



LUND UNIVERSITY

Aspects of sepsis/SIRS - An experimental study on fluid therapy, vitamin C and plasma volume in increased permeability

Bark, Björn

2014

[Link to publication](#)

Citation for published version (APA):

Bark, B. (2014). *Aspects of sepsis/SIRS - An experimental study on fluid therapy, vitamin C and plasma volume in increased permeability*. [Doctoral Thesis (compilation), Anesthesiology and Intensive Care]. Anaesthesiology and Intensive Care.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

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

Avdelningen för Anestesi och Intensivvård
Institutionen för Kliniska Vetenskaper, Medicinska Fakulteten,
Lunds Universitet

Aspects of sepsis/SIRS

An experimental study on fluid therapy, vitamin C
and plasma volume in increased permeability

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds universitet
för avläggande av doktorsexamen i medicinsk vetenskap i ämnet anestesiologi
och intensivvård, kommer att offentligens försvaras i
Belfragesalen (D1539a), BMC, Klinikgatan 32, Lund,
fredagen den 24 januari, kl. 13:15

av

Björn Bark



LUNDS UNIVERSITET
Medicinska fakulteten

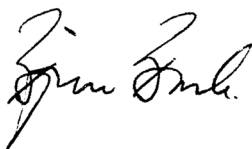
Handledare
Professor Per-Olof Grände

Fakultetsopponent
Professor Christer Svensén

Organization Lund University	Document name Doctoral dissertation	
	Date of issue January 24th 2014	
	Sponsoring organization	
Author(s) Björn Bark		
Title and subtitle Aspects of sepsis/SIRS – An experimental study on fluid therapy, vitamin C and plasma volume in increased permeability		
Abstract		
<p>In sepsis, after major surgery or severe trauma, the human body may suffer from various degrees of generalized inflammation, a syndrome called Systemic Inflammatory Response Syndrome (SIRS). One feature of SIRS is increased capillary permeability, caused by disruption of the capillary endothelium due to e.g. bacterial toxins, cytokines, pro-inflammatory hormones and free oxygen radicals. This will result in leakage of plasma fluid to the interstitium with subsequent intravascular hypovolemia and potentially harmful tissue oedema. Restoration of plasma volume with intravenous fluids is a cornerstone in the treatment of SIRS, but the infused fluids would be expected to leak through the capillary membrane to a greater extent, being less effective and further aggravating oedema. Thus, an important challenge in patients with increased capillary permeability will therefore be to achieve and maintain normovolemia with as little plasma volume substitution as possible. Also, finding a treatment that could seal the leaking capillaries would be of great value.</p> <p>Study I and II, performed in a sepsis/SIRS animal model, showed that the plasma volume expansion of 5% albumin, 6% HES 130/0.4, 4% gelatin and 6% dextran 70 measured 3 hours after start of infusion was larger when given with a slow infusion rate than when given with a fast infusion rate. This effect was not seen with 0.9% NaCl.</p> <p>In study III, performed in rat models, we compared the initial plasma volume expanding effect of 0.9% NaCl in sepsis/SIRS, after a standardized hemorrhage, and in a normal condition. It showed that the increase in plasma volume in relation to the infused volume of 0.9% NaCl (32 mL/kg) were 0.6% in in sepsis/SIRS, 20% after hemorrhage, and 12% when given to rats in a normal state. This means that efficacy of 0.9% NaCl is highly affected by pathophysiological changes in sepsis/SIRS, e.g. increased capillary permeability.</p> <p>In study IV, two different treatment regimes of high-dose vitamin C, initiated 3 hours after induction of sepsis, were investigated regarding their effect on plasma volume loss. None of the treatment regimes were found to have any effect on the loss of plasma volume, or any of the physiological parameters analysed, in the early stage of severe sepsis/SIRS in the rat.</p>		
Key words Sepsis, SIRS, permeability, plasma volume, colloid, crystalloid, vitamin C		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title 1652-8220		ISBN 978-91-87651-28-1
Recipient's notes	Number of pages	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature:



Date: 2013-12-05

Anaesthesiology and Intensive Care
Department of Clinical Sciences, Faculty of Medicine
Lund University

Aspects of sepsis/SIRS

An experimental study on fluid therapy, vitamin C and
plasma volume in increased permeability

Björn Bark



LUND UNIVERSITY
Faculty of Medicine

2014

Copyright © Björn Bark

Lund University
Faculty of Medicine
Department of Clinical Sciences
Anaesthesiology and Intensive Care
ISBN 978-91-87651-28-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2014



To my family

Table of contents

Original studies.....	1
Abbreviations.....	3
Background.....	5
Microcirculation.....	5
Endothelium and capillary permeability.....	6
Transcapillary exchange.....	7
The 2-pore model.....	8
Inflammation.....	9
Fluid therapy.....	10
Vitamin C.....	12
Aims of the studies.....	15
Materials and methods.....	17
Ethics.....	17
Animals.....	17
Anaesthesia and setup.....	17
Sepsis model.....	17
Plasma volume.....	18
Experimental protocols.....	19
Results.....	21
Study I.....	21
Study II.....	22
Study III.....	22
Study IV.....	23

General discussion.....	25
¹²⁵ I-albumin dilution technique.....	25
Colloids, NaCl and sepsis.....	26
Vitamin C and plasma volume.....	29
Main conclusions.....	31
Sammanfattning på svenska.....	33
Acknowledgements and grants.....	35
References.....	37
Appendix.....	45

Original studies

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals.

- I Bark BP, Persson J, Grände PO: Importance of the infusion rate for the plasma expanding effect of 5% albumin, 6% HES 130/0.4, 4% gelatin, and 0.9% NaCl in the septic rat. *Crit Care Med.* 2013; 41:857-66.

- II Bark BP, Grände PO: Infusion rate and plasma volume expansion of dextran and albumin in the septic guinea pig. *Acta Anaesthesiol Scand.* 2013; DOI: 10.1111/aas.12228. [Epub ahead of print]

- III Bark BP, Öberg CM, Grände PO: Plasma volume expansion by 0.9% NaCl during sepsis/systemic inflammatory response syndrome, after hemorrhage, and during a normal state. *Shock.* 2013; 40:59-64.

- IV Bark BP, Grände PO: The effect of vitamin C on plasma volume in the early stage of sepsis in the rat. *Resubmitted after revision. Intensive Care Medicine Experimental.*

Abbreviations

^{125}I	125-iodine
ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
Da	Dalton
HES	Hydroxyethyl starch
J_v	Transvascular fluid exchange rate
L_p	Hydraulic conductivity
P_a	Arterial pressure
P_c	Capillary hydrostatic pressure
P_i	Interstitial hydrostatic pressure
PV	Plasma volume
P_v	Venous pressure
R_a	Arterial vascular resistance
R_v	Venous vascular resistance
S	Microvascular surface area available for fluid exchange
SIRS	Systemic inflammation response syndrome
π_c	Capillary oncotic pressure
π_i	Interstitial oncotic pressure
σ	Reflection coefficient for macromolecules

Background

Microcirculation

The “modern” view of the blood circulating from arteries into veins by the motion of the heart was first described in 1628 by William Harvey (1578-1657) in *Exercitatio anatomica de motu cordis et sanguinis in animalibus* (An Anatomical Exercise on the Motion of the Heart and Blood in Living Beings) (Androustos et al. 2012). The peripheral arterio-venous connection, however, remained a mystery until Marcello Malpighi (1628-1694), with the help of the newly invented microscope, discovered capillaries in 1661 (West 2013).

The purpose of the blood circulation is to provide body tissues with nu-

trients, fluid and oxygen, and to carry away waste products produced by the metabolism, and the key parts in the exchange between blood and tissue are the capillaries (Guyton and Hall 2011). These vessels, the smallest in the body, have a diameter of only 4-8 μm (for comparison the diameter of an erythrocyte is 7.5 μm) and a length of 500-1000 μm . But as they exist in unimaginable numbers, in the order of 10^{10} , the result is a total capillary surface area of about 500-700 m^2 leaving almost no cell in the body farther away from a capillary than 20-30 μm (Guyton and Hall 2011, Levick 2010).

The arteries, carrying the blood from the heart, repeatedly divide to form smaller and smaller vessels, ending up

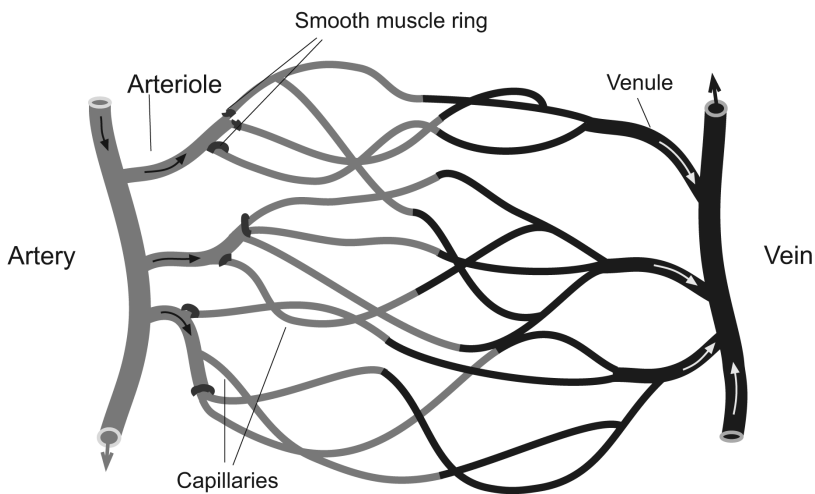


Figure 1. Schematic drawing of the microvascular network. Arrows indicate the direction of blood flow.

on the arterial side of the capillary with the terminal arterioles. The terminal arterioles are encircled by a single unit smooth muscle ring, which is the final contractile element before the capillaries (Fig. 1). The arterioles respond to the local tissue environment, e.g. concentrations of metabolites, oxygen and hydrogen ions, by either dilating or contracting, thereby closely regulating the tissue blood flow to match the demand. In many tissues at rest, a large part of the arterioles are contracted, leading to a small perfusion, but with an increase in tissue demand of nutrients and oxygen (e.g. physical activity), the number of open arterioles, and subsequently the number of perfused capillaries, will increase (Melander 1968, Guyton and Hall 2011). Under normal circumstances, a single terminal arteriole will not stay “open” or “closed” for very long, as the arterioles change states in a cyclic mode about every 15 seconds, a phenomenon known as vasomotion, resulting in cyclic perfusion of the capillaries as well (Ragan et al. 1988).

Endothelium and capillary permeability

The capillary wall consists of a single layer of endothelial cells surrounded by a continuous basement membrane, resulting in a total wall thickness, and thus diffusion distance, of only about 0.3-0.5 μm (Clough 1991). The endothelial cells are connected to each other by different adhesion proteins,

forming the intercellular cleft where transport of fluid and small solutes from plasma to the surrounding tissue takes place. The cleft is a dynamic structure that can be regulated by intracellular signals in response to environmental factors, increasing or decreasing the gap between the cells, thereby regulating the transcapillary transport of water and small solutes to the interstitium (Galley and Webster 2004). The luminal side of the endothelial cells is coated with a 0.1-0.5 μm thick layer of glycocalyx, a negatively charged hydrated gel of carbohydrate polymers, that acts as a barrier preventing plasma proteins from reaching the intercellular cleft, thereby maintaining them in the circulating plasma (Rippe et al. 2001, Levick and Michel 2010). In some endothelial cells transcellular holes, fenestrae, penetrate all the way through the cells resulting in another pathway for fluid and substances to pass from the circulation to the surrounding tissue (Michel and Curry 1999).

The constitution of the capillary endothelium differs between different organs and tissues depending on the specific local need for vascular integrity. The brain, for example, is protected from the circulation by extremely tight capillaries, allowing only small and lipid soluble substances, such as carbon dioxide and oxygen, to passively pass (Fenstermacher 1984). The capillary network of the liver, on the other hand, is highly permeable to almost everything dissolved in plasma, including plasma proteins (Levick 2010).

Transcapillary exchange

By far the most important ways by which substances are transported from the circulation to the surrounding tissue is diffusion and filtration. The diffusion process is passive, i.e. without energy consumption, and the driving force is differences in concentrations of the diffusing substance between the plasma and the interstitial fluid, where the flow of diffusion is directed down the concentration gradient (Renkin 1986). Lipid-soluble substances pass readily through the cell membranes of the endothelial cells. Water-soluble substances, on the other hand, cannot pass the lipid cell membrane but diffuse through the intercellular cleft and transcellular fenestrae. Water filtration is also passive but flows through the intercellular clefts and fenestrae down a pressure gradient - the net effect of the hydrostatic and the oncotic pressures. Together with the water, some substances are also swept along and thereby transported through the capillary membrane, a process called convection. Large lipid-insoluble molecules, e.g. plasma proteins, cross the capillary membrane through much less abundant larger intercellular gaps, and maybe to some extent also through a transcellular vesicular transport system (Levick 2010). The latter, however, most probably have a very limited capacity.

Different substances experience more or less difficulty to pass the capillary membrane due to physical properties

such as molecular size and charge in relation to the size and charge of the transcapillary pores and the membrane. The fraction of substance reflected by the capillary membrane is called the reflection coefficient (σ) (Rippe 1986). A substance that freely passes the membrane would have a reflection coefficient of zero ($\sigma=0$), while a substance not passing the membrane at all would have a reflection coefficient of one ($\sigma=1$). The semipermeable properties of the capillary membrane give rise to concentration differences of macromolecules, primarily proteins, between the intravascular space and the surrounding interstitium, resulting in a transcapillary force called colloid osmotic pressure or oncotic pressure. The plasma oncotic pressure is, in the normal case, in the range of 25 mmHg, and exerts a force directed in to the vascular space responsible for retaining fluid in the circulation (Holbeck 2006).

In 1896, a British physiologist, Ernest Henry Starling (1866-1927), described the forces involved in transvascular fluid exchange (Starling 1896). This was later summarized in the following equation, known as the Starling equation for transvascular fluid exchange (Renkin, 1986):

$$J_v = L_p S [(P_c - P_i) - \sigma(\pi_c - \pi_i)] \quad \text{Eq. 1}$$

where J_v is the volume filtered per time unit, L_p is the hydraulic conductivity, i.e. how easily fluid passes the membrane, S is the total membrane area available for filtration, P_c is the capil-

lary hydrostatic pressure, P_i is the interstitial hydrostatic pressure, σ is the reflection coefficient, π_c is the capillary oncotic pressure and π_i is the interstitial oncotic pressure. From this equation we can see that transcapillary flow is proportional to the hydrostatic pressure difference across the membrane minus the opposing oncotic pressure difference corrected for the permeability for macromolecules.

The most variable of the Starling forces is the capillary hydrostatic pressure (P_c). P_c is determined by arterial and venous pressure (P_a and P_v), and the relation between pre- and postcapillary resistance (R_a and R_v). Pappenheimer and Soto-Rivera (1948) summarized the relation in the following equation:

$$P_c = [(R_a/R_v)P_v + P_a] / [1 + (R_a/R_v)] \quad \text{Eq. 2}$$

In most tissues there is a net filtration of fluid from the intravascular space to the interstitium, transporting fluid, nutrients, etc. from the blood stream to the surrounding cells (Levick 2010).

It was previously believed to be a continuous reabsorption of fluid from the interstitium on the venous end of the capillaries, preventing tissue swelling. In the normal case, however, there is a net filtration pressure along the entire capillary (Levick 1991). Instead, the produced interstitial fluid is drained as lymph and returned to the circulation by the lymphatic system. The capacity of the lymphatic system is, in the normal case, large enough to drain the interstitial fluid produced and pre-

vents accumulation of fluid in the interstitium (Huxley and Scallan 2011, Scallan and Huxley 2010). All these factors described above contribute to maintain tissue fluid equilibrium. Alterations in Starling forces, such as increased P_c (e.g. heart failure), reduced π_c (e.g. malnutrition), increased L_p or reduced σ (e.g. systemic inflammation, sepsis), or impaired lymphatic drainage (e.g. post mastectomy) will disturb the homeostasis and might result in tissue oedema (Fishel et al. 2003).

The 2-pore model

The 2-pore model of transcapillary fluid exchange states that the endothelial membrane contains two types of pores: small pores and large pores (Rippe and Haraldsson 1994). The small pore would represent the normal intercellular cleft, has a radius of 4-6 nm and is permeable only to water and small solutes. The large pore, calculated to be 10.000-30.000 times less abundant, represents larger intercellular clefts, has a radius of about 20-30 nm and is also permeable to macromolecules such as proteins. The proteins are transported through the pore together with water via convection (Fig. 2). Both the small and the large pore were proposed to exist in the 1950s (Pappenheimer et al. 1951, Grotte 1956), and they were visualized with electronic microscope first in the 1980s (Bundgaard 1984) and 1990s (McDonald et al. 1999). Even though they have a smaller radius, under normal conditions the ma-

major part of transvascular fluid transport takes place through small pores (85-95%), as they by far outnumber the large pores (Michel and Curry 1999, Rippe et al. 2001). As the large pores are freely permeable to plasma proteins, the transcapillary oncotic pressure difference ($\pi_c - \pi_i$) across the pore is close to zero and the dominating factor determining the fluid flow through the pore will be the difference in transcapillary hydrostatic pressure ($P_c - P_i$). An increase in capillary pressure would therefore cause an increase in fluid flow through the large pore and a simultaneous increase in plasma protein loss to the interstitium via convection. The increased permeability in states of sepsis and systemic inflammatory response syndrome (SIRS) is suggested to be caused by an increased number of large pores (Levick and Michel 2010, McDonald et al. 1999), which would explain the protein and plasma volume loss seen in these conditions.

Inflammation

The classic signs of a localized inflammation are redness, heat, pain, swelling and loss of function. These symptoms are caused by a number of different pro-inflammatory mediators (e.g. TNF- α , interleukins, histamine, prostaglandins, serotonin, bradykinin, superoxide radicals, thrombin, substance P, etc.) released by the affected cells (Levick 2010). The swelling consists of a protein rich interstitial oedema, which is caused by increased microvascular permeability due to widening of the intercellular clefts, formation of transcellular gaps and degradation of the glycocalyx (Fishel 2003). This is what the 2-pore model would recognize as an increased number of large pores (Rippe 1994). Simultaneously, the underlying interstitial collagen is loosened by fibroblasts (Koller and Reed 1992). Transferred to the Starling equation (Eq. 1), this process results

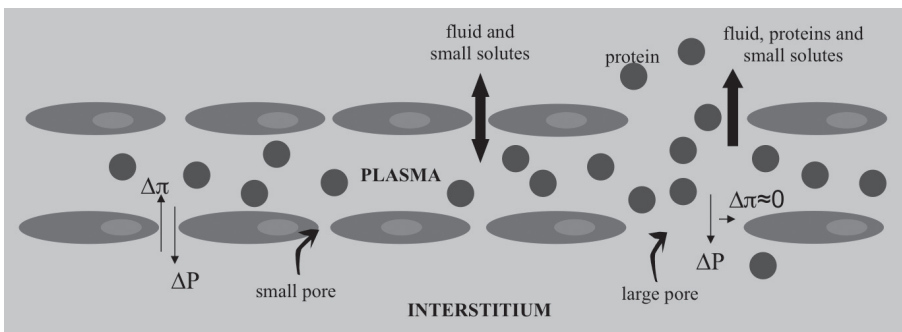


Figure 2. Schematic drawing of the 2-pore model. $\Delta P = P_c - P_i$, $\Delta\pi = \pi_c - \pi_i$.

in reduced reflection coefficient (σ), increased interstitial oncotic pressure as interstitial protein concentration increases (π_i) and decreased interstitial hydrostatic pressure (P_i). The redness and heat is caused by arteriolar vasodilation, which reduces R_a/R_v (Eq. 2) thereby increasing capillary hydrostatic pressure (P_c). The net result of these changes is a dramatic increase in fluid extravasation (van Hinsbergh 1997, McDonald et al. 1999, Webb 2000).

