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Dubniks, Maris

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

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Department of Anaesthesiology and Intensive Care

Faculty of Medicine

Lund University

Sweden

ASPECTS OF FLUID THERAPY

An experimental study of the effects of systemic inflammation, microvascular permeability, blood pressure and plasma volume

Maris Dubniks

LUND UNIVERSITY

Faculty of Medicine

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Original studies

This thesis is based on the studies reported in the following papers, referred to in the text by respective Roman numerals (I-V):

I Dubniks M, Persson J, Grände P-O. Comparison of the plasma volume-expanding effect of 6% dextran 70, 5% albumin, and 6% HES 130/0.4 after hemorrhage in the guinea pig. Submitted for publication.

II Dubniks M, Persson J, Grände P-O Plasma volume expansion of 5% albumin, 4% gelatin, 6% HES 130/0.4, and normal saline under increased microvascular permeability in the rat. Intensive Care Med 2007; 33:293-9.

III Dubniks M, Persson J, Grände P-O. Effect of blood pressure on plasma volume loss in the rat under increased permeability. Intensive Care Med 2007; 33:2192-8.

IV Dubniks M, Grände P-O. Change in plasma volume from a state of hyper-, normo- or hypovolaemia with or without noradrenalin infusion in the rat – implications of the 2-pore theory for transcapillary fluid exchange. Submitted for publication.

V Dubniks M, Grände P-O. The effects of activated protein C and prostacyclin on arterial oxygenation and protein leakage in the lung and the gut under endotoxaemia in the rat. Acta Anaesthesiol Scand 2008; 52:381-7.

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Introduction

Hypovolaemia

Adequate circulating blood volume and optimal heart preload are essential determinants of stable circulation. Hypovolaemia implies a reduced circulating blood volume and is one of the most common reasons of circulatory instability in surgical and critically ill patients. Hypovolaemia can be absolute or relative. Absolute hypovolaemia results from haemorrhage, external or internal fluid losses. Internal fluid loss because of increased microvascular permeability is one of the most common reasons for hypovolaemia in critically ill patients suffering from sepsis, shock or systemic inflammatory reaction syndrome (SIRS) of various ethological factors [1-5]. Relative hypovolaemia can result from vasoplegia, which is frequently observed in critically ill patients with sepsis/SIRS [1], or can be caused by pharmacologically-induced vasodilation. Fluid therapy aimed at restoring and maintaining circulating blood volume is, therefore, an important part of the complex circulatory management of perioperative and critically ill patients [3-5].

Hypovolaemia leads to reduced venous return and inadequate cardiac preload, decreased cardiac output and insufficient oxygen delivery to the tissues. In more severe cases, this is associated with arterial hypotension. The decrease in circulating volume triggers activation of the baroreflex originating from stretch receptors in the central veins, the right atrium, in the carotid sinus and in the aortic arch. This leads to increase in sympathetically mediated vasomotor tone in venous system aimed to preserve central blood volume, cardiac preload, and, therefore, cardiac output and systemic arterial pressure. Unloading of the arterial baroreceptors will result in an increased sympathetically-induced arterial vasoconstriction, which is selective to maintain perfusion in the vital organs such as the brain and the heart. Simultaneously, however, it will result in hypoperfusion in regional beds, such as splanchnic area, skin,

and muscle [6, 7]. The consequences of decreased oxygen delivery and impaired microcirculatory flow are tissue hypoxia and oxygen debt [8] which, if not corrected early [9], leads to cell damage, organ dysfunction, multiple organ failure (MOF), and death [3, 5, 7-10].

Adequate fluid therapy is, therefore, one of the keystones in the management of circulatory failure and shock, the aims being to increase and maintain intravascular fluid volume, restore effective tissue perfusion, and re-establish and maintain a balance between tissue oxygen demand and supply [11, 12]. Current evidence shows that prompt and optimally guided fluid therapy restores circulatory stability and improves outcome not only in situations when hypovolaemia is the only factor determining circulatory failure, but also in more complicated cases like severe sepsis and septic shock [9, 13]. It has been shown that the fluid therapy alone, or as a part of complex cardiovascular optimization, is able to improve outcome in the high risk surgical patients [14-18]. At the same time, fluid overload because of poorly controlled fluid administration [19] or intentional hypervolaemia guided by specific protocols [20], have deleterious physiological consequences, and can be associated with increased morbidity and mortality [19-22]. Thus, an early and correct diagnosis of hypovolaemia and fluid responsiveness, and a decision about the amount and the type of fluid which will be used, are important factors in the management of circulatory failure to affect the clinical outcome [9, 14-18, 23-25]

Treatment of hypovolaemia

Once the decision to give a fluid has been made, the next question arises: what type of fluid shall be given? Both colloids and crystalloids are used currently to correct intravascular volume deficit. It is still an ongoing dispute regarding the clinical use of crystalloids vs. colloids. Moreover, in recent years with the increasing number of clinically available colloid solutions, the crystalloid/colloid dispute has been enlarged to a colloid/colloid debate [26, 27].

Crystalloids

Crystalloids are solutions of small particles with molecular weight less than 30 kDa. For the purpose to restore intravascular volume, sodium-containing isotonic (or nearly isotonic) and hypertonic solutions are used. Normal saline and Ringer solutions (Ringer's lactate) represent the classical isotonic crystalloid solutions with the osmolality resembling the normal extracellular compartment. Solutes of crystalloids are freely permeable to most capillary membranes of the body, and are therefore rapidly distributed in the extracellular space proportionally to the volumes of intravascular and interstitial compartments [28, 29]. This means that, after about 15-20 min, only 20-25% of an isotonic crystalloid solution remains in circulation, whereas 75-80% extravasates into the interstitium. Thus, from a physiological point of view, crystalloids are not true plasma volume expanders. In clinical praxis, to replace 1 volume unit of blood or plasma loss, 4 to 5 times large volume of isotonic crystalloid solution must be infused [28, 29]. Hypertonic saline is a hypertonic crystalloid solution used for plasma volume expansion. Due to increased osmolality in extracellular compartment, water will be absorbed from intracellular compartment resulting in a transient but significant increase in extracellular fluid volume and thereby increase of plasma volume by 20-25% of the absorbed fluid [30].

In patients with increased microvascular permeability, the volume expanding capacity of crystalloids can be lower than under normal circumstances. Leakage of plasma can increase the volume of the interstitial compartment, resulting in a lower plasma volume relative to interstitial volume [31]. Consequently, a larger volume of the administered crystalloid will be distributed into the interstitial space and less will remain intravascularly. Infusion of a volume expander increases hydrostatic capillary pressure, which promotes the escape of fluid and proteins into the interstitial space under increased permeability more than under normal permeability. Infusion of a crystalloid solution will cause dilution of proteins which is expected to be larger in plasma than in the interstitium, leading to decreased colloid absorbing driving force [31].

Crystalloids are inexpensive and they are thought to be virtually free from significant side-effects, especially in the respect of coagulation. However, recent studies have indicated that also the use of crystalloids is associated with enhancement of coagulation [26]. Hyperchloremic acidosis is an another unfavourable side effect of normal saline with yet unclear clinical significance, and will occur if saline is used in larger amounts [26]. In comparison to colloids, volume resuscitation with larger volumes of crystalloids is less likely to restore microcirculatory blood flow [32], while the use of large fluid volumes often are associated with the development of tissue oedema which can compromise microcirculation and organ function [33, 34].

Colloids

Colloid solutions are defined as solutions containing molecules with a molecular weight above 30 kDa [35]. Colloid solutions used currently in clinical praxis are albumin, dextran, gelatin and hydroxyethyl starch. In comparison to crystalloids, they are more efficient plasma volume expanders both in the respect of the extent and the duration of the volume expanding effect [4, 26, 36, 37]. MW is an important but not the only factor determining volume expanding efficacy. Other factors of importance are MW distribution, size molecules, oncotic pressure, degradation rate, threshold for renal elimination, molecular shape and electrical charge, and also interaction with the endothelial glycocalyx, preventing extravasation [4, 26, 36-39].

Colloids may be described as monodisperse or polydisperse. Albumin, the only available natural colloid, is monodisperse. All synthetic colloids are polydisperse, which means that that the molecules are not uniform in size and weight. Colloid solutions can contain molecules ranging from 1 to 5000 kDa in MW [4, 26, 39]. The degree and pattern of dispersion determine the colloid osmotic pressure (COP), persistence and viscosity. Particles with a low MW (and small size) will easier leave the circulation through the capillary and glomerular membranes, while for a given concentration of the colloid they will exert a greater oncotic effect and a smaller effect

on viscosity than those with larger MW or size. In contrast, larger molecules for a given concentration will be presented in smaller number resulting in smaller, but more persistent volume expanding effect [4, 26, 39] The molecular weight distribution is presented in Fig.1.

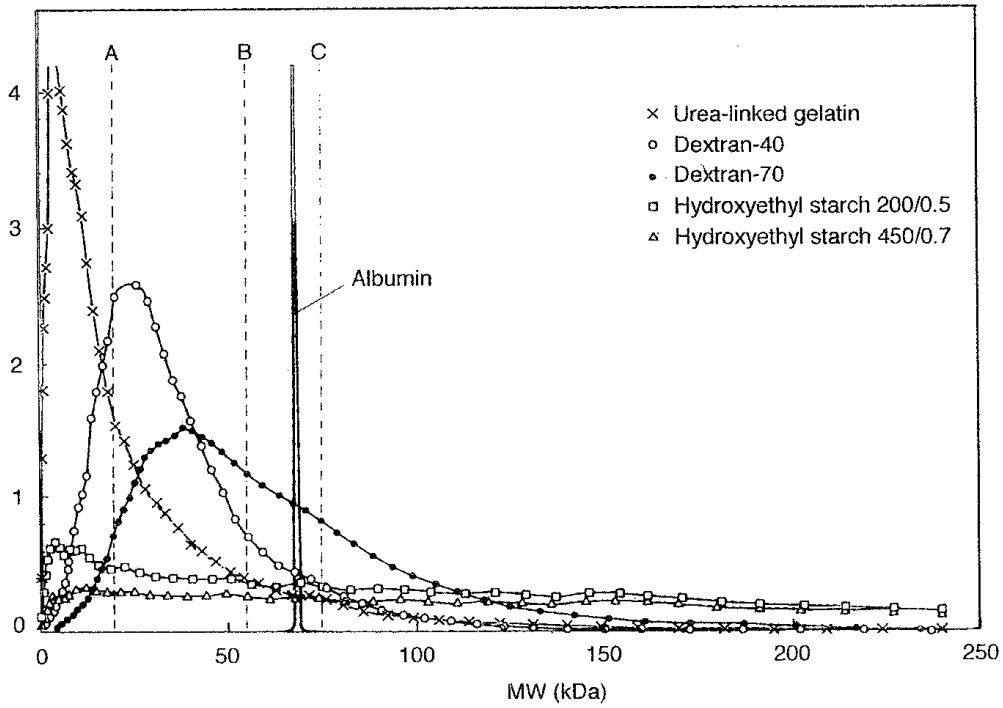

Fig. 1 *Molecular wight distribution of different colloid solutions.*

In general, the size of molecules in colloid solutions is described by the average molecular weight. This can be presented as the weight-averaged molecular weight (MWw), which is the number of molecules at each mass multiplied by the particle mass, divided by the total mass. It can also be presented as the number-averaged molecular weight (MWn), representing the arithmetic mean of all particle molecular weights [26]. For albumin, MWw and MWn are equal and 69 kDa. For the polydisperse colloids, MWw is always higher than MWn.

According to the 2-pore theory [38], which will be described in more detail below, there is a continue escape of macromolecules from the intra- to the extra-vascular space also under normal permeability. This extravasation is largely influenced by the prevailing microvascular permeability. Consequently, the intravascular stay of any colloid can be shortened in patients suffering from increased permeability, such as after trauma or during sepsis/SIRS. In these patients circulatory stability following fluid resuscitation is usually achieved at the expense of tissue oedema that may significantly influence vital organ function [12]. However, advocates of colloid therapy argue that by maintenance of an increased oncotic pressure, fluid is better retained in the intravascular space, even in the presence of increased permeability [3].

Albumin

Albumin is a natural colloid prepared from human plasma and historically it has been considered as the ‘’perfect’ colloid [1]. More than 95% of solute particles are of a uniform molecular size (MW 69 kDa). At normal concentration in plasma (35-45 g/l) it accounts for 70-80% of the plasma oncotic pressure. About 40% of total body albumin (4-5 g/kg) is situated intravascularly, and about 60% in the interstitium. Albumin molecules escape the circulation continuously with the rate of 5-7% per h (the transcapillary escape rate, TER) in health, whilst 90% returns back to the circulation via the lymphatic system [39]. It is known that after large surgical intervention TER of albumin can double, and it can be increased up to 300% in patients with sepsis [2, 39, 40].

Many pathological conditions are associated with decreased plasma albumin concentration, in many cases associated with decreased production, like in hepatic cirrhosis, malignancies, or malnutrition. In critically ill patients with sepsis/SIRS, the capillary leak with increased distribution of albumin in the extravascular space is the most important mechanism behind hypoalbuminaemia [2]. Low albumin level can be used as a non-specific marker of the seriousness of the critical illness and predictor of poor prognosis in intensive care (a very high mortality rate if the serum albumin level is <20 g/L) [40-42].

