LARGE VOLUME HEMODILUTION PERFUSION IN NEWBORN PIGS: ITS EFFECT ON PLASMA VOLUME, PLASMA PROTEINS, AND PLASMA ELECTROLYTES

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LARGE VOLUME HEMODILUTION PERFUSION IN NEWBORN PIGS: ITS EFFECT ON PLASMA VOLUME, PLASMA PROTEINS, AND PLASMA ELECTROLYTES

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ABSTRACT

Simultaneous evaluations of plasma volume, plasma protein and plasma electrolyte changes in response to large volume hemodilution perfusion in newborn pigs were made. These data are needed for the application of the technique of extracorporeal circulation using large volume hemodilution in the neonate.

Seven days old pigs were used in this study. Two groups of 15 animals underwent arteriovenous perfusion with the chest closed, at normothermia, and without an oxygenator in the system; perfusion was continued until complete mixing of the blood and priming fluid was accomplished. The extracorporeal circuit was primed in group I with a buffered Ringer-lactate solution, and in group II with a buffered solution of 2% Rheomacrodex in Ringer-lactate. Priming solutions were individually tailored to result in a (mixed blood and prime) hematocrit of 22%. The volume of the diluted blood in the circuit after perfusion, was identical to the volume of the priming fluid. A third group consisting of littermates of the above animals was subjected to the same surgical procedures but without perfusion.

Hematocrit, plasma volume, total plasma proteins and electrophoretic patterns of proteins, osmolarity, and

the major plasma electrolytes were measured immediately before and after perfusion, then every 8 hours over a period of 24 hours. Mean arterial blood pressure, heart rate and rectal temperature were monitored at the same time.

In both groups the hematocrit values came very close to the desired target of 22%. The hematocrit in group I showed a further drop until 16 hours following perfusion, whereas, that of group II rose steadily.

Immediately after perfusion the deficit in plasma volume was more pronounced in group I suggesting an internal loss from the intravascular space, since there was no corresponding reduction in body weight. The replenishment of plasma volumes followed an exponential curve and was faster in group I. The restoration of plasma proteins in both experimental groups was also exponential and was faster in group II.

Osmotic equilibrium was reached at the end of perfusion. There was a steady rise in osmolarity during the following 24 hours. Sodium and chloride followed the osmolarity pattern.

Newborn pigs can cope with a large volume hemodilution resulting in a hematocrit of 22%. Their ability to counteract plasma volume and plasma proteins losses is well developed so that restoration is accomplished in about 24 hours. The restoration of plasma volume occurs faster than the restoration of plasma proteins, but when Rheomacrodex is

present the reverse is the case.

The reappearance rate of proteins is about identical to the disappearance rate of Rheomacrodex. This substance binds water selectively, therefore it has a stabilizing effect on plasma volume shifts but not on plasma osmolarity. Two percent Rheomacrodex in Ringer-lactate enhances protein restoration considerably.

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CHAPTER I

INTRODUCTION

1. General Outline of the Problem

The technique of extracorporeal perfusion requires that the extracorporeal circuit be filled with a priming solution which will be subsequently mixed rapidly with the blood of the recipient. The use of homologous blood as a priming fluid has many disadvantages, such as the procurement of large quantities of fresh blood, the risk of disease transmission and "homologous blood reactions". The increased popularity of non-hemic fluids in replacement therapy for acute hemorrhage has encouraged their use as priming fluids in extracorporeal perfusion circuits. The mixing of these non-hemic fluids with the circulating blood, however, leads to the dilution of blood constituents. This is particularly true with the cellular elements and the plasma proteins, when non-hemic fluids with approximately the same electrolyte concentrations which are present in blood are used. This type of hemodilution results in a decrease of the hematocrit* level. A safe lower limit for survival has been obtained with hematocrit levels between 20 and 25 per cent. When such large volume hemodilution is induced with a protein-free priming solution, a marked

*The word "hematocrit" throughout is used to refer to the packed cell volume and not to the apparatus.

lowering in the concentration of plasma protein fractions can be expected.

The lowering in the concentration of these plasma protein fractions and their subsequent recovery following hemodilution was the primary aim of this study. The choice of the priming fluid was therefore reduced to a protein-free solution having approximately the same electrolyte concentrations as those of blood. Ringer-lactate solution has been successfully used experimentally and clinically, not only for blood replacement therapy following acute hemorrhage, but also as a diluent for extracorporeal perfusion. The addition of polysaccharide volume expanders such as Rheomacrodex to Ringer-lactate solution, has also been successfully used in replacement therapy for the treatment of hemorrhage and in extracorporeal perfusion, because of their colloid osmotic properties.

General readjustment patterns of plasma volume, plasma proteins and plasma electrolytes following hemorrhage treated immediately by substituting the lost blood with non-hemic fluids are being recognized in adult patients and mature animals. Studies of these readjustment patterns following hemodilution perfusion are still in the early investigative stages. The technique of hemodilution perfusion in adults is now universally accepted, but the application of this technique to neonates has been very limited. One serious obstacle has been the large size of

the extracorporeal circuits as compared to the size of the neonate; these circuits require that a large amount of priming solution be used in proportion to the newborn's total blood volume, hence making the hemodilution alone impractical. Another important obstacle is the immaturity of the physiologic systems of the newborn. During the neonatal period, the newborn is engaged in a series of physiological adjustments to adapt to extrauterine life.

No simultaneous evaluations of plasma volume, plasma protein and plasma electrolyte changes in response to large volume hemodilution perfusion in the newborn have been reported. This investigation was therefore undertaken to analyze simultaneously plasma volume, plasma protein, and plasma electrolyte changes following large volume hemodilution perfusion with an electrolyte and a colloid solution in newborn pigs. The newborn pig was selected as an experimental animal because of its close physiological similarity to the human neonate.

2. General Functions and Composition of Blood

Unique among tissues because of its fluid state, the blood is essentially the transport medium of the body. As a result of the circulation of blood, the cellular environment throughout the body remains relatively constant. To carry out the function of maintaining this constancy the blood must keep its composition within physiological

limits.

a) The cellular elements.

The composition of blood is exceedingly complex. It consists of cellular elements-erythrocytes, leucocytes and platelets suspended in a fluid medium, the plasma. The erythrocytes represent most of the cellular mass of the blood. Their specific function is the transport of oxygen from the lungs to the tissues. They also play a major role in the transport of carbon dioxide in the reverse direction. The delicate cell membrane of the erythrocyte is readily permeable to water, small uncharged organic particles and to certain monovalent anions, for example bicarbonate and chloride. Erythrocytes contain virtually no calcium and relatively little sodium, most of the cations being potassium and magnesium. The main function of the leucocytes is to combat any infectious agents that may try to invade the body. Blood platelets are essential for maintaining the integrity of the terminal vascular bed. They play an essential role in the hemostatic mechanism and a very important part in blood coagulation.

b) The blood plasma crystalloids.

The blood plasma or intravascular fluid is about 91 per cent water by weight, most of the solid content being plasma proteins, the remainder consisting of crystalloid substances (electrolytes and non-electrolytes).

Plasma volume accounts for 4 to 5 per cent of the body weight and makes up 55 to 60 per cent of the total circulating volume of blood (Edelman and Liebman, 1959). Plasma is separated from the intracellular fluid of blood cells by their respective cellular membrane and from the interstitial fluid by the capillary endothelium. The interstitial fluid is approximately three times the volume of the fluid in the intravascular compartment, together the plasma volume and the interstitial fluid volume, constitute the extracellular fluid volume.

Except for the difference in protein content, blood plasma and interstitial fluid are quite similar in ionic composition. The principal cation of blood plasma is sodium; the principal anions are chloride, bicarbonate and protein. In addition, plasma contains small quantities of potassium, calcium, magnesium, phosphate, sulfate, and organic acid ions.

The electrolytes contribute most of the osmotically active particles; they provide buffer systems and mechanisms for the regulation of acid-base balance and they maintain normal functions of nerves, muscles and parenchymal organs. The plasma non-electrolytes include glucose, and urea. Their total concentration is relatively small as compared with that of the plasma electrolytes.

c) The plasma proteins.

The plasma proteins are composed of a complex

mixture of many proteins (Simmons *et al.*, 1969). These include the three major groups referred to as albumin, globulins and fibrinogen, and a large number of proteins that function as enzymes, hormones and blood coagulation factors. Albumin, which is the most abundant of the plasma proteins, accounts for 55 to 65 per cent of the total plasma protein concentration. It has a molecular weight of about 68,000. It is responsible for about 75 to 80 per cent of the total colloid osmotic effect of the plasma proteins. It acts as a general transport protein for fatty acids, bilirubin, metals, enzymes, hormones and metabolites. The globulins consist of a heterogeneous population of proteins which may be divided into three major types: The alpha, beta, and gamma globulins. The alpha and beta globulins have molecular weights ranging from 45,000 to 3,000,000. They serve as carriers for a number of substances, among them lipids, vitamins, hormones and certain metal ions. The gamma globulins are a group of structurally related proteins with molecular weights of 90,000 to 1,300,000. Their principal function is to act as antibodies and to provide the body with a defense against infections. Fibrinogen (molecular weight 340,000) represents about 4 per cent of the total plasma protein concentration. It is intimately involved in the mechanism of blood coagulation. The general overall process of blood coagulation is concerned with the conversion of the proenzyme prothrombin to

the enzyme thrombin and the action of thrombin upon fibrinogen to form fibrin. Each of these stages represents a complicated set of reactions involving blood coagulation factors. (Mann, 1957; MacFarlane, 1964).

3. Formation of Plasma Proteins

The theories on the site of formation of plasma proteins have had a long history. As early as 1893, the site of origin of fibrinogen was attributed to various organs such as intestine, skin, and lung, while the liver was credited with the function of fibrinogen destruction (Dastre, 1893). Years later, Doyon and associates (1905), Whipple and Hurwitz (1911) and Smith, Belt and Whipple (1920) were able to show that there was a correlation between the extent of hepatic tissue injury and the fall of blood fibrinogen in dogs poisoned by chloroform and phosphorus. Observations made by Drury and McMaster (1929) on hepatectomized rabbits, gave supportive evidence that the liver was the sole source of fibrinogen production.

The role of the liver in albumin and globulin formation was demonstrated by Kerr and his collaborators in 1918. These workers noted that dogs with an Eck fistula have difficulty in regenerating blood serum protein following plasma depletion. The evidence pointing to the liver as the site of plasma protein production was given by Madden and Whipple (1940) who studied the ready replenishment

of plasma protein after plasmapheresis in dogs. Tarver and Reinhardt (1947) later confirmed this observation using radioactive isotope tracer techniques. They injected methionine labelled with radioactive sulfur into normal and hepatectomized dogs and calculated the rate of its incorporation into plasma protein fractions and tissue proteins. They found that the liver produced the plasma proteins, and that normal and hepatectomized dogs incorporated methionine into body tissue protein at the same rate, indicating that the production of body tissue proteins takes place in many extrahepatic tissues. In comparing normal and hepatectomized animals they concluded that the liver was the sole site of fibrinogen production. Subsequently, the formation of plasma proteins was demonstrated in vitro with liver slices (Marsh and Drabkin, 1958), with liver cells in tissue cultures (Hochwald *et al.*, 1961) and with isolated perfused livers (Miller *et al.*, 1951).

In 1954, Miller and his co-workers investigating perfused rat carcass and liver, showed that liver was responsible for the synthesis of most plasma proteins except the gamma globulins. Nine years later, Kukral and associates (1963) fractionated the labelled plasma proteins synthesized by normal and hepatectomized dogs and concluded that 10 to 25 per cent of the alpha globulin and about 50 per cent of the beta globulin were extrahepatic in origin. Experiments with hemopoetic and lymphoid tissues in vitro have clearly

demonstrated that spleen, lymph nodes and gastro-intestinal tract are involved in the formation of alpha and beta as well as gamma globulins (Askonas *et al.*, 1956; Dutton *et al.*, 1959; Asofsky and Thornbecke, 1961).

4. Plasma Colloid Osmotic Pressure and Fluid Distribution Between Plasma and Interstitial Fluid

The colloid osmotic pressure of the plasma proteins which averages approximately 28 mm. Hg., plays an exceedingly important role in fluid exchange across the capillary wall and therefore in the maintenance of normal blood plasma and interstitial fluid volumes. Starling (1896) first pointed out the importance of the plasma proteins in helping to regulate the distribution of fluid between the blood and tissues. The endothelial lining of the capillaries which acts as a semipermeable membrane is freely permeable to water and to the crystalloidal solutes, but relatively impermeable to the proteins. The crystalloids then exert no effective osmotic pressure across the capillary membrane unless their concentration is suddenly altered (Darrow and Yannet, 1935); on the other hand, the proteins exert a colloid osmotic effect which tends at all times to draw fluid from the interstitial space to the capillary lumen. At the arteriolar end of the capillaries the hydrostatic pressure is higher than the colloid osmotic

pressure so that approximately 0.5 per cent of the plasma entering the capillaries is filtered out. At the venous end of the same vessels, the hydrostatic pressure has fallen below the colloid osmotic pressure so that the fluid is reabsorbed into the capillaries. The absorption pressure causes about nine-tenths of the fluid that has filtered out of the arteriolar end to be absorbed at the venous end. The remaining one tenth returns to the vascular system as lymph along with the proteins that have entered the interstitial space.

5. Blood Replacement

Because of its vital importance to the maintenance of life, blood has occupied the attention of scientists for many centuries. The description of the circulatory system by William Harvey in 1628 provided a rational basis for transfusion.

a) Volume replacement with blood.

In 1666, in England, Richard Lower transfused a splenectomized dog with the blood of another dog. The following year, in France, Jean Baptiste Denis (1667) successfully transfused nine ounces of blood from the carotid artery of a sheep into a young boy who was dying from repeated hemorrhages. For the next one hundred and thirty years a great degree of apathy was exhibited with

regard to blood transfusion; then, in the early nineteenth century, an English obstetrician Blundell (1818), helpless in the face of so many postpartum hemorrhages, reinvestigated blood transfusion in animals. The good results he obtained justified his attempt to transfuse patients (Blundell, 1828). Publication of Blundell's work stimulated the interest of scientists and physicians who began, for the first time, to look seriously at the problems of transfusion (Doan, 1927; Zimmerman, 1942; Keynes, 1943). The studies on the blood types of man made by Landsteiner in 1901, the use of sodium citrate as an anticoagulant described by Hustin in 1914 and the addition of glucose for the preservation of citrated blood advocated by Rous and Turner in 1916, marked the beginning of the modern era of blood transfusion.

b) Volume replacement with biologic colloid solutions.

(i) Serum, plasma and albumin.

The substitution of blood serum for whole blood was appreciated as early as 1871 by Bowditch who perfused a frog's heart with sheep and rabbit serum. A few years later, Ringer (1885) accepted the view that the addition of serum in balanced salt solutions was beneficial to the heart. In 1919, Richet who subjected dogs to acute hemorrhage, was able to observe recovery in some animals after replacement of the blood with horse plasma. In the early days of World War II, a renewed interest in the use

of serum or plasma in the restoration of blood volume after hemorrhage was evidenced by a number of investigations (Magladery *et al.*, 1940; Best *et al.*, 1940; Levinson *et al.*, 1940; Brennan, 1940; Hill *et al.*, 1941). It was also during the Second World War that Cohn (1941) developed a method for the large scale separation of plasma proteins into purified fractions and suggested the use of albumin solutions "for the treatment of some, but not necessarily all, conditions associated with diminished plasma volume". The importance of albumin solutions in the replenishment of blood volume following acute hemorrhage in humans was shown in the experiments of Heyl *et al.*, (1943) and Warren *et al.*, (1944).

In spite of the advantage of blood, plasma and albumin preparations in the treatment of blood loss, the transmission of diseases, particularly hepatitis, was, and has remained, one of the greatest problems in the use of transfusions today (Beeson, 1943; Allen *et al.*, 1950; Sayman *et al.*, 1958, Committee, 1968).

(ii) Milk.

The substitution of blood by "another animal fluid" following hemorrhage was advocated by Gaillard Thomas in 1878. Thomas a general practitioner from New York, successfully infused fresh cow's milk into a patient who was dying from a profuse uterine hemorrhage. Encouraged by his results, he immediately predicted a "brilliant and

useful future for the Intra-venous Lacteal Injection". For a while the practice of milk infusion enjoyed a considerable popularity (Jennings, 1885) but the increase in failures and the development of blood transfusions led to the cessation of this practice (Doan, 1927).

c) Volume replacement with artificial colloid solutions.

Starling's discovery in 1896 of the significance of plasma protein in the maintenance of water balance between the intravascular and the extravascular compartment, led to the use of colloidal infusion solutions in the replacement of blood loss.

(i) Gum arabic.

The use of gum arabic (gum acacia) in physiological work was first introduced by Carl Ludwig in 1863 in his kidney perfusion studies. However, Ludwig did not state his reason for using it. More than forty years later, Morawitz (1906) demonstrated that dogs, who had been subjected to a large hemorrhage, were able to survive if the lost blood was replaced by a solution of 3 per cent gum saline. During the First World War, both Bayliss (1916) and Hurwitz (1917), strongly advocated the use of gum acacia in preference to gelatin in the treatment of severe hemorrhage. Although gum arabic had been employed extensively prior to 1937, it later fell into disrepute because of its toxicity, especially with respect to its antigenic action (Maytum and Magath, 1932), and its tendency to

produce liver dysfunction and hypoproteinemia (Andersch and Gibson, 1934; Yuile *et al.*, 1939).

(ii) Gelatin

Gelatin was first used as a perfusion fluid in the classic studies of Ringer (1885). In 1915, it became the first artificial colloid to be tested clinically (Hogen, 1915). However, the risk of contamination of gelatin solutions with tetanus and anthrax spores, the high viscosity and high gelation point of gelatin preparations, and finally the increasing use of gum arabic, led to a decrease in experimental and clinical investigations regarding these preparations (Gropper *et al.*, 1952). Since World War II, the possibility of gelatin as a replacement fluid has been seriously reconsidered (Parkins *et al.*, 1943; Fletcher *et al.*, 1945; Nahas and Estime, 1955). At the present time, many types of gelatin preparations are available, especially in Europe, but more studies need to be carried out on their therapeutic effectiveness in patients with hypovolemia.

(iii) Polyvinylpyrrolidone (PVP) and hydroxyethyl starch (HES).

The synthetic polymer PVP which was developed by Weese and Hecht was used extensively by the German Army Medical Service during the Second World War (Hassig and Stampfli, 1969). Today, PVP preparations are seldom administered to patients but they are still utilized in

experimental studies.

Hydroxyethyl starch was introduced as a plasma replacement substance in 1957 by Wiedersheim. On the basis of good results gathered from animal experimentation (Ballinger *et al.*, 1966; Vinegard *et al.*, 1966) clinical trials began in 1965 (Ballinger *et al.*, 1966). Preliminary reports revealed that HES was effective in the restoration of blood loss following hemorrhage (Lee *et al.*, 1968; Solanke *et al.*, 1968). At present, HES is still undergoing clinical appraisal (Gollub *et al.*, 1969; Solanke *et al.*, 1971).

(iv) Dextran.

Dextran is formed by the fermentative action of various bacterial strains of *Leuconostoc mesenteroides* upon a sucrose containing medium. The nature or raw dextran produced from culture of *Leuconostoc mesenteroides* consists of a mixture of polysaccharides molecules with an average molecular weight of several millions. In 1943, Grönwall and Ingelman tested native dextran as a colloid transfusion fluid in cats and rabbits subjected to hemorrhagic and traumatic shock (1944). Their initial experiments revealed that native dextran could not be used for infusion therapy. They subsequently reduced the size of the dextran molecules by means of partial hydrolytic fractionation and thus obtained a dextran with an average molecular weight of 75,000 (Grönwall and Ingelman, 1945). They introduced

this into clinical use in 1947 under the name of "Macrodex" (Thorsén, 1959). In Europe and in the United States some clinical centers became interested in dextran and soon, reports began to appear concerning the efficacy of this substance in the treatment of hemorrhage (Bowman, 1953; Craig *et al.*, 1951; Fayot and Huguenard, 1955; Lundy *et al.*, 1947). Since then, many improved dextran preparations, especially with respect to the standardization of the molecular weight-distribution and the concentration, have been produced. The average molecular weight of every batch of clinical dextran is determined by light scattering measurements, which give a value known as the "weight average molecular weight" ($\overline{M}_w$). To distinguish the different dextran preparations, the word "dextran" plus the weight average molecular weight expressed in thousands, is now used. For example, Macrodex is a solution of dextran 70 and Rheomacrodex (LMWD = low molecular weight dextran) is a solution of dextran 40. The dextran preparations primarily used today are 6 per cent Macrodex and 10 per cent Rheomacrodex in either 0.9 per cent sodium chloride or 5 per cent glucose. This selection has been made from the results of many years of extensive laboratory and clinical studies throughout the world. Although "Dextran" and "Rheomacrodex" are trade names, these names will be used in this study for reason of convenience.

d) Volume replacement with non-colloid solutions.

(i) Electrolytes.

At first, solutions of sodium chloride were the only electrolyte solutions used in an effort to avert the fatal consequences from a large blood loss. In 1879, Jolyet and Laffond in France and Kronecker and Sander in Germany studied the effect of saline infusion following hemorrhage in animals. Bull in 1884 advocated the use of saline infusions as a substitute for transfusion of blood in humans. The classic studies of Ringer 1882 during the same period brought new concepts in the preparation of physiologically equilibrated salt solutions as substitutes for blood (Amberson, 1937). Unfortunately the literature concerning the use of electrolytes solutions in the treatment of acute hemorrhage has reflected controversies ever since (Gruber, 1969; Rush *et al.*, 1969). This prolonged controversy has been centered not only on the kind of salt solutions utilized in the post hemorrhagic and post traumatic periods, but also on the question as to whether infusions of large volumes of electrolyte solutions are beneficial or not.

(ii) Non-electrolytes.

The intravenous administration of dextrose solutions in conditions associated with hypoglycemia or edema was advocated by Hartman in 1934. A decade later, Frank, Seligman and Fine (1945) tested the therapeutic value of

5 per cent glucose in Ringer-lactate solution for the treatment of acute hemorrhage in dogs. Dillon and his associates demonstrated the superiority of electrolyte solutions over glucose solutions (1966). At the present time, the therapeutic effectiveness of hypertonic glucose solutions in hemorrhagic shock is under investigation (Baue *et al.*, 1967; McNamara *et al.*, 1972).

6. The Use of Hemodilution in Extracorporeal Circulation

When the technique of extracorporeal circulation was first introduced in clinical practice, the "extracorporeal circuit" was filled with homologous blood. In 1960, Panico and Neptune described a technique which eliminated this. The pump oxygenator was primed with a liter of isotonic saline. A cannula was inserted into a systemic artery and the arterial pressure was then allowed to push blood retrogradely through the pump-oxygenator "displacing, laminating and trapping" the isotonic saline into a restricted reservoir above the level of the circuit. At the same time the patient was transfused intravenously with an equivalent amount of blood to accommodate the exact quantity of blood displaced into the pump oxygenator. They reported twenty five successful open cardiac operations on patients using this extracorporeal system primed with isotonic saline. The same year, Zuhdi and his group began

experimental studies of total body perfusion with intentional hemodilution in association with hypothermia. (Zuhdi *et al.*, 1960). In 1961, they reported successful clinical trials, using 5 per cent glucose in water as a prime, returning the content of the extracorporeal circuit to the patient at the end of the procedure (Zuhdi *et al.*, 1961). Since then, many reports have been published concerning the application of hemodilution in extracorporeal circulation and its subsequent effects on the organism. Unfortunately it is difficult to evaluate the published data because of the many differences in hemodilution techniques. These differences are:

(i) A multiplicity of priming solutions have been utilized to date (Schechter and Sarot, 1969). Most of these solutions, which are also employed for volume replacement after hemorrhage, have already been described in the preceding section.

(ii) These solutions are employed either singly (Beall and Cooley, 1965; Neville *et al.*, 1964) or in combination (Litwak *et al.*, 1965; Gollub *et al.*, 1969) or are sometimes supplemented with chemical adjuncts such as mannitol, sodium bicarbonate, (Dieter *et al.*, 1970) and calcium, potassium etc., (Baffes *et al.*, 1970).

(iii) These solutions are used in different quantities. For example the priming volume may be calculated according to body weight (Baffes *et al.*, 1970) or

body surface area (De Boer, 1969). It may also be calculated according to the pre-operative blood volume and hematocrit (Roe *et al.*, 1965) or on the basis of the patient's daily fluid requirement (De Wall *et al.*, 1962).

(iv) Blood is administered at times during, or after extracorporeal hemodilution perfusion (De Boer, 1969, Dieter *et al.*, 1970).

(v) The perfusate remaining in the pump oxygenator is sometimes infused upon completion of perfusion or it is centrifuged so that the separated blood cells can be auto-transfused (Cohn *et al.*, 1971).

(vi) Hypothermia is used in conjunction with hemodilution perfusion which, in turn, is performed under various flow rates (Cook and Webb, 1965).

(vii) Finally, many perfusion techniques of extracorporeal circulation, also called cardiopulmonary bypass, are performed in combination with hemodilution. Their selection and duration vary according to the needs of the individual patient (Clement *et al.*, 1971; Dorson *et al.*, 1969). For example, during the performance of intra-cardiac and great vessel surgery, total and partial cardiopulmonary bypass are used. In partial bypass, superior and inferior venae cavae are cannulated through the atrial appendage, and the cannulae connected to a common venous line. Most of the systemic venous blood is allowed to flow into the venous reservoir, where it is

oxygenated and returned to the patient via an arterial line. In total bypass, the walls of the venae cavae are cinched around the cannulae by the encircling tapes, thus diverting all of the systemic venous return to the heart-lung machine. In certain conditions where the heart or lungs require functional assistance only, the venous system is cannulated via jugular or femoral catheters, blood is oxygenated and returned to a systemic artery. This is called veno-arterial bypass or veno-arterial perfusion. Other techniques such as veno-venous and arteriovenous bypass perfusion are used in the support of infants and adults suffering from respiratory distress.

7. The Response of Blood Volume, Plasma Proteins and Plasma Electrolytes to Intentional Hemodilution

The effect of hemodilution on the blood volume, plasma proteins and plasma electrolytes after bleeding and immediate substitution with crystalloid and colloid solutions was studied in adult dogs by Rodionov and associates (1957), Reiger (1967a, b), and Liljedahl and Reiger (1967). Similar studies were conducted in patients by Moore and his group (1966), and Kaijser and Reiger (1968). These investigators demonstrated that, depending on the severity of the hemorrhage and immediate substitution, the replenishment of plasma volume is accomplished 24 to 48

hours after substitution whereas that of the plasma protein is accomplished only 48 to 36 hours after. They also showed that there are no significant changes in the plasma electrolytes during these physiological responses.

Only a few clinical and experimental animals studies have been reported relating to the plasma volume, plasma protein and electrolyte responses following hemodilution perfusion. In 1961, Long and associates measured changes in the concentration of each plasma protein fraction and electrolytes within 90 minutes following the completion of cardiopulmonary bypass with hemodilution in 124 patients; their ages varied from a few months to over 50 years. The patients were divided into three groups: In the first, the extracorporeal circuit was primed with blood alone, in the second it was primed with blood plus a 5 per cent solution of human albumin, and in the third, with blood plus a solution of 10 per cent Rheomacrodex in 0.9 per cent saline. These authors then compared average values of the above variables obtained for each of these groups. They reported a fall in plasma albumin levels in all three groups, with the largest fall in the Rheomacrodex group "due probably to an additional dilution effect". They also recorded a decrease in all plasma globulins with the least change in the Rheomacrodex group, and a decrease in the fibrinogen levels in all three groups, with a greater drop in the Rheomacrodex and albumin groups. No significant

change could be detected in the plasma electrolytes of the three groups, except for a fall in potassium levels.

In 1963, Hellström and Björk investigated serum protein and electrolyte changes in response to hemodilution with 10 per cent Rheomacrodex in 0.9 per cent saline during total body perfusion, at moderate hypothermia in patients from 3 to 56 years of age. They showed that the addition of Rheomacrodex to the priming volume produced no significant change in the serum proteins and electrolytes during extracorporeal perfusion.

Moffit, Maher and Kirklin, in 1965, described the behavior of plasma concentration of electrolytes and proteins during and after hemodilution perfusion at moderate hypothermia in 18 patients from 4 to 62 years of age. One group of patients was perfused with whole blood, the other group with whole blood diluted with a solution of dextrose and albumin in saline. The patients who received undiluted blood showed little deviation from normal levels of plasma electrolytes and proteins during, and 18 hours after perfusion. The hemodiluted group showed a decrease in electrolytes and total protein values during extracorporeal perfusion. This was followed by a return to normal levels soon after completion of the operation but there was, again, a trend to hypokalemia.

Gollub and associates (1969), studied intravascular responses to extracorporeal perfusion with large volume

hemodilution in five Jehovah's Witnesses who refused blood transfusion on religious grounds. The perfusate consisted of varying amounts of Ringer-lactate solution, 5 per cent glucose in water, Tris-hydroxymethylaminomethane (THAM) and Mannitol. Among their observations, these investigators found that the fall in blood volume and hematocrit value at the end of perfusion was followed by a partial restoration of blood volume and a further decrease of hematocrit value in the first 18 hours post-operatively. They also found that, at the end of perfusion, "the total measured protein content of plasma exceeded the expected value". They suggested that protein had been mobilized to the intravascular compartment from extravascular sources.

Wallace, Arai and Blakemore (1970) compared plasma protein electrophoretic patterns of patients undergoing open-heart surgery under moderate hypothermia and hemodilution, with those undergoing other extensive surgical procedures without hemodilution perfusion. The extracorporeal circuit of the open-heart group was primed with a mixture of whole blood, electrolyte solution and 10 per cent dextran in normal saline solution to which ascorbic acid and sodium bicarbonate were added. The results indicated that all surgical procedures caused a significant fall in all the plasma protein fractions which persisted during the next 24 hours. This was followed by a "marked elevation in alpha-I globulin and "slight decreases" in

beta- and gamma globulins" during the first post-operative week. On the second post-operative week the protein fractions were almost back to the pre-operative values. This response seemed to be more accentuated and prolonged in patients who underwent extracorporeal hemodilution perfusion.

English, Digerness and Kirklin (1971) measured plasma colloid osmotic pressure and total protein concentration in 12 patients from 6 to 62 years old, before, during and after extracorporeal circulation at moderate hypothermia. The priming volume consisted of a mixture of whole blood, 5 per cent dextrose in saline, sodium bicarbonate, calcium chloride and balanced salt solution. Colloid osmotic pressure and plasma protein concentration, which were reduced during bypass, began to return toward pre-operative levels after one hour; however they were still significantly lower than pre-operative values after 24 hours. These investigators suggested that the replenishment of plasma protein after extracorporeal perfusion followed the same pattern as that observed after hemorrhage with immediate volume replacement. Thorough studies on the changes in blood volume in response to extracorporeal circulation with or without hemodilution were done by Litwak and his associates (1963). These investigators observed significant deficits in the circulating blood volume of adult patients after perfusion, whether the

technique of hemodilution was used or not, and despite careful replacement of all measured blood loss during perfusion. Homologous blood mixed in a 3 to 1 ratio with 1 gram of human serum albumin per 100 ml. of 5 per cent dextrose in water was employed in the technique of hemodilution. They attributed these blood volume deficits to a temporary "sequestration of blood into areas not in measurable communication with the circulating blood volume".

Beall and Cooley (1965), and Yashar and associates (1971) also found blood volume alterations associated with extracorporeal perfusion at normothermia, using 5 per cent dextrose in distilled water as the priming solution. The age of the patients ranged from 14 months to 74 years. Immediately after perfusion and the return of all fluid in the extracorporeal circuit to the patients, the average total blood volume determinations were somewhat lower than pre-operative values. A further decline was shown within the first 24 hours after operation, followed by a return toward normal values during the next 8 days. This return was mainly due to an increase in plasma volume with little change in red cell mass. Other groups have reported similar results irrespective of the priming solution used and whether the content of the extracorporeal circuit was transferred to the patients or not, (Cohn *et al.*, 1971; De Boer, 1969).