This is a normal pathophysiological process aimed to protect and repair damaged tissue. In cases of severe trauma, general ischemia or serious infections (sepsis) the inflammatory reaction and the increased microvascular permeability can be widespread, affecting all parts of the body, resulting in systemic inflammatory response syndrome, SIRS. This is a serious and potentially lethal condition marked by hypovolemia, general oedema and inadequate tissue perfusion. To prevent further damage by hypoperfusion of the tissues and organs, restoration of normovolemia using plasma volume substitutes, crystalloids or colloids, is a central part in the treatment of these patients (De Backer et al. 2011, Dellinger et al. 2013). However, in a state of increased capillary permeability, the infused fluids would be expected to leak to a greater extent through the capillary membrane, being less effective and further aggravating oedema (Marx 2003).

Thus, an important challenge in patients with increased capillary permea-

bility would therefore be to achieve and maintain normovolemia with as little plasma volume substitution as possible, thereby minimizing transcapillary leakage and adverse oedema formation. Also, finding a way of treatment to seal the leaking capillaries would be of great value.

Fluid therapy

Intravenous injections are described as early as the 17th century, but it was not until the cholera epidemics in the 1830s that the first reported intravenous fluid treatment took place. The fluids used, however, were not sterile and often hypotonic, resulting in severe side effects, and even death from hemolysis and infections, and the therapy did not gain popularity (Cosnett 1989, Gamble 1953). Further research and experiments during the late 19th and early 20th century resulted in the development of sterile balanced salt solutions, what we today would recognize as crystalloid solutions. These solutions contain small particles, mainly electrolytes (e.g. Na^+ , Cl^-), sometimes buffers (e.g. lactate, acetate) and are mainly isotonic. The three dominating crystalloid solutions are: normal saline (0.9% NaCl), Ringer's acetate and Ringer's lactate (not registered in Sweden). As the capillary membrane is freely permeable to a crystalloid solution, it is rapidly distributed in the whole extravascular space, which means that the plasma volume-expanding effect is relatively poor, in the range 20-25% of the infused volume (Berne

and Levy 1993, Nolan 1999, Guyton and Hall 2011). This is because the ratio of the plasma volume and the interstitial volume is approximately 1:4. Large volumes of crystalloid solutions are therefore needed to replace intravascular volume losses (Lamke et al. 1976, Grathwohl et al. 1996). The need for other solutions, that better expanded the plasma volume, was early recognized and the search for substances that remained in the bloodstream after administration was started.

In 1915 a colloid solution containing gelatin was introduced into clinical practice, but it had severe side effects and was difficult to store (Haljamäe 2006). The gelatin products were gradually improved and reached their modern compositions in the early 1960s. Gelatin is derived from bovine collagen (Nolan 1999), and the solutions are polydisperse with a rather small mean molecular weight (30 kDa). It is rapidly cleared from the circulation and excreted through the kidneys, resulting in a short-lived volume expanding effect. It also carries the highest risk of all colloid solutions to induce allergic reactions (0.35%) (Laxenaire et al. 1994, Barron 2004).

In the early 1940s albumin was purified from human plasma and used massively in clinical practice during World War II. Albumin contributes to approximately 80% of plasma oncotic pressure, and has several physiological properties (e.g. transport function). It is the only natural colloid solution and has a rather uniform molecular size of

65-69 kDa (Imm and Carlson 1993). In the normal case, each hour 5-7% of the plasma albumin escapes the circulation to the interstitium and recirculate via the lymphatic system. The rate of escape can increase significantly in states of increased microvascular permeability (e.g. sepsis, trauma, major surgery) (Fleck et al. 1985, Groeneveld et al. 1987, Haskell et al. 1997) reducing the efficacy of the treatment with potential risk of accumulation of albumin in the interstitium, as the lymphatic system might get overloaded, and albumin is degraded slowly.

In 1947 dextran, a glucose polymer, was introduced. Several different solutions are available with different molecular sizes (40-70 kDa) in different concentrations dissolved in different fluids (e.g. dextrose, saline). Approximately half of infused dextran is excreted through the kidneys, while the rest is degraded to CO₂ and water by endogenous dextranase (Haljamäe and Hjelmqvist 2006). Dextran has good plasma volume expanding properties, but can cause anaphylactic reactions because of naturally occurring antibodies (0.3%). Pretreatment with dextran hapten (20 mL dextran 1) reduces this incidence markedly (0.0014%) (Hedin and Ljungström 1997). Dextran solutions also affect the coagulation system, a side effect that may increase the risk of bleeding.

Hydroxyethyl starch (HES) was introduced in 1957, but came in to clinical use first in the 1970s. HES is a branched glucose polymer derived from

maize or potatoes. Solutions are available with different molecular sizes and concentrations, and are all polydisperse. The smaller molecules (< 60-70 kDa) is eliminated by renal filtration, while the larger molecules are broken down by plasma amylase before eliminated in the urine (Jungheinrich et al. 2002, Vercueil et al. 2005). The last few years, however, the safety of HES solutions has been seriously questioned, as large studies have shown worse outcome in patients treated with HES (Perner et al. 2012, Myburgh et al. 2012, Brunkhorst et al. 2008).

Vitamin C

The clinical symptoms of scurvy were described as early as 1700 BC and repeatedly thereafter throughout history. Scurvy was a limiting factor when making long distance voyages, killing large numbers of sailors during the Age of Discovery. During the 300 years between 1500 and 1800, more than two million sailors are estimated to have died from the disease.

In 1753 a Scottish doctor, James Lind, published "A treatise of scurvy" where the importance of dietary intake of fresh vegetables, lemons and oranges were underlined in order to protect against and treat scurvy, and this sailor plague could finally be extinct (Carpenter 2012, Chatterjee 2009). The active substance of this treatment regime, the antiscorbutic factor, was discovered in 1927 by the Hungarian scientist Albert Szent-Györgyi (1893-

1986) working at the Hopkins Laboratory in Cambridge, UK. The precise nature of the substance he had discovered was, however, unknown to him and it was finally named hexuronic acid (Szent-Györgyi 1928). In 1932 a research team led by Charles Glen King (1896-1988) at Columbia University isolated vitamin C from lemon juice (King and Waugh 1932). Further studies revealed that it was identical to Szent-Györgyi's hexuronic acid (Svirbely and Szent-Györgyi 1932), and an infected controversy regarding the discovery followed. In 1937 the battle was finally won by Szent-Györgyi as he was awarded the Nobel Prize in Physiology or Medicine for his discoveries.

In the 1980s, free oxygen radicals were suggested to be a pathogenetic factor behind the massively increased microvascular permeability after burns (Till et al. 1989, Demling et al. 1987, Saez et al. 1984). Different substances with free-radical scavenging capabilities were tested, among them vitamin C, which showed beneficial effects in burns (e.g. preventing capillary leakage and fluid requirements) both experimentally and clinically (Matsuda et al. 1992, Tanaka et al. 1999, Tanaka et al. 2000, Kremer et al. 2010).

Sepsis, as well as burns, is known to cause oxidative stress by production of free oxygen radicals, with the subsequent consumption of antioxidant molecules (Wilson and Wu 2012, Tyml 2011) explaining why sepsis patients often have low levels of vitamin C (Galley et al. 1996). Recent experi-

mental studies have shown that high-dose vitamin C can reduce transcapillary leakage of plasma markers, reduce lymph flow and reduce oedema formation in animal sepsis models (Fisher et al. 2011, Zhou et al. 2012). The mechanism of action is not fully understood, but besides scavenging oxygen radicals, vitamin C also reduces endothelial adhesion molecules and modulates of nitric oxide production (Wu et al. 2004, Friedl et al. 1989), thereby improving microvascular function.

Aims of the studies

Study I

To compare the plasma volume expanding effect, in sepsis/systemic inflammatory response syndrome (SIRS), of a fast infusion rate with that of a slow infusion rate of a fixed volume of 5% albumin, of the synthetic colloids 6% HES 130/0.4 and 4% gelatin, and of 0.9% NaCl, and to compare the plasma expanding effect between these fluids.

Study II

To evaluate the plasma volume expanding effect, in sepsis/SIRS, of a fast infusion rate with that of a slow infusion rate of a fixed volume of 6% dextran 70 and 5% human albumin, and to compare the plasma expanding effect of these fluids.

Study III

To compare the degree of plasma volume expansion by 0.9% NaCl in sepsis/SIRS, after a standardized hemorrhage, and in a normal condition.

Study IV

To evaluate the effect of post-injury high-dose vitamin C-treatment on the loss of plasma volume in the early stage of sepsis

Materials and methods

Ethics

All studies were approved by the Ethical Committee on Animal Experiments, Lund, Sweden, and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals.

Animals

The experiments were performed on professionally bred male adult Sprague-Dawley rats (I, III, IV) or male adult Dunkin-Hartley guinea pigs (II).

Anaesthesia and set up

Anaesthesia was induced by placing the animals in a covered glass container with a continuous supply of 5% isoflurane in air (Forene® 100%; Abbot Scandinavia AB, Solna, Sweden). After induction, the animals were removed from the container, and anaesthesia was maintained with 1.5–1.8% isoflurane in air using a mask, while tracheostomy was performed. Then the animals were connected to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy), and ventilated in a volume-controlled mode with a positive end expiratory pressure

of 4 cmH₂O. Anaesthesia was maintained with 1.5–1.8% isoflurane in air throughout the experiment. End-tidal PCO₂ was continuously monitored (Capstar-100; CWE, Ardmore, PA). Rectally measured body temperature was kept at 37.1–37.3°C via a feedback-controlled heating pad. The left femoral artery (I, III, IV) or the left common carotid artery (II) was cannulated to monitor arterial blood pressure and to obtain blood samples for analysis of electrolytes, haematocrit, lactate, arterial blood gases (I-STAT; Abbot Point of Care Inc, Abbot Park, IL), and plasma volumes. The left femoral vein (I, III, IV) or the left internal jugular vein (II) was cannulated and used for infusions, and kept open with a continuous infusion of saline at 0.2 µL/min. The right internal jugular vein was cannulated and used for injection of ¹²⁵I-albumin for plasma volume measurements. After the experiments, the animals were killed with an intravenous injection of potassium chloride.

Sepsis model

A well-established model of severe sepsis (Scheiermann et al. 2009) was used in the present study. After a longitudinal midline skin incision over the abdominal wall with diathermia, a la-

parotomy was performed by incision along the linea alba. The caecum was ligated just below the ileo-caecal valve and an incision of 1 cm in length was made in the caecum, allowing leakage of faeces into the abdominal cavity, thereby inducing sepsis/SIRS. The abdominal wall and the skin were then closed with clips. After a few hours there was a significant decrease in plasma volume, indicating systemic inflammation with increased microvascular permeability.

Wallac, Turku, Finland). Free iodine was measured regularly following precipitation with 10% trichloroacetic acid.

Plasma volume

Plasma volume (PV) was determined by measuring the radioactivity in 100 μL of plasma taken 5 min after an intravenous 0.5-mL injection of human ^{125}I -albumin with a known amount of activity. To calculate the amount of radioactivity given (n_{tot}), the remaining activity in the used and emptied vial, syringe, and needle was measured and subtracted from the total activity in the prepared dose. The increase in plasma radioactivity was then calculated by subtracting the concentration of activity in a blood sample taken just before the injection (C_1) from that taken 5 min after the injection (C_2), thereby adjusting for any remaining radioactivity from previous measurements. PV could then be calculated:

$$\text{PV} = n_{\text{tot}} / (C_2 - C_1) \quad \text{Eq. 3}$$

Radioactivity was measured with a gamma counter (Wizard 1480; LKB-

Experimental protocols

Study I

The plasma volume expanding effects of a fixed volume of different plasma volume expanders were investigated when given at a slow (3 hrs) or a fast (15 mins) infusion rate in a rat model of severe sepsis. Treatment was given intravenously and started 3 hrs after the initiation of sepsis. The plasma volume expanders analysed were 5% albumin (Albumin Baxter, 50 g/L, Baxter Medical AB, Kista, Sweden), 6% HES 130/0.4 (Voluven[®], 60 mg/mL, Fresenius Kabi, Uppsala, Sweden), 4% gelatin (Gelofusine[®], 40 mg/mL, Braun Medical AB, Danderyd, Sweden) and 0.9% NaCl (Natriumklorid, 9 mg/mL, Fresenius Kabi, Uppsala, Sweden). Plasma volumes were measured at baseline, 3 hrs after initiation of sepsis and at the end of the experiment 3 hrs later. A control group that underwent the same surgical procedure, but received no treatment, was also included in the study.

Study II

The plasma volume expanding effects of a fixed volume of two different plasma volume expanders were investigated when given at a slow (3 hrs) or a fast (15 mins) infusion rate in a guinea pig model of severe sepsis. Treatment was given intravenously and started 3

hrs after the initiation of sepsis. The plasma volume expanders analysed were 6% dextran 70 (Macrodex[®], 60 mg/mL, MEDA AB, Solna, Sweden) and 5% albumin (Albumin Baxter, 50 g/L, Baxter Medical AB, Kista, Sweden). Plasma volumes were measured at baseline, 3 hrs after initiation of sepsis and at the end of the experiment 3 hrs later. A sham group that underwent the same surgical procedure, but received no treatment, was also included in the study.

Study III

The plasma volume expanding effect of 0.9% NaCl (Natriumklorid, 9 mg/mL, Fresenius Kabi, Uppsala, Sweden) was investigated in rats. A fixed volume (32 mL/kg) given to 3 different groups: a sepsis/SIRS group in which sepsis/SIRS was induced by cecal ligation and incision, a hemorrhage group, in which the rats were left without intervention for 4 hrs and bled 8 mL/kg thereafter, and a third group that was left without intervention. Then, 4 hrs after baseline, all 3 groups were given an infusion of 0.9% NaCl (32 mL/kg) for 15 mins. Baseline was defined as the time point when the surgical preparation was finished. Plasma volumes were measured at baseline, at 4 hrs just before start of the infusion, and finally 20 mins after the end of infusion.

Study IV

The effect of intravenous vitamin C (Askorbinsyra 100 mg/mL, APL, Stockholm, Sweden) on plasma volume was evaluated in the early stage of sepsis in the rat. We compared 2 different treatment regimes: one with a small bolus dose (66 mg/kg) followed by a continuous infusion (33 mg/kg/h) (Tanaka et al. 1999, Kremer et al. 2010), and one with a high bolus dose (200 mg/kg) as single treatment (Zhou et al. 2012, Fisher et al. 2011). Treatment was initiated 3 hrs after induction of sepsis. A sham group that underwent the same surgical procedure, but received no treatment, was also included in the study. Plasma volumes were measured at baseline, at 3 hrs after the end of surgical preparation, and at the end of the experiment another 3 hrs later.

Results

Study I

The plasma expansion of 5% albumin, 6% HES130/0.4, and 4% gelatin was larger 3 h after the start of infusion when given with a slow infusion rate than when given with a fast infusion rate. This difference was more pronounced with albumin than with the other colloids. Given in equal vo-

lumes, the plasma volume expanding effect 3 h after start of the infusion was better for 5% albumin than for 6% HES 130/0.4 and 4% gelatin. The plasma expanding effect of 0.9% NaCl was not affected by the infusion rate, and 0.9% NaCl was not more effective than any of the colloids, even though it was given in a 4 times larger volume (Fig 3).

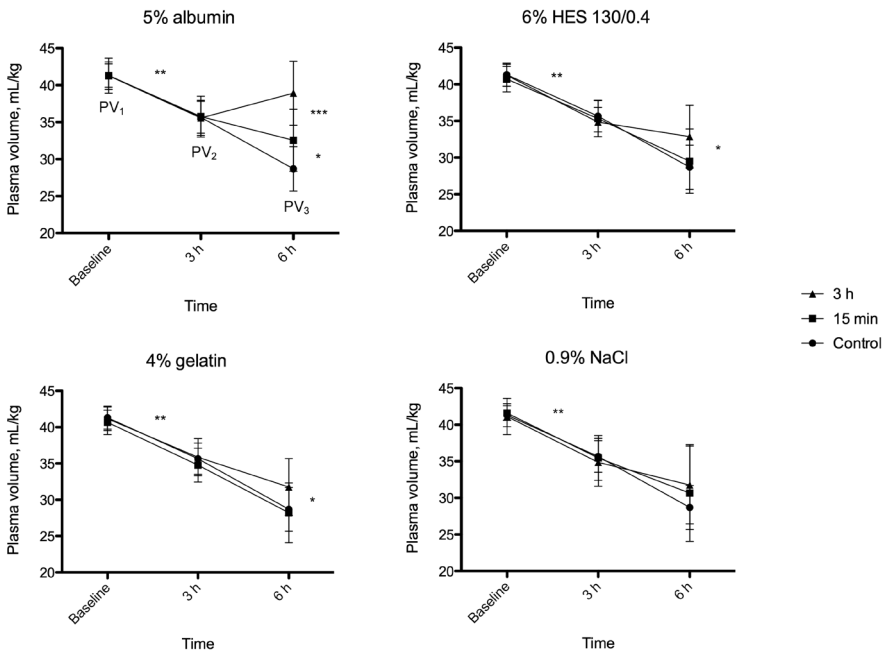


Figure 3. Plasma volumes (PV). PV at baseline (PV₁), 3 h after the preparation just before the start of infusion (PV₂), and at the end of the experiment (PV₃) given as a continuous (3-h) infusion or as a bolus (15-min) infusion of 5% albumin (n = 12 per group), 6% HES 130/0.4 (n = 10 per group), 4% gelatin (n = 10 per group) or 0.9% NaCl (n = 8 per group). Corresponding data for the control group (n = 8) are also shown. There was a significant difference between PV₁ and PV₂ for all groups and a significant difference between the continuous group and the bolus group for all solutions except 0.9% NaCl. There was a significant difference between the albumin bolus group and the control group. Two-way ANOVA with Bonferroni as post hoc test was used for the statistical analyses. (* p < 0.05, ** p < 0.01, *** p < 0.001).

Study II

The plasma volume expanding effects of a fixed volume of 6% dextran 70 and 5% human albumin were greater 3 h after the start of infusion when the fluid was given at a slow infusion rate rather than at a fast one. PV for all treatment groups differed significantly from that of the sham group at the end of the experiment, regardless of infusion rate (Fig 4).

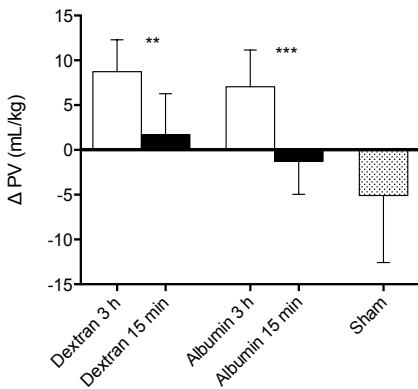


Figure 4. Change in plasma volume. Comparison of change in plasma volume from the start of infusion (PV_2) to the end of the experiment (PV_3) for the continuous (3 h) groups and the bolus (15 min) groups. There was a significant difference between the continuous (3 h) groups and the bolus (15 min) groups for both fluids analysed. There was also significant difference between both dextran groups and the continuous albumin group, and the sham group ($p < 0.05$). Student's t-test for unpaired observations was used for the statistical analyses (** $p < 0.01$, *** $p < 0.001$).

