: Denaturation of Plasma Proteins as a Cause of Morbidity and Death after Intracardiac Operations, Surg., 50:29, 1961.

16. Gelin, L. E.; Solvell, L.; and Zederfeldt, B.: The Plasma Volume Expanding Effect of Low Viscous Dextran and Macrodex^{R} Acta. Chir. Scand., 122:309, 1961.

17. Granath, K.: Personal communication, 1962.

18. Wallenius, G.: Renal Clearance of Dextran as a Measure of Glomerular Permeability, Almquist and Wiksells, Uppsala, 1954.

19. Finsterbusch, W.; Long, D. M., Jr.; Sellers, R. D.; Amphlatz, K.; and Lillehei, C. W.: Renal Arteriography During Extracorporeal Circulation in Dogs with a Preliminary Report upon the Effects of Low Molecular Weight Dextran, J. Thoracic Surg., 41:252, 1961.

20. Dintenfass, L.: Thixotrophy of Blood and Proneness to Thrombus Formation, Circ. Res., 11:233, 1962.

21. Bergentz, S. E.; Gelin, L. E.; Rudenstam, C. M.; and Zederfeldt, B.: Indications for the Use of Low Viscous Dextran in Surgery, Acta Chir. Scand., 122:343, 1961.

22. Bernstein, E. F., and Evans, R. L.: Low Molecular Weight Dextran, J.A.M.A., 174:1417, 1960.

23. Bjork, V. O.: Personal communication, 1962.

24. Ota, Y.; Camishion, R. C.; and Gibbon, J. H., Jr.: Dirofilariea Immitis (Heart Worms and Dipetalonema Species as Causes of Transfusion Reactions in Dogs), Surg., 51:518, 1962.

25. Jacobson, B. M.: Effects of Cortisone and Corticotrophin on Prolonged Bleeding Time; Treatment in Certain Hemorrhagic States (Pseudohemophilia), A.M.A. Arch. Int. Med., 92:471, 1953.

26. Bergentz, S. E.; Eiken, O.; Nilsson, I. M.: The Effect of Dextran of Various Molecular Weight on the Coagulation in Dogs, Thromb. Diath. Haem., 6:15, 1961.

27. Melrose, D. G.: The Use of Low Molecular Weight Dextran in Extracorporeal Circulation, Read at the XI Congress of the European Society of Cardio-vascular Surgery, 1962. In press.

28. Gelin, L. E.; Korsan-Bengtsen, K.; Ygge, J.; and Zederfeldt, B.: Influence of Low Viscous Dextran on the Hemostatic Mechanism, Acta Chir. Scand., 122:324, 1961.

29. Pettenkofer, H.: Zur Blutgruppebestimmung nach Verabreichung von kolloidalen plasmaersatzmitteln, Chirug, 30:25, 1959.

30. Ziegler, N. F.: Personal communication, 1960.

31. McClenathan, J. E.: Personal communication, 1961.

THE RED BLOOD CELL ENVELOPE IN INTRAVASCULAR HEMAGGLUTINATION*

The Role of Low Molecular Weight Dextran

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Since the early observations of intravascular hemagglutination by Fahraeus in 1921[1] and Knisely in 1941,[2,3] there has been considerable controversy regarding the physiological, pathological, and clinical significance of this phenomenon in health and disease. In addition, the mechanism producing "blood sludge" (as Knisely has termed the phenomenon) has remained obscure. Bloch,[4] and Knisely,[3] have postulated that the erythrocytes in sludged blood are stuck together by a "sticky coating" which could be only vaguely identified by Bloch[5] in his study on electron microscopy of sludged blood. Recently, Long, Rush, and others have revived a clinical interest in this phenomenon because of its obvious occurrence in cases associated with the use of extracorporeal circulation.[6,7,8]

The occurrence of intravascular hemagglutination in the syndromes of burn, trauma, hyperlipemia, prolonged pump oxygenator perfusion, shock states, hypothermia, and other disease states has been well documented by visual observation of the microcirculation.[4,6,7,8,9,10,11] However, there has been little attempt previously to estimate whether or not a given example of blood sludge represents an elastic agglutination of cells which can deform to pass through the smaller branches of the microvascular bed, or whether the sludge is rigid and produces occlusion of terminal arterioles and/or capillary beds. It would seem that this factor must be included in any quantitative estimate of the degree of severity of intravascular hemagglutination in an effort to define the clinical significance.

The clinical significance of intravascular hemagglutination would seem to present a three-fold potential: 1) occlusion of microvascular beds by rigid clumps of red cells; 2) a decrease in velocity of flow to the point of overt stasis secondary to semi-rigid sludges, and increased viscosity; 3) the settling phenomenon as described by Knisely[12] which may result in the perfusion of cellular elements with clear plasma in some areas, while capillary beds are impacted with sedimented erythrocytes in other areas.

Several sets of data, previously published or recently accumulated in this laboratory, strongly suggest that rigid intravascular hemagglutination produces clinically significant pathophysiology.

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1. In a recent study of intravascular hemagglutination associated with protein denaturation in pump oxygenator devices, rigid blood sludging was associated with the production of neurological damage, cardiovascular instability and shock, and death in experimental animals.[8] These data suggested that the sludge which occurred following prolonged pump oxygenator perfusion was caused by the adsorption of denatured globulin macromolecules onto the erythrocyte surfaces with subsequent intermolecular bridging of such molecules binding the cells together in a gel-like matrix. The abnormal coating of such erythrocytes was demonstrated clearly by electron microscopy.

2. In a recent pilot investigation in this laboratory, isolated renal perfusion of the kidneys of dogs (following contralateral nephrectomy) utilizing crosscirculation from the femoral artery and vein of a healthy anesthetized donor dog after one hour of perfusion produced no significant elevation of the blood-urea-nitrogen subsequently. However, in similar experiments, when the donor dog's circulation was altered by the production of a rigid sludge (produced by a thermal burn of the hind quarters or by infusion of 10 cc. per kilogram of body weight of thermally denatured plasma) there was a marked rise in BUN for one to two weeks following the procedure. Figure 1 summarizes the results of the BUN analyses of these experiments in 21 animals.[13]

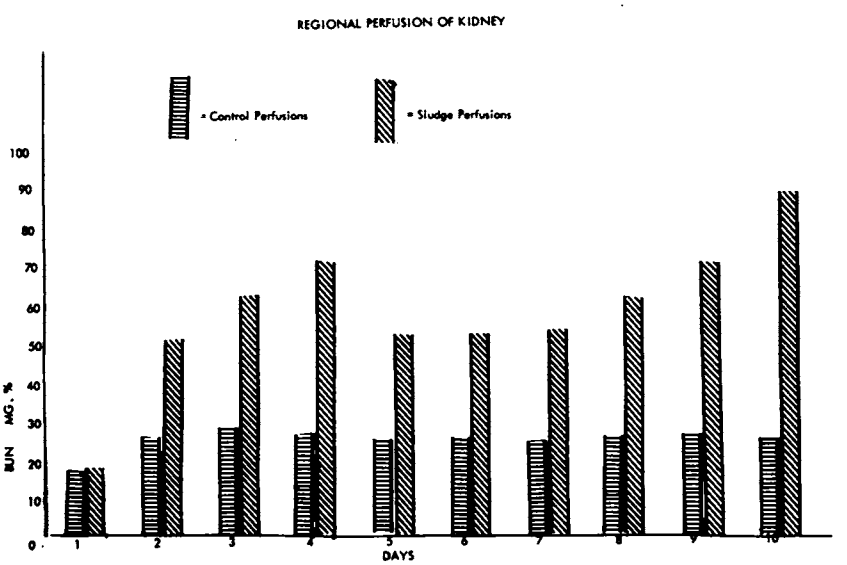

Figure 1

3. In another recent investigation of the effects of a standard thermal burn stimulus upon the microcirculation as visualized in the bulbar conjunctiva of the dog, there was a rough correlation between the degree of severity of rigid intravascular hemagglutination and acute mortality. Temperatures which were measured in the subcutaneous tissues of these burned animals (tissues which contained flowing blood) were recorded at levels demonstrated to produce measurable protein denaturation.[14]

4. Detailed observations of the conjunctival microcirculation of humans experiencing a rigid intravascular hemagglutination associated with prolonged pump

oxygenator perfusion, septic shock, or extensive thermal burns revealed a dramatic improvement in their clinical condition following amelioration of their rigid sludging by the administration of low molecular weight dextran in saline. Other investigators have reported the reversal of severe sludging by LMWD.[6,15,16,17]

On the basis of the above observations and considerations, it seems reasonable and justified to construct a working hypothesis: (a) that intravascular hemagglutination, if rigid, may produce clinically serious aberrations of microcirculatory physiology (e.g., microvascular occlusion, sequestration, and decreased tissue perfusion); (b) that at least some types of rigid sludge may result from the intermolecular bridging, or polymerization, of altered macroglobular proteins adsorbed onto the red cell membrane as a protein envelope.

In an effort to define further the character of such an envelope, the following experiments were done: Freshly drawn heparinized blood was centrifuged and the plasma separated. Aliquots of the plasma and of the cells were set aside for controls. The plasma was then heated in a water bath at 55 degrees C. for one minute. This maneuver had previously been determined to produce measurable gross denaturation of soluble plasma proteins both in vivo and in vitro.[14] Following this the plasma was remixed with its red cells and allowed to stand at room temperature for 30 minutes. The blood was again centrifuged, and the plasma removed. The control plasma and the thermal-denatured plasma were both analyzed by standard paper electrophoresis for proteins. The control red cells and the cells which had been incubated with the thermal-denatured plasma were then washed in isotonic sucrose solution until the supernatant washing gave a negative test for protein by the qualitative ninhydrin technique. The final protein-negative sucrose washing was saved for subsequent electrophoretic analysis. Identical aliquots of the cells were then washed, one each in the following solutions: .85 per cent sodium chloride, low molecular weight dextran in saline, and low molecular weight dextran in dextrose. Each of these washings was analyzed by electrophoresis for protein. Previous experiments using the differential washing technique reported by Ditzel[18] had established that the proteinacious material which could be extracted from the red cells was probably globulin in character, since it appeared to be insoluble in crystalloid solution but soluble in dilute saline solution and had the electrophoretic mobility of the globulins. Figure 2 is representative of the tracings obtained from such experiments. The normal globulin coating which is known to envelop the red cell membrane[19] is found to be greater in amount, has an altered electrophoretic mobility, and may vary considerably in specific identity as determined by electrophoresis.

Because of the high efficiency of extraction obtained in the saline washings, the question arises as to whether much of the antisludge or rheologic effect of low molecular weight dextran may be due to the saline vehicle in which it is suspended. Figure 3 demonstrates, however, that low molecular weight dextran in dextrose may also profoundly affect the protein capsule of red cells. The nature of the mechanism by which low molecular weight dextran influences the protein capsule is unknown. Rothman has previously demonstrated that dextrans are adsorbed to the erythrocyte membrane by an isotope tracer technique utilizing carbon 14 labeled dextran.[20] The results described above would tend to suggest that this adsorption does not merely represent a competitive affinity with protein for the erythrocyte membrane, but rather suggests that the dextran enters the colloidal system and exerts an active influence on the protein moiety itself. Bernstein's data would be

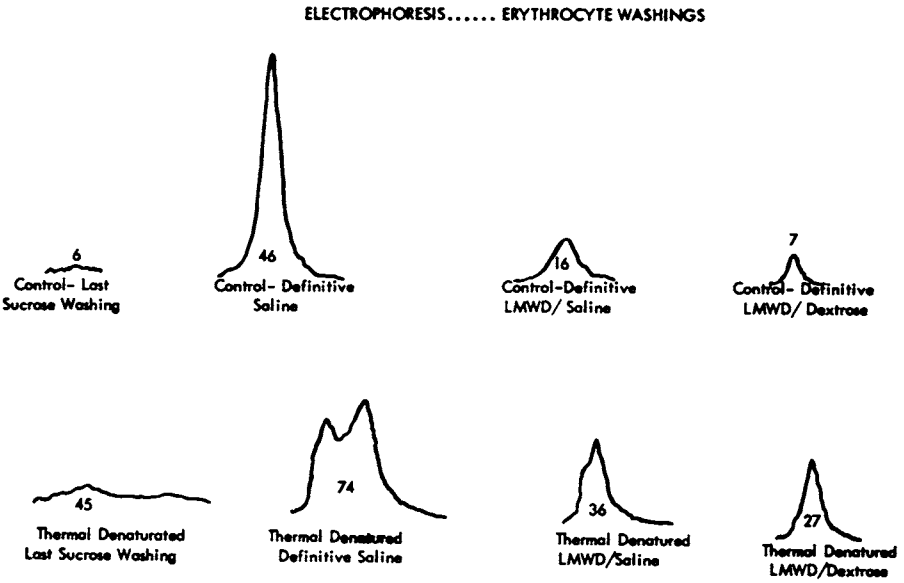

Figure 2

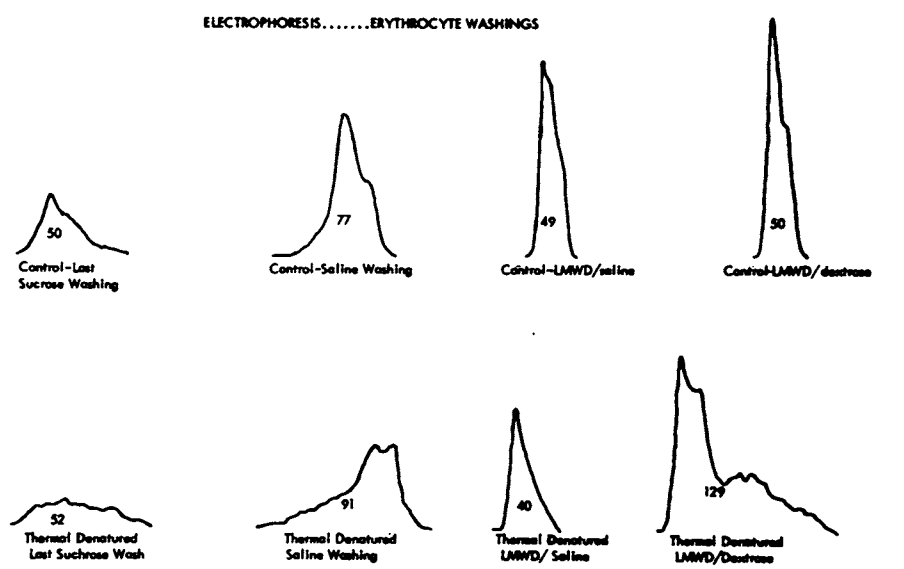

Figure 3

compatible with this hypothesis, demonstrating an increased electronegativity of erythrocytes in a charged polar field when low molecular weight dextran is added to the medium.[21]

In conclusion there would seem to be little doubt that a rigid intravascular hemagglutination may comprise an important and hazardous parameter of the total pathological physiology of several disease states. The fragmentary data which have been accumulated regarding the mechanism of intravascular hemagglutination and possible mechanisms for preventing or reversing this phenomenon provide little more than intriguing areas for future investigation.

REFERENCES

1. Fahraeus, Robin: The Suspension Stability of the Blood, Acta Med. Scand., 55:1, 1921.

2. Knisely, M H.; Stratman-Thomas, W. K ; and Eliot, T. S.: Capillary Circulation in the Malarial Infected Monkey, JAMA, 116:2430, 1941.

3. Knisely, M. H., and Bloch, E. H.: Microscopic Observations of Intravascular Agglutination of Red Cells and Consequent Sludging of the Blood in Human Diseases, Anat. Rec., 82:426, 1942.

4. Bloch, E. H.: Microscopic Observations of the Circulating Blood in the Bulbar Conjunctiva in Man in Health and Disease, Ergebnisse der Anatomic und Entwicklungs-Geschichte, 36:1, 1956.

5. Bloch, E. H., and Powell, A.: Electron Microscopy of Human Erythrocytes from Healthy and Sludged Blood, Science, 115:46, 1952.

6. Long, D. M.; Sanchez, L.; Varco, R. L.; and Lillehei, C. W.: The Use of Low Molecular Weight Dextran and Serum Albumin As Plasma Expanders in Extracorporeal Circulation, Surgery, 50:12, 1961.

7. Rush, B. F.; Wilder, R. J.; Fishbein, R.; and Ravitch, M. M.: Effects of Total Circulatory Standstill in Profound Hypothermia, Surgery, 50:40, 1961.

8. Lee, W. H., Jr.; Krumhaar, D.; Fonkalsrud, E. W.; Schjeide, O. A.; and Maloney, J. V., Jr.: Denaturation of Plasma Proteins as a Cause of Morbidity and Death after Intracardiac Operations, Surgery, 50:29, 1961.

9. Bigelow, W. G.; Heimbecker, R. O ; and Harrison, C. R.: Intravascular Agglutination (Sludged Blood), Vascular Stasis, and Sedimentation Rate of the Blood in Trauma, Arch. Surgery, 59:667, 1949.

10. Heimbecker, R. O., and Bigelow, W. G.: Intravascular Agglutination of Erythrocytes (Sludged Blood) and Traumatic Shock, Surgery, 28:461, 1950.

11. Higginbotham, A. C., and Williams, A. V.: Intravascular Agglutination of the Blood Following Fat Ingestion; a Possible Factor in the Production of Angina Pectoris and Coronary Thromboses and in the Pathogenesis of Atherosclerosis, J. South Carolina Med. Assoc., 53:1, 1957.

12. Knisely, M. H.; Warner, L.; and Harding, F.: Antemortem Settling, Angiology, 11:535, 1960.

13. Lee, W. H., Jr.: The Effects of Burn-Induced Blood Sludge Upon Renal Function (unpublished data).

14. Jackson, T., and Lee, W. H., Jr.: The Role of Plasma Protein Denaturation in the Thermal Burn Syndrome, Scheduled for presentation at the Southeastern Surgical Congress, March, 1963. (unpublished)

15. Löfström, B.: Induced Hypothermia and Intravascular Aggregation, Acta Anaes. Scand., Suppl 3, 1959.

16. Gelin, Lars-Erik, and Ingelman, B.: Rheomacrodex^{R} - A New Solution for Rheological Treatment of Impaired Capillary Flow, Acta Chir. Scand., 122:294, 1961.

17. Bernstein, E. F., and Evans, R. L.: Low Molecular Weight Dextran, JAMA, 174, 1417, 1960.

18. Ditzel, J : Relationships of Blood Protein Composition to Intravascular Erythrocyte Aggregation (Sludged Blood), Acta Med. Scand., Suppl 343, 1959.

19. Pirofsky, B.; Cordova, M.; and Imel, T. L.: The Non-Immunologic Reaction of Globulin Molecules with the Erythrocyte Surface, Vox Sanguinis, 7:334, 1962.

20. Rothman, S.; Adelson, E.; Schwebel, A.; and Langdell, R. D.: Adsorption of Carbon-14 Dextran to Human Blood Platelets and Red Blood Cells, in Vitro, Joint Report with the National Bureau of Standards, U. S. Dept. of Commerce, WRAIR - 191-56, Dec., 1956.

21. Bernstein, E. F.; Emmings, F. G.; Mackey, G. C.; Castaneda, A.; and Varco, R. L.: Effect of Low Molecular Weight Dextran on Red Blood Cell Charge During Extracorporeal Circulation, Transactions, American Society for Artificial Internal Organs, 8:23, 1961.

DISCUSSION

Dr. Strumia: Our interest in dextran is based on its profound protective effect on red cells which are subjected to freezing and thawing. In this respect, we found that dextrans are all similarly protective above a 10 per cent concentration. Above a 17 per cent concentration, dextran is highly protective in vitro but the erythrocytes disappear rapidly when re-transfused. It is necessary to utilize a higher concentration of the lower molecular weight material to achieve an equal protective effect on the erythrocyte. We have thus tested dextrans ranging from the molecular weight 40,000 to 300,000 but have not yet utilized 40,000 or LMWD.

Dr. Melvin Knisely: I would like to remind you that capillary rupture can occur following thrombosis of an artery or capillary due to necrosis of the blood vessel wall. Could it be that in Dr. Long's experiments the dextran could not reach the involved vessels due to plugging?

Dr. Long: We found that production of hypercapneic acidosis by respiring dogs on 30 to 40 per cent carbon dioxide produced severe intravascular erythrocyte aggregation. By chance I noted that animals that simultaneously underwent hepatectomy or resection of the gut did not develop such cellular aggregation despite their acidosis. The explanation to this is not clear.

We also observed, as did Dr. Knisely, that high doses of LMWD produced some cellular aggregation.

Dr. Pearson: Dr. Lee raised a point about the success with which these agglomerates pass through the microvessels. I wondered whether he had any evidence as to how successfully these agglomerates pass through the small blood vessels.

Dr. Lee: No, not quantitative evidence. It is my impression that the first sludge that forms following trauma is fairly elastic and does not cause much plugging of the vessels. It seems as though these masses become more rigid as time goes on. In pump perfusion the changes on the erythrocyte begin to appear in 30 minutes, within 15 minutes after a thermal burn, within 30 minutes following endotoxin or septic shock, and within an hour following metabolic acidosis or lactic acid infusions.

Dr. Boba: Dr. Gelin has shown that by giving high molecular weight dextran the cardiac output goes down and that by giving low molecular weight dextran cardiac output will increase. I would like to know what would have happened if he had not given the low molecular weight dextran? In other words, would the dogs have died in shock?

Also, did they really appear to be in shock? Did they look ashen gray or cyanotic, for instance?

Dr. Gelin: I would like to stress there is a progressive drop in cardiac output. If it were merely a matter of viscosity, the drop in cardiac output should reasonably have been maximum immediately after the infusion when the viscosity was its highest. But this was not the case so we can hypothesize an additional factor which progressively decreases cardiac output. Parallel with this effect was an increase in stagnation on the venular side of the capillary bed.

In answer to the second question, the animals looked ill, would not eat, moved little, and were oliguric. They had, however, an increased rather than a decreased blood pressure. If given 2 grams of high molecular dextran, they would die. We utilized 1 gram in our studies.

IN VITRO STUDIES OF THE INFLUENCE OF DIFFERENT COLLOIDS ON THE AGGREGATION OF ERYTHROCYTES

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The aim of this paper is to describe a simple quantitative in vitro method of estimating the erythrocyte aggregating properties of plasma or artificial colloids and present some results obtained concerning colloids not previously tested for their aggregating properties.

The method, first published by Thorsen and Hint in 1950, is based on the measurement of erythrocyte sedimentation rate in Westergren tubes using a series of dilutions of plasma or artificial colloids. One part of washed human erythrocytes is suspended in two parts of each dilution of suspension fluid. In this way a fixed hematocrit value is obtained. This is important because hematocrit is known to have a very great influence on the sedimentation rate of erythrocytes.

The logarithms of sedimentation rate after one hour are plotted on Figure 1 against the logarithms of the colloid concentrations in the respective tubes. As can be seen from the figure, the points lie approximately on straight lines with nearly the same slopes. The points where the lines cut the abscissa give the concentration of colloid which causes a sedimentation rate of 1 mm per hour. Observe that this parameter, called the critical concentration by Thorsen and Hint, is not dependent on the colloid concentrations in individual tubes and can, therefore, be considered as a quantitative measure for the erythrocyte aggregating power of plasma. When the colloid concentration is lower than critical, the erythrocyte aggregation disappears; and when the concentration is raised, aggregation and sedimentation rate rise very rapidly.

The relation between colloid concentration and sedimentation rate may be expressed by the formula seen in Figure 2 where C is concentration, C_{crit} is critical concentration and n represents the slope of the line on the graph.

The reproducibility of the method may be illustrated by experiments made with one commercial brand of gelatin manufactured for transfusion purposes. Three experiments with blood corpuscles from two different donors give very nearly the same values as seen from this slide.

We have no evidence that there is any variation between the aggregating properties of erythrocytes from different persons. On the other hand, erythrocytes from different species can vary greatly in this respect.