In 1964, Linder, Sakai and Paton studied the effects

of different perfusates on the plasma sodium and potassium concentrations in dogs. The priming solutions consisted of either 5 per cent dextrose in water, 5 per cent dextrose in 0.11 per cent saline, 5 per cent dextrose in 0.2 per cent saline or isotonic saline. The contents of the extracorporeal circuit were always returned to the animals at the end of perfusion. These investigators did not observe a statistically significant fall in electrolyte concentrations immediately after perfusion, except in the group perfused with 5 per cent dextrose in water. They suggested that 5 per cent dextrose in 0.2 per cent saline was the priming solution of choice because of the minimal electrolyte changes, low hemolysis levels and good post-operative urinary output associated with its use.

Cruz and Callagham, in 1966 analyzed the effects of increasing degrees of hemodilution on the serum electrolytes of dogs using Ringer-lactate solution as the priming solution. At the end of perfusion all the remaining perfusate was returned to the animals. Variations in serum electrolyte levels were more or less uniform among the different groups. Slight hyponatremia was observed immediately and 24 hours after perfusion with a return to normal levels at 48 hours. A moderate hyperchloremia was observed at the end of perfusion, this also returned to normal values 48 hours after the termination of perfusion. Plasma potassium levels remained constant throughout.

Miyauchi, Inoue and Paton (1966) compared the changes in electrolyte concentrations during 2-hour total cardiopulmonary bypass and during the 4 hour post-perfusion period in 7 groups of dogs. They also measured blood volumes before bypass and 10 minutes after the end of bypass. The extracorporeal circuit was primed with one of the following solutions: 1) 5 per cent dextrose in water, 2) 5 per cent dextrose in 0.2 per cent saline, 3) 5 per cent dextrose in normal saline, 4) Ringer solution, 5) lactated Ringer solution, 6) 10 per cent Rheomacrodex in 5 per cent dextrose solution and 7) heparinized whole blood. The contents of the extracorporeal circuit were infused into the dogs at the end of perfusion. In all instances a decrease in the concentrations of serum sodium and chloride was seen; this decrease was more marked in the group perfused with dextrose in water. There were no statistically significant changes in serum potassium concentration when either whole blood, Rheomacrodex or Ringer-lactate were used as perfusate; moderate hypokalemia was seen in the 5 per cent dextrose in 0.2 per cent saline group. A highly significant drop in potassium was found in the other groups. These investigators also observed a large deficit in blood volume in the group perfused with dextrose in water and no significant changes in the blood volumes of the other 6 groups. Miyauchi and associates concluded that changes in electrolyte levels during the perfusion depend primarily

upon the electroyte concentrations of the perfusate, and that lactated Ringer solution was the diluent of choice.

Serum electrolyte levels in 49 adult patients, immediately after extracorporeal perfusion and 7 days later, were studied by Dieter, Neville and Pifarré (1969). These investigators used Ringer-lactate solution as a priming solution. Their results indicated that plasma sodium and chloride levels were unaltered during and following perfusion, whereas potassium and calcium concentrations were significantly decreased.

8. Hemodilution Perfusion in the Newborn

The extent to which the blood volume in the newborn may be intentionally diluted without danger and the subsequent physiological responses to such a challenge are almost unknown. Not unnaturally there has been a tendency for the few clinical investigators who have approached this problem to focus on clinical benefits rather than physiological responses to hemodilution (Baffes *et al.*, 1964; Lemoine *et al.*, 1971). Other investigators who have attempted to analyze these responses have unfortunately combined the data obtained from their newborn patients with those obtained from patients of older age groups (Beall and Cooley, 1965; Long *et al.*, 1961; De Boer, 1969; Roe *et al.*, 1965).

Investigations in newborn animals relevant to the response of blood constituents to hemodilution perfusion are few. In 1965, Rosenkrantz and his associates gave preliminary reports on serum electrolyte changes during and after open chest extracorporeal circulation at normothermia in young puppies weighing 2 to 5 Kg. In one group of animals the extracorporeal circuit was primed with homologous blood; in a second group the prime was a mixture of homologous blood and 5 per cent dextrose in 0.1 per cent saline. The use of the latter priming solution resulted in a hematocrit of 30 per cent in the animals after thorough perfusion. During perfusion, no statistically significant differences were found in the serum electrolytes of either group.

In 1969, Dorson and his group investigated the effects of prolonged extracorporeal hemodilution perfusion on the plasma hemoglobin levels and on the hematocrit of puppies weighing from 1.5 to 4.5 Kg. A membrane oxygenator was primed with Ringer-lactate solution. The experiments consisted of arterio-venous perfusion in closed chest lasting from 21 to 25 hours, both with and without an extracorporeal pump. Among their observations, they found that the hematocrit values were continuously decreasing during perfusion and that the decrease was more pronounced in animals perfused without a pump. These results were attributed to the effect of the pump within the system on

the erythrocytes. Recently, Turina (1972) subjected 36 puppies, weighing 3 to 7.5 Kg. to total body perfusion with hemodilution at both normothermia and hypothermia. A membrane oxygenator was primed with a mixture of blood, glucose and Ringer's solution. One finding of particular interest was the fall in the serum albumin concentration to nearly one half the initial level during perfusion which lasted from 1 to 2 hours. He attributed this observation to the possible adherence of albumin to the surface of the membrane oxygenator. This decrease of albumin concentration was always followed by a rapid return to normal levels after the interruption of perfusion. Unfortunately, Turina did not mention the time it took for albumin to return to normal levels.

9. Experimental Design

In the preceding sections, the importance of the restoration of plasma proteins, plasma electrolytes and plasma volume after hemodilution perfusion have been outlined and the relevant literature has been reviewed. Three aspects of hemodilution perfusion have received little attention: The first is the ability of the neonate to cope with hemodilution perfusion, the second is the observation on the simultaneous changes of some of the major plasma constituents both colloids and electrolytes, finally the

third is the time course of the restoration of these plasma constituents, particularly of plasma proteins.

This study was undertaken to clarify these aspects, particularly in the newborn. Bearing in mind that in order to study the restoration of plasma proteins after hemodilution perfusion, it is desirable to provoke a large deviation from the normal protein concentration level. The largest deviation will be obtained with the largest possible dilution which is still compatible with life. A hematocrit of 20-25 per cent seems to be a safe limit in mature animals (Perasalo *et al.*, 1967) but it is not established whether this is also true for newborn animals. Since hematocrit values vary considerably in newborns it seems better to adjust the dilution volume to a target hematocrit rather than to use just one dilution volume or to determine the dilution by another physiological variable such as body surface area. This was the first guideline that was adopted for the study presented here.

It is likely that when one plasma constituent is upset, others will also be affected. The use of a perfusion fluid that largely differs from the plasma of the recipient with respect to its ionic content and pH, may elicit side effects that may hamper the interpretation of the recovery pattern of the plasma constituents, especially that of the proteins. Therefore, as a second guideline, a perfusion fluid with electrolyte concentrations close to those of

plasma was chosen. For comparative reasons a similar fluid with the addition of a colloid-osmotic agent was also used.

The experimental animal chosen was the piglet which appears to bear a close physiological resemblance to the human infant (Glauser, 1966). In the first week after birth, however, it shows a rapidly changing plasma protein profile (Miller *et al.*, 1961; Lardinois and Page, 1969). Therefore seven days old animals were used. Newborn pigs are poor subjects for general anesthesia to which they respond with a marked decrease in capillary blood flow (Simon and Olsen, 1969). Therefore, the perfusion was carried out after the animal had recovered from the anesthesia required for the surgery.

The above considerations thus led to the following experimental proposal:

Newborn pigs (7 days old) of either sex were used. They were divided into three groups. Two groups underwent arteriovenous perfusion in closed chest at normothermia and without an oxygenator in the system, until complete mixing of blood and perfusate was accomplished. The extracorporeal circuit was primed in the first group with a buffered Ringer-lactate solution, and in the second group with a buffered solution of 2 per cent Rheomacrodex in Ringer-lactate. A mixed (blood and prime) hematocrit ranging between 20 and 25 per cent immediately after perfusion was the target. A pilot study was carried out

to see whether a target value of 22 per cent was feasible (II, 1, p. 35). The third group, consisting of littermates of the above animals, was subjected to the same surgical procedures but without perfusion. This group was referred to as the "sham-operated group". During one experiment blood samples were collected at five different time intervals, namely: immediately before hemodilution perfusion, immediately after the termination of perfusion and every eight hours over a period of 24 hours. Only the blood losses equivalent to that amount taken for sampling were replaced by diluted blood remaining in the extracorporeal circuit after perfusion. The blood samples were used for the measurement of plasma volume and for the analysis of plasma proteins and electrolytes. Readings of blood pressure, heart rate and rectal temperature were made before and after the perfusion and subsequently when samples were taken. All piglets were allowed to survive for seven days.

CHAPTER II

MATERIAL AND METHODS

1. Animals

The experiments were performed on 45 newborn piglets of either sex. The animals were purebred Landrace or a cross of Landrace and York. All piglets were precisely seven days old at the beginning of the experiments and their weights ranged between 2.79 and 2.93 Kg. Pilot studies were performed on 4 other piglets to standardize the experimental method. Thirty piglets underwent closed chest extracorporeal circulation without an oxygenator in the system. Fifteen control piglets, each a littermate of an experimental animal were operated as described below but not perfused (sham-operated group). The blood of 3 other piglets was used for the identification of plasma protein fractions (II, 6a, iv, p. 53).

2. Experimental Routine

In planning and conducting cardiovascular experiments with piglets, it is very important to consider

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[1]Solution balance, Ohaus Model 1119, Union, New Jersey, U.S.A.

the excitability of these animals. They respond to handling with a sudden marked increase in blood pressure and heart rate; therefore, particular care in handling them is essential to obtain reliable data.

At the beginning of each experiment, the piglet was weighed to the nearest gram, anesthetized, operated upon (II, 3, p. 38), returned to its quarters and allowed to regain consciousness. At this time it was brought back to the operating room and secured loosely with adhesive tape on a restraining table in the supine position; its blood pressure and rectal temperature were monitored (II, 7, p. 67) and a transistor radio was tuned into music in order to cover all unfamiliar noises that would excite the animal. When blood pressure became stable, the piglet was given an intravenous injection of heparin[2] (300 I.U. per kilogram body weight). After this, the routine of the plasma volume determination (II, 5, p. 45) was carried out for the first time. This procedure included the collection of blood samples for the subsequent analyses (II, 6, p. 53). In the case of a control animal, the catheters were disconnected, closed and secured to its neck with adhesive tape. The animal was then returned to its quarters which were set at an ambient temperature of 32-34°C. This

[2]Panheparin, Abbott Laboratories Ltd., Montreal, Canada.

temperature is optimal for piglets. (McCance and Widdowson, 1959; Mount, 1963). In the case of a perfusion animal, the hemodilution perfusion was started (II, 4, p. 39). Immediately after the termination of the perfusion a blood smear was prepared, the routine for the plasma volume determination repeated and the animal returned to its quarters after the catheters were disconnected, closed, and secured. To avoid the untoward reactions which may be associated with the intravenous administration of protamine sulfate (Gourin *et al.*, 1971), heparin was not neutralized after perfusion. The diluted blood left in the extracorporeal circuit was treated as follows: a sample was taken to be analyzed in the same way as the other blood samples (II, 6, p. 53); six syringes were filled and put aside for blood replacement (II, 4a, p. 39). Eight hours after hemodilution perfusion, or in the case of the control animal eight hours after the first plasma volume determination, the animal was brought back to the operating room, weighed, restrained on the table, exposed to radio music, and connected to the measuring devices for the blood pressure and rectal temperature. The routine for the plasma volume determination was then repeated.

The procedure carried out at eight hour post-perfusion period was repeated again at 16 hours and 24 hours. During the 24 hour period the animal was not allowed to drink and eat. The animal was allowed to

survive and was sacrificed on the seventh post-operative day. The principles of laboratory animal care as promulgated by the Canadian Council on Animal Care were observed.

3. Surgical Procedures

Aseptic precautions were observed during the surgical procedures. Anesthesia was induced with sodium pentobarbital[3] (25 mg. per kilogram body weight) injected intraperitoneally. The anesthesia was maintained at a superficial level so that the animal would regain consciousness two to three hours after the conclusion of the surgical procedures. The piglet was restrained in the supine position on a warmed operating table by means of adhesive tape looped about its legs. A mid-line skin incision about 3 cm. long was made caudal to the lower border of the larynx with an electrosurgical blade.[4] The muscles in front on the trachea were separated longitudinally in the mid-line by blunt dissection, and 2 cm. of the left common carotid artery was exposed. The artery was ligated distally and a small bulldog clamp was applied centrally. A ligature was then applied loosely about the

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[3]Nembutal, Abbott Laboratories Ltd., Montreal, Canada.

[4]Birtcher Electro-Surgical Set, The Birtcher Corporation, Los Angeles, California, U.S.A.

vessel between the upper ligature and the clamp. A size 5 French polyvinyl plastic tube[5] was cut to a 10 cm. length and filled with a solution of heparin sodium (200 U.S.P. units per ml.). The catheter was fed past the clamp down the artery through a transverse arteriotomy to the level of the aortic arch and secured in a place by the ligature. The bulldog clamp was then removed. A second incision 1.0 cm. long, was made laterally, 1.5 cm. to the right and parallel to the first incision. The right external jugular vein was exposed and cannulated by a similar technique. The catheters were closed with plastic stoppers which fitted tightly in the conical ends. The incisions were closed either with 9 mm. autoclips or #00 surgical silk. The stoppered end of the catheters which protruded 2 to 3 cm. from the closed incision were taped to the skin of the animal to prevent damage. The animal was then allowed to regain consciousness in its quarters.

4. Hemodilution Perfusion

a) General description.

Hemodilution perfusion in closed thorax was accomplished with an infant type arterial bubble trap[6]

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[5]Bardic Feeding Tube, C. R. Bard, Inc., Murray Hill, N. J., U.S.A.

[6]Sarns Inc., Ann Arbor, Michigan, U.S.A.

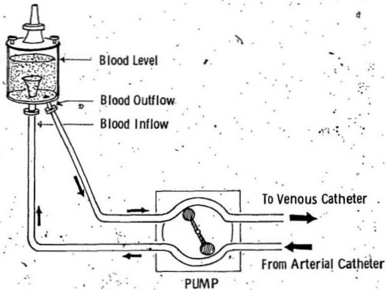

FIGURE 1

THE EXTRACORPOREAL CIRCUIT

which acted as the reservoir for the priming solution, and a roller pump[7] operating on both arterial and venous lines. Two sterilized 1/8" I.D. x 1/4" O.D. medical vinyl tubes[8] of equal length were connected to the bubble trap (Figure 1, p. 40). This vessel has a trumpet-shaped inlet emerging from the bottom and an outlet situated in the bottom. One tube was connected to the trumpet, the other to the outlet. The reservoir was filled by pushing the priming solution through the two tubes with a large polypropylene syringe to remove any air bubbles in the tubes. The two tubes were then closed with stopcocks and inserted in the roller pump head. A check mark was made on the reservoir to indicate the level of the perfusion fluid. Before the piglet was perfused, the two tubes were clamped and the stopcocks removed. The piglet's catheters were also clamped and the stoppers removed. The tube running to the trumpet was then connected to the arterial catheter, the other to the venous catheter. At the onset of extracorporeal circulation the four clamps were removed and the pump started. The arterial blood was pumped into the reservoir at a rate of 50 ml. per minute and pumped back into the animal at about the same rate. Slight alterations in the level of the

[7]Sarns Modular Pump, Travenol Laboratories, Inc., Morton Grove, Illinois, U.S.A.

[8]Bard-Parker Company, Inc., Rutherford, N.J., U.S.A.

diluted blood in the reservoir during perfusion were immediately corrected by gently clamping either the venous line when blood was falling below the initial level, or the arterial line when blood was rising above the initial level. Hence the volume of perfusate remaining in the pump at the termination of extracorporeal circulation was always equal to the priming volume. At the end of perfusion the arterial and the venous catheters were simultaneously clamped and the pump stopped. Two I.U. of heparin per ml. of diluted blood were mixed gently with the blood remaining in the reservoir. Six 10-ml. syringes were filled up to the 5.5 ml.-mark and stored at 4°C. in a refrigerator for subsequent blood replacements after plasma volume determinations and analyses.

b) Calculation of the priming volume (v_{pr}).

An estimation of each piglet's blood volume (EBV) based on 90 ml. per kilogram body weight (Engelhardt, 1966; Talbot and Swenson, 1970) and a determination of its corrected hematocrit (LVH) were made before perfusion. The volume of the priming solution was then calculated by means of the following formula in order to achieve an hematocrit of 22 per cent when the piglet's blood and the priming solution were thoroughly mixed:

$$V_{pr} = \left(\frac{LVH}{22} - 1 \right) EBV$$

The priming volume thus calculated ranged between 45 to 180 ml.

c) Calculation of the perfusion time.

The construction of the bubble trap was such that the arterial blood flowing in could readily mix with the priming solution. Therefore the time required to mix the priming solution completely with the animal's blood could be calculated by dividing the sum of the blood volume and the priming volume by the pump flow rate (50 ml. per minute). This time was doubled, not only to ensure complete mixing but also to simulate an hypothetical surgical procedure. The perfusion times were in the order of 14 to 19 minutes.

d) Preparation of the two priming solutions.

The same batch of Ringer-lactate[9] was used throughout this entire investigation. When using Ringer-lactate alone as the priming fluid, the pH of the solution was raised from an initial value of 6.5 to 7.4 by adding 0.31 ml. of 7.5% sodium bicarbonate solution[10] into 250 ml. of Ringer-lactate. The 2% Rheomacrodex in Ringer-lactate was prepared by mixing 50 ml. of 10% Rheomacrodex in normal

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[9] Lot A 79, six 250 ml. bottles per case, Baxter Laboratories of Canada Limited.

[10] Abbott Laboratories Limited, Montreal, Canada.

saline[11] with 200 ml. of Ringer-lactate. This mixture which had a pH of 5.9 was adjusted to a final pH of 7.4 by the addition of 0.41 ml. of a 7.5% sodium bicarbonate solution, in order to buffer a possible hypoxic acidosis which is reported to occur in hemodilution procedures (Najafi *et al.*, 1965; Cruz and Callaghan, 1966). The final pH of these solutions was checked with a pH meter.[12]

The priming solution was always warmed up at a temperature of 38°C. before it was introduced into the extracorporeal circuit. During bypass, the perfusate was maintained between 37 and 38°C. by intermittently warming the reservoir with an infra-red lamp.

e) Hematocrit determinations.

Hematocrit values play a very important role in this study. They are used to calculate the priming volume as described above and also for the calculation of blood volume in association with plasma volume as will be described below. Blood samples for hematocrit determinations were taken from the venous catheter. Hematocrit values are therefore large vessel hematocrit (LVH) values (Swan and Nelson, 1971). Three heparinized capillary tubes per labelled culture tube were filled with blood which was

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[11] Pharmacia (Canada) Ltd., Montreal, Canada.

[12] Radiometer pH meter Type pHM296, Sweden.

collected during the plasma volume determination. The capillaries were then sealed and centrifuged in a micro-hematocrit centrifuge[13] at 13,500 g for 5 minutes. With this apparatus it is possible to read the hematocrit value to the nearest 0.1%. The triplicate readings did not differ more than this amount. (Table II-1, p. 54). The values obtained were corrected for the 3% trapped plasma (Talbot and Swenson, 1963).

5. Plasma Volume Determination and the Calculation of Blood Volume

The determination of plasma volume is complicated by the fact that it takes time for the injected indicator to mix thoroughly with the circulating blood and also because the indicator starts to disappear from the circulation as soon as it is injected. In order to obviate these difficulties, several samples must be taken with known time intervals.

a) Dye injection and dye sampling.

The plasma volume was determined by the T-1824 dye dilution technique (Gregersen and Rawson, 1959). The same batch of ampouled 0.5% Evans Blue Solution[14] was used.

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[13]Adams Autocrit centrifuge, Clay Adams, Parsippany, N.J., U.S.A.

[14]Warner-Chilcott Laboratories, Division of Warner-Lamber Canada Ltd., Toronto, Canada, Lot No. 0358040.

throughout the study.

Assuming that the specific gravity of the dye is unity (Strumia *et. al.*, 1968), it was then possible to fill the syringe with 1.0 ml. of the dye by weighing.

A 2.2 ml. sample of blood was withdrawn from the venous catheter with a 2.5 ml. disposable syringe. This sample was placed into a labelled 10 x 75 mm. culture tube containing 0.02 ml. of heparin (20,000 I.U. per ml.) and was later utilized for the preparation of the "blank" and the "standard" used in the dye assay. The tuberculin syringe containing the known amount of T-1824 was connected to the catheter which was clamped and had its stopper removed. In the following manipulations the clamps were opened and closed according to demand. The dye was pushed in at a rate of about 0.5 ml. per second; the plunger of the syringe was then moved gently in and out three times. The tuberculin syringe was removed, filled with 0.1 ml. of heparinized saline and emptied into the catheter. The last manoeuvre was also repeated three times to wash out the content of the catheter (the volume of the catheter was about 0.1 ml.). Finally, a new 2.5 ml. syringe was attached to the catheter in preparation for the next blood sampling.

Blood samples were withdrawn at exactly 5, 10 and 15 minutes after completion of the injection and in the following manner: the heparinized saline in the catheter was flushed by moving the plunger in and out, displacing a

volume of about 0.5 ml. Thereafter, a 1.1 ml. sample of blood was withdrawn and put into a second labelled culture tube containing 0.01 ml. of heparin. The catheter was filled again with 0.1 ml. of heparinized saline and connected to a new 2.5 ml. syringe for the next blood sampling. The same procedures were repeated at 10 and 15 minutes following dye injection. The total amount of blood withdrawn from the animal at each plasma volume determination amounted to 5.5 ml. (2.2 ml. for the "blank" and the "standard" and 3 times 1.1 ml. for the samples). This amount was replaced immediately after the termination of the above procedures (except for the pre-perfusion period) with diluted blood taken from the reservoir (II, 4a, p. 39). This blood was warmed up to 38°C. After this, the catheter was flushed with heparinized saline and the end was closed with the plastic stopper. Finally the catheter was secured to the animal with adhesive tape. The four culture tubes containing the heparinized blood samples were stoppered and inverted five times. Thereafter, three capillary tubes for the hematocrit determination were filled with blood from each tube. The culture tubes were then balanced and spun for 30 minutes at 1,500 g in a centrifuge.[15] The plasma samples were removed from the culture tubes with disposable

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[15]Model HN, International Equipment Co. Massachusetts, U.S.A.

capillary Pasteur pipettes and transferred into similarly labelled test tubes. The first test tube contained plasma obtained from the 2.2 ml. blood sample before dye injection. Two equal volumes of 0.3 ml. plasma were taken from this test tube and transferred to two labelled 10 ml. beakers. The standard was prepared by adding 0.1 ml. of a 1:50 dilution of T-1824 solution in one of these beakers; the mixture was gently swirled and allowed to stand for 2 minutes. The blank was left as it was. An amount of 0.3 ml. of each dye-tinged plasma sample, obtained from the 5, 10 and 15 minutes blood samples, were also pipetted into labelled 10 ml. beakers.

b) Dye extraction.

The technique of Campbell *et al.*, (1958) was used to extract the dye T-1824 from the plasma, but using smaller volumes than these authors employed. Each plasma volume determination yielded 5 plasma samples in 5 beakers. During one experiment, 5 such plasma volume determinations were made. Therefore 25 labelled extraction columns were set in racks.

About 80 mg. of pyrex glass wool were firmly packed with a long glass rod above the small constriction of the extraction columns. Eight ml. of a suspension of purified cellulose[16] in 2% Na_2HPO_4 were transferred to the extraction

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[16]Solka Floc SW-40-A was kindly supplied by G. H. Wood Co. Ltd., Montreal, Quebec, Canada.

columns. The suspension fluid was allowed to drain out, thus leaving an even column of cellulose about 3 cm. high above the glass wool. Each prepared column was washed with 10 ml. of 2% Na_2HPO_4.

Five ml. of Teepol-phosphate (3 ml. of Teepol[17] in 100 ml. of a 2% Na_2HPO_4 solution) were added to each beaker to precipitate all plasma proteins and to dissociate the dye from the albumin. The contents were mixed by gentle swirling and poured very gently on the top of their respective column. Each beaker was rinsed out with 5 ml. of Teepol-phosphate and rinsings were also transferred into the columns. When the drainage was almost complete, 10 ml. of 2% Na_2HPO_4 were poured on the top of the column. During the adsorption of the dye by the cellulose, the flow through the column never exceeded 3 to 4 ml. a minute. As soon as the drainage was complete, a labelled 5 ml. volumetric flask containing 0.25 ml. of 8% KH_2PO_4, was placed under the delivery needle. A 1:1 mixture of acetone and distilled water was prepared and adjusted to a pH of about 8 with a few drops of 1 N NaOH. About 2 ml. of this solution was poured on the top of the column. The eluted dye was collected in the volumetric flask. Thirty minutes later, a second portion of the same acetone-water solution was poured on the top of the column and the eluate was

[17]G. H. Wood Co. Ltd., Montreal, Quebec, Canada.

collected until the flask was almost filled to the 5 ml. mark. The volume was then brought to the mark with acetone-water, the flask was stoppered, and its contents were mixed by inversion.

The optical densities of the acetone-water extracts were measured with a spectrophotometer[18] at 620 mμ in 12.5 x 45 mm. absorption cells.

In order to check the efficiency of the extraction method one dye solution was made in the same way as described above but in Teepol-phosphate solution instead of plasma. It was run through an additional extraction column, washed with phosphate and eluted with acetone-water as already described. The optical density of the eluate was compared with that of a second solution containing the same quantity of dye added to a mixture of 1 ml. of 2% Na_2HPO_4 and 0.25 ml. of 8% KH_2PO_4 which was then brought to a volume of 5.0 ml. with acetone-water at pH 8.0. There was no more than 2 to 3 per cent difference in the optical densities of these two solutions.

c) Calculation of plasma volume.

The apparent plasma volume at time t (PV_t) was

[18]Unicam SP500 Series 2, Unicam Instruments Ltd., Cambridge, England.

calculated from the volume of the dye injected, the optical density of the dye standard, the plasma blank and the sample with a formula given by Campbell *et al.*, (1958), but re-arranged in the following form:

$$PV_t = V \times DF \left[\frac{OD_{std} - OD_{blk}}{OD_s - OD_{blk}} \right]$$

where,

V = volume of dye solution injected

DF = "relative" dilution factor = 500 (the standard contained 10 μg. of dye; 5,000 μg. were injected into the animal).

OD_{std} = optical density of the standard

OD_{blk} = optical density of the blank

OD_s = optical density of the sample

The logarithms of the apparent plasma volumes at 5, 10, and 15 minutes were then plotted against time as illustrated in Figure 2, p. 52. A straight line was drawn through the measured points and extrapolated back to zero time. The relationship was so linear that a best fitting straight line could be drawn by hand. The intercept at zero time represents the plasma volume that would have existed if mixing of the dye in the porcine circulatory system were instantaneous and before any loss of dye. The value obtained at the extrapolated point was referred to as the plasma volume (PV). In Figure 2, this value is 188 ml.

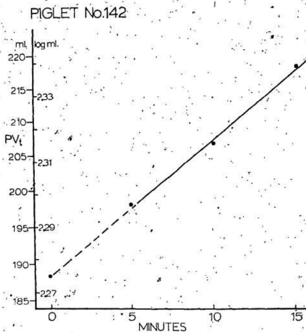

FIGURE 2

CALCULATION OF PLASMA VOLUME

The measured points represent plasma volumes at time t (PV_t) calculated from blood samples collected at 5, 10 and 15 minutes after injection of T-1824. The extrapolated point (PV) represents the calculated plasma volume at the instant of dye injection.

The above procedure is only valid when there are no fluid shifts present in the animal during the period of sampling (Gregersen and Rawson, 1959). The absence of fluid shift can be verified by observing the hematocrits and plasma protein concentrations simultaneously. The following table which contains arbitrarily chosen data from four piglets, illustrates that neither the hematocrit nor the protein concentration changed during the sampling period (Table II-1, p. 54). This check was made routinely in all plasma volume determinations. No shift could be detected and therefore average hematocrit and plasma protein concentrations could be calculated using all values obtained.

The blood volume (BV) was calculated using the plasma volume and the hematocrit (LVH):

$$BV = PV \frac{100}{(100-LVH)}$$

6. Blood Analyses

In the process of the plasma volume determination, four blood samples were taken and each sample was subsequently centrifuged. From the plasma thus obtained, a part was used for the measurement of the dye concentration. The remainder was used for the blood analyses.

a) Plasma proteins.

Two methods were used to determine plasma protein

TABLE II-1

LARGE VESSEL HEMATOCRITS AND PLASMA PROTEIN CONCENTRATIONS DURING THE PERIOD OF SAMPLING FOR THE PLASMA VOLUME DETERMINATION

Piglet #	Hematocrit percent Sample #				Plasma protein concentration g./100 ml. Sample #			
	1	2	3	4	1	2	3	4
	29.9	29.8	30.0	29.9				
79 "Before"	29.9	29.9	29.9	30.0	4.8	4.8	4.9	4.9
	30.0	30.0	29.9	29.8				
	21.3	21.4	21.3	21.3				
117 "After"	21.3	21.3	21.3	21.2	4.4	4.5	4.4	4.4
	21.4	21.3	21.3	21.2				
	35.5	35.5	35.4	35.4				
132 8 hours	35.5	35.4	35.4	35.3	5.9	5.8	5.7	5.8
	35.4	35.5	35.3	35.4				
	24.4	24.2	24.3	24.2				
141 24 hours	24.3	24.3	24.3	24.2	5.5	5.6	5.5	5.5
	24.3	24.3	24.2	24.3				

1 = Before dye injection
2 = 5 minutes after
3 = 10 minutes after
4 = 15 minutes after.

concentration depending on the absence or presence of Rheomacrodex in the plasma sample. If there was no Rheomacrodex present, the biuret method was used as developed by Kingsley (1939, 1942) and modified by Weichselbaum (1946). For the plasma samples containing Rheomacrodex, the filter paper method of Mans *et al.*, (1961) was used.

(i) Measurement of plasma protein concentration and total circulating plasma protein in the absence of Rheomacrodex.

Biuret Method.

The analysis was carried out on all plasma samples obtained; this was necessary because a check must be made on fluid shift during the period of sampling for the plasma volume determination as mentioned above (II, 5c, p. 50).

One hundred microliters of each sample were pipetted and transferred to labelled 12 x 75 mm. test tubes; they were then mixed with 4.0 ml. biuret reagent. Blanks for each sample were prepared by mixing 0.1 ml. plasma samples with 4.0 ml. tartrate-iodide. A protein standard was prepared in duplicate using 0.1 ml. of 1.0% bovine albumin[19] in isotonic saline solution, which was then added to 4.0 ml. biuret reagent. A blank for the standard was prepared by mixing 0.1 ml. bovine albumin with 4.0 ml. tartrate-iodide. Two reference blanks were prepared by adding 0.1 ml. isotonic

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[19] Sigma Chemical Company, St. Louis, Mo., U.S.A.

saline into two test tubes containing 4.0 ml. biuret reagent and 4.0 ml. tartrate iodide solution respectively. The former reference blank was used for the zero setting of the spectrophotometer before reading the optical density of the biuret-containing samples; the latter was used before the blanks were measured.

The optical density of the standard and its blank, and the samples and their blanks were read with a spectrophotometer set at a wave-length of 555 mμ. Plasma protein concentration was calculated as follows:

$$PPC = \frac{OD_s - OD_{sb}}{OD_{st} - OD_{stb}} \times \text{ g. protein/100 ml. standard}$$

where,

PPC = plasma protein concentration in grams per 100 ml. plasma

OD_s = optical density of sample biuret

OD_{sb} = optical density of sample blank

OD_{st} = optical density of protein standard

OD_{stb} = optical density of protein standard blank

Table III-7, p. 110, of the results illustrates the accuracy of the method. Twenty four samples were each analyzed eight times.