Albumin is extensively studied with very few known adverse side effects, and reported incidence of anaphylaxis is only about 1.5%. Albumin exerts several favourable physiological effects; it is a binding protein for many endogenous and exogenous substances, and can modify capillary permeability and positively influence the microcirculation; it also has scavenger, antioxidant and antiapoptotic properties [39]. Though being almost an ''ideal colloid'' without contraindications for the use in critically ill hypovolemic patients, human albumin is still a matter of hot discussions regarding its use in clinical praxis [43].

Dextran.

Dextran is a product of hydroxylation of polysaccharides by bacterial source [26, 39]. Two main types of dextrane formulations exist: 10% dextran-40 and 6% dextran-70, referring to concentration and the average MW.

Dextran has a very high colloid oncotic power due to high water binding capacity. One gram of dextran 40 retains 30 ml of water and 1 g of dextran 70 about 20-25 ml of water. The renal threshold for dextran is between 50 and 55 kDa, and the elimination of dextran occurs almost exclusively by the kidneys, whilst non-negligible amounts are either metabolised to CO_2 and H_2O by endogenous dextranase in the liver or cleared via gastrointestinal tract. Dextran has no toxic metabolites. Up to 40% of dextrane-40 and 70% of dextrane-70 remains in the circulation at 12 h after the infusion. Because naturally occurring dextran antibodies, dextran, however, can cause anaphylactoid reactions (0.3% in patients receiving dextran-70), but severe cases are relatively uncommon. The incidence of these reactions can be significantly decreased (less than 1 in 70 000 patients) by injecting 20 ml of dextran-1 (monovalent dextran hapten with MW of 1 kDa) a few minutes before the dextran infusion, putting dextran as the synthetic colloid with the best safety-profile [44]. Dextran possesses beneficial hemorhelogic properties, which are used to maintain microcirculatory dynamics during various types of shock and in the ischemia-reperfusion injury [26, 39]. Because of interaction with platelets and coagulation factors, dextran reduces postoperative

thrombembolism, but this property can increase the risk of perioperative bleeding complications. Similarly to other hyperoncotic colloid solutions, dextran can induce renal dysfunction in patients with specific risk factors. This type of dysfunction has been attributed mainly to production of hyperviscous urine since no chemical cytotoxicity has been related to the dextran molecule [26, 39]. Taken together, dextran is a useful and safe colloid for the treatment of hypovolaemia in critically ill patients.

Gelatin

Gelatins are polydispersed polypeptides produced by degradation of bovine collagen. The MW ranges from 5 to 50 kDa, with the weight-average MW of 30-35kDa [26, 39]. There are 2 most used types of gelatin products: urea-cross-linked gelatin (Heamoacel®), and succinated or modified fluid gelatin (Gelofusine®), but the latter is the dominating solution. All the solutions have similarly poor volume-restoring efficacy (intravascular half-life of about 2 h) due to rapid escape to the interstitial space and passage through the glomerular membrane. In a lesser part, gelatine molecules are degraded by proteases. Gelatins do not accumulate in the body, and no adverse effects on renal function have been reported [26, 39]. Gelatin solutions carry significant risk of hypersensitivity reactions (0.345%) [44, 45], and also affect haemostasis [46]. The clinical significance of coagulation impairment, however, is still uncertain.

HES

Hydroxyethyl starch (HES) is a modified natural polysaccharide obtained from maize and potatoes, and, by its chemical structure, is similar to glycogen [47]. In the process of synthesis, substitution of hydroxyl groups with hydroxyl ethyl groups leads to highly increased solubility and resistance to hydrolysis of the compounds by plasma amylase, delaying its degradation and elimination from the circulation [26, 39]. Beside of solute concentration and weight averaged mean MW (the arithmetic mean of MW of all HES molecules), the pharmacokinetics of HES is, in contrast to other colloids, influenced also by other physical-chemical characteristics: MS, the molar ratio of the total number of hydroxyl ethyl groups to the total number of glucose units; DS, the

degree of substitution defined as the ratio of substituted glucose units to the total number of glucose molecules; and, the C2/C6-ratio, which is the ratio of the number of substituted hydroxyl ethyl groups in glucose molecule in C2 position to the number of hydroxyl ethyl groups in C6 position. Higher molecular weight and more extensive degree of substitution result in slower elimination. The C2/C6 ratio is a factor which modifies HES resistance for degradation by alpha-amylase, and possible responsible for its side-effects (*e.g.* accumulation, tissue accumulation, bleeding complications) [26, 39, 48].

The water binding capacity of HES ranges between 20 and 30 ml/g, and the expanded volume initially is higher than the volume infused. However, following the infusion of HES, larger molecules rapidly undergo amylase-dependent breakdown, and molecules smaller that 50-60 kDa are eliminated by glomerular filtration [39].

Anaphylaxis is not frequent, and the reported incidence is of less than 0.1%. HES alters coagulation in a dose dependent manner. The tissue storage in the reticuloendothelial system is more relevant for higher MW, and may account for hypersensitivity reactions and pruritus [44, 48]. In addition, it has been suggested that HES solutions may have the permeability decreasing properties which can be advantageous in critically ill patients with increased permeability [26, 39]. However, there are some concerns about the possible negative effects of HES on renal function, as increased incidence of renal failure in critically ill patients has recently been reported [49, 50].

Microcirculation and microvascular exchange of fluid and macromolecules

Capillaries and small vessels

The purpose of the circulation is to deliver essential nutrients to the cells and remove waste products of cell metabolism. [51] This is accomplished through a series of increased number of smaller vessels, progressing from the artery, to the arteriole (diameter less than 30-50 μm), to the metaarteriole, and finally to the capillary. The capillary (diameter less than 9 μm) continues to become a venule (diameter 7-30 μm) that ultimately coalesces with other venule into larger veins. Arterioles are composed of endothelium, basal membrane and smooth muscle cells. In there most distal parts before the capillary they form the precapillary sphincters. Precapillary sphincters have a rythmic and spontaneous behaviour denoted vasomotion, which is important for an even distribution of microcirculatory flow in the tissue [52]. By this mechanism, the blood flow is not continuous through the capillaries, but is intermittently turned on and off with different time intervals varying from few seconds to minutes. This vasomotion is provided (assured) by spontaneous myogenic activity and metabolic needs of the tissues [51, 53] and effectuated by the single unit type of the smooth muscles. The capillary wall consists of endothelial monolayer with its basal membrane. Venules also possess smooth muscle cells surrounding endothelium. Although these muscle cells are fewer in comparison to arteriolar side, they still are able contract affecting the flow and especially hydrostatic capillary pressure [51].

There are several types of capillaries [54]. Continuous capillaries are tightest with very few discontinuities in the junctions. This type is most common and is found in skeletal muscle, cardiac muscle, lungs, skin, subcutis, kidneys, and in the brain. Fenestrated capillaries are found in glands, glomeruli and gastrointestinal tract. In this type of capillaries endothelium forms transcellular openings, often containing a membrane diaphragm. Discontinuous (sinusoidal) capillaries are found in liver, spleen and bone marrow. They have large gaps with interrupted or absent basement membrane, which

make possible free passage of all blood elements. Postcapillary venules have simpler intercellular junctions than capillaries. [51]

Endothelium

Endothelium [55] is lining the inside of all vessels of the body and by its total weight of almost 2 kg is the second largest endocrine organ of the body. It has a key role in the maintenance of vascular homeostasis by producing several vasoactive substances, affecting blood flow, vascular permeability, coagulation, inflammation, immunological reactions, angiogenesis, and endocrine processes [56-59]. Various vasoactive substances, such as prostacyclin and nitric oxide are produced and released from the endothelial cell, affecting both vascular resistance and capillary permeability.

Endothelium consists of 0.3-2 μm thick layer of endothelial cells. These cells are enwrapped by a negatively charged layer of glycoproteins, called glycocalyx. The glycocalyx normally is 0.1-0.2 μm thick, and extents from the cell surface into the vessel lumen and the interendothelial junctions. The negatively charged glycocalyx is believed to form an important part of the fluid and molecular permeability-barrier in the exchange vessels [60, 61].

Endothelial cells also have intraendothelial contractile microfilaments, such as actin and myosin, forming a cytoskeleton capable an of cellular shape changes [62, 63].

Increased microvascular permeability

Increased microvascular permeability is an important aspect of the large picture of microcirculatory disturbances associated with endothelial activation and damage, and bidirectional interaction between coagulation and inflammation. Both morphological damage of the vascular structures and conformational changes affecting interendothelial junctions [61-64] in microvascular endothelium have been suggested being responsible for the increase in microvascular permeability.

Damage to the endothelial cells begins with an insult such as endotoxin exposure, ischemia-reperfusion, vessel injury with platelet deposition, or mechanical stress [65]. Subsequent release of mediators activates neutrophils and the endothelial cells of the capillaries. The activated neutrophils release proteases, oxygen radicals, and other molecules that are toxic to the endothelial cells and lead to further structural damage. Products of activated neutrophils cause alterations in heparan sulfate proteoglycans, components of the structural matrix of the capillary membrane and basement membrane, and result in an increased distance between endothelial cells, thus promoting the protein leak [66-68].

It has been suggested that endothelial contraction which affects the width of intercellular clefts and hence the diameter of pores, is an important permeability increasing factor [69]. The contraction of the endothelial cells is promoted by calmudin and myosin kinase and inhibited by cyclic adenosine monophosphate [70-72]. The activation of calmudin and myosin kinase leads to raised intracellular Ca concentration and contraction of endothelial cell, whilst the increase in intracellular cyclic adenosine monophosphate counteracts it [73].

The permeability increasing properties have been attributed to many substances participating in the inflammatory cascade such as tumour necrosis factor-α, interleukin-1β, platelet activating factor, thromboxanes, complement, histamine, bradykinin, and vascular endothelial growth factor [74].

Both apoptotic and necrotic death of endothelial cells may contribute to the capillary leak [74] by further weakening the structure and integrity of the capillary. Apoptosis is increased in response to mediators associated with the septic response, whereas activated protein C and prostacyclin can prevent it [75].

Autoregulation.

Microcirculation is protected from large swings in systemic blood pressure by autoregulation [76]. Because of autoregulation, the variations both in blood flow to a

tissue and hydrostatic pressure in microcirculatory vessels upon variation in arterial pressure are restricted, and are smaller than the variations in systemic arterial pressure. Autoregulation of flow is a result of changes in vascular resistance accomplished by function of precapillary arterioles, while the autoregulation of capillary pressure is a consequence of changes in the post-precapillary resistance ratio. These autoregulatory mechanisms are present in microvascular beds of most organs, but the degree of autoregulation varies both between organs and between different conditions in an organ. It is absent in the lung, while it is more effective in the brain, the skeletal muscle, the kidney, and in the intestinal circulation. It has also been shown that autoregulation of hydrostatic capillary pressure is more effective than autoregulation of the flow [76].

Hydrostatic pressure in capillaries, Pc, is important and a most variable factor determining fluid movement through the capillary wall [77]. Pc depends on the ratio between pre- and postcapillary resistance, and can be described by the following equation by Pappenheimer and Soto-Rivera [78]:

$$P_c = [(R_v/R_a) \times P_A + P_V]/[1 + (R_v/R_a)]$$

Where R_v is postcapillary resistance, R_a is precapillary resistance, P_A is arterial pressure, and P_V is venous pressure.

Autoregulation becomes seriously affected in conditions associated with systemic inflammation (SIRS) such as sepsis, severe trauma, extensive surgery, burns, pancreatitis, extracorporeal circulation, as well as anaphylaxis [79]. Because of impaired vasomotor activity of precapillary arterioles, the basal capillary hydrostatic pressure will be increased, and all changes in systemic arterial pressure will cause relatively larger changes in capillary hydrostatic pressure than under normal circumstances. According to the Starling's law for transcapillary fluid exchange [61, 77, 80] (see below), the hydrostatic capillary pressure is one of the determinants of net fluid filtration across the capillary wall. This means that depressed autoregulation can

be associated with increased filtration of fluid, and increase in arterial pressure will further increase the filtration of plasma fluid in larger extent than under intact autoregulation. Inflammatory conditions such as sepsis or SIRS increase the microvascular permeability via different mechanisms [2, 54, 61-64, 70, 74]. Increased microvascular permeability implies an increase in the filtration of plasma fluid. As soon as the net rate of fluid filtration exceeds the lymph flow, oedema evolves. The classical Starling equation does not explain, however, the relation between loss of fluid and proteins, which instead can be explained if extending the Starling hypothesis with the so called 2-pore theory for transcapillary fluid exchange [38]. Both the Starling hypothesis and the 2-pore theory will be described in more details below.