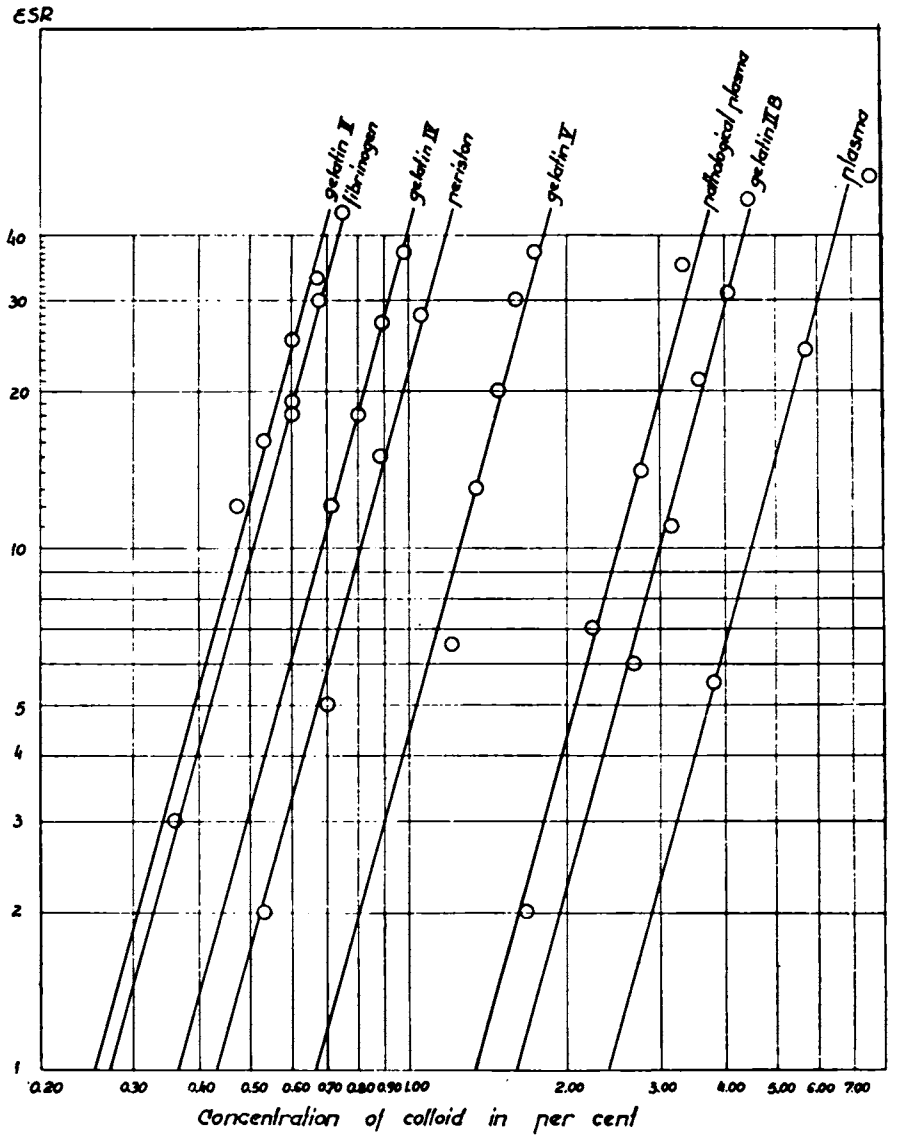

Figure 1. Relation between the logarithms of the colloid concentration and the sedimentation rate.

The values of critical concentrations obtained for some of the colloids tested are presented in the table. A sample of pathological plasma shows a critical concentration of 1.3 per cent. This means that by diluting such a plasma five to six times, aggregation disappears.

Fibrinogen shows a critical concentration of 0.28 per cent, and this is very near to the normal value of fibrinogen in plasma. A rise of fibrinogen concentration to two or three times its normal value causes a high sedimentation rate; this corresponds well with clinical experience.

The critical concentrations for dextran fractions depend on the molecular weight of the fraction in question. Swedish clinical dextran gives no aggregation if

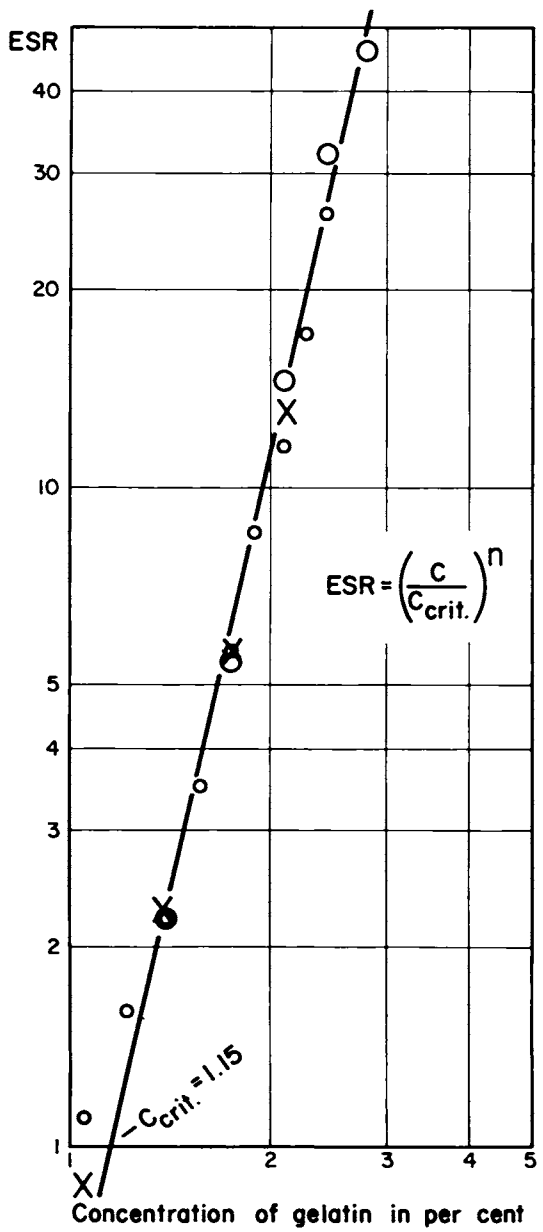

Figure 2. Relation between colloid concentration and sedimentation rate.

concentration is kept below 0.9 per cent. Low molecular dextran with a mean molecular weight of 40,000 as in Rheomacrodex^R gives no aggregation of erythrocytes in vitro and diminishes aggregation if added to pathological plasma.

Thorsen and Hint proposed in their publication of 1950 the hypothesis that the aggregating properties of a colloid depend mainly on the size and asymmetry of the colloidal molecules, the elongated molecules having a very high aggregating power while spherical ones have a much lower aggregating power.

In hitherto unpublished experiments performed by Dr. Richter and Hint, the following were investigated:

a) the cellulose derivative Cellosize, known to consist of very asymmetrical and stiff molecules, and

b) Ficoll, a sucrose polymer, which is known to have nearly spherical molecules.

As can be seen from the table, fractions of cellosize are the most powerful aggregating colloids known, the highest fraction showing a critical concentration of about 5 mg per cent. Ficoll, on the other hand, shows a very low aggregating power in spite of its high molecular weight.

Experiments done with cellosize are of interest because these reveal a possibility that very low concentrations of some specific plasma colloids may theoretically play a considerable part in the total aggregating power of plasma.

The method described above gives a parameter for the aggregating powers of colloids, colloid fractions or mixtures of colloids—as, for example, plasma or plasma-containing dextran. While sedimentation rate by the Westergren's method is influenced also by concentration of colloids in plasma and by hematocrit, the critical concentration estimated by our method gives a parameter for the aggregating power of the various colloids which is not influenced by hematocrit or colloid concentration in the individual tubes.

TABLE

Colloid	Molecular Weight	Critical Concentration
Cellosize 4400	---	0.005
Cellosize 300	250,000	0.010
Cellosize 3	---	0.020
Cellosize 09	---	0.030
Fibrinogen	500,000	0.28
Swedish clinical dextran	75,000	0.90 - 1.3
Ficoll	280,000	1.0
Ficoll	2,000,000	0.4
Pathological plasma	---	1.3
Normal plasma	---	2.5 - 5.0

EFFECT OF THE LOW MOLECULAR WEIGHT DEXTRAN ON THE SUSPENSION OF THE BLOOD CELLS IN VITRO WITH SPECIAL REFERENCE TO THE "SLUDGING PHENOMENON" CAUSED BY POLYBASIC MOLECULES

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The stability of the suspension of the blood cells was evaluated by the sedimentation rate and the measurement of the cell charge.

Red cells became more negatively charged in the presence of low molecular weight dextran. The negative charges were found to increase by 30 to 50 per cent. The negative charge on the granulocytes and lymphocytes doubled in magnitude. The effect was more apparent with the lymphocytes.

The increase in the negativity was enhanced by the addition of surface acting agents. Pluronic and Triton were used for this purpose.

Polyacidic molecules like EDTA, heparin, and other polysulfonic acid compounds were either found to cause no change in the charge of the red cells or sometimes even to decrease it. This apparent decrease in the charge was associated with folding of the cell membrane. On the other hand, these polyacidic molecules were found to increase the negativity of the white cells, thus enhancing the dextran effect.

Few polybasic compounds have been tested. The effects of Protamine^{R} Sulfate and Polybrene^{R} have been reported. These compounds are known to cause agglutination or "sludging" of the red cells. This phenomenon is enhanced by the presence of the low molecular weight dextran. Sludging tends to start with less polybasic material when the concentration of dextran in the blood increases above 1 - 1-1/2 per cent.

The increase in the "sludging" tendency with Polybrene^{R} and dextran on one hand and the prevention of the clinically observed "sludging" of the red cells in cases of trauma and burns by dextran suggest that the two phenomena are probably different in nature although they have the same visual appearance, which is red cell agglutination.

METHODS OF MEASUREMENT OF RED BLOOD AGGREGATION

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I. Introduction

The phenomenon of red blood cell aggregation, or clumping, has been described by a number of investigators,[11,29,37,38] and has been associated with various pathological or diseased states. However, others[25] have described a similar phenomenon associated with normal physiological events, such as pregnancy. These observations of RBC aggregation have generally been made by relatively low power direct microscopy, particularly of the bulbar conjunctival microcirculation.

Many causative factors have been implicated in changing the microcirculation from homogeneous capillary flow to a flow pattern containing clumps of red blood cells with clear areas of plasma between, associated with slow flow, hyperchromatic blood cells, and sludging or stasis of these red blood cell clumps. Because intravital microscopy and cinematography are both subjectively interpreted phenomena, a need for more precise and quantitative means of measuring red blood cell aggregation has become apparent. Other properties of whole blood, and of its constituents, have therefore been measured in attempts to obtain a single quantitative test for determining the presence and degree of red blood cell aggregation. That none of these has succeeded is evident from the multiplicity of procedures still in use. Further, simple identification and quantitation of the phenomenon of aggregation is inadequate to determine the causative mechanism of the aggregation, whether it is pathologic, or it requires treatment. This review attempts to summarize the currently available methods of studying red blood cell aggregation, and offers some suggestions regarding unfilled needs and new methods and approaches to this problem.

II. The Microcirculation

Knisely and his associates[38,39,40,41] used high power stereobinocular microscopy, with magnifications up to 600x, in their initial observations of blood sludging. This has remained the classical method of observing the phenomenon, both clinically and experimentally. Such observations have included the microcirculation of the conjunctiva, omentum, mesentery, pia mater, and subcutaneous tissues. Various investigators have added cinematography,[11] and in some instances television cinematography,[33] in order to obtain permanent records of their observations.

Intravital microcirculatory studies remain the only method of identifying the aggregation phenomenon (by definition) in the intact animal and in the human. As such, they constitute the basis for comparison with any other method. In general, good quality stereobinocular microscopes capable of magnification up to 100 diameters appear to offer the best balance between accurate detailed observation and stable visual fields.

III. Erythrocyte Sedimentation Rate

The first classical studies of the "suspension stability" of the blood, involving correlation with the erythrocyte sedimentation rate, were by the Swedish pathologist, Reuben Fahraeus, in 1921.[26] The sedimentation rate is dependent upon the number of red blood cells present, their state of agglutination, and the quantity of fibrin or fibrinogen present. Fahraeus found it markedly increased in patients who were pregnant and in those who had infectious diseases. He realized the importance of the factors in Stoke's Law*[52] and tried to clarify the relative roles of density, viscosity, and red blood cell particle size. Of these, he thought that red blood cell particle size, particularly when increased (during aggregation), was the most important. In addition, he felt that the larger plasma proteins (globulin and especially fibrinogen) were important in increasing the sedimentation rate, while albumin appeared to decrease it. In a later review of this problem,[25] Fahraeus said that red blood cell aggregation was not pathological when seen in association with pregnancy. While the sedimentation rate was a measure of suspension stability of the blood, he felt that rouleaux formation was a related non-pathological process, which was a surface tension, dependent phenomenon.

The technique, as described by Westergren,[58] involves the use of a citrate anti-coagulant and sedimentation tubes which are 30 cm. long with a 2.5 mm. diameter. These are filled with the blood sample and permitted to stand in a vertical position. Timed observations are made at one, two, and 24 hours, although the single figure at one hour is most commonly employed. Sedimentation rates greater than 7 mm/hr were considered probably pathologic by Westergren, while any sedimentation rate greater than 12 mm/hr was definitely pathologic. Other discussions of this procedure have been presented by Eastham[22] and Ham.[32]

The sedimentation rate, then, was the earliest in vitro technique thought to be a quantitative means of identifying red blood cell aggregation and entirely comparable to in vivo microcirculatory observations. That this association does not always exist has recently been demonstrated by Salzman,[7] who has demonstrated a lack of correlation between the development of marked red blood cell aggregation following the injection of various radiopaque substances, and a decrease in the erythrocyte sedimentation rate (Table). Similarly, Long[43] has described a lack of correlation between the development of red cell aggregation during extracorporeal

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* $V = \frac{2}{9} g \frac{S-S}{\eta} r^2$, where V = velocity (cm/sec), g = gravity (cm/sec^2), S = density (gm/cm^3), η = suspension fluid viscosity (Poise) and r = falling particle diameter (cm).

TABLE

Erythrocyte sedimentation rates following in vitro mixtures of canine blood and several radiopaque contrast media (0.02 cc contrast material +5 cc canine blood), demonstrating the marked decrease in sedimentation rate seen, despite the development of RBC aggregation.

	Mean ESR (10 expts.) (mm 1 hr.)
1. Control blood	15.9
2. Cholografin 52%	0.4
3. Hypaque 90%	15.1
4. Ditriokon 68%	12.8
5. Urokon 70%	0.9
6. Diodrast 35%	5.4

circulation and changes in the sedimentation rate. Therefore, changes in sedimentation rate appear to be associated with the more chronic conditions associated with red blood cell aggregation, but may not be a reliable indicator of the state of aggregation in several more acute situations.

IV. Viscosity

Among the earliest studies of blood viscosity were those of Burton and Opitz[17,18] who used a U-tube or capillary tube viscometer. Most measurements of viscosity have been based on the theory of Poiseuille[48] which described the relationship of the viscosity of a moving fluid to the tube in which the fluid was flowing, and particularly the importance of the diameter of this tube.* In 1931, Fahraeus and Lindquist,[27] in a classical paper, demonstrated that the viscosity of blood appears to decrease when very narrow capillary tubes are used for such studies. Therefore, (1) Poiseuille's Law does not apply to the flow of blood in tubes of diameters smaller than 0.3 mm. (such as capillaries); and (2) blood is not an ideal or Newtonian fluid. Another important series of studies of blood viscosity were performed by Bingham and Roepke in the early 1940's.[8,9,10] These demonstrated the importance of fibrinogen in determining the fluidity of blood, as well as the role of the hemoglobin content and the albumin/globulin ratio, but basically they reemphasized the non-Newtonian nature of blood. The use of an isolated hind-limb preparation as a viscometer was described by Whittaker and Winton[58] in 1933, but almost all other studies have involved the use of capillary tube techniques.[5,23,47,53]

* $Q = \frac{\pi R^4 \Delta P}{8 \eta L}$ where Q = flow rate (cm^3/sec), R = tube radius (cm^2), ΔP = pressure ($dynes/cm^2$) difference along length L(cm), and η = fluid viscosity (Poise).

Studies with such simple capillary tube measurements have been carried out in our laboratory and have proven quite useful and informative, as demonstrated by depicted results during partial extracorporeal circulation in the dog (Figure 1).

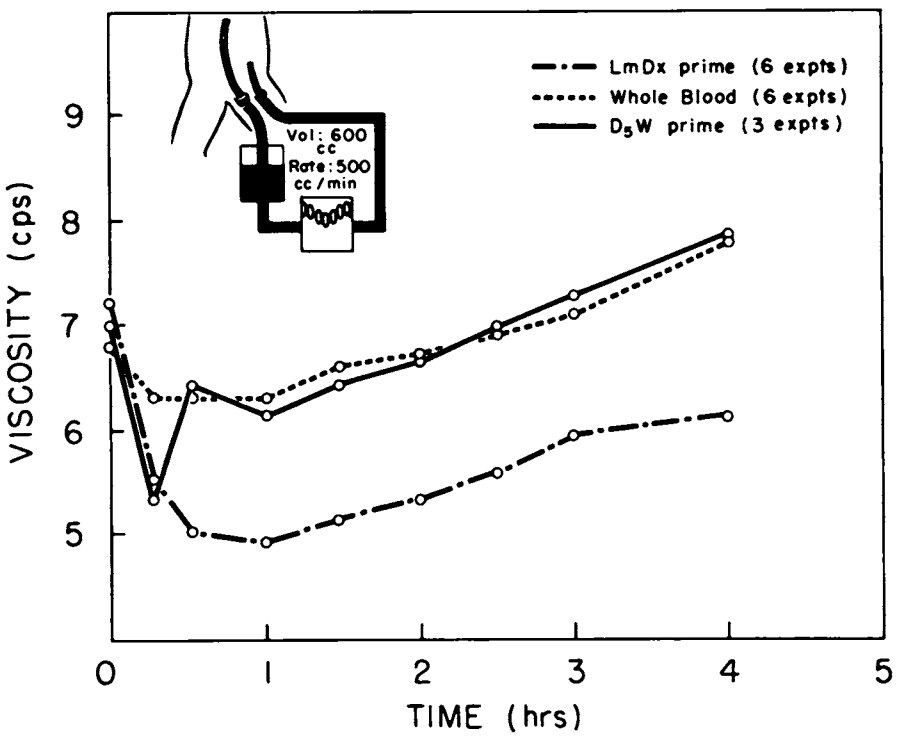

Figure 1. Viscosity data obtained with a simple capillary tube viscometer, indicating the ability of low molecular weight dextran to cause prolonged depression in whole blood viscosity in vivo, during partial extracorporeal perfusions.

More recently, the non-Newtonian nature of whole blood has been re-emphasized by Wells and Merrill,[57] and by Czerny[20] and Dintenfass[21] and Haynes.[34] These authors have stressed the importance of shear rate viscometers in measurements of blood viscosity.

Shear stress is the tangential force per unit area on a fluid plane causing sliding, and one which is related to the frictional forces during flow and determined in magnitude by the viscous characteristics of the fluid. The shear rate is the velocity gradient between adjacent planes of layers of fluid during flow. The ratio of shear stress over shear rate is a quantitative indication of the flow property of any fluid. If this ratio is constant over any shear rate, the fluid is simple and Newtonian. However, since blood is not Newtonian, shear stress must be measured at specified rates of shear.

Certainly, more sophisticated studies of viscosity, with a consideration of the shear rate, are indicated, and for these, the Brookfield* viscometers appear most suitable.

*Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

V. "Apparent RBC Viscosity" and "Stokes' Radius"

These two mathematical concepts have been developed by Evans[5,7] in an effort to derive some simple numerical indices, based on measurements of viscosity, density, hematocrit, and sedimentation rate, which may be of value in quantitating red blood cell aggregation. They are evidence of the inability of the measurements themselves to accurately distinguish degrees of aggregation, much less its etiologic mechanism.

"Apparent RBC viscosity" is based on the assumption that the viscosity of whole blood is the sum of the viscosity of the red blood cell portion and the viscosity of the plasma fraction.* An example of its usefulness in demonstrating marked changes in apparent red blood cell viscosity, which correlated well with changes in in vivo red blood cell aggregation but not with changes in the sedimentation rate, is presented in Figure 2, which compares measurements taken before and after the injection of massive amounts of intravenous radiopaque contrast media in dogs. Prior premedication with low molecular weight dextran was effective in maintaining an essentially normal "apparent RBC viscosity" and also prevented or markedly diminished the development of red blood cell aggregation and death in these animals. Another example of the usefulness of this technique was demonstrated by Salzman in in vitro and in vivo studies with Cholografin, a biliary tract radiopaque. This substance also produces red blood cell aggregation in vivo, which correlates well with the apparent red blood cell viscosity measurements. However, as demonstrated in the table, mixtures of Cholografin and blood result in a marked depression in sedimentation rate, probably the first documented instance of the simultaneous observation of a decrease in sedimentation rate with an increase in red blood cell aggregation.

"Stokes' radius"** is simply the calculated radius of the falling particle as determined by measurement of all of the other variables of Stokes' Law,[52] including red blood cell and plasma density, plasma viscosity, and the velocity of the falling particles as measured by the sedimentation rate. It should represent the "effective radius" of the falling RBC particles, whether falling separately or as aggregates. In the Cholografin studies, such measurements are inversely proportional to the apparent red blood cell viscosity, and appear to parallel measurements of the sedimentation rate per se. Whether this is a useful concept, and one which may prove to be of value in future studies of aggregation under various conditions remains to be seen.

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* $\eta RBC = \frac{\eta BL - (I-H)\,\eta\, PL}{H}$, where η = viscosity (Poise), H = hematocrit (%), BL = whole blood, PL = plasma, and RBC = red blood cell.

** "Stokes' radius": "Active gravity" vs "Stokes' resistance"

$$\frac{4}{3}\pi r^3 (S-S_1)\, g = 6\pi r v \eta \qquad r = \sqrt{\frac{9}{2} \cdot \frac{v\eta}{(S-S_1)\, g}}$$

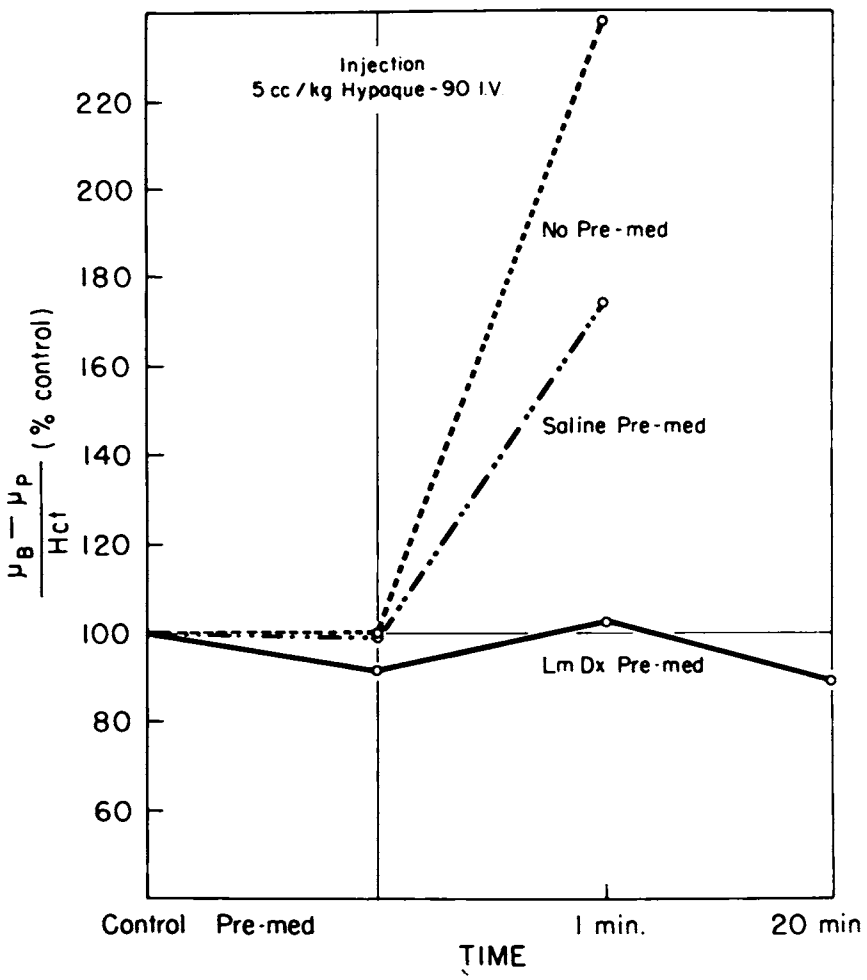

Figure 2. "Apparent RBC viscosity" changes following the intravenous injection of 5 cc Hypaque -M-90/kg body weight in the dog. Premedication with saline has little beneficial effect, but low molecular weight dextran prevents these changes entirely.

VI. Red Blood Cell Charge

The importance of static electrical charge in the mutual repulsion of red blood cells as a source of suspension stability was first suggested by Jevons in 1870.[36] However, practical means for the measurement of this charge were not available until the work of Northrup and his associates,[44] and the re-emphasis and refinement of these techniques by Abramson, Moyer, and Gorin in the 1930's. Such measurements are performed in a microelectrophoresis chamber with a known electrical potential gradient placed across it. Samples of diluted red blood cells in their own plasma are placed in the chamber, and the velocity of the individual RBC's are measured with the aid of darkfield microscopy and a calibrated micrometer

eyepiece. If the viscosity of the plasma is measured, the charge of the red cells can be calculated. *

However, red blood cell charge is not the only, or dominant, factor in all forms of aggregation reactions. Evidence to this effect has been presented by Sachtleben,[49] and has been confirmed by our own group in studies with various radiopaque agents,[7] antiheparin agents,[19] extracorporeal circulation,[6] and low molecular weight dextran.[6] Our technique of measurement of RBC charge and the results of several experiments are presented in Figures 3 and 4.