A control measurement, to test the accuracy of the method, was always performed at each series of

analysis with human serum standard Moni-Trol I[20] which has a known protein concentration of 7 g. per 100 ml. These measurements never deviated more than 0.1 g. per 100 ml.

(ii) Measurement of plasma protein concentration in the presence of Rheomacrodex and its detection in plasma.

Since Dextrans in plasma interfere with the biuret method (Jacobsson and Hansen, 1953; Aronsson *et al.*, 1966), the plasma protein concentration in samples containing Rheomacrodex was determined according to the method of Mans *et al.*, (1961).

Disks of No. 3 Whatman filter paper (4.0 cm. diameter) were numbered, weighed to the nearest 0.1 mg. with an analytical balance and mounted on 26 gauge needles. A sample of 0.1 ml. plasma was delivered with a calibrated pipette to each disk which was then dried up under an infrared lamp at 40°C for 5 minutes. The disks were placed into correspondingly labelled 100 ml. beakers and immersed in 50 ml. of ice cold 10% trichloroacetic acid for 10 minutes in order to precipitate the protein quantitatively into the matrix of the cellulose fibers. The disks were subsequently suspended twice for 5 minutes in 50 ml. of ice cold 5% trichloroacetic acid. They were immersed in 30 ml. of 95%

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[20]Dade, Canadian Laboratory Supplies Limited, Montreal, Canada.

ethanol for 2 minutes, then suspended in 30 ml. of ether-ethanol mixture (1:1) which was subsequently evaporated at 37°C. for 15 minutes. The disks were resuspended in ether-ethanol mixture for 5 minutes and immersed in ethyl-ether for another 10 minutes at room temperature to remove alcohol and water. Finally they were dried under the infra-red lamp at 40°C. for 5 minutes. The pins were removed and the disk weighed again. Plasma protein concentrations were calculated from the two weights and expressed as gram protein per 100 ml. plasma.

A check of the method was always made with each determination using a standard human plasma protein Moni-Trol 1 (7 g. per 100 ml.). These determinations were within 0.1 g. of this value.

The presence of dextran in plasma samples was determined by a modification of the method described by Jacobsson and Hansen (1952): two ml. of 8% trichloroacetic acid were added to 0.20 ml. plasma to precipitate the proteins. The mixture was shaken, allowed to stand for 10 minutes and centrifuged at 1,500 g for 5 minutes. One ml. of the supernatant was mixed with 5.0 ml. of 95% ethyl alcohol. A "blank" was made by mixing another 1.0 ml. of supernatant with 5.0 ml. of distilled water. Both the optical density of the alcoholic solution (OD_{alc}) and the blank solution (OD_{bl}) were measured one hour later with a spectrophotometer, at 720 mμ and against the "blank". The

amount of Rheomacrodex corresponding to the corrected optical density (OD_{alc} - OD_{bl}) was read from a previously determined standard curve.

(iii) Separation and measurement of plasma protein fractions.

Electrophoretic separation of plasma protein into five major fractions was performed on 2.5 x 15 cm. cellulose acetate strips[21] (Briere and Mull, 1964). The analysis was carried out on each plasma sample obtained before dye injection (II, 5a, p. 45).

The cellulose acetate membranes were labelled for identification and allowed to float at the surface of a high resolution buffer[22] (tris-barbital-sodium barbital, pH 8.8, ionic strength 0.05 M) until they were uniformly wet: they were then thoroughly soaked by immersion for a period of 15 minutes. After saturation, the membranes were placed on the surface of 30 x 25 cm. glass plate and blotted lightly to remove excess buffer. When droplets of liquid were no longer visible on the surface, the strips were positioned across the dividers of an electrophoretic chamber[23] containing 800 ml. of high resolution buffer.

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[21]Sepraphore 111 Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.

[22]HR Buffer, Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.

[23]Gelman Deluxe Electrophoretic Chamber, Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.

Flat tissue forceps were used routinely to handle the membranes except in the final step of mounting the membranes within the chamber. Three microliters of plasma were loaded on the push button serum applicator with a capillary tube. The applicator was laid across the chamber ridge and the plasma sample was applied to the cellulose acetate strips, 1.35 cm. from the outer cathode support bridge of the chamber. The lateral edges of the cellulose acetate membranes were always kept free of the sample. A second application was made at exactly the same point as the first sample, the chamber was covered and the power supply turned on. A constant voltage of 250 volts D.C. was applied for 45 minutes. After that, the strips were removed from the chamber and stained in a bath of 0.5% Ponceau S in 5% trichloroacetic acid for 10 minutes. This staining procedure was followed by four rinses of 5% acetic acid until the background stain was removed. The cellulose acetate membranes were dehydrated by immersion in 100% methanol for 2 minutes and cleared in a solution of 10% acetic acid in methanol for another two minutes. They were then laid out on a glass plate and dried at 60°C. in an oven[24] for 15 minutes. The strips were peeled off the plate and placed between two sheets of paper using a heavy object for

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[24]Gallenkamp Hotbox Model OV-010 Canadian Laboratory Supplies Limited, Montreal, Canada.

weight to prevent curling. Examples of electrophoretograms obtained with this procedure are given in Figures 8 - 12 pp. 91-95.

The transparent electrophoretic strips were scanned with an automatic recording and integrating densitometer[25] equipped with a slit aperture of 0.1 x 0.6 mm. and a No. 525 filter (absorption maximum at 525 mμ). Duplicate scannings were done on each strip. Protein fraction counts obtained from the densitometer printout were added, each subtotal was divided by the total, and expressed as a percentage. Four arbitrarily chosen duplicate scannings, each composed of five percentages representing the five protein fractions, showed a mean difference of 0.4 between two readings. Knowing the total protein concentration of the sample (II, 6a, i-ii, p. 55) the protein concentration of each fraction could then be calculated.

(iv) Identification of piglet's plasma protein fractions.

Piglet's plasma for this study was obtained from three piglets separate from those used in the main study.

The electrophoretic migrations of the plasma protein fractions of these piglets was compared with those of the fractions of porcine plasma protein which are

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[25]Densicord, Model 552, Photovolt, New York, N.Y., U.S.A.

commercially available as Cohn fractions.[26]

Two series of five 75 x 10 mm. culture tubes were labelled respectively I_1 to V_1 and I_2 to V_2. Thirty-five mg. of the first Cohn fraction were added to tube I_1 and tube I_2; 35 mg. of the second Cohn fraction were added to tube II_1, and tube II_2 etc. . . . Each tube of the first series received 1.0 ml. of isotonic saline. Tubes of the second series received 1.0 ml. of newborn pig plasma. The tubes were allowed to stand for 10 minutes, they were then stoppered, slowly inverted 10 times and centrifuged at 2,500 g for 20 minutes at room temperature. The supernatant of each tube was subjected to electrophoretic separation. A concomitant electrophoresis was made of the same piglet plasma that was used for the mixtures in the tubes of the second series. Thus three electrophoretograms were obtained: one from the piglet plasma alone, one from the Cohn fraction alone, and one from the mixture of the two (see Figures 8 - 12, pp. 91 - 95). Triplicate electrophoretograms were made on each sample according to the method described above. The identification of the albumin fraction was made by a visual comparison between the distance travelled by the Cohn albumin fraction alone (middle electrophoretogram), the

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[26]Research Products Division, Miles Laboratories, Inc., Kankakee, Illinois, U.S.A.

first band of the piglet's plasma (top electrophoretogram) and the first band of the mixture (bottom electrophoretogram) (see Figure 8, p. 91). Since the distance travelled by a fraction may vary from one electrophoretogram to another, these distances were related to the distance travelled by the albumin fraction which is always the largest. In the top and bottom electrophoretograms of Figures 8 - 12, the relative mobility (Mr) of the fraction being analyzed was expressed as the ratio of the mobility of this fraction to that of its own albumin fraction. The mobility was measured as the distance travelled by the fraction from the point of sample application to the center of the electrophoretic band representing this fraction. If the ratios were similar, the fractions in the piglet's plasma were identified with their corresponding Cohn fractions.

(v) Total amount of circulating plasma protein and its fractions.

The total amount of circulating plasma protein was calculated as the product of the total protein concentration and the plasma volume. Likewise, the total amount of a particular protein fraction was the product of the concentration of this fraction and the plasma volume.

b) Electrolytes.

(i) Determination of total plasma osmolar concentration.

Plasma osmolarity was measured cryoscopically using an osmometer.[27] The instrument was calibrated with 500 and 100 milliosmol standard solutions prior to each series of analysis. Two hundred microliter plasma samples were pipetted in microtubes which were then inserted into the refrigerator well of the osmometer. The samples were supercooled and seeded by mechanical stirring and the osmolarity of the plasma samples read directly from the hand dial after bringing the indicator needle back to zero. Plasma osmolarity was expressed in milliosmols per liter solution (mOsm/l). All measurements were made in duplicate. Duplicate readings did not differ more than 3 units.

(ii) Determination of sodium and potassium concentrations.

Plasma sodium and potassium concentrations were determined with a flame photometer[28] equipped with an automatic dilutor. The instrument was set at zero using "Li-diluent" which consists of a solution of lithium stock concentrate (1,500 milliequivalents per liter) diluted in distilled water in a one to 200 ratio. It was calibrated with a standard solution of 140 milliequivalents sodium and 5 milliequivalents potassium per liter distilled water.

[27] Osmette Model 2007, Precision Systems, Waltham, Massachusetts, U.S.A.

[28] Il Model 143, Instrumentation Laboratory Inc., Boston, Massachusetts, U.S.A.

which had been diluted 200 times with "Li-diluent". A 75 microliter sample was introduced in the dilutor and sodium and potassium concentrations were read directly from the digital display in milliequivalents per liter (mEq/l). All measurements were made in duplicate. The sodium reading did not differ more than 2 milliequivalents and those of potassium not more than 0.1 milliequivalent per liter.

(iii) Determination of chloride concentration.

Chloride ion concentration was determined using an automatic electrometric titration device,[29] the principle of which is as follows (Lehmann, 1939): Chloride in diluted acetic acid solution is potentiometrically titrated with silver nitrate ($AgNO_3$) using a silver and a potassium sulfate electrode. The device stops the instillation of $AgNO_3$ automatically when the required ion concentration, i.e. the equivalence point is reached.

About 10 ml. of a 0.002 M $AgNO_3$ solution was introduced into the burette and the proper outflow from the burette was checked. A beaker containing 20 ml. of 50% acetic in distilled water was placed on an electromagnetic stirrer under the burette. The electrodes were

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[29]Radiometer Titrator Type TTT, titration assembly Type TTA1 Radiometer A/S, Copenhagen, Denmark.

immersed in the solution and the equivalence point was selected on the potentiometer (100 millivolts). The titration was calibrated by titrating 20 µl. of a chloride standard (100 mEq/1) in the acetic acid solution; the burette reading was taken, the titration carried out automatically until the equivalence point was reached, and a second burette reading was taken. The value for the chloride ion contentration (in mEq/1) was obtained by subtracting the initial burette reading from the final reading at the end point and multiplying the result by 100. Plasma chloride analysis was performed in the same way on 20 µl. plasma samples. The analyses were made in duplicate; the results did not differ more than 2 milliequivalents per liter.

(iv) Determination of calcium concentration.

The determination of free calcium ions in plasma was assessed by fluorometric titration method[30] (Jackson *et al.*, 1962), the principle of which is as follows: Di-sodium-ethylene-diamine-tetra-acetate (EDTA) removes calcium from the calcium-calcein complex; when all the calcium ions are bound by EDTA the original fluorescence is quenched. The end point of the titration is this quenching of the fluorescence.

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[30]Oxford Titrator Model 301, Oxford Reagents, San Mateo, California, U.S.A.

Fifty microliters (50 μl.) of a 10 mg. per 100 ml. calcium standard, were titrated in 0.25 N potassium hydroxide with EDTA using calcein as an indicator. The titration volume was recorded from the dial reading. The same procedure was then repeated on 50 μl. plasma samples. Calcium concentration was calculated as follows:

$$[Ca] = \frac{\text{Titration value for the unknown}}{\text{Titration value for the standard}} \times 10 \text{ mg./100 ml.}$$

where,

[Ca] = Calcium concentration in mg. per 100 ml. plasma

The results were converted to milliequivalents per liter. All measurements were made in duplicate. Duplicate values did not differ more than 0.2 milliequivalent per liter. Another example of the accuracies of the above determinations is given by the analysis of the Ringer-lactate solution (Table III-8, p. 111).

7. Blood Pressure, Heart Rate and Body Temperature Measurements

The arterial blood pressure was recorded from the carotid artery catheter by means of a pressure transducer,[31] a pre-amplifier and a polygraph recorder.[32] Static

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[31]Statham Type P23AA, Gilson Medical Electronics, Middleton, Wisconsin, U.S.A.

[32]Model M5P, Gilson Medical Electronics, Middleton, Wisconsin, U.S.A.

calibrations were carried out regularly with a mercury-filled manometer. The heart rate was obtained from the blood pressure tracing by counting the number of systolic peaks that occurred in one minute.

Rectal temperature was measured with a thermistor probe[33] inserted to a depth of 3 centimeters.

8. Statistical Analyses and the Exponential Curve Fitting

All variables investigated were tabulated (Appendices page 173 to 199). Means, standard deviations (s.d.) and standard errors of the means (s.e.m.) were calculated after insuring that the values were more or less normally distributed. The level of significance of the differences between the initial values and the observations at various times within one group was determined by the paired observation t-test. The significance of the differences of the mean values between groups at various times was determined by the t-test for the difference between the two sample means (Snedecor and Cochran, 1969). Calculations were carried out on a table calculator.[34]

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[33] Thermistemp, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio, U.S.A.

[34] Model 9810 Hewlett Packard calculator, Hewlett-Packard (Canada Ltd.), Halifax, Canada.

The following significance levels were used:

** significant $0.01 > P > 0.001$

*** highly significant $P < 0.001$

In this study, the terms insignificant, significant and highly significant, will be used only in the statistical sense as defined by the above probability levels. The correlation coefficient was used to measure the intensity of a relationship between two variables. The data were plotted in a form of a scatter-diagram; the graph was then studied for any apparent linear relation, and the correlation coefficient was calculated.

When the recovery of a physiological variable after hemodilution perfusion could be expected to follow an exponential time course and when the observed values showed an indication of such a time course, an exponential curve fitting procedure was carried out.

The observed values (Yt) were made non-parametric by expressing each as a fraction $y = \left(\frac{Y\infty - Yt}{Y\infty - Yo}\right)$ of the total change ($Y\infty - Yo$), where, $Y\infty$ is the value before the start of perfusion or the value at 24 hours, whichever was greater, and Yo is the minimum value obtained immediately after perfusion. The logarithms of these fractions were then plotted against time. If the dots appeared to lie on a straight line and the line appeared to intercept the ordinate at its origin ($\log y = 0$), a straight line was fitted using the method of least squares but

constrained to run through the origin. This line represented a single exponential relationship. If a linear relationship was present but the line did not go through the origin, a double exponential relationship could be assumed. In that case the linear equation of the slow component was calculated with the method of least-squares and the fast component estimated from a second plot made by reading the differences between the calculated line and the interpolation between the fractions. The slopes of the lines were converted into time constants ($\tau = \frac{1}{e}$), where, τ is the time required for a change of 36.8 per cent. This time constant indicated the rate of restoration of the variable under study.

If no linear relationship could be recognized, no attempt was made to express the changes of the variable under study in any mathematical form. On the graphs, the observed values were then simply connected by straight lines.

All values plotted on the graphs were mean values plus or minus one standard deviation or standard error of the mean whichever was applicable. This standard deviation or standard error of the mean was indicated either upward or downward for the purpose of clarity.

CHAPTER III

RESULTS

1. Pilot Study

A pilot study based on 4 animals was undertaken to test the feasibility of lowering the piglet hematocrit to a value between 20 and 25% (I, 9, p. 31) and to practise on the experimental procedures. Each animal was subjected to surgical procedures and to hemodilution perfusion as described in sections 3 and 4 of Chapter II. The volume of the priming solution was determined by the piglet blood volume and its hematocrit (II, 4b, p. 42). A hemodilution resulting in a mixed hematocrit of 23% was the first target. Only buffered Ringer-lactate was used as a priming solution. The priming volumes varied from 31.0 to 97.0 ml. The hematocrit values immediately after the termination of perfusion ranged between 23.3 and 23.9%. All animals survived this hemodilution perfusion and were in good condition after seven days. The target hematocrit was then brought down to 22% and these animals also survived. They were subsequently included in the present study.

2. Presentation of Material

The results presented in this chapter are shown graphically, the graphs being prepared from mean values obtained from 15 piglets in each of the three groups.

The actual values obtained in each experiment for each of the 23 variables that were either measured directly (17) or calculated (6) from these values are presented in Tables 1-23 of the Appendix A, pages 173 to 195.

The mean values of circulating amounts of plasma proteins, also obtained from this data, are presented in Tables 1-3 of Appendix B, pages 196 to 198.

The estimations of plasma dextran concentrations after hemodilution are given in Appendix C-Table 1, page 199. All tables in Appendix A have the same format. Within one group of 15 animals the piglet numbers and the data of each individual piglet are shown as they were collected before perfusion (before) immediately after (after), 8, 16 and 24 hours after perfusion; moreover, the values observed in the extracorporeal circuit immediately after perfusion (pump) are also given.

In the present investigation the emphasis is on averages obtained from groups rather than on the results obtained from each individual animal. The tables, however contain all data of all experimental animals in order to make comparisons within a group and also to demonstrate the

variability existing within a group. Statistical comparisons were made within each group on the differences in values between the values before perfusion and the subsequent values (II, 8, p. 68). The results are indicated in the tables by I, for the comparison with the intial value (paired observation t-test).

For the statistical comparison between groups, means and variances were calculated. The comparisons between the experimental groups and the sham-operated group and the comparisons between the Ringer-lactate and the Rheomacrodex group are indicated in the tables by S and R respectively.

Because plasma volume has an unduly high variance in a group if body weight is not taken into account (Table III-1, p. 74), plasma volume and blood volume are expressed per kilogram body weight (Appendix A, Tables 3 and 4). Amounts of proteins which are the product of concentration and plasma volume are expressed in grams per kilogram body weight (Appendix B).

TABLE III-1

REDUCTION OF VARIANCE OF PLASMA VOLUMES
BY TAKING BODY WEIGHT INTO ACCOUNT
The Coefficient of Variance
(c.v. = s.d./m x 100)*
is reduced to more than one half

"Before"						
	Sham		RL		LMWD	
	PV	PV/Kg	PV	PV/Kg	PV	PV/Kg
m. (ml.)	183.0	64.0	178.4	64.0	186.1	63.5
s.d.	24.2	4.0	18.9	3.1	27.6	3.9
c.v.	13.2%	6.3%	10.5%	4.8%	14.8%	6.1%

*m = mean; s.d. = standard deviation;
c.v. = coefficient of variance.

Although the priming volume was prepared for each piglet individually (Table III-2, p. 75) taking not only body weight but also hematocrit into account (II, 4b, p. 42) an average priming volume was required for subsequent considerations (III, 6a, p. 80). These averages were 33.7 ml./Kg. for the Ringer-lactate group and 36.0 ml./Kg. for the Rheomacrodex group.

In the subsequent text the sham-operated group will be referred to as sham group; the Ringer-lactate group and the Rheomacrodex group as RL group and LMWD group respectively.

TABLE III-2

PRIMING VOLUMES (ML.) IN THE TWO EXPERIMENTAL GROUPS

Ringer-lactacte		2% Rheomacrodex	
Piglet #		Piglet #	
65	130.9	114	113.0
70	59.2	115	157.3
71	96.7	116	73.5
74	100.8	117	80.1
76	98.0	122	69.8
79	85.0	125	141.0
80	100.8	127	110.0
82	72.0	129	121.8
83	55.3	131	189.9
85	105.9	133	115.0
95	120.8	136	109.4
98	90.7	138	89.4
99	112.1	141	77.2
102	82.0	142	92.1
105	89.4	144	44.2

3. The Thoroughness of the Mixing During Hemodilution Perfusion

The successfulness of mixing the animal's blood with the priming fluid was examined by comparing the hematocrits and the concentrations of the blood constituents observed in the animal and in the extracorporeal circuit immediately after the termination of perfusion; Table

III-3, p. 76 shows the results. The mean differences between the two values and the standard deviations are close to zero, indicating that the mixing was indeed complete for all experimental animals.

TABLE III-3

MEANS AND STANDARD DEVIATIONS OF THE DIFFERENCES BETWEEN THE VALUES OF THE VARIABLE OBSERVED IN THE ANIMAL AND IN THE EXTRACORPOREAL CIRCUIT IMMEDIATELY AFTER PERFUSION

	RL Group		LMWD Group	
	Mean of diff.	s.d. of diff.	Mean of diff.	s.d. of diff.
LVH	0.2	0.29	0.0	0.03
PPC	0.0	0.03	0.0	0.02
Albumin	-0.02	0.07	0.02	0.07
Globulins				
Alpha-	0.02	0.03	0.00	0.04
Beta -	0.00	0.06	-0.01	0.03
Gamma-	0.03	0.07	-0.01	0.06
Fibrinogen	0.01	0.11	0.00	0.10
Osmolarity	0.5	1.2	0.3	1.2
Sodium	-1.1	3.7	-1.4	3.0
Potassium	0.0	0.8	0.0	0.2
Chloride	0.0	1.4	-0.3	4.1
Calcium	0.0	0.3	0.0	0.2

4. Body Weight

There was a decrease in body weight in all three groups during the 24 hour experiment due to starvation and

dehydration (Appendix A-Table 1, p. 173, Figure 3, p. 78). The average weight losses expressed as the difference between initial and final weight were 262 g. for the sham group, 278 g. for the RL group and 280 g. for the LMWD group.

5. Large Vessel Hematocrit

All hematocrit values are presented in Appendix A-Table 2, p. 174. Each value is an average of 12 readings. (II, 5c, p. 50). Averages of the groups are shown in Figure 4, p. 79.

The degree of hemodilution which occurred during the period of perfusion was reflected by a decrease in the hematocrit value immediately after perfusion. The target value was 22% (II, 4b, p. 42). The hematocrit of the RL group was however significantly higher than this value, whereas that of the LMWD group was significantly lower (paired observation t-test).

Despite the fact that the priming volumes were calculated the same way for both groups, the decrease in the hematocrit was significantly greater in the LMWD group than in the RL group. The former showed a continuous and highly significant rise during the 24 hours while the latter showed a highly significant fall until 16 hours and then no change between 16 and 24 hours. The significant

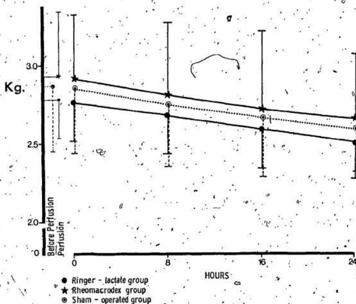

FIGURE 3

BODY WEIGHT BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard deviations within the group of 15 animals. The reader is referred to Appendix A-Table 1 for statistical comparisons.

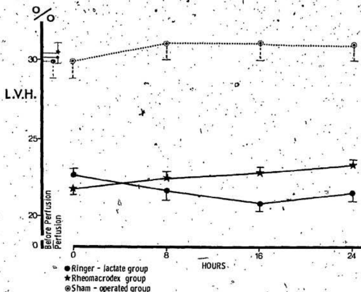

FIGURE 4

LARGE VESSEL HEMATOCRIT (L.V.H.) BEFORE
AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix A-Table 2 for statistical comparisons.

rise in large vessel hematocrit observed at 8 hours in the sham group was maintained until 24 hours. The differences between the LMWD and the RL group at 0, 16 and 24 hours were significant. Miscroscopic inspections of the erythrocytes did not reveal any sign of damage.

6. Plasma Volume

a) Changes in plasma volume within hemodilution perfusion period.

All observations are presented in Appendix A-Table 3. The averages of the groups are shown in Figure 5, p. 81

i - There was a drastic decrease in the plasma volume during perfusion in both experimental groups; the largest fall was observed in the RL group (14.2 ml./Kg.) which was significantly different from that in the LMWD group (7.8 ml./Kg.). This is at first sight surprising since the volume of the extracorporeal circuit was kept constant and since there was no blood loss during the period of perfusion. The constancy of the animal's fluid volume was also reflected in the constancy of his body weight: the difference between body weight before and after the perfusion was 8 g. (s.d. = 1) for the LMWD group and 7 g. (s.d. = 2) for the RL group. (In the latter group, piglets 70, 76, 99 and 102 were left out from the calculation because they urinated and/or defaecated during perfusion).

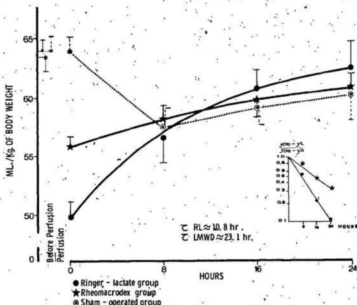

FIGURE 5

PLASMA VOLUME BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix A-Table 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

The above differences come very close to the amount of blood (6 g.) which was withdrawn from the animal but could not be replaced (II, 5, p. 45).

It may be argued that plasma volume determinations immediately after hemodilution perfusion are in error and that the observed deficits are artifacts. To investigate the validity of the plasma volume determination immediately after perfusion another approach was made: plasma volume was calculated using the concentrations of blood constituents, and assuming that the total amount of a particular plasma constituent (protein or electrolyte) before perfusion equals the total amount after perfusion. This assumption is valid when kidney function is negligible during the perfusion period and when there is no loss of the constituents into the extravascular space during that period.

The balance equation for the total amount of a particular constitutent before and after perfusion can be written as follows:

$$V_i \cdot C_i + V_{pr} \cdot C_{pri} = V_a \cdot C_a + V_{pr} \left(\frac{100 - Ht}{100} \right) \cdot C_{pra}$$

where:

$V_i \cdot C_i$ is the amount of the constituent in the animal before perfusion,

$V_{pr} \cdot C_{pri}$ is the amount of the constituent in the extracorporeal circuit before perfusion,

$V_a \cdot C_a$ is the amount of the constituent in the animal after

perfusion,

$V_{pr}\left(\frac{100 - Ht}{100}\right) \cdot C_{pra}$ is the amount of the constituent left behind in the extracorporeal circuit after perfusion, in which:

V_i, V_{pr} and V_a are initial plasma volume, priming volume and plasma volume after perfusion respectively;

C_i and C_a are the concentrations of the constituent before and after perfusion respectively,

C_{pri} and C_{pra} are the concentrations of the constituent in the priming volume and in the perfusate respectively;

$V_{pr}\left(\frac{100 - Ht}{100}\right)$ represents the "plasma volume" of the perfusate.

The equation can be rearranged to solve for V_a. Using average values of initial plasma volumes, priming volumes and concentrations of various blood constituents, the following values for V_a were obtained and compared with the plasma volume determinations made by the T-1824 dye dilution technique immediately after perfusion (V_o). This value was 49.8 ml./Kg. for the RL group and 55.7 ml./Kg. for the LMWD group. The results are indicated in Table III-4, p. 84.

TABLE III-4

ESTIMATES OF PLASMA LOSS DURING HEMODILUTION PERFUSION BASED ON THE CONCENTRATION OF PLASMA AND PERFUSATE CONSTITUENTS

Constituents	RL Group		LMWD Group	
	V_a	V_o-V_a	V_a	V_o-V_a
	ml./Kg.		ml./Kg.	
Albumin	57.0	- 7.2	64.5	- 8.8
Globulin	54.5	- 4.7	60.0	- 4.3
Fibrinogen	51.5	- 1.7	60.1	- 4.5
Sodium	71.1	-21.3	69.0	-13.3
Potassium	71.4	-21.6	67.5	-11.8
Chloride	76.3	-26.5	77.4	-21.7
Calcium	66.3	-16.5	58.5	- 2.8
Osmolarity	68.4	-18.6	71.6	-15.9

It can be seen that the agreement between the calculated (V_a) and observed plasma volume (V_o) was close when constituents of large molecular size were used for the calculation, and it appeared that the larger the molecule, the closer was the agreement. This agreement was poor when ion concentrations were used; ions did not comply with the assumption stated above, as could be expected. It can be concluded that the above approach validated the plasma volume determination by the T-1824 technique immediately after perfusion and the subsequent detection of plasma volume deficits during perfusion.

b) Changes in plasma volume after hemodilution perfusion.

The plasma volume values of the three groups were very similar at the beginning of the experiment (Figure 5, p. 81). The sham group showed an unexpected fall in the plasma volume after 8 hours. This fall was likely to be a gradual one because a repetition of plasma volume determination about 20 minutes after the initial one would probably have yielded a same value. Therefore the value shown for this group at time zero is the same plot as the "before perfusion" value. This method of plotting was consistent for all such data. Immediately after perfusion, the two experimental groups showed a marked decrease in plasma volume, as already discussed. At 8 hours the plasma volume values of the three groups were similar. From there on, plasma volumes seemed to be restored progressively. The restoration in the sham group followed the same pattern as that in the LMWD group whereas the restoration in the RL group was somewhat faster. This was also indicated by the time constants calculated for the experimental groups. These time constants (τ) were calculated using the initial value as the target value to which plasma volume tends to come back ($y\ \infty$). Further explanation of this technique is given in Chapter II, 8, p. 68). This calculation of time constants seems to be better than to compare the data of the experimental groups with those of the sham group and to conclude that the experimental animals are "back to normal" after 8 hours. In the RL group and the LMWD group, the

replenishment of the lost plasma volumes followed a single exponential curve and had a time constant of 10.8 hours and 23.1 hours respectively. In the RL group, the plasma volume did not differ significantly from the initial value at 16 and 24 hours whereas in the LMWD group it remained still significantly lower.

7. The Disappearance of Rheomacrodex From the Plasma After Hemodilution Perfusion

In Appendix C, the observed concentrations of dextran in plasma are presented. Immediately after perfusion, a mean concentration of 0.49 g. per 100 ml. was found. After 8 hours, only 0.06 g. per 100 ml. was left in the circulation and at 24 hours, no dextran could be detected anymore (Figure 6, p. 87). The disappearance of dextran followed a double exponential curve; the time constant of the fast component was in the order of 2.6 hours and that of the slow component 16 hours. A considerable amount of Rheomacrodex must have disappeared during the period of perfusion because it can be calculated that the concentration of the Rheomacrodex in the circulation would have been 0.7 g. per 100 ml. plasma if mixing had been instantaneous and no Rheomacrodex was lost from the circulation. (This concentration can be obtained by dividing the known amount of Rheomacrodex administered to the animal by the sum of plasma volume and priming volume).

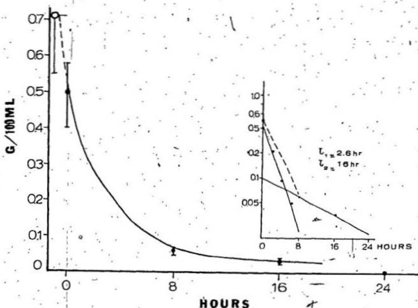

FIGURE 6
THE DISAPPEARANCE OF RHEOMACRODEX FROM PLASMA AFTER HEMODILUTION PERFUSION

The bars shown for each value represent the standard deviations within the group of 15 animals. The reader is referred to Appendix C-Table 1 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68. The open circle represents the calculated concentration which would have existed if mixing was instantaneous and no dextran was lost from the circulation.

8. Blood Volume

The observed blood volumes as calculated from plasma volumes and hematocrits are presented in Appendix A-Table 4, p. 176. The average values per group are shown in Figure 7, p. 89 .

The pre-operative blood volume values averaged 91.4 ml./Kg., 91.7 ml./Kg. and 90.8 ml./Kg. in the sham, RL, and LMWD groups respectively. A mean blood volume deficit of 27.2 ml./Kg. in the animals perfused with Ringer-lactate and 19.6 ml./Kg. in those perfused with Rheomacrodex was observed immediately after the termination of bypass. During the next 24 hours the replenishment of blood volume in both groups was consistent with a single exponential curve. The curve had a time constant of 28.3 hours and 45.3 hours in the RL and LMWD groups respectively. Consequently, at the 24 hour reading, the blood volumes in both groups were still significantly lower than their initial values. In the sham-operated group the blood volume was markedly reduced at 8 hours, but at 16 and 24 hours it had sufficiently increased to approach the initial value. During this fall and subsequent rise it remained however, significantly larger than the blood volumes of the two perfused groups.