Taken together, the increased escape of fluid and proteins from the intravascular- to the interstitial compartment under inflammation is determined not only by increased microvascular permeability but also by depressed autoregulation. It has been also suggested that inflammatory conditions are associated with structural changes in the interstitial matrix and reduced intersticial pressure what further favours filtration of plasma fluid and proteins. [81-83]

Starling equation

In 1896 the British physiologist E. H. Starling identified and described the transcapillary hydrostatic and osmotic pressures as responsible for movement of fluid through the exchange vessel wall [80]. The Starling equation can be written as:

$$J_v = L_p A[(P_c - P_i) - \sigma(\Pi_c - \Pi_i)]$$

Where J_v is transvascular fluid exchange, L_p is fluid conductivity, A is the surface area available for fluid exchange, P_c is hydrostatic capillary pressure, P_i is hydrostatic interstitial pressure, Π_c is plasma colloid osmotic pressure, Π_i is interstitial colloid osmotic pressure, and σ is the reflection coefficient for macromolecules.

The microvascular fluid or hydraulic permeability is represented by the product of L_pA and called fluid conductance (ml/min/mmHg/100g tissue). It has been measured in laboratory as the capillary filtration coefficient (CFC) [77, 84, 85]. L_p varies considerably between different organs in the body, and is higher at the venous than at the arterial side of the capillary [38].

The protein permeability is described by reflection coefficient for macromolecules, σ. The reflection coefficient quantifies the resistance at which a solute passes membrane relative to water [86, 87]. It also can be considered as a fraction of the colloid osmotic pressure gradient that counteracts the force dependent on hydrostatic pressure gradient. Its value can vary between 0 and 1, where a value of 0 implies that macromolecules pass through the membrane without limitation, and a value of 1 means that the membrane is totally impermeable for the solute. Only in the microvascular bed of the brain this value is 1 while in other organs of the body it is below 1. The reflection coefficient for albumin in skeletal muscles is 0.9-0.95, 0.9 in the gut, 0.5-0.6 in the lung, but it is very low in the liver and in the spleen [31].

The 2-pore model

As described above, the Starling equation explains how hydrostatic and osmotic forces regulate fluid fluxes across the capillary membrane. However, it does not elucidate the mechanisms behind the transfer of proteins and other macromolecules from the intra- to the extra-vascular space. With this purpose the 3-pore model was developed, and offers a functional basis for understanding of both hydraulic and macromolecular permeability [38, 60]. According to this model, the capillary wall functionally can be considered as a membrane containing three different types of pores, classified according their size. It has been suggested that the total pore area comprises less than 0.1% of the total capillary surface area.

Thus, the capillary wall contains a high number of small interendothelial pores with radius of 4-6 nm, distributed along the entire capillary bed. These pores are exclusively permeable for water and small solutes. The existence of the small pores

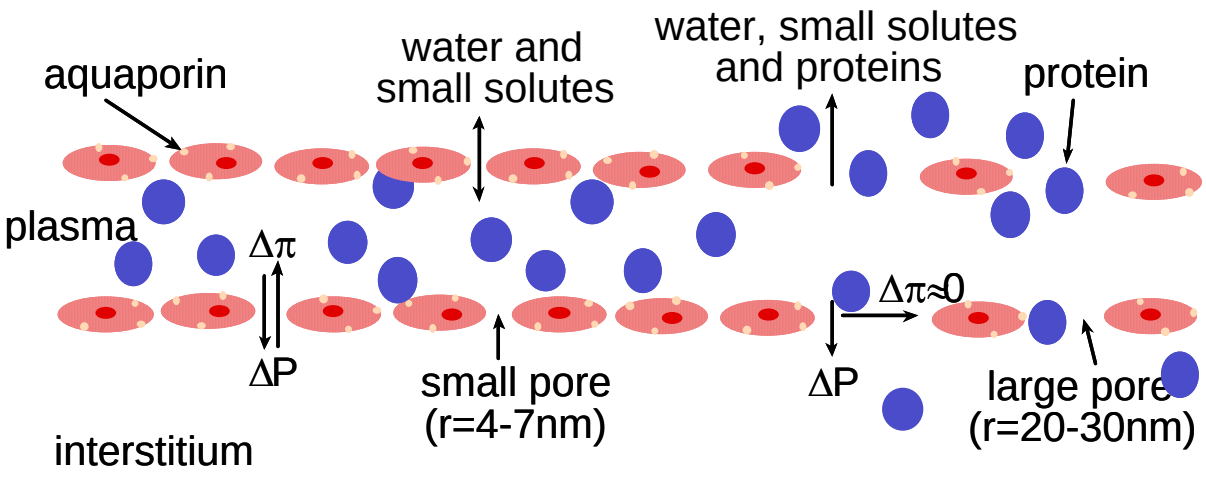

Fig. 2. *Schematic illustration of the pores in the exchange vessel. Large pores allow free passage of proteins providing that the colloid osmotic pressure gradient in the large pore area is zero. The main driving force for fluid escape from intra- to extravascular space is the hydrostatic pressure. Proteins escape circulation passively via the convection.*

was suggested by Pappenheimer in 1951, and visualized by electron microscope by Bundgaard in 1984 [88]. They comprise less than 1% of total capillary surface area, but still 85-95% of the transfer of fluid and small solutes occurs mainly through the small pores. The capillary membrane contains also large pores with radius of 20-30 nm, which are 10 000 to 30 000 times less frequent and are located at the venous side of capillary bed as well as in proximal venules [38]. The presence of large pores was proposed by Grotte in 1956, and later visualized by electron microscopy under inflammatory condition by McDonald in 1999 [38, 63]. Normally, they comprise only 0.2 to 0.4% of total pore area, and only 5 to 10% of the net fluid flux from the intravascular compartment to the interstitium have been attributed to the large pores. Aquaporins are the third type of pores which are very small. In fact, these structures are transcellular proteins, forming channels permeable exclusively to water, They account for small amounts of transvascular water exchange, about 1-5% of the total transvascular fluid flow [38, 60, 77].

The presence of three morphologically and functionally different pores in the capillary membrane creates a background for three-pore model of microvascular permeability, and is schematically depicted in Fig. 2. Aquaporins can be neglected from a

functionally point of view because of their small contribution, and the model can be simplified to the two-poor model including only the small and large pores.

As mentioned previously, the small pores are passively permeable only for water and small solutes, thus being responsible for hydraulic permeability. Consequently, the colloid osmotic pressure gradient can be developed across the small pores, counteracting the hydrostatic force for filtration. In turn, protein molecules can easily pass through the large pores resulting in nearly equal concentration of proteins on both sides of capillary membrane within the large pore and, consequently, the colloid osmotic pressure gradient across the large pore is small. It means that the escape of proteins occurs passively, via convection when the macromolecules follow the fluid flux through the large pore. According to this theory, convection is a main mechanism responsible for protein loss and, in normal condition, and it seems to accounts for two-third of total protein loss from the circulation. Diffusion is another mechanism explaining protein loss from the circulation, which means that even in situations of no net fluid flow or net fluid absorption, there still is a loss of macromolecules through the large pores.

It follows, that the main driving force for transvascular escape of fluid and macromolecules is a hydrostatic capillary pressure, and any process that increases capillary hydrostatic pressure will increase protein clearance from the circulation. It also means that during increased permeability when the number and size of the large pores are increased, the increase in the capillary hydrostatic pressure will cause larger net filtration of fluid and proteins. This may occur, for example, by an increase in arterial and/venous pressures.

Prostacyclin.

Prostacyclin (PGI_2) is an endogenous substance with numerous biological functions. It was discovered by Vane, Moncada and colleagues in 1976 [89, 90]. While there is growing amount of studies available about its biological and pharmacological effects, still many questions remain to be answered regarding its clinical application.

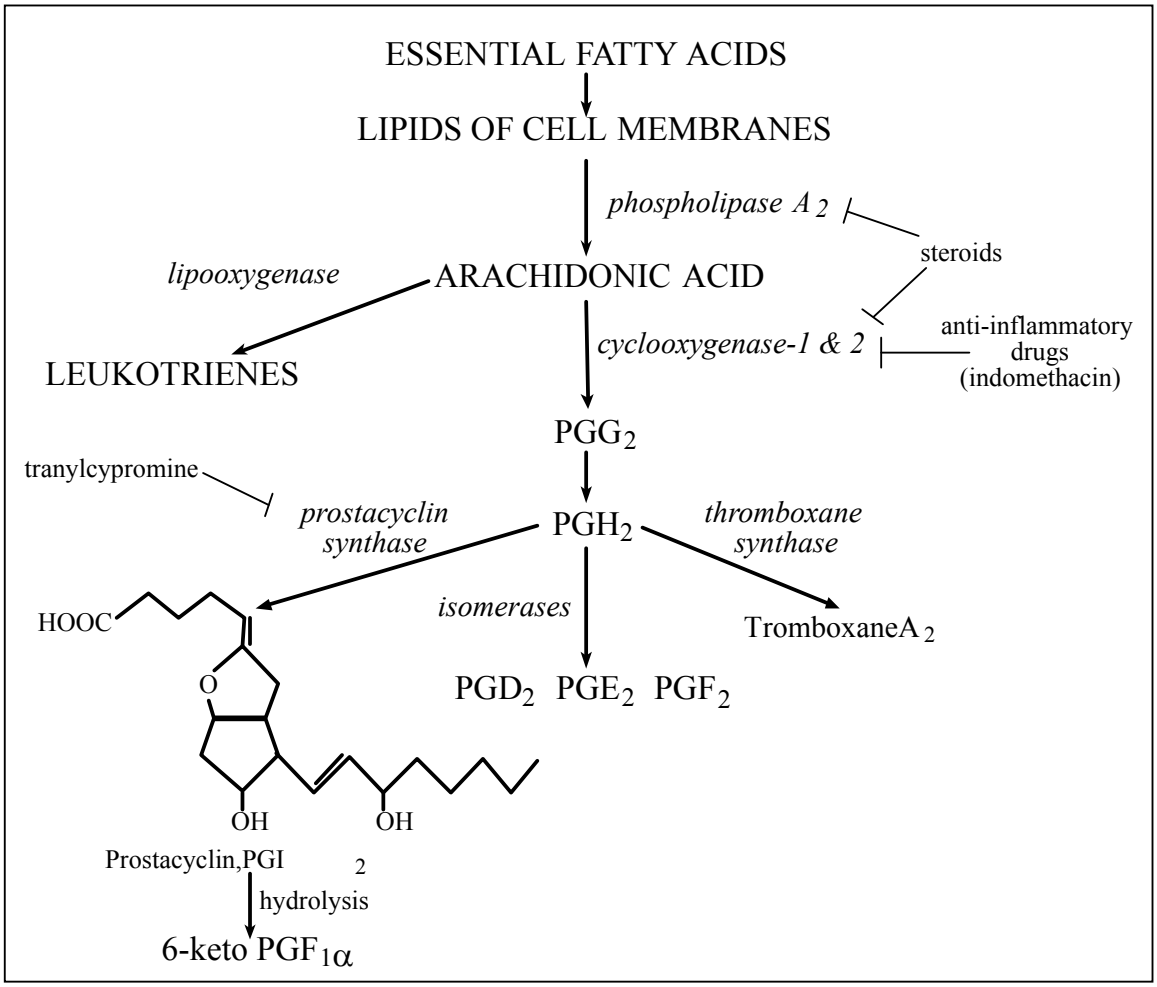

Fig. 3. *Structure and biosynthesis of prostacyclin. Substances that can inhibit synthesis of prostacyclin are indicated (⊥).*

Prostacyclin is mainly synthesised by vascular endothelial and vascular smooth muscle cells, and is the main product of the arachidonic acid metabolism in the vascular tissue [89, 90]. The synthesis of prostacyclin in the endothelial cells starts with the release of arachidonic acid from cell membrane phospholipids, which subsequently is converted into to prostaglandin H2 (PH2), the precursor to all prostaglandins. Further, the enzyme prostacyclin synthase catalyses conversion of prostaglandin H2 to prostacyclin (Fig. 3). Prostacyclin is chemically unstable with the half-life in the circulation of only 2-3 minutes. It hydrolyses spontaneously to a stable metabolite 6-keto-prostaglandin F_1alfa which is eliminated by the kidneys [90].

Most of the biological effects of prostacyclin are mediated through a G-protein-linked receptor called IP receptor through the activation of adenylate cyclase [90, 91]. The

activation leads to activation of cyclic adenosine monophosphate (cAMP) in vascular smooth muscle cells as well as in endothelial cells, (54, 73), subsequent activation of KATP-channels, in turn decreasing the concentration of intracellular calcium concentration, [Ca2+]i. Recent evidence suggests that prostacyclin affects also cyclic guanosine monophosphate (cGMP) [92].

Prostacyclin is the substance of outmost importance in the respect of the maintenance of microcirculatory homeostasis and the regulation of the microcirculatory flow. It is known that prostacyclin is the most potent inhibitor of platelet aggregation yet discovered, and it is also a potent dose-dependent vasodilator, having impact on modulation of the basal vascular tone [89, 90]. Prostacyclin inhibits endothelial adhesion of platelets and leucocytes, has direct anti-inflammatory and anti-thrombotic activity, and inhibits the synthesis of tumour necrosis factor-α [89-93]. Well described is the ability of prostacyclin to reduce microvascular permeability to both fluid and macromolecules [93-95]. The suggested mechanism behind the permeability reducing properties is an increase in intracellular adenosine monophosphate which influences the contractile state of intraendothelial filaments and the width of the intraendothelial pores [59].