Recent improvements in chamber design permitting the use of smaller specimens with good temperature control have been designed by Bangham[2] and Seaman.[50]

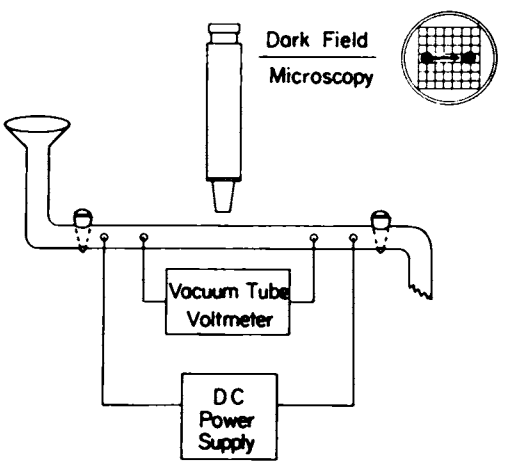

Figure 3. Schematic diagram of red blood cell microelectrophoresis technique, indicating the essential components of the system, and the use of darkfield microscopy and a calibrated viscometer eyepiece to measure the velocity of individual red blood cells under the influence of an electrical potential gradient.

VII. Electron Microscopy

Electron microscopy of red blood cells has been performed by several groups interested in the aggregation phenomenon, principally the groups of Bloch,[13,14,15] Katchalsky,[37] and Lee.[42] This appears to be one of the best and most reliable means of measuring a specific characteristic of the red blood cell commonly seen with aggregation. Bloch, in 1953, indicated that he felt electron microscopy was the only true in vitro indicator of red blood cell aggregation. He found that sludged cells have a coating which also forms bridges between them. In vivo, even in situations in which aggregation is quite evident, such an RBC coating cannot be seen with light microscopy. However, electron microscopic studies have shown a fuzzy red blood cell surface under all circumstances associated with red blood cell aggregation.

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* $Q = \frac{6\pi r \eta v}{Ex}$ where Q = charge (coulombs), r = radius of RBC (cm), η = plasma viscosity (Poise), v = RBC velocity (cm/sec) and Ex = potential gradient (volts/cm).

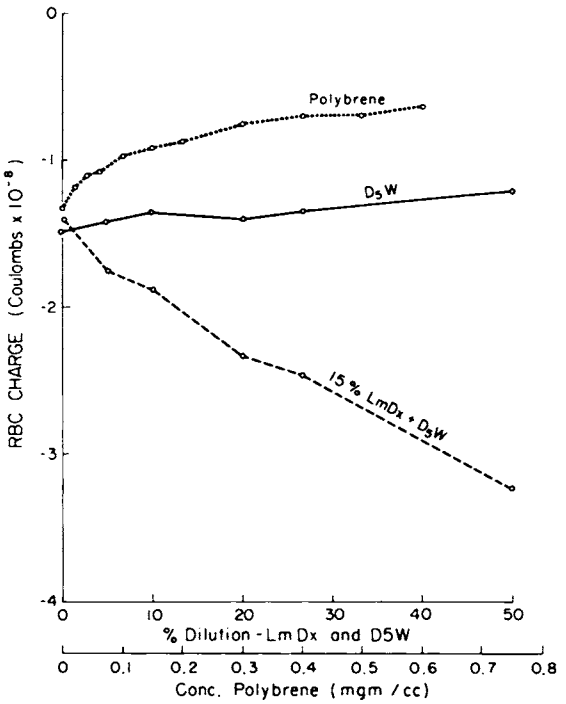

Figure 4. Summary of RBC charge determinations following in vitro mixtures of blood and low molecular weight dextran, which results in a marked increase in red blood cellular electronegativity. The addition of Polybrene, a polybasic substance, results in decreasing the normal RBC electrical charge.

Bloch also describes a milder form of aggregation, in which only slowly moving red cells aggregate and re-separate when the velocity of the stream increases. These cells frequently still appear sharp and clear in the electron microscope.

Electron density of the fuzzy edge of the red blood cell is identical to the appearance of hemoglobin. However, simple hemolysis of normal red blood cells does not produce this fuzzy edge on other cells.

Katchalsky[37] and his group have studied both intact red blood cells and ghosts with the electron microscope, as well as with other physico-chemical tools. They found marked changes in membrane texture, as well as a film of polybasic molecules which may bridge red cells, following the addition of polybasic substances. Lee[42] has demonstrated a similar coating of red blood cells following prolonged extracorporeal pumping and oxygenation. Prankerd[46] has also demonstrated 50 Angstrom thick placques of protein or lipid substance on the outside of red blood cells. He feels they may be combinations of antigens and antibodies, or may be the result of electrical charge phenomena.

Electron microscopy appears to be an extremely useful if not simple tool which may provide much more information in the future when combined with other methods of investigation. However, it certainly does not appear to offer an answer to the clinician's need for a readily applicable clinical tool.

VIII. Surface Tension

While a great deal has been written about the role of surface or interfacial tension between red blood cells, no direct measurements of this force have been

made. The surface tension between plasma and air can be measured, and that between plasma and the blood vessel wall can be measured. However, there is still no means of measuring the surface of primary interest, which is that between the red blood cell and plasma. Until the development of such a technique the free surface energy per unit area of red cell surface must remain an estimated but unknown quantity.

IX. Optical Density

Several recent studies have demonstrated that transparent solutions such as hypertonic salts or radiopaque compounds are capable of producing changes in optical density in the reverse direction of those which would be expected from simple dilution. That is, they produce an increase in optical density. This has been attributed to changes in cell size, shape, and orientation, by Sinclair[51] and his associates. It has been suggested that such techniques might be used as a simple in vivo method of studying red blood cell aggregation, and several pilot studies in this direction have been performed by Castaneda[19] using densitometers and recorders designed for hemodynamic indicator dilution measurements. Since a number of factors, including hemoglobin content, also affect the density of blood, simultaneous measurements of these are required. In spite of these drawbacks, use of densitometry as an indicator of the degree of aggregation appears worthy of further study. Figure 5 demonstrates such an increase in density following the

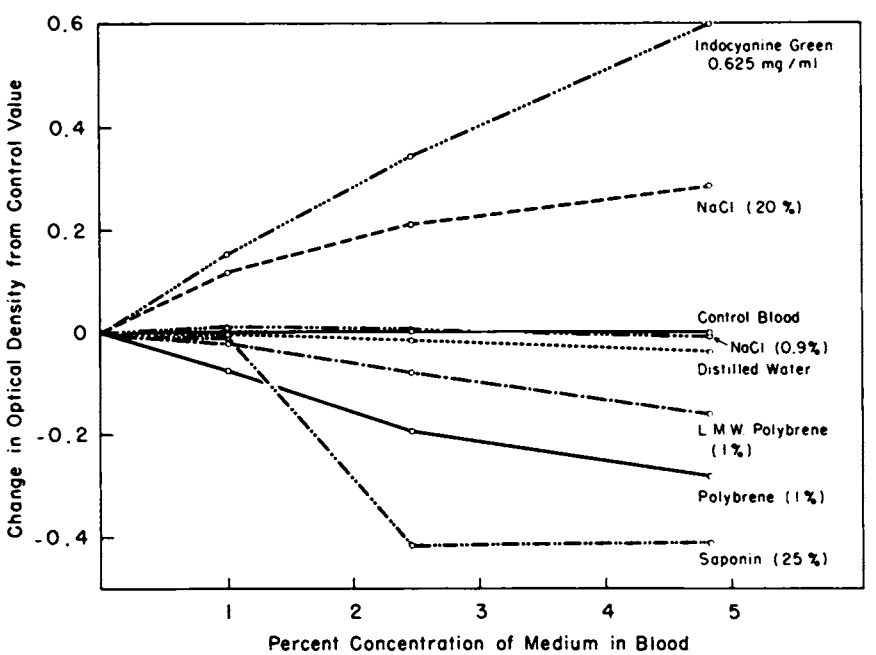

Figure 5. Summary of optical density measurements following in vitro mixtures of blood with equal volumes of hypertonic saline (20 per cent), isotonic saline, and distilled water, demonstrating a marked increase in O. D. with the hypertonic material. Polybrene causes a marked decrease in O. D., without changes in hemoglobin content.

in vitro addition of 20 per cent NaCl to blood, and a surprisingly marked decrease in O. D. measured after the addition of polybrene, a polybasic substance capable of causing very marked RBC aggregation, both in vitro and in vivo.

X. Filtration

While many authors have alluded to the presence of a sticky or glue-like substance in relation to red cell aggregation, a few have performed experiments indicating the presence of such a substance. In 1960, Fahraeus[24] used filter paper and suction to demonstrate the presence of large rouleaux or red cell aggregates. Similarly, Gaarder[28] demonstrated the effectiveness of adenosine diphosphate in causing adhesion or clumping of platelets, and implicated this substance as a trigger for platelet sticking and the subsequent development of thrombosis. Weiss,[56] in 1961, presented a summary of the currently available techniques for the measurement of cell adhesion. Of these, he felt that the older methods of detaching cells from surfaces with quantitative forces were probably inadequate. Instead, he advocated the measurement of cell adhesion by shearing distraction. Such a technique was employed with cells in cultured chambers and involved a rotating disk and counting the percentage of cells detached with a microscope. With this method, accurately determined shearing forces can be transmitted through the fluid to detach cells adherent to disks of various materials in vitro. Both the magnitude of the distractive force and the rate at which it is applied are considered to be important. Studies similar to those of Fahraeus were also reported by Swank[54] in 1952 in relation to acute exsanguination. This area appears to be a relatively undeveloped and possibly quite significant field for future studies of red blood cell aggregation, but presently is inadequately developed for routine clinical or laboratory use.

XI. Surface Chemistry

Chemical studies of the red blood cell surface and its reactions appear to be the most rewarding single area for future investigations. Other methods of study consider only the end result of one or more of a series of reactions, but are not sufficiently revealing to permit identification or quantitation of the specific process resulting in aggregation. Only surface chemistry can do this.

Aggregation may be related to the lipophilic colloidal properties of the red cell, or to red cell coating with substances released from damaged tissue cells,[39,40,41] or to the ability of multivalent cations to bridge such cells as a result of ionic charge phenomenon.[35] Certainly, it is in this area that the role of the various agents in the prevention or treatment of such aggregation must also be studied. The physical chemist will probably have to unravel the still unsolved problems of the relationship of various known etiologic and therapeutic agents to the general problem of red blood cell aggregation.

XII. Summary and Conclusions

A number of methods of identifying and attempting to quantitate the presence and degree of red blood cell aggregation have been described. These vary markedly

in their simplicity, clinical applicability, and reliability. However, all suffer from the inadequacies of being unable to identify or quantitate the mechanism resulting in, or preventing, red blood cell aggregation. Future studies of the surface chemistry of the red blood cell appear to be the most promising area for the further elucidation of this problem.

REFERENCES

1. Abramson, H. A.; Moyer, L. S.; and Gorin, M. H.: Electrophoresis of Proteins. Rheinhold Pub. Corp., New York, 1942.

2. Bangham, A. D.; Flemans, R.; Heard, D. H.; and Seaman, G. V. F.: An Apparatus for Microelectrophoresis of Small Particles, Nature, 182:642, 1958.

3. Bangham, A. D.; Seaman, G. V. F.; and Pethica, B. A.: The Charged Groups at the Interface of Some Blood Cells, Biochem. J. 69:12, 1958.

4. Bangham, A. D., and Pethica, B. A.: The Adhesiveness of Cells and the Nature of the Chemical Groups at their Surfaces, Proc. Roy. Phys. Cos. 28:43, 1960.

5. Bernstein, E. F.; Evans, R. L.; Blum, J. A.; and Avant, R. A.: Further Experimental and Early Clinical Observations of the Protective Action of Low Molecular Weight Dextran Upon Intravenous Hypaque Toxicity, Radiology 76:260, 1961.

6. Bernstein, E. F.; Emmings, F. G.; Mackey, G. C.; Castaneda, A.; and Varco, R. L.: Effect of Low Molecular Weight Dextran on Red Blood Cell Charge During Extracorporeal Circulation, Trans. Am. Soc. for Art. Int. Org. 8:23, 1962.

7. Bernstein, E. F.; Evans, R. L.; and Saltzman, S. F.: Experimental Studies of Certain Physico-Chemical Properties of Blood Following Exposure to Methylglucamine Iodopamide, and other Parenteral Contrast Media, Acta Radiol. Scand. (In Press)

8. Bingham, E. C., and Roepke, R. R.: The Rheology of the Blood. II. Effect of Fibrinogen on the Fluidity of Blood Plasma, J. Am. Chem. Soc. 64:1204, 1942.

9. Bingham, E. C., and Roepke, R. R.: The Rheology of the Blood. III. J. Gen. Physiol. 28:79, 1944.

10. Bingham, E. C., and Roepke, R. R.: The Rheology of the Blood. IV. The Fluidity of Whole Blood at 37°C, J. Gen. Physiol. 28:131, 1944.

11. Bloch, Edward H.: A Quantitative Study of the Hemodynamics in the Living Microvascular System, Am. J. Anat. 110:125, 1962.

12. Bloch, E. H.: Physical and Chemical Properties of Sludged Blood, Abstr. of Papers from Platform, Anat. Rec. 109:271, 1951.

13. Bloch, E. H.; Warner, L.; Kafig, E.; and Meryman, H. T.: Studies of the Mammalian Erythrocyte Surface by Electron Microscopy, Anat. Rec. 115:284, 1953.

14. Bloch, E. H., and Powell, A.: Electron Microscopy of Human Erythrocytes From Health and Sludged Blood, Science 115:46, 1952.

15. Bloch, E. H.; Powell, A.; Meryman, H. T.; Warner, L.; and Kafig, E.: A comparison of the Surfaces of Human Erythrocytes from Health and Disease by In Vivo Light Microscopy and In Vitro Electron Microscopy. Angiology, 7:479, 1956.

16. Bloch, Edward H.; McCuskey, R. S.; Tucker, G.; and Mencin, J.: The Effect of Cellular Aggregation on Pressure-Flow Relationships in the Microvascular System, Angiology 12(10):473, 1961.

17. Burton, A. C., and Opitz, R.: The Viscosity of the Blood, JAMA 57:353, 1911.

18. Burton, A. C., and Opitz, R.: The Effect of Changes in Temperature Upon the Viscosity of "Living" Blood, J. Exp. Med. 8:59, 1906.

19. Castaneda, A.; Bernstein, E. F.; Emmings, F. G.; and Fox, I. J.: Decreases in RBC Charge and in Optical Density (O. D.) of Blood Produced by Polybrene, The Physiologist 5:117, 1962.

20. Czerny, L. C.; Cook, F. B.; and Walker, C. C.: Rheology of Blood, Am. J. Phys. 202 (6):1188, 1962.

21. Dintenfass, L.: Considerations of the Internal Viscosity of Red Blood Cells and Its Effect on the Viscosity of Whole Blood, Angiology, 13:333, 1962.

22. Eastham, R. D.: Red Cell Factors and Erythrocyte Sedimentation Rate, Acta Med. Scand. 158:375, 1957.

23. Eckstein, R. W.; Book, D.; and Gregg, D. E.: Blood Viscosity Under Different Conditions and Its Effect on Blood Flow, Am. J. Physiol. 135:772, 1941.

24. Fahraeus, R.: Tissue Injury Due to Reduced Filterability of the Erythrocytes in the Biochemical Response to Injury, Blackwell Scientific Publications, Oxford, 1960, pp. 161-174.

25. Fahraeus, R.: The Suspension Stability of the Blood, Physiol. Rev. 9:241, 1929.

26. Fahraeus, R.: The Suspension Stability of the Blood, Acta Med. Scand. 55:1, 1921.

27. Fahraeus, R., and Lindquist, T.: Viscosity of Blood in Narrow Capillary Tubes, Am. J. Physiol. 96:562, 1931.

28. Gaarder, A.; Jonsen, J.; Laland, S.; Hellem, A.; and Owren, P. A.: Adenosine Diphosphate in Red Cells as a Factor in the Adhesiveness of Human Blood Platelets, Nature 192(4802):531, 1961.

29. Gelin, L., and Lofstrom, B.: Study of Peripheral Circulation During Deep Hypothermia. Observations on Decreased Suspension Stability of Blood and Its Prevention, Acta Chir. Scand. 108:402, 1955.

30. Gelin, L. E., and Shoemaker, W. C.: Hepatic Blood Flow and Microcirculatory Alterations Induced by Dextran of High and Low Viscosity, Surgery 49:713, 1961.

31. Gelin, L. E.: Studies in Anemia of Injury, Acta Chir. Scand. Suppl. 210, 1956.

32. Ham, T. H., and Curtis, F. C.: Sedimentation Rate of Erythrocytes: Influence of Technical, Erythrocyte, and Plasma Factors and Quantitative Comparison of Five Commonly Used Sedimentation Methods, Medicine 17:447, 1938.

33. Harders, Harald, and Fehse, Friedrich: Television Microcinematography of Human Bulbar Conjunctiva, Angiology 12(10):477, 1961.

34. Haynes, R. H.: Physical Basis of the Dependence of Blood Viscosity on Tube Radius, Am. J. Physiol. 198:1193, 1960.

35. Jandl, J. H., and Simmons, R. L.: Agglutination and Sensitization of Red Cells by Metallic Cations: Interactions Between Multivalent Metals and the Red Cell Membrane, Brit. J. Hemat. 3:19, 1957.

36. Jevons, W.: Tr. Manchester Phil. Soc. 1870, 78.

37. Katchalsky, A.; Danon, D.; and Nevo, A.: Interactions of Basic Polyelectrolytes with the Red Blood Cell, Biochem. et Biophys. Acta 33:120, 1959.

38. Knisely, M. H.; Bloch, B. H.; Eliot, T. S.; and Warner, L.: Sludged Blood, Science, 106:431, 1947.

39. Knisely, M. H.; Eliot, T. S.; and Bloch, E. H.: Sludged Blood in Traumatic Shock, Arch. Surg. 51:220, 1945.

40. Knisely, M. H., and Bloch, E. H.: Microscopic Observations of Intravascular Agglutination of Red Cells and Consequent Sludging of the Blood in Human Diseases, Anat. Rec. 82:426, 1942.

41. Knisely, M. H.: The Settling of Sludge During Life. First Observations, Evidences, Significances, Acta Anatomica, Vol. 44, Suppl. 41, 1961.

42. Lee, W. H., Jr.; Krumhaar, D.; Fonkalsrud, E. W.; Schjeide, O. A.; and Maloney, J. V., Jr.: Denaturation of Plasma Proteins as a Cause of Morbidity and Death After Intracardiac Operations, Surgery 50:29, 1961.

43. Long, D. M., Jr.; Sanchez, L.; Varco, R. L.; and Lillehei, C. W.: The Use of Low Molecular Weight Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation, Surgery 50:12, 1961.

44. Northrup, J. H., and Kunitz, M.: An Improved Type of Microscopic Electrocataphoresis Cell, J. Gen. Physiol 7:729, 1924.

45. Pethica, B. A.: The Physical Chemistry of Cell Adhesion, Exp. Cell Res. Suppl. 8:123, 1961.

46. Prankerd, T. A. J.: The Red Cell, Chas. C. Thomas, Springfield, 1961.

47. Pirofsky, B.: The Determination of Blood Viscosity in Man by a Method Based on Poiseuille's Law, J. Clin. Invest 32:292, 1953.

48. Poiseuille, J. L. M.: Recherches Experimentales Sur le Movements des Liquides Dans les Tubes de Tres Petits Diameters, Comptes. Rend. Acad. d. Sc. 11:961, 1041, 1840.

49. Sachtleben, P., and Ruhenstroth-Bauer, G.: Agglutination and the Electrical Surface Potential of Red Blood Cells, Nature 192(4806):982, 1961.

50. Seaman, G. V. F., and Beard, D. H.: A Microelectrophoresis Chamber of Small Volume for Use with Biological Systems, Blood 15(5):599, 1961.

51. Sinclair, J. D.; Sutterer, W. F.; Fox, I. J.; and Wood, E. H.: Apparent Dye-Dilution Curves Produced by Injection of Transparent Solutions, J. Appl. Physiol. 16:669, 1961.

52. Stokes, G. G.: Trans. Camb. Phil. Soc. 9:8, 1850.

53. Swank, R. L., and Roth, J. G.: Apparatus for Measuring Relative Blood Viscosity, Rev. Scient. Instruments 25:1020, 1954.

54. Swank, R. L.: Adhesiveness of Platelets and Leukocytes During Acute Exsanguination, Amer. J. Phys. 202(2):261, 1962.

55. Thorsen, G., and Hint, H.: Aggregation, Sedimentation and Intravascular Sludging of Erythrocytes, Acta Chir. Scand. Suppl. 154, 1950.

56. Weiss, L.: The Measurement of Cell Adhesion, Exp. Cell Res. Suppl. 8:141, 1961.

57. Wells, R. E., and Merrill, E. W.: The Variability of Blood Viscosity, Am. J. Med. 31:505, 1961.

58. Westergren, A.: Studies of the Suspension Stability of the Blood in Pulmonary Tuberculosis, Acta Med. Scand. 54:247, 1920.

59. Whittaker, S. R. F., and Winton, F. R.: The Apparent Viscosity of Blood Flowing in the Isolated Hind Limb of the Dog and Its Variation with Corpuscular Concentration, J. Physiol. 78:339, 1933.

60. White, H. L., and Monaghan, B.: Properties of Red Cell Surfaces Influencing Rouleaux Formation, Am. J. Physiol. 115:31, 1936.

DISCUSSION

Dr. Hint: I agree with Dr. Hilal that there are different causes of blood cell aggregations, some of which are counteracted by LMWD while others are not. In the latter category lies aggregation following administration of Polybrene. In the former are aggregations caused by addition of pathological plasma of varying types. For the latter anti-aggregation effect, relatively high concentration of LMWD was required.

There is wide species variation in the effect of LMWD on erythrocyte aggregation. Horse erythrocytes are relatively easily aggregated by LMWD while even dextran with a molecular weight of several million will not aggregate cattle red cells.

Dr. R. E. Semple: There is no doubt that the dextran macromolecule affects the surface properties of the red blood cell. The behavior of these cells in vivo must therefore be affected to some extent. This is shown by the changes in erythrocyte sedimentation rate (ESR) that can be correlated to some extent with both dextran concentration and with the mean molecular weight of the dextran used. It is possible that these changes in ESR are an indication of changes in the surface activity of red cells profound enough to affect the physiological activity of these cells in the circulation. However, the evidence available indicates that dextrans in concentrations (in plasma) up to three times those normally encountered clinically, and ranging in mean molecular weight from 30,000 to 150,000, do not do so to any measurable extent. Indeed the evidence from experiments carried out in a number of laboratories, including our own, indicates that some circulatory problems that are associated with haemorrhagic shock (e.g. stagnant anoxia, local anoxias) disappear or are ameliorated by adequate infusions of established clinical dextran preparations. These infusions are associated with improved liver and kidney function; both haematocrit and tagged-cell measurements give no indication of local accumulation or sequestration of erythrocytes.

A really satisfactory study of the effect of dextrans on red cells is very difficult because it is so hard to set up satisfactory controls. I would like to describe some experiments of ours in which we tried to correlate the molecular weight of the dextran used with its effect on ESR. Dextran fractions having mean molecular weights of 10, 30, 50, 90 and 150 x 10^3 were used and in all experiments every effort was made to carefully control temperature, osmotic pressure and red cell concentration. Our results were similar to those presented earlier by Dr. Gregersen. The two lowest molecular weight fractions had very little effect on ESR, the 50,000 fraction had a measurable effect and there were marked increases in ESR with the 90,000 and 150,000 molecular weight fractions. The

controls were a problem. These consisted of blood diluted with saline, blood diluted with its own plasma, as well as undiluted blood. There was no increase in ESR in the blood samples that were diluted with saline but in nine of twelve experiments, when 5 ml of blood was diluted with 1 ml of its own plasma, the ESR increased markedly; these increases being of the same order of magnitude as those observed when the same blood was diluted to the same extent with the 90,000 molecular weight dextran. I wondered if anyone else had ever noticed this phenomenon; we thought the controls were adequate but we are not at all sure now that they were.

I would like to point out, in this connection, that when dealing with the effect of macromolecules on the properties of blood, very often one finds that the results of in vivo experiments are quite different from the results obtained in vitro. Thus we tried sedimentation experiments, analogous to the in vitro tests, in which the dextran was mixed with blood in vivo. Controls were even more difficult under these conditions but the results, which are admittedly hard to evaluate, seem to indicate that the effect on ESR of high molecular weight dextran is somewhat less when the dextran is mixed with blood in the animal than it is when the mixing is carried out in vitro and, on the other hand, the effect of low molecular weight is, if anything, somewhat greater. This difference between results obtained in vivo and in vitro was brought out in another study in our laboratory in which we were investigating the mechanisms responsible for the wound bleeding often seen when the blood contains very high concentrations of dextran. We observed that blood, with high dextran concentrations (the dextran added in vitro), showed an increased prothrombin consumption time and that clots formed by it were weaker than those formed by blood containing no dextran. When the dextran concentration was of the same order of magnitude, after mixing in vivo, both prothrombin consumption time and clot strength were not significantly different from controls.