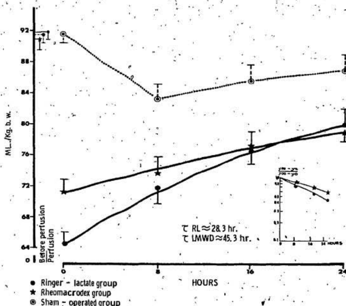

FIGURE 7

BLOOD VOLUME BEFORE AND FOLLOWING
HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix A-Table 4 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

9. Identification of Piglet's Plasma Protein Fractions

In order to determine concentrations of various protein fractions in newborn pigs (III, 10 b, p. 96) it was first necessary to identify these fractions in electrophoretograms since this has not been reported previously in the literature.

The electrophoretograms of the piglet's plasma, the porcine Cohn fraction and the mixture of the two are presented in Figures 8 to 12, pp. 91 to 95.

The relative electrophoretic mobility of piglet's plasma protein fractions without and with the addition of porcine Cohn fractions is shown in Table III-5, below. The results indicated that the electrophoretic migration of each plasma protein fraction remained the same when the relevant Cohn fraction was added.

TABLE III-5

RELATIVE ELECTROPHORETIC MOBILITY (Mr) OF PLASMA PROTEIN FRACTIONS FROM PIGLETS, WITHOUT (Mr_1) AND WITH (Mr_2) ADDITION OF PORCINE COHN FRACTIONS (MEANS AND RANGES OF VALUES)

	Globulin			Fibrinogen
	Alpha	Beta	Gamma	
Mr_1	0.62 (0.61-0.64)	0.46 (0.43-0.50)	0.14 (0.13-0.16)	0.31 (0.29-0.35)
Mr_2	0.62 (0.60-0.64)	0.46 (0.42-0.52)	0.14 (0.13-0.16)	0.32 (0.30-0.36)

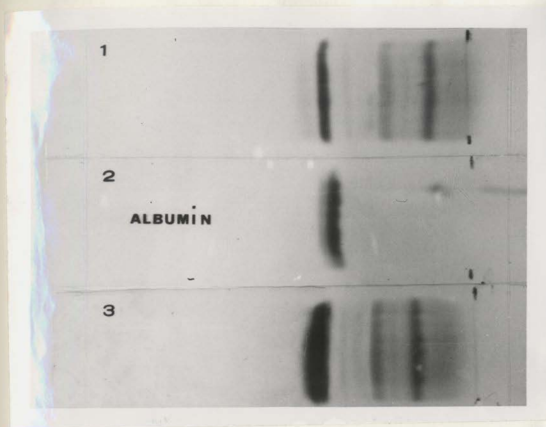

FIGURE 8

CELLULOSE ACETATE ELECTROPHORETIC SEPARATIONS OF PIGLET PLASMA PROTEIN FRACTIONS, WITHOUT AND WITH THE ADDITION OF A PORCINE COHN FRACTION (ALBUMIN)

Legend:

1. Plasma protein fractions from seven days old piglet
2. Porcine Cohn fraction
3. Piglet plasma protein fractions after addition of the porcine Cohn fraction

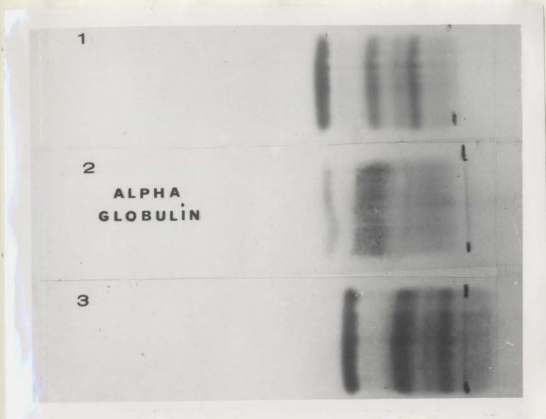

FIGURE 9

CELLULOSE ACETATE ELECTROPHORETIC SEPARATIONS OF PIGLET PLASMA PROTEIN FRACTIONS, WITHOUT AND WITH THE ADDITION OF A PORCINE COHN FRACTION (ALPHA GLOBULIN)

Legend:

1. Plasma protein fractions from seven days old piglet
2. Porcine Cohn fraction
3. Piglet plasma protein fractions after addition of the porcine Cohn fraction

FIGURE 10

CELLULOSE ACETATE ELECTROPHORETIC SEPARATIONS OF PIGLET PLASMA PROTEIN FRACTIONS, WITHOUT AND WITH THE ADDITION OF A PORCINE COHN FRACTION (BETA GLOBULIN)

Legend:

1. Plasma protein fractions from seven days old piglet
2. Porcine Cohn fraction
3. Piglet plasma protein fractions after addition the the porcine Cohn fraction

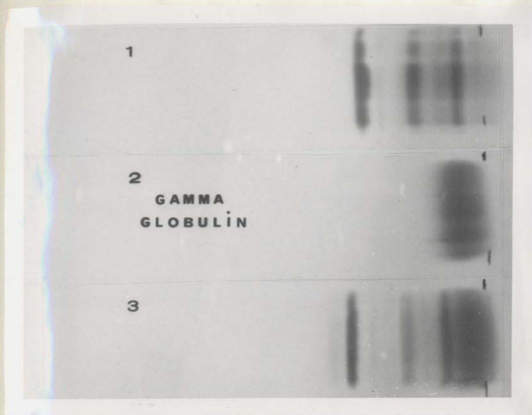

FIGURE 11

CELLULOSE ACETATE ELECTROPHORETIC SEPARATIONS OF PIGLET PLASMA PROTEIN FRACTIONS, WITHOUT AND WITH THE ADDITION OF A PORCINE COHN FRACTION (GAMMA GLOBULIN)

Legend:

1. Plasma protein fractions from seven days old piglet
2. Porcine Cohn fraction
3. Piglet plasma protein fractions after addition of the porcine Cohn fraction

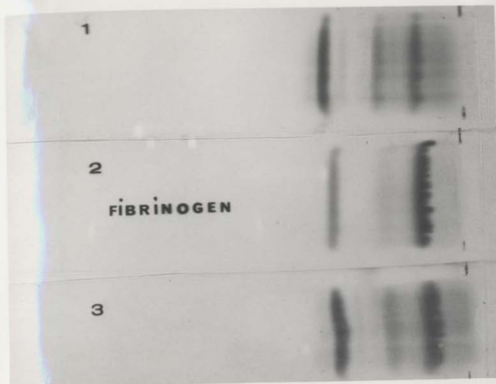

FIGURE 12

CELLULOSE ACETATE ELECTROPHORETIC SEPARATIONS OF PIGLET PLASMA PROTEIN FRACTIONS, WITHOUT AND WITH THE ADDITION OF A PORCINE COHN FRACTION (FIBRINOGEN)

Legend:

1. Plasma protein fractions from seven days old piglet
2. Porcine Cohn fraction
3. Piglet plasma protein fractions after addition of the porcine Cohn fraction

10. Plasma Protein Concentrations

a) Total plasma protein concentrations.

The observed total plasma protein concentrations are presented in Appendix A-Table 5, p. 177. Each value is the average of four determinations (II, 5c, p. 50). The average values per group are shown in Figure 13, p. 97.

There was a marked fall in the total plasma protein concentration of the two experimental groups immediately after the termination of bypass; this fall was significantly greater in the LMWD group. During the next 24 hours, total plasma protein concentrations in both groups were restored exponentially. The rate of restoration was much faster in the LMWD group (τ = 1.6 hours) than in the RL group (τ = 15.3 hours). Only in the former did total plasma protein concentration return to the initial value; as early as eight hours after perfusion, no difference could be found. The total plasma protein concentration in the sham-operated group remained the same throughout the experiment.

b) Concentration of each plasma protein fraction.

The relative percentages of the electrophoretically separated plasma protein fractions are presented in Appendix A-Tables 6-10, pp. 178-182. The concentration of each plasma protein fraction was calculated by multiplying the relative percentage of each fraction by the total plasma protein

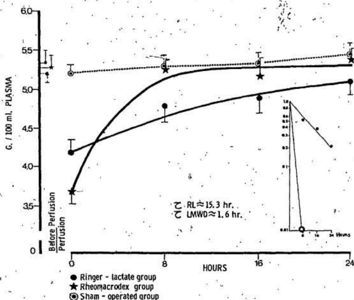

FIGURE 13

TOTAL PLASMA PROTEIN CONCENTRATIONS BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix A - Table 5 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

concentration. These results are given in Appendix A-Tables 11 to 15. The average values for each group are not presented graphically because their restoration patterns were essentially similar to that of the total plasma protein concentration, i.e. there was no change in the sham group, a return to normal values at 8 hours in the LMWD group and a slower restoration in the RL group.

11. Circulating Amounts of Plasma Proteins

The total circulating amount of a particular protein is the product of the plasma volume and the concentration of this protein. For subsequent considerations, these total amounts have been calculated for each individual piglet at all sampling times, but only averages per group are presented in Appendix B-Tables 1-3, pp. 196-198. Each table represents one group. The format is the same as in Appendix A, except that the individual values are left out. The comparisons are indicated by the letters I, S and R: I for the comparison with the initial value, S for the comparison with the sham operated group and R for that between the two experimental groups. The results are also shown in Figures 14 to 19 pp. 101-106. Although the relative percentages of the protein fractions did not change significantly during the experiment (Appendix A-Tables 6 to 10, pp. 178-182) and the concentrations of the fractions followed more or less the same restoration pattern as the total protein

concentration during the 24 hour post-perfusion period, the products of the primary variables revealed three distinct recovery patterns: a) a slow, single exponential recovery in the RL group and a double exponential recovery in the LMWD group for the total amount of proteins and albumin (Figures 14 and 15, pp. 101-102), b) a single exponential recovery in both experimental groups for the alpha and beta globulins, and fibrinogen (Figures 16, 17 and 19, pp. 103, 104, 106), c) no consistent pattern for the gamma globulin (Figure 18, p. 105).

a) Total amounts of proteins and albumin.

In the sham-operated group there was a significant fall in the total amounts of proteins and albumin at 8 hours (Figures 14 and 15, p. 101 and p. 102). The plasma proteins showed no significant difference at 16 hours and thereafter, while the albumin was still significantly lower at 16 hours and returned to the initial value only after 24 hours. In the RL group the exponential restoration of the total amount of proteins had a time constant of 14.6 hours and was similar to that of albumin (τ = 14.7 hours). At the 24 hour reading, the total amount of circulating proteins showed no significant difference from the initial value, whereas that of albumin remained significantly lower. In the LMWD group, the restoration of the total amounts of plasma-proteins and albumin followed a double exponential pattern. The time

constants of the fast components were 2.8 hours for the total plasma proteins and 2.4 hours for the albumin. The time constants of the slow components were 15.7 and 16.2 hours respectively. Both total proteins and albumin were back to their initial value within 24 hours.

b) Alpha and beta globulins and fibrinogen.

The exponential recovery of alpha and beta globulins and fibrinogen in the LMWD group showed time constants of 8.2, 5.1 and 7.2 hours respectively (Figures 16, 17 and 19, pp. 103, 104, and 106). In the RL group, the time constants appeared to be longer and were 11.4, 13.1 and 12.8 hours respectively. At 24 hours, the circulating amounts of these proteins in the two experimental groups were not significantly different from their initial levels. The sham group showed only insignificant changes.

c) Gamma globulin.

In the LMWD group, at 24 hours after hemodilution perfusion, the amount of gamma globulin showed no significant difference from the initial value (Figure 18, p. 105, Appendix B-Table 3). The fall in the RL group remained significant throughout the whole experimental period. No significant changes in gamma globulin could be found in the sham group during the 24 hours.

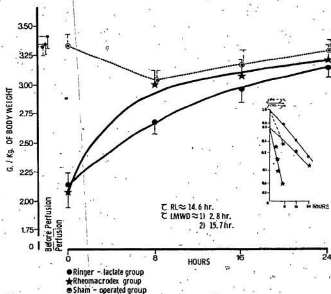

FIGURE 14

TOTAL AMOUNT OF CIRCULATING PLASMA PROTEINS BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

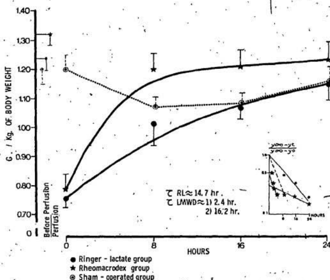

FIGURE 15

TOTAL AMOUNT OF CIRCULATING PLASMA ALBUMIN BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

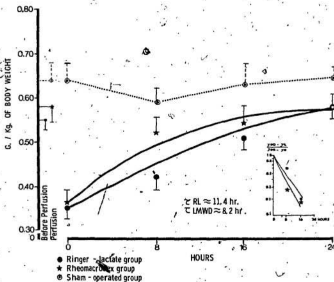

FIGURE 16

TOTAL AMOUNT OF CIRCULATING PLASMA ALPHA GLOBULIN BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

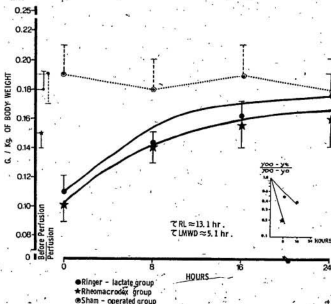

FIGURE 17

TOTAL AMOUNT OF CIRCULATING PLASMA BETA GLOBULIN BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also the time constants τ were read. This technique is described in Chapter II, 8, p. 68.

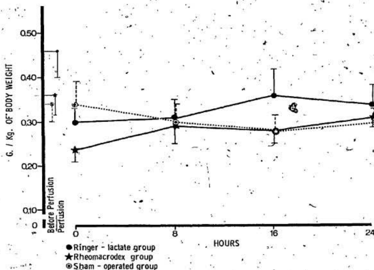

FIGURE 18

TOTAL AMOUNT OF CIRCULATING PLASMA GAMMA GLOBULIN BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

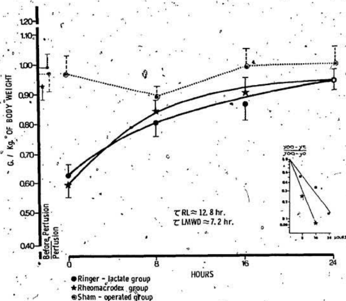

FIGURE 19

TOTAL AMOUNT OF CIRCULATING PLASMA FIBRINOGEN BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix B - Tables 1 to 3 for statistical comparisons. The curved lines given in the main graph were obtained from the linear relationships shown in the insert from which also time constants τ were read. This technique is described in Chapter II, 8, p. 68.

12. Loss of Plasma Proteins During Hemodilution Perfusion

Immediately after the termination of perfusion, the observed total amount of circulating plasma proteins was lower than before perfusion (Figure 14). This is not surprising since proteins are left behind in the extracorporeal circuit. An actual analysis of the amount of plasma protein remaining in the animal and in the circuit revealed, however, that protein was lost from the circulation. This is shown in Table III-6, p. 108. Total amounts of proteins before and immediately after perfusion were calculated for each individual animal, using the observed plasma volume (not plasma volume per Kg.) and plasma protein concentration. The table shows the averages of these values for each experimental group, together with the amount of protein left in the extracorporeal circuit (pump volume minus red cell volume multiplied by protein concentration). The circulating amount of proteins before perfusion minus the amount of proteins left in the extracorporeal circuit was the amount expected to be found in the circulation immediately after perfusion. This value was higher than the actually observed circulating amount of proteins. The differences were highly significant (paired observation t-test) for the two groups and indicated a loss of proteins during perfusion.

TABLE III-6

LOSS OF PLASMA PROTEINS DURING HEMODILUTION PERFUSION

TOTAL AMOUNT OF PROTEIN IN GRAMS

(AVERAGES OF ALL ANIMALS)

	Animal before	Pump	Expected in animals	Animal after	Amount lost
RL Group	9.57	3.00	6.57	5.79	0.76
LMWD Group	9.82	2.92	6.90	6.06	0.84

In order to ascertain that this loss occurred in the animal and not in the extracorporeal circuit, the following additional investigation was made:

Lyman *et al.*, (1968) have reported that proteins are adsorbed on hydrophobic polymer surfaces such as polyvinyl and teflon. Since the tubing of the extracorporeal circuit was made of polyvinyl, an investigation was undertaken to measure quantitatively any possible loss due to adsorption of plasma proteins in this circuit. The study was carried out on eight units of blood (about 250 ml. each) obtained from 2 piglets and 6 adult pigs. The piglets were anesthetized, heparinized (200 I.U./Kg.) and exsanguinated from a carotid artery catheter. Adult pig blood was collected in 6

heparinized plastic bottles at the local abattoir. Hematocrits were determined and samples for plasma protein determination were taken using the same techniques as described (II, 4e, p. 44; II, 5a, p. 45). The blood was diluted to a mixed hematocrit of 22% (II, 4b, p. 42) with Ringer-lactate or Rheomacrodex solution and samples were taken once more. The diluted blood was then warmed to 38°C, introduced into the extracorporeal circuit and recirculated for the same length of time as used in the actual experiments. At the conclusion of the run, a final sample was collected. Each sample was analyzed for plasma protein concentration eight times by the technique described in II, 6a, p. 53. Means and standard deviations are shown in Table III-7, p. 110. Since no change in plasma protein concentration could be detected after recirculation, it was concluded that there was no loss of plasma protein in the extracorporeal circuit due to adsorption.

TABLE III-7

THE EFFECT OF RECIRCULATING DILUTED BLOOD IN THE EXTRACORPOREAL APPARATUS ON ITS PLASMA PROTEIN CONCENTRATION. MEANS (N=8) AND STANDARD DEVIATIONS

		Grams per 100 ml. plasma		
		Before dilution	After dilution	After re-circulation
RL Group	Piglet # 1	5.4 (0.08)	4.0 (0.05)	4.0 (0.08)
	Adult pig # 1	5.8 (0.05)	3.4 (0.08)	3.5 (0.09)
	2	6.7 (0.11)	4.8 (0.08)	4.7 (0.05)
	3	6.1 (0.08)	3.1 (0.05)	3.0 (0.08)
LMWD Group	Piglet #124	4.5 (0.04)	3.4 (0.10)	3.4 (0.09)
	Adult pig# 4	5.7 (0.05)	2.7 (0.12)	2.8 (0.10)
	5	6.0 (0.05)	2.8 (0.06)	2.8 (0.09)
	6	6.3 (0.08)	3.0 (0.05)	2.9 (0.09)

13. Plasma Osmolarity and Plasma Electrolytes

a) The composition of priming solutions.

The analyses of the priming solutions were done according to the method described in II, 6b, p.63. The results which are shown in the following table, were obtained from duplicate analyses. The analysis of the Ringer-lactate solution was in accordance with that reported by the manufacturer.

TABLE III-8

COMPOSITION OF THE PRIMING SOLUTIONS. (FOR COMPARISON THE AVERAGES OF 45 PIGLETS BEFORE PERFUSION AND THE MANUFACTURER'S ANALYSIS ARE ADDED)

	Ringer-lactate			2% Rheomacrodex in Ringer-lactate
	Animals	Manufacturer	Analysis	Analysis
pH	7.4	6.0-7.5	7.4	7.4
Osmolarity (mOsm/l)	297	-	261	284
Sodium (mEq/l)	152	130	132	141
Chloride "	100	109	110	131
Potassium "	4	4	4	3
Calcium "	5	3	3	3
Lactate "	-	28	28	(22)
Sodium bicarbonate "	-	-	(5.6)*	(7.3)*
*( ) Calculated				

b) Plasma osmolar and electrolyte concentrations.

All plasma osmolarity and plasma electrolyte values are given in Appendix A-Tables 16-20, pp. 188-192. The averages of the groups are presented in Figure 20, p. 112.

There was a marked fall in the plasma osmolarities of both experimental groups immediately after hemodilution perfusion. The subsequent significant rise which was observed in these two groups during the next 24 hours, was also observed in the sham group.

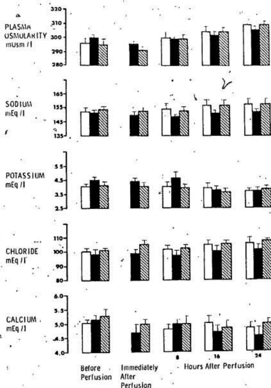

FIGURE 20

PLASMA OSMOLARITY AND PLASMA ELECTROLYTES
BEFORE AND FOLLOWING HEMODILUTION PERFUSION

Sham-operated group — Ringer-lactate group — Rheomacrodex group

The bars shown for each value represent the standard error of the means within the group of 15 animals. The reader is referred to Appendix A - Tables 16-20 for statistical comparisons.

Only in the RL group, was plasma sodium significantly decreased 8 hours after perfusion. At 16 and 24 hours it was back to its initial value. In the LMWD and sham groups plasma sodium showed very little change immediately after perfusion or at 8 hours. At 16 and 24 hours it was significantly higher than the initial value and also significantly higher than that of the RL group.

There was a significant drop in the potassium levels of the two experimental groups 16 hours after perfusion. At 24 hours potassium remained significantly lower than the initial value in the RL group whereas it returned to its normal level in the LMWD group. No significant changes were established in the sham group during the 24 hour period.

The variations in the plasma chloride concentration of the RL group throughout the experiment were not significant. In contrast, a significant increase of chloride was seen in the LMWD group from the termination of the perfusion period until the end of the 24 hours. In the sham group, plasma chloride rose steadily during the entire experiment but only at 16 and 24 hours was this rise significant.

There was a significant fall in the plasma calcium concentration of the RL group after perfusion. At 8 hours calcium returned to the initial levels but was again significantly lower at 16 and 24 hours. Not significant changes in the calcium levels of both the LMWD and sham groups were noted throughout the experiment.

14. Hemodynamics

In Appendix A-Tables 21 and 22, pp. 193 - 194, the values for mean arterial blood pressure and heart rate are presented. The average values per group are shown in Figures 21 and 22, p. 115 and p. 116.

a) Mean arterial blood pressure

No significant changes in the mean arterial blood pressure were recorded throughout the experiment in all three groups, except the reading at 24 hours in the RL group: at 24 hours the mean arterial blood pressure in this group had sufficiently increased to be significantly higher than the initial value.

b) Heart rate.

In the LMWD group, there was a highly significant increase in heart rate immediately after the termination of perfusion. This increase was maintained until 8 hours, after which heart rate returned to normal levels. There was a significant rise in heart rate in the RL group 8 hours after perfusion, followed by a return to normal levels at 16 hours. No significant changes in heart rate were recorded throughout the entire experiment in the sham-operated group.

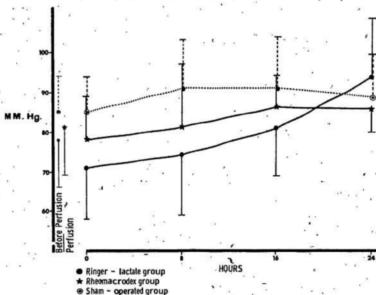

FIGURE 21
MEAN ARTERIAL BLOOD PRESSURE BEFORE AND
FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard deviations within the group of 15 animals. The reader is referred to Appendix A-Table 21 for statistical comparisons.

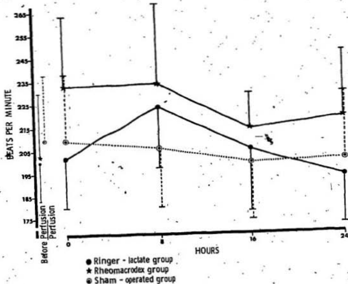

FIGURE 22

HEART RATE BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard deviations within the group of 15 animals. The reader is referred to Appendix A-Table 22 for statistical comparisons.

15. Rectal Temperature

Appendix A-Table 23, p. 195 presents the values for rectal temperature; Figure 23, p. 118 illustrates the variations in rectal temperature for the three groups.

The patterns of rectal temperature alterations following hemodilution perfusion were similar in the two experimental series. There was a highly significant fall in rectal temperature immediately after the termination of perfusion; this fall was in the magnitude of 0.6°C. and 0.5°C. in the RL and LMWD groups respectively. At 8 hours post-perfusion, rectal temperatures were back to initial levels and remained there until the end of the experiment. In the sham-operated group no significant changes in rectal temperature were observed throughout the experiment.

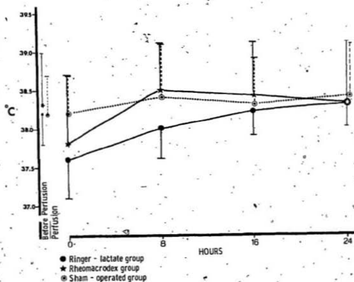

FIGURE 23

RECTAL TEMPERATURE BEFORE AND FOLLOWING HEMODILUTION PERFUSION

The bars shown for each value represent the standard deviations within the group of 15 animals. The reader is referred to Appendix A-Table 23 for statistical comparisons.

IV DISCUSSION

The object of this study was to investigate plasma volume, plasma protein and plasma electrolyte changes following large volume hemodilution perfusion with a crystalloid and a colloid solution in newborn piglets.

The main feature of the technique of hemodilution perfusion is that the extracorporeal circuit is primed with a non-hemic solution and that this solution, mixing with the blood of the recipient, dilutes the constituents of the blood. The immediate changes in the intravascular space are therefore reflected not only in the hematocrit but also in the plasma electrolytes and proteins, depending of course, upon the composition of the diluent fluid.

The T-1824 dye dilution technique has been reported to be accurate in determining the circulating plasma volume in man and many other mammals (Gregersen and Rawson, 1959), including the swine (Talbot and Swenson, 1963). Precise measurements of plasma volume using T-1824 require that, in addition to dye concentration, plasma protein concentration and hematocrit values be measured on all blood samples, including the blank in order to detect volume alterations during the sampling period and to make appropriate corrections for these alterations if necessary (Gregersen and Rawson, 1959).

In the present investigation no significant change in the plasma proteins or hematocrit values could be found between the blank and the unknown samples (Table II-1, p. 54), consequently, no correction was made on the dye readings. This investigation has confirmed that the T-1824 dye dilution technique is an accurate method. Confirmation of the plasma volume losses immediately after hemodilution perfusion could be obtained from the balance equation for plasma proteins before and after perfusion (III-6a, p. 80). The best agreement was found with large size proteins such as fibrinogen (Table III-4, p. 84). Raminez and co-workers (1963) reported blood volume estimates for 169 pigs from birth to 5 weeks of age based on T-1824 plasma volume and hematocrit measurements. Talbot and Swenson (1970) determined total blood volume by the simultaneous measurements of ^{51}Cr-erythrocyte volume, and T-1824 plasma volume in 176 pigs from birth to six weeks of age. In the present study, the plasma volume and blood volume of one week-old pigs immediately before hemodilution perfusion were in agreement with those reported by the above two groups of investigators.

Although the hematocrits in both experimental groups immediately after perfusion, were within the limits of the target values and thus indicated that their reduction was entirely due to intentional hemodilution, it became apparent from the plasma volume measurements that hypovolemia

was present in both groups at the termination of perfusion. In the RL group the loss was 14.2 ml./Kg., in the LMWD group 7.8 ml./Kg. (III, 6a, p. 80). The falls in the plasma volumes were in fact even larger than those observed when the hematocrits of the perfusates (diluted blood remaining in the extracorporeal circuit after perfusion) were taken into consideration; Figures 24 and 25, p. 122 and 123, illustrate this aspect. Before perfusion there are two volumes to consider: the plasma volume and the priming volume. In the RL group these volumes were 64.0 ml./Kg. and 33.7 ml./Kg. respectively, making a total volume of 97.7 ml./Kg. After perfusion, there are three volumes to take into account: the plasma volume in the animal, the red cell volume in the perfusate, and the "plasma volume" of the perfusate. The perfusate volume was kept the same as the priming volume (II-4, p. 39) and therefore "plasma volume" and cell volume of the perfusate could be calculated using the perfusate hematocrit. In the RL group these three volumes were 49.8 ml./Kg. plasma volume, 26.1 ml./Kg. "plasma volume" of the perfusate and 7.6 ml./Kg. red cell volume. The total fluid volume after perfusion was therefore 75.9 ml./Kg. resulting in a total plasma volume deficit of 21.8 ml./Kg. In the Rheomacrodex group, this deficit was 15.6 ml./Kg. (Figure 25, p. 123). Similarly it can be calculated that in the RL group there is a loss of RCV of 5.4 ml./Kg.; this deficit was 4.0 ml./Kg. in the LMWD group.

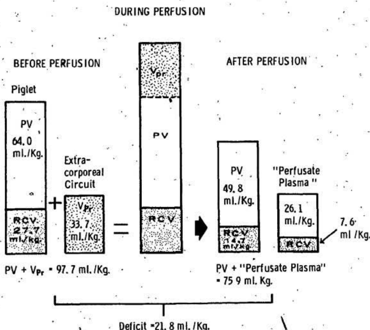

FIGURE 24

THE LOSS OF PLASMA VOLUME DURING HEMODILUTION PERFUSION IN THE RINGER-LACTATE GROUP

This figure illustrates how the plasma volume (PV) red cell volume (RCV) and priming volume (V_{pr}) change following perfusion. The center bar represents the process of mixing between the animal's blood and the Ringer-lactate solution. After perfusion some of the RCV is left behind in the perfusate. This RCV can be calculated from the perfusate volume and its hematocrit.

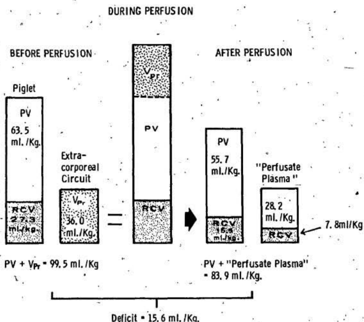

FIGURE 25

THE LOSS OF PLASMA VOLUME DURING HEMODILUTION PERFUSION IN THE RHEOMACRODEX GROUP

This figure illustrates how the plasma volume (PV) red cell volume (RCV) and priming volume (V_{pr}) change following perfusion. The center bar represents the process of mixing between the animal's blood and the Rheomacrodex solution. After perfusion some of the RCV is left behind in the perfusate. This RCV can be calculated from the perfusate volume and its hematocrit.

Since there was no sign of external hemorrhage throughout the surgical procedures and the perfusion, and since the volume in the reservoir was maintained at a constant level, moreover, since no significant loss in the body weight could be found immediately after extracorporeal circulation, the conclusion was reached that the loss of plasma was an internal one. These losses are by no means small; they constitute about one third of the total plasma volume in the RL group, and about one fourth in the LMWD group. If it were only a plasma loss, one would expect much higher hematocrit values than the calculated target immediately after hemodilution perfusion, however, this was not the case. In both groups the values were close to the target although the small differences from the target were statistically significant (III, 5, p. 77). Therefore it is concluded that whole blood must have disappeared from the circulation. Bernstein *et al.*, (1965) have shown that destruction of red blood cells in pumps currently in use for extracorporeal circulation may take place during and even after perfusion. However, damage to the erythrocytes which led to their disruption, usually occurred after prolonged periods of perfusion. In the present study, microscopic examinations of blood samples collected after the short period of perfusion did not reveal any sign of disruption of red blood cells (III, 5, p. 77).

Litwak and associates (1963) observed a decrease in both plasma and red cell volumes in their patients immediately after extracorporeal circulation with or without hemodilution. This decrease continued into the first post-operative day despite careful replacement of blood loss during perfusion and whether hemodilution was used or not. These authors coined the term "sequestration" and suggested that the post-perfusion blood volume deficit was due to the "sequestration of blood in sites that render the sequestered volume unavailable to the circulation at that time". They attributed the subsequent increase in blood volume during the first post-operative week to the return of this sequestered blood to the circulation. Davidson and Farthmann (1965) demonstrated that considerable pooling may occur in the splanchnic bed after hemodilution perfusion in dogs with Dextran 70, this was directly related to an elevation in the central venous pressure, with a parallel increase in the portal venous pressure. However, these authors administered blood to the animals in order to correct their hypotension, thus adding another factor that may affect the central venous pressure.