Study III

The plasma volume-expanding effect 20 mins after the end of an infusion of 0.9% NaCl (32 mL/kg) differed significantly between sepsis/SIRS and the normal state, and after a short period of hemorrhagic hypovolemia in rats. The increases in plasma volume in relation to the infused volume of 0.9% NaCl (32 mL/kg) were 0.6% in sepsis/SIRS, 20% after hemorrhage, and 12% when given to rats in a normal state.

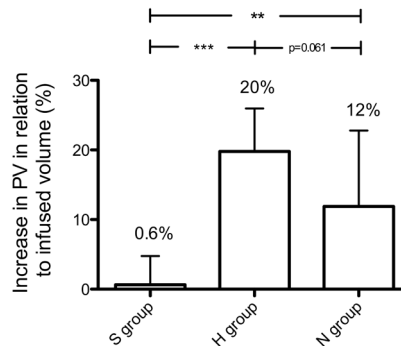


Figure 5. Increase in plasma volume (PV) in relation to the infused volume for the sepsis group (S group), the normovolemic group (N group), and the hemorrhage group (H group). There were significant differences between the S 110group and the N group, and the S group and the H group. Data are mean \pm SD. Two-tailed Student's t-test for unpaired observations was used for the statistical analyses (** $p < 0.01$, *** $p < 0.001$).

Study IV

The two investigated vitamin C treatment regimes, initiated 3 hrs after induction of sepsis, had no effect on the loss of plasma volume (Fig. 6), or any of the physiological parameters

analysed, in the early stage of sepsis in the rat. High-dose bolus of vitamin C (200 mg/kg) caused an increase in urine production (Fig. 7).

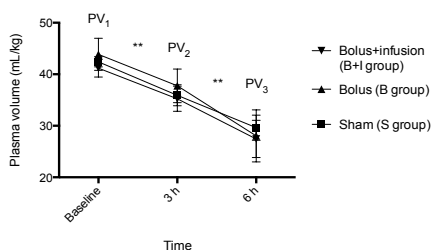


Figure 6. Plasma volumes. Plasma volumes at baseline (PV₁), 3 hrs after the surgical preparation just before the start of treatment (PV₂), and at the end of the experiment (PV₃). There was no significant difference between any of the groups at any time points. There was a significant difference between PV₁ and PV₂, and PV₂ and PV₃ for all groups. Two-way ANOVA for repeated measures followed by Bonferroni post hoc test was used for the statistical analyses (** p < 0.01).

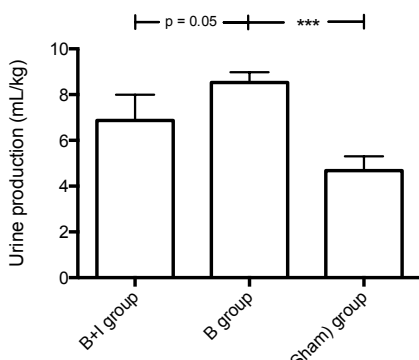


Figure 7. Urine production. Data for urine production (mL/kg) from the end of surgical preparation to the end of the experiment. There was a significantly larger urine production in the B group compared to the S (Sham) group. Student's t-test was used for the statistical analyses (***) p < 0.001).

General discussion

Caecal ligation and incision causes an acute bacterial peritonitis, with subsequent SIRS and organ failure, and provides a good predictable model for acute severe sepsis in rodents (Scheiermann 2009).

Sprague-Dawley rats (I, III, IV) and Dunkin-Hartley guinea pigs (II) were used, all male to rule out potential gender differences.

The surgical technique to induce sepsis was carefully standardized, since an equal distribution of the intensity of sepsis in the groups is of importance. Guinea pigs are more sensitive to the caecal ligation and incision procedure than rats, resulting in a more aggressive systemic response and a higher intra-experimental mortality, but in contrast to rats they are not allergic to dextran.

A problem with all animal models is that they are just animal models, and therefore the results cannot be directly transferred to man.

Also, the homogeneity of both the studied population and the severity of disease are of importance for the experiments, but it does not reflect the case mix and variability of sepsis in clinical practice.

¹²⁵I-albumin dilution technique

The dilution technique using ¹²⁵I-albumin as tracer is well established for the calculation of plasma volume in experimental and clinical studies with reproducible results in both normal and inflammatory states (Magarson and Soni 2005, Dubniks et al. 2007). However, free iodine in the tracer injected can result in some overestimation of the plasma volume (Valeri et al. 1973), as free iodine is distributed quickly to the whole extracellular space. Free iodine was measured regularly following precipitation with 10% trichloroacetic acid, and as it was found to be small in the prepared samples, it must have had minor influence on the results.

There might also have been overestimation of plasma volume because of transcapillary escape of radioactive albumin during the 5-min period between injection of the tracer and collection of the blood sample, especially at states of increased permeability (Magarson and Soni 2005, Valeri et al. 1973). This means, that there will be a larger overestimation of the plasma volume after initiation of sepsis than at baseline, but this overestimation would have been small, considering the short period of time (5 min) between the

injection of ^{125}I -albumin and measurement. This time period was chosen, as it has previously been shown to be sufficient for complete mixture of the tracer in plasma both in cat and in human (Persson and Grände 2006, Imm and Carlson 1993).

Finally, remaining radioactivity in the syringe, the vial, and the needle used was subtracted from the radioactivity initially calculated, and did not contribute to any error in the plasma volume measurement.

All this taken together means, that the expected overestimation of the plasma volume measurements with the design of the dilution technique used is small. Independent of this, in study I, II and IV remaining errors will have no influence on the conclusions made, as they will be of the same magnitude for all groups. In study III, the increased microvascular permeability in the septic group might have resulted in a larger overestimation of plasma volume compared to the 2 other groups. However, again considering the short time period of 5 min between the injection of ^{125}I -albumin and the measurement, the error must be small.

Colloids, NaCl and sepsis

Study I and II showed that the degree of plasma volume expansion of a fixed volume of the colloid solutions 5% albumin, 6% HES 130/0.4, 4% gelatine and 6% dextran 70 measured 3 h after

start of the infusion is larger when it is given at a slow infusion rate than if it is given at a fast rate in animal sepsis models. The differences in plasma volumes at the end of the experiment were reflected in the difference in hematocrit values for all studied colloids.

The synthetic colloid dextran 70 has previously been shown to have good plasma volume expanding properties in states of normal capillary permeability (Dubniks et al. 2009, Zdolsek et al. 2011) and in states of increased capillary permeability (Karanko 1987, Persson and Grände 2006). Dextran 70 could not be included in study I, as rats are allergic to dextran (Voorhees et al. 1951). Therefore, it was investigated in a separate study in guinea pigs (II).

The better plasma volume expanding effect with a slow infusion rate than with a fast infusion rate is compatible with the Starling equation (Eq. 1) and the 2-pore model of transcapillary fluid exchange (Fig. 2). A bolus infusion would be expected to cause a transient increase in capillary pressure (P_c) from the transient increase in systemic arterial pressure (P_a), a decrease in precapillary resistance (R_a) due to activation of the baroreceptor reflex, and decrease in hematocrit, all of which can be expected to lead to an increase in transcapillary fluid loss (J_v) (Nygren et al. 2010, Dubniks et al. 2007). Our results of a significantly larger increase in mean arterial pressure and decrease in Hct in the bolus groups during the first time period after start of the infusion (I) are

compatible with these proposals. This was also demonstrated in a separate experiment, where the plasma volume loss was shown to be fast after end of the bolus infusion of albumin, falling to the same plasma volume as that obtained with a continuous infusion after slightly more than 1 h (I).

The release of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) from the heart may be larger with a bolus infusion than with a continuous infusion, resulting in more urine production and smaller plasma volume (Woodard and Rosaldo 2008). These hormones may also cause an increase in microvascular permeability (Huxley and Tucker 1987). The results in these studies were not influenced by an increased urine production, as urine production was very small in relation to the volumes infused. However, the possibility that the permeability-increasing effect of ANP and BNP resulted in plasma volume loss (Huxley and Scallan 2011) cannot be excluded.

It has also been suggested that isoflurane may contribute to edema formation and tissue damage in sepsis/SIRS (Soehnlein et al. 2010), and the vasodilatory effect of isoflurane might have contributed to an increase in transcapillary leakage in all groups by an increase in P_c , which may have aggravated the plasma volume loss.

With HES and gelatin there were poorer plasma volume expansion, and smaller differences in plasma volumes between the continuous and the bolus

groups (I) compared to albumin (I, II) and dextran (II). Tentative explanations are given below.

HES is degraded by amylase resulting in halving of the molecular weight within 20–30 min. The initial half-life of plasma elimination of HES 130/0.4 is thought to be approximately 30–45 min after infusion in man (Waitzinger et al. 1998). The degradation rate can be expected to be even faster in the rat than in man because of a higher plasma concentration of amylase in the rat (Tuba and Wiberg 1953), most likely resulting in extensive degradation within the 3-h study period. Thus, the results for HES in the present study cannot be directly extrapolated to humans. The much smaller degradation products could also be expected to pass the endothelial membrane to the interstitium more easily, a mechanism probably even more pronounced in a state of increased microvascular permeability such as sepsis/SIRS.

Gelatin has a relatively low mean MW of 30 kDa. Being a polydisperse colloid, a large part of the molecules are small enough to pass not only through the large pores, but also through the small pores. This, and the fact that there is degradation of the molecules, means that there may be a relatively fast and continuous transcapillary leakage of gelatin during the 3-h period after the start of the infusion, especially when there is an increase in capillary permeability. This might explain the poor plasma volume expanding effect of gelatin in the present study.

For the crystalloid 0.9% NaCl there was no significant difference in plasma volume expanding effect 3 hrs after start of infusion, regardless of infusion rate. Plasma volumes did not even differ significantly from the control group, even though NaCl was given in a 4 times larger volume than the colloid solutions (I). This was further investigated in study III, which showed an initial plasma volume expanding effect of a bolus infusion of 32 mL/kg of only about 1% 20 mins after the end of infusion in the rat sepsis model. In hypovolemia, but with preserved microvascular permeability after acute haemorrhage, treated according to the same protocol (III), the plasma volume-expanding effect was found to be 20% of the infused volume.

The lack of difference in plasma volume expansion in sepsis/SIRS between the NaCl-groups (study I) is to be expected, since the capillaries are freely permeable to crystalloids with a fast distribution of the solution to the whole extracellular space. The lack of difference in PV expansion compared with the control group (I) and the result of a close to none-existing plasma volume expanding effect (III) is surprising, considering the traditional view that about 20-25% of the infused volume (Guyton and Hall 2011, Nolan 1999) should stay intravascularly, which, indeed, was the result with normal microvascular permeability (III). This indicates that pathophysiological changes in sepsis/SIRS also influence the plasma volume-expanding effect for a crystalloid.

When giving a crystalloid solution, under normal circumstances approximately 75% of the infused volume is distributed quickly to the interstitial space. With sepsis/SIRS, when plasma has already been lost to the extravascular space, the ratio between the plasma volume and the interstitial volume is reduced. This means that relatively more of the infused volume would be distributed to the interstitial space compared to the normal case. Further, as the infused saline is flowing through the large pores of the capillary membrane, there may also be a subsequent loss of proteins via convection (Gandhi and Bell 1992). This means that the part of a crystalloid infusion that passes through the large pores will increase the loss of proteins when the infused fluid is distributed from the intravascular space to the interstitial space (Mullins and Bell 1982). The loss of proteins by this mechanism will be aggravated in sepsis, since the number of large pores is increased (Smith et al. 1987), and a relatively greater proportion of the infused volume will therefore pass through the large pores.

Also, the large volumes of 0.9% NaCl will transiently dilute plasma proteins, resulting in a reduction in capillary oncotic pressure (π_c), which will increase fluid transfer to the extravascular space (Rippe and Haraldsson 1987).

Finally, the 4 times higher infusion rate for 0.9% NaCl than for the colloids (I) results in a transient increase in hydrostatic capillary pressure (P_c). All these mechanisms may lead to in-

creased leakage of fluid through the capillary pores, and increased leakage of proteins by convection through the large pores, explaining the poor plasma volume expanding effect.

For the colloid groups and the control group, the lactate concentrations followed the expected pattern in relation to the plasma volumes, which means that the lowest concentrations at the end of the experiment were seen for the group with the highest plasma volume, except for the dextran bolus group (II). Any explanation to why this agreement was not seen for this group has not been found. For 0.9% NaCl, the lactate concentrations were lower at the end of the experiment than in the colloid groups, despite the fact that the plasma volumes were low and did not even differ from the control group. Most likely this does not reflect a smaller lactate production in the 0.9% NaCl groups, but rather that lactate was diluted in a larger extracellular volume in these groups because of the 4 times larger volumes infused.

Vitamin C and plasma volume

Several experimental studies have shown that vitamin C is beneficial in burns by preventing capillary leakage and fluid requirements. In guinea pigs, vitamin C reduced the water content of the skin and decreased the need of resuscitation volume, and in dogs it decreased protein leakage and lymph

flow in the early phase after burns (Matsuda et al. 1992). Vitamin C therapy has also been shown to counteract the negative interstitial pressure, oedema formation and endothelial damage after burn in the rat (Kremer et al. 2010, Tanaka et al. 1999). A study in humans showed a reduction in resuscitation volume with vitamin C treatment after severe burn (Tanaka et al. 2000). In contrast, one study in dogs found no changes in microvascular permeability or in oedema formation when vitamin C was given after burn in the dog (Aliabadi-Wahle et al. 1999).

Vitamin C has also been shown to have beneficial effects on the microcirculation in moderate sepsis in the rat (Tymil et al. 2005). In 2006, on a meeting on vitamin C it was concluded that there were arguments based on experimental studies for the hypothesis that high-dose vitamin C improves microvascular endothelial function in sepsis (Lehr et al. 2006). This hypothesis has found further support in some recent studies in septic mice, where vitamin C has been shown to have positive effects on various pathophysiological changes in sepsis, including the microvasculature of the lung and capillary leakage of different injected tracers (Fisher et al. 2011 and 2012, Zhou et al. 2012).

Sepsis, as well as burns, cause transcapillary leakage of plasma, reducing the circulating plasma volume (Bark et al. 2013, Demling 2005). We therefore tested the hypothesis that vitamin C

would reduce the loss of plasma volume in the early stage of sepsis (IV), but in our study none of the two investigated treatment regimes of intravenous vitamin C had any effect on plasma volume loss in the early stage of sepsis in the rat (IV). Neither were there any differences in any of the physiological parameters analysed.

In most studies found in the current literature, vitamin C-treatment was started either before or closely after injury (e.g. sepsis, burns). The fact that we started the treatment 3 hrs after injury, which is a more clinically relevant approach, might explain our negative results. However, in one study (Zhou et al. 2012), treatment with vitamin C (200 mg/kg) was initiated 3 hrs after injury, as in our study, and they demonstrated a positive effect, in terms of reduced capillary leakage of Evans blue after 12 hrs in the septic mouse. Our negative results may also be partly explained by the fact that caecal ligation and incision used in the present study probably resulted in a more severe sepsis than in the caecal ligation and puncture model used in many other studies. We also chose to evaluate the effect on plasma volumes 3 hrs after initiation of treatment, a time period shorter than most other previous studies, and it cannot be excluded that this might have contributed to our negative results.

The larger urine production in the B group was in accordance with previous studies, both in humans and in dogs,

showing a diuretic effect of vitamin C (Kenawy 1952, Abbasy 1937), although the mechanism of action is unclear.

Main conclusions

- In animal models of severe sepsis, the plasma volume expanding effect of 5% albumin, 6% HES130/0.4, 4% gelatin and 6% dextran 70 is larger 3 h after the start of infusion when given with a slow infusion rate than when given with a fast infusion rate.

- In animal models of severe sepsis, the plasma volume expanding effect of 5% albumin and 6% dextran 70 is better than that of 6% HES130/0.4 and 4% gelatin.

- In a rat model of severe sepsis, the plasma volume expanding effect of 0.9% NaCl is negligible regardless of infusion rate.

- In a rat model of haemorrhagic hypovolemia the initial plasma volume expanding effect of a bolus infusion of 0.9% NaCl is 20%.

- The plasma volume expanding effect of 0.9% NaCl is highly dependent on patho-physiological changes in systemic inflammation.

- Intravenous vitamin C-treatment started 3 hrs after initiation of sepsis does not decrease the loss of plasma volume in the early stage of sepsis in the rat.

- High-dose vitamin C has a diuretic effect.

Sammanfattning på svenska

Blodcirkulationens uppgift är att förse kroppens vävnader med näringsämnen, vätska och syre, samt att transportera bort slaggprodukter från energiomsättningen. Hörnstenen i detta utbyte mellan vävnader och cirkulation är kapillärerna, kroppens minsta och tunnaste blodkärl, som består av ett enda lager sammanhängande celler. Mellanrummen mellan cellerna är mer eller mindre täta, beroende på omgivande organs och vävnaders behov. Kapillärerna fungerar på detta sätt som ett filter, där vissa ämnen i stor utsträckning hålls kvar i blodbanan (t ex proteiner, blodkroppar) medan andra fritt passerar mellan kapillären och omgivningen (t ex syre, koldioxid, vatten). Normalt flödar därför en näringsrik, proteinfattig vätska från blodet till omgivande vävnad. För att förhindra vätskeansamling i vävnaden, s.k. ödem, finns ett annat system som transporterar tillbaka vätskan till blodcirkulationen, nämligen lymfsystemet.

För att förstå kapillärfiltret kan man schematiskt se det som en sil bestående av två olika typer av hål. Små hål, som släpper igenom små ämnen och vatten, och stora hål som också släpper igenom proteiner. De små hålen finns det enorma mängder av, medan de stora hålen, i normala fall, är mycket sällsynta. När något skadas i kroppen uppstår ibland inflammation, då det skadade området blir rött

och svullet. Anledningen till detta är bland annat att kapillärfiltret blir mer genomsläppligt, det blir fler stora hål i silen, och större mängder vätska och proteiner läcker ut för att hjälpa till att reparera den skadade vävnaden. I samband med svår blodförgiftning, svåra olyckor eller omfattande operationer kan hela kroppen drabbas av inflammation i varierande grad (systemisk inflammation=SIRS). Stora mängder vätska kan då läcka ut från blodbanan, lymfsystemet blir överbelastat och det bildas ödem, som är skadligt för patienten. Samtidigt töms blodcirkulationen på vätska och hjärtat får för små volymer blod att pumpa ut, och blodcirkulationen kan inte längre förse vävnader och organ med syre och näringsämnen.

För att normalisera blodcirkulationen och förhindra skador på vävnaderna är en viktig del i behandlingen av dessa patienter, att ersätta den förlorade vätskan med vätskelösningar, dropp, som ges rakt in i blodet. Det finns flera olika typer av vätskelösningar, men de kan grovt delas in i två grupper, kristalloider, som består av vatten och småämnen (t ex salter) och kolloider, som också innehåller större ämnen. Kristalloider passerar fritt genom kapillärfiltret, medan kolloiderna bara kan passera genom de stora hålen. Problemet vid behandlingen av patienter med SIRS är att också de vätskelösningar

som ges läcker ut och bildar ytterligare ödem.

vi inte påvisa någon påverkan på läckaget av vätska från blodbanan.

I delarbete I och III kunde vi visa att genom att ge kolloida vätskor långsamt till råttor med SIRS stannade en större del kvar i blodcirkulationen, än om samma mängd vätska gavs snabbt. Om detta går att överföra till patienter med SIRS, så skulle man kunna öka effekten, och därigenom minska de givna mängderna och ödembildningen, genom ge kolloida vätskor långsammare. Den kristalloid, 0.9% NaCl (koksalt), vi också testade kunde vi, till vår förvåning, inte se någon effekt av alls.