Low molecular weight dextran may have fewer of the negative aspects of higher molecular weight (100 to 125 x 10^3) but perhaps it has not the essential positive factors, either. There are a number of studies in the literature of survival time in haemorrhagic and traumatic shock in which it is rather convincing to me that dextrans of a molecular weight of 100,000 or greater are more effective than are low molecular weight dextran preparations. In this connection I would like to draw your attention to at least one report, this by Dr. R. Haist of the University of Toronto. In this investigation traumatic shock was produced in rats by a clamping technique. Controls (no treatment) all died in about 20 hours. There were between 10 and 25 to 30 per cent survivals among animals which received saline or dextrans which had molecular weights up to 90 and 100 x 10^3. None of these treated groups was significantly different from any other, although all were significantly different from the controls. However, a group which received dextran with a molecular weight of 150,000 had 50 per cent survivals; this group was significantly different from all of the other treated animals and controls.

Practical experience indicates that most of the problems that are associated with higher molecular weight dextrans are problems associated with high concentrations (2 to 3 grams/100 ml plasma). You would need to give two or more bottles of dextran to come even close to 2 grams per cent in the average human patient. The overall effectiveness of the higher molecular weight material has not been questioned, to my knowledge, today, and I would like to hear some more discussion on this point.

Dr. Lee: I would like to ask Dr. Semple if in his controls the plasma in which the cells were suspended was drawn into a vacuum? There has been evidence accumulated that the drawing of blood into a vacuum produces sufficient alteration in protein molecules to form macromolecules to a measurable degree.

Dr. Eiseman: How much of a vacuum?

Dr. Lee: A blood bank bottle vacuum, which is about 750 millimeters negative pressure, depending on the atmosphere in which you live.

I would also like to ask Dr. Hint and Dr. Hilal whether or not all of the LMWD that they used in their experiments was made up in saline or in dextrose, because I think there is considerable difference in the activity of the substance, depending upon whether it is suspended in one of these two. In fact, saline has a unique solvation effect on the gelled protein capsules of sludged cells and has a transient but very effective anti-sludge effect when infused rapidly.

Dr. Semple: The blood was drawn into syringes, not into a vacuum.

Dr. Hilal: The dextran was dissolved in a phosphate buffer and autoclaved. The solutions were prepared from powders supplied by the Swedish company. The osmolarity was controlled by measurement on a viscometer and the pH was kept at 7.35 in all the experiments.

Dr. Hint: My experiments were all made with saline. We first utilized a buffer but found that even slight hypertonicity produced no effect on the sedimentation rate; but the very slight hypotonicity had a very great effect. Therefore, we utilized slightly hypotonic saline solutions.

Maybe I can add the comment that dextran experiments with rats are totally unsuitable because these animals are extremely sensitive to the drug producing an epileptic shock with any kind of dextran. LMWD is actually more potent than ordinary dextran in this regard.

EVIDENCE THAT INTRACAPILLARY PLUGGING IS IMPORTANT IN SHOCK*#

Colonel Robert M. Hardaway, MC, USA
Walter Reed Army Institute of Research
Washington, D. C.

If one injects diluted bovine thrombin intra-aortically into dogs, the result is an immediate and dramatic fall in systemic arterial pressure. This persists for approximately 15 minutes and then a gradual climb towards normal begins which is reached usually in 30 to 60 minutes. However, under proper circumstances, a secondary fall in blood pressure begins which terminates in death in less than 24 hours (Fig. 1). There is a dramatic fall in platelets following injection of thrombin, and they almost disappear 20 minutes after injection. There is a recovery following this. Similarly there is a dramatic fall in fibrinogen after thrombin administration; the level is quite low after 20 minutes and there is a rapid recovery thereafter (Fig. 2). Lee-White clotting times and prothrombin times are both prolonged after injection of thrombin. The clot which forms will usually lyse on incubation. Figure 3 shows the preinjection sample still clotted, as is the 20-minute sample. However, the one-hour sample has completely lysed and denotes the development of endogenous fibrinolysin some time between 20 and 60 minutes following thrombin injection. Under proper circumstances these thrombin-injected dogs will die in less than 24 hours. At autopsy their gastrointestinal mucosa shows the typical mucosal hemorrhagic necrosis seen in canine shock (Fig. 4).

The injection of unfiltered human amniotic fluid results in a similar dramatic fall in systemic arterial blood pressure (Fig. 5). If the animal is heparinized before injection of amniotic fluid, this blood pressure fall does not take place (Fig. 6).

The injection of incompatible (human) blood under circumstances similar to bovine thrombin results in a similar dramatic fall in systemic arterial blood pressure with a gradual return towards normal within an hour. In this case also, under the proper circumstances, a secondary fall soon starts and the animal usually dies in less than 24 hours. After injection of incompatible blood the portal pressure rises. In a series of ten dogs the portal pressure rose from an average preinjection level of 8.9 cm. water to a postinjection average of 24.2 cm. water. The pulmonary artery pressure also rises. The vena cava pressure during this same time falls. Fibrinogen levels fall after incompatible blood injection although not as dramatically as after thrombin. The fibrinogen drop is more dramatic in dogs prepared with cortisone. There is a prolonging effect of the clotting time of the injected dog's

*From the Div. of Clinical Surgery, Walter Reed Army Institute of Research, Washington, D. C.

#The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

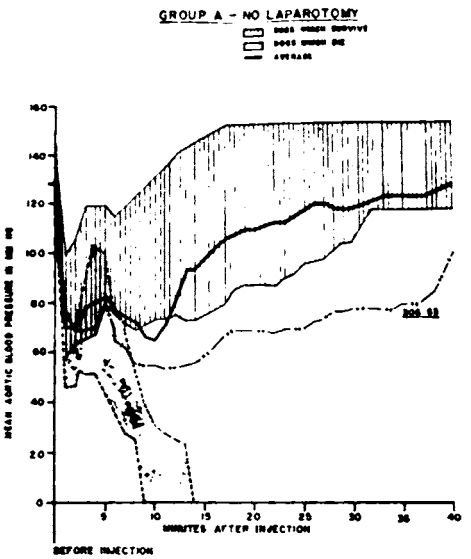

Figure 1. Range of mean aortic blood pressures of dogs given intra-aortic bovine thrombin immediately following "0" line.

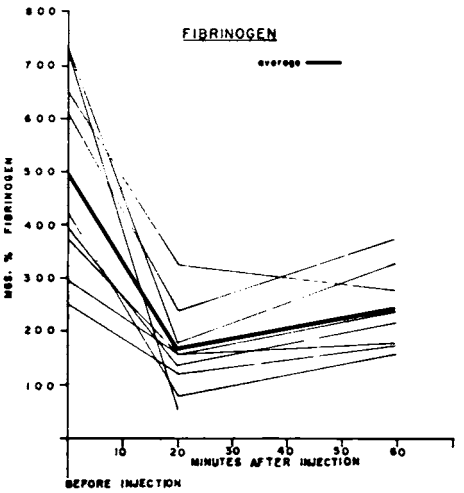

Figure 2. Fibrinogen levels of dogs given intra-aortic bovine thrombin immediately following "0" line.

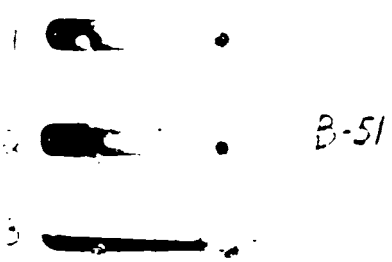

Figure 3. Blood samples were taken immediately before (first tube) and 20 minutes (2nd tube) and 60 minutes (3rd tube) following intra-aortic thrombin administration. All specimens were allowed to clot and then were incubated at 37 degrees C. The first two samples did not lyse; the third sample shows lysis as a result of endogenous fibrinolysin.

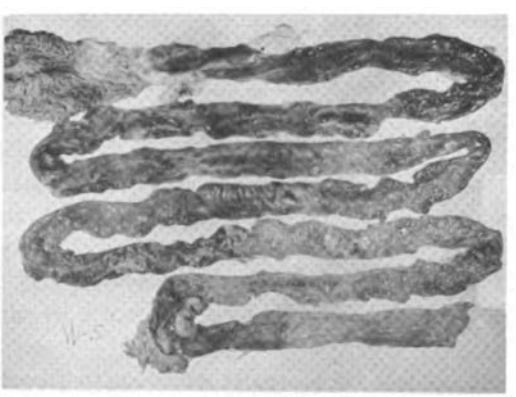

Figure 4. Entire gastrointestinal tract of dog dying 12 hours after intra-aortic injection of thrombin. Note hemorrhagic necrosis of nearly the entire mucosa.

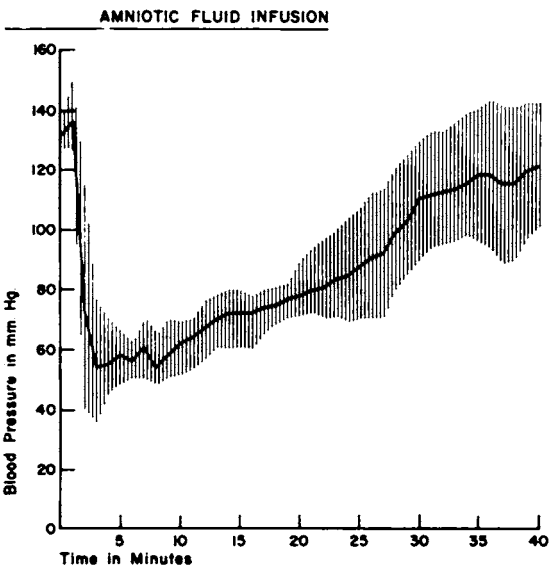

Figure 5. Range of mean aortic blood pressures of dogs given unfiltered amniotic fluid immediately following "0" line.

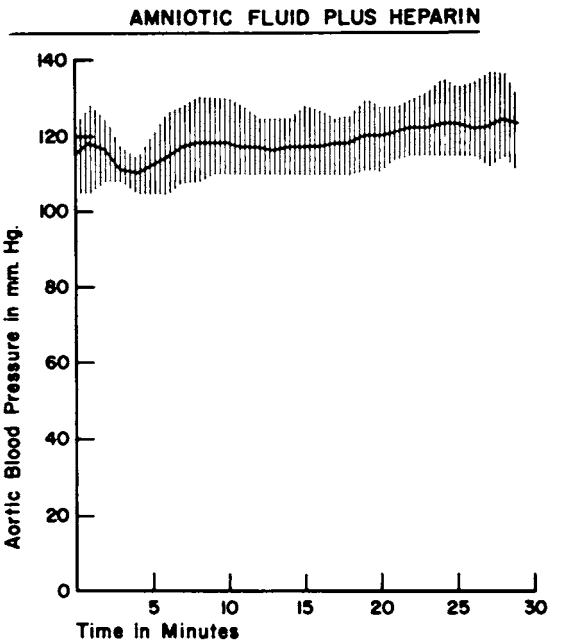

Figure 6. Range of mean aortic blood pressures of preheparinized dogs given unfiltered fluid immediately following "0" line.

blood on normal dog's blood. This heparin-like effect is most prominent in the second and third hours postinjection. These incompatible-blood-injected dogs die in a similar manner to the thrombin-injected dogs, and at autopsy show the same hemorrhagic necrosis of the gastrointestinal mucosa. A photomicrograph of the liver shows a platelet thrombus filling a central vein of the liver with central necrosis surrounding it (Fig. 7). Figure 8 shows a thrombus filling a small pulmonary vessel. There are thrombi in small vessels penetrating the gastrointestinal muscularis mucosa. Figure 9 shows gastrointestinal mucosa intact on the right side; however, on the left side there is early necrosis of the tips of the villi and a thrombus is noted in the mucosa underlying this. Figure 10 shows a thrombus in an omental vessel. There are thrombi in other organs including the kidneys.

The injection of E. coli endotoxin produces similar clinical, hematological, and pathological results. Figure 11 shows the dramatic fall of the systemic arterial pressure immediately following endotoxin injection. Note that the vena caval pressure shows a fall at the same time. Fibrinogen levels after endotoxin injection show a moderate but significant fall (Fig. 12). Figure 13 shows six tubes in which blood has been allowed to clot and then incubated at 37 degrees C. The upper three tubes are from a single dog, the first being before injection of endotoxin, the second and third at 20 minutes and 60 minutes after injection. Blood in the second and third tubes lysed on incubation denoting the development of endogenous fibrinolysin. This does not always occur, however, and the lower three tubes in another dog show no fibrinolysin. Figure 14 shows three thromboelastograms taken before injection of endotoxin and 30 and 60 minutes following injection. Note a normal spike and torpedo shape in the first specimen whereas the second and third show no torpedo shape and did not clot during the entire run. In another animal the normal clotting mechanism is denoted before injection whereas the postinjection specimen denotes delayed

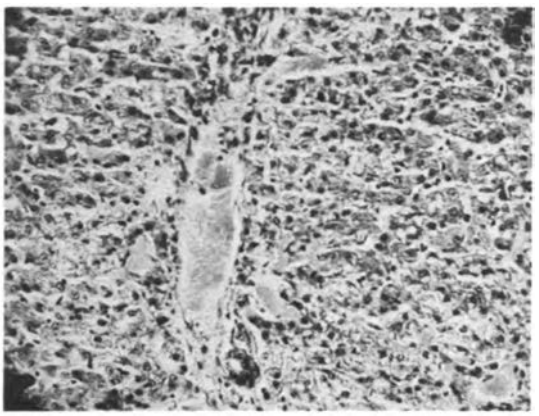

Figure 7. Photomicrograph of liver of dog dying after incompatible transfusion. Note a platelet thrombus occupying the central vein and early liver necrosis surrounding.

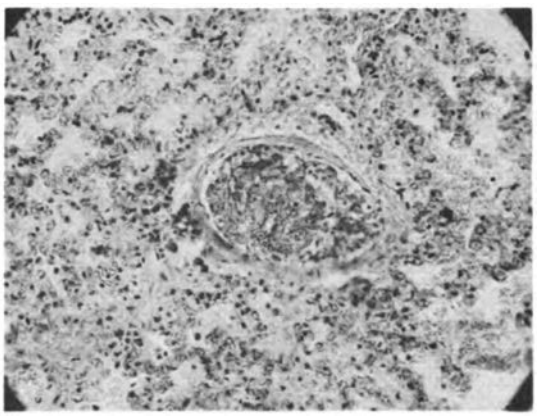

Figure 8. Photomicrograph of lung of dog dying after incompatible transfusion. Note a small arteriole filled with thrombus.

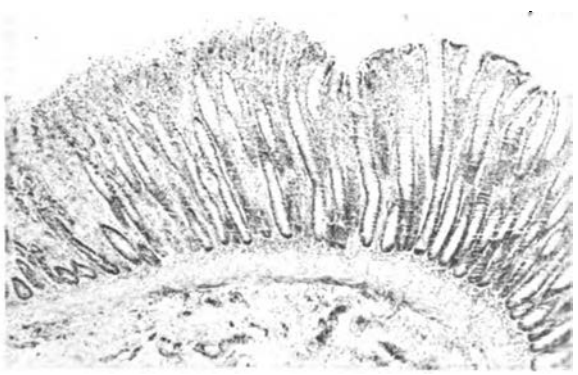

Figure 9. Photomicrograph of gut of dog dying after incompatible transfusion. Note that the mucosa is intact on the right but shows early superficial necrosis on the left. There is a thrombus in a small vessel on the left.

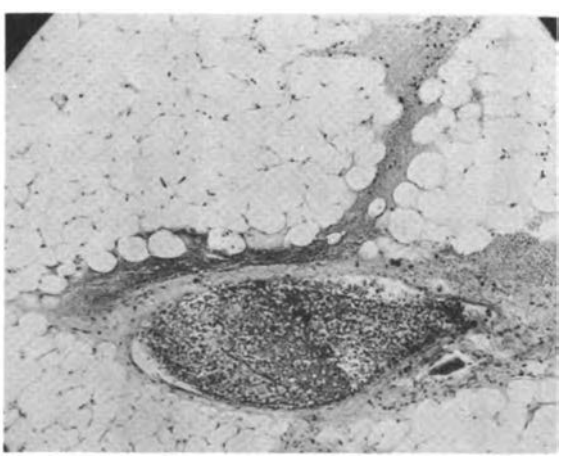

Figure 10. Photomicrograph of omentum of dog dying after incompatible transfusion. Note thrombus occluding a blood vessel.

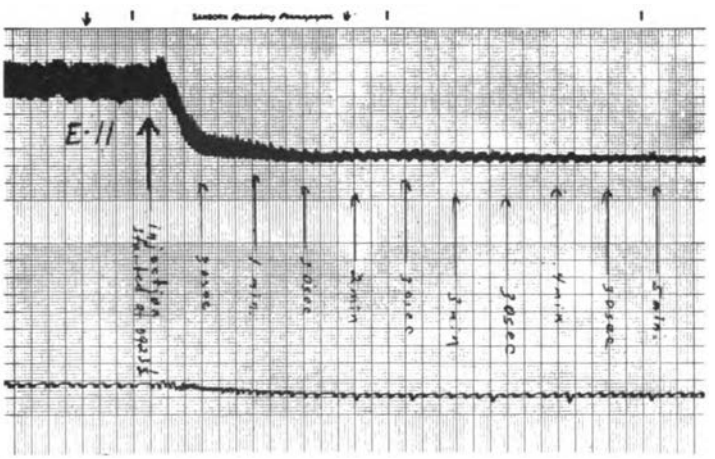

Figure 11. Recording of aortic blood pressure (above) and inferior vena caval pressure (below) of dog given E. coli endotoxin at point of big arrow. Note a dramatic fall in aortic pressure which is accompanied by a fall in vena caval pressure.

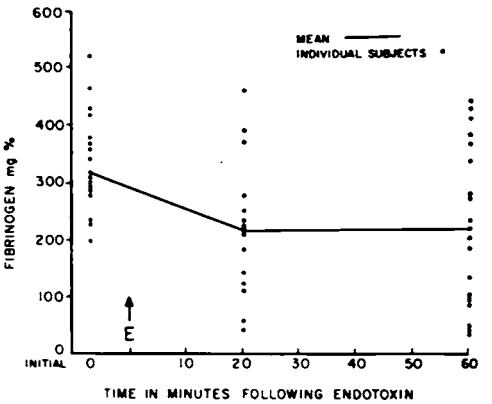

Figure 12. Plasma fibrinogen levels of dogs given E. coli endotoxin at point of arrow. Note that following endotoxin there are many readings below 200 mg. % whereas there are none before endotoxin.

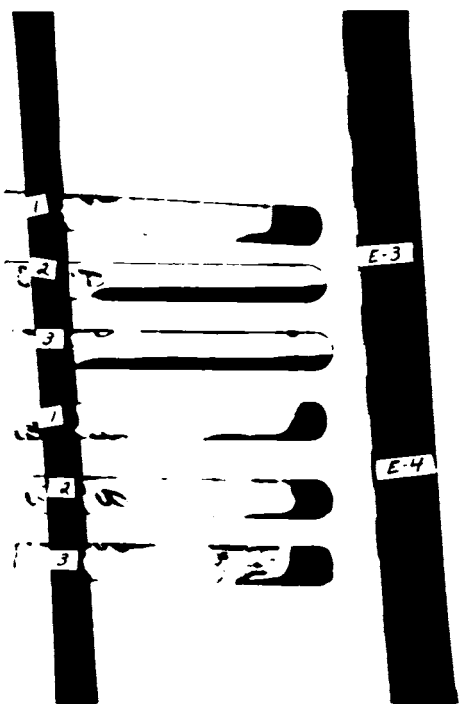

Figure 13. Blood samples on two dogs given E. coli endotoxin. The upper three tubes are from one dog and show no lysis in the preinjection sample but complete lysis in samples drawn 20 and 60 minutes following endotoxin. The lower three tubes are from another dog and show no endogenous fibrinolysin.

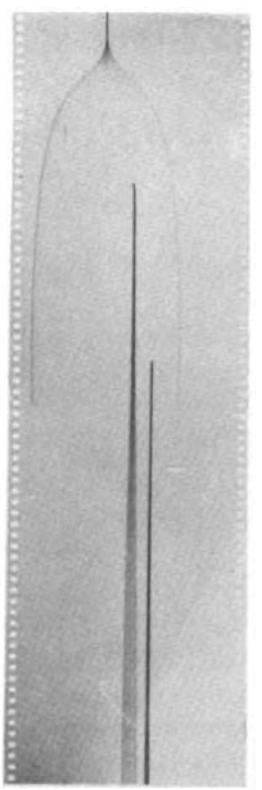

Figure 14. Thromboelastograms of blood of dog given E. coli endotoxin. The upper figure is blood taken before injection. The two lower figures are taken following injection and show absence of clotting.

clotting with almost immediate fibrinolysis (Fig. 15). These animals practically always die within 24 hours and at autopsy show the typical gastrointestinal mucosal hemorrhage necrosis also noted in the dogs injected with thrombin and incompatible blood. A photomicrograph of this intestine shows a superficial necrosis of the mucosa with mucosal vessels filled with masses of eosinophilic staining material (Fig. 16). Photomicrographs of the lung show most vessels to be plugged with this material. These plugs are composed of what appears to be masses of red cells agglutinated and fused together into one solid mass (Fig. 17). Figure 18 shows the liver. Note that the central veins are occluded with these plugs and that there is central necrosis surrounding them.

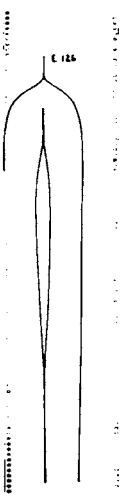

Figure 15. Thromboelastograms of blood of dog given E. coli endotoxin. The upper figure shows normal clotting before injection, the lower figure shows a prolonged clotting time with a poor clot and early fibrinolysis.

Figures 19 and 20 are illustrations taken from human clinical material. Figure 19 is a section of kidney from a patient dying after septic shock. It shows a vessel completely occluded with thrombus. Figure 20 shows the liver from the same patient showing a central vein occluded by the thrombus and surrounding central necrosis. A pregnant woman suffered a premature separation of the placenta followed by shock and incoagulable blood. This was effectively treated with fibrinogen and intravenous therapy. However, she became anuric and remained so. Her blood chemistries were kept within normal limits with the aid of peritoneal dialysis. However, after nine days of anuria she developed a fever and shock. This was thought to be due to gram negative septicemia although no positive culture was obtained. In spite of vigorous antishock therapy including norepinephrine, she died two days later in deepening shock. Autopsy showed a hemorrhagic necrosis of a large part of the gastrointestinal mucosa. This is very similar to that seen in the shocked dogs. Photomicrographs of this bowel show a superficial necrosis of the mucosa and occlusion of capillaries with thrombi. There were also thrombi in the pulmonary, renal, hepatic and pituitary microvessels. There was focal necrosis of all these organs except the lungs.

These findings are interpreted as follows: In all of these conditions of shock there occurs an episode of intravascular coagulation resulting in the using up of all blood clotting elements including fibrinogen, prothrombin, and platelets. Possibly as a protective mechanism against this event, the body liberates endogenous heparin and fibrinolysin in order to prevent more clotting and dissolve thrombi already present. These intracapillary thrombi consist of platelets or fibrin, or more commonly, masses of red cells firmly bound together (sludged red cells bound together with fibrin?). These plugs occlude the microcirculation in the viscera

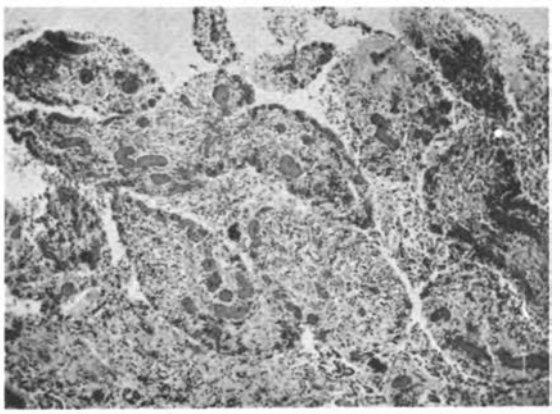

Figure 16. Photomicrograph of gastrointestinal mucosa of dog dying after E. coli endotoxin. Note superficial necrosis of the villi and occlusion of nearly all capillaries with eosinophilic staining plugs.

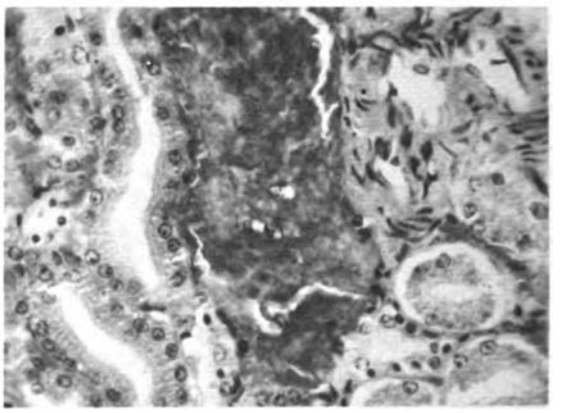

Figure 17. Photomicrograph of kidney of dog dying after E. coli endotoxin injection. Note a vessel occluded with a plug apparently consisting of red cells firmly bound together and fused into one solid mass.