Long and associates (1961, 1962) found that anemia after experimental and clinical extracorporeal circulation was not only due to mechanical injury of the red blood cells by pumps and oxygenating apparatus, but also to the aggregation of cells in the micro-circulation. After 30 to

60 minutes of total body perfusion without intentional hemodilution, an examination of conjunctival and mesenteric capillaries revealed an early increase in the number of red cell aggregates, with temporary occlusion of venules and arterioles. They reported that the addition of 2 g. of Rheomacrodex per Kg. body weight to the priming fluid (blood in their studies) resulted in a significant decrease in the destruction of red blood cells and prevented or minimized intravascular aggregation. Björk *et al.*, (1964a, b) and Raison (1964), in dog experiments, confirmed these observations.

The blood loss reported in the present study could be accounted for by the aggregation of erythrocytes as suggested by Long and associates. The decrease in plasma volume which will be discussed later (p. 130) causes aggregation of erythrocytes in the microcirculation. This occurs in both groups but it is more pronounced in the RL group. In this group, the plasma volume is rapidly replenished and leads to further dilution of the circulating erythrocytes as evidenced by a further drop in the hematocrit (Figure 4, p. 79), but disaggregation of the other erythrocytes trapped in the capillaries occurs more slowly. In the LMWD group, the aggregation is less pronounced, and the further dilution of the erythrocytes by the replenishment of the plasma volume is counteracted by the reappearance of the trapped erythrocytes; this results in a steady linear

increase of the hematocrit. Such an explanation would assume that erythropoesis was similar in both groups and was not affected by the composition of the perfusion fluids. The decrease in plasma volume observed immediately after hemodilution perfusion in the two experimental groups can be explained by a number of factors: The first one is the slight hypotonicity of the priming solutions (261 mOsm/l. for the Rheomacrodex solution and 284 mOsm/l. for the Ringer-lactate solution), as compared to plasma osmolarities of 295.5 and 299.1 mOsm/l. respectively. This causes a shift of fluid to the extravascular compartment. The second one is the acute decrease in the plasma protein concentration. This causes a decrease in oncotic pressure and consequently a similar outward movement of water. This is likely to have occurred in the RL group whose perfusion fluid did not contain an oncotic agent. Other factors which are not associated with the composition of the priming fluid must also play a role. Fox (1969), who has spent great care in preparing isotonic solutions with the proper electrolyte concentration and pH for replacement therapy in hemorrhage, has nevertheless found small plasma volume deficits immediately after replacement of the blood loss. Surgical stress must certainly play a role as can be seen in the sham-operated group of the present study (Figure 5, p. 81).

A quantitative approximation of body fluid osmolarity that could be expected after mixing with the hypotonic

priming fluids was made, using the equation given below and by making the following assumptions:

(a) Total body water in 7 days old piglets is about 75% of the total body weight (Friis-Hansen, 1961; Houpt, 1970).

(b) Before perfusion, the osmolarity of various body fluids is uniform (Robinson, 1960).

(c) The activity of the kidneys is negligible during the 14 to 19 minutes hemodilution perfusion period.

The formula used for these estimations of total body water osmolarity was:

$$mOsm/1_{TBW} = \frac{(TBW \times mOsm/1._{p}) + (V_{pr} \times mOsm/1._{pr})}{TBW + V_{pr}}$$

where,

$mOsm/1._{p}$ and $mOsm/1._{pr}$ represent the osmolarities of plasma and priming solution respectively, and TBW and V_{pr} are total body water and priming volume respectively.

The expected and observed osmolarities after perfusion are shown in Figure 26 p. 129 which illustrates the above calculation graphically.

It can be seen that in the case of the RL group the expected and observed osmolarity values were close to each other, but that in the LMWD group, there was a consistently lower observed value. This can be verified by comparing the expected values with the observed values given in Appendix A-Table 16, p. 188. It is likely that in the RL group

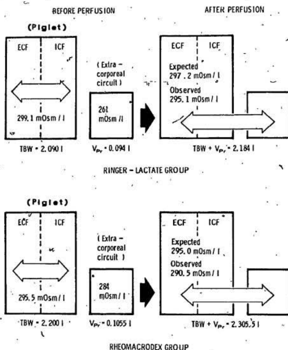

FIGURE 26
THE EXPECTED AND OBSERVED PLASMA OSMOLARITIES AFTER HEMODILUTION PERFUSION IN THE RINGER-LACTATE AND RHEOMACRODEX GROUPS

ECF = Extracellular fluid TBW = Total body water
ICF = Intracellular fluid V_{pr} = Priming volume

This schematic figure illustrates the shift in body water to various compartments in the animal and perfusion system. The size of the compartments is not indicated by the height or width of the figure.

equilibrium is reached by the end of the perfusion period as there is evidence that such fluid shift can occur rapidly. Flexner *et al.*, (1942), who studied the rate of distribution of water throughout the body in guinea pigs, found that 73% of the water in blood is exchangeable with extravascular water every minute. Even distribution of water has been reported in less than 30 minutes for rabbits (Hevesy and Jacobsen, 1940), and 2 to 3 hours for man (Schloerb *et al.*, 1950). Moore *et al.*, (1966) estimated that each minute the capillaries can exchange amounts of water and salt in excess of those found in the entire plasma volume. This net outflow of water accounts for the larger fall in plasma volume and the smaller fall of the hematocrit value as compared with that seen in the LMWD group.

After perfusion, there was a large movement of water and electrolytes back to the circulation accounting for the rapid replenishment of the plasma volume and a further decrease in the hematocrit. (Figures 4 and 5 pp. 79 and 81). Again, evidence for the movement of water and electrolytes is indicated by the osmolarity which shows the same pattern as that of the sham-operated group (Figure 20, p. 112, Appendix A-Table 16, p. 112) i.e., a steady rise over the 24 hours probably due to dehydration. The dilution which caused the decrease in hematocrit did not, however, cause a decrease in the plasma protein concentration (Figure 13, p. 97).

In the LMWD group, the difference in osmolarities between animal and priming fluid was less pronounced than in the RL group. This, in itself, may account for a smaller decrease in plasma volume. Moreover, the presence of Rheomacrodex in the priming fluid had a moderating effect on fluid movement. The reduced loss of intravascular water during perfusion, can be attributed to its water binding capacity (Grönwall, 1969), consequently the drop in plasma volume in the LMWD group was less, and the hematocrit and osmolarity values were lower than in the RL group.

The binding of water did not appear to be associated with the binding of electrolytes; dextran binds water selectively. This accounted for the discrepancy between the expected and observed osmolarities immediately after perfusion. Dextran again had a moderating effect in the movement of fluid into the circulation after perfusion, thus accounting for the slow recovery rate of plasma volume and the steady increase of the hematocrit over the subsequent 24 hours. Dextran is known to diffuse into the extravascular space (Arturson, 1965), thus reducing the influx of fluid into the circulation by its oncotic action.

Since dextran binds water selectively, it does not have a stabilizing effect on osmolarity. The large fall in plasma osmolarity observed immediately after perfusion was followed by a sharp rise during the subsequent 8 hours

(Figure 20, p. 112, Appendix A- Table 16, p. 188). Thereafter the osmolarity rose in a manner similar to that noted in the RL and the sham-operated group.

Studies on the disappearance of dextran from the circulation after infusion of 500 ml. of 10% Rheomacrodex in normovolemic humans were reported by Arturson (1965). He demonstrated a very rapid initial fall in total dextran concentration in the serum during the first hour after infusion followed by a slower decrease. On a semilogarithmic plot against time, this decline in concentration turned out to be a double exponential function. Ringer (1967), who investigated the elimination of dextran from the circulation in dogs after 30% blood loss and immediate substitution with Rheomacrodex in glucose, noted that less than 17% of the original plasma levels remained in the blood stream after 5 hours and less than 5% after 24 hours.

Arturson (1965) found that the time course of the elimination of dextran was affected by several factors: (1) Smaller molecules which pass the capillary membrane return to the blood stream by way of the lymphatic system. (2) Simultaneously, molecules which pass through the glomerular membrane are excreted in the urine. (3) Some of the dextran molecules are taken up by the cells of the reticuloendothelial system and are slowly metabolized. The overall results of these processes are, a falling dextran concentration in the serum and a steady increase

of the average molecular weight of circulating dextran. Dextran molecules with a molecular weight of 14,000 - 18,000 have an intravascular half-life of about 15 minutes and are entirely cleared from the plasma within two hours.

In the present investigation, the fall in plasma dextran concentration during the 24 hour post-perfusion period followed a pattern similar to that observed by these investigators. After 24 hours, dextran could no longer be detected in the plasma.

The marked decrease of the total amount of circulating plasma proteins is explicable because proteins are left behind in the extracorporeal circuit at the termination of perfusion. There is, however, an additional loss of protein (Table III-6, p. 108). This is not due to adsorption at the surface of the vinyl tubing (III-12, p. 107), but due to a movement of proteins in the animal from its vascular bed into extravascular space. On the other hand, by calculating the plasma volume immediately after perfusion from plasma constituents (Table III-4, p. 84), it was found that the plasma proteins predicted the plasma volume reasonably well and that the larger the plasma protein molecule, the better the prediction. These two facts imply that protein disappearance must be larger with smaller protein molecules. This is reflected by a small decrease in the percentage of albumin and a slight increase in the percentage of fibrinogen (Appendix A,

Tables 6-10). Since the amount of protein lost into the animal is small (0.76 and 0.84 g., in the RL and LMWD group respectively), the changes in relative percentages could not reach a level of significance.

Lee and associates (1961), who recirculated dog and human plasmas in various oxygenators such as disc, bubble, or the screen oxygenator from 3 to 20 hours reported a decrease in the percentage of the albumin fraction and a corresponding rise in the alpha, beta and gamma globulins. Wright *et al.*, (1962) studied the effect of prolonged recirculation of dog plasma on the plasma proteins. Two types of oxygenators were used in their experiments, the screen and the membrane oxygenators. These authors found small changes in the electrophoretic pattern of plasma proteins after prolonged perfusion. Siltanen *et al.*, (1968) reported a small but distinct denaturation of albumin, a pronounced alteration of fibrinogen and no change in gamma globulin during 90 minutes of extracorporeal circulation.

The rate of reappearance of the circulating plasma proteins in the LMWD group had a fast and a slow component. The slow component had a time constant in the same order of magnitude as that observed in the RL group. The fast component had a time constant which had a striking similarity with that of the fast component of the disappearance rate of dextran. Thus, in the LMWD group, proteins returned

to the circulation at the same rate as Rheomacrodex disappeared from the circulation. The possible mechanism for this relationship is at present not clear.

It appears that two mechanisms are involved in the replenishment of the plasma proteins after blood loss: (1) mobilization of preformed proteins and (2) synthesis of proteins. It is widely agreed that most of the restored proteins entering the vascular space during the first few hours following blood loss and immediate substitution with non-hemic fluids in dogs (Rodionov *et al.*, 1957; Rieger, 1967a, b; and Liljedahl and Rieger, 1967) and in man (Moore *et al.*, 1966), or following total volume plasmapheresis in baboons (Osteen and Klebanoff, 1971) are preformed proteins. The rapidity of protein refill in response to large volume hemodilution in adult man (Gollub *et al.*, 1969) also implies the need for preformed proteins. These proteins which are present in extravascular spaces, in tissues, such as skin, muscles and viscera (Rothschild *et al.*, 1972a, b) and in the lymphatic system (Wasserman and Mayerson, 1951; Mayerson *et al.*, 1960; Woolley and Courtice, 1962) are mobilized to the intravascular pool when this pool happens to be reduced. Replacement proteins may enter the circulation by transcapillary movement. Direct transcapillary migration of proteins was suggested by Moore *et al.*, (1966), Skillman *et al.*, (1967) and Osteen and Klebanoff (1971). On the other hand, Cope and Litwin (1962) and Liljedahl and Rieger

(1967) postulated that in the replenishment process, protein mainly returned by the thoracic duct.

From the observations made in the present study, it cannot be asserted which of the two mechanisms, mobilization of preformed proteins or synthesis, is dominant in the restoration of plasma proteins. It can be concluded however, that the restoration took place without a preference for a particular protein fraction as evidenced by the constancy of the relative percentages (Appendix A-Tables 6 to 10, pp. 178 - 182). In the RL group, the restoration of the total amounts of each protein fraction occurred at the same time. All time constants in this group were in the same order of magnitude.

The presence of dextran at the low concentration used in this study enhanced the restoration of all protein fractions: the restoration of albumin followed a double exponential function; the restoration of all other fractions showed time constants which were considerably shorter than those of the RL group (Figures 14 to 19, pp. 101 to 106).

Rothschild and associates (1961, 1962, 1965) observed a decrease of the serum albumin concentration in experimentally produced hypergammaglobulinemia and also following prolonged administration of high molecular weight dextran in rabbits. They suggested that albumin synthesis may be controlled by an osmotic regulatory system. In later studies, Rothschild and his group (1969) investigated the effect of

varying the colloid content of the perfusate on the albumin production of the isolated perfused rabbit liver. When an albumin concentration of 0.5 g./100 ml. was present in the perfusate, albumin production increased; when the perfusate was made hyperosmotic with 7.1 g. albumin/100 ml. or 2.7 g. albumin plus 1% sucrose, albumin production was drastically depressed. Thus, their in vitro studies confirmed the existence of a colloid osmotic regulatory mechanism in the control of albumin production and indicated that this control mechanism was located within the hepatic tissue (Rothschild *et al.*, 1972a).

By comparing the restoration patterns of plasma volume and total amount of plasma proteins in the RL group (Figures 5 and 14, p. 81 and 101) it is apparent that the former is faster than the latter. Plasma proteins seem to play only a secondary role in the replenishment of plasma volume. Some alternative system capable of sensing alterations of the circulating blood volume might be involved.

In the LMWD group, the restoration of plasma proteins is much faster than that of the plasma volume. This complete reversal suggests that an oncotic regulatory system plays an important role in the control of plasma volume (Hyman and Steinfeld, 1967).

Farrell and his associates (1956, 1958, 1959) found that the muscle of the right atrium contains stretch

receptors responsive to an increase or decrease of blood volume. These receptors appear to influence the release of aldosterone. Shu'ayb *et al.*, (1965) found that distention of the left atrium induces a decrease in the blood anti-diuretic hormone level and a parallel increase in urine flow. They suggested that the left atrium may be of particular importance in regulating water balance whereas the right atrium may be more concerned with changes in electrolyte concentration.

Direct evidence for the participation of the adrenal cortex in extracorporeal circulation is still lacking, but the involvement of the adrenal cortex in the response to blood loss (Skillman *et al.*, 1967) and the relationships between the renin angiotensin system and the adrenal cortex have been documented (Davis, 1961; Mulrow and Ganong, 1962; Bartter *et al.*, 1964). Vander (1967) reviewed in detail evidence which suggests that at least four factors control the rate of release of renin: change in intraluminal pressure of the afferent renal arterioles, change in the composition of the tubular fluid at the macula densa, sympathetic nerve impulses, catecholamines and other known and unknown hormonal agents. Since there was a decrease in the effective circulating blood volume immediately after perfusion, this is most likely to have resulted in a peripheral vasoconstriction and a decrease in blood flow in the capillaries. A decrease

in renal blood flow would stimulate the release of renin and thereby promote the secretion of aldosterone.

The present study casts some light on the processes involved in the restoration of plasma volume, plasma proteins and blood cells following hemodilution perfusion. Further studies are required to investigate the underlying mechanisms of these processes. The movements of body fluids, proteins and electrolytes should be studied using isotope markers. Direct observation of capillary circulation would be useful for gaining further evidence in support of an aggregation hypothesis being involved. Furthermore, the problem of whether the restoration of the proteins is due to the mobilization of preformed proteins, or to protein synthesis needs further explanation. The use of pharmacological agents inhibiting protein synthesis in conjunction with such experiment may yield decisive results.

The results in the present study refer to minimal surgical trauma in conscious animals; but when hemodilution perfusion is applied to the newborn infant undergoing open-heart surgery, additional effects of the open chest, respiratory assistance, anaesthesia and the use of suitable oxygenators have to be considered.

Pilot studies have been carried out to establish an experimental routine which includes these technical aspects.

CHAPTER V

CONCLUSIONS

The newborn pig is able to cope with a large volume hemodilution resulting in a hematocrit of 22%. Moreover, the newborn pig shows a well developed regulatory mechanism to counteract plasma volume and plasma protein losses. The replenishment of plasma volume and restoration of each circulating plasma protein fraction, except gamma globulin, are accomplished in about 24 hours. This appears to be a faster response than in human adults and mature animals.

The restoration of plasma volume occurs faster than the restoration of plasma proteins, but when dextran is present the reverse is the case. Therefore, oncotic receptors must play a role in the control of plasma volume. All protein fractions are restored at about the same rate. There is no preferential restoration of any particular protein fraction.

Osmotic equilibrium between intravascular and extravascular compartment is reached by the time the perfusion is completed. During perfusion, plasma volume, erythrocytes and proteins are lost from the circulation. Since the restoration of plasma volume, plasma proteins, and red blood cells as indicated by the hematocrit, do not show the same time constant, sequestration of blood is

unlikely. It is tentatively suggested that fluid moves into the extravascular space, causing aggregation of erythrocytes in the capillaries.

The reappearance rate of proteins is almost identical to the disappearance rate of dextran. Dextran binds water selectively, therefore, it has a stabilizing effect on plasma volume shifts but not on plasma osmolarity. Two percent Rheomacrodex in Ringer-lactate enhances protein restoration considerably.

BIBLIOGRAPHY

Allen, J. G., Sykes, C., Enerson, D. M., Moulder, F. V., Elghammer, R. M., Grossman, B. J., McKeen, C. L., and Galluzi, N. J. (1950) Homologous Serum Jaundice and its Relation to Methods of Plasma Storage. JAMA. 144 : 1069-1074.

Amberson, W. R. (1937) Blood Substitutes. Biol. Rev. 12 : 48-86.

Andersch, M., and Gibson, R. B. (1934) Studies on the Effects of Intravenous Injections of Colloids. I Deposition of Acacia in the Liver and Other Organs and its Excretion in Urine and Bile. J. Pharmacol. Exp. Ther. 52 : 390-407.

Aronsson, T., Arturson, G., and Wallenius, G. (1966) Determinations of Serum Protein in the Presence of Dextran. Scand. J. Clin. Lab. Invest. 18 : 458-460.

Arturson, G., and Wallenius, G. (1964) The Intravascular Persistence of Dextran of Different Molecular Sizes in Normal Humans. Scand. J. Clin. Lab. Invest. 1 : 76-80.

Arturson, G. (1965) The Change of Molecular Weight Distribution of Infused Dextran. Scand. J. Clin. Lab. Invest., Suppl. 86 : 69-79.

Askonas, B. A., Humphrey, J. H., and Porter, R. R. (1956) On the Origin of the Multiple Forms of Rabbit γ - Globulin. Bioch. J. 63 : 412-419.

Asofsky, R., and Thornbecke, G. L. (1961) II Sites of Formation of Immune Globulins and of a Component of C'_3. Production of Immuno-electrophoretically Identified Serum Proteins by Human and Monkey Tissues in Vitro. J. Exp. Med. 114 : 471-483.

Baffes, T. G., Riker, W. L., and DeBoer, A. (1964) Open-Heart Surgery for Infants and Small Children. Arch. Surg. 88 : 675-680.

Baffes, T. G., Fridman, J. L., Bicoff, J. D., and Whitehill, J. L. (1970) Extracorporeal Circulation for Support of Palliative Cardiac Surgery in Infants. Ann. Thorac. Surg. 10 : 354-363.

Ballinger, W. F., II, Solanke, T. F., and Thompson, W. L. (1966) The Effect of Hydroxyethyl Starch Upon Survival of Dogs Subjected to Hemorrhagic Shock. Surg. Gynec. Obstet. 122 : 33-36.

Ballinger, W. F., II, Murray, G. F., and Morse, E. E. (1966) Preliminary Report on the Use of Hydroxyethyl Starch Solution in Man. J. Surg. Res. 6 : 180-183.

Bartter, F. C., Barbour, B. H., Carr, A. A., Delea, C. S., and Slater, J. D. H. (1964) Control of Adreno-cortical Steroid Secretion Dependent Upon and Independent of Pituitary and Kidneys. Can. Med. Assoc. J. 90 : 240-242.

Baue, A. E., Tragus, E. T., and Parkins, W. M. (1967) A Comparison of Isotonic and Hypotonic Solutions and Blood on Blood Flow and Oxygen Consumption in the Initial Treatment of Hemorrhagic Shock. J. Trauma. 7 : 743-756.

Bayliss, W. M. (1916) Methods of Raising a Low Arterial Pressure. Proc. Roy. B. 89 : 380-393.

Beall, A. C., Jr., and Cooley, D. A. (1965) Physiologic Studies During Cardiopulmonary Bypass Eliminating Heparinized Blood. Dis. Chest. 47 : 7-16.

Beeson, B. P. (1943) Jaundice Occuring One to Four Months After Transfusion of Blood or Plasma; Report of Seven Cases. JAMA. 121 : 1332-1334.

Berger, R. L., Boyd, T. F., and Marcus, P. S. (1964) A Pattern of Blood Volume Responses to Open-Heart Surgery. New. Eng. J. Med. 271 : 59-63.

Bernstein, E. F., Castaneda, A. R., Blackshear, P. L., and Varco, R. L., (1965) Prolonged Mechanical Circulatory Support : Analysis of Certain Physical and Physiologic Considerations. Surgery, 57 : 103-122.

Best, C. H., and Solandt, D. Y. (1940) Use of Plasma or Serum as a Substitute for Whole Blood. Brit. Med. J. 2 : 116-117.

Björk, V. O., Intonti, F., and Nordlund, S. (1964a) Correlation Between Sludge in the Bulbar Conjunctiva and the Mesentery. Ann. Surg. 159 : 428-431.

Björk, V. O., Intonti, F., and Nordlund, S. (1964b) Effect of Haemodilution Upon Erythrocyte Aggregation. Studies on Dogs Infused With Different Dextran Fractions. Acta. Chir. Scand. 128 : 466-470.

Blundell, J. (1818) Experiments on the Transfusion of Blood by the Syringe. Med. Chir. Trans. London. 9 : 56-59.

Blundell, J. (1828) Observations on Transfusion of Blood. Lancet, 2 : 321-325.

Bowditch, H. P. (1871) Arbeit. a.d. Physiol. Anstalz zu Leipzig. 6 : 139. Cited by Amberson, W. R. (1937) Blood Substitutes. Biol. Rev. 12 : 48-86.

Bowman, H. (1953) Clinical Evaluation of Dextran as a Plasma Volume Expander. JAMA 153 : 24-26.

Brennan, H. T. (1940) Plasma Transfusions in the Treatment of Haemorrhage. Brit. Med. J. I : 1047-1048.

Briere, R. O., and Mull, J. D. (1964) Electrophoresis of Serum Protein with Cellulose Acetate. A Method for Quantitation. Amer. J. Clin. Path. 42 : 547-551.

Bull, W. T. (1884) On the Intraveneus Injection of Saline Solutions as a Substitute for Transfusion of Blood. Med. Rec. N.Y. 25 : 6-8.

Campbell, T. J., Frohman, B., and Reeve, E. B. (1958) A Simple, Rapid and Accurate Method of Extracting T-1824 From Plasma, Adapted to the Routine Measurement of Blood Volume. J. Lab. Clin. Med. 52 : 768-777.

Clarke, G. M. (1969) Statistics and Experimental Design. American Elsevier Publishing Company Inc., New York, pp. 2-19.

Clement, A. J. (1971) The Physiological Background of an Apparatus Used for Extracorporeal Circulation. Brit. J. Anaesth. 43 : 233-243.

Cohn, E. J. (1941) The Properties and Functions of the Plasma Proteins with a Consideration of the Methods for their Separation and Purification. Chem. Rev. 28 : 395-417.

Cohn, L. H., Angell, W. W., and Shumway, N. E. (1971) Body Fluid Shifts After Cardiopulmonary Bypass. I. Effects of Congestive Heart Failure and Hemodilution. J. Thorac. Cardiovasc. Surg. 62 : 423-430.

Committee on Plasma and Plasma Substitutes (1968) Statement on Normal (Whole Pooled) Human Plasma. Transfusion. 8 : 57-59.

Cook, W. A., and Webb, W. R. (1965) Pump Oxygenators and Hypothermia. Ann. Thorac. Surg. I : 466-504.

Cope, O., and Litwin, S. B. (1962) Contribution of the Lymphatic System to the Replenishment of the Plasma Volume Following Hemorrhage. Ann. Surg. 156 : 655-667.

Craig, W., Gray, H. K., and Lundry, J. S. (1951) Present Status of Plasma Volume Expanders in the Treatment of Shock : Clinical Results in Surgery. Arch. Surg. 63 : 742-749.

Cruz, A. B., and Callaghan, J. C. (1966) Hemodilution in Extracorporeal Circulation : Large or Small Non-Blood Prime? J. Thorac. Cardiovasc. Surg. 52 : 690-697.

Darrow, D. C., and Yannet, H. (1935) The Changes in the Distribution of Body Water Accompanying Increase and Decrease in Extracellular Electrolyte. J. Clin. Invest. 14 : 266-275.

Dastre, A. (1893) Contribution à l'Etude de L'Evolution du Fibrinogène dans le Sang. Arch. Phys. Norm. Path. 5 : 327-331.

Davidson, A. I., and Farthmann, E. H. (1965) Splanchnic Pooling During Left Heart Bypass. Surg. Gynec. Obstet. 121 : 1277-1281.

Davis, J. O. (1961) Mechanisms Regulating the Secretion and Metabolism of Aldosterone in Experimental Secondary Hyperaldosteronism. Recent Prog. Horm. Res. 17 : 293-352.

DeBoer, A. (1969) Body Fluid Compartment Changes Following Large Volume Hemodilution. Comparison With and Without Heart Failure. Arch. Surg. 98 : 602-606.

Denis, J. B. (1667) Phil. Trans. Roy. Soc., London, No. 27, July 22. Cited by Keynes, G. (1943) in : The History of Blood Transfusion, 1628-1914. Brit. J. Surg. 31 : 38-50.

De Wall, R. A., Lillehei, R. C., and Sellers, R. D. (1962) Hemodilution Perfusions for Open Heart Surgery. New. Engl. J. Med. 266 : 1078-1084.

Dieter, R. A., Jr., Neville, W. E., and Pifarré, R. (1970) Serum Electrolyte Changes After Cardiopulmonary Bypass With Ringer's Lactate Solution Used for Hemodilution. J. Thorac. Cardiovasc. Surg. 59 : 168-177.

Dillon, J., Lynch, L. J., Myers, R., Butcher, H. R., and Moyer, C. A. (1966) A Bioassay of Treatment of Hemorrhagic Shock. I The Role of Blood, Ringer's Solution With Lactate, and Macromolecules (Dextran and Hydroxyethyl Starch) in the Treatment of Hemorrhagic Shock in the Anesthetized Dog. Arch. Surg. 93 : 537-555.

Doan, C. A. (1927) The Transfusion Problem. Physiol. Rev. 7 : 1-84.

Dorson, W. J., Jr., Baker, E., Hull, H., Molthan, M., Meyer, B., Fargotstein, R., and Cohen, M. L. (1969) A Long-Term Partial Bypass Oxygenation System. Ann. Thorac. Surg. 8 : 297-312.

Dorson, W., Jr., Meyer, B., Baker, E., Cohen, M., Elgas, R., Molthan, M., and Trump, D. (1970) Response of Distressed Infants to Partial Bypass Lung Assist. Trans. Amer. Soc. Artif. Int. Organs, 16 : 345-351.

Doyon, M. M., Morel, A., and Kareff, N. (1905) Action du Phosphore sur la Coagulabilité du Sang. Originé du Fibrinogène. C. R. Soc. Biol. (Paris) 58 : 493-495.

Drury, D. R., and McMaster, P. D. (1929) The Liver as the Source of Fibrinogen. J. Exp. Med. 50 : 569-578.

Dutton, A. H., Dutton, R. E., and Vaughan, J. H. (1959) The in Vitro Uptake of C^{14} Amino Acid Into Spleen Cell Fractions Which Travel as Globulins on Starch Electrophoresis. Fed. Proc. 18 : 565.

Eldelman, I. S., and Liebman, J. (1959) Anatomy of Body Water and Electrolytes, Amer. J. Med. 27 : 256-277.

English, T. A. H., Digerness, S., and Kirklin, J. W. (1971) Changes in Colloid Osmotic Pressure During and Shortly After Open Intracardiac Operation. J. Thorac. Cardiovasc. Surg. 63 : 338-431.

Engelhardt, W. V. (1966) Swine Cardiovascular Physiology--A Review. In: Swine in Biomedical Research. Bustad, L. K., and McClellan, R. D., Editors, Frayne Printing Co., Seattle, pp. 307-329.

Farell, G. L., Rosnagle, R. S., and Rauschkolb, E. W. (1956) Increased Aldosterone Secretion in Response to Blood Loss. Circ. Res. 4 : 606-611.

Farell, G. L. (1958) Regulation of Aldosterone Secretion. Physiol. Rev. 38 : 709-728.

Farell, G. L. (1959) Physiological Factors Which Influence Secretion of Aldosterone. Recent Prog. Horm. Res. 15 : 275-310.

Fayot, G., and Huguenard, P. (1955) Impressions Cliniques après 350 Perfusions Veineuses de Dextran. Anesth. Analg. Reanim. 12 : 517-523.

Fletcher, A. G., Hardy, J. D., Reigel, C., and Koop, C. E. (1945) Gelatin as a Plasma Substitute : The Effect of Intravenous Infusion of Gelatin on Cardiac Output and Other Aspects of the Circulation of Normal Persons, of Chronically Ill Patients, and of Normal Volunteers Subjected to Large Hemorrhage. J. Clin. Invest. 24 : 405-415.

Flexner, L. B., Gellhorn, A., and Merrell, M. (1942) Rates of Exchange of Substances Between the Blood and Extravascular Fluid. I. The Exchange of Water in the Guinea Pig. J. Biol. Chem. 144 : 35-40.

Fox, C. L., Jr. (1970) Physiological Basis for Balanced Electrolyte Solution in Surgery. In: Body Fluid Replacement in the Surgical Patient. Fox, C. L., Jr. and Nahas, G. G., Grune and Stratton, New York, pp. 256-269.

Frank, H. A., Seligman, A. M., and Fine, J. (1945) Traumatic Shock. X. The Treatment of Hemorrhagic Shock Irreversible to Replacement of Blood Volume Deficiency. J. Clin. Invest. 24 : 435-444.

Friis-Hansen, B. (1961) Body Water Compartments in Children : Changes During Growth and Related Changes in Body Composition. Pediatrics, 28 : 169-181.

Gentz, J., Bengtsson, G., Hakkarainen, J., Hellström, R., and Persson, B. (1970) Metabolic Effects of Starvation During Neonatal Period in the Piglet. Am. J. Physiol. 218 : 662-668.

Glauser, E. M. (1966) Advantages of Piglets as Experimental Animals in Pediatric Research. Exp. Med. Surg. 24 : 181-190.

Gollub, S., Schechter, D. C., Hirose, T., and Bailey, C. P. (1969) The use of Hydroxyethyl Starch in Extensive Surgical Operation. Surg. Gynec. Obstet. 128 : 725-728.

Gollub, S., Schechter, D. C., Schaefer, C., Svigals, R., and Bailey, C. P. (1969) Absolute Hemodilution Cardiopulmonary Bypass : Free Water Distribution and Protein Mobilization in Body Compartments. Amer. Heart. J. 78 : 626-634.

Gourin, A., Streisand, R. L., Greineder, J., and Stuckey, J. H. (1971) Protamine Sulfate Administration and the Cardiovascular System. J. Thorac. Cardiovasc. Surg. 62 : 193-204.

Gregersen, M. I., and Rawson, R. A. (1959) Blood Volume. Physiol. Rev. 39 : 307-342.

Grönwall, A., and Ingelman, B. (1944) Nagra nya Kolloidlösningar för Infusionsändamal. Nord. Med. 21 : 247-249.