Prostanoid-dependent part of vascular homeostasis is negatively affected in inflammatory conditions like sepsis/SIRS [92, 93]. In early stages of endotoxemia the endothelium modifies its normal homeostasis, and, consequently, decreases the ability to synthesize nitric oxide (NO) and prostacyclin, thus acquiring a prothrombotic phenotype favouring expression of the tissue factor and triggering the coagulation response. It also leads to vasoconstriction in microcirculatory bed. In later stages of sepsis/SIRS prostacyclin synthesis may return to normal or can be even increased. However, through the all course of sepsis/SIRS, the thromboxane A_2/prostacycllin ratio still remains increased in favour of thromboxane A2 [93, 97-99], which is a potent vasoconstrictor and pro-aggregatory substance [90, 91]. Several mechanisms may be responsible for the decrease in prostacyclin synthesis in sepsis/SIRS. Activated neutrophils decrease endothelial prostacyclin production by damaging the endothelium

through liberation of neutrophil elastase [100]. In later stages, decreased synthesis of prostacyclin can result in endothelial damage because of unresolved inflammation and persistent hypoxia [93]. It is well known that antithrombin III (ATIII) is a potent stimulator of endothelial production of prostacyclin [101]. In fact, ATIII is an important inhibitor of serine proteases generated within coagulation cascades; it prevents vascular injury by inhibiting leukocyte activation thanks to profound increase in endothelial prostacyclin production [101, 102]. Sepsis and other severe systemic inflammatory conditions can be associated with decreased production and increased consumption of AT III, leading to decreased the prostacyclin synthesis in the endothelial cells.

Experimental studies have shown that the infusion of prostacyclin or its analogues increases splanchnic blood flow, increases oxygen gut oxygen delivery and uptake under endotoxemia [103-105], and reduces capillary permeability [93-96]. Favourable effects of prostacyclin have been shown in perioperative and critically ill patients in terms of improved splanchnic circulation [106, 107], improved renal function [108], and improved splanchnic and systemic oxygen delivery and consumption [109-111]. Prostacyclin has become an established treatment for pulmonary hypertension and other vascular disorders requiring vasodilation, and it has been used for anticoagulation under extracorporeal circulation [112-114]. Even thought that prostacyclin appears to be the best vasodilating agent studied so far in experimental sepsis and has shown an improved tissue perfusion, oxygen extraction and attenuation of acidosis, the role of prostacyclin in the treatment of other pathological conditions with disturbed microcirculation and increased microvascular permeability like sepsis/SIRS, is still not clear [115, 116]

Taken together, prostacyclin is a substance with many favourable effects on microcirculation such as suppression of inflammation and inhibition of cellular interactions, vasodilation, resolution of microthrombosis [93, 117], and direct reduction of microvascular permeability [94-96]. These effects, in association with an

absolute and relative deficiency of prostacyclin in sepsis/SIRS, can be a rationale for its clinical use in the selected population of critically ill patients.

Protein C pathway and activated protein C (APC)

Endogenous protein C is a vitamin K dependent glycoprotein synthesized by the liver [118]. It plays an important role in the maintenance of homeostasis and modulation of inflammation. Protein C circulates in the blood in inactivated form, and is converted into activated protein C by thrombin-thrombomodulin complexes. [118, 119] Activated protein C acts in concert with its co-factor, protein S, and inactivates essential coagulation factors Va and VIIIa, thereby inhibiting further thrombin generation [119-121]. Endothelial cells have protein C receptors (EPCR), which both accelerate activation of protein C and amplify anticoagulant and anti-inflammatory effects of activated protein C [122]. Activated protein C is indirectly profibrinolytic by inhibiting the plasminogen activator inhibitor-1 [123]. In addition to its role as anticoagulant, a number of anti-inflammatory effects and anti-apoptotic properties have been described [118, 119, 124]. Other important properties of activated protein C are inhibition of neutrophil chemoataxis, leukocyte-endothelial interaction, and maintaining endothelial integrity, as well as direct cytoprotective effects [121]. Recently, it has been shown that activated protein C improves sepsis-induced microvascular alterations by recruiting capillaries and maintaining higher density of perfused capillaries both in experimental and clinical conditions [125, 126]. Activated protein C upregulates the cyclo-oxyganase-2 (COX-2) synthesis, which results in the increased synthesis of prostacyclin, a fact is of special interest for our study [127, 128]. Thus, it is possible that infusion of not only prostacyclin but also of activated protein C might favourably affect the disturbed balance between prostacyclin and thromboxane A2.

The activated protein C system malfunctions at all levels in patients with sepsis (Fig. 4), and there is an evidence that insufficient functioning of protein C pathway contributes to the derangement of coagulation in sepsis, which ranges from subtle activation of coagulation to fulminant DIC and widespread microvascular thrombosis

[129-131] First, plasma levels of zymogen protein C are low because of impaired synthesis [130], increased consumption [131-133] and degradation by proteolytic enzymes as neutrophil elastase [134] Second, significant downregulation of thrombomodulin caused by proinflammatory cytokines, such as tumor necrosis factor-alfa and interleukin-1, diminishes activation of protein C [135]. Low levels of protein S, what usually take place in septic conditions, also compromises optimal function of protein C system and further contribute to a procoagulant state [136].

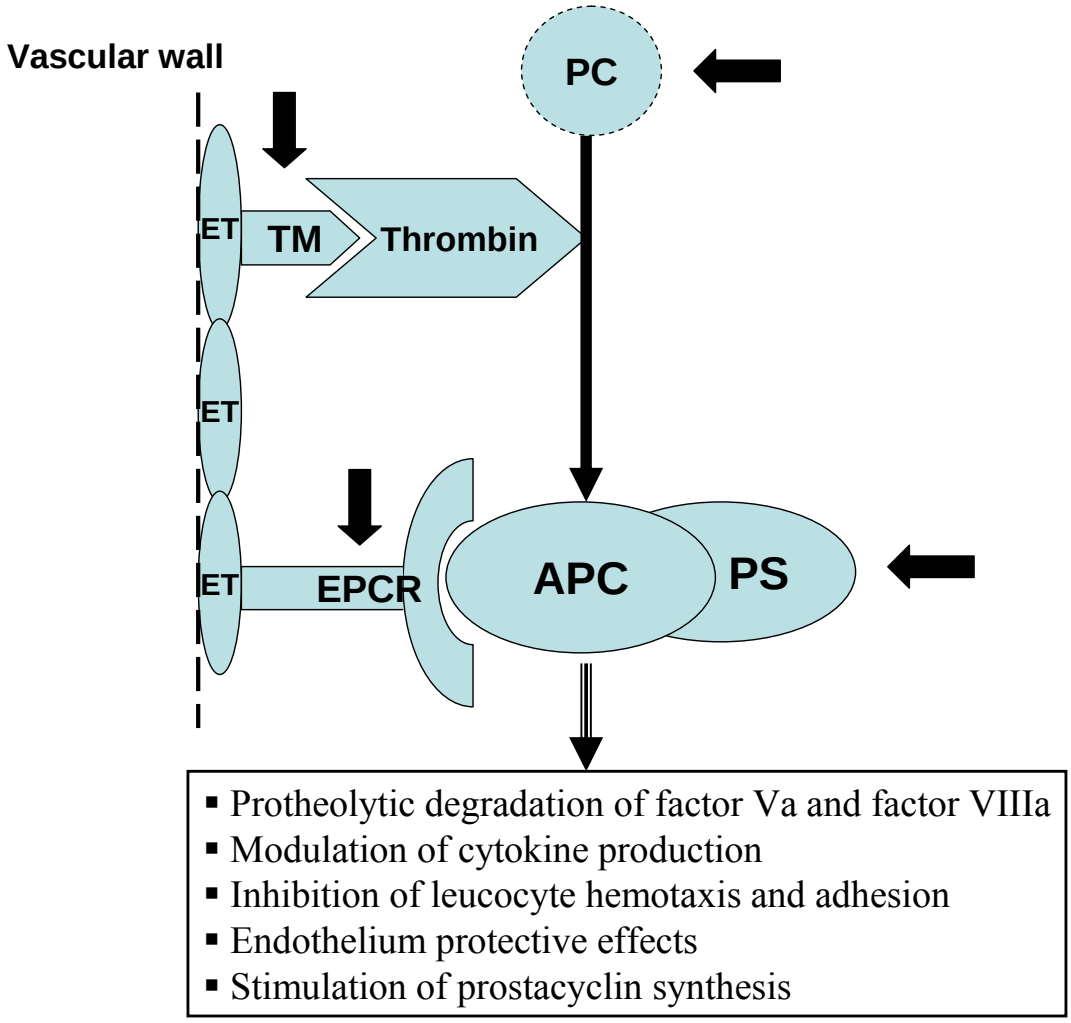

Fig. 4. *The protein C system. Solid arrows indicate 4 main mechanisms by which protein C system malfunctions in sepsis. ET – endothelium, PC – protein C (zymogen), APC – activated protein C, TM – thrombomodulin, EPCR – endothelial protein C receptor, PS – protein S.*

Both clinical and experimental studies have shown that low levels of protein C and activated protein C are associated with poor outcome in sepsis [137]. In turn, treatment with activated protein C has been shown to improve outcome in severe sepsis in the terms of preserved organ function and reduced mortality [138].

The goals of the studies

Study I

To compare the plasma expanding effect of 6% dextran 70 with that of 5% albumin and HES130/0.4 after a standardised haemorrhage during normal permeability.

Study II

To evaluate the plasma volume expanding effect of 5% albumin, 4% gelatin, 6% HES 130/0.4, and normal saline during increased microvascular permeability.

Study III

To evaluate the extent to which arterial pressure influences plasma volume loss under inflammatory state with increased microvascular permeability, and compare results with those obtained under non-inflammatory state with normal permeability.

Study IV

To evaluate the changes in plasma volume from hypervolaemic and hypovolaemic state, and how these changes are influenced by the increase in arterial pressure.

Study V

To evaluate and compare the ability of activated protein C and prostacyclin to reduce protein leakage in the lung and in the gut, and to improve blood oxygenation under endotoxin-induced inflammation in the rat.

Methods

Material and anaesthesia

The experiments were performed on professionally bred adult male Sprague-Dawley rats (II-V) and adult male Guinea pigs (I). All studies were approved by the Ethics Committee for Animal Research at Lund University, Sweden, and the animals were treated in accordance with the Guidelines of the National Institutes of Health for Care and Use of Laboratory Animals.

Anaesthesia was induced by placing the animals in a covered glass container with a continuous supply of isoflurane (Forene; Abbot, Stockholm) and maintained by inhalation of 1.5% isoflurane throughout the experiment, initially by means of facemask and later by a tracheal cannula after tracheotomy The animals were placed on a heating pad to maintain a body core temperature of 37.1–37.2ºC via a feedback circuit measured rectally. After tracheotomy the animals were connected to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy). End-tidal PCO_2 was monitored continuously and kept between 4.7 and 5.6 kPa (Capstar-1000, CWE, Ardmore, PA).

Model of increased microvascular permeability (Studies II and III)

A standardized increase in permeability was induced by 0.5 ml of dextran 70, based on the fact that dextran induces an anaphylactic reaction in the rat [78, 79]. Increased permeability with transcapillary leakage was confirmed by the visually observed marked peripheral oedema developed shortly after the dextran injection, by the equally large reduction in plasma volume for all groups, by the reduction in mean arterial pressure and by the increase in haemoglobin concentration.

Determination of plasma volume (Studies I-IV)

The plasma volume (V) was calculated by measurement of the increase in radioactivity per ml of plasma (ΔC_2) after an intravenous injection of a known amount of activity of ^{125}I-albumin (C_1) [139, 140]

$$V = C_1/\Delta C_2$$

Radioactivity was measured with a gamma counter (Wizard 1480, LKB-Wallace, Turku, Finland). The increase in radioactivity of ^{125}I-albumin was determined by subtracting the activity in a blood sample taken before the injection from that taken 5 min after the injection. The blood was centrifuged and the radioactivity in a fixed volume of plasma was determined with the gamma counter. The radioactivity of the syringe and the needles used for injection was subtracted from the total radioactivity measured.

Determination of transcapillary leakage of albumin (Paper V)

Transcapillary leakage of albumin was assessed by calculating the ratio between the tissue radioactivity (cpm/g tissue; cpm – counts per minute) and the actual amount of radioactivity given (cpm/g body weight). At the start of the experiment a known amount of activity of iodinated ^{125}I-albumin (in the range of 1100-1200 cpm/g body weight) in 0.2 ml of normal saline was injected in the central venous catheter. The blood was removed from the tissue before measurement of tissue radioactivity by replacing the blood with saline via pressure controlled pump perfusion via the cannulated right ventricle. The blood left the circulation via a small perforation in the right arterial wall. The saline-perfusion procedure continued under ongoing spontaneous circulation and mechanical ventilation until the fluid running from the right atrium was clear of blood and the lungs and the gut were pale and homogenous in colour. After end of the perfusion, the lungs and 5–6 cm of the small intestine were collected. The tissues were weighed and radioactivity was determined with a gamma counter (Wizard 1480, LKB-Wallace Turku, Finland).