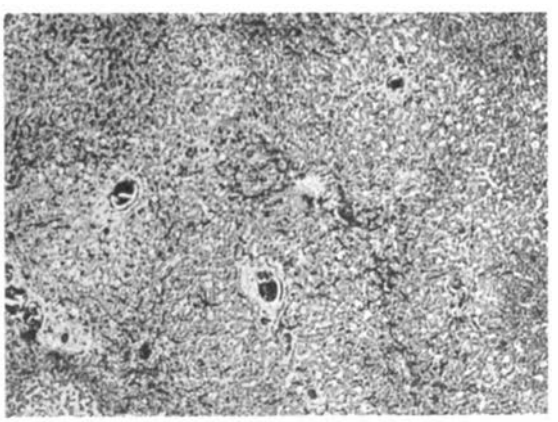

Figure 18. Photomicrograph of liver of dog dying after E. coli endotoxin injection. Note occlusion of central veins by plugs with surrounding central necrosis.

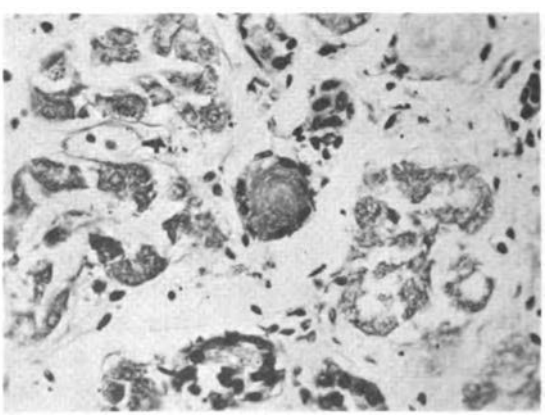

Figure 19. Photomicrograph of kidney of clinical case dying in septic shock. Note a vessel occluded with what is apparently a fibrin plug.

resulting in hemodynamic changes as seen in Figure 21. Blocking of blood flow in the liver produces portal hypertension and a decreased blood return into the vena cava. There is a decreased cardiac return to the right heart. Capillary plugs in the lung block the pulmonary flow and result in pulmonary hypertension. Decreased cardiac return to both the right and left heart results in decreased cardiac output and a resulting decreased systemic arterial pressure. These changes are reversible and these plugs may be washed out, possibly with the aid of endogenous

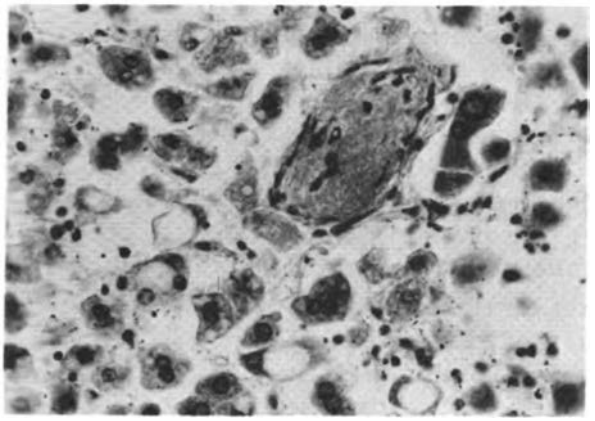

Figure 20. Photomicrograph of liver from clinical case dying after septic shock. Note occlusion of central vein with a platelet and fibrin plug and surrounding liver damage.

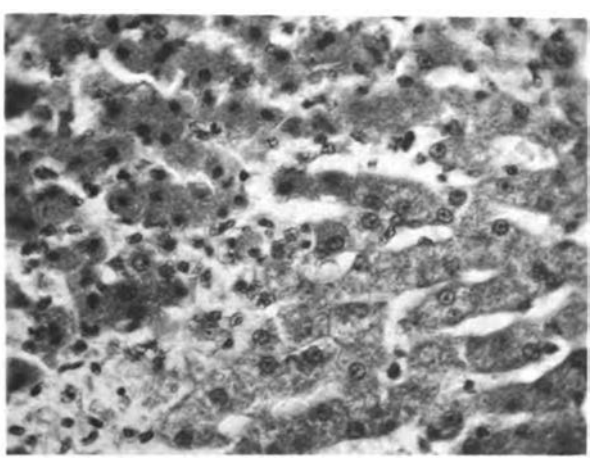

Figure 22. Photomicrograph of liver of dog dying of "irreversible" hemorrhagic shock. Note normal cells at lower right with early necrosis at upper left. This represents the central necrosis of the liver seen in animals with any of the types of shock described.

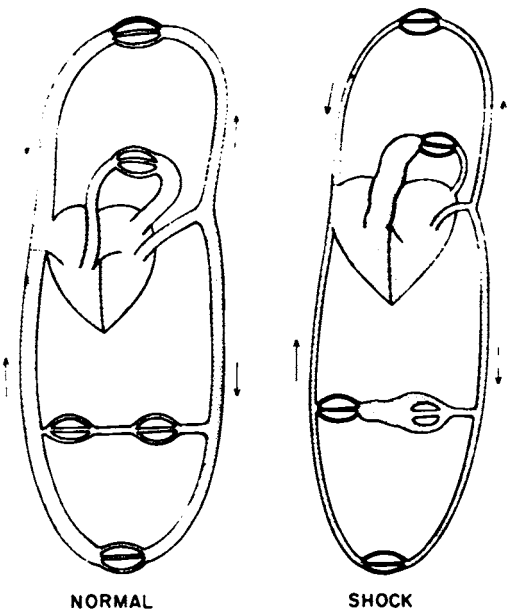

Figure 21. Diagram of hemodynamic changes in "reversible" shock. On the left is the normal condition. The width of the vessels is roughly analogous to actual diameter and pressure and blood flow through them. The diagram on the right shows partial occlusion of capillaries of the lung and liver with resulting piling up of blood behind, creating portal and pulmonary hypertension, decreased vena caval pressure and decreased aortic pressure due to decreased cardiac return.

fibrinolysin, with return of blood pressure to normal. However, if these microcirculatory occlusions persist long enough they result in focal tissue necrosis as seen in the liver and other organs. In Figure 22 part of the liver cells are intact and part show early necrosis. These same areas of necrosis occur in kidney, pancreas, intestine, and other organs. If this process is widespread enough, the shock is irreversible and death results.

EXPERIMENTAL STUDIES OF THE ANTITHROMBOGENIC PROPERTIES OF LOW MOLECULAR WEIGHT DEXTRAN

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Following a year in Sweden with Dr. Gelin, we initiated a number of experimental studies with low molecular weight dextran (LMDX). The results of four of these investigations seem particularly pertinent to the present discussion; the following is a brief summary of these investigations:

1. Portal Vein Grafting—Vein graft replacement of segments of the canine portal vein was found feasible with aid of vigorous heparin therapy beginning at the time of implantation and during the ensuing week; 11 of 12 such grafts proved successful. Without heparin therapy 5 of 7 grafts thrombosed. When LMDX therapy was substituted for heparin, 6 of 10 grafts remained patent. Thus heparin therapy seemed better than LMDX in preserving patency of portal vein grafts; however, LMDX does seem to have a definite beneficial effect as compared to the controls.

2. Small Artery Surgery—A standardized method of inducing thrombosis in a small canine artery (2-4 mm.) was found in a 2 centimeter arteriotomy and intimectomy; this procedure yielded thrombosis in less than 24 hours. Three treatment groups were then created: (1) LMDX (10% in saline), (2) normal saline, and (3) canine plasma. The latter two groups served as controls for the LMDX group. Each of the solutions was given in the amount of 500 cc over the 24 hours following the arteriotomy and intimectomy. After 24 hours the operative incision was opened and the artery examined for evidence of patency or thrombosis. In the saline group 11 of 12 of the vessels thrombosed, in the plasma group 5 of 10 of the vessels thrombosed while in the LMDX group only one thrombosed while 19 remained patent. Thus, LMDX seemed to have a marked beneficial effect in preventing early thrombosis following direct small artery surgery; this beneficial effect seems to be beyond that of plasma volume expansion.

3. Tourniquet Palsy—Application of a pneumatic tourniquet to the hind limb of a dog at 650 mm Hg for 5 hours produces a standard, reversible nerve injury (foot drop) and soft tissue injury (swelling). The influence of LMD and heparin on these sequelae were measured. It was found that both LMDX and heparin measurably decreased the amount of swelling and duration of the foot drop if the drugs were administered before the tourniquet was applied and just before it was released. This might be interpreted as evidence of prevention of sludging of blood in the small capillaries and venules, however the tissue studies do not clearly support this hypothesis.

4. Coronary Artery Occlusion—The amount of myocardium rendered ischemic by acute ligation of the circumflex branch of the left coronary artery was investigated. Two hours following ligation the animal was sacrificed and the left and right coronary arteries injected with a color indicator; the area not stained was considered ischemic. A paired animal received preligation treatment with low molecular weight dextran, while the control animal received an equivalent amount of saline. Observations to date do not indicate any significant difference in the quantitative amount of myocardial rendered ischemic in the control and dextran treated groups. This study is incomplete.

Summary

Our studies to date would indicate that low molecular weight dextran does provide protection against early thrombosis following vascular surgical procedures; this has been most striking in the small artery group.

DISCUSSION

Dr. Bryant: I would like to mention the results obtained from some experiments comparing the effectiveness of dextrans of various molecular weights in preventing thrombosis in small arteries subjected to a standardized mechanical trauma.

The experimental preparation that we used was the same as the one described by Dr. Foster. In 100 such control preparations we had a thrombotic rate of 95 per cent, so we felt that we had an excellent standardized experimental preparation in order to study various substances that would influence this rate. By giving a 1 per cent infusion of clinical dextran prior to restoring a normal pulsatile flow of blood through the traumatized segment, we were able to reduce the thrombotic rate to 5 per cent in these controls. We then used various low molecular weight dextrans with an average molecular weight of 10,000, 20,000, 30,000, and the Rheomacrodex^{R}, which has been mentioned here today. With these low molecular weight dextrans, our thrombotic rate was 50 per cent. So in our hand, at least, clinical dextran has been more effective in preventing postoperative thrombosis in small arteries than low molecular weight dextran.

Dr. Moncrief: We have had essentially the same experience as did Dr. Bryant. We utilized venous grafts in arterial segments rather than arteriotomy and found that the 70,000 molecular weight dextran was much more efficacious in preventing thrombosis than was low molecular weight dextran.

Some of the work we have been doing in preventing venous thrombosis with dextran leaves me puzzled after hearing some of the papers this morning. Using gold electrodes and a 5 milliamp current, for one hour, we can produce thrombosis in a vessel in 100 per cent of the experimental animals. If we give the animal clinical dextran, the thrombosis is prevented. The low molecular dextran, however, has not been as effective in preventing thrombosis. The curious thing is (and until today I thought I knew why), the thrombus always occurs at the positive electrode when the two half-shell electrodes are placed around the vessel. It puzzles me why dextran which provides an even more negative charge to the erythrocyte should prevent such thrombosis at the positive electrode.

Unidentified: I would like to address a question to Colonel Hardaway. He has shown that the injection of thrombin is capable of generating fibrolytic activity. Since this is so, it has been demonstrated that fibrin breakdown products are capable of inhibiting the polymerization of fibrinogen to fibrin. In addition, it is well known that hyperplastinemia is associated with the destruction of Factor V. Either of these components is capable of rendering blood incoagulable and consequently the incoagulability of blood

can't be used as an argument that heparin is present, and is produced by intravenous injection of thrombin.

I wonder if Colonel Hardaway has any other evidence that intravenous injection of thrombin causes hyper-heparinemia?

Col. Hardaway: I don't think that we have any sure evidence that heparin is produced. There are a number of things that point toward it, however, such as its inactivation by protamine.

Dr. Eirich: I want to give a possible explanation for the fact that in the presence of dextran there is no agglutination of erythrocytes on the positive electrode. I don't think it is so much a matter of charge because, after all, you don't bring the particles right up to the electrode which lies outside the vessel. I think it is only that the erythrocytes are in a more stable suspension when coated with dextran as they concentrate around the anode.

Dr. Howard: Dr. Foster's paper was interesting from the standpoint of replacing veins. As you notice, he used low molecular weight dextran every eight hours for a week, and his venous replacements were successful. Dr. Ronald Tower and I had done the same thing, but gave the animal two liters of the 40,000 molecular weight dextran only on the day of replacement. Our replacements were all unsatisfactory.

It seems as we talk more and more about the clinical application of low molecular weight dextran, we need to remember that because of its low molecular weight it is excreted very rapidly by the kidney. If you give a normal person a liter of the 20,000 molecular weight dextran, he would have excreted the majority of it by the time you had finished the one or two hour infusion.

Dr. Berman: The effects of different molecular weight fractions of dextran (40,000, 150,000 and 500,000) on platelet thrombosis and vascular fragility were determined in vivo by a microelectrode test.[1,2] The minimum milliamperage required to produce a platelet thrombus observable at 120 magnifications in the intact blood vessel and the minimum current that would break the blood vessel wall were determined in vessels in the hamster cheek pouch. Experimental animals were tested 24 hours after replacement of 3 ml of blood with 3 ml of a 6% solution of the specified MW dextran dissolved in isotonic saline. The average threshold for each experimental group was divided by that of normal untreated controls and the ratio expressed as a single figure in the table. A study of the data in the table shows that within the range of the experimental parameters the effect of dextran appears to depend on its molecular weight and concentration in plasma. An increase in the concentration of dextran in plasma caused an increase in the current required to produce the threshold thrombus, until with the 500,000 MW dextran fraction, no platelet thrombi formed in 50% of the experimental animals after application of 0.80 milliamperes for 1 millisecond at the maximum output (150 volts) of the Grass stimulator.

TABLE

Different Molecular Weight Fractions of Dextran*

Molecular Weight	Thrombus Ratio (I)	Time for Thrombus To Form (sec.)	Fragility Ratio	Tail Puncture Bleeding Time (sec.)	Platelet Count ($x10^5$ mm^3)	Clotting Time (sec.)	Conc. of Dextran mg/cc
Untreated Controls	1.00	27	1.00	94	9.09	22	--
40,000	1.38	52	.81	165	9.35	31	0.57
150,000	2.84	57	.72	(8) 400** (2) 300	8.77	57	6.8
450,000	(5) -- (5) 2.95	62	.35	400**	8.42	50	9.4

*Supplied by Pharmacia, Uppsala, Sweden
**10 animals per group

The time required for the platelet thrombus to form and reach its maximum size also increased. The lengthening of the bleeding time paralleled the increase in the platelet thrombus threshold and to a lesser extent the thrombus formation time. The vessel wall was weakened in hamsters that received infusions of the higher MW dextrans and consequently had greater plasma dextran concentrations. The platelet count was relatively normal in all experimental groups at the time of testing but the whole blood clotting time was extended in those hamsters that received high MW dextrans.

The data must be interpreted with care because of limited experience with the microelectrode test. Both the extent of the blood loss and the concentration of dextran in plasma are important. Some abnormalities were observed with the lowest MW dextran fraction and these were greatly accentuated with the high MW fractions. Furthermore, with the highest MW fraction erythrocytes were observed to aggregate. These circulating masses, however, readily separated in the capillary bed only to reform on the venous side. Our limited observations indicate that a more precise statement of the experimental conditions and measurement of important variables such as the blood pressure, flow rate, osmotic pressure, pCO_2, pO_2 and lactic acid, would facilitate a clearer understanding of the problem and comparison of experimental results.

What causes dextrans of different molecular weights to act either as a dispersing or aggregating agent? This diverse property may be related to molecular size. A smaller dextran molecule may only be able to bind or react with one of the formed elements in blood. As the molecule becomes larger its increased size may enable it to react with two particles and thus, for example, form a bridge between two erythrocytes. The reaction could also occur as a two or multistage process. A macromolecule could be absorbed to a formed element and react with a second macromolecule attached to a second formed element. Intervention of additional dextran molecules would tend to weaken the link and low MW molecules, because of their size and therefore more limited capacity to react, would tend to break the bridge. Such a simple physical explanation could possibly account for the capacity of high MW dextrans to cause red cells to aggregate and low MW dextrans to cause these aggregates to disperse.

References

1. Berman, H. J. and G. P. Fulton, 1961. Platelets in the Peripheral Circulation. Blood Platelets. S. A. Johnson, R. W. Monto, J. W. Rebuck and R. C. Horn, editors. pp. 7-20. Little, Brown & Company, Boston.

2. Berman, H. J., 1961. Anticoagulant-induced Alterations in Hemostasis, Platelet Thrombosis, and Vascular Fragility in the Peripheral Vessels of the Hamster Cheek Pouch. Anticoagulants and Fibrinolysins. pp. 95-107. R. L. MacMillan and J. F. Mustard, editors. Macmillan of Canada, Toronto.

EFFECT OF LOW MOLECULAR WEIGHT DEXTRAN ON ORGAN PERFUSION AND SLUDGING

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Extracorporeal perfusion of a parenchymatous organ such as the kidney is complicated by several technical and physiologic problems. In our laboratories arterial spasm and insufficient flow of perfusate has been a difficult problem to combat both in normothermic and hypothermic perfusions of dog, sheep, and baboon kidneys. Composition of the perfusate now appears important for successful profound hypothermic extracorporeal storage of the kidney and also for normothermic extracorporeal perfusion of the kidney in a pump oxygenator.

Success in both areas of work in our laboratories has been concomitant with use of low molecular weight dextran (molecular weight of 40,000) in the perfusates. We are presenting our experience in both hypothermic extracorporeal storage of the kidney and also normothermic extracorporeal perfusion of the kidney, and will demonstrate how both methods have been combined to accomplish extracorporeal perfusion of the kidney with subsequent autografting and resumption of adequate function.

Extracorporeal Hypothermic Storage of the Kidney

The obvious need for a simple technique to successfully store parenchymal organs prior to homografting led us to review and test many techniques. At the beginning nine different combinations of perfusate were tested in dogs to arrive at a satisfactory solution of the problem:

1. Cold autologous whole blood (250 cc.) with 10 mg. heparin (1-4 degrees C.).
2. Cold autologous whole blood (250 cc.) with 10 mg. heparin + procaine (50 cc. of 2%), (1-4 degrees C.).
3. Cold autologous whole blood (250 cc.) with 200 mg. heparin (1-4 degrees C.).
4. Cold autologous whole blood (250 cc.) with 200 mg. heparin + procaine (50 cc. of 2%), (1-4 degrees C.).
5. Normal saline solution (300 cc.) (1-4 degrees C.).
6. Ringer's lactate solution (300 cc.) (1-4 degrees C.).

7. Tisusol (modified Gey's physiologic solution) (300 cc.) (1-4 degrees C.).

8. Homologous plasma (300 cc.) (1-4 degrees C.).

9. Cold homologous or autologous blood (150 cc.) + low molecular weight dextran (150 cc. of 10% in saline), + procaine (50 cc. of 2%) and heparin (15 mg.) (1-4 degrees C.).

After exposure and mobilization of the right kidney through a flank incision, the vessels and ureter were quickly clamped and the organ removed for perfusion. The various composite mixtures previously cooled to 1-4 degrees C. in a refrigerator were perfused through the organ with a cannula in the renal artery. A simple hand-bulb was used to provide 180 mm. of Hg pressure in the perfusate bottle during the three to five minutes of hypothermic perfusion. The resulting temperature of the kidney was approximately 10 degrees C. The kidney was immersed in the perfusate mixture and stored in a standard refrigerator at 4 degrees C.

After varying periods of time, the kidneys were autografted into the original site in the right flank using an end-to-side anastomosis of the renal artery to the aorta, and end-to-side anastomosis of the renal vein to the vena cava, and an end-to-end anastomosis of the ureter over a #20 polyethylene stent. Contralateral nephrectomies were performed in all animals either initially or several weeks after autografting of the refrigerated kidney.

When saline, Ringer's lactate solution, Gey's solution, or homologous plasma were used as the cold perfusate (and storage bath) the kidneys consistently failed to function. Severe edema was evident in these organs after several hours in the perfusate mixture and histologic sections indicated extensive damage to tubular cells after two or more hours of storage. No animals in these early experiments survived following contralateral nephrectomy.

When the perfusate contained cold autologous blood in various mixtures, kidneys were less edematous and the blood flow could be re-established at the time of autografting. However, the renal arteries frequently thrombosed and subsequent sections of the kidneys revealed areas of ischemia secondary to renal artery thrombi within the parenchyma of the organ. None of these dogs survived a contralateral nephrectomy performed several weeks following autografting of the cold and stored kidney for more than a few days.

The addition of low molecular weight dextran to homologous or autologous blood and including 20 mg. of heparin and 50 cc. of 2 per cent procaine resulted in consistently successful autografting. In an initial group of five dogs the ischemia time lasted from 45 minutes to 1-1/4 hours and contralateral nephrectomy was performed immediately after autografting the "cold" kidney. These animals all demonstrated some disturbances in renal function which lasted for three to ten days. Studies at six months postgrafting indicated normal values for serum, creatinine, sodium, potassium, chloride, carbon dioxide, and BUN. These autografted kidneys were capable of concentrating urine to a specific gravity of 1.043 to 1.050.

In a second group of nine dogs with renal ischemia times of 4-1/2 to 7 hours, eight animals survived for prolonged periods. These animals had contralateral

nephrectomies at 21 days postautografting, at which time there was moderate rise in blood urea nitrogen and creatinine. At six months the renal function in this group of animals was normal except for one animal with a 4-1/2 hour ischemia time and a second animal with a 5 hour ischemia time where the BUN was 55 and 87, respectively. In both of these animals a hypertension was present (225/125 mm. Hg, and 190/120 mm. Hg). Urinary concentration tests performed six months following autografting in this group of animals indicated an ability to concentrate with a specific gravity from 1.030 to 1.045, and only one animal showed a 1+ albumin in his urine. These animals lived for prolonged periods of time with repeated urinalyses and blood chemistries within normal ranges.

One dog (#489) had a simultaneous contralateral nephrectomy when the right kidney (cooled for 4-1/2 hours at 4 degrees to 10 degrees C.) was autografted. The BUN rose to 150 mg. per cent and the creatinine rose to 6 mg. per cent with return to normal in seven days. At six months this animal was able to concentrate urine to a specific gravity of 1.038.

Histologic studies showed a disappearance of the brush border of the tubular cells beginning approximately one to two hours after perfusion with the autologous blood-low molecular weight dextran mixture and immersion in the perfusate bath. Hyaline granular degeneration of tubular cells with a tendency to hydropic degeneration occurred but these changes were all reversible and subsequent needle biopsies at three and six months postgrafting showed near-normal histologic appearance. Dog kidneys cooled for eight hours had the most severe changes occurring in tubular cells but a return of normal function was attended by a change toward normal of these cells.

Primate Experiments (Baboon)—Extracorporeal Hypothermic Storage

Because there are significant physiologic gaps between canines and primates, it seemed reasonable to attempt this type of kidney storage in a suitable primate. The animal chosen was suggested by several investigators for its physiologic similarities to man. The Kenya baboon, as studied by anthropologists, appears to be a close physiologic relative to man and this animal grows to a size permitting vascular surgery with ease.

Six baboons had extracorporeal storage of a kidney up to eight hours and four are now surviving 24 months. Six animals had extracorporeal renal hypothermic storage for 20 to 24 hours and two of these animals are now surviving 18 months. Of the six animals that died, three had no apparent cause of death, one had venous thrombosis at the time of death five days postgrafting, and one had sepsis in the region of the graft.