Grönwall, A., and Ingelman, B. (1945) Untersuchungen über Dextran und Sein Verhalten bei Parenteraler Zufuhr. II Acta Physiol. Scand. 9 : 1-27.

Grönwall, A. (1969) Biological Effects of Dextran. In: Properties and Applications of Dextrans. Ingelman, B., Grönwall, A., Gelin, L. E., and Eliasson, R., Editors, Almqvist and Wiksell, Stockholm, pp. 24-42.

Gropper, A. L., Raisz, L. G., and Amspacher, W. H. (1952) Plasma Expanders. Surg. Gynec. Obstet. 95 : 521-542.

Gruber, U. F. (1969) Blood Replacement. Springer-Verlag, Berlin, pp. 104-135.

Gruber, U. F. (1969) Blood Replacement. Springer-Verlag, Berlin, pp. 146-154.

Hartman, A. F. (1934) Theory and Practice of Parenteral Fluid Administration. JAMA 13 : 1349-1354.

Harvey, W. (1628) Exercitatio Anatomica, De Mortu Cordis Et Sanguinis In Animalibus. London, pp. 20-72.

Hässig, A., and Stampfli, K. (1969) Plasma Substitutes Past and Present. Blibl. Haemat. 33 : 1-8.

Hellström, G., and Björk, V. O. (1963) Hemodilution with Rheomacrodex During Total Body Perfusions. J. Thorac. Cardiovasc. Surg. 45 : 395-401.

Hevesy, G., and Jacobsen, C. F. (1940) Rate of Passage of Water Through Capillary and Cell Wall. Acta Physiol. Scand. I : 11-18.

Heyl, J. T., Gibson, J. G. II, and Janeway, C. A. (1943) Studies on the Plasma Proteins. V. The Effect of Concentrated Solutions of Human and Bovine Serum Albumin on Blood Volume After Acute Blood Loss in Man. J. Clin. Invest. 22 : 763-773.

Hill, J. M., Muirhead, E. E., Ashworth, C. T., and Tigertt, W. D. (1941) The Use of Desiccated Plasma with Particular Reference to Shock. JAMA 116 : 395-402.

Hochwald, G. M., Thorbecke, G. J., and Asofsky, R. (1961) Sites of Formation of Immune Globulins and of a Component of C'$_3$. I. A New Technique for the Demonstration of the Synthesis of Individual Serum Proteins by Tissues in Vitro. J. Exp. Med. 114 : 459-470.

Hogen, J. J. (1915) The Intravenous Use of Colloidal Gelatin Solutions in Shock. JAMA 54 : 721-722.

Houpt, R. T. (1970) Water Electrolytes and Acid-Base Balance. In: Duke's Physiology of Domestic Animals, 8th Edition, M. J. Swenson Editor, Cornell University Press, Ithaca, New York, p. 744.

Hurwitz, S. H. (1917) Intravenous Injections of Colloidal Solutions of Acacia in Hemorrhage; Preliminary Report. JAMA 68 : 699-701.

Hustin, A. (1914) Principe d'une Nouvelle Méthode de Transfusion. J. Med. de Brux. 2 : 436-439.

Hyman, C., and Steinfeld, J. L. (1967) Regulation of Plasma Volume. Am. Heart J. 74 : 436-438.

Jackson, J. E., Breen, M., and Chen, C. (1962) Fluorometric Titration of Calcium. J. Lab. Clin. Med. 60 : 700-708.

Jacobsson, L., and Hansen, H. (1952) A Simple Method for Determination of Dextran in Blood and Urine. Scand. J. Clin. Lab. Invest. 4 : 352-354.

Jacobsson, L., and Hansen, H. (1953) Determination of Serum Protein and Dextran in Treatment with "Macrodex". Nord. Med. 49 : 362.

Jennings, C. E. (1885) The Intravenous Injection of Milk. Brit. Med. J. I : 1147-1149.

Jolyet, F., and Laffond, R. (1879) Sur les Effets des Injections d'Eau Salée dans le Système Circulatoire des Animaux Exsangues. Soc. Biol. Gaz. Med. Paris, 101-103. Cited by Hayem, G. In: Leçons sur les Modifications du Sang sous l'Influence des Agents Medicamenteux et des Pratiques Therapeutiques. Emission Sanguines, Transfusion du Sang. G. Masson Cie., Paris, 1882.

Kaijser, L., and Rieger, A. (1968) Blood Volume and Plasma Protein. V. Changes in Blood Volume and Plasma Proteins on Administration of Different Substitutes after Operations in Man. Acta. Chir. Scand. 134 : 15-26.

Kerr, W. J., Hurwitz, S. H., and Whipple, G. H. (1918) Regeneration of Blood Serum Protein. III. Liver Injury Alone : Liver Injury and Plasma Depletion. The Eck Fistula Combined With the Plasma Depletion. Am. J. Physiol. 47 : 379-391.

Keynes, G. (1943) History of Blood Transfusion, 1628-1914. Brit. J. Surg. 31 : 38-50.

Kingsley, G. R. (1939) The Determination of Serum Total Protein, Albumin and Globulin by the Biuret Reaction. J. Biol. Chem. 131 : 197-200.

Kingsley, G. R. (1942) The Direct Biuret Method for the Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry. J. Lab. Clin. Med. 27 : 840-845.

Kronecker, H., and Sander, J. (1879) Bemerkungen Über Lebensrettende Transfusion mit anorganischen Salzlösungen bei Hunden. Berliner Klin. Wochens. NO 57. 5. 768. Cited by von Ott (1883) In: Ueber den Einfluss der Kochsalzinfusion auf den Verbluteten Organismus im Vergleich mit anderen zur Transfusion Verwendeten Flüssigkeiten. Virchovs Arch. Path. Anat. 93 : 114-167.

Kukral, J. C., Sporn, J., Louch, J., and Winzler, R. J. (1963) Synthesis of Alpha- and Beta-Globulins in Normal and Liverless Dog. Am. J. Physiol. 204 : 262-264.

Landsteiner, K. (1901) Über Agglutinationserscheinungen Normalen Menschlichen Blutes. Wien. Kli. Wschr. 14 : 1132-1134.

Lardinois, R., and Page, L. (1969) Serum Albumin, Prealbumin and Postalbumin in Perinatal Pigs. Dev. Biol. 19 : 261-280.

Lee, W. H. Jr., Krumhaar, D., Fonkalsrud, E. W., Schjeide, O. A., and Maloney, J. V. Jr. (1961) Denaturation of Plasma Proteins as a Cause of Morbility and Death After Intracardiac Operation. Surgery, 50 : 29-39.

Lee, W. H. Jr., Cooper, N., Weidner, M. G. Jr., and Murner, E. S. (1968) Clinical Evaluation of a New Plasma Expander, Hydroxyethyl Starch. J. Trauma 8 : 381-393.

Lehmann, J. (1939) Elektrometrische Mikrobestimmug von Chlor in Voll Blut, Serum and Harn. Acta. Paediat. Uppsala, 26 : 258-267.

Lemoine, G., Imbert, E., Cornu, P., Glaser, F., Neveux, J. Y., Galey, J. J., and Mathey, J. (1971) Circulation Extracorporelle Chez le Nourrisson. Ann. Chir. Thorac. Cardiovasc. 10 : 249-258.

Levinson, S. O., Rubovits, F. E., and Necheles, H. (1940) Human Serum Transfusions. JAMA 115 : 1163-1169.

Liljedahl, S. O., and Reiger, A. (1967) Blood Volume and Plasma Protein. IV. Importance of Thoracic Duct Lymph in Restitution of Plasma Volume and Plasma Proteins After Bleeding and Immediate Substitution in the Splenectomized Dog. Acta. Chir. Scand. Suppl. 379 : 39-51.

Linder, E., Sakai, Y., and Paton, B. P. (1964) Electrolyte Changes During Dilution Perfusion. Arch. Surg. 88 : 39-43.

Litwak, R. S., Slonim, R., Wisoff, G., and Gadboys, H. L. (1963) Homologous Blood Syndrome During Extracorporeal Circulation in Man. II. Phenomena of Sequestration and Desequestration. New. Eng. J. Med. 268 : 1377-1382.

Litwak, R. S., Gadboys, H. L., Kahn, M., and Wisoff, B. G. (1965) High Flow Total Body Perfusion Utilizing Diluted Perfusate in a Large Prime System. J. Thorac. Cardiovasc. Surg. 49 : 74-90.

Long, D. M., Sanchez, L, Varco, R. L., and Lillehei, C. W. (1961) The Use of Low Molecular Weight Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation. Surgery 50 : 12-28.

Long, D. M., Jr., Myer, M. W., Brown, E. B., and Lillehei, C. W. (1962) Myocardial Necrosis and Electrocardiographic Changes Related to Microcirculatory Abnormalities. Am. J. Cardiol. 10 : 695-705.

Lower, R. (1666) Tractus de Corde. In: Gunther's Early Science in Oxford, Oxford, 1932, Cited by Keynes, G. (1943) Brit. J. Surg. 31 : 38-50.

Ludwig, C. (1863) Einige neue Beziehungen Zwischen dem Bau und der Function der Niere. Report on the Meetings of the Section of Mathematics and Natural Sciences of the Imperial Academy of Science, Vienne, Vol. 48, Part 2, Journal VI to X, pp. 725-733.

Lundy, J. S., Tuchy, E. B., Adams, R. D., Mousel, L. H., Seldon, T. H., and Pender, J. W. (1947) Annual Report for 1946 of the Section on Anesthesiology Including Data and Remarks Concerning Blood Transfusion and the Use of Blood Substitutes. Proc. Mayo Clin. 22 : 357-368.

Lyman, D. J., Brash, J. L., Chaikin, S. W., Klein, K. G., and Carini, M. (1968) The Effect of Chemical Structure and Surface Properties of Synthetic Polymers on the Coagulation of Blood. II. Protein and Platelet Interaction with Polymer Surfaces. Trans. Amer. Soc. Artif. Intern. Organs. 14 : 250-255.

Madden, S. C., and Whipple, G. H. (1940) Plasma Proteins: Their Source, Production and Utilization. Physiol. Rev. 20 : 194-217.

Magladery, J. W., Solandt, D. Y., and Best, C. H. (1940) Serum and Plasma in Treatment of Haemorrhage in Experimental Animals. Brit. Med. J. 2 : 248-250.

Mann, F. D. (1957) Blood Clotting. Ann. Rev. Physiol. 19 : 205-230.

Mans, J. R., and Novelli, G. D. (1961) Measurement of the Incorporation of Radioactive Amino Acids into Protein by a Filter-Paper Disk Method. Arch. Biochem. Biophys. 94 : 48-53.

Marsh, J. B., and Drabkin, D. L. (1958) Metabolic Channeling in Experimental Nephrosis. IV. Net Synthesis of Plasma Albumin by Liver Slices from Normal and Nephrotic Rats. J. Biol. Chem. 230 : 1073-1081.

Mayerson, H. S., Wolfram, C. G., Shirley, H. H., and Wasserman, K. (1960) Regional Differences in Capillary Permeability. Am. J. Physiol. 198 : 155-160.

Maytum, C. K., and Magath, T. B. (1932) Sensitivity to Acacia. JAMA 99 : 2251-2252.

McCance, R. A., and Widdowson, E. M. (1959) The Effect of Lowering the Temperature on the Metabolism of the Newborn Pig. J. Physiol. 147 : 124-134.

MacFarlane, R. G. (1964) An Enzyme Cascade in the Blood Clotting Mechanisms and its Function as a Biological Amplifier. Nature, 202 : 498-500.

McNamara, J. H., Molot, M. D., Dunn, R. A., and Stremple, J. F. (1972) Effect of Hypertonic Glucose in Hypovolemic Shock in Man. Ann. Surg. 176 : 247-250.

Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoeffer, J. A., and Luecke, R. W. (1961) Swine Hematology From Birth to Maturity. I. Serum Proteins. J. Anim. Sci. 20 : 31-35.

Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F. (1951) The Dominant Role of the Liver in Plasma Protein Synthesis. A Direct Study of the Isolated Perfused Rat Liver with the Aid of Lysine - Σ - C^{14}. J. Exp. Med. 94 : 431-453.

Miller, L. L., and Bale, W. F. (1954) Synthesis of All Plasma Protein Fractions Except Gamma Globulins by the Liver. J. Exp. Med. 99 : 125-132.

Miller, L. L., Bly, C. G., and Bale, W. F. (1954) Plasma and Tissue Proteins Produced by Non-Hepatic Rat Organs as Studied with Lysine - Σ - C^{14}. Gamma Globulin the Chief Plasma Protein Fraction Produced by Non-Hepatic Tissues. J. Exp. Med. 99 : 133-153.

Miyauchi, Y., Inoue, T., and Paton, B. C. (1966) Comparative Study of Priming Fluids for Two-Hour Hemodilution Perfusion. J. Thorac. Cardiovasc. Surg. 52 : 413-421.

Moffitt, E. A., Maher, F. T., and Kirklin, J. W. (1965) Effect of Cardiopulmonary Bypass and Haemodilution on Some Constituents of Blood. Can. Anaes. Soc. J. 12 : 458-464.

Moore, F. D., Dagher, F. J., Boyden, C. M., Lee, C. F., and Lyons, J. H. (1966) Hemorrhage in Normal Man: I. Distribution and Dispersal of Saline Infusions Following Acute Blood Loss : Clinical Kinetics of Blood Volume Support. Ann. Surg. 163 : 485-503.

Morawitz, P. (1906) Beobachtungen über den Wiederersatz der Bluteiweisskörper. Beitr. Chem. Physiol. Path. 7 : 153-164.

Mount, L. E. (1963) Environmental Temperature Preferred by the Young Pig. Nature, 199 : 122-123.

Mulrow, P. J., and Ganong, W. F. (1962) Role of Kidney and Renin-Angiotensin System in Response of Aldosterone Secretion to Hemorrhage. Circulation, 25 : 213-220.

Nahas, G. G., and Estime, A. (1955) Etude Expérimentale des Gélatines Fluides Modifiées dans le Traitement des Hémorragies Massives (40% du Volume Sanguin Total) Rev. Hemat. 10 : 725-732.

Najafi, H., Neville, W. E., Banuchi, F., Peacock, H., and Meeks, M. F. (1965) Comparison of High- and Low-Volume Hemodilution for Cardiopulmonary Bypass. Surgery, 58 : 989-994.

Neville, W. E., Spinazzola, A., Banuchi, F., Scicchitano, L. P., and Peacock, H. (1964) Clinical Experience With Buffered Ringer's Lactate Solution for Total Prime of the Disc Oxygenator During Cardiopulmonary Bypass. J. Thorac. Cardiovasc. Surg. 48 : 101-111.

Osteen, R. T., and Klebanoff, G. (1971) Early Plasma Protein Recovery After Total Volume Plasmapheresis in Baboons. Surgery, 69 : 276-283.

Panico, F. G., and Neptune, W. B. (1960) A mechanism to Eliminate The Donor Blood Prime From the Pump-Oxygenator. Surg. Forum. 10 : 605-609.

Peräsalo, O., Siltanen, P., and Merikallio, E. (1967) Use of Hemodilution in Total Body Perfusion. J. Cardiovasc. Surg. 8 : 442-445.

Parkins, W. M., Koop, C. E., Riegel, C., Vars, H. M., and Lockwood, J. S. (1943) Gelatin as Plasma Substitute with Particular Reference to Experimental Hemorrhage and Burn Shock. Ann. Surg. 118 : 193-214.

Pluth, J. R., Cleland, J., Tauxe, W. N., and Kirklin, J. W. (1968) Late Changes in Body Fluid and Blood Volume After Intracardiac Surgery. J. Thorac. Cardiovasc. Surg. 56 : 108-113.

Raison, J. C. A. (1964) The Effect of Improving Tissue Perfusion in Extracorporeal Circulation by the Use of Low Molecular Weight Dextran. Isr. J. Exper. Med. 19 : 128-135.

Raminez, C. G., Miller, E. R., Ullrey, D. E., and Hoefer, J. A. (1963) Swine Hematology From Birth to Maturity. III. Blood Volume of the Nursing Pig. J. Animal. Sci. 22 : 1068-1093.

Reiger, A. (1967a) Blood Volume and Plasma Protein. II. Changes in Blood Volume and Plasma Proteins After Bleeding and Immediate Substitution with Physiologic Saline, Dog Plasma and Dog Albumin in the Splenectomized Dog. Acta. Chir. Scand., Suppl. 379: 5-21.

Reiger, A. (1967b) Blood Volume and Plasma Protein. III. Changes in Blood Volume and Plasma Proteins After Bleeding and Immediate Substitution with Macrodex, Rheomacrodex and Physiogel in the Splenectomized Dog. Acta. Chir. Scand. Suppl. 379 : 22-38.

Richet, C. (1919) Injection de Gomme ou de Plasma après Hémorragie. C. R. Acad. Sci. Paris. 169 : 1072-1074.

Ringer, S. (1882) Concerning the Influence Exerted by Each of the Constituents of the Blood on the Contraction of the Ventricle. J. Physiol. 3 : 380-393.

Ringer, S. (1885) Regarding the Influence of the Organic Constituents of the Blood on the Contractibility of the Ventricle, J. Physiol. 6 : 361-369.

Robinson, J. R. (1960) Metabolism of Intracellular Water. Physiol. Rev. 40 : 112-149.

Rodionov, V. M., Upsenskaja, V. D., and Zamjatkuna, O. G. (1957) Reconstitution of Plasma Proteins After Severe Hemorrhage in Dogs. Inst. Biol. Med. Chem. (U.S.S.R.) 3 : 255-267.

Roe, B. B., Hutchinson, J. C., and Swenson, E. E. (1965) High-Flow Body Perfusion With Calculated Hemodilution. Ann. Thorac. Surg. 5 : 581-589.

Rosenkrantz, J. G., MacMahon, R., Frost, W. W., Paton, B. C. and Swan, H. (1965) Extracorporeal Circulation in Puppies. Ann. Surg. 91 : 513-515.

Rothschild, M. A., Oratz, M., Wimer, E., and Schreiber, S. S. (1961) Studies on Albumin Synthesis: The Effects of Dextran and Cortisone on Albumin Metabolism in Rabbits Studied with Albumin - I^{131}. J. Clin. Invest. 40 : 545-554.

Rothschild, M. A., Oratz, M., Mongelli, J., and Schreiber, S. S. (1965) Albumin Metabolism in Rabbits During Gamma Globulin Infusions, J. Lab. Clin. Med. 66 : 733-740.

Rothschild, M. A., Oratz, M., Mongelli, J., and Schreiber, S. S. (1969) The Effect of Albumin Concentration on Albumin Synthesis in the Perfused Liver. Am. J. Physiol. 216 : 1127-1130.

Rothschild, M. A., Oratz, M., Mongelli, J., Fishman, L., and Schreiber, S. S. (1969) Amino Acid Regulation of Albumin Synthesis. J. Nutr. 98 : 395-403.

Rothschild, M. A., Oratz, M., Franklin, E. C., and Schreiber, S. S. (1962) The effect of Hypergammaglobulinemia on Albumin Metabolism in Hyperimmunized Rabbits Studied with Albumin - I^{131}. J. Clin. Invest. 41 : 1564-1571.

Rothschild, M. A., Oratz, M., and Schreiber, S. S. (1972a) Albumin Synthesis (First of Two Parts). N. Engl. J. Med. 286 : 748-757.

Rothschild, M. A., Oratz, M., and Schreiber, S. S. (1972b) Albumin Synthesis (Second of Two Parts). N. Engl. J. Med. 286 : 816-820.

Rous, P., and Turner, J. R. (1916) The Preservation of Living Red Blood Cells In Vitro. J. Exp. Med. 23 : 219-239.

Rush, B. F., Richardson, J. D., Bosomworth, P., and Eiseman, B. (1969) Limitations of Blood Replacement with Electrolyte Solutions. A Control Clinical Study. Arch. Surg. 98 : 49-52.

Sayman, W. A., Gauld, R. L., Star, S. A., and Allen, J. G. (1958) Safety of Liquid Plasma - A Statistical Appraisal. JAMA 168 : 1735-1739.

Schechter, D. C., and Sarot, I. A. (1969) The Potential Hazards of Immoderate Hemodilution in Cardiac Surgery. Dis. Chest, 55 : 94-95.

Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Solomon, A. K., and Moore, F. D. (1950) The Measurement of Total Body Water in the Human Subject by Deuterium Oxide Dilution, With a Consideration of the Dynamics of Deuterium Distribution. J. Clin. Invest. 29 : 1296-1310.

Shu'ayb, W. A., Moran, W. H., and Zimmermann, B., (1965) Studies on the Mechanism of Antidiuretic Hormone Secretion and the Post-Commissurotomy Dilutional Syndrome. Ann. Surg. 162 : 690-701.

Siltanen, P., Kekki, M., Merikallio, E., Myllylä, G., Somer, T., and Tala, P. (1968) Alteration of Plasma Proteins During Extracorporeal Circulation. I. Experimental Studies With ^{131}I-Labelled Plasma Protein Fractions. Ann. Chir. Gyn. Fenn. 57 : 1-8.

Simmons, P., Penny, R., and Goller, I. (1969) Plasma Proteins, A Review. Med. J. Aust. 2 : 494-506.

Simon, M. A., and Olsen, W. R. (1969) Capillary Flow in Hemorrhagic Shock. II. Hemorrhage in the Anesthetized Pig. Arch. Surg. 99 : 634-636.

Skillman, J. J., Lauler, D. P., Hickler, R. B., Lyons, J. H., Olson, J. E., Ball, M. R., and Moore, F. D. (1967) Hemorrhage in Normal Man : Effect on Renin, Cortisol, Aldosterone and Urine Composition. Ann. Surg. 166 : 865-885.

Skillman, J. J., Awwad, H. K., and Moore, F. D. (1969) Plasma Protein Kinetics of the Early Transcapillary Refill After Hemorrhage in Man. Surg. Gynecol. Obstet. 5 : 983-996.

Smith, H. P., Belt, A. E., and Whipple, G. H. (1920) Rapid Blood Plasma Protein Depletion and the Curve of Regeneration. Am. J. Physiol. 52 : 54-71.

Snedecor, G. W., and Cochran, W. G. Statistical Methods. The Iowa State University Press, Ames, Iowa, 1969, pp. 91-116 and pp. 172-195.

Solanke, T. F. (1968) Clinical Trial of 6% Hydroxyethyl Starch (A new Plasma Expander). Brit. Med. J. II : 783-785.

Solanke, T. F., Khwaja, M. S., and Madojemu, E. I. (1971) Plasma Volume Studies with Four Different Plasma Volume Expanders. J. Surg. Res. II : 140-143.

Starling, E. H. (1896) On the Absorption of Fluids From the Connective Tissue Spaces. J. Physiol. 19 : 312-326.

Strumia, M. M., Strumia, P. V., and Dugan, A. (1968) Significance of Measurement of Plasma Volume and of Indirect Estimation of Red Cell Volume. Transfusion, 8 : 197-209.

Swan, H., and Nelson, W. A. (1971) Blood Volume I : Critique : Spun vs. Isotope Hematocrits; [125]RIHSA vs. [51]CrRBC. Ann. Surg. 173 : 487-495.

Taguchi, K., Sasaki, N., Matsuura, Y., Yoskizaki, E., Tamura, M., and Uemura, R. (1968) Clinical Experiences With Hemodilution Total Cardiopulmonary Bypass : An Analysis of 650. Surgery, 63 : 252-260.

Talbot, R. B., and Swenson, M. J., (1963) Measurement of Porcine Plasma Volume, Using T-1824 Dye. Am. J. Vet. Res. 24 : 467-471.

Talbot, R. B., and Swenson, M. J. (1970) Blood Volume of Pigs from Birth Through 6 Weeks of Age. Am. J. Physiol. 218 : 1141-1144.

Tarver, H., and Reinhardt, W. O. (1947) Methionine Labeled With Radioactive Sulfur as an Indicator of Protein Formation in the Hepatectomized Dog. J. Biol. Chem. 167 : 395-400.

Thomas, G. T. (1878) The Intra-Venous Injection of Milk as a Substitute for the Transfusion of Blood. N.Y. Med. J. 27 : 449-465.

Thorsen, G. (1959) The Use of Dextrans as Infusion Fluids. Surg. Gynec. Obstet. 109 : 43-52.

Turina, M. (1972) Une Machine Coeur-Poumon pour Nouveaux-Nés et Nourrissons. Nouv. Med. 5-6 : 61-76.

Vander, A. J. (1967) Control of Renin Release. Physiol. Rev. 47 : 359-382.

Vinegard, G. C., Bradley, B. E., Defalco, A., Lawson, D., Wagner, E. T. A., Pastis, W. K., Nardella, F. A., and Hayes, J. R. (1966) Effect of Hydroxyethyl Starch on Plasma Volume and Hematocrit Following Hemorrhagic Shock in Dogs. Comparison with Dextran, Plasma and Ringer's. Ann. Surg. 164 : 891-899.

Wallace, H. W., Arai, K., and Blakemore, W. S. (1970) Glycoprotein Response to Open-Heart Surgery. J. Thorac. Cardiovasc. Surg. 59 : 376-381.

Warren, J. V., Stead, E. A. Jr., Merrill, A. J., and Brannon, E. S. (1944) Chemical, Clinical and Immunological Studies on the Products of Human Plasma Fractionation. IX. The Treatment of Shock with Concentrated Human Serum Albumin : A Preliminary Report. J. Clin. Invest. 23 : 506-519.

Wasserman, K., and Mayerson, H. S. (1951) Exchange of Albumin Between Plasma and Lymph. Am. J. Physiol. 165 : 15-26.

Weichselbaum, T. E. (1946) An Accurate and Rapid Method for the Determination of Proteins in Small Amounts of Blood Serum and Plasma. Am. J. Clin. Path. 10 : 40-49.

Whipple, G. H., and Hurwitz, S. H. (1911) Fibrinogen of the Blood as Influenced by the Liver Necrosis of Chloroform Poisoning. J. Exp. Med. 13 : 136-161.

Wiedersheim, M. (1957) An Investigation of Oxyethylstarch as a New Plasma Volume Expander in Animals. Arch. Int. Pharmacodyn. III : 353-361.

Woolley, G., and Courtice, F. C. (1962) The origin of Albumin in Hepatic Lymph. Aust. J. Exp. Biol. Med. Sci. 40 : 121-128.

Wright, E. S., Sarkozy, E., Harpur, E. R., Dobell, A. R. C., and Murphy, D. R. (1962) Plasma Protein Denaturation in Extracorporeal Circulation. J. Thorac. Cardiovasc. Surg. 44 : 550-556.

Yashar, J. J., Hallman, G. L., Leachman, R. D., and Cooley, D. A. (1971) Cardiac Output and Blood Volume Changes After Open-Heart Surgery. J. Thorac. Cardiovasc. Surg. 61 : 724-728.

Yuile, C. L., and Knutti, R. E. (1939) Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia. Production of Chronic Hypoproteinemia. J. Exp. Med. 70 : 605-613.

Zimmerman, L. M. (1942) Evolution of Blood Transfusion. Amer. J. Surg. 55 : 613-620.

Zuhdi, N., McCollough, B., Carey, J., and Greer, A. (1960) The Use of Routine Citrated Banked Blood for Open-Heart Surgery. Anesthesiology, 21 : 496-501.

Zuhdi, N., McCollough, B., Carey, J., and Greer, A. (1961) Double-Helical Reservoir Heart-Lung Machine Designed for Hypothermic Perfusion; Primed With 5% Glucose in Water; Including Hemodilution. Arch. Surg. 82 : 320-325.

APPENDIX A - Table 1

Body weight (Kg.)

Piglet No.	Sham-operated before	8	16	24	Piglet No.	Ringer-lactate before	after	8	16	24	Piglet No.	Rheomacrodex before	after	8	16	24
84	3.692	3.602	3.472	3.392	65	2.630	2.623	2.585	2.500	2.460	114	2.635	2.625	2.475	2.445	2.370
86	2.412	2.372	2.302	2.232	70	2.385	2.372	2.345	2.230	2.198	115	2.736	2.729	2.620	2.545	2.472
96	2.280	2.200	2.118	2.067	71	2.930	2.925	2.835	2.665	2.640	116	2.943	2.936	2.792	2.705	2.650
103	2.868	2.738	2.630	2.600	74	2.500	2.490	2.400	2.290	2.215	117	2.955	2.948	2.807	2.740	2.670
106	3.275	3.250	3.140	3.070	76	2.780	2.755	2.710	2.653	2.605	122	2.200	2.194	2.150	2.089	2.035
123	2.240	2.131	2.120	2.050	79	2.640	2.630	2.500	2.438	2.346	125	3.740	3.730	3.685	3.545	3.470
128	3.120	2.970	2.915	2.845	80	2.850	2.843	2.733	2.628	2.553	127	3.820	3.812	3.701	3.590	3.510
130	2.910	2.865	2.785	2.720	82	2.420	2.414	2.310	2.235	2.180	129	2.915	2.909	2.865	2.785	2.765
132	3.455	3.292	3.160	3.050	83	2.610	2.606	2.505	2.420	2.350	131	3.275	3.267	3.150	3.050	3.010
134	2.533	2.445	2.390	2.345	85	3.012	3.006	2.842	2.757	2.672	133	2.755	2.748	2.595	2.532	2.465
137	2.990	2.870	2.725	2.658	95	2.750	2.740	2.589	2.489	2.421	136	2.790	2.782	2.595	2.488	2.420
139	2.840	2.668	2.610	2.505	98	3.057	3.025	3.000	2.960	2.802	138	2.770	2.764	2.661	2.505	2.420
140	2.845	2.738	2.640	2.580	99	2.980	2.960	2.920	2.757	2.652	141	2.665	2.658	2.551	2.406	2.335
143	2.990	2.866	2.735	2.660	102	2.950	2.922	2.838	2.663	2.653	142	3.140	3.132	2.973	2.895	2.022
145	2.545	2.460	2.345	2.290	105	3.290	3.282	3.200	3.100	3.025	144	2.670	2.660	2.595	2.460	2.400
Mean	2.866	2.764	2.672	2.604		2.786	2.773	2.682	2.586	2.518		2.934	2.926	2.814	2.719	2.654
s.d.	0.418	0.400	0.375	0.267		0.248	0.246	0.244	0.243	0.240		0.421	0.406	0.409	0.399	0.420
I		***	***	***			n.s.	***	***	***			n.s.	***	***	***
S						n.s.		n.s.	n.s.	n.s.		n.s.		n.s.	n.s.	n.s.
R												n.s.	n.s.	n.s.		n.s.

APPENDIX A - Table 2

Large vessel hematocrit (%)

Sham-operated

Piglet No.	before	8	16	24
84	33.5	34.0	32.8	32.6
86	29.6	32.5	32.5	31.5
96	33.2	32.0	32.0	30.6
103	21.3	24.7	25.2	25.2
106	29.9	30.1	30.6	30.7
123	33.0	32.5	34.0	33.5
128	29.1	29.6	29.7	29.1
130	34.4	36.9	34.0	34.7
132	34.9	35.5	34.9	33.8
134	33.6	34.0	35.5	34.9
137	32.0	33.5	33.5	34.0
139	27.6	29.1	29.1	29.6
140	24.3	25.7	26.2	27.2
143	29.6	32.0	32.5	32.5
145	22.8	22.8	23.2	23.8
Mean	29.9	31.0	31.0	30.9
s.e.m.	1.1	1.0	1.0	0.9
I		**	**	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	32.5	22.1	22.1	23.0	24.3	24.3
70	28.0	21.8	21.8	21.3	21.8	22.8
71	30.0	22.8	22.8	22.3	22.3	22.8
74	32.0	21.8	22.3	20.3	18.9	18.7
76	30.6	20.9	21.3	19.1	18.6	19.9
79	29.9	22.1	21.8	19.9	22.3	22.3
80	31.5	22.3	22.7	22.8	21.8	22.3
82	29.1	22.3	22.8	18.4	19.5	19.2
83	27.2	22.2	22.3	18.7	19.2	18.9
85	30.6	21.5	21.9	22.3	22.3	22.3
95	32.5	24.1	24.9	19.9	17.9	19.9
98	29.1	22.6	22.6	23.1	19.9	19.4
99	31.1	25.2	25.2	25.7	19.1	22.3
102	28.6	22.7	23.1	21.8	21.3	22.8
105	28.6	24.5	24.7	24.7	23.3	24.3
Mean	30.1	22.6	22.8	21.6	20.8	21.5
s.e.m.	0.4	0.3	0.3	0.6	0.5	0.5
I		***		***	***	***
S	n.s.			***	***	***
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	31.5	21.8	21.8	22.3	22.3	23.3
115	35.6	20.9	21.0	20.9	21.3	23.3
116	28.1	21.3	21.3	21.8	21.8	22.3
117	28.6	21.3	21.3	20.4	19.4	20.9
122	29.6	21.8	21.8	23.7	23.8	24.0
125	31.0	21.8	21.8	23.8	23.8	23.3
127	28.9	21.9	21.9	20.4	21.3	22.8
129	32.0	21.9	21.9	25.2	24.3	22.6
131	34.9	21.3	21.3	22.3	24.3	24.4
133	32.0	21.0	21.0	21.8	21.8	22.3
136	31.5	22.3	22.3	22.8	22.8	23.6
138	29.1	21.8	21.8	22.1	22.3	23.3
141	28.1	22.3	22.3	21.3	22.3	24.3
142	29.1	21.8	21.8	22.8	23.8	24.7
144	26.0	22.3	22.3	23.9	24.7	24.7
Mean	30.4	21.7	21.7	22.4	22.7	23.3
s.e.m.	0.6	0.1	0.1	0.4	0.4	0.3
I		***		***	***	***
S	n.s.			***	***	***
R	n.s.	***		n.s.	**	**

APPENDIX A - Table 3

Plasma volume (ml./Kg.)