I delarbete II undersökte vi hur stor del av en given mängd 0.9% NaCl som fanns kvar i blodcirkulationen 20 minuter efter avslutad behandling vid SIRS och efter en akut blödning. I blödningsgruppen fanns 20 % kvar, medan det i gruppen med SIRS bara fanns knapp 1 % kvar, trots att båda grupperna hade lika stor vätskebrist från början. Detta tyder på att sjukliga förändringar vid SIRS påverkar effekten av behandling med kristalloida vätskor.

I delarbete IV undersökte vi om stora doser C-vitamin givet tre timmar efter insjuknande i SIRS kunde påverka vätskeläckaget från blodbanan. I tidigare djurförsök har C-vitamin visat sig vara effektivt för just detta vid SIRS, men i de flesta försök har man har börjat behandlingen redan innan djuren blivit sjuka, vilket är mindre intressant ur klinisk synpunkt. Med vår behandling, startad i ett senare skede, kunde

Acknowledgements and grants

Many people have inspired and helped me during my work with this thesis. For this, I thank you all!

In particular I would like to thank:

Per-Olof Grände, my supervisor, scientific mentor, and friend, for giving me the opportunity to enter the world of medical research, for sharing your knowledge on both science and life, and for teaching me what being a real scientist is all about. This has been a most memorable journey.

Johan Persson, my co-supervisor, clinical mentor, colleague, and friend, for your everlasting guidance and support, for always sharing your professional and personal insights, and for memorable runs, though that marathon is yet to come.

Helené Axelberg, our laboratory technician, for your untiring and invaluable work in the laboratory, for organizing and always keeping track of papers, binders and data. This thesis would never have seen the light of day without you.

Peter Bentzér, roommate, colleague, and friend, for stimulating discussions on science, fish ethics and recycling. Your dedication and enthusiasm is truly inspiring.

The Rippe group, our institutional neighbors: Bengt Rippe, Anna Rippe, Josefin Axelsson, and Kristinn Örn Sverrisson, for interesting discussions on various academic and worldly subjects during countless coffees and lunch breaks. Carl M. Öberg, co-writer, for clarifying the mathematics on the holes and the flows in study III.

Cornelia Lundblad, for statistical advice, and for informative talks on everything from digging holes to molecular biology.

Eva Ranklev-Twetman, Görel Nergelius and Bengt Roth, past heads of the Department of Anaesthesia and Intensive care, for giving me the opportunity to train and develop, both clinically and scientifically.

Dag Lundberg, professor emeritus, for once upon a time introducing me to the magnificent world of anaesthesia and intensive care.

Per Flisberg, my mentor, for great advice and for your excellent sense of humour.

Colleagues and friends at the Department of Anaesthesia and Intensive Care, Lund University Hospital, for sharing everyday work.

All my friends for keeping me sane.

Lars-Arne, Elisabeth and Mattias Lindvall with family, my family-in-law, for good company, and for helping out in everyday life.

Åke and Ethel Bark, my parents, for bringing me up and for always letting me choose and walk my own path in life, but at all times still present when needed.

Stefan Bark, my brother, and his family, for great brotherhood through life and for the early soccer training.

Erik and Elsa, the lights and joys of my life, for being who you are, and for constantly, by your mere presence, reminding me of what life is really all about.

Therese Lindvall, my love, for sharing life with me, for your leniency and patience with me and my whims, for being an excellent partner and mother, and for lighting up my life. I could not have had better.

Grants

This work was supported by the Swedish Research Council, Stockholm (#11581), Region Skåne (ALF #18401), the Faculty of Medicine, Lund University, and the Department of Anaesthesia and Intensive Care, Lund University Hospital, Sweden. This is gratefully acknowledged.

References

- Abbasy MA. The diuretic action of vitamin C. *Biochem J* 1937;31:339-42.
- Aliabadi-Wahle S, Gilman DA, Dabrowski GP, Choe EU, Flint LM, Ferrara JJ. Postburn vitamin C infusions do not alter early postburn edema formation. *J Burn Care Rehabil* 1999;20:7-14.
- Androustos G, Karamanou M, Stefanadis C. William Harvey (1578-1657): discoverer of blood circulation. *Hellenic J Cardiol* 2012;53:6-9.
- Bark BP, Persson J, Grände PO. Importance of the infusion rate for the plasma expanding effect of 5% albumin, 6% HES 130/0.4, 4% gelatin, and 0.9% NaCl in the septic rat. *Crit Care Med* 2013;41:857-66.
- Bark BP, Öberg CM, Grände PO. Plasma volume expansion by 0.9% NaCl during sepsis/Systemic Inflammatory Response Syndrome, after hemorrhage, and during a normal state. *Shock* 2013;40:59-64.
- Barron ME, Wilkes MM, Navickis RJ. A systematic review of the comparative safety of colloids. *Arch Surg* 2004;139:552-63.
- Berne RM, Levy MN (Eds). *Physiology*. St. Louis, USA: Mosby - Year Book, 1993, pp 754-758
- Brunkhorst FM, Engel C, Bloos F, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med* 2008;358:125-39.
- Bundgaard M. The three-dimensional organization of tight junctions in a capillary endothelium revealed by serial-section electron microscopy. *J Ultrastruct Res* 1984;88:1-17.
- Clough G. Relationship between microvascular permeability and ultrastructure. *Prog Biophys Mol Biol* 1991;55:47-69.
- Cosnett JE. The origins of intravenous fluid therapy. *Lancet* 1989;1:768-71.
- Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013;39:165-228.
- Demling RH. The burn edema process: current concepts. *J Burn Care Rehabil* 2005;26:207-27.
- Demling RH, Katz A, Lalonde C, Ryan P, Jin LJ. The immediate effect of burn wound excision on pulmonary function in sheep: the role of prostanoids, oxygen radicals, and chemoattractants. *Surgery* 1987;101:44-55.

- De Backer D, Donadello K, Taccone FS, Ospina-Tascon G, Salgado D, Vincent JL. Microcirculatory alterations: potential mechanisms and implications for therapy. *Ann Intensive Care* 2011;1:27.
- Dubniks M, Persson J, Grände PO. Comparison of the plasma volume-expanding effects of 6% dextran 70, 5% albumin, and 6% HES 130/0.4 after hemorrhage in the guinea pig. *J Trauma* 2009;67:1200-4.
- Dubniks M, Persson J, Grände PO. 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.
- Dubniks M, Persson J, Grände PO. Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 2007;33:2192-8.
- Fenstermacher JD. Volume regulation of the central nervous system. In: Edema. Staub NC, Taylor AE (Eds). New York, Raven Press, 1984, pp 383-404.
- Fishel RS, Are C, Barbul A. Vessel injury and capillary leak. *Crit Care Med* 2003;31:S502-11.
- Fisher BJ, Kraskauskas D, Martin EJ, et al. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L20-32.
- Fisher BJ, Seropian IM, Kraskauskas D, et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. *Crit Care Med* 2011;39:1454-60.
- Fleck A, Raines G, Hawker F, et al. Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985;1:781-4.
- Friedl HP, Till GO, Trentz O, Ward PA. Roles of histamine, complement and xanthine oxidase in thermal injury of skin. *Am J Pathol* 1989;135:203-17.
- Galley HF, Webster NR. Physiology of the endothelium. *Br J Anaesth* 2004;93:105-13.
- Galley HF, Davies MJ, Webster NR. Xanthine oxidase activity and free radical generation in patients with sepsis syndrome. *Crit Care Med* 1996;24:1649-53.
- Gamble JL. Early history of fluid replacement therapy. *Pediatrics* 1953;11:554-67.
- Gandhi RR, Bell DR. Influence of saline infusions on transvascular protein transport. *Am J Physiol* 1992;262:H443-50.
- Grathwohl KW, Bruns BJ, LeBrun CJ, Ohno AK, Dillard TA, Cushner HM. Does hemodilution exist? Effects of saline infusion on hematologic parameters in euvoletic subjects. *South Med J* 1996;89:51-5.

- Groeneveld AB, Teule GJ, Bronsveld W, van den Bos GC, Thijs LG. Increased systemic microvascular albumin flux in septic shock. *Intensive Care Med* 1987;13:140-2.
- Grotte G. Passage of dextran molecules across the blood-lymph barrier. *Acta Chir Scand Suppl* 1956;211:1-84.
- Guyton AC, Hall JE. The Circulation. In: Guyton and Hall: Textbook of Medical Physiology. 12th Edition. Hall JE (Ed). Philadelphia, Elsevier, 2011, pp 157-301
- Haljamäe H. Historisk tillbakablick. In: Vätsketerapi. Hjelmqvist H, Haljamäe H (Eds). Stockholm, Lieber AB, 2006, pp 9-17.
- Haljamäe H, Hjelmqvist H. Basalt om kristalloider och kolloider. In: Vätsketerapi. Hjelmqvist H, Haljamäe H (Eds). Stockholm, Lieber AB, 2006, pp 61-78.
- Haskell A, Nadel ER, Stachenfeld NS, Nagashima K, Mack GW. Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 1997;83:407-13.
- Hedin H, Ljungstrom KG. Prevention of dextran anaphylaxis. Ten years experience with hapten dextran. *Int Arch Allergy Immunol* 1997;113:358-9.
- Holbeck S. Cellulärt och mikrovaskulärt vätskeutbyte. In: Vätsketerapi. Hjelmqvist H, Haljamäe H (Eds). Stockholm, Lieber AB, 2006, pp 18-26.
- Huxley VH, Scallan J. Lymphatic fluid: exchange mechanisms and regulation. *J Physiol* 2011;589:2935-43.
- Huxley VH, Tucker VL, Verburg KM, Freeman RH. Increased capillary hydraulic conductivity induced by atrial natriuretic peptide. *Circ Res* 1987;60:304-7.
- Imm A, Carlson RW. Fluid resuscitation in circulatory shock. *Crit Care Clin* 1993;9:313-33.
- Jungheinrich C, Scharpf R, Wargenau M, Bepperling F, Baron JF. The pharmacokinetics and tolerability of an intravenous infusion of the new hydroxyethyl starch 130/0.4 (6%, 500 mL) in mild-to-severe renal impairment. *Anesth Analg* 2002;95:544-51, table of contents.
- Karanko MS. Effects of three colloid solutions on plasma volume and hemodynamics after coronary bypass surgery. *Crit Care Med* 1987;15:1015-22.
- Kenawy MR, El N, El-Mohandis MM, El-D Rohayem HK, El-Sheehy AW. Studies on the diuretic action of vitamin C in normal animals and human beings, and its clinical value in pathological retention of water. *Int Z Vitaminforsch* 1952;24:40-61.

- King CG, Waugh WA. The Chemical Nature of Vitamin C. *Science* 1932;75:357-8.
- Koller ME, Reed RK. Increased negativity of interstitial fluid pressure in rat trachea in dextran anaphylaxis. *J Appl Physiol* 1992;72:53-7.
- Kremer T, Harenberg P, Hernekamp F, et al. High-dose vitamin C treatment reduces capillary leakage after burn plasma transfer in rats. *J Burn Care Res* 2010;31:470-9.
- Lamke LO, Liljedahl SO. Plasma volume changes after infusion of various plasma expanders. *Resuscitation* 1976;5:93-102.
- Laxenaire MC, Charpentier C, Feldman L. Anaphylactoid reactions to colloid plasma substitutes: incidence, risk factors, mechanisms. A French multicenter prospective study. *Ann Fr Anesth Reanim* 1994;13:301-10.
- Lehr HA, Germann G, McGregor GP, et al. Consensus meeting on "Relevance of parenteral vitamin C in acute endothelial dependent pathophysiological conditions (EDPC)". *Eur J Med Res* 2006;11:516-26.
- Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 2010;87:198-210.
- Levick JR. Capillary filtration-absorption balance reconsidered in light of dynamic extravascular factors. *Exp Physiol* 1991;76:825-57.
- Levick JR. *Cardiovascular Physiology*. 5th Edition. Levick JR (Ed). London, Hodder Arnold, 2010, pp 147-219
- Margarson MP, Soni NC. Plasma volume measurement in septic patients using an albumin dilution technique: comparison with the standard radio-labelled albumin method. *Intensive Care Med* 2005;31:289-95.
- Marx G. Fluid therapy in sepsis with capillary leakage. *Eur J Anaesthesiol* 2003;20:429-42.
- Matsuda T, Tanaka H, Shimazaki S, et al. High-dose vitamin C therapy for extensive deep dermal burns. *Burns* 1992;18:127-31.
- McDonald DM, Thurston G, Baluk P. Endothelial gaps as sites for plasma leakage in inflammation. *Microcirculation* 1999;6:7-22.
- Mellander S, Johansson B. Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacol Rev* 1968;20:117-96.
- Michel CC, Curry FE. Microvascular permeability. *Physiol Rev* 1999;79:703-61.

- Mullins RJ, Bell DR. Changes in interstitial volume and masses of albumin and IgG in rabbit skin and skeletal muscle after saline volume loading. *Circ Res* 1982;51:305-13.
- Myburgh JA, Finfer S, Bellomo R, et al. Hydroxyethyl starch or saline for fluid resuscitation in intensive care. *N Engl J Med* 2012;367:1901-11.
- Nolan J. Fluid replacement. *Br Med Bull* 1999;55:821-43.
- Nygren A, Redfors B, Thoren A, Ricksten SE. Norepinephrine causes a pressure-dependent plasma volume decrease in clinical vasodilatory shock. *Acta Anaesthesiol Scand* 2010;54:814-20.
- Pappenheimer JR, Soto-Rivera A. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. *Am J Physiol* 1948;152:471-91.
- Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. *Am J Physiol* 1951;167:13-46.
- Perner A, Haase N, Guttormsen AB, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. *N Engl J Med* 2012;367:124-34.
- Persson J, Grände PO. Plasma volume expansion and transcapillary fluid exchange in skeletal muscle of albumin, dextran, gelatin, hydroxyethyl starch, and saline after trauma in the cat. *Crit Care Med* 2006;34:2456-62.
- Ragan DM, Schmidt EE, MacDonald IC, Groom AC. Spontaneous cyclic contractions of the capillary wall in vivo, impeding red cell flow: a quantitative analysis. Evidence for endothelial contractility. *Microvasc Res* 1988;36:13-30.
- Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. *Am J Physiol* 1986;250:H706-10.
- Rippe B, Haraldsson B. Capillary permeability in rat hindquarters as determined by estimations of capillary reflection coefficients. *Acta Physiol Scand* 1986;127:289-303.
- Rippe B, Haraldsson B. Fluid and protein fluxes across small and large pores in the microvasculature. Application of two-pore equations. *Acta Physiol Scand* 1987;131:411-28.
- Rippe B, Haraldsson B. Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 1994;74:163-219.
- Rippe B, Rosengren BI, Venturoli D. The peritoneal microcirculation in peritoneal dialysis. *Microcirculation* 2001;8:303-20.

- Saez JC, Ward PH, Gunther B, Vivaldi E. Superoxide radical involvement in the pathogenesis of burn shock. *Circ Shock* 1984;12:229-39.
- Scallan JP, Huxley VH. In vivo determination of collecting lymphatic vessel permeability to albumin: a role for lymphatics in exchange. *J Physiol* 2010;588:243-54.
- Scheiermann P, Hoegl S, Revermann M, et al. Cecal ligation and incision: an acute onset model of severe sepsis in rats. *J Surg Res* 2009;151:132-7.
- Smith L, Andreasson S, Thoren-Tolling K, Rippe B, Risberg B. Sepsis in sheep reduces pulmonary microvascular sieving capacity. *J Appl Physiol* 1987;62:1422-9.
- Soehnlein O, Eriksson S, Hjelmqvist H, et al. Anesthesia aggravates lung damage and precipitates hypotension in endotoxemic sheep. *Shock* 2010;34:412-9.
- Starling EH. On the Absorption of Fluids from the Connective Tissue Spaces. *J Physiol* 1896;19:312-26.
- Svirbely JL, Szent-Gyorgyi A. The chemical nature of vitamin C. *Biochem J* 1932;26:865-70.
- Szent-Gyorgyi A. Observations on the function of peroxidase systems and the chemistry of the adrenal cortex: Description of a new carbohydrate derivative. *Biochem J* 1928;22:1387-409.
- Tanaka H, Lund T, Wiig H, et al. High dose vitamin C counteracts the negative interstitial fluid hydrostatic pressure and early edema generation in thermally injured rats. *Burns* 1999;25:569-74.
- Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: a randomized, prospective study. *Arch Surg* 2000;135:326-31.
- Till GO, Guilds LS, Mahrougui M, Friedl HP, Trentz O, Ward PA. Role of xanthine oxidase in thermal injury of skin. *Am J Pathol* 1989;135:195-202.
- Tuba J, Wiberg GS. On rat serum amylase. I. Studies in the normal and the alloxan diabetic animal. *Can J Med Sci* 1953;31:377-86.
- Tyml K. Critical role for oxidative stress, platelets, and coagulation in capillary blood flow impairment in sepsis. *Microcirculation* 2011;18:152-62.
- Tyml K, Li F, Wilson JX. Delayed ascorbate bolus protects against maldistribution of microvascular blood flow in septic rat skeletal muscle. *Crit Care Med* 2005;33:1823-8.
- Valeri CR, Cooper AG, Pivacek LE. Limitations of measuring blood volume with iodinated I 125 serum albumin. *Arch Intern Med* 1973;132:534-8.

- van Hinsbergh VW. Endothelial permeability for macromolecules. Mechanistic aspects of pathophysiological modulation. *Arterioscler Thromb Vasc Biol* 1997;17:1018-23.
- Voorhees AB, Baker HJ, Pulaski EJ. Reactions of albino rats to injections of dextran. *Proc Soc Exp Biol Med* 1951;76:254-6.
- Waitzinger J, Bepperling F, Pabst G, Opitz J, Muller M, Francois Baron J. Pharmacokinetics and Tolerability of a New Hydroxyethyl Starch (HES) Specification [HES (130/0.4)] after Single-Dose Infusion of 6% or 10% Solutions in Healthy Volunteers. *Clin Drug Investig* 1998;16:151-60.
- Webb AR. Capillary leak. Pathogenesis and treatment. *Minerva Anesthesiol* 2000;66:255-63.
- West JB. Marcello Malpighi and the discovery of the pulmonary capillaries and alveoli. *Am J Physiol Lung Cell Mol Physiol* 2013;304:L383-90.
- Wilson JX, Wu F. Vitamin C in sepsis. *Subcell Biochem* 2012;56:67-83.
- Woodard GE, Rosado JA. Natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol* 2008;268:59-93.
- Wu F, Wilson JX, Tynl K. Ascorbate protects against impaired arteriolar constriction in sepsis by inhibiting inducible nitric oxide synthase expression. *Free Radic Biol Med* 2004;37:1282-9.
- Zdolsek HJ, Vegfors M, Lindahl TL, Tornquist T, Bortnik P, Hahn RG. Hydroxyethyl starches and dextran during hip replacement surgery: effects on blood volume and coagulation. *Acta Anaesthesiol Scand* 2011;55:677-85.
- Zhou G, Kamenos G, Pendem S, Wilson JX, Wu F. Ascorbate protects against vascular leakage in cecal ligation and puncture-induced septic peritonitis. *Am J Physiol Regul Integr Comp Physiol* 2012;302:R409-16.