The technique of perfusion was changed in the baboon experiments by deleting whole blood from the perfusate and using only 10 per cent low molecular weight dextran in saline (500 cc.), to which was added procaine hydrochloride (50 cc. of 1 per cent) and heparin (30 mg.). This mixture was cooled to approximately 1 degree C. and quickly perfused through the renal artery of the excised kidney during a three to five minute perfusion. As in the previous dog experiments, the perfusion

pressure was maintained below 180 mm. Hg. Specimens, as before, were stored in stainless steel basins in the effluent as it came from the renal vein. Upon reimplantation of the kidney as autografts 20 to 24 hours later, the previous incision in the right flank was opened and end-to-side anastomoses of the renal artery to the aorta and renal vein to the vena cava were performed and the ureter was anastomosed end-to-end over a polyethylene stent (Fig. 1). Contralateral nephrectomies were

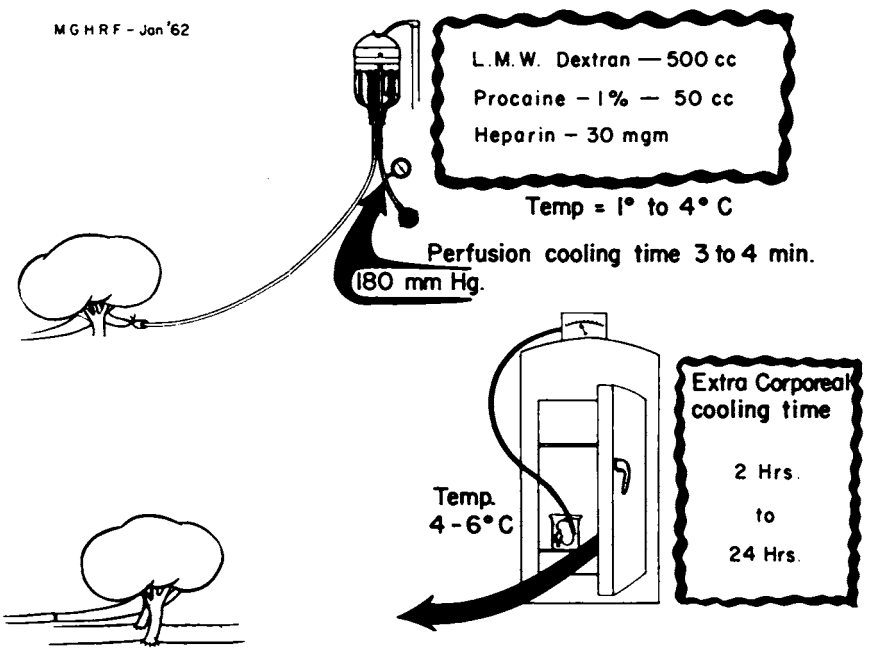

Figure 1. Technique for cold perfusion of the primate kidney.

performed one month following autografting in the animals with kidneys stored four to eight hours, and at five months following autografting in the baboons with kidneys stored extracorporally for over 20 hours. Two animals had only slight rise in the blood urea nitrogen following contralateral nephrectomy (kidneys cooled for 7 hours and 21-1/2 hours) and the remaining four had an elevation of the blood urea nitrogen varying between 75 and 130 mg. per cent. All values returned to normal by eight days post-contralateral nephrectomy and have remained within normal range for the entire period of observation. Repeated histologic studies have been performed on needle biopsy specimens and a pattern of cellular response in the kidney tubules similar to that seen in the dog was present.

No obvious changes appeared in the glomeruli on the histologic sections but tubular cells became thinner and the brush border disappeared as in the dog studies. A mild degree of hydropic degeneration was evident in biopsies performed at one month post-cooling, but those changes were reversible and sections taken at 9 months, 18 months, and 24 months postgrafting demonstrated only some residual tubular dilatation and an occasional inflammatory infiltrate in scattered areas of the kidneys. Figure 2 shows a section from a baboon made 18 months after the kidney had been cooled for 21-1/2 hours and autografted; the animal had survived in a normal manner on this kidney for 13 months. Blood pressure in this animal was 115/80 mm. Hg.

The urine concentrated to a specific gravity of 1.017 on an overnight specimen and the BUN was 11 mg. per cent. There was no protein in the urine.

Hypertension developed in four of these animals as demonstrated in Figure 3. In our dog studies two of nine animals had hypertension six months post-autografting of kidneys cooled for 4-1/2 and 5 hours but, each of these animals had a persistent elevation of the blood urea nitrogen to 55 and 87 mg. per cent, respectively. The baboons with hypertension all had normal blood urea nitrogen levels as indicated in

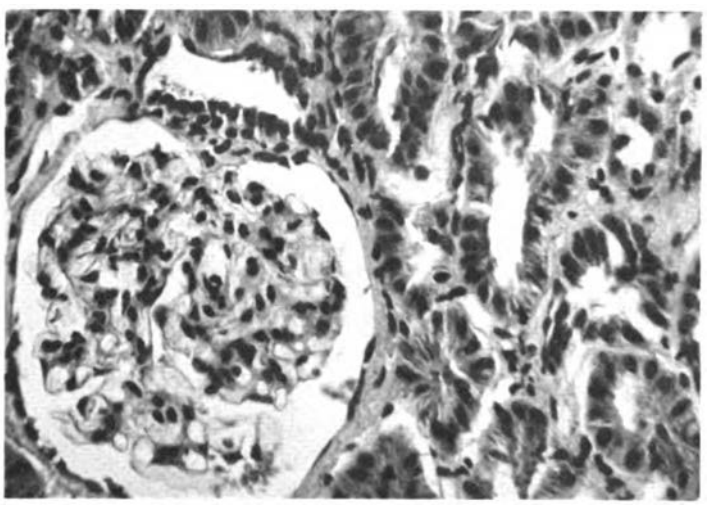

Figure 2. Section of baboon kidney sacrificed 18 months following removal, cooling for 21-1/2 hours and replacement.

Baboon Number	Ischemia Time (Hours)	Blood Pressure (mmHg)	B.U.N. meq./L	Urine	
				Sp.Gravity	Protein
A 233	4	185 / 120	13	1.013	0
A 229	5	160 / 110	10	1.033	0
A 227	7	135 / 75	12	1.015	0
A 232	8	195 / 110	14	1.016	0
311	21 1/2	115 / 80	11	1.017	0
398	22 1/2	170 / 135	19	1.021	0

All with contralateral nephrectomy
Studies at 18-24 mos. post graft

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Figure 3. Blood pressure and renal function after refrigeration of baboon kidneys.

Figure 3 and the specific gravity of the urine on overnight specimens was satisfactory. None of the animals spilled protein in the urine. Aortograms performed on these baboons indicated widely patent anastomoses with good renal blood flow in smaller parenchymal vessels. Renal artery stenosis in the area of the anastomosis has been ruled out as a possible cause for the hypertension.

Renal clearance studies were performed on the baboons with glomerular filtration rates studied by means of inulin clearance, renal plasma flow studied by means of PAH clearance, and maximum tubular excretion studied by a PAH technique.

Glomerular filtration rate varied from 28 per cent to 53 per cent of expected normal. The renal flow varied between 39 per cent and 58 per cent of expected normal (standards were based on animals with two kidneys). Since maximal tubular excretion values varied between 50 per cent and 84 per cent of expected normal values in animals with two kidneys, a satisfactory degree of tubular excretion was apparent. The etiology of the hypertension in these baboons is under further study.

Normothermic Renal Perfusion in the Baboon and Sheep

Our interest in normothermic perfusion of the kidney stems from two sources; first is the desire to achieve an actively metabolizing organ permitting possible chemical and metabolic alterations of the tissues; second is the desire to have a better laboratory preparation for detailed studies of renal function with kidneys removed from the influences of the parent organism. Although isolated kidney perfusions have been attempted for more than 100 years, the duration of successful perfusions has been limited to a maximum of seven hours and always a marked impairment of function has resulted. Replantation of the kidney after perfusion has not been successful in the past.

The character of the perfusate in these studies was changed after the initial experimental period to include low molecular weight dextran. Prior to the use of low molecular weight dextran prolonged "runs" in the specialized pump oxygenator system resulted in extensive tubular damage and edema of the kidneys. These changes are demonstrated in Figure 4 which shows a baboon kidney after a 24-hour run with a perfusate consisting of autologous blood, physiologic salt solution, essential amino acids and vitamins, and penicillin and streptomycin. As in our previous work with hypothermic extracorporeal storage, the perfusability and functional capacity of the kidneys improved significantly.

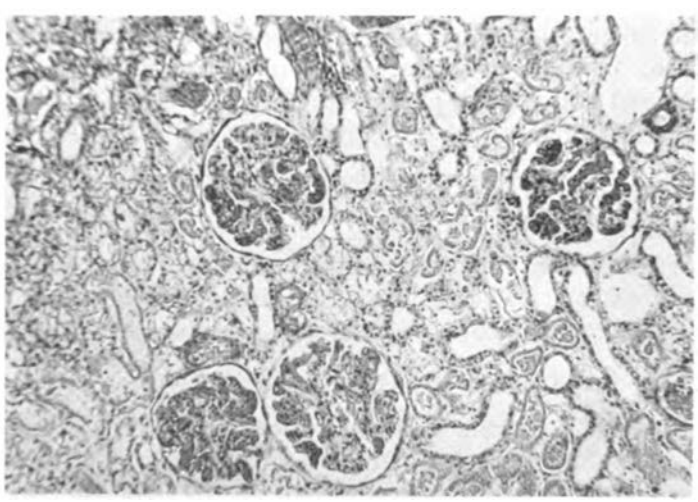

Figure 4. Section of baboon kidney demonstrating tubular damage and edema after perfusion for 24 hours with solution not containing dextran.

The perfusion apparatus was constructed of glass tubing and special glass chambers with rubber tubing for connections. A reciprocating pump provided a pulsatile pressure via a large rubber cot through which the blood flows. The perfusate was oxygenated as it descended in a film on the surface of a large glass

reservoir. The successful perfusate and the method of replenishing the pump oxygenator system is shown in Figure 5. The perfusate consisted of: 1) Fresh, heparinized, autologous or homologous blood - 250 cc.; 2) Balanced salt solution - 250 cc. (this contained the 10 essential amino acids and 13 vitamins); 3) Low molecular weight dextran - 75 cc.; and 4) Antibiotics - (100,000 units crystalline penicillin and 250 mg. streptomycin). The pump oxygenator system was charged with this perfusate and at approximately three hour intervals during a course of a 24 hour perfusion the system was replenished with 50 to 75 cc. of balanced salt solution and 20 to 40 cc. of low molecular weight dextran.

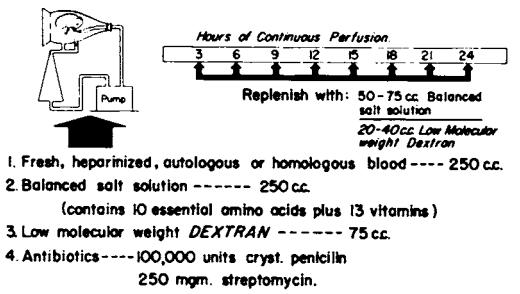

Figure 5. Perfusates employed successfully for normothermic perfusion of baboon and sheep kidneys.

During perfusions the following determinations were made: Perfusion pressure, observed renal blood flow, urine flow, pH, hematocrit, blood glucose, creatinine clearance, and serum hemoglobin. Using the perfusate containing Rheomacrodex^{R}, five baboon kidneys were perfused for 24 hours with adequate renal blood flow and urine flow during the entire perfusion. The hematocrit of the system varied from 20 to 25. Histologic sections of these kidneys revealed changes in the tubules but they were minimal, consisting of mild interstitial edema and slight swelling of the proximal tubules. Figure 6 demonstrates the near normal appearance of such a kidney after 24 hours of perfusion. Note the retention of the brush border (a feature that consistently disappeared in hypothermic extracorporeal storage) and the clear-cut cellular outlines. The glomeruli retained a normal morphology including the region of the juxtaglomerular apparatus.

A group of seven sheep underwent perfusion of their left kidney with successful replantation as autografts. Duration of these perfusions ranged from 5-1/4 to 7 hours and contralateral nephrectomies were performed at 5 to 10 days post-autografting. The glomerular filtration rate and effective renal blood flow were 50 per cent or more of the total preoperative function of both kidneys in these animals.

Five baboons had perfusion of the left kidney for periods of time ranging from 5-1/4 to 24 hours followed by autografting. Contralateral nephrectomy was performed 14 to 21 days post-graft in these animals. Two animals (with kidneys perfused for 5-1/2 and 7 hours) had a transient rise of their BUN, but it returned to normal in several days. The glomerular filtration rate and the effective renal blood flow again were at least 50 per cent of the total preoperative function of both kidneys. Three of these baboons were living and well at 10 months.

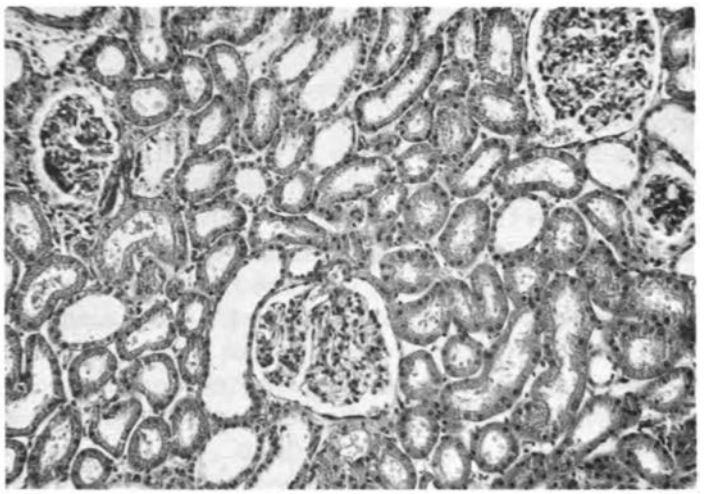

Figure 6. Section of baboon kidney perfused 24 hours with solution containing Rheomacrodex^{R}.

Usefulness of Extracorporeal Hypothermic Storage Combined with Normothermic Extracorporeal Perfusion

The simplified technique for rapid cooling of the kidney parenchyma with low molecular weight dextran was used to facilitate transfer of the kidney from the experimental animal into the pump oxygenator system and again from the pump oxygenator into the animal at autografting. This sequence with approximate time intervals is shown in Figure 7. Thus, the baboon and sheep kidneys now functioning as autografts following normothermic extracorporeal perfusion retained satisfactory functional capacity during two periods of hypothermia to 4 to 10 degrees C., and varying periods of time at normal body temperature in the pump oxygenator system. The photomicrograph in Figure 6 is representative of the appearance of the baboon kidney after 24 hour perfusion under these circumstances. Neither of these two types of perfusion experiments was successful in our laboratory prior to use of low molecular weight dextran in the procedures.

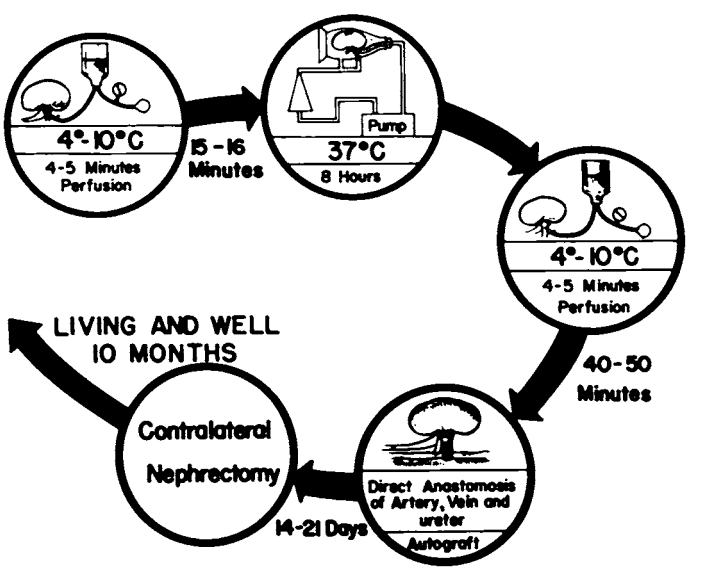

Figure 7. Procedure for normothermic extracorporeal renal perfusion.

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Summary and Conclusions

Several years ago Lapchinsky of the Research Institute in Moscow reported successful preservation of renal function during extracorporeal storage of dog kidneys for 24 hours. It is reported that he pumped cold blood through kidneys for approximately one hour and stored them at 2 to 4 degrees C. and then pumped warm blood through the organ for one hour prior to autografting. Other investigators utilizing complicated and difficult techniques have failed to satisfactorily preserve renal function during varying storage periods outside the body. Humphries and his group from the Medical College of Georgia recently reported experiments in the dog utilizing a complicated system of continuous perfusion at 4 degrees to 10 degrees C. The kidneys in their studies were subjected to increased ambient pressure to 3 atmospheres with 100 per cent oxygen and one such kidney maintained life in an animal for approximately 13 days.

The simplicity of the techniques that have proved successful in our laboratories using low molecular weight dextran in the extracorporeal hypothermic storage of kidneys is appealing. In this work technical aspects of the surgical procedures have been of lesser importance since experience was gained early in the experimental period with smaller vessel anastomoses and ureteral anastomoses. Technical factors probably are of somewhat greater significance in the successful long-term extracorporeal normothermic perfusions.

The pump oxygenator equipment used in the normothermic perfusions has been successfully applied to perfusions for organs such as bovine ovaries and baboon aortas for as long as two and three weeks by other investigators. However, our initial experiments with the sheep and baboon kidneys consistently failed until low molecular weight dextran was added to the perfusate. Comparison between Figure 4 and Figure 6, showing the histology of baboon kidneys perfused with and without Rheomacrodex^{R}, seems to show the value of this material in affording protection to delicate tubular and glomerular cells. We believe the anti-sludging effect of Rheomacrodex^{R} is important in this work. In addition, low molecular weight dextran seems to prevent cellular edema and interstitial edema even with prolonged storage of delicate parenchymal tissue in this material at low temperatures.

1. Low molecular weight dextran as a perfusate with the addition of heparin and procaine appears important in the successful extracorporeal hypothermic storage of dog and baboon kidneys.

2. The addition of low molecular weight dextran to the perfusate in a pump oxygenator system appears to have brought success out of repeated failures with normothermic perfusions of baboon and sheep kidneys for periods up to 24 hours.

3. The combination of techniques for hypothermic extracorporeal storage for short periods of time, and normothermic extracorporeal perfusion for periods up to eight hours, has permitted autografting of normothermic perfused kidneys with successful resultant function.

4. The anti-sludging effect of low molecular weight dextran and its beneficial high "oncotic" qualities appear important in both hypothermic perfusions and normothermic perfusions of kidneys.

BIBLIOGRAPHY

1. Kiser, J.C.; Telander, R.L.; Peterson, T.A.; Coe, J.I.; and Hitchcock, C.R.: Canine Renal Autografts. Studies of Reversible Histopathologic Changes Following Prolonged Extracorporeal Refrigeration, Surg., 83:502, 1961.

2. Kiser, J.C.; Farley, H.H.; Mueller, G.F.; Strobel, C.J.; and Hitchcock, C.R.: Successful Renal Autografts in the Dog After Seven Hour Selective Kidney Refrigeration, Surg. Forum XI:26, 1960.

3. Lapchinsky, A.F.: Recent Results of Experimental Transplantation of Preserved Limbs and Kidneys and Possible Use of this Technique in Clinical Practice, Ann. New York Acad. Sc. 87:539, 1960.

4. Telander, R.L.: Prolonged Normothermic Perfusion of the Isolated Baboon and Sheep Kidney with Maintenance of Function, Surg. Forum XIII:378, 1962.

DISCUSSION

Dr. Eiseman: Dr. Hitchcock, you have developed your techniques for organ perfusion over a number of years. You now claim that your more recent switch to LMWD was the vital factor that has provided success. Might this be post hoc reasoning and could it be that you might achieve equal success utilizing saline, plasma, or some other fluid in combination with your obviously expert techniques that have evolved over the years?

Dr. Hitchcock: I don't think we need to redo any of this work. So far as we are concerned, it is a foregone conclusion that we could not achieve success until we added low molecular weight dextran. Previous failure has been so consistent in attempts to get organs back into the animal following prolonged periods of pump oxygenator perfusion that our current success with 17 animals in which we haved used LMWD makes us want to stick to this method.

Dr. Hint: We used isolated rabbit ears to study the flow of blood suspensions and of colloids. The apparatus involved included a centrifugal pump which holds the suspension going around all the time, so no sedimentation can occur in the chamber. Investigating the pressure-flow relationship for two dextran solutions of exactly the same viscosity, but of very different colloidal osmotic pressures, we observed that the dextran with high osmotic pressure flowed more readily through the ears than the dextran of lower osmotic pressure. We found by weighing the rabbit ears that dextran of low molecular weight caused fluid seep from the ear; probably this is the cause for the improvement of flow.

We compared the flow of agglutinated human blood in isolated rabbit ears with the flow of normal human blood. Agglutination was produced by suspending erythrocytes from Group A blood in citrated plasma from Group O blood. Normal blood consisted of Group A erythrocytes in their own plasma. Both plasmas had the same flow rate. Agglutination of erythrocytes caused a relatively small increase in apparent relative viscosity in higher flow rates as compared with the flow of normal blood.

In lower flow rates a difference in apparent relative viscosity, however, increased and resulted in a considerable rise of so-called critical closing pressure. If the experiment was immediately repeated, the apparent relative viscosity was high also in highest flow rates, indicating that some change had occurred in the vascular bed. Afterwards the flow of saline was considerably diminished, but could be partly restored by forced injections through the vessel. I think this may indicate that the low flow rate caused some trapping, which also inhibited the passage of saline.

Dr. William Knisely: I wonder what happens to the dextrans in a living animal? Is there merely a removal of the lowest molecular components or are they potentially altered chemically within the body?

Dr. Gelin: There is rapid loss of the smallest molecules in the urine. The remaining molecules equilibrate in the extravascular compartment. Then there is a short storage period of molecules in the reticular epithelial systems for perhaps ten days, and finally the polymer is broken down and metabolized since labeled dextran has been identified as carbon dioxide in the expired air.

EVIDENCE OF EFFECT OF LOW MOLECULAR WEIGHT DEXTRAN ON SHOCK IN EXPERIMENTAL ANIMALS

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Irreversible shock in the dog whether due to hemorrhage, endotoxins, epinephrine or occlusion of the superior mesenteric artery is associated with characteristic visceral pathology of which acute hemorrhagic necrosis of the intestinal mucosa is the principal and most striking pathological finding. Although pathological changes are also found in the brain, heart, lungs, liver and kidneys, these changes are usually mild, frequently variable and cannot be related directly to mortality. The opposite is true of the small bowel lesion, and measures which ameliorate or prevent the bowel lesions also prevent the death of the dog. This similarity of gross pathological changes in various types of shock suggests that at some stage there are common physiological derangements which cause deleterious effects in the intestine. This common denominator in irreversible shock in the dog is believed by the authors to be a mechanical limitation of blood flow to the intestine of the dog. The decrease in intestinal blood flow appears to be due both to a loss of circulating blood volume and to an increased secretion of catecholamines leading to severe visceral vasoconstriction.

A number of studies by the authors as well as by others have shown that pretreatment of shock with adrenergic blocking agents will in most cases prevent the onset of irreversible shock induced by the diverse methods noted above. Such agents act by blocking or ameliorating vasospasm and hence also prevent the loss of fluid from the vascular system which inevitably accompanies vasospasm. While such studies using pretreatment enable us to further confirm our thoughts on the basic mechanisms of shock, the pressing clinical need is for methods to treat severe shock already present.

Using the principles described above, shock was induced in dogs by endotoxins, hemorrhage or vasoactive drugs as described previously: after the shock was well established, treatment was instituted. This consisted of adrenergic blocking agents alone, fluids alone, hydrocortisone alone, or combinations of these.

Adrenergic blocking agents used after shock existed, often resulted in a further depression of blood pressure and death. Low molecular weight dextran (LMWD), plasma or saline were used exclusively in another group of experiments. Volumes of each of these substances were given intravenously in sufficient amounts to maintain the blood pressure at normal or near normal levels. While the duration of survival could be significantly prolonged by large volumes of plasma or LMWD (50 to 75 ml/kg), death occurred when the fluid therapy was stopped. In the case of

LMWD, when such large volumes as 50 to 75 ml/kg were given, a bleeding diathesis resulted in diffuse hemorrhage in all the tissues of the body. Such findings did not occur when plasma alone was used.

The combination of intravenous plasma (50 to 75 ml/kg) and dibenzyline (0.5 to 1 mg/kg) was then tried and found to be an effective treatment for irreversible endotoxin shock if given early after its inducement. Moreover, hydrocortisone, 50 mg/kg, given intravenously 30 minutes after induction of irreversible endotoxin shock also prevented death in dogs so treated. These studies and those cited above were correlated with individual organ blood flows measured with the electromagnetic flow meter, cardiac outputs, peripheral resistance determinations and plasma catecholamine measurements.

THE EFFECT OF LOW MOLECULAR WEIGHT DEXTRAN ON HEMORRHAGIC HYPOTENSION

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We were first interested in LMWD from the standpoint of superior mesentery venous occlusion. Therefore, we set up an experiment in dogs in which we occluded for two hours the superior mesenteric vein, just at its junction with the splenic vein. Immediately after occlusion, we infused test solutions in the amount of 20 ml. per kilogram of body weight five minutes after occlusion. There were 15 dogs in each group utilized. Group 1 received normal saline; group 2 received high molecular dextran (this is clinical dextran available in America) and saline; and group 3 received low molecular dextran, or Rheomacrodex^{R}. Serial measurements of blood pressure, hemoglobin, hematocrit were made; and the vascular status of the small intestine was observed throughout and at death or sacrifice.

The pattern we saw in the dogs given saline was universal; they had venous infarctions of the bowels. In the group given LMWD, the pattern was quite different; there were small punctate hemorrhages in the mesentery, sometimes none at all. Occasionally there was some duskiness which would disappear on releasing the occlusion.

The high molecular weight dextran group was variable in that some bowels went on to infarction and death of the gut, and some of them responded the same as the LMWD group.

Hematocrit changes showed hemoconcentration in the saline group, slight hemoconcentration in the high molecular but none in the LMWD group.

The blood pressure response in the control group and the high molecular dextran group was very significantly lowered; whereas there was little change in the LMWD group.

Infarction in those treated with normal saline was 100 per cent; in those treated with high molecular weight dextran, there was no evidence of infarction. All the surviving animals were autopsied three weeks later; there was nothing evident in the gut in those that had not infarcted.