Sham-operated

Piglet No.	before	8	16	24
84	58	57	52	52
86	64	57	61	70
96	60	49	57	56
103	68	57	63	67
106	65	53	47	62
123	64	60	59	62
128	65	62	61	59
130	68	55	52	52
132	59	51	58	61
134	67	66	67	60
137	61	56	60	58
139	68	60	56	57
140	68	57	61	62
143	62	55	60	56
145	71	68	73	69
Mean	64.0	57.4	59.0	60.0
s.e.m.	1.0	1.3	1.6	1.5
I		***	**	**
S				
R				

Ringer-lactate

Piglet No.	before	after	8	16	24
65	65	48	52	54	60
70	63	53	51	67	73
71	63	50	62	58	61
74	57	44	55	62	64
76	63	43	56	62	60
79	65	51	70	73	72
80	65	52	52	53	60
82	67	59	61	64	69
83	70	58	64	64	78
85	64	51	60	62	65
95	61	47	58	64	57
98	65	48	45	52	52
99	68	45	47	64	57
102	61	50	63	62	57
105	64	50	49	52	54
Mean	64.0	49.8	56,3	60.8	62.5
s.e.m.	0.8	1.2	1.8	1.5	1.9
I		***	***	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R					

Rheomacrodex

Piglet No.	before	after	8	16	24
114	58	55	57	57	62
115	56	50	53	52	60
116	66	51	54	56	61
117	61	51	54	57	58
122	66	61	52	65	64
125	60	57	58	58	56
127	67	61	57	61	65
129	61	53	60	55	57
131	62	58	57	56	56
133	65	55	63	65	64
136	67	60	58	64	63
138	66	60	58	66	64
141	67	58	61	64	62
142	60	51	52	57	55
144	70	58	61	65	66
Mean	63.5	55.7	57.0	59.8	60.8
s.e.m.	1.0	1.0	0.9	1.2	0.9
I		***	***	***	***
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	***	n.s.	n.s.	n.s.

APPENDIX A - Table 4

Blood volume (ml./Kg.)

Sham-operated

Piglet No.	before	8	16	24
84	87	86	77	76
86	90	84	90	102
96	89	71	84	80
103	86	76	84	90
106	93	76	67	89
123	96	89	89	93
128	92	88	86	83
130	91	87	79	79
132	91	78	90	92
134	100	101	103	93
137	90	84	91	88
139	94	84	79	81
140	90	77	82	86
143	88	81	89	83
145	92	88	96	90
Mean	91.4	83.3	85.6	86.8
s.e.m.	0.9	1.8	2.2	1.8
I		***	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	8	16	24
65	96	62	68	71	79
70	87	67	65	86	94
71	90	64	79	75	79
74	84	57	69	76	79
76	91	54	69	76	75
79	92	65	88	93	93
80	95	67	67	68	77
82	94	76	74	79	85
83	96	74	78	79	97
85	93	66	77	79	83
95	91	62	72	78	71
98	92	62	59	65	65
99	99	61	65	79	74
102	88	64	81	79	73
105	89	67	66	68	72
Mean	91.7	64.5	71.8	76.8	79.6
s.e.m.	1.0	1.5	2.0	1.8	2.4
I		***	***	***	***
S			***	**	**
R					

Rheomacrodex

Piglet No.	before	after	8	16	24
114	80	70	74	74	80
115	87	63	68	66	78
116	92	65	69	71	78
117	85	65	68	70	75
122	94	78	68	85	84
125	88	72	77	76	73
127	94	78	71	77	84
129	90	68	81	73	74
131	96	73	73	74	74
133	95	69	81	83	82
136	98	77	75	83	82
138	94	76	75	85	83
141	94	74	77	83	82
142	85	65	68	75	73
144	95	74	80	86	88
Mean	90.8	71.2	73.5	77.3	79.3
s.e.m.	1.3	1.3	1.2	1.7	1.3
I		***	***	***	***
S	n.s.		***	**	**
R	n.s.	**	n.s.	n.s.	n.s.

APPENDIX A - Table 5

Total plasma protein concentrations (g./100ml. plasma)

Sham-operated

Piglet No.	before	8	16	24
84	5.1	4.9	4.8	4.7
86	5.5	5.4	5.9	5.9
96	5.6	5.7	6.0	5.9
103	5.3	5.3	5.5	5.5
106	4.7	4.8	4.3	5.0
123	4.9	4.9	4.8	4.9
128	5.1	5.2	5.2	5.2
130	4.7	4.9	4.7	4.8
132	5.9	5.8	5.9	5.9
134	4.7	5.0	5.1	5.1
137	5.2	5.4	5.4	5.5
139	5.6	6.3	6.0	6.8
140	5.8	5.5	5.6	5.8
143	5.1	5.5	5.6	5.6
145	5.0	4.8	5.4	5.2
Mean	5.21	5.29	5.34	5.45
s.e.m.	0.10	0.11	0.15	0.14
I	—	n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	5.2	3.9	3.9	4.7	[illegible]	4.4
70	5.6	4.8	4.8	5.8	5.8	5.8
71	5.5	4.6	4.6	5.2	5.1	5.5
74	5.4	4.1	4.1	4.7	5.2	4.9
76	4.8	3.7	3.7	4.3	4.6	4.9
79	4.9	3.7	3.7	4.3	4.7	4.8
80	5.1	3.9	3.8	3.8	4.3	4.5
82	4.6	3.7	3.6	3.8	3.9	4.1
83	4.4	3.4	3.4	3.9	3.9	4.2
85	6.0	4.3	4.4	5.3	5.3	5.6
95	6.1	4.3	4.3	5.3	5.6	6.2
98	6.3	5.3	5.4	5.7	5.8	6.2
99	5.7	4.6	4.5	5.0	4.9	5.4
102	5.4	4.6	4.6	5.2	5.1	5.4
105	5.4	4.0	4.0	4.8	5.1	5.0
Mean	5.34	4.20	4.18	4.79	4.88	5.11
s.e.m.	0.13	0.13	0.13	0.17	0.16	0.17
I		***		***	***	**
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	4.8	2.9	2.9	4.7	4.1	4.7
115	5.3	2.2	2.2	5.0	4.8	5.6
116	4.5	3.4	3.5	4.1	4.3	4.3
117	5.2	4.4	4.5	5.1	4.8	5.3
122	5.3	4.1	4.1	5.3	5.3	5.3
125	5.3	3.8	3.8	5.1	5.1	5.3
127	5.0	3.5	3.5	4.3	4.3	4.9
129	5.9	3.9	3.9	6.1	6.0	5.9
131	6.1	3.7	3.6	5.7	6.1	6.3
133	5.2	3.2	3.2	5.9	5.7	5.2
136	5.5	4.3	4.3	5.5	5.5	5.7
138	5.6	4.3	4.3	6.4	6.1	6.4
141	5.9	4.5	4.5	5.6	5.1	5.5
142	5.1	3.7	3.7	5.6	5.7	5.5
144	4.3	3.7	3.7	4.4	4.5	4.7
Mean	5.27	3.70	3.70	5.26	5.16	5.37
s.e.m.	0.14	0.16	0.16	0.17	0.17	0.14
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	**		n.s.	n.s.	n.s.

APPENDIX A - Table 6

Relative percentages of albumin

Sham-operated

Piglet No.	before	8	16	24
84	46.8	47.5	46.8	46.6
86	35.9	33.2	33.0	34.8
96	35.8	34.5	32.1	29.6
103	26.3	25.0	27.5	27.5
106	49.0	44.6	47.2	45.8
123	30.5	27.4	28.7	28.9
128	33.9	30.6	33.0	32.5
130	41.8	40.4	38.9	42.5
132	34.3	37.7	36.4	37.7
134	36.2	37.6	32.1	34.8
137	34.0	33.6	34.8	35.6
139	42.6	44.4	41.0	45.2
140	32.3	35.7	34.6	33.5
143	38.3	33.9	35.0	35.9
145	24.9	23.3	23.1	22.7
Mean	36.18	35.29	34.94	35.52
s.d.	6.76	7.06	6.57	7.00
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	42.2	47.1	47.1	41.3	40.1	36.2
70	29.0	28.3	27.8	28.7	29.0	28.8
71	29.3	36.0	35.1	40.8	31.7	31.2
74	36.0	36.6	33.8	37.1	36.6	35.6
76	35.6	35.7	37.3	36.9	36.8	35.2
79	40.7	35.6	36.8	43.0	[illegible]	39.9
80	43.0	43.6	42.0	43.8	44.1	44.9
82	39.9	42.5	45.0	45.3	38.0	43.1
83	41.3	39.8	41.5	48.5	43.4	35.0
85	38.0	31.0	33.7	40.1	41.2	38.5
95	33.5	34.2	34.5	37.2	35.4	36.8
98	31.9	30.2	32.9	32.4	31.8	36.9
99	27.1	25.4	26.7	25.6	30.4	26.5
102	39.2	33.6	34.0	33.3	31.5	35.6
105	42.6	37.8	38.0	40.5	38.5	38.5
Mean	36.62	35.83	36.40	38.30	36.45	36.19
s.d.	5.36	5.85	5.66	6.26	4.74	4.82
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	40.0	37.5	37.2	45.6	43.1	43.2
115	50.1	46.5	47.3	50.2	46.1	46.7
116	46.9	48.2	48.2	44.8	46.7	43.1
117	42.8	33.6	35.7	38.6	41.9	40.9
122	36.8	37.5	37.7	36.1	33.7	34.1
125	40.4	37.3	39.3	38.0	37.0	38.5
127	37.5	35.9	35.5	34.8	34.6	36.2
129	38.4	34.3	34.2	33.6	37.9	38.7
131	35.0	33.5	31.4	36.4	34.4	32.1
133	33.1	34.3	33.6	36.4	35.7	36.0
136	38.7	36.1	35.6	34.1	40.4	33.9
138	44.0	43.5	42.9	46.5	43.0	42.2
141	38.2	42.8	44.4	49.9	41.1	38.0
142	41.0	42.8	44.2	44.4	41.9	39.9
144	32.3	34.6	35.6	34.1	35.4	35.1
Mean	39.68	38.35	38.85	40.22	39.52	38.39
s.d.	4.85	4.77	5.24	5.98	4.30	4.09
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 7

Relative percentages of alpha globulin

Sham-operated

Piglet No.	before-	8	16	24
84	14.0	15.2	17.5	17.2
86	14.7	15.6	16.4	16.4
96	18.3	18.9	18.1	20.7
103	19.4	17.9	19.2	18.4
106	15.6	19.[illegible]	17.0	18.6
123	24.8	24.6	26.3	23.6
128	16.6	17.7	18.4	17.6
130	21.0	20.4	22.6	22.2
132	17.7	17.5	16.9	16.0
134	24.5	22.5	23.3	24.9
137	19.9	18.4	17.3	20.3
139	13.6	15.2	16.2	13.2
140	20.4	19.2	18.3	19.4
143	20.1	22.3	21.9	20.4
145	26.4	24.7	28.7	27.2
Mean	19.13	19.29	19.86	19.72
s. d.	3.95	3.08	3.83	3.58
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	16.1	12.7	12.7	15.8	18.4	28.6
70	14.0	14.4	14.9	14.6	15.7	18.0
71	17.7	14.4	14.9	13.5	19.4	19.0
74	19.7	19.1	18.3	17.3	18.4	21.1
76	21.1	18.4	18.1	17.3	18.2	19.4
79	15.9	16.0	14.2	13.7	17.4	17.3
80	16.0	14.2	15.3	15.3	15.3	14.1
82	18.0	17.7	17.3	16.2	17.6	18.2
83	16.0	16.8	16.3	14.3	17.8	19.8
85	11.7	13.3	13.0	13.2	13.4	15.5
95	17.1	18.3	17.1	17.5	19.2	17.7
98	16.7	17.5	16.4	17.1	19.0	16.5
99	15.4	17.4	17.2	17.3	16.5	16.8
102	11.4	14.1	14.4	15.1	15.1	15.3
105	15.8	22.5	22.0	18.4	17.5	17.5
Mean	16.16	16.43	16.14	15.78	17.27	18.33
s. d.	2.55	2.63	2.38	1.68	1.72	3.39
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	25.7	23.1	28.4	21.3	21.1	20.9
115	16.3	18.3	17.7	15.1	16.4	15.1
116	15.0	14.9	14.9	16.6	13.2	17.7
117	17.8	19.0	18.1	12.9	13.7	14.3
122	22.6	20.6	20.2	21.0	25.8	23.8
125	12.7	11.7	11.8	15.4	17.0	17.0
127	14.6	15.8	16.0	19.2	19.0	17.9
129	14.6	14.6	14.7	17.7	17.3	15.6
131	1.07	12.5	13.7	12.9	14.1	15.1
133	23.3	22.3	22.7	22.3	22.9	23.2
136	20.4	22.4	19.7	20.0	14.3	20.5
138	11.3	11.7	12.8	13.1	13.6	12.9
141	19.0	16.3	16.6	13.4	18.3	18.3
142	18.1	16.5	16.2	16.5	17.8	19.2
144	20.2	18.3	19.5	25.3	19.0	19.2
Mean	17.51	17.21	17.23	17.50	17.56	18.03
s. d.	4.49	3.83	3.49	3.87	3.66	3.19
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 8

Relative percentages of beta globulin

Sham-operated

Piglet No.	before	8	16	24
84	5.1	4.8	5.0	5.2
86	8.8	5.6	5.5	5.5
96	4.5	4.7	5.6	5.1
103	8.8	8.9	9.0	9.3
106	4.8	5.7	5.9	5.6
123	6.1	6.3	5.4	5.9
128	7.1	7.1	5.7	5.5
130	4.2	4.9	5.5	3.7
132	4.2	4.1	4.1	3.8
134	5.4	5.4	5.9	3.9
137	5.9	5.4	5.3	6.4
139	4.7	4.4	5.2	3.8
140	4.4	4.4	5.0	5.2
143	3.6	7.0	5.4	4.7
145	8.1	9.5	8.9	9.5
Mean	5.71	6.00	5.82	5.53
s.d.	1.72	1.99	1.34	1.76
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	3.7	3.0	3.0	4.4	4.6	3.7
70	5.5	5.5	4.7	6.4	6.0	7.1
71	5.4	5.3	5.3	4.3	5.4	5.7
74	7.4	7.7	6.2	7.1	7.7	6.5
76	7.6	7.0	6.6	6.1	6.2	6.9
79	4.6	6.0	5.1	5.9	4.3	6.4
80	4.5	5.2	5.4	4.4	6.3	6.2
82	4.9	4.3	4.3	5.2	5.4	3.7
83	4.8	4.7	5.6	2.7	3.0	4.2
85	4.0	3.4	6.3	3.2	4.9	5.5
95	5.6	6.8	6.0	6.1	7.3	7.3
98	4.0	2.9	5.0	4.5	3.9	4.0
99	5.3	5.4	5.9	3.7	5.1	4.8
102	4.6	6.8	5.3	5.6	5.6	6.1
105	6.0	7.0	6.3	7.4	6.6	6.5
Mean	5.20	5.39	5.42	5.15	5.46	5.63
s.d.	1.14	1.51	0.92	1.39	1.26	1.26
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	5.2	5.5	5.5	5.0	5.1	5.4
115	5.5	5.3	5.5	6.9	3.9	5.4
116	3.9	4.8	4.8	5.4	8.1	4.2
117	4.6	5.4	5.4	6.7	4.5	4.4
122	4.4	3.7	3.6	4.9	4.4	4.6
125	3.5	6.0	5.6	3.9	3.7	2.3
127	5.8	5.7	5.9	5.1	4.5	4.7
129	5.1	4.6	5.5	4.4	4.3	5.1
131	4.3	4.7	3.4	3.7	4.5	3.5
133	4.4	5.4	4.6	6.0	6.1	4.9
136	6.6	6.6	6.4	6.3	5.6	5.9
138	3.1	4.3	4.0	3.3	4.1	3.8
141	2.4	2.3	2.5	2.9	4.2	3.9
142	3.1	4.0	4.5	3.4	4.2	4.5
144	7.6	6.5	4.7	2.4	5.7	6.2
Mean	4.63	4.98	4.80	4.68	4.85	4.58
s.d.	1.38	1.13	1.05	1.40	1.13	0.99
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 9

Relative percentages of gamma globulin

Sham-operated

Piglet No.	before	8	16	24
84	12.0	8.6	5.7	8.3
86	19.8	19.8	12.7	17.3
96	13.9	12.5	14.8	13.2
103	10.1	13.1	9.9	10.4
106	4.8	4.5	3.6	2.9
123	12.0	14.4	12.5	12.1
128	15.4	15.4	14.1	14.4
130	7.4	4.6	6.0	6.8
132	16.3	14.7	10.7	11.8
134	5.0	5.6	5.1	2.7
137	8.8	9.0	7.0	6.2
139	8.9	6.9	6.6	8.8
140	7.3	10.1	9.0	7.7
143	7.5	6.3	6.5	6.7
145	3.7	4.3	4.3	4.1
Mean	10.18	9.98	8.56	8.89
s. d.	4.66	4.79	3.67	4.27
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	9.0	11.1	11.1	10.4	8.8	8.2
70	21.0	19.1	19.5	22.2	17.6	16.8
71	19.6	15.0	14.9	11.3	12.2	11.5
74	9.1	7.9	7.7	6.7	6.7	8.3
76	5.8	8.2	8.5	10.7	10.6	10.2
79	8.8	12.8	13.3	11.0	8.2	9.1
80	8.9	11.0	10.3	7.6	7.2	6.4
82	11.5	9.2	8.2	7.5	8.3	6.9
83	11.5	12.3	7.5	6.2	2.8	7.7
85	19.7	23.8	20.5	15.3	16.6	14.3
95	11.6	12.4	12.1	8.2	9.7	6.3
98	18.7	14.3	15.9	13.2	14.2	11.3
99	22.0	22.7	19.2	19.0	18.4	17.6
102	11.3	13.8	13.8	11.8	14.0	13.2
105	9.7	9.7	9.6	8.3	8.0	9.3
Mean	13.19	13.54	12.81	11.30	10.87	10.47
s. d.	5.37	4.86	4.42	4.58	4.48	3.59
I		n.s.		n.s.	n.s.	**
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	5.8	7.5	5.3	5.2	4.6	3.5
115	7.7	7.8	7.6	7.6	5.0	10.0
116	5.5	5.6	5.6	5.1	4.6	6.7
117	9.3	14.1	11.9	8.5	8.1	8.3
122	14.5	12.6	14.0	11.8	10.7	11.5
125	13.5	12.8	13.1	10.9	10.9	10.3
127	15.4	14.7	14.4	15.6	11.8	10.8
129	11.1	18.1	14.8	14.6	12.3	12.4
131	22.8	24.0	22.9	19.6	16.8	17.0
133	6.4	7.1	6.5	7.4	6.7	6.0
136	5.3	4.6	6.7	4.5	4.6	7.5
138	12.5	10.5	11.1	7.5	8.8	9.4
141	6.1	7.0	8.8	5.6	7.2	8.5
142	10.8	11.9	9.9	9.5	7.1	9.4
144	14.1	12.5	12.0	13.7	15.3	12.9
Mean	10.72	11.37	10.96	9.80	8.9[illegible]	9.61
s. d.	4.90	5.16	4.63	4.46	3.89	3.21
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.			n.s.	n.s.	n.s.

APPENDIX A - Table 10

Relative percentages of fibrinogen

Sham-operated					Ringer-lactate							Rheomacrodex						
Piglet No.	before	8	16	24	Piglet No.	before	after	pump	8	16	24	Piglet No	before	after	pump	8	16	24
84	22.1	24.0	25.0	23.3	65	29.0	26.1	26.1	28.2	28.1	23.2	114	23.4	26.5	28.1	22.8	26.2	27.0
86	20.9	25.9	32.5	26.1	70	30.3	32.8	33.1	28.1	31.7	29.3	115	20.4	22.2	22.0	20.3	28.5	22.9
96	27.4	29.5	29.5	31.4	71	28.1	29.4	29.9	30.2	31.4	32.7	116	28.6	26.5	26.5	28.1	27.4	28.3
103	35.4	35.0	34.4	34.5	74	27.8	28.8	34.0	31.7	30.7	28.5	117	25.5	27.9	29.0	33.3	31.8	32.1
106	25.9	25.8	26.4	27.1	76	29.9	30.8	29.5	29.0	28.2	28.3	122	21.6	25.6	24.5	26.2	25.4	26.0
123	26.6	27.3	27.2	29.5	79	30.1	29.7	30.6	26.4	32.0	27.4	125	30.0	32.3	30.3	31.8	31.5	32.0
128	27.0	29.3	28.9	30.1	80	27.6	25.9	27.1	28.9	27.2	28.4	127	26.6	27.9	28.1	25.3	30.2	30.3
130	25.5	29.7	27.2	24.9	82	25.8	26.4	25.2	25.7	30.7	28.1	129	30.8	28.3	30.8	29.8	28.3	28.3
132	27.5	25.9	31.9	30.7	83	26.5	26.4	29.0	28.2	33.0	33.2	131	27.3	25.3	28.6	27.4	30.1	32.2
134	29.0	29.1	33.7	33.7	85	26.6	28.5	26.5	28.1	23.9	26.2	133	32.8	30.9	32.6	27.9	28.6	29.9
137	31.5	33.6	35.6	31.6	95	32.3	28.3	30.3	30.9	28.5	31.9	136	29.0	30.3	31.7	35.2	35.1	32.2
139	30.3	29.2	31.0	29.1	98	28.8	35.2	29.7	32.8	31.1	31.3	138	29.1	30.1	29.2	29.7	30.5	31.7
140	35.7	30.6	33.1	34.3	99	30.3	29.1	31.1	34.3	29.5	34.3	141	34.4	34.6	27.7	28.2	29.4	31.3
143	30.5	30.5	31.2	32.4	102	33.4	31.8	32.5	34.2	33.9	29.8	142	27.0	24.8	25.2	26.3	29.1	27.1
145	36.9	36.3	35.0	36.5	105	25.8	23.1	24.1	25.4	29.4	28.2	144	26.0	28.1	28.3	24.6	24.6	26.7
Mean	28.80	29.44	30.82	30.34		28.84	28.81	29.24	29.48	29.95	29.39		27.48	28.09	28.17	27.80	29.11	29.20
s. d.	4.68	3.49	3.34	3.78		2.21	3.05	2.92	2.82	2.54	2.90		3.87	3.18	2.80	3.89	2.67	2.85
I		n.s.	n.s.	n.s.			n.s.		n.s.	n.s.	n.s.			n.s.		n.s.	n.s.	n.s.
S						n.s.			n.s.	n.s.	n.s.		n.s.			n.s.	n.s.	n.s.
R													n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 11

Albumin concentrations (g./100ml. plasma)

Sham-operated

Piglet No.	before	8	16	24
84	2.37	2.31	2.25	2.14
86	1.96	1.80	1.96	2.05
96	2.02	1.96	1.92	1.75
103	1.39	1.34	1.52	1.51
106	2.28	2.15	2.04	2.27
123	1.51	1.35	1.38	1.41
128	1.73	1.59	1.71	1.69
130	1.96	1.96	1.83	2.04
132	2.04	2.19	2.15	2.24
134	1.71	1.88	1.64	1.76
137	1.76	1.81	1.88	1.95
139	2.40	2.81	2.45	3.07
140	1.86	1.97	1.93	1.96
143	1.97	1.87	1.95	2.01
145	1.24	1.11	1.24	1.18
Mean	1.88	1.87	1.86	1.94
s.e.m.	0.09	0.11	0.08	0.11
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	2.17	1.84	1.84	1.95	1.68	1.60
70	1.63	1.37	1.33	1.65	1.68	1.66
71	1.60	1.66	1.61	2.13	1.60	1.70
74	1.93	1.49	1.37	1.75	1.91	1.75
76	1.70	1.33	1.38	1.59	1.69	1.71
79	1.98	1.33	1.38	1.84	1.80	1.89
80	2.18	1.69	1.60	1.66	1.88	2.02
82	1.82	1.55	1.63	1.72	1.47	1.76
83	1.80	1.35	1.40	1.89	1.69	1.48
85	2.27	1.35	1.48	2.11	2.17	2.15
95	2.04	1.47	1.48	1.96	1.97	2.29
98	1.99	1.61	1.76	1.84	1.83	2.28
99	1.55	1.16	1.21	1.28	1.48	1.42
102	2.14	1.55	1.57	1.74	1.59	1.91
105	2.29	1.52	1.52	1.95	1.95	1.92
Mean	1.94	1.49	1.50	1.80	1.76	1.84
s.e.m.	0.07	0.04	0.04	0.05	0.05	0.07
I		***		n.s.	***	n.s.
S	n.s.			n.s.		n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	1.92	1.07	1.06	2.14	1.78	2.03
115	2.65	1.01	1.03	2.53	2.20	2.61
116	2.11	1.66	1.66	1.85	2.03	1.86
117	2.21	1.49	1.59	1.97	2.03	2.15
122	1.94	1.54	1.55	1.92	1.78	1.81
125	2.15	1.43	1.51	1.95	1.89	2.03
127	1.88	1.26	1.24	1.49	1.49	1.70
129	2.28	1.35	1.34	2.04	2.27	2.30
131	2.15	1.22	1.14	2.06	2.10	2.02
133	1.73	1.09	1.07	2.14	2.02	1.89
136	2.15	1.54	1.52	1.89	2.21	1.92
138	2.47	1.86	1.83	2.99	2.62	2.68
141	2.24	1.77	1.98	2.77	2.11	2.10
142	2.10	1.57	1.61	2.50	2.37	2.19
144	1.39	1.28	1.31	1.51	1.60	1.64
Mean	2.09	1.41	1.43	2.12	2.03	2.06
s.e.m.	0.08	0.07	0.08	0.11	0.07	0.08
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	**			n.s.	n.s.