Experimental protocols

Study I

Plasma volume expansion after standardized haemorrhage (20 ml/kg) was performed either by 6% dextran 70 or 5% albumin, or HES130/0.4. Plasma volume was measured at the baseline before haemorrhage and 3 hours after infusion of the colloid solution.

Study II

Microvascular permeability was increased by inducing anaphylactoid reaction by i.v injection of 0.5 ml dextran 70. One hour after dextran injection, a bolus of 5% albumin, 4% gelatin, 6% HES 130/0.4 and normal saline was infused. All colloids were given in the dose of 20 ml/kg, while normal saline was given in a dose of 80 ml/kg. Plasma volume was measured at baseline, directly before and 3 hours after the bolus infusion of the plasma volume expander. Corresponding plasma volume measurements were performed in a control group also during increased permeability, but no volume expansion was given.

Study III

Microvascular permeability was increased by dextran followed by albumin infusion after 1 h to create normovolaemia. Directly after the albumin infusion, the animals were randomized to 3 groups: 1) a group receiving no pharmacological intervention; 2) a group receiving noradrenalin infusion and 3) a group receiving metoprolol/clonidine infusion. Two additional groups were analysed: 1) no albumin was given after the increase in permeability, and plasma volume was measured before and 2.5 h after the start of noradrenalin infusion; 2) noradrenalin infusion was given at a state of normal permeability for 2.5 h after the animals were bled and substituted to normovolaemia by a bolus dose of albumin; plasma volumes were measured at the baseline before haemorrhage and after noradrenalin infusion.

Study IV

The study included 5 groups. Plasma volume was measured at the baseline and at the end of the experiments after the group-specific interventions: group 1 - 2.5 h of noradrenalin infusion; group 2 - infusion of 5% albumin followed by 2.5 h of noradrenalin infusion; group 3 – infusion of 5% albumin followed by an observation period of 2.5 h; group 4 – standardized haemorrhage followed by noradrenalin infusion for 2.5 h; group 5 – standardized haemorrhage followed by an observation period of 2.5 h.

Study V

Transcapillary albumin leakage in the lung and in the gut, as well as arterial oxygenation, was assessed at the end of the experiments in 5 groups of rats, 4 of which given LPS (endotoxin) for 30 h at a dose of 240 000 U/kg/h to simulate a severe inflammatory condition: 1) sham group; 2) LPS group given no treatment; 3) APC group given LPS in combination with activated protein C (8 µg/kg/min) ; 4) PGI_2 group given LPS in combination with infusion of prostacyclin (2 ng/kg/min). Infusion of activated protein C or prostacyclin was started 6 h after the start of the LPS infusion and continued for 24 h.

Results

Effects of dextran, albumin and HES on plasma volume after haemorrhage in guinea pig (Study I)

Plasma volume at baseline was 48.7 ± 3.8 ml/kg (n = 33), which is normal in the adult guinea pig [13]. The remaining increase in plasma volume (ΔPV) measured 3 h after the bolus colloid infusion is shown in Fig. 5. It was 36.5 ± 2.3 ml/kg in the dextran group, 26.8 ± 5.6 ml in the albumin group, and 17.6 ± 3.5 ml/kg in the HES group. The plasma expanding effect was larger in the dextran group than in the other groups, and larger in the albumin group than in the HES group ($p<0.05$).

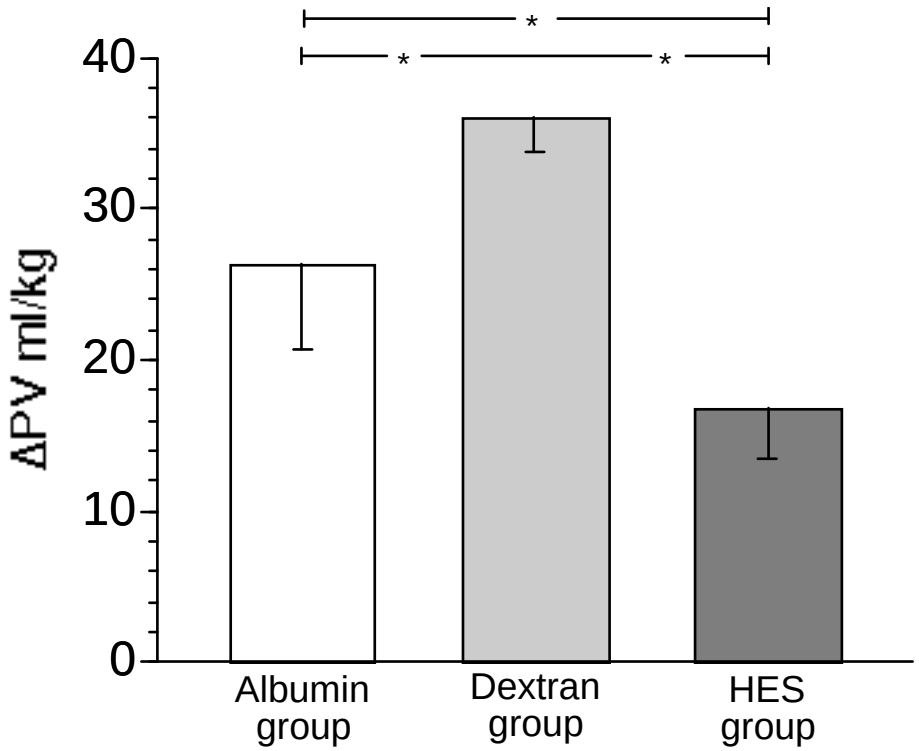

Fig.5. *Increase in plasma volume (ΔPV) by the colloid infusion (20 ml/kg) after hemorrhage, as measured 3 h after the infusion, for the 3 groups The volume-expanding effect in the dextran group was better than that in the albumin group, which was better than that in the HES group. * $p<0.05$.*

Effects of the increase of microvascular permeability on plasma volume, haemoglobin concentration, and blood pressure (Study III)

1 h after i.v. injection of 0.5 ml dextran 70 which caused an anaphylactoid reaction and increased microvascular permeability, the plasma volume decreased from 41.2 ± 2.1 (baseline value) to 32.1 ± 3.3 ml/kg ($p<0.001$) (Fig. 6). Plasma leakage was also reflected in the changes of haemoglobin concentration which decreased from 129 ± 4 g/l (baseline) to 146 ± 5 g/l ($p<0.001$) and in the decrease in blood pressure (Fig. 6).

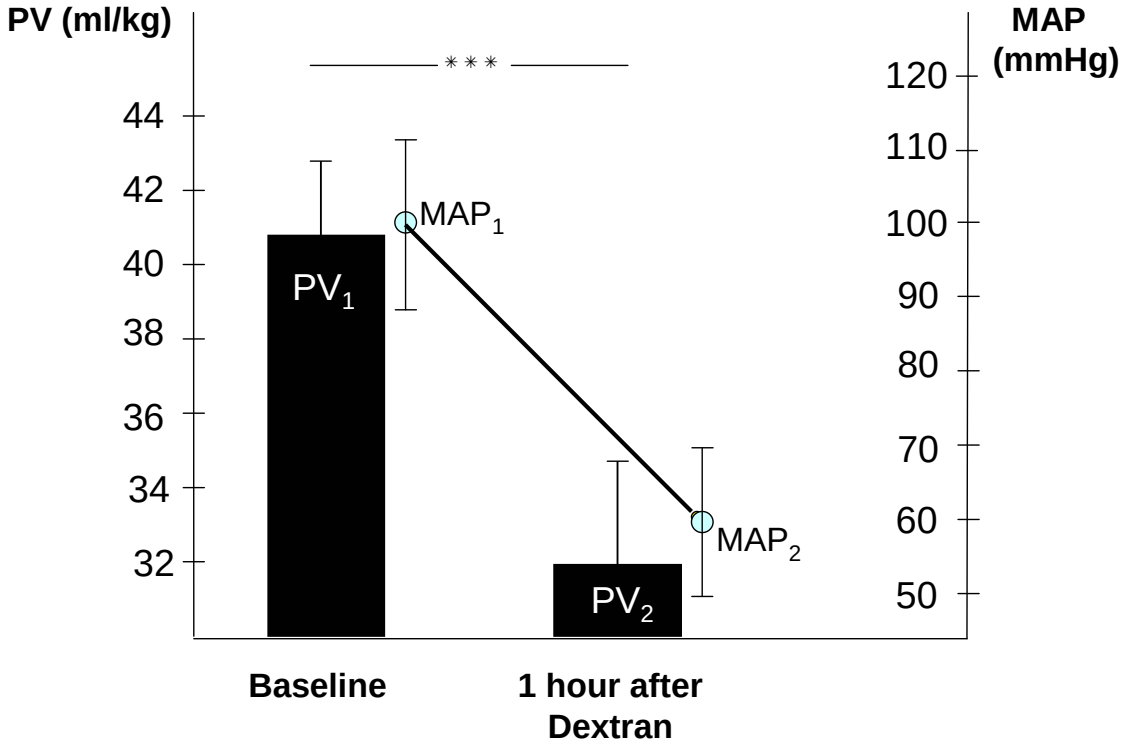

Fig.6. *Plasma volume (PV) and mean arterial blood pressure (MAP) at the baseline and 1 h after dextran-70 injection for the whole population in study III (n=30). ***p<0.001 for plasma volume and for mean arterial pressure.*

Effects on plasma volume of albumin, gelatin, HES and saline under a state of increased microvascular permeability (Study II)

Baseline plasma volume for was 41.1 + 1.9 ml/kg. The remaining increase in plasma volume 3 h after infusion of plasma volume expander (20 ml/kg for the colloids and 80 ml/kg for saline) is shown in Fig. 7; it was 17.1 ± 3.4 ml/kg for albumin , 7.9 ± 3.6 ml/kg for gelatin, 7.4±4.4 ml/kg for HES, and 12.2 ± 3.1 ml/kg for normal saline. The plasma expanding effect was larger in the albumin group than in the other groups ($p<0.05$). There were no differences between the HES, the gelatin and the saline groups. There was no change in plasma volume in the control group from 1 h after the dextran injection to the end of the experiment.

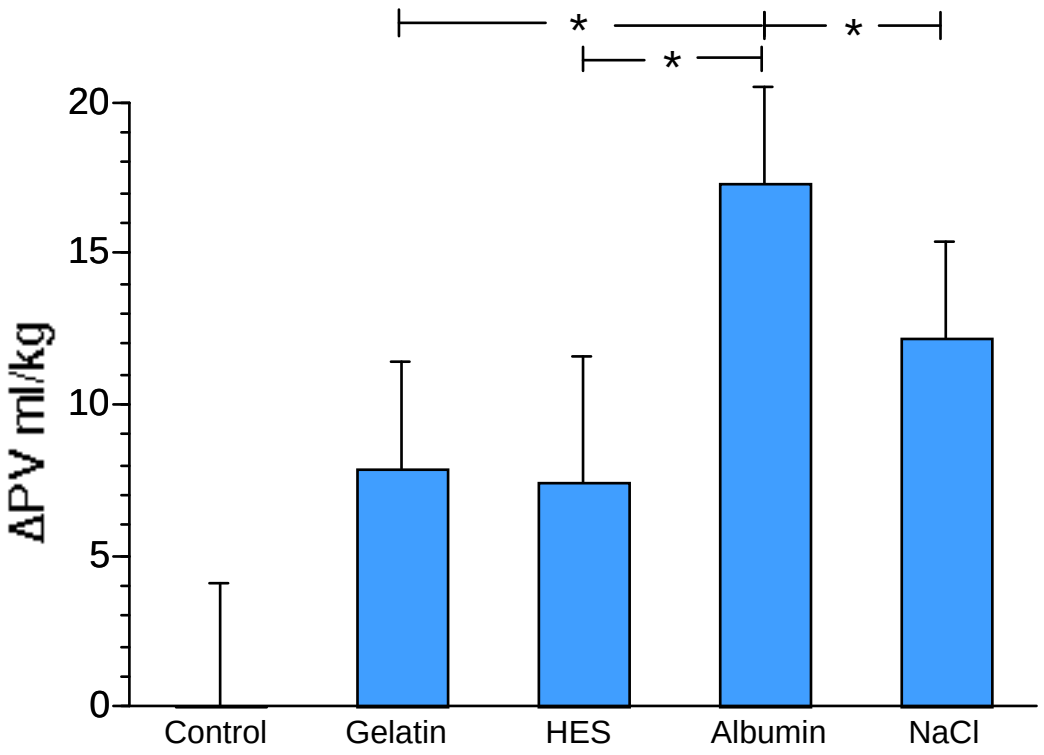

Fig. 7. *Increase in plasma volume, ΔPV, 3 h after the bolus infusion of 20 ml/kg for colloids and 80 ml/kg for saline. No plasma volume expansion was given in the control group. Albumin showed a better volume expanding effect than gelatin, HES and normal saline.*=p<0.05.*

Effects of arterial blood pressure on change in plasma volume during increased permeability (Study III)

The remaining increase in plasma volume (ml/kg) for the different groups 2.5 h after albumin infusion (15 ml/kg) is shown in the Fig. 8. In the control group where no medications affecting blood pressure were given, the remaining increase in plasma volume was 11.8 ± 3.6 ml/kg. In the group treated with noradrenalin after the albumin bolus it was 0.5 ± 6.3 ml/kg ($p<0.01$), and 12.6 ± 4.9 ml/kg in the group given metoprolol/clonidine infusion (ns). In the separate experiments, where noradrenalin was given without plasma volume substitution during increased permeability, the plasma volume had decreased by 3.5 ± 3.0 ml/kg at the end of the experiment.