Appendix

- I** Bark BP, Persson J, Grände PO: Importance of the infusion rate for the plasma expanding effect of 5% albumin, 6% HES 130/0.4, 4% gelatin, and 0.9% NaCl in the septic rat. *Crit Care Med.* 2013; 41:857-66.

Editorial comment in: Ishihara H. Impact of fluid infusion rate on restoring plasma volume in sepsis. *Crit Care Med.* 2013; 41:931-2.

- II** Bark BP, Grände PO: Infusion rate and plasma volume expansion of dextran and albumin in the septic guinea pig. *Acta Anaesthesiol Scand.* 2013; DOI: 10.1111/aas.12228. [Epub ahead of print]

- III** Bark BP, Öberg CM, Grände PO: Plasma volume expansion by 0.9% NaCl during sepsis/systemic inflammatory response syndrome, after hemorrhage, and during a normal state. *Shock.* 2013; 40:59-64.

- IV** Bark BP, Grände PO: The effect of vitamin C on plasma volume in the early stage of sepsis in the rat. *Resubmitted after revision. Intensive Care Medicine Experimental.*

Paper I

Importance of the Infusion Rate for the Plasma Expanding Effect of 5% Albumin, 6% HES 130/0.4, 4% Gelatin, and 0.9% NaCl in the Septic Rat*

Björn P. Bark, MD; Johan Persson, MD, PhD; Per-Olof Grände, MD, PhD

Objectives: To compare the plasma volume (PV) expanding effect of a fast infusion rate with that of a slow infusion rate of a fixed volume of 5% albumin, of the synthetic colloids, 6% hydroxyethyl starch 130/0.4 and 4% gelatin, and of 0.9% NaCl in a rat sepsis model and to compare the plasma-expanding effect among these fluids.

Design: Prospective, randomized animal study.

Setting: University hospital laboratory.

Subjects: One hundred and twelve adult male rats.

Interventions: Sepsis was induced by cecal ligation and incision followed by closure of the abdomen. After 3 hrs, an infusion of the PV expander under study was started at a volume of 12 mL/kg for the colloids and of 48 mL/kg for 0.9% NaCl, either for 15 mins or for 3 hrs. A control group underwent the same experimental procedure but no fluid was given.

Measurements and Main Results: Three hours after start of the infusion (end of experiment), the plasma-expanding effect

was better with a slow than a fast infusion rate for the colloids, especially albumin, but the NaCl groups did not differ significantly from the control group. The PV for the control group was 28.7 ± 3 mL/kg. In the slow and the fast infusion groups, it was 38.9 ± 4.3 and 32.6 ± 4.2 mL/kg for albumin ($p < 0.001$), 32.9 ± 4.3 and 29.5 ± 4.4 mL/kg for hydroxyethyl starch 130/0.4 ($p < 0.05$), 31.8 ± 3.9 and 28.2 ± 4.1 mL/kg for gelatin ($p < 0.05$), and 31.8 ± 5.3 and 30.7 ± 6.6 mL/kg for NaCl (n.s), respectively.

Conclusions: The study showed that the PV expansion by a colloid was greater when given at a slow than at a fast infusion rate, an effect more pronounced for albumin. This difference was not seen for NaCl. The PV-expanding effect was poor for NaCl and better for albumin than for the other colloids. (*Crit Care Med* 2013; 41:857–866)

Key Words: albumin; colloids; gelatin; hydroxyethyl starch; infusion rate; plasma volume; plasma volume expander.

Plasma is continuously transferred from the intravascular space to the extravascular space and returned back to the circulation via the lymphatic system. The rate of the transfer is denoted “the transcapillary escape rate” (TER). TER for albumin is normally 5%–7% of total albumin per hour in man, but it can increase by a factor of 2–3 times

during inflammatory conditions such as sepsis/systemic inflammatory response syndrome (SIRS) and after trauma (1–3). A TER above the capacity of the lymphatic system will result in accumulation of interstitial fluid and hypovolemia, with activation of the baroreceptor reflex. This may cause compromised tissue perfusion, increased tissue pressure, and reduced transcapillary oncotic pressure with altered Starling fluid equilibrium, longer diffusion distances, and pulmonary insufficiency (4, 5). Treatment of hypovolemia with plasma volume (PV) substitution under these conditions will cause further accumulation of interstitial fluid. Thus, in the restoration of PV in these patients, it would be favorable to reduce transfer of fluid to the extravascular space.

According to the two-pore theory of transcapillary fluid exchange, the capillary membrane contains small pores that are permeable only to small solutes and the large pores—which are more than 10,000 times less abundant—that are also permeable to proteins (6). Transcapillary leakage of proteins not

***See also p. 931.**

All authors: Department of Anesthesia and Intensive Care, Lund University and Lund University Hospital, Lund, Sweden.

This study was supported by the Swedish Research Council, Stockholm, Sweden (11581), and Region Skåne (ALF 18401).

The authors have not disclosed any potential conflicts of interest.

Address requests for reprints to: Per-Olof Grände, MD, PhD, Department of Anesthesia and Intensive Care, Lund University Hospital, SE-221 85 Lund, Sweden. E-mail: per-olof.grande@med.lu.se

Copyright © 2013 by the Society of Critical Care Medicine and Lippincott Williams & Wilkins

DOI: 10.1097/CCM.0b013e318274157e

only depends on the permeability of large pores but also on the hydrostatic transcapillary pressure. Hydrostatic capillary pressure can increase through an increase in arterial pressure, an increase in venous pressure, or an increase in postcapillary/precapillary resistance ratio (7). It was recently confirmed, both experimentally in the rat (8) and in man (9), that a moderate increase in arterial pressure from noradrenaline infusion results in a significant decrease in PV under conditions of increased capillary permeability. As a high infusion rate of a PV expander most likely will cause a transient increase in arterial and venous pressures and also a transient decrease in precapillary resistance, it can be expected that there will be greater transcapillary leakage when a PV expander is administered at a high infusion rate than when it is administered at a low infusion rate. A fast infusion will also cause a transient dilution of the red blood cells, which will increase the exposure of the extended plasma column between two erythrocytes to the large pores, with the potential of an increase in plasma leakage. This hypothesis is supported by experimental studies on the dog and the rat, showing increase in PV after the transfusion of erythrocytes (10, 11). Furthermore, the release of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) from the heart may be larger with a bolus infusion than with a continuous infusion, resulting in more urine production and smaller PV (12). There are indications from the literature that these hormones may also cause an increase in microvascular permeability (13).

Correction of hypovolemia is an important therapeutic measure, and both crystalloids and colloids are used (14). Crystalloids are distributed passively within the entire extracellular compartment in relation to the volume of plasma and interstitium. Under normal circumstances, approximately 70%–80% of the infused volume will be distributed relatively quickly to the interstitial space and about 20%–30% acts as PV expander (15). Thus, treatment with crystalloids is associated with a significant increase in interstitial fluid volume. Colloid solutions consist of macromolecules and, according to the two-pore theory, leak mainly through the large pores, a leakage that increases under increased permeability (8, 16). This means that treatment with colloids can be associated with aggravation of adverse interstitial accumulation of macromolecules and fluid, especially in inflammatory conditions such as sepsis/SIRS.

To our knowledge, there are no studies specifically analyzing the importance of the infusion rate for PV expansion. We have only found two studies, which showed some relation to this issue. One study showed a higher survival rate and lower morbidity with a slow infusion rate than with a fast infusion rate in acute pancreatitis (17). The other study showed higher mortality in children suffering from a severe febrile illness if given a bolus infusion at the time of admission to the hospital instead of a standard fluid administration (18). Even though there is no consensus regarding infusion rates, a PV expander is often given at a fast rate to treat a suspected hypovolemia without delay.

According to the considerations above, the smallest possible volumes for PV resuscitation to maintain normovolemia should be used to reduce the risk of simultaneous interstitial fluid accumulation. Using a model of rat sepsis, we tested the hypothesis that a slow infusion rate of a PV expander results in better plasma expansion than a fast infusion rate. This was done by comparing the PVs 3 hrs after start of the infusion of a fixed volume of the natural colloid 5% albumin, the synthetic colloids 6% HES 130/0.4 and 4% gelatin, and the crystalloid 0.9% NaCl when given at a fast and at a slow rate. We also compared the PV expansion for the different fluids for each infusion rate.

MATERIALS AND METHODS

Anesthesia and Set-Up

The study was approved by the Ethical Committee for Animal Research at Lund University, Sweden (application no. M180-10), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats ($n = 112$) weighing 354 ± 21 g (mean \pm SD) were used. Anesthesia was induced by placing the animals in a covered glass container with a continuous supply of 5% isoflurane in air (Isoba vet; Intervet, the Netherlands). After induction, the animals were removed from the container and anesthesia was maintained with 1.5%–1.8% isoflurane in air using a mask, followed by tracheostomy and connection to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy). Ventilation was performed in a volume-controlled mode using a positive end expiratory pressure of 4 cm H₂O. End-tidal PCO₂ was continuously monitored and kept between 4.9 and 5.5 kPa (Capstar-100; CWE, Ardmore, PA). Body temperature, measured rectally, was kept at 37.1°C–37.3°C via a feedback-controlled heating pad. The left femoral artery was cannulated to record arterial blood pressure (BP) and to obtain blood samples for analysis of arterial blood gases, electrolytes, lactate, hematocrit (I-STAT; Abbot Point of Care Inc, Abbot Park, IL), and PVs. The left femoral vein was cannulated and used for infusions and kept open with a continuous infusion of saline at 0.2 μ L/min. The right internal jugular vein was cannulated and used for injection of ¹²⁵I-albumin to measure PVs. After the experiments, the animals were killed with intravenous injection of potassium chloride.

Experimental Procedure

A model of severe sepsis as described previously (19, 20) was used in this study. After a longitudinal midline skin incision over the abdominal wall with diathermia, a laparotomy was performed by incision along the linea alba. The cecum was ligated just below the ileocecal valve, and an incision of 1 cm in length was made in the cecum, allowing leakage of fecal material into the abdominal cavity, thereby inducing sepsis/SIRS. The abdominal wall and the skin were then closed with clips. There were no bleedings.

Plasma Volume

PV was determined by measuring the increase in radioactivity in 100 μ L of plasma taken 5 mins after an intravenous injection of human 125 I-albumin with a known amount of activity. The increase in radioactivity was calculated by subtracting the activity in a blood sample taken just before the injection from that taken 5 min after the injection. Each PV measurement was thereby adjusted for any remaining radioactivity from previous measurements. To calculate the amount of radioactivity given, the remaining activity in the emptied vial, syringe, and needle used was calculated and subtracted from the total activity in the prepared dose. This is a reliable and established technique for PV measurements, giving reproducible results (16, 21, 22). As will be discussed, sources of error are small with the design of the technique used in this study (see Discussion). Free iodine was measured regularly following precipitation with 10% trichloroacetic acid and was found to be less than 1.2% in the prepared samples. Radioactivity was measured with a gamma counter (Wizard 1480; LKB-Wallace, Turku, Finland).

Experimental Protocol

In this study, we compared the PV-expanding effect of a fixed volume of a PV expander when given at a slow or a fast infusion rate. The PV expanders analyzed were 5% albumin (MW 69 kDa; $n = 12$ per group), 6% HES 130/0.4 (MW 20–250 kDa, mean 130 kDa; $n = 10$ per group), 4% gelatin (0–150 kDa, mean MW 30 kDa; $n = 10$ per group), and 0.9% NaCl ($n = 8$ per group) given intravenously. The time scale of the experimental procedure is shown schematically in **Figure 1**. The infusions of 12 mL/kg for the colloids and 48 mL/kg for saline were started 3 hrs after the end of the surgical intervention. Pilot experiments have shown that a systemic inflammation with plasma leakage has developed at this time point, shown by an increase in hematocrit and a marked decrease in PV. An infusion volume of 12 mL/kg was selected in this study as previous experiments have shown a PV reduction 3 hrs after sepsis induction of 7–9 mL/kg, and to this, we then added a few mil-

liliters to compensate for the anesthesia-induced vasodilation, and the blood samples taken just before start of the infusion. A 4 times larger volume for saline was given as saline is quickly distributed to the whole extracellular space, which is about 4 times larger than the PV.

Two groups were formed at random for each fluid. In one group, the fluid was given over 15 mins (the “bolus” group), and in the other group, the same volume was given over 3 hrs (the “continuous” group). The investigators were blinded to the bolus or the continuous treatment. In a “control” group, the animals underwent the same experimental procedure but no PV expander was given. PVs were measured at baseline (PV₁), 3 hrs after the surgical preparation (PV₂), and 3 hrs after the start of the infusion (at the end of the experiment; PV₃). Blood samples for measurements of arterial pH, PaCO₂, PaO₂, hematocrit, lactate, sodium, and potassium were taken at the same time points. Urine was collected in a glass vial placed at the external meatus of the urethra from the start of the infusion until the end of the experiment, and the bladder was emptied by external compression at the end of the experiment. Animals, that did not show a decrease in PV 3 hrs after the preparation were considered to be nonseptic and were excluded from the study. These animals and animals that died before the end of the experiment were replaced with new animals. PVs of blood samples were of the same size for all groups and therefore had no influence on the conclusions made.

To evaluate if there was a difference in BP between the bolus and the continuous groups the nearest time after the infusion, which could have contributed to a difference in PV between the groups (see introductory section), the mean of mean arterial BP during the 30-min period just before start of the infusion were compared with that during the 30-min time period just after start of the infusion for the different groups.

To evaluate if there was a difference in hematocrit, between the bolus and the continuous group, which could have contributed to a difference in transcapillary leakage between the groups affecting PV (see introductory section), a special

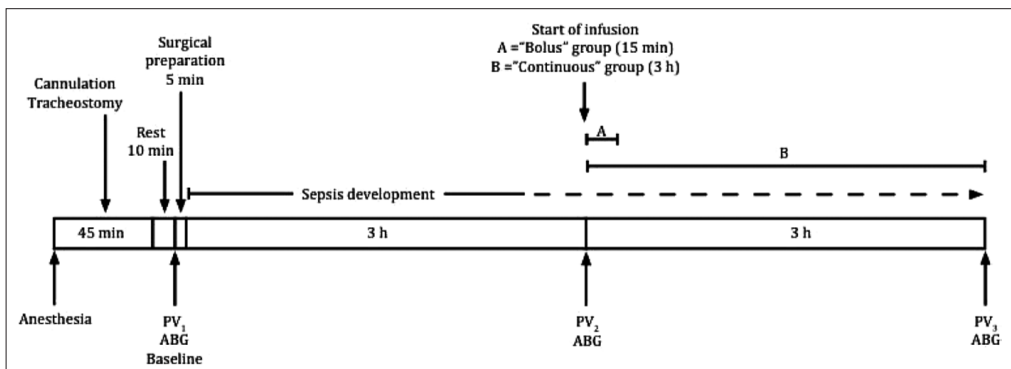


Figure 1. Time scale of the experimental protocol. PV₁ = plasma volume at baseline; PV₂ = plasma volume 3 hrs after surgical preparation, just before the start of infusion; and PV₃ = plasma volume at the end of the experiment; and ABG = arterial blood sample for analysis of blood gases, hematocrit, and electrolytes.

analysis was performed in 12 separate animals (6 per group) given albumin. In these experiments, hematocrit was measured 20, 40, and 60 mins after start of the infusion and compared with the value just before the start of the infusion.

Our results of a faster loss of albumin in the bolus group initiated additional experiments to evaluate how fast PV was lost after end of the albumin bolus infusion. The results were compared with corresponding results when given as a continuous infusion ($n = 6$ per group). The experiments were similar to the main experiments except that PV was measured only just before the start of the infusion and at 1 and 1.5 hrs thereafter.

Statistical Analysis

Statistical comparisons between the groups regarding difference in PV, difference in physiological variables, and difference in hematocrit were performed with a two-way analysis of variance followed by Bonferroni post hoc test. Student's t test for unpaired observations was used to evaluate the difference between groups in the change in mean arterial pressure after start of the infusion. One-way analysis of variance followed by Bonferroni post hoc test was used for analysis of urine production. p values of less than 0.05 were considered significant. All data were normally distributed. The results are presented as mean \pm sd. GraphPad Prism version 5.0 for Mac OS was used (GraphPad Software, San Diego, CA).

RESULTS

Four animals died in the control group (mortality rate, 33%), three animals died in the HES groups (13%), and one animal died in the gelatin groups (5%) before end of the experiment. Four animals were considered to be nonseptic and were excluded from the study, as they did not show any decrease in PV and any increase in hematocrit and lactate concentration 3 hrs after the preparation.

Physiological Data

Data from arterial blood samples for sodium (Na^+), potassium (K^+), hematocrit, lactate, pH, PaCO_2 , and PaO_2 are summarized in **Table 1** for the bolus groups, the continuous groups, and the control group. There were no differences among the control group, the continuous groups, and the bolus groups in any of the physiological variables analyzed at baseline and at 3 hrs after the surgical preparation.

For all solutions analyzed, there was a tendency of higher hematocrit in the bolus groups than in the continuous groups at the end of the experiment, but this difference did not reach the stipulated significance level in any group. There was a significant difference in hematocrit between the continuous and the control group for all colloids analyzed ($p < 0.05$), while the differences for the bolus groups and the control group reached significance only for albumin ($p < 0.05$).

Results from the separate experiments, in which the hematocrit values for the bolus and the continuous groups for albumin were compared during a 60-min period just after start of the infusion (at 20, 40, and 60 mins), are presented in

Figure 4A ($n = 6$ per group). There was a significantly lower hematocrit at 20- and 40-min period in the bolus group than in the continuous group.

At the end of the experiment, the differences in lactate levels among all groups were small, but they reached the stipulated level of significance between the NaCl continuous group and the HES bolus and continuous groups and between the gelatin bolus group and the control group ($p < 0.05$).

Data for mean arterial BP at baseline, 3 hrs after the preparation, and 1.5 and 3 hrs after the start of the infusion are given in **Table 2**. There were no significant differences in BP between the groups at any of these time points. The difference between mean of mean BP during a 30-min period after the start of the infusion with that during a 30-min period just before the start of the infusion for the bolus and the continuous groups for the solutions analyzed are presented in Figure 4B. There was a significant difference between the bolus and the continuous groups for all fluids analyzed. This difference in mean BP between the bolus and the continuous groups was transient as mean BP after 1.5 hrs did not differ between the groups (**Table 2**).

Plasma Volume

PV at baseline (PV_1), 3 hrs after the preparation (PV_2), and 3 hrs later at the end of the experiment (PV_3) for the fluids analyzed and also for the control group are shown in **Figure 2**. At baseline and 3 hrs after the preparation, there were no differences in PVs among the control group, the continuous groups, and the bolus groups. At the end of the experiment, there were significant differences between the albumin continuous group and the albumin bolus group ($p < 0.001$), between the HES continuous group and the HES bolus group ($p < 0.05$), and between the gelatin continuous group and the gelatin bolus group ($p < 0.05$). There was no significant difference between the NaCl bolus and the NaCl continuous group. There was a significant difference compared with the control group for the albumin continuous group ($p < 0.001$), the HES continuous group ($p < 0.01$), and the albumin bolus group ($p < 0.05$).

A comparison of the change in PVs among the different solutions analyzed from the start of infusion (PV_1) to the end of the experiment (PV_3) for the continuous group and the bolus group is shown in **Figure 3A** and **B**. The plasma expansion was significantly better for the albumin continuous group than for the other groups analyzed ($p < 0.001$). There was a significant difference between the albumin bolus group and the gelatin bolus group ($p < 0.01$) and between the albumin bolus group and the control group ($p < 0.05$).