APPENDIX A - Table 12

Alpha globulin concentrations (g./100ml. plasma)

Sham-operated

Piglet No.	before	8	16	24
84	0.71	0.74	0.84	0.80
86	0.80	0.84	0.97	0.97
96	1.03	1.07	1.08	1.22
103	1.03	0.96	1.06	1.01
106	0.73	0.94	0.74	0.92
123	1.22	1.21	1.26	1.15
128	0.85	0.92	0.95	0.91
130	0.98	0.99	1.06	1.06
132	1.05	1.02	0.99	0.95
134	1.16	1.13	1.19	1.26
137	1.03	0.99	0.93	1.11
139	0.77	0.96	0.97	0.89
140	1.17	1.06	1.02	1.13
143	1.03	1.23	1.22	1.14
145	1.31	1.18	1.54	1.41
Mean	0.99	1.02	1.06	1.06
s.e.m.	0.05	0.04	0.05	0.04
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	0.83	0.49	0.49	0.75	0.77	1.26
70	0.79	0.69	0.71	0.84	0.91	1.04
71	0.97	0.66	0.68	0.70	0.98	1.03
74	1.05	0.77	0.74	0.82	0.96	1.04
76	1.01	0.69	0.67	0.75	0.84	0.94
79	0.77	0.60	0.53	0.59	0.82	0.82
80	0.81	0.55	0.58	0.58	0.65	0.64
82	0.82	0.65	0.63	0.62	0.68	0.74
83	0.70	0.57	0.55	0.56	0.69	0.84
85	0.70	0.58	0.57	0.70	0.71	0.87
95	1.04	0.79	0.73	0.93	1.06	1.10
98	1.04	0.93	0.88	0.97	1.09	1.02
99	0.88	0.79	0.78	0.87	0.80	0.90
102	0.62	0.65	0.66	0.79	0.76	0.82
105	0.85	0.91	0.88	0.89	0.89	0.87
Mean	0.86	0.69	0.67	0.76	0.84	0.93
s.e.m.	0.04	0.03	0.03	0.03	0.04	0.04
I		***		**	n.s.	n.s.
S	n.s.		***	**		n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	1.24	0.66	0.68	0.99	0.87	0.98
115	0.86	0.40	0.39	0.76	0.79	0.84
116	0.67	0.51	0.52	0.69	0.57	0.76
117	0.91	0.85	0.81	0.66	0.66	0.75
122	1.19	0.85	0.83	1.12	1.36	1.27
125	0.67	0.45	0.45	0.79	0.87	0.90
127	0.73	0.55	0.56	0.82	0.82	0.93
129	0.87	0.58	0.58	1.07	1.03	0.93
131	0.66	0.46	0.50	0.73	0.86	0.94
133	1.22	0.70	0.72	1.30	1.30	1.21
136	1.13	0.96	0.84	1.11	0.78	1.16
138	0.64	0.50	0.55	0.85	0.83	0.82
141	1.11	0.73	0.74	0.74	0.94	1.01
142	0.93	0.61	0.59	0.93	1.00	1.05
144	0.87	0.67	0.72	1.12	0.86	0.90
Mean	0.91	0.63	0.63	0.91	0.90	0.96
s.e.m.	0.06	0.04	0.04	0.05	0.06	0.04
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 13

Beta globulin concentrations (g./100ml./plasma)

Sham-operated

Piglet No.	before	8	16	24
84	0.26	0.23	0.24	0.24
86	0.48	0.30	0.32	0.32
96	0.47	0.27	0.33	0.30
103	0.47	0.48	0.50	0.51
106	0.22	0.28	0.25	0.28
123	0.30	0.31	0.26	0.29
128	0.36	0.37	0.29	0.29
130	0.20	0.24	0.26	0.18
132	0.25	0.24	0.24	0.22
134	0.25	0.27	0.30	0.20
137	0.30	0.29	0.29	0.35
139	0.27	0.28	0.31	0.26
140	0.25	0.24	0.28	0.30
143	0.19	0.39	0.30	0.26
145	0.40	0.55	0.48	0.49
Mean	0.30	0.32	0.31	0.30
s.e.m.	0.02	0.02	0.02	0.02
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	0.19	0.12	0.12	0.21	0.19	0.16
70	0.31	0.27	0.23	0.37	0.35	0.41
71	0.30	0.24	0.24	0.23	0.27	0.31
74	0.40	0.31	0.25	0.34	0.40	0.32
76	0.36	0.26	0.25	0.26	0.29	0.34
79	0.22	0.22	0.19	0.25	0.20	0.30
80	0.23	0.20	0.21	0.17	0.27	0.28
82	0.22	0.16	0.16	0.20	0.21	0.15
83	0.21	0.16	0.19	0.11	0.12	0.18
85	0.24	0.15	0.28	0.17	0.26	0.31
95	0.34	0.29	0.25	0.32	0.40	0.45
98	0.25	0.16	0.27	0.26	0.23	0.25
99	0.30	0.25	0.27	0.19	0.25	0.26
102	0.25	0.31	0.25	0.29	0.28	0.33
105	0.32	0.28	0.25	0.36	0.33	0.32
Mean	0.28	0.23	0.23	0.25	0.27	0.29
s.e.m.	0.02	0.02	0.01	0.02	0.02	0.02
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	0.35	0.16	0.16	0.23	0.21	0.25
115	0.29	0.11	0.12	0.35	0.19	0.30
116	0.18	0.16	0.16	0.23	0.35	0.18
117	0.24	0.24	0.24	0.34	0.22	0.23
122	0.23	0.15	0.15	0.26	0.23	0.24
125	0.19	0.23	0.21	0.20	0.19	0.12
127	0.29	0.20	0.21	0.22	0.19	0.28
129	0.30	0.18	0.22	0.27	0.25	0.30
131	0.26	0.17	0.13	0.21	0.27	0.22
133	0.23	0.17	0.14	0.35	0.35	0.26
136	0.36	0.28	0.27	0.39	0.31	0.34
138	0.17	0.18	0.17	0.21	0.25	0.24
141	0.14	0.10	0.11	0.16	0.21	0.22
142	0.16	0.15	0.16	0.19	0.24	0.25
144	0.33	0.24	0.17	0.11	0.26	0.29
Mean	0.24	0.18	0.18	0.25	0.25	0.25
s.e.m.	0.02	0.01	0.01	0.02	0.01	0.01
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 14

Gamma globulin concentrations (g./100ml.plasma)

Sham-operated

Piglet No.	before	8	16	24
84	0.61	0.42	0.27	0.39
86	1.08	1.07	0.75	1.02
96	0.53	0.71	0.88	0.78
103	0.53	0.70	0.55	0.57
106	0.22	0.22	0.15	0.14
123	0.59	0.71	0.60	0.59
128	0.79	0.80	0.73	0.75
130	0.35	0.22	0.28	0.32
132	0.97	0.86	0.63	0.70
134	0.24	0.28	0.26	0.14
137	0.45	0.48	0.38	0.34
139	0.50	0.44	0.40	0.60
140	0.42	0.56	0.50	0.45
143	0.38	0.34	0.36	0.37
145	0.18	0.20	0.23	0.21
Mean	0.54	0.53	0.47	0.49
s.e.m.	0.07	0.07	0.06	0.06
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	0.46	0.43	0.43	0.49	0.37	0.36
70	1.18	0.92	0.93	1.28	1.01	0.97
71	1.07	0.69	0.68	0.59	0.62	0.63
74	0.49	0.32	0.31	0.32	0.35	0.40
76	0.28	0.31	0.32	0.46	0.49	0.49
79	0.43	0.48	0.50	0.47	0.39	0.43
80	0.45	0.42	0.39	0.29	0.31	0.29
82	0.53	0.34	0.30	0.29	0.32	0.28
83	0.50	0.42	0.26	0.24	0.11	0.33
85	1.18	1.03	0.90	0.80	0.88	0.80
95	0.71	0.54	0.52	0.43	0.54	0.39
98	1.17	0.76	0.85	0.75	0.82	0.70
99	1.26	1.03	0.87	0.95	0.89	0.94
102	0.62	0.63	0.63	0.62	0.71	0.71
105	0.52	0.39	0.39	0.40	0.40	0.46
Mean	0.72	0.58	0.55	0.56	0.55	0.55
s.e.m.	0.09	0.05	0.06	0.07	0.07	0.06
I		***		**	***	***
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	0.28	0.21	0.15	0.25	0.19	0.17
115	0.41	0.17	0.17	0.38	0.24	0.56
116	0.25	0.19	0.19	0.21	0.20	0.29
117	0.48	0.63	0.53	0.43	0.39	0.44
122	0.77	0.52	0.58	0.63	0.56	0.61
125	0.72	0.49	0.50	0.56	0.56	0.54
127	0.77	0.52	0.51	0.67	0.51	0.57
129	0.66	0.72	0.58	0.88	0.74	0.73
131	1.40	0.88	0.83	1.10	1.03	1.06
133	0.34	0.23	0.21	0.44	0.38	0.32
136	0.29	0.20	0.29	0.25	0.25	0.42
138	0.70	0.45	0.47	0.48	0.54	0.60
141	0.36	0.31	0.39	0.31	0.37	0.47
142	0.55	0.43	0.36	0.53	0.40	0.51
144	0.60	0.46	0.44	0.61	0.69	0.60
Mean	0.57	0.43	0.41	0.52	0.47	0.53
s.e.m.	0.07	0.05	0.05	0.06	0.06	0.06
I		***		n.s.	**	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 15

Fibrinogen concentrations (g./100ml./plasma)

Sham-operated

Piglet No.	before	8	16	24
84	1.12	1.17	1.20	1.08
86	1.14	1.40	1.93	1.54
96	1.54	1.68	1.76	1.86
103	1.88	1.87	1.90	1.89
106	1.21	1.24	1.14	1.34
123	1.31	1.34	1.31	1.44
128	1.38	1.52	1.49	1.56
130	1.20	1.44	1.28	1.19
132	1.63	1.51	1.88	1.82
134	1.37	1.46	1.72	1.70
137	1.63	1.80	1.92	1.73
139	1.71	1.85	1.86	1.98
140	2.05	1.69	1.85	2.00
143	1.57	1.68	1.74	1.81
145	1.83	1.73	1.88	1.90
Mean	1.51	1.56	1.66	1.66
s.e.m.	0.07	0.06	0.07	0.07
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	1.49	1.02	1.02	1.33	1.18	1.02
70	1.70	1.58	1.58	1.62	1.83	1.69
71	1.54	1.35	1.37	1.58	1.59	1.78
74	1.49	1.17	1.38	1.50	1.60	1.40
76	1.43	1.15	1.10	1.25	1.29	1.38
79	1.46	1.11	1.14	1.13	1.52	1.30
80	1.40	1.00	1.03	1.09	1.16	1.28
82	1.17	0.96	0.91	0.98	1.19	1.15
83	1.16	0.90	0.98	1.10	1.29	1.40
85	1.59	1.24	1.17	1.48	1.26	1.46
95	1.97	1.22	1.30	1.63	1.58	1.98
98	1.80	1.88	1.59	1.87	1.79	1.94
99	1.74	1.32	1.40	1.72	1.44	1.84
102	1.82	1.46	1.49	1.78	1.71	1.60
105	1.39	0.93	0.96	1.23	1.49	1.41
Mean	1.48	1.22	1.23	1.42	1.46	1.51
s.e.m.	0.09	0.07	0.06	0.07	0.05	0.07
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	1.12	0.76	0.80	1.07	1.08	1.27
115	1.08	0.48	0.48	1.02	1.36	1.28
116	1.29	0.91	0.92	1.16	1.19	1.22
117	1.31	1.24	1.30	1.70	1.54	1.69
122	1.14	1.06	1.01	1.40	1.34	1.38
125	1.60	1.24	1.16	1.64	1.61	1.69
127	1.33	0.98	0.99	1.08	1.30	1.42
129	1.83	1.12	1.21	1.81	1.69	1.68
131	1.67	0.93	1.04	1.55	1.84	2.02
133	1.72	0.98	1.03	1.64	1.62	1.56
136	1.61	1.30	1.36	1.95	1.92	1.82
138	1.63	1.29	1.25	1.91	1.85	2.01
141	2.02	1.54	1.24	1.57	1.51	1.73
142	1.39	0.91	0.92	1.48	1.64	1.49
144	1.11	1.04	1.04	1.09	1.12	1.25
Mean	1.46	1.05	1.05	1.47	1.51	1.57
s.e.m.	0.07	0.06	0.05	0.08	0.07	0.07
I		***		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 16

Plasma Osmolarity (mOsml./1.)

Sham-operated

Piglet No.	before	8	16	24
84	305	310	312	318
86	298	302	308	310
96	302	300	304	313
103	289	290	296	293
106	298	302	307	309
123	297	304	306	309
128	290	290	298	308
130	308	310	314	318
132	304	311	314	318
134	293	294	304	303
137	300	304	308	312
139	291	301	305	312
140	286	295	295	313
143	290	296	304	305
145	288	308	306	308
Mean	295.9	301	305.4	309.9
s.e.m.	1.8	1.8	1.5	1.7
I		***	***	***
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	297	297	297	299	303	308
70	295	293	293	295	300	302
71	293	289	289	292	296	298
74	306	303	303	306	308	308
76	302	298	300	296	302	308
79	298	298	300	302	305	308
80	305	298	300	302	300	310
82	298	293	295	298	303	306
83	297	296	296	298	303	306
85	292	285	285	292	292	302
95	298	290	289	305	308	310
98	305	295	295	299	305	310
99	306	301	303	301	305	310
102	293	290	289	292	296	293
105	302	300	299	304	302	310
Mean	299.1	295.1	295.5	298.7	301.9	305.9
s.e.m.	1.3	1.3	1.5	1.2	1.2	1.3
I		[illegible]		n.s.	**	***
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	303	284	284	305	307	307
115	304	291	290	305	304	304
116	287	280	284	303	305	310
117	292	290	290	290	297	304
122	297	295	295	304	306	309
125	295	295	295	302	307	312
127	286	288	288	293	299	308
129	307	305	304	308	314	318
131	301	296	296	312	320	325
133	297	293	294	296	299	308
136	295	289	289	300	307	310
138	299	288	288	299	310	309
141	289	287	288	288	297	313
142	285	281	281	290	299	302
144	295	291	291	297	302	305
Mean	295.5	290.5	290.5	299.5	304.9	309.6
s.e.m.	1.7	1.5	1.5	1.8	1.3	1.5
I	**	**		**	***	***
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 17

Plasma sodium concentrations (mEq/1.)

Sham-operated					Ringer-lactate							Rheomacrodex						
Piglet No.	before	8	16	24	Piglet No.	before	after	pump	8	16	24	Piglet No	before	after	pump	8	16	24
84	140	147	146	140	65	152	139	146	149	151	154	114	153	148	149	153	154	155
86	160	156	153	154	70	146	146	141	145	146	149	115	146	153	146	149	150	150
96	147	148	153	152	71	145	145	142	145	146	147	116	149	151	147	148	151	151
103	127	148	148	148	74	151	146	146	147	158	158	117	148	146	145	146	150	152
106	152	150	156	153	76	152	150	146	148	159	156	122	159	159	159	159	169	169
123	156	154	162	165	79	142	145	139	145	147	149	125	154	149	149	154	155	154
128	152	152	155	156	80	147	145	144	147	149	153	127	152	154	150	150	153	156
130	160	161	163	158	82	157	153	153	150	162	149	129	160	153	153	153	155	155
132	152	156	156	161	83	160	150	152	150	151	147	131	152	155	151	152	155	159
134	163	166	166	166	85	147	142	146	140	143	149	133	161	156	154	156	159	161
137	150	155	163	165	95	147	146	144	146	146	154	136	153	155	155	156	164	162
139	159	160	162	162	98	154	154	154	154	155	157	138	160	159	156	160	163	162
140	156	160	163	167	99	160	152	151	151	154	150	141	158	158	164	160	164	168
143	161	166	165	170	102	151	156	149	149	149	152	142	157	154	151	159	158	160
145	144	145	148	151	105	156	152	152	149	151	153	144	147	147	147	151	152	155
Mean	151.9	154.9	157.3	157.9		151.1	148.1	147.0	147.7	151.1	151.8		153.9	153.1	151.7	153.7	156.8	157.9
s.e.m.	2.5	1.7	1.7	2.1		1.4	1.2	1.2	0.9	1.4	0.9		1.3	1.0	1.3	1.2	1.5	1.5
I		n.s.	**	**			n.s.		**	n.s.	n.s.			n.s.		n.s.	**	**
S						n.s.			**	**	**		n.s.			n.s.	n.s.	n.s.
R													n.s.	***		***	**	**

APPENDIX A - Table 18

Plasma potassium concentrations (mEq./1.)

Sham-operated

Piglet No.	before	8	16	24
84	4.3	5.1	4.8	3.9
86	3.7	5.1	5.4	3.5
96	4.6	3.8	3.8	4.0
103	3.6	3.5	3.9	3.7
[illegible]	4.2	3.8	3.8	4.2
123	3.9	3.6	3.6	3.5
128	4.0	3.8	4.2	3.8
130	4.0	4.2	3.3	3.8
132	4.0	4.6	3.5	3.6
134	4.5	4.4	4.0	3.6
137	3.7	4.1	4.4	4.5
139	3.7	3.3	3.2	3.9
140	3.9	3.2	3.5	3.3
143	3.8	3.5	3.9	3.3
145	3.8	3.8	3.5	3.6
Mean	4.0	4.0	3.9	3.8
s.e.m.	0.1	0.2	0.2	0.1
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	3.8	4.9	3.7	4.9	3.4	3.4
70	3.9	3.4	3.5	5.6	3.2	3.3
71	4.6	4.3	3.9	5.5	3.7	5.1
74	3.6	4.1	4.1	6.6	3.2	3.2
76	4.5	4.4	4.4	5.5	3.7	3.6
79	5.7	3.5	5.5	3.5	3.3	3.6
80	3.9	5.9	5.1	4.4	3.3	3.6
82	5.5	3.7	5.0	3.8	3.9	3.7
83	5.7	5.0	5.0	3.8	3.7	3.6
85	3.7	4.2	4.2	3.6	6.5	3.9
95	5.2	5.0	5.4	4.1	3.6	3.8
98	4.2	4.1	4.1	5.2	4.0	3.5
99	4.1	4.6	4.1	5.5	3.7	3.5
102	4.3	4.3	4.0	3.4	3.4	3.9
105	4.3	[illegible]	4.1	3.6	3.4	3.7
Mean	4.5	4.4	4.4	4.6	3.7	3.7
s.e.m.	0.2	0.2	0.2	0.2	0.2	0.1
I		n.s.		n.s.	**	**
S	n.s.			**	n.s.	n.s.
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	4.8	4.8	4.4	3.6	3.6	6.4
115	6.0	4.2	4.1	3.8	3.2	5.8
116	4.0	4.0	4.1	3.9	3.8	3.7
117	4.0	4.3	4.3	4.4	3.9	3.3
122	4.5	4.5	4.4	3.7	3.7	3.8
125	3.7	3.9	4.0	3.5	3.3	3.3
127	3.9	4.0	3.9	3.4	3.6	3.7
129	3.6	3.7	3.6	4.1	3.1	3.1
131	3.7	4.0	4.0	3.9	3.7	3.6
133	4.2	3.9	3.9	4.1	4.2	3.7
136	4.3	3.3	3.4	4.0	4.0	3.7
138	3.5	4.0	3.7	3.4	3.0	3.1
141	4.0	3.9	4.5	3.2	2.7	3.1
142	4.1	4.2	4.3	3.7	4.0	3.6
144	3.7	4.1	4.1	3.8	3.6	3.6
Mean	4.1	4.0	4.0	3.8	3.6	3.8
s.e.m.	0.2	0.1	0.1	0.1	0.1	0.2
I		n.s.		n.s.	**	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		**	n.s.	n.s.

APPENDIX A - Table 19

Plasma chloride concentrations (mEq./1.)

Sham-operated

Piglet No.	before	8	16	24
84	90	90	95	89
86	109	108	110	110
96	104	105	109	111
103	94	100	105	105
106	106	102	111	110
123	102	96	101	104
128	100	105	106	108
130	96	101	100	100
132	98	97	100	102
134	100	100	101	101
137	102	109	114	117
139	104	110	115	112
140	95	100	103	106
143	95	101	103	106
145	106	107	111	112
Mean	100.1	102.1	105.6	106.2
s.e.m.	1.4	1.4	1.5	1.7
I		n.s.	**	**
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	91	90	90	94	91	95
70	88	87	87	90	93	96
71	92	90	90	90	94	93
74	90	91	91	87	96	96
76	88	89	90	89	92	92
79	93	90	90	92	94	98
80	90	92	91	92	93	98
82	106	102	105	103	111	102
83	109	104	104	102	105	100
85	96	101	100	98	102	105
95	107	106	105	104	103	112
98	108	107	108	107	105	112
99	106	107	105	103	106	101
102	101	102	100	100	105	110
105	110	107	109	104	108	112
Mean	98.3	97.7	97.7	97.0	99.9	101.5
s.e.m.	2.2	1.9	1.8	1.7	1.7	1.8
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			**	**	**
R						

Rheomacrodex

Piglet No	before	after	pump	8	16	24
114	102	109	109	106	108	111
115	103	109	108	104	107	108
116	105	108	104	108	106	109
117	105	106	116	103	107	108
122	100	100	106	100	103	101
125	101	102	102	103	104	104
127	100	104	99	98	100	105
129	103	105	102	105	106	106
131	98	103	104	102	107	108
133	101	103	105	102	101	107
136	100	121	117	104	112	110
138	102	105	100	105	108	112
141	96	100	100	103	107	110
142	99	102	102	100	103	105
144	100	102	102	100	108	107
Mean	101.0	105.3	105.0	102.9	105.8	107.4
s.e.m.	0.7	1.3	1.4	0.7	0.8	0.8
I		**		**	***	***
S	n.s.	n.s.		n.s.	n.s.	n.s.
R	n.s.	***		**	**	**

APPENDIX A - Table 20

Plasma calcium concentrations (mEq./1.)

Sham-operated

Piglet No.	before	8	16	24
84	4.6	4.4	4.4	4.3
86	5.3	4.5	5.4	4.4
96	4.8	4.6	5.0	5.0
103	5.0	4.4	4.7	4.8
106	5.2	4.9	5.0	5.0
123	4.5	3.8	3.5	4.3
128	5.0	5.0	4.7	3.9
130	4.8	5.0	5.0	4.9
132	5.5	4.8	5.0	5.5
134	4.5	5.3	5.0	4.9
137	5.0	5.0	5.3	5.7
139	5.7	5.3	5.8	5.7
140	5.2	5.4	5.7	4.4
143	5.3	5.5	6.2	5.6
145	5.3	4.2	5.2	4.7
Mean	5.0	4.8	5.1	4.9
s.e.m.	0.1	0.1	0.2	0.2
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	pump	8	16	24
65	5.1	3.7	4.0	4.5	2.9	3.0
70	5.3	4.9	5.0	5.4	5.3	5.3
71	4.8	4.8	4.8	4.8	4.8	4.2
74	4.6	3.3	3.5	4.4	4.2	3.5
76	4.6	4.6	4.5	5.2	5.3	4.9
79	5.1	4.6	4.6	5.0	4.1	4.4
80	5.0	4.4	4.4	5.0	4.9	3.7
82	5.4	4.1	4.8	5.3	4.6	5.1
83	5.6	5.3	4.9	4.7	4.4	4.6
85	5.1	4.8	4.4	4.7	4.7	4.5
95	5.0	4.7	4.5	4.5	4.6	4.4
98	5.9	5.9	5.9	6.0	5.3	5.6
99	5.4	4.9	4.7	5.4	5.0	4.9
102	5.4	5.4	5.4	5.5	5.1	5.4
105	5.2	4.7	4.7	4.9	4.9	4.7
Mean	5.2	4.7	4.7	5.0	4.7	4.6
s.e.m.	0.1	0.2	0.1	0.1	0.2	0.2
I		**		n.s.	**	**
S	n.s.			n.s.	n.s.	n.s.
R						

Rheomacrodex

Piglet No.	before	after	pump	8	16	24
114	5.6	4.7	4.5	4.8	4.0	3.3
115	5.1	4.5	4.4	5.0	4.1	3.1
116	5.0	4.6	4.7	3.4	3.6	5.0
117	5.2	4.7	4.7	4.1	3.6	4.5
122	4.6	4.6	4.6	4.4	4.5	4.6
125	4.8	4.6	4.3	4.7	5.0	5.0
127	4.7	4.8	4.9	4.5	5.0	5.1
129	6.2	5.8	5.7	6.2	6.1	5.8
131	6.4	5.1	6.0	5.8	5.3	5.9
133	5.0	5.1	5.2	4.6	5.3	4.9
136	4.9	4.5	4.5	5.0	4.9	5.2
138	5.6	5.6	5.4	5.6	5.8	5.9
141	6.2	5.3	5.5	6.3	5.7	5.8
142	5.6	5.8	5.5	5.8	5.5	5.7
144	4.8	4.9	5.1	5.1	4.8	4.9
Mean	5.3	5.0	4.9	5.0	4.9	5.0
s.e.m.	0.2	0.1	0.1	0.2	0.2	0.2
I		n.s.		n.s.	n.s.	n.s.
S	n.s.			n.s.	n.s.	n.s.
R	n.s.	n.s.		n.s.	n.s.	n.s.

APPENDIX A - Table 21

Mean arterial blood pressure (mm.Hg.)

Sham-operated

Piglet No.	before	8	16	24
84	87	95	90	87
86	80	100	85	100
96	95	70	95	85
103	80	110	110	100
106	70	90	95	90
123	90	95	105	85
128	95	95	98	95
130	70	75	80	70
132	105	90	90	90
134	85	100	95	95
137	85	85	75	75
139	85	100	85	92
140	85	75	65	75
143	85	110	115	110
145	75	80	85	80
Mean	85	91	91	89
s.d.	9	12	13	11
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	8	16	24
65	70	70	85	90	110
70	75	65	75	75	87
71	105	85	90	82	95
74	65	80	93	100	80
76	85	105	69	45	67
79	65	50	55	75	110
80	80	60	55	90	85
82	70	70	80	90	110
83	90	72	70	85	115
85	87	50	55	75	115
95	85	67	80	80	85
98	85	75	60	80	95
99	65	70	65	80	95
102	75	70	90	85	90
105	67	70	95	85	75
Mean	78	71	74	81	94
s.d.	12	13	15	12	15
I		n.s.	n.s.	n.s.	**
S	n.s.			n.s.	n.s.
R					

Rheomacrodex

Piglet No.	before	after	8	16	24
114	95	67	50	90	100
115	75	67	70	80	90
116	105	85	90	100	87
117	85	80	95	95	87
122	75	85	57	100	92
125	90	85	82	83	85
127	80	90	90	80	80
129	70	60	70	90	75
131	85	80	120	83	88
133	75	65	80	85	75
136	100	95	80	75	75
138	60	65	80	85	100
141	75	75	85	75	85
142	75	90	85	92	90
144	75	85	80	80	80
Mean	81	78	81	86	86
s.d.	12	11	16	8	6
I		n.s.	n.s.	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	n.s.

APPENDIX A - Table 22

Heart rate (beats per minute)

Sham-operated

Piglet No.	before	8	16	24
84	186	156	162	153
86	210	225	222	258
96	168	188	204	180
103	204	210	210	218
106	196	228	198	228
123	186	198	192	180
128	264	246	186	168
130	207	198	198	192
132	276	204	174	218
134	198	210	180	180
137	216	224	240	210
139	228	228	216	240
140	185	165	156	192
143	192	240	250	210
145	222	174	192	174
Mean	209	206	199	200
s.d.	29	27	26	29
I		n.s.	n.s.	n.s.
S				
R				

Ringer-lactate

Piglet No.	before	after	8	16	24
65	186	186	216	216	210
70	192	204	234	204	186
71	252	264	254	210	228
74	198	204	224	264	198
76	192	230	234	225	210
79	192	186	174	170	198
80	198	198	214	216	192
82	210	192	200	210	198
83	210	210	255	222	196
85	212	186	174	156	156
95	204	204	240	196	204
98	204	172	240	234	198
99	192	198	268	180	168
102	184	198	222	192	174
105	180	192	210	180	186
Mean	200	202	224	205	193
s.d.	17	22	27	27	18
I		n.s.	**	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.

Rheomacrodex

Piglet No.	before	after	8	16	24
114	198	192	180	204	180
115	192	204	234	204	186
116	252	264	254	216	210
117	246	270	252	228	198
122	186	240	174	222	222
125	252	288	198	200	210
127	180	205	252	192	210
129	198	258	240	228	198
131	192	240	210	204	210
133	156	192	234	204	228
136	198	204	222	240	234
138	198	258	258	234	234
141	185	190	288	228	246
142	204	230	290	198	302
144	210	240	204	204	198
Mean	203	232	233	214	218
s.d.	27	31	35	15	29
I		***	**	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	**	n.s.	n.s.	n.s.

APPENDIX A - Table 23

Rectal Temperature (°C)

Piglet No.	Sham-operated before	8	16	24	Piglet No.	Ringer-lactate before	after	8	16	24	Piglet No.	Rheomacrodex before	after	8	16	24
84	38.5	38.3	38.4	38.5	65	38.6	38.6	38.0	38.2	38.4	114	38.2	37.5	38.5	38.5	38.7
86	38.5	38.5	38.3	39.0	70	38.0	37.0	38.3	38.5	38.5	115	38.3	37.7	38.5	38.5	38.2
96	38.3	38.4	38.5	38.5	71	38.0	37.5	37.5	38.0	38.0	116	39.0	38.5	38.5	38.5	38.7
103	38.0	38.5	38.4	38.5	74	38.8	38.0	37.7	38.5	38.0	117	38.0	37.5	38.2	38.3	38.5
106	38.5	38.8	38.5	38.4	76	38.0	37.3	37.6	37.8	37.8	122	38.5	38.0	38.5	38.5	39.0
123	38.5	38.5	38.5	39.5	79	38.8	38.0	37.7	38.3	38.5	125	39.5	39.0	39.5	39.5	39.5
128	39.5	40.0	39.6	39.8	80	38.0	37.5	38.5	38.3	38.2	127	39.5	39.0	39.4	40.0	40.0
130	37.2	38.0	37.2	37.3	82	38.0	37.3	38.3	38.2	38.4	129	37.5	36.6	37.3	37.5	37.5
132	37.8	37.0	37.6	39.0	83	37.3	37.5	38.0	38.5	38.5	131	37.6	36.5	38.5	37.0	37.5
134	37.8	38.0	38.5	37.0	85	38.5	38.0	37.7	38.5	38.3	133	37.5	36.5	38.0	38.5	37.0
137	37.8	38.0	38.0	38.0	95	37.5	37.3	37.8	37.5	38.0	136	38.0	37.0	38.5	38.0	38.0
139	38.5	39.5	38.0	38.0	98	38.3	37.8	38.0	38.5	38.5	138	38.0	37.5	39.5	38.0	38.0
140	38.5	38.6	38.0	38.5	99	38.0	36.8	37.5	38.0	38.5	141	38.5	38.5	38.0	38.0	38.1
143	38.3	38.3	39.0	38.5	102	39.0	38.0	38.5	38.5	38.7	142	38.0	38.0	38.0	39.0	38.0
145	38.0	38.0	38.0	38.0	105	38.3	37.8	38.8	38.4	38.0	144	38.5	38.5	38.0	38.0	38.0
Mean	38.2	38.4	38.3	38.4		38.2	37.6	38.0	38.2	38.3		38.3	37.8	38.5	38.4	38.5
s.d.	0.5	0.7	0.6	0.7		0.4	0.5	0.4	0.3	0.3		0.7	0.9	0.6	0.7	0.8
I		n.s.	n.s.	n.s.			***	n.s.	n.s.	n.s.			***	n.s.	n.s.	n.s.
S						n.s.		n.s.	n.s.	n.s.		n.s.		n.s.	n.s.	n.s.
R												n.s.	n.s.	n.s.	n.s.	n.s.

APPENDIX B - Table 1

Sham-operated group. Mean values and standard errors of the mean of the circulating amounts of plasma proteins (g/kg body weight).

	Initial	8 hours	16 hours	24 hours
Total circulating proteins	3.34 ± 0.08	3.03 ± 0.08	3.17 ± 0.13	3.27 ± 0.12
I		***	n.s.	n.s.
Albumin	1.20 ± 0.05	1.07 ± 0.06	1.08 ± 0.04	1.15 ± 0.06
I		***	**	n.s.
Alpha globulin	0.64 ± 0.04	0.59 ± 0.03	0.63 ± 0.05	0.64 ± 0.03
I		n.s.	n.s.	n.s.
Beta globulin	0.19 ± 0.02	0.18 ± 0.02	0.19 ± 0.02	0.18 ± 0.02
I		n.s.	n.s.	n.s.
Gamma globulin	0.34 ± 0.04	0.30 ± 0.04	0.28 ± 0.03	0.30 ± 0.04
I		n.s.	n.s.	n.s.
Fibrinogen	0.97 ± 0.06	0.89 ± 0.04	0.99 ± 0.06	1.00 ± 0.06
I		n.s.	n.s.	n.s.

I, S, R; comparison with Initial value, Sham group and Ringer-lactate group.

APPENDIX B - Table 2

Ringer-lactate group. Mean values and standard errors of the mean of the circulating amounts of plasma proteins (g/kg body weight).

	Initial	After	8 hours	16 hours	24 hours
Total circulating proteins	3.42 ± 0.09	2.14 ± 0.09	2.68 ± 0.10	2.97 ± 0.12	3.18 ± 0.11
I		***	***	**	n.s.
S	n.s.		n.s.	n.s.	n.s.
Albumin	1.24 ± 0.04	0.76 ± 0.03	1.03 ± 0.05	1.07 ± 0.04	1.14 ± 0.04
I		***	***	**	**
S	n.s.		n.s.	n.s.	n.s.
Alpha globulin	0.55 ± 0.02	0.35 ± 0.02	0.42 ± 0.02	0.51 ± 0.02	0.58 ± 0.03
I		***	***	n.s.	n.s.
S	n.s.		***	n.s.	n.s.
Beta globulin	0.18 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
I		***	***	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
Gamma globulin	0.46 ± 0.06	0.30 ± 0.04	0.31 ± 0.04	0.36 ± 0.05	0.34 ± 0.04
I		***	***	**	**
S	n.s.		n.s.	n.s.	n.s.
Fibrinogen	0.99 ± 0.04	0.62 ± 0.04	0.80 ± 0.04	0.86 ± 0.06	0.94 ± 0.04
I		***	***	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.

APPENDIX B (cont'd) - Table 3

Rheomacrodex group. Mean values and standard errors of the mean of the circulating amounts of plasma proteins (g/kg body weight).

	Initial	After	8 hours	16 hours	24 hours
Total circulating proteins	3.35 ± 0.09	2.07 ± 0.11	2.99 ± 0.12	3.06 ± 0.14	3.22 ± 0.09
I		***	***	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	n.s.
Albumin	1.32 ± 0.04	0.79 ± 0.04	1.20 ± 0.06	1.21 ± 0.05	1.23 ± 0.04
I		***	n.s.	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	**
Alpha globulin	0.58 ± 0.04	0.36 ± 0.03	0.52 ± 0.03	0.54 ± 0.04	0.58 ± 0.03
I		***	n.s.	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	**	n.s.	n.s.
Beta globulin	0.15 ± 0.01	0.10 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
I		***	n.s.	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	n.s.
Gamma globulin	0.36 ± 0.04	0.24 ± 0.03	0.29 ± 0.04	0.28 ± 0.03	0.31 ± 0.03
I		***	**	**	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	n.s.
Fibrinogen	0.93 ± 0.05	0.59 ± 0.04	0.84 ± 0.05	0.90 ± 0.05	0.94 ± 0.04
I		***	**	n.s.	n.s.
S	n.s.		n.s.	n.s.	n.s.
R	n.s.	n.s.	n.s.	n.s.	n.s.

APPENDIX C - Table 1

The fall in plasma dextran concentration after hemodilution perfusion with 2% Rheomacrodex.

Piglet No.	g./100 ml. plasma			
	After	8 hours	16 hours	24 hours
114	0.59	0.08	0.05	None
115	0.74	0.11	0.06	"
116	0.37	0.05	0.03	"
117	0.44	0.06	0.03	"
122	0.43	0.07	0.04	"
125	0.52	0.06	0.04	"
127	0.44	0.04	0.03	"
219	0.55	0.05	0.03	"
131	0.71	0.12	0.07	"
133	0.54	0.07	0.05	"
136	0.50	0.05	0.04	"
138	0.46	0.05	0.03	"
141	0.40	0.04	0.03	"
142	0.45	0.05	0.03	"
144	0.27	0.03	0.00	"
Mean	0.49	0.06	0.04	"
s.d.	0.09	0.01	0.01