Mortality followed infarction in each instance. All 15 of the saline group died, anywhere up to 48 hours. In the high molecular weight dextran group, 9 of 15 or 60 per cent died. In the LMWD group, 3 died or 20 per cent of the total; these were not related to bowel status, since there was no infarction of the bowel.

From these studies we concluded that we could offer protection to animals subjected to mesenteric venous occlusion. We postulated that this was because we were able to keep capillary circulation open. We studied mesenteric circulation, and found that it was true that venal circulation did continue after occlusion. There was cessation at first, then resumption to about half-speed flow; and at the time of release, most of the capillary bed was open. In the control group, there was massive hemorrhage, massive loss of blood into the bowel and into the peritoneum, and the animals were really in hemorrhagic hypotension.

From this point we went to the study of hemorrhagic hypotension in dogs. We studied 39 dogs. These animals were kept for three weeks during which time they were dewormed, vaccinated, and freed of disease, and then they were splenectomized. They were sent to a farm for three weeks and then returned to our laboratory which is temperature and humidity controlled. We did hematocrits, and rejected one of the 39 because of a low value.

Anesthesia was performed with pentobarbital. The femoral artery was cannulated and attached through a three-way stopcock to a mercury manometer for continuous monitoring of systemic pressure. The cannula was also used for bleeding or for reinfusion of blood or test solution as indicated.

We used 30 of these animals. After we anesthetized the animal, we allowed the blood pressure to stabilize within 10 mm. Hg for 30 minutes. Then we did an I-131 bound albumin blood volume.

An average of two samples obtained at 15 and 20 minutes were used as the calculated blood volume. Thirty minutes more were allowed to elapse, and then the animal was bled 15 ml. per kilogram of body weight, and the animals divided into three groups of ten each.

In Group 1, the blood was returned.

In Group 2, LMWD, a 15 per cent solution in 5 per cent glucose and water was administered in the amount of 15 ml. per kilo.

In Group 3, dextran 80, average molecular weight 75,000, 15 ml. per kilo of 15 per cent solution in 5 per cent glucose and water was administered.

We waited 15 minutes for mixing, and then removed 40 per cent of the animal's calculated blood volume. We observed the animal for two hours, monitoring blood pressure. The blood pressure response was severe in the control animals in that it dropped to 20 mm. Hg immediately after bleeding, and then rose to the 60 to 70 range at the end of one to two hours.

The high molecular dextran group dropped to 60 mm. Hg, then back to the 70 to 80 millimeter range.

The LMWD went down to 90 mm. Hg and then back to above 100 after one to two hours.

We found that the difference was significant between the LMWD and the control group on analysis to the 1 percent level.

The hematocrit changes showed a severe hemodilution in the dextran treated dogs, both high and low, with drops to 19.6 and 21 per cent, respectively. However, the control group only dropped to 35 per cent; this would represent a relative hemoconcentration in that 40 per cent of the blood volume was removed.

The oxygen tension in the LMWD group did not drop below 90 at any time, although controls immediately after bleeding were down to 73 from an average of 95 in the beginning, and were at extremely low levels at the end of the experiment. The high molecular dextran group responded to a lesser extent.

pH changes were significant. The control group dropped progressively from an onset level of 7.37 down to 7.15 one and a half hours after hemorrhage, and the high and LMWD groups dropped to a much lesser degree. This did not have a respiratory component, as in no instance did the mean pCO_2 level rise above 40 mm. Hg.

Two of the animals survived in the control group, five of ten, or 50 per cent in the high molecular dextran group, and nine of ten, or 90 per cent in the LMWD treated animals.

We took the remaining eight animals and tried to find out why the difference. We did exactly the same thing up to the point of reinfusion of a test solution. At this point, we divided the eight animals into two groups of four. In one group we returned the blood; in the second group we gave LMWD as before. We allowed 15 minutes for mixing, did a repeat I-131 blood volume and sampling at 15 and 20 minutes later compared to a background count drawn before reinjection of I-131.

We found from this that the blood volume in the LMWD group was expanded 15 ml. per kilogram of body weight above the control value found in the beginning; this indicated that we did have a massive increase in blood volume by injecting this material.

We also drew serum samples for osmolarity and determined these by the freezing point depression method. We found that sampling at 10, 15, 20, and 30 minutes, we could find no significant difference in the osmolarity of the serum in the LMWD and the control groups.

The injected material, however, the low molecular weight dextran in this 15 per cent solution, had an osmolarity of 385 millimols per liter. This simply represented rapid dilution and balancing out at the times that we were sampling.

We also studied the microcirculation. We found that in the control group, as we found in all shock studies in observing the microcirculation in the conjunctiva in about one-third of the control animals, there was a slowing of blood in the venules and capillaries which eventually went on to complete stasis in almost all the fields observed. This did not return.

In the high molecular weight dextran group, there was more slow flow occurring in most of the animals studied and less complete stasis than in the control group.

In the LMWD group, we saw slowing but we did not see a large amount of complete stasis. Usually we saw restoration of flow in practically all fields before we put the animal back in the cage.

Clumping and sludging was obvious in the control group and also in the high molecular weight dextran group; it was present in some animals to a lesser degree in the LMWD group.

From this we concluded that the administration of LMWD offers protection to dogs subjected to severe hemorrhagic shock. Ninety per cent of the animals so treated survived, whereas 50 per cent of those treated with high molecular weight dextran, and only 20 per cent of the controls, survived. The possible reasons are:

1. Increased blood volume due to increased osmolarity.

2. Maintenance of patency in capillary beds ordinarily sequestered in hemorrhagic shock.

DISCUSSION

Col. Johnston: How did you handle the 15 cc. of shed blood per kilo prior to reinfusion? It has been my experience that a 40 per cent bleed in a dog is not highly lethal, as the figure suggested, even though nothing is reinfused. We have many dogs that have been bled up to 60 per cent without reinfusion and have survived. Such untreated dogs might serve as better controls for the dogs receiving low molecular weight dextran.

Dr. Lepley: All the animals received 15 milligrams of heparin in addition to the reinfused blood. This included animals receiving high or low molecular weight dextran. The factor of 40 per cent of the blood volume was based on Swan's work, in which he found a mean lethal dose of hemorrhage of about 41.6 per cent. There is a difference in our findings and his, in that we had a much higher mortality. However, remember that we bled these animals twice.

Dr. Eiseman: You kept that blood sterile, no doubt?

Dr. Lepley: Yes.

Col. Teschan: I would like to ask Dr. Hitchcock whether, in the hypothermic storage experiments, he has any serial data on the changes in rates of urine flow and microscopic appearance of the urine shortly after reimplantation of the kidneys as autografts. A good deal of this has to do with analogies between what may have happened in terms of the urinary output relative to the BUN rise in comparison to what we might be looking for in human acute renal failure.

Dr. Hitchcock: The dogs that had immediate contralateral nephrectomy following an autograft stored for four and one-half hours in an ice box put out good quantities of urine, although the specific gravity dropped significantly. They regained their ability to concentrate, usually by seven days. They all had proteinuria and glycosuria, which cleared within a week.

Dr. Lewis: I can confirm Dr. Lepley's findings from a slightly different type of experimental approach, showing the striking increased plasma volume produced by LMWD infusion. We studied hypothermic pump oxygenator perfusions in dogs utilizing saline, plasma and LMWD as a prime. There was minimal plasma volume change with saline or plasma but when LMWD was employed the plasma volume increased markedly. I cannot say whether this was due to its prevention of sludging or to some other effect of the dextran, but the changes were marked.

We also have measured vascular resistance in the dog mesentery at 20°C in control conditions and following addition of saline, plasma and LMWD. During such hypothermia we found the major change in resistance to be on the arterial side of the circulation in contradiction to reported studies that in the dog's forepaw at least such changes were due to alterations on the venous side of the capillary bed.

Saline diminished resistance slightly; plasma decreased both arterial and total resistance a bit more; but LMWD was more effective than either. In none was there any change in resistance on the venous side.

Curiously, the addition of LMWD increased viscosity of the blood even though it decreased vascular resistance.

Dr. Moore: In these discussions we must be very careful to distinguish between total osmotic pressure and colloid osmotic pressure. The total solute strength of dextran solutions is usually miniscule because of its huge molecular weight. These substances do have a huge colloid osmotic pressure or oncotic pressure. Such a colloid osmotic pressure can only be measured when faced with a membrane impermeable to the molecule. The colloid osmotic pressure of 15 per cent LVD I will leave to Charlie Gelin, since he has measured it in millimeters of mercury, but it is just about three times that of plasma because, gram for gram, it is similar to albumin.

The reason that fluid is pulled into the vascular space has nothing to do with the total osmotic pressure in milliosmols per liter. It has all to do with the colloid osmotic pressure.

Dr. Rhoads: Has anyone compared the effects of dextrans on an equal osmolar basis; in other words, utilizing the same number of molecules to compare the inherent effects of the different sizes?

Dr. Gelin: I don't think it is possible to do it.

Dr. Eiseman: Why not?

Dr. Gelin: Because if we should make comparative studies between LMWD with a weight of 40,000 and repeat the experiment with a molecule weighing 800,000, that animal should die.

Unidentified: You could make different solutions of various dextrans that had the same oncotic pressure. If I remember correctly, such an experiment was carried out in Sweden, and it worked out pretty much the way you would expect. If you don't utilize huge, toxic macromolecules, the plasma volume increment is a linear function of the colloid osmotic pressure.

Dr. Eiseman: What would the relationship be in oncotic pressure between the LMWD and ordinary 75,000 molecular weight dextran?

Dr. Ingelman: I expect the oncotic pressure would be about the same.

Dr. Moore: It depends solely on the membrane the molecule is facing. In the liver, for instance, which is almost perfectly permeable to albumin, human albumin exerts practically no oncotic pressure. In the periphery, of course, it has an important oncotic property in holding fluid within the vascular space.

Dr. Melvin Knisely: Dr. Moore's statement concerning the nature of the membrane is extremely important. A number of times here today people have asserted that something put into the vascular system would pull water in with it. This assumes a healthy, normal endothelial lining. Following burns or other forms of trauma, vessels far from the site of injury may change in such a way as to become completely permeable to all blood plasma. Everything seems to leak out. You can watch it happen in the experimental animals.

Dr. Richard Lillehei: Concern has been raised as to the evidence that LMWD diminishes peripheral vascular resistance. Not only has total resistance been measured by following total cardiac output, but individual organ resistance can be monitored by utilizing the flow meter. I think that differential changes in vascular resistance among the viscera will be found by such studies following trauma.

USE OF LOW MOLECULAR WEIGHT DEXTRAN IN BURNS

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Several years ago it was shown by Knisely that a certain amount of intravascular agglutination accompanied burning injury. Since ordinary dextran had proved to be very beneficial in burned patients as a plasma volume expander, it was felt that a trial of Rheomacrodex^{R} would be indicated. It has always been interesting how well patients treated with ordinary dextran progressed. Some investigators have felt that one of the values of dextran was the small molecules which might break up intravascular agglutination and also serve as an osmotic diuretic to promote a better urinary output.

Because of known intravascular agglutination and thromboses immediately beneath the burned surface, a study was undertaken several years ago with the use of heparin. In rats, heparin was very beneficial in the treatment of burns. On the basis of this, several patients received heparin early after burn injury and it was believed to be of some value.

The studies with Rheomacrodex^{R} in burns have been divided into two parts: (1) clinical experiences; and, (2) evaluation of the material as an osmotic diuretic in burned dogs.

Rheomacrodex^{R} was given to 12 burned patients in the early period after injury. All burned patients received 1,000 ml. or more. Most of the patients received 2,000 ml. over a one- or two-day period. In one instance, a 20-year-old man with an 83 per cent burn was given 5,500 ml. over a period of five days. One unit was given about every 12 hours without any particular difficulty. This patient eventually died on his 8th postburn day, and there was no evidence of any untoward reaction from the Rheomacrodex^{R}. In one extensive electrical burn, 2,000 ml. of Rheomacrodex^{R}, given over a period of 24 hours, seemed to improve the patient's urinary output. The obvious difficulty in the administration of Rheomacrodex^{R} is the dosage. It has been impossible to tell when the proper level of Rheomacrodex^{R} is being maintained in the blood stream. In spite of repeated attempts to observe the conjunctival vessels, not enough experience has been gained to use this as a technique of evaluating proper levels.

In a series of 21 mongrel dogs, standard lactated Ringer's therapy after a 30 per cent third-degree burn was compared with a like amount of Rheomacrodex^{R}. The average urinary output per 24-hour period after trauma was 269 ml. in the standard treatment group, and with Rheomacrodex^{R} was 402 ml. The kidneys of the dogs, examined at autopsy, showed that those treated with Rheomacrodex^{R} had less thrombosis and less edema than those in the control group. It would appear that in these animals Rheomacrodex^{R} caused an increased urinary output in burned animals.

The current status of Rheomacrodex^{R} in burns seems to be that there may be many theoretical advantages to its use and that the administration to patients thus far has shown no untoward reaction. The chief problem to be delineated is the proper dosage and further clinical evaluation is advisable.

DISCUSSION

Dr. Haynes: The following experience with the use of low molecular weight dextran in severe burns is concerned primarily with an evaluation of the potential of this material to improve urinary output when oliguria is a clinical problem.

There were 12 patients in this series with 10 patients having in excess of 50 per cent second degree and third degree burns. There were two survivors in the group (Table). From the point of view of the effectiveness of low molecular weight dextran as an osmotic diuretic, the patients in this series were divided into three general groups.

Group I included patients R. B. and H. T. and was characterized by having relatively normal renal function and circulation and increased urine volumes following the use of LMW dextran.

Group II included patients W. B., C. S., R. K., and E. P. There was no known evidence of pre-existing renal damage but hypotension and thus decreased renal blood flow was considered a primary explanation for falling urine volumes and failure of dextran to produce diuresis. However, patients W. B. and C. S. demonstrated rising BUN's, so that some renal damage, whatever its genesis, may have been present.

Group III included patients C. L., S. B., W. M., A. H., and I. R., and was characterized by some evidence of pre-existing renal damage antedating the burn injury, a rising BUN, and lack of response to LMW dextran. One patient, V. P., resisted classification since she had excellent renal function but failed to respond to LMW dextran.

These data suggest that the patient with severe burns and reasonably adequate renal function will respond to low molecular weight dextran to produce increased urine volumes. It is likely that this same patient would respond to blood volume expansion with many agents, including clinical dextran though not with the same amount of urine flow. The hypotensive patient with poor renal blood flow does not demonstrate improved urinary output after low molecular weight dextran has been administered. Adequate renal blood flow is essential to good renal function and the hypotensive burn patient with poor renal perfusion is not aided by LMW dextran. Finally, the burn patient with evidence of pre-existing renal damage who is a clinical problem in the management of post-burn oliguria is not improved by the use of low molecular weight dextran. This observation suggests that the damaged kidney with reduced glomerular and tubular function additionally insulted by burn injury producing reduced renal blood flow, hemoglobinuria, and further tubular damage may be incapable of responding to LMW dextran. No untoward reactions have been observed.

TABLE

Low Molecular Weight Dextran In Burns

		Sex	Age	Total Burn %	% 3°	Low Molecular Weight Dextran				Remarks
						Day Given	Amount	Urine Vols. Before (4 hrs.)	Urine Vols. After (4 hrs.)	
1.	C. L.	M	45	50	32	1 BUN-75	500cc	No response		Died Day 3. Chr. Pyelonephritis at post.
2.	S. B.	F	72	95	95	1 BUN-68	1000cc on day 2	0 0 0 2	45 45 60 30	Died Day 3. Old glomerular disease, tubular nephrosis, pulm. edema on post.
3.	W. B.	M	68	65	55	3 BUN-98	1000cc	No response (1 hr. before death, hypotensive)		
4.	C. S.	M	30	90	90	10 BUN-90 Cr. 3.2	750cc	No response (Falling blood pressure)		Died same day.
5.	W. M.	M	45	50	50	3 BUN-41	500cc	40 45 65 30 (Died 4 hrs. later)	135 80 80 60	H. C. V. D., hemiplegia. Died day 3 with cerebrovascular accident, falling B. P.
6.	A. H.	M	57	60	50	1 BUN-55	1000cc on day 2	No response		Died Day 6, rising BUN to 155, falling B. P. terminally. HCVD, B. P. 210/130
7.	I. R.	F	59	58	58	1	1000cc	No response		Died Day 7, rising BUN to 225, arrived in shock, oliguric throughout course.

TABLE (Continued)

Low Molecular Weight Dextran in Burns

		Sex	Age	Total Burn %	% 3°	Low Molecular Weight Dextran: Day Given	Amount	Urine Vols. Before (4 hrs.)	Urine Vols. After (4 hrs.)	Remarks
8.	R.K.	M	20	20	20 Severe	0	1000cc	No response (hypotensive - 60-70 mm. Hg. systolic)		Intra-abdominal injury, electrical. Died Day 0, electrical burn of stomach, liver, colon jejunum & abd. & thoracic wall.
9.	E.P.	F	17	73	65	3 BUN-21	500cc	No response		Died Day 3, in pulm. edema. Urine vols. 600-1000cc/daily 1st 2 days.
10.	V.P.	F	30	55	50	1 BUN-12	500cc	No response (Urine flows before and after were good, 40-50cc/hr.)		Survived.
11.	R.B.	F	61	65	65	0 BUN-15	500cc	25cc 5 5 19	110cc 200 120 120	Died Day 4, with good urine vols. throughout course. Death due to respiratory obstruction inspissated tracheal secretions.
12.	H.T.	F	49	26	15	3 BUN-15	500cc	45cc 45 20 20	200cc 875 300 450	Survived. Adequate urine volumes throughout course.

These data were collected for the most part one or more days after injury. Further study using LMW dextran immediately after injury to determine its potential to prevent progressive renal insufficiency should be done in view of its effectiveness in the experimental animal in preventing acute renal insufficiency following transfusion reaction (Atik, and Gutierrez Saenx, Surgical Forum 12:48, 1961).

I might summarize briefly by saying that in this brief experience we think probably that the patient who responds dramatically to low molecular weight dextran, who has a serious burn injury, might respond well to plasma volume expansion with other agents, as well, including clinical dextran.

There is some suggestion from our experience that perhaps the volume of diuresis occurring after low molecular weight dextran is somewhat greater initially than that coming from the same volume of clinical dextran. And secondly, the patient who is a serious problem following his injury in terms of low urine volumes, rising BUN and so forth, and a goodly number of these patients seem to have some pre-existing evidence of renal damage prior to their injury, do not appear to be helped by low molecular weight dextran.

Dr. Hint: In a small series of burns studied in Lund, we had 31 consecutive cases treated with low molecular weight dextran as the only colloid. Two of five cases of 40 per cent burn died. Below 40 per cent burn there was but one death using low molecular weight dextran, and this in an 86 year old man having a 10 per cent burn area who died one week after injury.

We have used three grams per kilogram of body weight as the total amount over a period of 48 to 72 hours. We did not have any bleeding with the 15 or 10 per cent solution.

May I offer a warning in utilizing LMWD in burns? These substances as we have seen have a high oncotic pressure and draw a great deal of water into the intravascular space and usually produce diuresis. However, if they haven't enough available water given by mouth or intravenously, they will become very dehydrated. The urine will become very viscous, so viscous one may think there is mechanical obstruction in the distal tubules causing oliguria and threatening anuria. It is contraindicated, in our opinion, to give LMWD in large doses unless adequate water is given simultaneously.

THE USE OF LOW MOLECULAR WEIGHT DEXTRAN IN ACUTE MYOCARDIAL INFARCTION

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Pathologic study shows a rather poor correlation between evident coronary occlusion and myocardial infarction and implies that the former may not necessarily result in the latter. Poorly understood observations in acute infarction such as elevated sedimentation rate, leukocytosis, falling hematocrit, and serum enzyme reactions are to a degree, similar to those observed in experimentally produced intravascular sludging. Sludging has been clearly described in acute myocardial infarction. Sludging can be to a degree prevented or reversed by intravenous low molecular dextran.

Consecutive cases of acute myocardial infarction were treated in the usual manner, plus intravenous low molecular dextran (1.5 to 3.0 gm./Kg/24 hrs.) for the initial 76 hours. Controls consisted of the previous 5 years mortality in acute infarction at this institution and the established laboratory and EKG evolution of this disease.

The following observations were made:

1. The electrocardiograms varied from the usual pattern in that the injury potential commonly persisted up to 10 days; the initial T wave changes of ischemia were commonly reversed, only to reappear in about 10 days; and Q waves rarely developed after institution of this therapy.

2. There was an absence of the usual leukocyte response.

3. The elevation of the sedimentation rate was slightly delayed.

4. The SGOT levels remained elevated unusually long.

The mortality rate compared favorably with controls. There have been no deaths or significant complications on the very brief current follow up.

HEMODILUTION IN EXTRACORPOREAL CIRCULATION

Comparative Study of Low Molecular Weight Dextran and Five Per Cent Dextrose

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The antisludging property of low molecular weight dextran* (LMWD) has been established in previous reports;[1] therefore, its use has been recommended as beneficial in preventing or minimizing these phenomena during and after cardiopulmonary bypass.

To date over 800 open-heart operations have been performed at the University of Minnesota Hospital using LMWD combined with fresh blood or alone for priming volume in the pump oxygenator. Observations made during this experience have corroborated the advantages of the basic principle of hemodilution in extracorporeal circulation.[8] Zuhdi[2,3] and DeWall[4] have substituted 5 per cent dextrose in water (5 per cent DW) for LMWD. Experimentally, Gollan[5,6] has utilized normal saline, and Siegal[7] has advocated an artificial plasma mixture for priming purposes.

Since two of these techniques, namely LMWD and 5 per cent DW have been broadly utilized clinically at the University of Minnesota Hospital, we have sought to objectively analyze the perfusion records of patients belonging in each of these groups.

Method of Study

Three groups of patients were studied as follows:

1. 5 per cent DW used as the entire priming volume in the pump oxygenator.

*The low molecular weight dextran used was Rheomacrodex^{R}, Pharmacia, Uppsala, Sweden. The average molecular weight was 41,000 and the intrinsic viscosity 0.19. The low fraction has an average molecular weight of 14,500 and is 11.5 per cent of the total, and the high fraction has a molecular weight of 68,000 and is 10.6 per cent of the total. The solution is a 10 per cent Rheomacrodex^{R} in 0.9 per cent saline solution. It has a buffer capacity of 1.5 ml. of 0.1 N. sodium hydroxide per liter and a pH of 5.1.

2. Equal volumes of LMWD and fresh blood as the priming volume.

3. LMWD alone as the priming volume.

For each of the three groups the total priming volume was calculated to be 15 to 30 cc./kg. of body weight. All operations were performed with the same helix reservoir bubble oxygenator.

All cases of 5 per cent DW were studied with the exception of those who died within the first 12 hours and several patients with laboratory workup too incomplete to permit evaluation. Thus, 43 patients remained about whom there was adequate data and sufficient survival time for objective analysis. We matched these 43 patients with a similar number operated upon at the same general time and in whom LMWD and blood were used as priming volume. The criteria for this matching were the following: similar heart lesion, body weight, age, and duration of the perfusion. Thus, we were able to couple 86 patients for comparative analysis.

The third group, a recently initiated study, includes those patients in whom LMWD was used exclusively as the priming volume. These patients have had atrial septal or ventricular septal defects. At this time 12 patients constitute this group (Table 1).

TABLE 1

Lesion Distribution

	Priming Solution		
	5% Dextrose in Water	Dextran & Blood (Equal Parts)	Dextran
I. Congenital			
1. Atrial Septal Defect	9	9	6
2. Ventricular Septal Defect	17	17	6
3. Tetralogy of Fallot	7	7	
4. Pulmonic Stenosis	2	2	
5. Aortic Stenosis	4	4	
II. Acquired			
1. Mitral Stenosis & Insufficiency	3	3	
2. Aortic Stenosis & Insufficiency with Total Valve Replacement	1	1	
Total	43	43	12

Results

We have studied some or all of the following parameters in these groups of patients:

1. Microcirculation.
2. Electronegativity of the erythrocytes.
3. Hematologic changes.
4. Blood chemistry alterations.
5. Total postoperative chest drainage during the first 12 hours.
6. Total urine output and specific gravity during the first 24 hours.

Microcirculation

This subject was extensively studied in a previous report from our group[1] and LMWD was found to be significantly beneficial in minimizing this reaction to trauma.

Alterations in the Negative Charge of Red Cells

These changes have been studied by Bernstein, et al,[7] in our patients. He found that in 26 of 27 patients in whom blood and dextran were used to prime the oxygenator, there was an increase in the electronegativity of the red blood cells. In all instances except one there was a rapid and marked increase in negative red blood cell charge from control values averaging 1.23×10^{-8} Coulombs/cell to averages of 1.85×10^{-8} Coulombs/cell 15 minutes after perfusion. The increase in cellular electronegativity was maintained throughout the course of these perfusions.

Meanwhile, in the group of 5 per cent DW alone no significant change was seen. From 12 patients, one showed no change, four had a slight decrease, three had an important decrease, and three had a significant increase on the electronegativity of their red blood cells.