PVs in the additional experiments analyzing how fast the PV was lost after end of the infusion after a bolus infusion of albumin are shown in Figure 4C. As seen, PV was reduced to the same level as in the group with continuous infusion after slightly more than 1 hr after start of the infusion, a time point when only 35%–40% of the continuous infusion volume had been given.

TABLE 1. Data (Mean \pm SD) for Sodium, Potassium, Hematocrit, Lactate, pH, P_aCO₂, and P_aO₂

	Sodium (mmol/L)	Potassium (mmol/L)	Hematocrit (%)	Lactate (mmol/L)	pH	P _a CO ₂ (kPa) ^a	P _a O ₂ (kPa)
Albumin							
15 min (n = 12)							
Baseline	135 \pm 2	4.7 \pm 0.4	43 \pm 2	2.0 \pm 0.2	7.52 \pm 0.02	4.6 \pm 0.2	12.1 \pm 1.3
3 h after preparation	132 \pm 3	5.0 \pm 0.5	44 \pm 1	2.4 \pm 0.6	7.45 \pm 0.04	4.8 \pm 0.4	12.1 \pm 1.2
3 h after start of infusion	134 \pm 2	5.6 \pm 0.8	46 \pm 3 ^a	2.4 \pm 0.9	7.43 \pm 0.04	4.4 \pm 0.5	12.2 \pm 1.3
3 h (n = 12)							
Baseline	136 \pm 1	4.7 \pm 0.4	43 \pm 2	1.8 \pm 0.6	7.51 \pm 0.02	4.6 \pm 0.3	12.0 \pm 0.8
3 h after preparation	134 \pm 2	4.9 \pm 0.4	44 \pm 2	2.3 \pm 0.6	7.47 \pm 0.04	4.7 \pm 0.4	11.5 \pm 0.9
3 h after start of infusion	135 \pm 1	4.9 \pm 0.7	44 \pm 3 ^a	2.1 \pm 0.5	7.45 \pm 0.03	4.7 \pm 0.3	11.3 \pm 0.9
Hydroxyethyl starch							
15 min (n = 10)							
Baseline	135 \pm 2	4.5 \pm 0.5	42 \pm 2	1.9 \pm 0.4	7.50 \pm 0.03	5.1 \pm 0.3	11.4 \pm 1.1
3 h after preparation	133 \pm 2	4.9 \pm 0.6	43 \pm 4	2.3 \pm 0.4	7.45 \pm 0.04	5.3 \pm 0.4	11.3 \pm 0.5
3 h after start of infusion	134 \pm 3	5.4 \pm 0.8	48 \pm 5	2.7 \pm 0.9	7.41 \pm 0.05	4.8 \pm 0.5	11.6 \pm 0.9
3 h (n = 10)							
Baseline	136 \pm 2	4.4 \pm 0.6	43 \pm 3	2.0 \pm 0.7	7.51 \pm 0.03	4.9 \pm 0.2	11.8 \pm 0.6
3 h after preparation	133 \pm 3	5.2 \pm 0.7	44 \pm 3	2.2 \pm 0.6	7.47 \pm 0.03	5.0 \pm 0.5	11.5 \pm 0.9
3 h after start of infusion	134 \pm 2	5.7 \pm 1.1	45 \pm 4 ^a	2.5 \pm 0.9	7.42 \pm 0.03	4.7 \pm 0.6	11.4 \pm 0.7
Gelatin							
15 min (n = 10)							
Baseline	136 \pm 2	4.9 \pm 0.4	43 \pm 3	2.1 \pm 0.4	7.51 \pm 0.03	4.7 \pm 0.4	11.5 \pm 0.6
3 h after preparation	134 \pm 3	5.2 \pm 0.6	44 \pm 4	2.5 \pm 0.5	7.45 \pm 0.03	4.9 \pm 0.4	11.3 \pm 0.5
3 h after start of infusion	135 \pm 2	5.8 \pm 1.0	47 \pm 5	2.5 \pm 1.0	7.42 \pm 0.04	4.6 \pm 0.6	11.7 \pm 0.9
3 h (n = 10)							
Baseline	135 \pm 2	4.6 \pm 0.3	43 \pm 2	2.1 \pm 0.3	7.50 \pm 0.03	5.0 \pm 0.5	11.5 \pm 0.9
3 h after preparation	133 \pm 2	5.1 \pm 0.7	45 \pm 2	2.8 \pm 0.3	7.45 \pm 0.02	4.9 \pm 0.5	11.5 \pm 1.0
3 h after start of infusion	134 \pm 2	5.6 \pm 1.0	46 \pm 3 ^a	2.6 \pm 0.6	7.42 \pm 0.05	4.5 \pm 0.6	11.6 \pm 0.9
NaCl							
15 min (n = 8)							
Baseline	134 \pm 2	4.9 \pm 0.4	43 \pm 2	2.0 \pm 0.4	7.52 \pm 0.02	4.9 \pm 0.2	12.1 \pm 0.7
3 h after preparation	132 \pm 2	5.6 \pm 0.6	44 \pm 3	2.7 \pm 0.4	7.46 \pm 0.02	4.9 \pm 0.4	11.9 \pm 0.7
3 h after start of infusion	135 \pm 1	5.8 \pm 1.0	47 \pm 6	2.2 \pm 0.5	7.41 \pm 0.04	4.4 \pm 0.6	12.3 \pm 0.8
3 h (n = 8)							
Baseline	135 \pm 2	4.9 \pm 0.3	42 \pm 2	1.9 \pm 0.4	7.50 \pm 0.02	5.0 \pm 0.5	11.5 \pm 0.9
3 h after preparation	133 \pm 2	5.4 \pm 0.9	46 \pm 2	2.6 \pm 0.6	7.46 \pm 0.02	4.9 \pm 0.5	11.5 \pm 1.0
3 h after start of infusion	136 \pm 2	5.5 \pm 1.3	45 \pm 5 ^a	1.8 \pm 0.4 ^b	7.41 \pm 0.04	4.5 \pm 0.6	11.6 \pm 0.9
Control (n = 8)							
Baseline	136 \pm 2	4.7 \pm 0.5	44 \pm 2	1.9 \pm 0.3	7.49 \pm 0.02	5.3 \pm 0.3	11.1 \pm 1.2
3 h after preparation	133 \pm 2	5.2 \pm 0.3	45 \pm 3	2.5 \pm 0.4	7.46 \pm 0.02	5.2 \pm 0.1	10.8 \pm 1.0
3 h after start of infusion	133 \pm 1	5.9 \pm 0.4	51 \pm 4	2.6 \pm 0.6	7.45 \pm 0.02	4.6 \pm 0.3	12.0 \pm 1.1

^ap < 0.05 compared with the control group.

^bp < 0.05 compared with the control group, the 3-h and 15-min HES groups, and the 15-min gelatin group; Two-way analysis of variance with Bonferroni post hoc test were used for the statistical analysis.

TABLE 2. Data (Mean \pm SD) for Mean Arterial Blood Pressure (mm Hg) at Baseline, 3 hr After the Surgical Preparation, 1.5 hr After the Start of Infusion, and 3 hr After the Start of Infusion With 5% Albumin, 6% Hydroxyethyl Starch 130/0.4, 6% Gelatin, 0.9% NaCl, and the Control Group

	Baseline	3 h After Surgical Preparation	1.5 h After Start of Infusion	3 h After Start of Infusion
Albumin				
15 min (<i>n</i> = 12)	97 \pm 8	90 \pm 11	95 \pm 11	95 \pm 12
3 h (<i>n</i> = 12)	100 \pm 11	88 \pm 8	96 \pm 8	103 \pm 8
Hydroxyethyl starch				
15 min (<i>n</i> = 10)	104 \pm 11	90 \pm 14	96 \pm 13	97 \pm 15
3 h (<i>n</i> = 10)	107 \pm 10	94 \pm 10	101 \pm 12	99 \pm 18
Gelatin				
15 min (<i>n</i> = 10)	95 \pm 18	97 \pm 10	98 \pm 11	99 \pm 14
3 h (<i>n</i> = 10)	101 \pm 14	102 \pm 12	108 \pm 9	109 \pm 8
NaCl				
15 min (<i>n</i> = 8)	102 \pm 11	95 \pm 16	95 \pm 16	103 \pm 18
3 h (<i>n</i> = 8)	100 \pm 9	97 \pm 15	106 \pm 12	108 \pm 17
Control (<i>n</i> = 8)	101 \pm 14	95 \pm 10	97 \pm 13	98 \pm 12

Urine Production

Urine production from the start of the infusion to the end of the experiment was 5.9 ± 1.3 mL/kg in the albumin continuous group, 6.3 ± 0.9 mL/kg in the albumin bolus group, 3.2 ± 0.9 mL/kg in the HES continuous group, 3.8 ± 1.2 mL/kg in the HES bolus group, 3.2 ± 0.9 mL/kg in the gelatin continuous group, 3.3 ± 1.2 mL/kg in the gelatin bolus group, 4.0 ± 0.6 mL/kg in the NaCl continuous group, 4.9 ± 2.2 mL/kg in the NaCl bolus group, and 2.6 ± 1.4 mL/kg in the control group. There was significantly more urine production in the albumin groups and the bolus NaCl group than in the control group ($p < 0.05$).

DISCUSSION

This study has shown that the degree of PV expansion of a fixed volume of a colloid solution measured 3 hrs after start of the infusion is larger when it is given at a slow infusion rate than if it is given at a fast rate. However, this difference was greater for albumin than for HES and gelatin, and albumin was the most effective PV expander. For NaCl, there was no significant difference in PV-expanding effect at the end of the experiment for the bolus group and the continuous group. The NaCl groups did not differ significantly from the control group, even though NaCl was given in a 4 times larger volume than the colloid solutions. The differences in PV at the end of the experiment were reflected in the difference in hematocrit values for all colloids, in the sense that there were lower hematocrit values in all the continuous groups than in the control group.

The dilution technique using ^{125}I -albumin as tracer is well established for the calculation of PV in experimental and clinical studies with reproducible results in both normal and inflammatory states (21, 22). As has been discussed previously (21, 23), this technique, however, means some overestimation of the PV. Free iodine in the tracer injected can result in some overestimation, as free iodine is distributed quickly to the whole extracellular space, but the free iodine was small in this study ($< 1.2\%$) and therefore must have had minor influence on the results. There might have been overestimation of PV because of transcapillary escape of radioactive albumin during the 5-min period between injection of the tracer and collection of the blood sample and especially at states of increased permeability (21, 23). This means a larger overestimation of the PV after initiation of sepsis than at baseline. This overestimation, however, must be small in this study by the short time period of 5 min between the injection of ^{125}I -albumin and the measurement, a time period shorter than the 10–15 min used in the referred studies (21, 23). A time period of 5 min has previously been shown to be sufficient for complete mixture of the tracer in plasma both in cat and in human (14, 21). Finally, remaining radioactivity of the syringe, the vial, and the needle used was subtracted from the initially calculated radioactivity and therefore will not contribute to an overestimation of the PVs. All this taken together means, that the expected overestimation of the PV measurements with the design of the dilution technique used in this study is small. Independent of this, remaining errors will have no influence on the conclusions made, as they will be of the same magnitude for all groups.

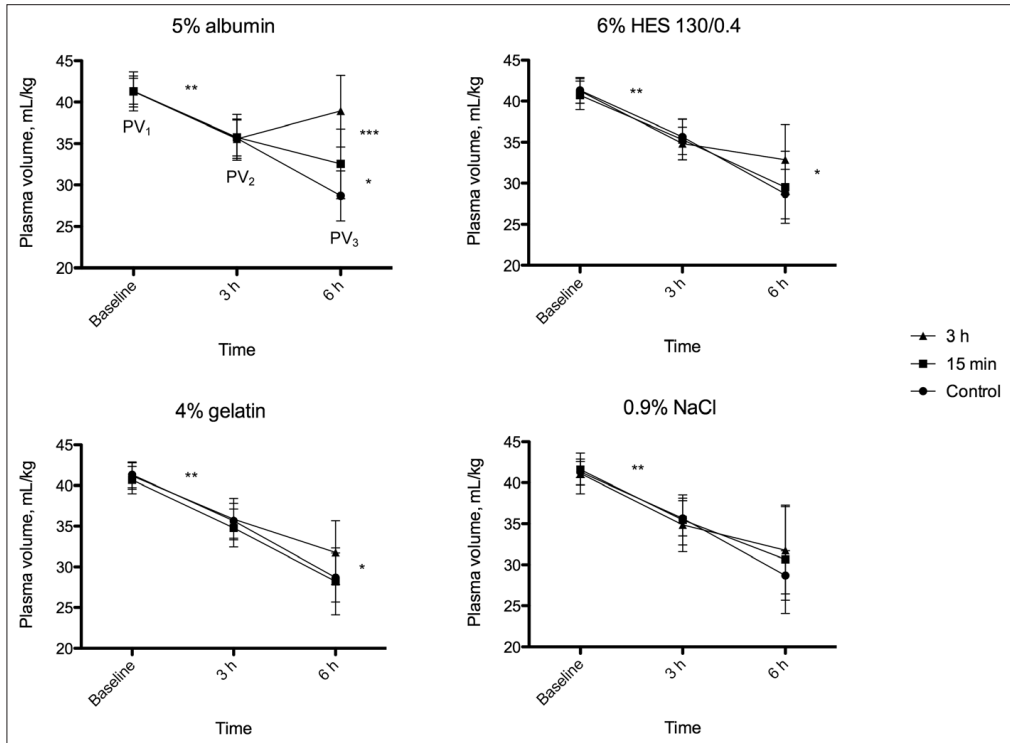


Figure 2. Plasma volumes (PVs) at baseline (PV₁), 3 hrs after the preparation just before the start of infusion (PV₂), and at the end of the experiment (PV₃) given as a continuous (3-hr) infusion or as a bolus (15-min) infusion of 5% albumin ($n = 12$ per group), 6% hydroxyethyl starch 130/0.4 ($n = 10$ per group), 4% gelatin ($n = 10$ per group), or 0.9% NaCl ($n = 8$ per group). Corresponding data for the control group ($n = 8$) are also shown. There was a significant difference between PV₁ and PV₂ for all groups and a significant difference between the continuous group and the bolus group for all solutions except 0.9% NaCl. There was a significant difference between the albumin bolus group and the control group. Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The better PV-expanding effect with a slow infusion rate than with a fast infusion rate is compatible with the two-pore theory of transcapillary fluid exchange. As suggested in the introductory section, a bolus infusion would be expected to cause a transient increase in capillary pressure from the transient increase in systemic arterial pressure and decrease in precapillary resistance and decrease in hematocrit, all of which can be expected to lead to an increase in transcapillary fluid loss (8). Our results of a significantly larger increase in mean arterial pressure and decrease in hematocrit in the bolus groups during the first time period after start of the infusion are compatible with these proposals (Fig. 4A and B). It is unlikely that the release of ANP and BNP after a bolus infusion, as discussed in the introductory section, would have influenced the results through an increase in urine production, as urine production was very small in these experiments in relation to the volumes infused. However, we cannot exclude the possibility that the permeability-increasing effect of ANP and BNP resulted in PV loss (5).

Tentative explanations can be given regarding the bad plasma expansion, and the smaller differences in PVs between the continuous and the bolus groups for HES and gelatin compared with albumin. HES is degraded by amylase resulting in halving of the molecular weight within 20–30 min (24). The initial half-life of plasma elimination of HES 130/0.4 is thought to be approximately 30–45 mins after infusion in man (24). Degradation of the HES molecules causes an increase in leakage of the smaller degradation products to the extravascular space: The degradation rate can be expected to be even faster in the rat than in man because of a higher plasma concentration of amylase in the rat (25), most likely resulting in extensive degradation within the 3-hr study period. This might be one explanation of the poor PV-expanding effect of HES, and the fact that the bolus group did not even differ significantly from that of the control group. Thus, the results for HES in this study cannot be directly extrapolated to humans.

Gelatin has a relatively low mean MW of 30kDa. Being a polydisperse colloid, a large part of the molecules are small

enough to pass not only through the large pores but also through the small pores. This, and the fact that there is degradation of the molecules, means that there may be a relatively fast and continuous transcapillary leakage of gelatin during the 3-hr period after the start of the infusion, especially when there is an increase in capillary permeability. This fact might explain the poor PV-expanding effect of gelatin in this study.

The lack of any difference in PV expansion between the bolus and the continuous groups for 0.9% NaCl is to be expected, since the capillaries are freely permeable to crystalloids with

a fast distribution of the solution to the whole extracellular space. One would expect, however, that the plasma-expanding effect of 0.9% NaCl would be better than that of HES and gelatin, considering that about 25% of the infused volume of 48 mL/kg should stay intravascularly and that the urine production was small.

Unexpectedly, the PV expansion for 0.9% NaCl did not even differ significantly from that of the control group, and we can

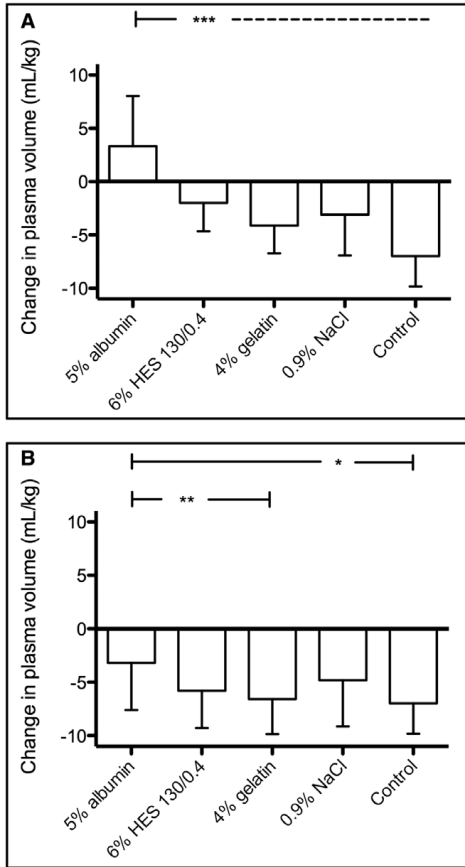


Figure 3. Comparison of the different solutions analyzed regarding change in plasma volume (PV) from the start of infusion (PV₀) to the end of the experiment (PV₃) for the continuous (3-hr) groups (A) and the bolus (15-min) groups (B). There was a significant difference between the albumin continuous group and the other groups, between the albumin bolus group and the gelatin bolus group, and between the albumin bolus group and the control group. Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

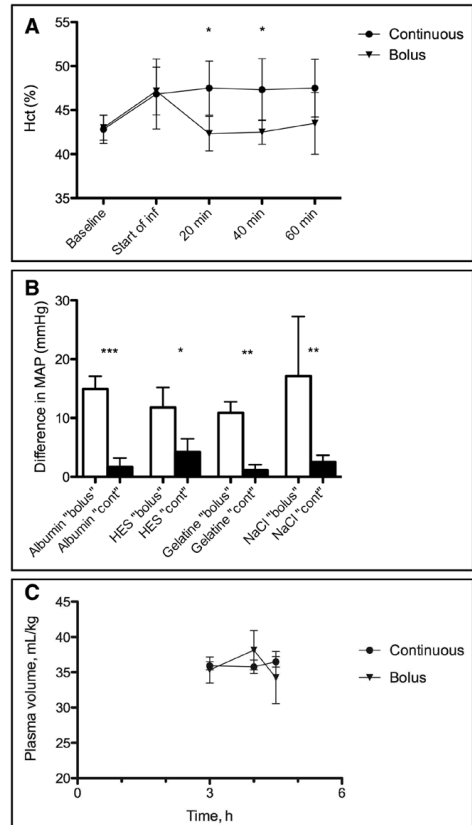


Figure 4. A, Hematocrit at baseline; just before start of the infusion for the bolus group and for the continuous group for albumin. The analysis was performed in a separate series of animals ($n = 6$ per group). Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses (* $p < 0.05$). B, Mean of mean arterial blood pressure (MAP) during a 30-min period just after start of the infusion subtracted with that 30 min just before start of the infusion for the bolus and the continuous groups for the different solutions analyzed. Student's t test for unpaired observations was used for the statistical analyses (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). C, Plasma volume (PV) 3 hrs after initiation of sepsis just before start of the infusion and 1 and 1.5 hrs after start of the infusion for the bolus group and the continuous group. The analysis was performed in a separate series of animals after end of the main study ($n = 6$ per group).

only speculate about possible explanations. When giving a crystalloid solution, under normal circumstances approximately 75% of the infused volume is distributed quickly to the interstitial space. With sepsis, when plasma has already been lost to the extravascular space, the ratio between the PV and the interstitial volume is reduced. This means that relatively more of the infused volume would be distributed to the interstitial space. While the infused saline is passing the large pores of the capillary membrane, there may also be a subsequent loss of proteins via convection (26). The large volumes of saline will transiently dilute plasma proteins, resulting in a reduction in transcapillary oncotic pressure, which will increase fluid transfer to the extravascular space and also reduce the absorbing forces across the capillary membrane (27). Finally, the 4 times higher infusion rate for saline than for the colloids results in a transient increase in hydrostatic capillary pressure. All these mechanisms may lead to increased leakage of fluid through the capillary pores, and increased leakage of proteins by convection through the large pores, especially under a state of increased number of large pores, such as in sepsis/SIRS.