In the group with normal permeability, where albumin bolus (15 ml/kg) was given after haemorrhage (15 ml/kg), the remaining increase in plasma volume 2.5 h after volume the albumin infusion and noradrenalin infusion was 13.7 ± 3.4 ml/kg.

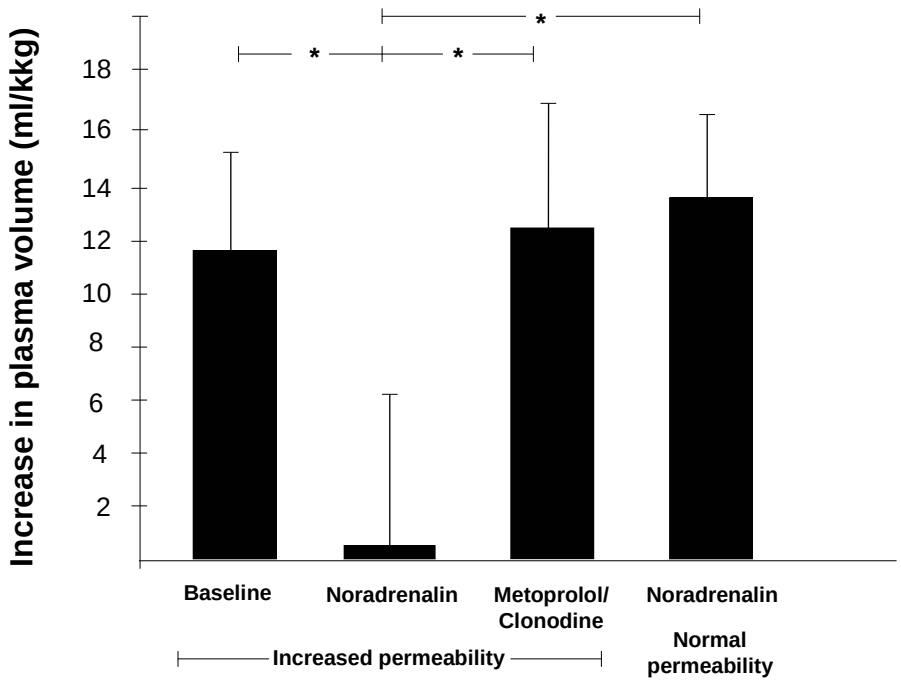

Fig. 8. *Increase in plasma volume 2.5 h after the albumin infusion compared to the plasma volume just before albumin bolus for three groups during increased permeability and for the group given albumin and noradrenalin after haemorrhage during normal permeability.*

Effects of prevailing plasma volume and arterial blood pressure on changes in plasma volume loss (Study IV)

Plasma volume at baseline was 42.0 ± 3.1 ml/kg (n = 59), which is normal in the adult male Spraque-Dawley rat. The plasma volume loss for the 5 groups is shown in Fig. 9. In group 1 given only noradrenalin, there was a plasma volume loss of 2.0 ± 3.6 ml/kg. The plasma volume loss was 11.1 ± 4.0 ml/kg in group 2 given both albumin and noradrenalin. In group 3 given only albumin, there was a plasma volume loss of 5.0 ± 3.4 ml/kg. In group 4 given noradrenalin after haemorrhage, there was a tendency of reabsortion of 2.0 ± 2.7 ml/kg (ns). In group 5 (only haemorrhage) there was a reabsorption of 6.2 ± 1.7 ml/kg.

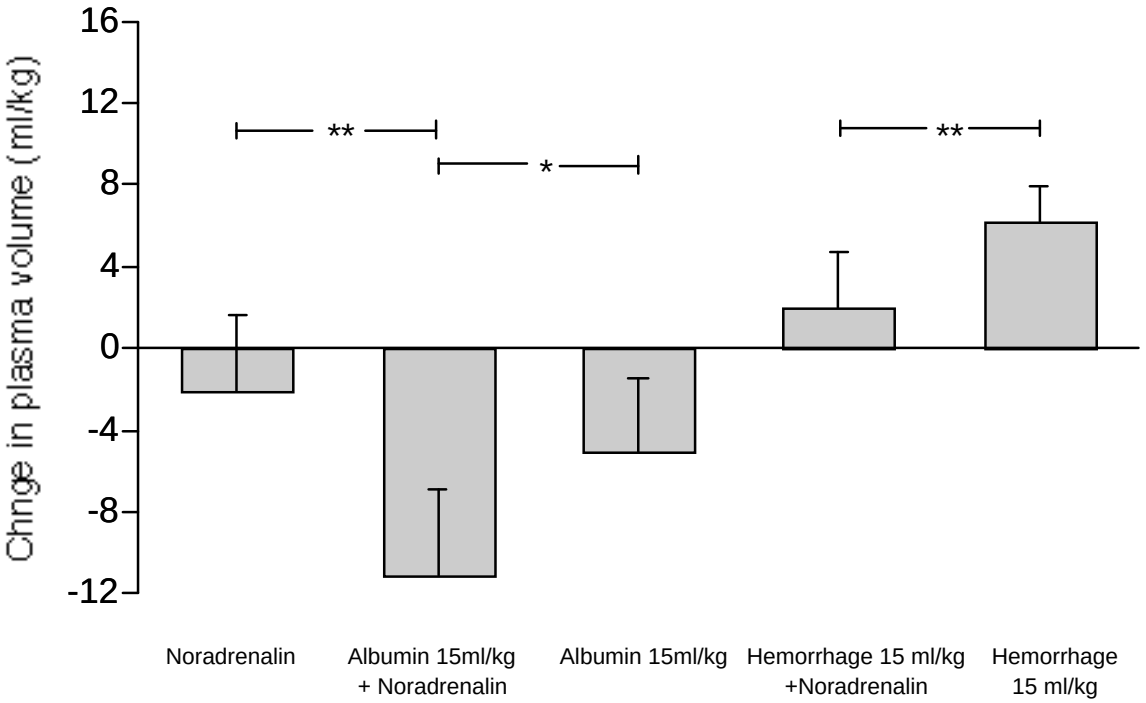

Fig. 9. *Plasma volume change 2.5 h after the start of the noradrenalin infusion (group 1), 2.5 h after the start of the noradrenalin infusion and a bolus infusion at 5% albumin of 15 ml/kg (group 2), 2.5 h after a bolus infusion 15 ml/kg of 5% albumin (group 3), 2.5 h after hemorrhage of 15 ml/kg and noradrenalin infusion (group 4), and 2.5 h after hemorrhage of 15 ml/kg (group 5). * p< 0.05, ** p < 0.01.*

Effects of activated protein C and prostacyclin on protein leakage in the lung and in the gut (Study V)

Inflammatory condition achieved by an infusion of endotoxin (LPS) in sub-lethal dose for 30 hours, produced an increase in albumin leakage both in the lung and in the gut (control group vs. sham group). Fig. 10 and Fig. 11 show the albumin leakage in the gut lung and in the lung, respectively. As seen, the leakage in the sham groups given no LPS was much smaller than in the control groups given only LPS both in the lung and the gut. In the group given LPS and prostacyclin (PGI_2), but not in the group given LPS and activated protein C (APC), there was a smaller leakage in the lung than in the control group. Both in the group given prostacyclin and activated protein C, there was a smaller leakage in the gut than in the control group given LPS only. Albumin leakage is shown as the ratio between the tissue radioactivity relative to the tissue weight (cpm/g tissue) and the injected radioactivity relative to body weight (cpm/g body weight). There was a better arterial oxygenation both in the PGI_2 and in the APC group compared to the control group given LPS only, Fig. 12.