Hematologic Changes

These were studies by Long[1] and also by Gans.[10] They found a statistically significant decrease in the amount of destruction of erythrocytes and platelets when LMWD was used. This lessened reduction of platelets and decreased destruction of erythrocytes was interpreted as being a reflection of a better status in the microcirculation which in turn was studied by direct observation of the mesenteric and conjunctival circulation in dogs and patients, respectively.

Hemoglobin levels were studied during the perfusions. These changes are portrayed in Figure 1. Comparing the preoperative and immediate postoperative

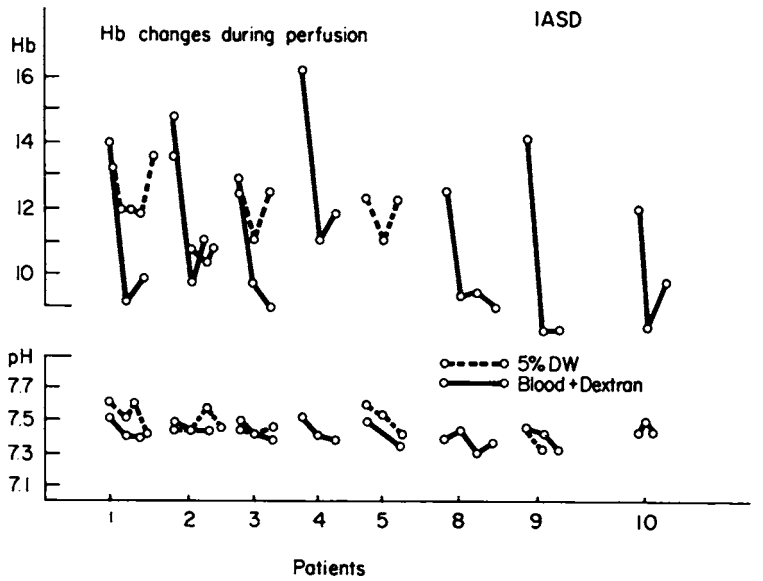

Figure 1

levels indicates that the latter value closely approaches the initial in all three groups (Table 2). These differences between the pre and postoperative hemoglobin contents in Table 2 were not statistically significant. However, when the initial or immediate postoperative hemoglobin levels were compared with those sampled midway during the perfusion, the blood dextran group showed the widest spread. This difference was probably due to the greater diffusion of 5 per cent DW out of the intravascular compartment.

TABLE 2

Hematologic Alterations in Hemodilution Perfusions

Comparing Preoperative and Postoperative Samples*
(Mean Variations)

	5% Dextrose in Water	Dextran and Blood	Dextran
Hb. (Gms. %)	- .326	+ .851	- .317
Na (Meq.)	- .326	+2.29	+3
Cl (Meq.)	+6.46	+7.06	+9
CO_2 (Meq.)	+3.46	+3.11	+2.45
K (Meq.)	+1.17	+ .956	+ .245

*Postanesthetic recovery room approximately 1/2 hour after patient left operating room.

Plasma Hemoglobin

In the group of patients in whom 5 per cent DW was used as the priming volume, the plasma hemoglobin per minute of perfusion was the highest (1.85 ± 0.74 mg./min.). The blood and dextran group had a mean plasma hemoglobin level of 1.57 ± 0.35 mg./min. The group of straight dextran had the lowest plasma hemoglobin per minute of perfusion (1.06 ± 0.74 mg./min.)(Table 3). This result is in agreement with a series of in vitro experiments performed to study the hemolysis per unit of different solutions. In this study we compared the hemolysis in vitro caused by mixing different solutions in equal proportions with fresh blood at room temperature (24 degrees C.) and at body temperature (37 degrees C.) under sterile conditions. Five per cent dextrose in water showed a progressive and consistent increase in the plasma hemoglobin attaining levels of 250 mg. per cent in one hour. LMWD produced no significant hemolysis even 16 hours after being mixed with equal parts of fresh blood. Other data pertaining to this study are shown in Table 4.

TABLE 3

Plasma Hemoglobin

Mgs./Minute of Perfusion

Patient Defect	Priming Solutions		
	5% Dextrose in Water	Blood and Dextran	Dextran
Ventricular Septal Defect	2.18±0.97	1.52±0.32	0.98±0.32
Atrial Septal Defect	1.51±0.48	1.47±0.79	1.23±1.07
Overall	1.85±0.74	1.57±0.35	1.06±0.55

Blood Chemistry Changes

Serum electrolytes were analyzed preoperatively and immediately postoperatively. None of the three groups showed any significant difference from the other. The changes during the actual perfusion time were extensively studied by DeWall and Lillehei[11] in a previous report. They found a moderate reduction in the Na and Cl during the period of hemodilution and a return to near normal values by the time the patient had returned to the postanesthetic recovery room.

In this study the previously observed[11] potassium decrease was not seen. In all three groups there were slight increases in the serum K (see Table 2).

Acid base balance included studies on the arterial pH and carbon dioxide combining power. The arterial pH vartations before, during, and immediately after the perfusion were measured. In all three groups there was a slight decrease of the

TABLE 4

Hemolysis Study

	Temp. Degrees C.	Control Plasma Hemoglobin	5% Dextrose in Water and Blood 1:1 Plasma Hemoglobin	Dextran and Blood 1:1 Plasma Hemoglobin
1 Hour	24	9	232	9.5
		12	250	14.5
4 Hours		45	318	14.5
		49	322	12.0
16 Hours		21.5	388	14.5
		22	388	18.0

pH during the perfusion with a return towards the preperfusion levels. These mean values were as follows: The 5 per cent DW group had a preperfusion mean value of 7.54 and a post-perfusion pH of 7.50. The blood and dextran group had a preperfusion pH of 7.49 and a post-perfusion pH of 7.47. The straight dextran group had a mean preperfusion pH of 7.44 and a post-perfusion pH of 7.45. All of these patients had received a moderate empirical dose (1 to 2 Meq./kg body weight) of Na bicarbonate near the end of the perfusion.

The CO_2 combining power was measured preoperatively and postoperatively in the postanesthetic recovery room. In all three groups there was a mean rise from the preoperative level (5 per cent DW + 3.46 Meq./L., blood and dextran + 3.11 Meq./L., and dextran + 2.45 Meq./L.) which could be explained by the Na bicarbonate which they receive at the end of the perfusion.

Chest Drainage

The total serosanguinous output through the chest catheters during the first 12 hours was measured in all of the 98 patients; this figure was divided by the patient's body weight and compared as cc./kg. of body weight. The group of 5 per cent DW had the lowest overall amount of chest drainage, 17.72 ± 11.24 cc./kg. The blood dextran group had 23.09 ± 12.06 cc./kg. and the straight dextran group had 24.53 ± 14.40 cc./kg. This was consistent in the overall result and in the individual lesion groups with the exception of the atrial septal defect groups. The overall mean values of the chest drainage between the blood dextran and the 5 per cent DW group were submitted to statistical analysis. A two sample t test was performed and the difference was statistically significant. ($p > .04$.)

Urine Output and Specific Gravity

During the first 24 hours, as expected, the highest specific gravity was found in the straight dextran group and in the dextran blood group as the dextran is eliminated via the kidneys. The urine output during the first 24 hours was divided by the patient's body weight and analyzed as ml./kg. body weight (Table 5). The mean values were slightly favorable to the blood dextran group 26.91 ± 11.81 cc./kg. against 24.54 ± 11.77 cc./kg. for the 5 per cent DW group. The straight dextran group had the lowest urine output: 19.45 ± 11.87 cc./kg. However, these differences were not significant statistically.

TABLE 5

Postoperative Urine Output

First 12 Hours, cc./kg.

Patient Defect	Priming Solutions		
	5% Dextrose in Water	Blood and Dextran	Dextran
Ventricular Septal Defect	26.68 ± 12.76	24.15 ± 12.75	
Atrial Septal Defect	29.29 ± 9.89	33.63 ± 9.83	
Aortic Stenosis	20.54 ± 7.60	25.25 ± 15.82	
Overall	24.54 ± 11.77	26.30 ± 11.81	19.45 ± 11.87

Discussion

From other reports and from our data it has been clearly demonstrated that hemodilution is feasible and safe utilizing 5 per cent DW, dextran and blood, or dextran only. Thus, a considerable reduction of the priming volume of the pump oxygenator is achieved and, consequently, much less homologous blood is necessary during extracorporeal circulation.

The advantages of the hemodilution technique are significant. Clotting abnormalities are decreased when less foreign blood is used. The hazards of over or undertransfusion, homologous blood syndrome,[12] renal shutdown, hepatitis, and infection are reduced. Fewer blood donors are needed, and the application and development of open heart surgery is more readily available as well as safer.

Five per cent DW may not be the best blood substitute since it diffuses out of the vascular compartment very rapidly. This could be detrimental in long perfusions. It increases cell fragility and does not specifically inhibit cellular sludging. From these preliminary studies it is easier to define an ideal blood substitute. Low molecular weight dextran appears to have many of the qualities needed for a blood substitute

in extracorporeal circulation. Further experimental studies are necessary to define objectively the optimum degree of hemodilution, as well as to study other solutions as blood substitutes for priming the pump oxygenator.

Summary and Conclusions

1. From previous studies and from these data, low molecular weight dextran (LMWD) appears to minimize the effects of cellular sludging during open heart surgery.

2. LMWD lessens hemolysis during extracorporeal circulation.

3. There was no significant difference in the urine output of those patients who had LMWD or 5 per cent DW as priming volumes.

4. In this present series of patients, dextran produced a slightly greater quantity of chest drainage in the first 12 hours when compared with 5 per cent DW. This difference was statistically significant.

5. LMWD has a definite place in extracorporeal circulation, especially for longer perfusions and for acquired valvular surgery where a low postoperative cardiac output and a high plasma hemoglobin may lead to oliguria or anuria.

REFERENCES

1. Long, D.M.; Sanchez, L.; Varco, R.L.; and Lillehei, C.W.: The Use of Low Molecular Weight Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation. Surgery 50:12, 1961.

2. Zuhdi, N.; McCollough, B.; Carey, J.; Krieger, C.; and Greer, A.: Hypothermic Perfusion for Open Heart Surgical Procedures. Internat. Coll. Surg. 35:319, 1961.

3. Zuhdi, N.; McCollough, B.; Carey, J.; and Greer, A.: Double Helical Reservoir Heart-Lung Machine. Arch. Surg. 82:320, 1961.

4. DeWall, R.A.; Lillehei, R.C.; and Sellers, R.D.: Hemodilution Perfusion for Open Heart Surgery. New England J. Med. 266:1078, 1962.

5. Gollan, F.: Physiology of Cardiac Surgery. Springfield, Illinois, Charles C. Thomas, 1959.

6. Gollan, F.; Hoffman, J.E.; and Jones, R.M.: Maintenance of Life of Dogs Below 10 Degrees Without Hemoglobin. Am. J. Physiol. 179:640, 1954.

7. Siegal, D.; Bilgutay, A.M.; Sanchez, L.; and Lillehei, C.W.: Experience with an Artifical Plasma for Use with Extracorporeal Circulation. J.A.M.A. 179:206, 1960.

8. Bernstein, E.; Castaneda, A.; Evans, R.L.; and Varco, R.L.: Alterations in Red Blood Cell Charge with Extracorporeal Circulation. Surg. Forum 13:193, 1962.

9. Gans, H.; Lillehei, C.W.; and Krivit, W.: Problems in Hemostasis During Open Heart Surgery. I. On the Release of Plasminogen Activator. Ann. Surg. 154:915, 1961.

10. Gans, H.; Siegal, D.L.; Lillehei, C.W.; and Krivit, W.: Problems in Hemostasis During Open Heart Surgery. II. On the Hypercoagulability of Blood During Cardiac Bypass. Ann. Surg. 156:19, 1962.

11. DeWall, R.A., and Lillehei, C.W.: Simplified Total Body Perfusion: Reduced Flows, Moderate Hypothermia, and Hemodilution. J.A.M.A. 179:130, 1962.

12. Gadboys, H.L.; Slonim, R.; and Litwak, R.S.: Homologous Blood Syndrome. Ann. Surg. 156:793, 1962.

DISCUSSION

Dr. Maloney: The work by a number of investigators in our laboratory over the past several years confirms and expands the studies of Doctors Varco, Long, Knisely, Gelin, Hardaway, and their associates. The work at the University of Minnesota has served a valuable purpose in this country by inviting attention to intravascular sludging. Although the intravascular agglutination of erythrocytes has long been recognized, its importance has been ignored by the clinician, perhaps because this phenomenon has been demonstrated to occur in so many benign conditions.

The principal points to arise from the work in our laboratory on this phenomenon are the following:

1. A demonstration of the cause of sludging with pump oxygenators (the denaturation of plasma protein with the formation of an adhesive gel of colloid on the surface of the erythrocytes. This abnormal protein can be demonstrated by a variety of techniques such as electrophoresis, viscosimetry, and electronmicroscopy.).

2. The demonstration that the mechanism of sludging is similar in other conditions in which studies have been made.

3. The demonstration that sludging is undesirable *per se* by virtue of the fact that it produces evidence of anaerobic tissue metabolism.

4. The development of a means of measuring sludging *in vitro* in a manner which is more objective and convincing than the microscopic observations of capillaries.

5. The proposition that on a biochemical basis there would appear to be four general classes of compounds which will prevent or ameliorate sludging:

   (a) surface active agents

   (b) molecular fixatives

   (c) biological reduction

   (d) inert, low molecular weight colloids.

A brief screening effort quickly produced four effective, non-toxic agents. A little effort in this area would likely produce many superior agents.

Dr. Lewis: We started using low molecular weight dextran to supplement the pump prime in extracorporeal circulation combined with profound hypothermia. We found that it was very effective in increasing perfusion in these animals, in some cases almost doubling it. Simultaneously body temperature could be lowered much more effectively and rapidly, and in all viscera, not just in the central organs. Muscle temperature, for example, went down far more quickly than in cooling experiments without the use of low molecular weight dextran.

We then decided to use only low molecular weight dextran in the priming volume. In these dog experiments we encountered a high incidence of bleeding, so we have not subsequently used a total dextran prime in any of our extracorporeal circulation experiments.

I would like to ask Dr. Cuello if the clinical experiments in total dextran prime were preceded by experiments in animals, and whether there was any excessive bleeding in the animals.

I also wish to raise another question which has puzzled me. We have observed sludging within a plastic shunt between the femoral artery and vein of dogs utilizing a binocular microscope. To our surprise we found some apparently normal dogs that sludged even when clinically healthy and prior to any experimental manipulation. For example in a recent series of 11 dogs, 4 sludged before we started the experiment. LMWD decreased the sludge in 6 of the 7 animals where sludging was produced by profound cooling. In the 4 animals that sludged to begin with, some erythrocyte agglomeration remained despite the use of low molecular weight dextran.

Dr. Atik: We have had experimental experience with low molecular weight dextran for about three years, and for the past two years some clinical experience. In our clinical use of this material in about 40 cases of peripheral vascular surgery, we have seen no evidence of its producing hemorrhage or thrombosis. We have also utilized LMWD in about 75 cases following trauma, utilizing a dose of 10 cc./kilo.

There are two other aspects of our work that I would like to mention briefly. The first is the use of LMWD in the prevention of acute renal failure produced in the dog by incompatible blood transfusion following hemorrhagic shock. Dogs were bled 30 to 40 cc./kilo B.W. and then given 400 to 500 cc. of human blood. Two of 5 dogs that survived developed acute renal failure. Four others treated with saline, dextrose and water, and macrodex (that is, 75,000 molecular weight) developed renal failure. None of those treated with low molecular weight dextran developed renal failure.

We have similarly found that LMWD will diminish or abolish the hypotension, diminution in renal blood flow and oliguria or anuria that occurred in dogs given a similar volume of saline (50 cc.).

Dr. Eiseman: Have you noticed any toxic symptoms after giving LMD?

Dr. Atik: No. In one case we stopped it because of a possible allergic response, but subsequent readministration two days later was well tolerated.

Dr. Eiseman: What is the maximum amount you have given to a patient within a short period of time?

Dr. Atik: We have given 1,000 cc. of 10 per cent LMWD the day of surgery, and 500 cc. three days later.

Dr. Richard Lillehei: Cardiopulmonary bypass is analogous in many ways to traumatic shock. We have found elevated levels of both epinephrine and norepinephrine after an hour of bypass so that it would seem reasonable to utilize vasodilators as well as means to unsludge the blood during such bypass.

It has been my subjective impression that patients in whom LMWD is used as a pump prime have more difficulty with hemostasis than when glucose is utilized, but I cannot prove this.

Similarly, LMWD becomes much more viscous than does glucose at profound hypothermic levels of perfusion.

Dr. Hehre: In this conference we have been speaking of dextran from the Leuconostoc mesenteroides, that is the B5 12 strain. I would like to point out that a material of parallel physical type can readily be produced through the direct fermentation of streptococcus 50. I would hope that some advantage would be taken of this second source of dextran to make parallel experiments on material of comparable molecular size, and yet of slightly different chemical structure.

I also want to make a plea for the development of a somewhat better nomenclature for these dextran products. Actually, the name dextran historically belongs to the natural biological material. One would not think of using the term glycogen or starch or cellulose for a hydrolysate or a fraction of these materials. And yet dextran magically enough stays dextran no matter what you do with it.

Dr. Walton Lillehei: Much of the prejudice against LMWD concerning its propensity to cause bleeding is a hold-over from effects of high molecular weight material and should be ignored.

Dr. Varco and I carried out a study in 150 consecutive open heart pump runs. We randomly selected by the drawing of a card whether we would prime with pure blood; with blood plus 3 grams/kilo B. W. of serum albumin; or blood plus 3 grams/kilo B. W. of LMWD. There was no significant difference between the postoperative bleeding in any of the groups.

More recently our interest has been in utilizing a 5 per cent glucose pump prime. It has the advantage of being cheap and easily available but leaves the circulation more readily than does LMWD. Secondly, it does not have the anti-sludging properties of LMWD. Finally, glucose seems to affect red cell fragility whereas LMWD does not.

There is value to use of hemodilution in pump oxygenators whether the prime be of glucose or LMWD.

Dr. William Knisely: I would like to comment on several of today's presentations.

It was stated that supposedly normal dogs demonstrated erythrocyte aggregation. We examined the circulation of 21 greyhounds which were raised outdoors and found that essentially all showed erythrocyte aggregation when they first were received by us. As the sores and abrasions cleared, so did the erythrocyte agglutination disappear.[1] I would say that "by definition" dogs are not normal if they have some aggregation or agglutination.

In regard to species differences, it has been mentioned that erythrocytes from horses have a rapid sedimentation rate *in vitro* but do not aggregate *in vivo*.[2]

Much of what has been discussed today was published by Ploman in both the French[3] and Swedish[4] literature but largely overlooked in this country. He observed intravascular sedimentation rates *in vivo* in the retinal circulation and tried to relate *in vivo* and *in vitro* observations.

Finally, I want to make the point that the inactivating mechanisms of lung be considered in evaluating the efficacy of intravenously administered substances since they must traverse the pulmonary bed before reaching the periphery. Future investigation might include comparison of the effect of drugs given both intravenously and intra-arterially. We know, for example, that serotonin is thus inactivated by pulmonary tissue.[5]

BIBLIOGRAPHY

1. Ezell, D.; Garlick, M.; Harding, F.; Knisely, W.H.; Saunders, W.; and Weis-Fogh, J.: Duration of Burning as a Factor Affecting the Peak Concentration of Free Hemoglobin Found in Blood Plasma After Severe Thermal Burns. Anat. Rec. 115:303, 1953.

2. Knisely, M.H.; Bloch, E.H.; Brooks, F.; and Warner, L.: Microscopic Observations of the Circulating Blood of Nine Healthy Normal Horses, All of Which had Unagglutinated Circulating Blood Cells and High In Vitro Erythrocyte Sedimentation Rates. Am. J. Med. Sci. 219:249, 1950.

3. Ploman, K.G.: Oftalmoskopiskt pavisande av variationer i blodkroppssuspensionens stabilitet. Hygiea, 7:363, 1920.

4. Ploman, K.G.: Demonstration ophtalmoscopique des variations de stabilite dans la suspension des globules rouges. Ann. d'ocul. 157:569, 1920.

5. Knisely, W.H.; Wallace, J.M.; and Addison, W.A.: "Temporary" Pulmonary Embolization Caused by Intravenous Injections of 5-hydroxytryptamine. Fed. Proc. 17:88, 1958.

CLOSING COMMENTS

Francis D. Moore, M.D.
Harvard Medical School

Dr. Eiseman asked me if I would offer one or two thoughts about where we wind up at the end of this interesting conference.

Low viscous dextran is a special purpose fluid. It is supposed to provide something that is new in our therapeutic vocabulary and in our armamentarium. It is supposed to decrease the blood viscosity in patients in whom the viscosity has increased by abnormal concentration of large asymmetrical protein molecules, or by an increase in the hematocrit.

These effects are still open to some question. There are evidences that the viscosity effects of low viscous dextran are merely due to the dilution of all the elements of blood by the large amount of low-viscosity interstitial fluid which is attracted into the plasma volume from across the capillary wall when low viscous dextran is administered.

I believe that this conference has shown clearly that many of the viscosity effects of low viscous dextran are indeed due to a lowering of the concentration of red cells (the hematocrit) and the attraction of low viscosity aqueous solutions of electrolyte, from across the capillary. In addition however, there is a direct effect of low viscous dextran on the electrical charge on the surface of the red cell itself. This factor more than any other probably reduces the tendency to aggregation.

Dr. Eiseman has asked what is the maximum safe dose. I believe this should be paraphrased to the question, "what is the minimum effective dose". This is not a fluid like blood where you are trying to pile in as much as you possibly can when pressure is low. Instead it is a special purpose fluid in a sense analogous to insulin or digitalis. It is being given for a special purpose—namely, to decrease blood viscosity. The question is, therefore, not "how much can we give and have the patient live?" It is instead "how little can we give and obtain the desired effect?"

Normal human plasma and American commercial dextran both hold fluid in the plasma volume for a longer time than the low molecular weight low viscous material. The reason for this is perfectly obvious and I shall not dwell on it further. But it is important to emphasize that the long term treatment of burn shock with low viscous dextran alone is irrational. Each dose of dextran is given and after an hour or two it leaks into the burn area rather than attracting fluid back into the plasma for excretion through the kidney.

As far as being an osmotic diuretic, mannitol is much more effective. Mannitol is a very low molecular weight material (180). It is excreted into the glomerular filtrate in very large quantities and holds fluid there very effectively. By contrast, the principal renal effects of low viscous dextran appear to be those of an increase in glomerular filtration rate.

Future studies in man should be devoted to critical observations on tissue perfusion, blood viscosity and blood flow. These must be studied with measurements of tissue oxygen tension and peripheral rates of blood flow rather than blood pressure.

I believe that low viscous dextran is here to stay and that it will find a secure place in the treatment of hypovolemic shock, along with trisbuffer and mannitol.

RESUME

Ben Eiseman, M. D.
University of Kentucky
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The purpose of this conference was to clarify present evidence of the actions of LMWD in the management of the severely injured and to determine fruitful lines of future investigation.

My role is to summarize our findings:

1. There seems to be sufficient evidence that this material is of sufficient promise and proven safety to warrant further clinical and laboratory investigation in the management of various types of trauma and shock in man. The maximum safe dose is as yet uncertain.

2. Although there has been a good deal of clinical trial with the material, its major toxic properties are not completely known. There should be more study of its effects on the clotting mechanism as they may prolong bleeding. Effect in clinical treatment should be differentiated from prevention of renal vascular or tubular lesion. All such studies should be correlated with the state of hydration of the patient or experimental subject.

3. The mechanism by which low molecular weight dextran is effective is not totally clear. It apparently has properties different from those of high molecular weight dextran. In all likelihood its activity depends on altering the surface charge of the erythrocyte and its consequent anticoagulant and anti-sludging activity. In other respects its activity might largely be due to its oncotic effect. More work is required to elucidate the mechanism by which LMWD is effective so that even more efficient agents might be found.

4. In the many clinical and laboratory studies that are certain to continue, objective evaluation of LMWD will require careful controls. The first will be in comparison with a comparable degree of hemodilution attained by administering a similar volume of glucose or physiologic saline. The second control group must utilize a comparable degree of anticoagulation. A third group might combine both pure hemodilution and anticoagulation. A fourth must include an infusion of high molecular weight dextran and other substances of an equal oncotic pressure.

In evaluating the role of LMWD during such complex procedures as pump-oxygenator perfusion during open heart surgery, objective evidence of benefit must be sought. Clinical benefit and survival statistics alone depend upon too many other factors that might simultaneously be operative.

5. The National Research Council inevitably will be asked its recommendations concerning future use and study of this material. Personally, it would seem that it is of sufficient proven benefit to warrant its use in the management of the injured. It has not such proven superior attributes that other time-tested methods should be abandoned.

Conclusions drawn by the individual surgeon treating the occasional injured patient with LMWD will probably be of little value. The pitfalls of such uncritical evaluation of a complex clinical problem are too obvious to require elaboration.

I do believe that critical shock teams such as have been sponsored by the National Research Council would do well to include LMWD in their protocol studies.

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