The capacity of a colloid to maintain a normal PV is essential for its effectiveness. However, the relatively fast degradation rates of HES and gelatin can be compensated for by repeated infusions, while the low degradation rate of albumin compared with synthetic colloids may be negative because albumin will linger in the interstitium for a longer time.

As seen from Figure 4C, the PV loss after end of the bolus infusion of albumin was rather fast, reaching the same PV as that obtained when the infusion was given continuously after slightly more than 1 hr. This result supports the hypothesis presented in the introductory section that the PV loss can be related to hemodynamic effects of the bolus infusion, such as the transient decrease in hematocrit (Fig. 4A), the transient increase in arterial pressure (Fig. 4B) and the precapillary vasodilation.

We cannot tell from this study for how long time the slow rate of albumin infusion is favorable after it has been completed. However, if assuming about the same leakage of albumin after end of the infusion as occurring after initiation of sepsis (the same TER), the low rate of albumin infusion will be favorable during the subsequent 2–3 hrs after end of the infusion. If so, the continuous infusion will be favorable up to 5–6 hrs after start of the infusion, while the bolus infusion is more favorable than the continuous infusion only up to 1–1.5 hrs after start of the infusion (Fig. 2 and 4C).

As seen from Table 1, the direction of the changes in hematocrit follows the pattern expected from the articulated hypothesis as presented in the introductory section, in the sense that the continuous groups generally have lower hematocrit values than the bolus groups.

For the colloid groups and the control group, the lactate concentrations follow the expected pattern in relation to the PVs, which means that the lowest concentrations at the end of the experiment were seen for the group with the highest PV, that is, the continuous group of albumin. For 0.9% NaCl, the lactate concentrations were lower at the end of the experiment

than in the colloid groups, despite the fact that the PVs were low and did not even differ from the control group. It is most likely that this does not mean that there was less lactate production in the 0.9% NaCl groups but rather that lactate was diluted in a larger interstitial volume in these groups because of the larger (4 times) volumes infused.

As the differences in urinary production were small between the continuous and the bolus groups, it is unlikely that the urine production influenced the difference in PV. The highest production of urine was seen in the albumin groups, which also had the largest PVs—most likely due to a lesser degree of hypovolemia in these groups.

Our finding of a generally better PV-expanding effect with 5% albumin than with the other solutions tested is in agreement with previous studies on rat and cat (16, 22).

The fact that there was a higher mortality rate before the end of the experiment in the control group compared with the other groups, supports the idea that fluid infusion is of importance for outcome in sepsis/SIRS.

Even though this study with its strict protocol has few clear limitations, one limitation is that the experiments were performed on the rat and therefore cannot be directly transferred to man. Especially, the results with HES suffer from limitations as HES is degraded by amylase, the concentrations of which are higher in rat than in man. Further, there may be some variations in the degree of sepsis and thus in degree of increase in microvascular permeability among animals, even if the surgical technique to induce sepsis was carefully standardized. An equal distribution of the intensity of sepsis in the groups is therefore of importance as plasma clearance for colloids is dependent on permeability.

In summary, this study in the septic rat showed that the plasma expansion of 5% albumin, 6% HES 130/0.4, and 4% gelatin was larger 3 hrs after start of infusion when given with a slow infusion rate than when given with a fast infusion rate. This difference was more pronounced with albumin than with the other colloids. Given in equal volumes, the PV-expanding effect 3 hrs after start of the infusion was better for 5% albumin than for 6% HES and 4% gelatin. The plasma-expanding effect of 0.9% NaCl was not affected by the infusion rate, and 0.9% NaCl was not more effective than any of the colloids, even though it was given in a 4 times larger volume. If these results can be transferred to clinical practice, the total volume needed of colloids to maintain normovolemia in patients with sepsis/SIRS would be significantly reduced if given at a slow instead of a fast infusion rate.

ACKNOWLEDGMENT

We thank Helene Axelberg for skilled technical assistance.

REFERENCES

1. Fleck A, Raines G, Hawker F, et al: Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985; 1:781–784
2. Groeneveld AB, Teule GJ, Bronsveld W, et al: Increased systemic microvascular albumin flux in septic shock. *Intensive Care Med* 1987; 13:140–142

3. Haskell A, Nadel ER, Stachenfeld NS, et al: Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 1997; 83:407-413
4. Fishel RS, Are C, Barbul A: Vessel injury and capillary leak. *Crit Care Med* 2003; 31(8 Suppl):S502-S511
5. Huxley VH, Scallan J: Lymphatic fluid: exchange mechanisms and regulation. *J Physiol (Lond)* 2011; 589(Pt 12):2935-2943
6. Rippe B, Haraldsson B: Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 1994; 74:163-219
7. Mellander S, Johansson B: Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacol Rev* 1968; 20:117-196
8. Dubniks M, Persson J, Grände PO: Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 2007; 33:2192-2198
9. Nygren A, Redfors B, Thorén A, et al: Norepinephrine causes a pressure-dependent plasma volume decrease in clinical vasodilatory shock. *Acta Anaesthesiol Scand* 2010; 54:814-820
10. Valeri CR, Donahue K, Feingold HM, et al: Increase in plasma volume after the transfusion of washed erythrocytes. *Surg Gynecol Obstet* 1986; 162:30-36
11. Persson J, Grände PO: Volume expansion of albumin, gelatin, hydroxyethyl starch, saline and erythrocytes after haemorrhage in the rat. *Intensive Care Med* 2005; 31:296-301
12. Woodard GE, Rosado JA: Natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol* 2008; 268:59-93
13. Huxley VH, Tucker VL, Verburg KM, et al: Increased capillary hydraulic conductivity induced by atrial natriuretic peptide. *Circ Res* 1987; 60:304-307
14. Imm A, Carlson RW: Fluid resuscitation in circulatory shock. *Crit Care Clin* 1993; 9:313-333
15. Grocott MP, Mythen M: Fluid therapy. In: Bailliére's Clinical Anaesthesiology. Harcourt Publishers Ltd, 1999, pp 363-381
16. Persson J, Grände PO: Plasma volume expansion and transcapillary fluid exchange in skeletal muscle of albumin, dextran, gelatin, hydroxyethyl starch, and saline after trauma in the cat. *Crit Care Med* 2006; 34:2456-2462
17. Mao EQ, Tang YQ, Fei J, et al: Fluid therapy for severe acute pancreatitis in acute response stage. *Chin Med J* 2009; 122:169-173
18. Maitland K, Kiguli S, Opoka RO, et al; FEAST Trial Group: Mortality after fluid bolus in African children with severe infection. *N Engl J Med* 2011; 364:2483-2495
19. Hubbard WJ, Choudhry M, Schwacha MG, et al: Cecal ligation and puncture. *Shock* 2005; 24 Suppl 1:52-57
20. Scheiermann P, Hoegl S, Revermann M, et al: Cecal ligation and incision: an acute onset model of severe sepsis in rats. *J Surg Res* 2009; 151:132-137
21. Margaron MP, Soni NC: Plasma volume measurement in septic patients using an albumin dilution technique: comparison with the standard radio-labelled albumin method. *Intensive Care Med* 2005; 31:289-295
22. Dubniks M, Persson J, Grände PO: 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; 33:293-299
23. Valeri CR, Cooper AG, Pivacek LE: Limitations of measuring blood volume with iodinated I 125 serum albumin. *Arch Intern Med* 1973; 132:534-538
24. Waitzinger J, Bepperling F, Pabst G, et al: Pharmacokinetics and Tolerability of a New Hydroxyethyl Starch (HES) Specification [HES (130/0.4)] after Single-Dose Infusion of 6% or 10% Solutions in Healthy Volunteers. *Clin Drug Investig* 1998; 16:151-160
25. Tuba J, Wiberg GS: On rat serum amylase. I. Studies in the normal and the alloxan diabetic animal. *Can J Med Sci* 1953; 31:377-386
26. Gandhi RR, Bell DR: Influence of saline infusions on transvascular protein transport. *Am J Physiol* 1992; 262(2 Pt 2):H443-H450
27. Rippe B, Haraldsson B: Fluid and protein fluxes across small and large pores in the microvasculature. Application of two-pore equations. *Acta Physiol Scand* 1987; 131:411-428

Impact of Fluid Infusion Rate on Restoring Plasma Volume in Sepsis*

Hironori Ishihara, MD

Department of Anesthesiology
Hirosaki University Graduate School of Medicine
Hirosaki, Japan

Correction of hypovolemia is fundamental, but it is one of the most important factors in managing critically ill patients. In patients with sepsis, intensivists and investigators are generally interested in the type of colloids and crystalloids, as well as the total volume of fluids required to overcome hypovolemia. However, they may have been overlooking the infusion rate of administered fluids, since it is believed that hypovolemia should be treated as early as possible to improve survival (1). In this issue of *Critical Care Medicine*, Bark et al (2) from Lund University Hospital in Sweden report their experiment highlighting the importance of the infusion rate of different types of fluids for maintaining normal plasma volume in septic rats. A fixed volume of different types of colloids and a crystalloid including 5% albumin, 6% hydroxyethyl starch 130/0.4, 4% gelatin, and 0.9% NaCl was administered via fast or slow infusion. In the fast-infusion group, fluids were administered over 15 mins, while in the slow-infusion group, fluids were administered over 3 hrs. Measurement of plasma volume was carefully performed at 3 hrs after the start of volume resuscitation using human iodine-125 (¹²⁵I) albumin, even though this volume marker is known to overestimate plasma volume in critical illness associated with increased capillary protein leakage (3). Although the interval between the end of fluid administration and measurement was apparently different between the two infusion groups, the authors demonstrate the negligible effect of the interval by additional experiments (Fig. 4C). The results show that plasma volume expansion by a colloid is greater when infused slowly, an effect more pronounced with albumin. The authors also speculate the mechanism mediating the increased transcapillary fluid loss due to the fast infusion of colloids in the presence of increased permeability. A transient increase in arterial pressure and hemo dilution would have a significant impact on transcapillary fluid loss, but this mechanism was not observed in the absence of increased permeability (4). In a clinical setting, Wills et al (5) showed that the effect of fast infusion of hydroxyethyl starch or dextran is obvious only during the first 2 hrs in children with dengue shock syndrome characterized by severe vascular

leakage as judged by changes in hematocrit. Considering the results of the experimental study by Bark et al (2) as well as human studies indicating that a lower net fluid balance is associated with fewer days of ventilator use and fewer days in the ICU (6, 7), a slow rather than a fast infusion of colloids, particularly albumin, would have the potential to improve survival, even though evidence that colloids provide better survival is lacking (8).

Bark et al (2) gave three-fold more 0.9% NaCl than colloids to achieve a similar effect on plasma volume expansion but resultant expansion was poor regardless of the infusion rate because crystalloids were rapidly distributed from the intravascular to the interstitial fluid compartment (9). In a clinical setting, a three- to four-fold higher volume of crystalloids than colloids is generally required during fluid resuscitation to achieve similar plasma volume expansion. However, the reported administered fluid volume ratio (crystalloids to colloids) was only 1.1–1.4 over the first 4 days in patients with severe sepsis (10). Presumably, the effects of plasma volume expansion by crystalloids remain unchanged for a longer period, even though a large volume of crystalloids is required in the early phase of fluid resuscitation. The result of the experimental study by Bark et al (2) wherein similar poor plasma volume expansion by 0.9% NaCl was observed, regardless of the infusion rate, also suggests such pathophysiology, even though a volume kinetic study suggests a favorable effect of slow rather than fast infusion of crystalloid (11).

Slow infusion of colloids, especially albumin, may have not only hemodynamic benefits, but also cost-effectiveness. However, further clinical studies are required to determine whether such hemodynamic benefits are sustainable and sufficient for improving survival, even though Bark et al (2) speculated that the hemodynamic benefit after starting infusion of colloids can be maintained for 5–6 hrs with slow infusion, compared only 1–1.5 hrs with fast infusion. Furthermore, it remains unclear whether currently available clinical markers or other variables can be used to monitor the adequacy of a slow-infusion rate of fluids during fluid volume resuscitation, since the magnitude of sepsis-induced hypovolemia and hemodynamic states varies considerably. On the other hand, the hemodynamic state in the present controlled experimental study did not severely deteriorate according to arterial blood lactate levels. Several concerns should be addressed before clinical application with a slow-infusion regimen. Nevertheless, the concept of slow infusion provides an important message that should resonate with intensivists. It is time to take into account not only fluid type, but also the infusion rate for fluid resuscitation in patients with sepsis.

*See also p. 857.

Key Words: colloid; crystalloid; fluid resuscitation; infusion rate; plasma volume; sepsis

The author has not disclosed any potential conflicts of interest.

Copyright © 2013 by the Society of Critical Care Medicine and Lippincott Williams & Wilkins

DOI: 10.1097/CCM.0b013e3182770ffe

REFERENCES

1. Dellinger RP, Levy MM, Carlet JM, et al: Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; 36:296–327
2. Bark B, Persson J, Grände P-O: Importance of the Infusion Rate for the Plasma Expanding Effect of 5% Albumin, 6% HES 130/0.4, 4% Gelatin and 0.9% NaCl in the Septic Rat. *Crit Care Med* 2013; 41:857–866
3. Valeri CR, Cooper AG, Pivacek LE: Limitations of measuring blood volume with iodinated I 125 serum albumin. *Arch Intern Med* 1973; 132:534–538
4. Dubniks M, Persson J, Grände PO: Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 2007; 33:2192–2198
5. Wills BA, Nguyen MD, Ha TL, et al: Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005; 353:877–889
6. The National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network: Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;354:2564–2575
7. Renal Replacement Therapy Study Investigators, Bellomo R, Cass A, et al: An observational study fluid balance and patient outcomes in the Randomized Evaluation of Normal vs. Augmented Level of Replacement Therapy trial. *Crit Care Med* 2012; 40: 1753–1760
8. Reinhart K, Perner A, Sprung CL, et al: Consensus statement of the ESICM task force on colloid volume therapy in critically ill patients. *Intensive Care Med* 2012; 38: 363–383
9. Levick JR, Michel CC: Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 2010; 34:2157–2168
10. Bayer O, Reinhart K, Kohl M, et al: Effects of fluid resuscitation with synthetic colloids or crystalloids alone on shock reversal, fluid balance, and patient outcomes in patients with severe sepsis: a prospective sequential analysis. *Crit Care Med* 2012; 40:2543–2551
11. Hahn RG: Volume kinetics for infusion fluids. *Anesthesiology* 2010; 113:470–481

Paper II

Infusion rate and plasma volume expansion of dextran and albumin in the septic guinea pig

B. P. BARK and P.-O. GRÄNDE

Department of Anaesthesia and Intensive Care, Lund University and Lund University Hospital, Lund, Sweden

Background: Intravenous fluid treatment of hypovolaemia in states of increased capillary permeability, e.g. sepsis, is often accompanied by adverse oedema formation. A challenge is therefore to achieve and maintain normovolaemia using as little plasma volume substitution as possible to minimise interstitial oedema. In the present study, we evaluated the importance of infusion rate for the plasma volume expanding effects of 6% dextran 70 and 5% human albumin in a guinea pig sepsis model. **Methods:** In this prospective, randomised study, 50 anaesthetised adult male Dunkin-Hartley guinea pigs were used. After laparotomy, sepsis was induced by caecal ligation and incision. Three hours later, an infusion (12 ml/kg) of one of the studied fluids was given either over 15 min (bolus group) or over 3 h (continuous group). A sham group underwent the same surgical procedure but did not receive any fluid.

Results: At the end of the experiment 3 h after the start of infusion, plasma volumes in the continuous group and the bolus group, respectively, were: 47.2 ± 5.3 ml/kg and 36.5 ± 3.9 ml/kg ($P < 0.001$) for 6% dextran 70, and 47.3 ± 7.5 ml/kg and 39.7 ± 2.8 ml/kg ($P < 0.01$) for 5% albumin. Plasma volume for the sham group at the same time point was 29.9 ± 3.3 ml/kg.

Conclusions: The study performed on a guinea pig sepsis model showed that the plasma volume expanding effects of fixed volumes of 6% dextran 70 and 5% albumin were greater when given at a slow than at a fast infusion rate.

Accepted for publication 10 October 2013

© 2013 The Acta Anaesthesiologica Scandinavica Foundation.
Published by John Wiley & Sons Ltd

PLASMA fluid and proteins are continuously lost from the intravascular space to the interstitium through the capillary membrane. In the normal state in humans, the transcapillary escape rate (TER_{alb}) of albumin to the interstitium is 5–7% of the total plasma pool per hour.¹ Interstitial fluid and proteins are returned to the circulation via the lymphatic system.²

In sepsis and other states of systemic inflammation, permeability of the capillary membranes is increased, with a subsequent increase in plasma loss to the interstitial space.^{3,4} When it exceeds the capacity of the lymphatic system, plasma leakage will result in accumulation of interstitial fluid and intravascular hypovolaemia. This may cause altered Starling fluid equilibrium, tissue oedema, and compromised tissue perfusion.² Furthermore, as a result of systemic inflammation, interstitial pressure may be reduced, resulting in additional loss of plasma to the interstitium.^{5,6}

To prevent further damage – or even death – by hypoperfusion of the tissues and organs, restoration of normovolaemia using plasma volume (PV) substitutes, crystalloids or colloids, is a central part in the treatment of patients with increased capillary permeability. Colloid solutions, which consist of macromolecules, are of benefit in the normal state, as they remain in the intravascular space, at least initially.⁷ However, in a state of increased capillary permeability, the infused colloids would be expected to leak to a greater extent through the capillary membrane, being less effective and further aggravating oedema.⁸ Thus, an important challenge in patients with increased capillary permeability would therefore be to achieve and maintain normovolaemia with as little PV substitution as possible, by minimising transcapillary leakage and adverse oedema formation.