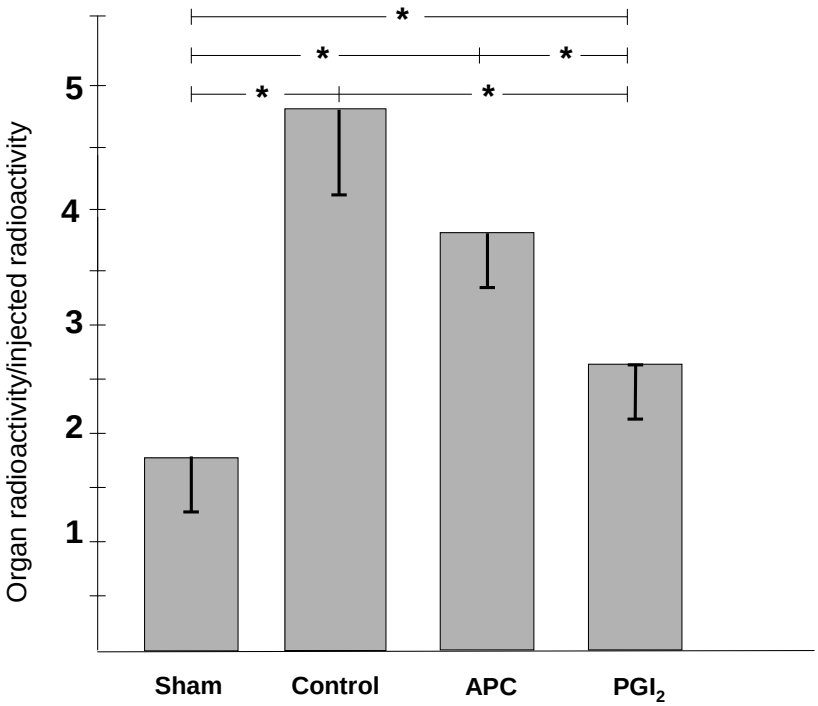

Fig. 10. *Albumin leakage in the lung.*

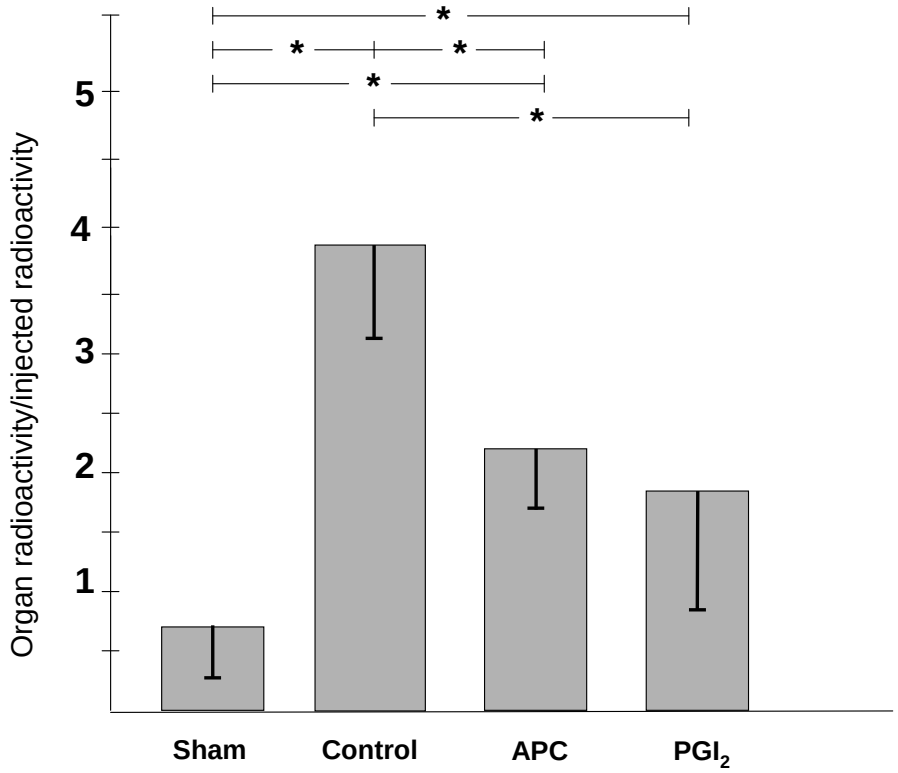

Fig. 11. *Albumin leakage in the gut.*

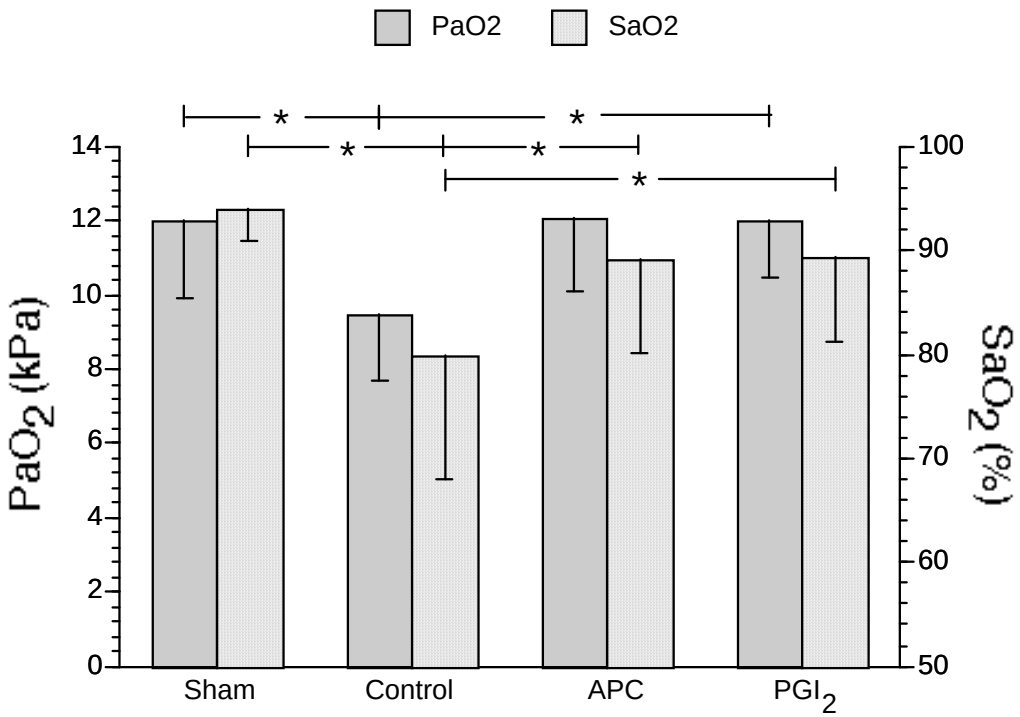

Fig. 12. *Arterial oxygenation.*

PaO_2, arterial oxygen partial pressure; SaO_2, arterial oxygen saturation.

General discussion

Plasma volume expansion during normal and increased microvascular permeability

The extent and the duration of plasma volume expanding effects of administered fluids are of outmost importance in the perioperative and critical care setting. In comparison to crystalloid solutions, the defining properties of colloids are greater plasma volume expansion and more prolonged intravascular persistence. In our studies we have compared plasma volume expanding capacity of currently used colloid solutions and normal saline during normal (I) and increased microvascular permeability (II) in the standardized models of hypovolaemia using direct plasma volume measurements.

The study I performed on the guinea pig was designed to compare 6% dextran 70, with 5% albumin and 6% HES 130/0.4 in haemorrhage induced hypovolemia. It is unlikely that a short period of haemorrhage-induced hypovolemia would cause a systemic inflammatory reaction resulting in increased permeability. Therefore, the model most likely represents a condition with normal permeability. The study showed that 3.0 h after the infusion given in equal volumes, dextran had the best volume expanding capacity of the solutions analysed, and that albumin had better capacity than the HES solution. After the infusion of dextran and albumin there was an increase in plasma volume after 3 h beyond the 20 ml/kg of volume infused, which means a net absorption. The absorption was about 17 ml/kg for dextran to be compared with about 7 ml/kg for albumin. In the HES group no net absorption was observed, and the plasma volume had instead decreased by 2-3 ml/kg after 3 h.

Regarding the plasma volume expanding effect of albumin and HES, the results of the present study (I) performed on the guinea pig are in agreement with results from a

previous study on the rat [141]. The absolute values of increase in plasma volume 3 h after the colloid bolus infusion were higher in the experiments on the guinea pig than those observed for the rat. Thus, while plasma volume had increased by 21-22 ml/kg in the rat 3h after infusion of 20 ml/kg of albumin [141], the corresponding value was almost 27 ml/kg in the guinea pig. The HES infusion of 20 ml/kg induced a plasma volume increase by 14 ml/kg in the rat 3 h after the infusion [141] and 17 ml/kg in the guinea pig. Most likely, these differences can be referred to the species used, as the normal plasma volume is higher in the guinea pig (48-49 ml/kg) [142] than in the rat (40-41 ml/kg) [143]. Thus, the increase in plasma volume relative to baseline plasma volume is similar in the guinea pig and in the rat both for albumin and HES. Nevertheless, the relative difference between albumin and HES was the same in the rat as in the guinea pig.

The relative differences in plasma volume expanding capacity between dextran, albumin and HES in the present study also agree with those in a previous study on the cat with post-traumatic increased permeability [144], in the sense that treatment of hypovolemia with dextran was more effective than that of albumin and HES.

In the study II we have compared clinically available plasma expanders (albumin, gelatin, HES and normal saline) regarding their capacity to restore reduced intravascular volume during increased microvascular permeability. The permeability was increased in a standardized manner by inducing anaphylactic reaction with a small (0.5 ml) fixed bolus injection of dextran [82, 83]. Within one hour after the dextran injection, the anaphylactic reaction resulted in reduction in plasma volume from baseline values of about 41 ml/kg to 29-30 ml/kg. There was a simultaneous increase in Hb concentration and decrease in arterial blood pressure. This model of a dextran-induced increase in permeability has limitations in the sense that it does not represent the whole complexity of inflammation associated with sepsis or other types of critical illness. This fact, however, is of advantage in our studies (II and III) as the purpose was to evaluate changes of plasma volumes under isolated increase in permeability.

The results showed that, under a condition of increased permeability when the colloids were given in equal volumes (20 ml/kg) and saline in a 4 times larger volume (80 ml/kg), 5% albumin is a more effective plasma volume expander than 4% gelatine or 6% HES130/0.4, and that gelatine, HES and saline were equally effective.

As mentioned above, the plasma volume expanders analysed in the present study (II) have been examined in the rat previously in our laboratory, but under the condition of normal permeability [141]. In that study, blood volume was reduced before the infusion by a standardized haemorrhage of 20 ml/kg. While plasma volume had increased by 21.1 ml/kg 3 h by the infusion of 20 ml/kg of 5% albumin in that study, it increased by 17.1 ml/kg in the present study. Corresponding values for gelatine were 13.1 versus 7.9 ml/kg, and 13.8 versus 7.4 ml/kg for HES 130/0.4. For saline, after the infusion of 80 ml/kg, the values were 16.0 and 12.2 ml/kg, respectively. The difference between plasma volume expanding capacity of the solutions studied can be explained by the increase in permeability. The relative difference between the colloid solutions and normal saline in the present study is similar to that in the study performed under a condition of normal permeability [141].

In general terms, the difference in plasma expanding capacity of the colloids may have several explanations. Factors of importance are average MW, MW distribution, oncotic pressure, degradation rate of the solution infused, threshold value for renal elimination, molecular shape, electrical charge, and interference with glycocalyx [4, 11, 12, 26, , 37, 39]. The higher urine production observed in our study (I) of 4-5 ml/kg in the HES group compared to 2-3 ml/kg the albumin and dextran groups can explain about 1 ml/kg of the larger plasma volume loss in the HES group. The renal elimination and enzymatic degradation rate of dextran (via dextranase) is slower than that for HES (via amylase) [145-147]. The fact that 6% dextran 70 has a colloid osmotic pressure twice that of albumin in combination with its relatively slow disappearance rate may contribute to its larger and more persistent plasma volume expanding effect [148].

Taken together, these results clearly showed that both during normal and increased microvascular permeability dextran is a superior plasma volume expander, and albumin has a better volume expanding capacity than gelatine and HES. Moreover, especially dextran, but also albumin have significant absorbing effect during normal permeability. Our findings demonstrated that the plasma volume expanding capacity of both colloids and normal saline is decreased if microvascular permeability is increased.

The results may not be directly transferred to man as, for example, the degradation rate of a colloid solution can be species dependent. The degradation rate of HES may be dependent on the amylase activity in plasma and, thus, can be faster in rodents with their higher plasma amylase activity than in man. If HES130/0.4 has a relatively lower plasma expanding effect than dextran also in clinical practice, it can be partly compensated by the fact that HES can be given in higher daily volumes.

Effects of permeability, blood pressure and prevailing plasma volume on changes in plasma volume

According to the 2-pore theory of transcapillary fluid exchange [38], the escape of plasma fluid and proteins into the interstitial space depends not only on the large pore area but also on the hydrostatic capillary pressure. It follows that the plasma volume loss will increase if the hydrostatic capillary pressure increases, especially when microvascular permeability is increased.

In the study III on a rat model of increased microvascular permeability we have evaluated to what extent the increase in arterial blood pressure will increase the loss of plasma volume. In a normovolaemic condition, the plasma volume loss associated with the increase in microvascular permeability was about 3 ml/kg for 3 h, and it increased up to 14 ml/kg when the blood pressure was moderately increased by noradrenalin infusion. In uncorrected hypovolaemia caused by increased permeability

(decrease in circulating plasma volume by about 25%), the plasma volume loss associated with an increase in blood pressure was only 3.5 ml/kg. The much smaller corresponding loss of plasma volume under normal permeability of only about 2 ml/kg illustrates the importance of the degree of permeability for plasma volume loss.

According to the 2-pore theory, even a small increase in large pore area may cause a marked increase in leakage of fluid and proteins a leakage also dependent on the hydrostatic capillary pressure, and thus arterial and venous pressures and the degree of impaired autoregulation. Our results showed that the plasma volume after an increase in permeability is moderate if blood pressure is low, in this case following the anaphylaxis-induced hypovolaemia and decrease in vascular resistance, but increased significantly when blood pressure was increased to normal or slightly supranormal values. In fact, in the state of systemic inflammation and impaired autoregulation, the microcirculatory dynamics is no longer so well protected from the large pressure swings in the systemic circulation. It means that the capillary hydrostatic pressure is already increased at the baseline because of low arteriolar resistance, and further increase in systemic arterial pressure will produce relatively larger increase in hydrostatic capillary pressure and plasma volume loss than can be expected under intact autoregulation. In addition, the capillary hydrostatic pressure is also a function of the post-/precapillary resistance ratio. It is well known that noradrenalin induces vasoconstriction not only in small arteries and arterioles but also in venules and even larger venous capacitance vessels. This means that the reduction in post-/precapillary resistance ratio will be limited in spite of a large increase in total vascular resistance. This means that the noradrenalin-induced increase in arterial pressure results in a net increase in hydrostatic capillary pressure in spite of its precapillary vasoconstrictor effect. Further, the increase in venous pressure due to vasoconstriction in venous capacitance vessels will be transferred to the capillaries (by about 80%), also contributing to the increase in hydrostatic capillary pressure.

We also showed that plasma volume loss associated with an increase in arterial blood pressure was influenced by the prevailing circulating plasma volume (study III and

IV). In other words, the plasma volume loss was larger from a hypervolaemic state compared to a normovolaemic state, which in turn was larger than from a hypovolaemic state. This was the case both during increased and normal permeability. It means that the higher is the plasma volume, the larger is the blood pressure-induced plasma volume loss. The possible explanation can be a larger increase in hydrostatic capillary pressure when noradrenalin was given at a hyper and normovolaemic state than at a hypovolaemic state. The difference in urine production between groups (2-6 ml/kg) can explain only minor parts of the difference in plasma volume loss.

The hydrostatic capillary pressure, which is an important component in the control of transcapillary fluid exchange, may increase via an increase in arterial pressure and an increase in venous pressure. While the effects of an increase in arterial pressure on hydrostatic capillary pressure will depend on the degree of inflammation via variation in autoregulatory capacity, the effects of an increase in venous pressure will be approximately equal under normal and inflammatory conditions. Our results in study IV that the loss of plasma volume when given noradrenalin under normovolaemia is relatively small may be explained by a preserved autoregulation and that the venous pressure did not increase in this group, while the larger loss with and without noradrenalin after infusion of albumin may be explained by a larger increase in venous pressure because of plasma volume expansion. It seems that noradrenalin and hypervolaemia occurring simultaneously complemented each other regarding their effects on plasma volume loss, perhaps due to a combination of the increase in arterial and venous pressures.

Hypervolaemia will also lead to unloading of the baroreceptor reflex activity, resulting in reduced sympathetic discharge and increase in post/pre-capillary resistance ratio and increased hydrostatic capillary pressure [50].

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Hypovolemia after hemorrhage is normally followed by refill of the intravascular space from the interstitial space, suggested as an effect of reduced transcapillary hydrostatic pressure and increased extracellular glucose osmolality [149, 150]. The existence of this

mechanism was also strengthened in the present study IV by showing restitution of plasma volume after hemorrhage of about 6 ml/kg within 2.5 h. However, also in this situation noradrenalin will reduce plasma volume by preventing the normal physiological plasma volume refill after hemorrhage. Also this effect may be caused by a higher hydrostatic capillary pressure, both by the higher arterial pressure, and a higher venous pressure.

Even after optimal correction of hypovolaemia, the blood pressure still can be low in patients suffering from sepsis/SIRS because of low peripheral vascular resistance. In these situations noradrenalin is used to increase the blood pressure. The current evidence suggests that the mean arterial pressure of 65 mmHg can be considered to be safe in most septic patients [151], and the increase of mean arterial pressure up to 85 mmHg has not shown any improvement in systemic oxygen uptake, skin microcirculation, metabolic function, renal function and urine output [152, 153]. Our study shows that restoration of blood pressure to the normal or supranormal values can have negative consequences in the sense of increased plasma leakage that inevitably leads to decrease in circulating blood volume and impaired oxygen delivery. The increased loss of plasma volume predetermines repeated and more massive fluid administration which leads to more positive fluid balance, tissue edema, and impaired organ function. It means, that the avoidance of unnecessarily high blood pressure in patients with increased permeability may preserve organ function, while the lower safe limit of perfusion pressure must be determined in every patient on the individual basis.

Not only excessive use of vasopressors but also too generous and poorly guided fluid therapy can be deleterious. In our study we have showed that the increase of plasma volume above the normal even during normal permeability causes relatively rapid escape of plasma volume from intravascular to the interstitial compartment, and this process is promoted by the use of noradrenalin. This finding is in agreement with other studies performed using crystalloid solutions [154, 156]. The consequences can be tissue oedema and impaired organ function [19-23, 25]. Too restrictive use of hemodynamic monitoring in clinical praxis often leads to unmotivated fluid

administration as the every episode of circulatory instability, inadequate oxygen delivery and impaired tissue perfusion can be interpreted as the deficit of the circulating volume. In other words, often fluid is given in the situations when patients are preload unresponsive, and, consequently, will not increase cardiac output in the response to fluid bolus. Interestingly, that in preload unresponsive subjects the fluid infused leaves circulation much faster than in the situations of preload responsiveness [156].

Effects of activated protein C (APC) and prostacyclin (PGI_2) on protein leakage.

Increased microvascular permeability for fluid and proteins is a general feature of sepsis/SIRS, and is a result of activation of the complex inflammatory cascade and disturbed microcirculation [2, 74]. It leads to hypovolaemia, which negatively affects systemic oxygen delivery and organ perfusion. Another consequence is tissue edema with further worsening of microcirculation and tissue oxygenation [157, 158]. Moreover, tissue edema directly compromises specific organ functions such as blood oxygenation in the lung, and it can cause various types of compartment syndrome [22].

Microcirculatory dysfunction plays a central role in the pathogenesis of organ failure in the sepsis/SIRS [158-160]. Because of many similarities in the action of activated protein C and prostacyclin (see Introduction) both substances, directly and indirectly, have a potential to counteract sepsis-related microcirculatory dysfunction by increasing and preserving the number of perfused capillaries. Recent clinical studies have shown that the degree of impairment of microcirculatory perfusion correlates not only with the severity of sepsis but also with the severity of other critical illness-related conditions [161-164]. Moreover, the ability to increase the number of capillaries in the progress of the disease is associated with improved outcome [164]. Importantly, the suggested beneficial effect of vasodilators in sepsis in the sense of improvement of microcirculatory perfusion [115, 116] has been confirmed in clinical volume resuscitated sepsis as increased density of perfused capillaries after the use of nitroglycerin [166]. In this respect prostacyclin, because of its vasodilator properties, is

a substance of particular interest. It has been shown that the use of activated protein C improves outcome in patients with severe sepsis and septic shock [138]. However, the recommended dose of activated protein C is associated with significant impairment of coagulation and risk of bleeding, which in many circumstances can be limiting factors in the use of this substance [138, 166, 167]. In contrast, prostacyclin in the dose used (2 ng/kg/min) can be clinically effective in the sense of improvement of microcirculatory perfusion and reduction of permeability, and is virtually free from negative side effects.

In the study V we have analyzed and compared the efficacy of activated protein C and prostacyclin regarding their ability to counteract transcapillary protein leakage in the lung and in the gut in LPS-induced inflammation. We also analyzed the impact of these substances on arterial oxygenation as a marker of lung function. The results of our study V showed that both activated protein C and prostacyclin reduced protein leakage in the gut and improved arterial oxygenation, and prostacyclin reduced protein leakage in the lung.

Both activated protein C and prostacyclin are substances of interest in the sense of their ability to suppress inflammation and improve microcirculation. These properties may theoretically cause a decrease in microvascular permeability and protein leakage, which was confirmed in the present study. The fact that prostacyclin was more effective than activated protein C to reduce protein leakage in the lung may be attributed to greater permeability-reducing effect of prostacyclin. The permeability-reducing effect of prostacyclin has also been demonstrated in several previous studies. Prostacyclin also reduces permeability by direct cyclic adenosine monophosphate-mediated permeability-reducing effect through active relaxation of contractile intra-endothelial filaments. The permeability-reducing effect of activated protein C may be more indirect via improved microcirculation and secondary release of prostacyclin. The mechanisms behind improvement of oxygenation by activated protein C and prostacyclin may be reduction of inflammation, endothelial damage and edema, and also prevention and dissolution of microthrombosis in the lung. Based on our results,

both and prostacyclin may be beneficial in critically ill patients as a part of ‘’microcirculatory activated protein C resuscitation’’. Fewer side effects and a more favorable effect on the lung give some advantages for prostacyclin.

Main conclusions

Three hours after the infusion of 20 ml/kg, 6% dextran 70 had better plasma volume expanding effect than 5% albumin and 6% HES 130/0.4, and 5% albumin was more effective than 6% HES 130/0.4 as shown in a standardized model of haemorrhage (20 ml/kg) in the guinea pig during normal permeability (I).

Both 6% dextran 70 and 5% albumin caused net fluid absorption 3 h after haemorrhage and the colloid infusion, during normal permeability, whereas HES 130/0.4 caused a slight net filtration (I). The absorption effect was not seen under a state of increased permeability.

Three h after the infusion under a state of increased permeability, 5% albumin was more effective than 4% gelatin and 6% HES 130/0.4, and normal saline given in four times larger volume, as plasma volume expanders. Gelatin and HES were equally effective. (II)

All colloid solutions studied, as well as normal saline, were less effective as plasma volume expander during increased than normal microvascular permeability (I-II).

Noradrenalin-induced increase in arterial blood pressure causes plasma volume losses, which are larger during increased than during normal permeability. The loss of plasma volume was influenced by the prevailing plasma volume, and was smaller under hypovolaemia than under normovolaemia, which in turn was smaller than under hypervolaemia (III and IV).

The refill of plasma volume normally seen after hemorrhage is counteracted by noradrenalin infusion (IV).

In LPS-induced inflammation in the rat, prostacyclin reduces protein leakage in the lung and the gut and activated protein C reduce protein leakage in the gut. Arterial oxygenation was improved by both prostacyclin and activated protein C (V).

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Summary in Swedish

Populärvetenskaplig sammanfattning på svenska

Bevarande av normal blodvolym är en av de viktigaste åtgärderna vid behandling av patienter inom intensivvården. För liten blodvolym innebär att cirkulationen i olika vävnader minskar, vilket i värsta fall kan innebära syrebrist i vitala organ som tarm, lever och njure. Blodvolymsförluster uppkommer efter blödning, men den vanligaste orsaken hos patienter med blodförgiftning eller efter trauma är att plasma överföres till vävnaden pga ökad genomsläpplighet av vätska och proteiner över kapillärmembranet. Man kompenserar för dessa förluster genom att tillföra olika plasmavolymsexpanderare eller blod. Kunde man finna någon farmakologisk behandling som motverkade plasmaförluster till vävnaden vore detta en värdefull kompletterande terapi. Vi saknar dock etablerade sätt att på farmakologisk väg minska plasmaförluster över kapillärmembran. Denna avhandling, som består av 5 delarbeten, berör effektiviteten av olika i kliniken använda plasmavolymsexpanderare, samt utvärderar ett par substanser med potentialen att minska vätskeförluster över kapillärmembranet.

I delarbete I värderas den volym effekten av våra vanligaste typer av plasmavolymsexpanderare, nämligen albumin, dextran och stärkelselösning (HES). Vi har valt att analysera de i kliniken vanligaste lösningarna nämligen 5% albumin, 6% dextran 70 och HES 130/0.4. Denna studie är gjord på marsvin och inte råtta, eftersom råtta utvecklar en anafylaktisk reaktion av dextran. I övriga studier användes råtta som försöksdjur. Plasmavolymer mättes med en 125I-albumin utspädningsteknink (delarbeten I-IV). Kolloiden infunderas som en bolusdos med en volym av 20 ml/kg efter att marsvinet har blött 20 ml/kg. Vi finner att efter 3 timmar har kolloidinfusionen inneburit att plasmavolymen ökat med 36 ml/g i dextrangruppen, med 27 ml/kg i albumingruppen samt minskat med 2.5 ml/kg i HES gruppen inräknat

infusionen på 20 ml/kg. Sålunda i denna modell var dextran bäst som plasmavolymsexpanderare och albumin var något bättre än HES.

I delarbete II analyseras den plasmavolymsexpanderande effekten av kolloiderna 5% albumin, HES 130/0.4, samt gelatin i en modell med ökad permeabilitet. Gelatin användes mycket lite i Sverige men är en vanlig plasmavolymsexpanderare i andra länder. Kolloiderna gavs i en volym av 20 ml/kg. Resultaten jämföres även med motsvarande effekter av fysiologisk koksalt givet i 4 gånger större volym. I denna studie göres analysen vid ökad permeabilitet åstadkommet genom att ge en mindre dos (0.5 ml) av dextran 70, utnyttjande att råttan utvecklar en anafylaktisk reaktion med permeabilitetsökning av dextran. Efter 3 timmar återstod 17 ml av infunderat albumin, ca 8 ml av gelatin och HES samt 12 ml av koksalt. I denna modell var albumin en bättre plasmavolymsexpanderare än övriga lösningar.

Den sk 2-porsteorin för transkapillärt vätskeutbyte innebär att plasmaförluster av protein till vävnaden går via ett mindre antal stora porer belägna mot vensidan av kärlbädden. Enligt denna teori sker proteinförluster framför allt genom konvektion via vätskeflödet genom stora porer. I själva poren påverkas detta flöde mindre av det transkapillära onkotiska trycket och drivkraften är därför det hydrostatiska kapillärtrycket. Detta innebär att såväl ökat artärblodtryck och ventrycksökning skulle öka plasmavolymsförluster och speciellt vid ökad permeabilitet. Denna hypotes testades i arbete III på råtta med dextraninducerad ökad permeabilitet. Plasmavolymsförluster efter en albumininfusion vid ökat blodtryck jämfördes med motsvarande förluster vid lägre blodtryck. Blodtrycket ökades medels noradrenalininfusion. Resultatet visade att noradrenalin kraftigt ökade plasmavolymsförluster efter transfusion med albumin jämfört med situationen då inget noradrenalin gavs. Motsvarande förluster var betydligt lägre vid normal permeabilitet.

I delarbete IV studerades effekter av hypervolemi, alternativt hypovolemi på plasmaförluster vid normal permeabilitet med och utan noradrenalin att jämföra med en normovolem situation. Det visade sig att noradrenalin från ett normovolemt

tillstånd inducerade bara måttliga plasmavolymsförluster, medan dessa förluster var kraftigt ökade om man utgick ifrån ett hypervolemt tillstånd efter albumininfusion. Även hypervolemi utan noradrenalin innebar att plasmavolymen strävade mot normovolemi. Studien visade också att noradrenalininfusion från ett hypovolemt tillstånd efter blödning motverkade kroppens normala mekanismer för återfyllning av plasma från vävnaden.

Delarbete V utvärderar i vilken omfattning ämnena prostacyklin och aktiverat protein C (APC) i kliniskt relevanta doser kan motverka ödembildning i tarm och lunga och förbättra syresättning vid endotoxininducerad blodförgiftning på råtta. Detta göres mot bakgrunden av att dessa ämnen kan förbättra vävnaders mikrocirkulation genom att minska bildandet av mikroproppar och att vita blodkroppar fastnar på kärlväggen. Dessutom har speciellt prostacyklin visats ha egenskapen att minska kapillärpermeabilitet. Resultaten visade att ödembildningen i lunga minskade med prostacyklin, och att den minskade i tarm både med prostacyklin och APC. Resultaten visar även på en förbättrad syresättning både i prostacyklin och APC gruppen.
deras som en bolusdos med en volym av 20 ml/kg efter att marsvinet har blött 20 ml/kg. Vi finner att efter 3 timmar har kolloidinfusionen inneburit att plasmavolymen ökat med 36 ml/g i dextrangruppen, med 27 ml/kg i albumingruppen samt minskat med 2.5 ml/kg i HES gruppen inräknat infusionen på 20 ml/kg. Sålunda i denna modell var dextran bäst som plasmavolymsexpanderare och albumin var något bättre än HES.

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minska bildandet av mikroproppar och att vita blodkroppar fastnar på kärlväggen. Dessutom har speciellt prostacyklin visats ha egenskapen att minska kapillärpermeabilitet. Resultaten visade att ödembildningen i lunga minskade med prostacyklin, och att den minskade i tarm både med prostacyklin och APC. Resultaten visar även på en förbättrad syresättning både i prostacyklin och APC gruppen.

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