The two-pore theory of vascular fluid exchange postulates that plasma fluid passes through two types of pores in the capillary membrane: the small pores, which are permeable only to small solutes and water, and the more than 10,000 times less

Setting: All experiments were carried out in a university hospital laboratory

abundant large pores, which are also permeable to proteins.⁹ According to this theory, the increased capillary permeability under inflammatory conditions, such as sepsis or systemic inflammatory response syndrome (SIRS), is mainly the result of an increased number of large pores. As the large pores are freely permeable to proteins and other macromolecules, the differences in oncotic pressure on either side of the pores must be small, so that the major force determining the flow through the large pore would be differences in transcapillary hydrostatic pressure.^{9,10} This may explain results from previous studies that an increase in arterial blood pressure can result in an increased loss of albumin from the intravascular space and a decrease in PV.^{11–13} If PV substitution – crystalloid or colloid – was to be given quickly, the transient increase in capillary hydrostatic pressure would be greater, causing a greater leakage of fluid resulting in a less effective treatment and aggravation of potentially harmful oedema. Furthermore, in an inflammatory state with increased numbers of large pores, relatively more fluid would pass through the large pores, resulting in an increased loss of macromolecules via convection.

Fast infusion of a PV substitute would also result in a greater increase in ventricular filling pressures, which might cause release of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), resulting in further increase in capillary permeability and urine production.^{14,15}

Based on these considerations, we recently performed a study in rats comparing the PV expanding effect of a fixed volume of 5% albumin, 6% hydroxyethyl starch (HES) 130/0.4, 4% gelatin, and 0.9% NaCl during sepsis, when given at either a slow infusion rate (over 3 h) or a fast infusion rate (over 15 min).¹⁶ The results from that study showed that the plasma expansion for the colloid solutions was larger 3 h after the start of infusion when given at a slow infusion rate than when given at a fast rate, but with great difference between the colloids studied. As rats are allergic to dextran,¹⁷ dextran 70 was not included.

The synthetic colloid dextran 70 is mainly used in Scandinavia. It has been shown to have good PV expanding properties, in some studies even superior to that of all other colloids studied, in states of normal capillary permeability^{18,19} and in states of increased capillary permeability.^{20,21} Based on recent studies, the use of HES is no longer recommended in sepsis,²² which has reduced the synthetic colloid treatment alternatives in the critically ill patients to a

minimum. This could mean an increased interest in the use of dextran. To test the hypothesis that a fixed volume of 6% dextran 70 would have a greater PV expanding effect when given as a continuous infusion, than when given as a bolus infusion, by analogy with our previous study, we therefore performed this experiment in the guinea pig, which tolerates dextran. To better compare the results from the present study with those from our previous study on the rat, we also used 5% albumin as a reference.

Materials and methods

Anaesthesia and set-up

The study was approved by the Ethical Committee on Animal Experiments, Lund, Sweden (application nos. M180-10 and M309-12), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Fifty adult male Dunkin-Hartley guinea pigs weighing 378 ± 49 g [mean \pm standard deviation (SD)] were used. Anaesthesia was induced by placing the animals in a covered glass container with a continuous supply of 5% isoflurane in air (Forene® 100%; Abbot Scandinavia AB, Solna, Sweden). After induction, anaesthesia was maintained using a mask with 1.5–1.8% isoflurane in air, while tracheostomy was performed. Then the animals were connected to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy), and ventilated in a volume-controlled mode with a positive end expiratory pressure of 4 cmH₂O. End-tidal PCO₂ was continuously monitored (Capstar-100; CWE, Ardmore, PA, USA). Rectally measured body temperature was kept at 37.1–37.3°C via a feedback-controlled heating pad. The left common carotid artery was cannulated to continuously record arterial blood pressure and to obtain blood samples for analysis of arterial blood gases, electrolytes, lactate, haematocrit (I-STAT; Abbot Point of Care Inc., Abbot Park, IL, USA), and PVs. The left internal jugular vein was cannulated and used for infusions, and kept open with a continuous infusion of saline at 0.2 μ l/min. The right internal jugular vein was cannulated and used for injection of ¹²⁵I-albumin for PV measurements. After the experiments, the animals were killed with an intravenous injection of potassium chloride.

Experimental procedure

We used a model of severe sepsis in the guinea pig that had been described previously in the rat.^{16,23}

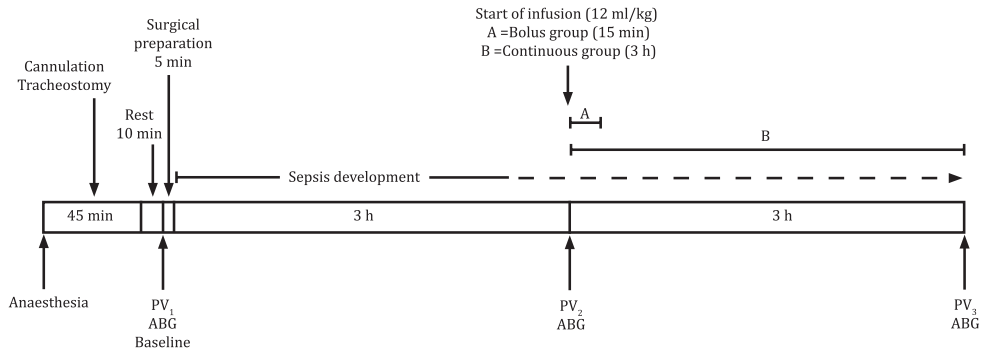


Fig. 1. Time scale of the experiment. PV_1 = plasma volume at baseline. PV_2 = plasma volume 3 h after surgical preparation, just before the start of infusion. PV_3 = plasma volume at the end of the experiment. ABG = arterial blood sample for analysis of blood gases, haematocrit, lactate, and electrolytes.

After making a longitudinal midline skin incision with diathermia in the abdominal wall, laparotomy was performed by incision along the linea alba. The caecum was ligated just below the ileo-caecal valve and an incision of 1 cm in length was made in the caecum, allowing leakage of faecal material into the abdominal cavity, thereby inducing sepsis/SIRS. The abdominal wall and the skin were then closed with clips. There was no bleeding.

PV

PV was measured with a well-established technique with small sources of error (see Discussion), which has been shown previously to give reliable and reproducible results.^{12,16,21,24} As previously described,¹⁶ PV was determined by measuring the radioactivity in 100 μ l of plasma taken 5 min after an intravenous 0.5 ml injection of human ¹²⁵I-albumin with a known amount of activity. The increase in radioactivity was then calculated by subtracting the activity in a blood sample taken just before the injection from that taken 5 min after the injection, thereby adjusting for any remaining radioactivity from previous measurements. To calculate the amount of radioactivity given, the remaining activity in the used and emptied vial, syringe, and needle was measured and subtracted from the total activity in the prepared dose. Free iodine was measured continuously following precipitation with 10% trichloroacetic acid, and it was found to be $1.5 \pm 0.6\%$ in the prepared samples. Radioactivity was measured with a gamma counter (Wizard 1480; LKB-Wallac, Turku, Finland).

Experimental protocol

In this study, we compared the PV-expanding effect of a fixed volume of a PV expander when given at a slow infusion rate (over 3 h) or a fast infusion rate (over 15 min). The PV expanders analysed were 6% dextran 70 (Macrodex[®], 60 mg/ml; MEDA AB, Solna, Sweden; mean MW 70 kDa) ($n = 20$) and 5% human albumin (Albumin Baxter, 50 g/l; Baxter Medical AB, Kista, Sweden; MW 69 kDa) ($n = 20$). All fluids were given intravenously. The time scale of the experimental procedure is shown in Fig. 1. Three hours after the end of the surgical procedure, infusion of the fluid under study was started (12 ml/kg). A previous study on the rat using this sepsis model showed that a systemic inflammation has developed by this time point, marked by a significant decrease in PV. The infused volume used in this study (12 ml/kg) was chosen because that was the volume used in our previous study.¹⁶ As described previously,¹⁶ this volume was selected as previous experiments have shown a PV reduction 3 h after sepsis induction of 7–9 ml/kg, and to this, we then added a few millilitre to compensate for the anaesthesia-induced vasodilation and the blood samples taken just before start of the infusion.

For each fluid, the animals were randomised into two groups. In one group, the fluid was given as a slow infusion over 3 h (the continuous groups), and in the other group, the same volume was given as a fast infusion over 15 min (the bolus groups). The investigators were blind regarding fast or slow treatment. In a sham group ($n = 10$), the animals

underwent the same surgical procedure but no PV expander was given. PV was measured at baseline (PV₁), 3 h after the surgical preparation (PV₂), and 3 h after the start of infusion (at the end of the experiment) (PV₃). Blood samples for measurement of arterial pH, PaCO₂, PaO₂, haematocrit, lactate, sodium, and potassium were taken at the same time points. PV lost by the blood samples was of the same size for all groups. Urine was collected in a glass vial placed at the external meatus of the urethra from the start of the infusion until the end of the experiment, when the bladder was emptied by external compression. Animals that did not show a decrease in PV 3 h after the preparation were considered to be non-septic and were excluded from the study. These animals and animals that died before the end of the experiment were replaced with new animals.

Statistical analysis

Statistical comparisons between the groups regarding differences in PV, differences in physiological parameters, and differences in haematocrit were performed with a two-way analysis of variance for repeated measures followed by the Bonferroni post-hoc test. Student's *t*-test for unpaired observations was used for analyses of differences in mean of mean arterial blood pressure, urine production, and changes in PV. Probability values less than 0.05 were considered significant. To achieve a statistical power of 90% with a difference in PV of 6 ml/kg between the bolus and continuous groups, the calculated sample size for each group was 9.3. All data were normally distributed. The results are presented as mean ± SD. We used GraphPad Prism software version 5.0c for Mac OS (GraphPad Software, San Diego, CA, USA).

Results

One animal from the dextran groups died, seven animals from the albumin groups died, and six animals from the sham group died before the end of the experiment. Eight animals were considered to be non-septic and were excluded from the study, as they did not show a decrease in PV 3 h after the preparation.

Physiological data

Sodium (Na⁺), potassium (K⁺), haematocrit (Hct), lactate (Lac), pH, PaCO₂, and PaO₂ data from arterial blood samples are summarised in Table 1.

At the end of the experiment, there was a significantly lower haematocrit in all groups that received

fluids than in the sham group ($P < 0.05$). There was also a significantly lower haematocrit in the dextran continuous group than in the dextran bolus group ($P < 0.05$), while the difference in haematocrit between the albumin continuous and bolus groups did not reach the stipulated level of significance. Potassium concentrations were higher at the end of the experiment in all groups compared with baseline. Lactate was significantly lower in the albumin groups and in the continuous dextran group than in the sham group at the end of the experiment ($P < 0.01$). There was also a significantly lower lactate in the dextran continuous group than in the dextran bolus group at the same time point ($P < 0.05$).

The remaining physiological parameters showed great individual variations, both within and between groups, and no specific conclusions could be drawn from the data obtained (Table 1).

The difference between mean of mean arterial blood pressure (MAP) during a 30-min period after the start of the infusion and the mean of MAP during a 30-min period just before the start of the infusion for the bolus and continuous groups for both solutions analysed are presented in Fig. 4. There was a significantly higher MAP in the bolus group than in the continuous groups for both fluids analysed ($P < 0.05$). There were no significant differences in MAP at the end of the experiments between the continuous and bolus groups in neither the albumin or the dextran group.

PV

PV values at baseline (PV₁), 3 h after the preparation (PV₂), and 3 h later at the end of the experiment (PV₃) for the treatment groups and for the sham group are shown in Fig. 2. PV₂ was significantly lower than PV₁ in all groups ($P < 0.01$). PV values at the end of the experiment (PV₃) were 47.2 ± 5.3 ml/kg and 36.5 ± 3.9 ml/kg ($P < 0.001$) for 6% dextran 70 and 47.3 ± 7.5 ml/kg and 39.7 ± 2.8 ml/kg ($P < 0.01$) for 5% albumin in the continuous groups and bolus groups, respectively. PV for the sham group at the end of the experiment was 29.9 ± 3.3 ml/kg.

The differences between PV₃ and PV₂ for all groups are shown in Fig. 3. There was a significant difference between the continuous group and the bolus group for both fluids analysed ($P < 0.01$). There was significant difference between the dextran groups and the continuous albumin group, and the sham group ($P < 0.05$).

Table 1

Physiological parameters. Data (mean \pm SD) for sodium (Na⁺), potassium (K⁺), haematocrite (Hct), lactate (Lac), pH, arterial partial pressure of carbon dioxide (PaCO₂), arterial partial pressure of oxygen (PaO₂).

	Na ⁺ , mmol/l	K ⁺ , mmol/l	Hct, %	Lac, mmol/l	pH	Paco ₂ , kPa	Pao ₂ , kPa
6% dextran 70							
15 min (n = 10)							
Baseline	134 \pm 1	4.7 \pm 0.5	42 \pm 2	1.9 \pm 0.4	7.39 \pm 0.03	4.8 \pm 0.3	8.9 \pm 1.9
3 h after prep	134 \pm 1	6.4 \pm 0.9	47 \pm 3	4.4 \pm 1.5	7.34 \pm 0.10	4.8 \pm 0.8	10.5 \pm 1.1
3 h after start of inf	132 \pm 2	8.4 \pm 0.6	41 \pm 3*	8.6 \pm 3.3	7.27 \pm 0.08	3.9 \pm 0.8	11.9 \pm 1.0
3 h (n = 10)							
Baseline	135 \pm 2	4.5 \pm 0.6	42 \pm 2	2.2 \pm 0.7	7.40 \pm 0.07	4.9 \pm 0.5	9.0 \pm 1.5
3 h after prep	134 \pm 2	5.6 \pm 0.7	44 \pm 2	3.5 \pm 1.6	7.43 \pm 0.09	4.7 \pm 0.6	9.9 \pm 1.2
3 h after start of inf	135 \pm 2	7.0 \pm 1.2	37 \pm 3*,†	5.8 \pm 1.8†,§	7.39 \pm 0.08	4.4 \pm 0.6	11.2 \pm 1.3
5% albumin							
15 min (n = 10)							
Baseline	135 \pm 3	4.4 \pm 0.6	40 \pm 3	2.2 \pm 0.7	7.36 \pm 0.08	4.6 \pm 0.3	12.1 \pm 0.9
3 h after prep	135 \pm 2	6.1 \pm 1.4	41 \pm 9	4.6 \pm 2.0	7.37 \pm 0.10	3.7 \pm 0.5	13.4 \pm 0.7
3 h after start of inf	134 \pm 3	7.5 \pm 1.5	41 \pm 4‡	6.3 \pm 2.1§	7.32 \pm 0.11	3.3 \pm 0.4	13.9 \pm 0.8
3 h (n = 10)							
Baseline	135 \pm 2	4.8 \pm 0.7	40 \pm 3	2.2 \pm 0.7	7.34 \pm 0.07	5.3 \pm 0.6	11.6 \pm 1.2
3 h after prep	134 \pm 3	6.4 \pm 1.4	45 \pm 4	5.4 \pm 3.9	7.36 \pm 0.19	3.7 \pm 0.6	12.8 \pm 1.8
3 h after start of inf	134 \pm 2	7.7 \pm 1.0	39 \pm 4§	5.7 \pm 1.4§	7.39 \pm 0.06	3.8 \pm 0.4	12.9 \pm 1.3
Sham (n = 10)							
Baseline	135 \pm 2	4.8 \pm 0.8	42 \pm 3	1.9 \pm 0.6	7.38 \pm 0.06	5.3 \pm 0.8	10.4 \pm 1.7
3 h after prep	135 \pm 2	5.8 \pm 0.8	46 \pm 4	3.2 \pm 1.4	7.41 \pm 0.09	4.1 \pm 0.4	12.0 \pm 1.5
3 h after start of inf	131 \pm 4	8.0 \pm 1.4	47 \pm 3	9.2 \pm 4.3	7.25 \pm 0.15	3.2 \pm 0.6	13.5 \pm 1.6

*P < 0.001 compared with the sham group.

†P < 0.05 compared with the dextran bolus group (15 min).

‡P < 0.05 compared with the sham group.

§P < 0.01 compared with the sham group.

inf, infusion; prep, preparation.

Urine production

Urine production from the start of infusion to the end of the experiment were very low (less than 0.5 ml/kg/3 h) for all groups, and there were no significant differences between the groups.

Discussion

This study in guinea pigs showed that the PV-expanding effects of a fixed volume of 6% dextran 70 and 5% human albumin were greater when the fluid was given at a slow infusion rate than at a fast one. PV for all treatment groups differed significantly from that of the sham group at the end of the experiment, regardless of infusion rate.

Compared with the sham group, the lower haematocrit at the end of the experiment for all treatment groups was in accordance with the greater PV.

The ¹²⁵I-dilution technique for measurement of PV is a well-established and reliable technique. There might have been overestimation of PV because of transcapillary escape of radioactive albumin during the 5-min period between injection of the tracer and collection of the blood sample. However, as described in detail previously,^{12,16,21,24}

potential sources of error with this method are small, and as they would have been of the same magnitude in all groups, they would have little or no influence on the conclusions made.

That there was a larger PV-expanding effect of the fluids analysed when given at a slow infusion rate rather than a fast one is in accordance with the results from our previous study in rats.¹⁶

Possible explanations for our results of a better PV-expanding effect with a slow infusion are given in detail in our previous publication.¹⁶ Briefly, as shown in both this study and our previous study, a bolus infusion causes a transient increase in systemic arterial pressure (Fig. 4) and a subsequent transient increase in capillary pressure.¹² Furthermore, inhibition of the baroreceptor reflex will reduce the precapillary resistance, also resulting in an increase in capillary pressure.²⁵ As a fast infusion causes a transient dilution of red blood cells, seen from a decrease in haematocrit in the bolus groups during the first time period after the start of infusion,¹⁶ exposure of the extended plasma column between two erythrocytes to the pores in the capillary membrane will increase. All of the above can be expected to lead to an increase in transcapillary fluid loss.

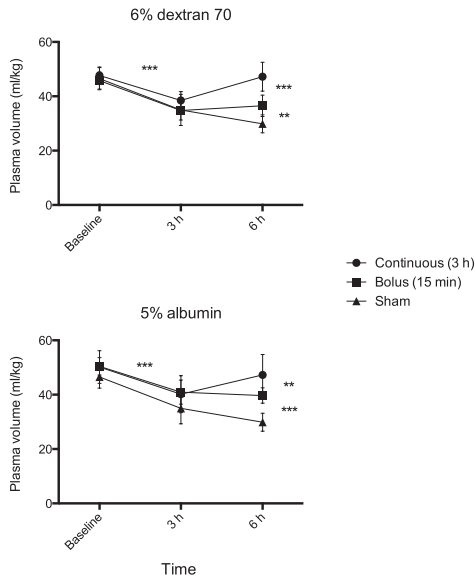


Fig. 2. Plasma volumes. PV at baseline (PV₁), 3 h after the preparation and just before the start of infusion (PV₂), and at the end of the experiment (PV₃). The animals were given a continuous (3 h) infusion or a bolus (15 min) infusion of 6% dextran 70 (n = 10 per group) and 5% albumin (n = 10 per group). Corresponding data for the sham group (n = 10) are also shown. There was a significant difference between PV₁ and PV₂ in all groups. There was a significant difference in PV₃ between the continuous group and the bolus group for both solutions, and between all treated groups and the sham group. Two-way analysis of variance for repeated measures followed by Bonferroni post hoc-test was used for the statistical analyses. (**P < 0.01; ***P < 0.001).

A larger increase in blood pressure may also result in an increased release of ANP and BNP. However, this did not influence the results through an increase in urine production, as urine production was very low in these experiments relative to the volumes infused. We cannot, however, exclude the possibility that the permeability-increasing effect of ANP and BNP contributed to the loss of PV.^{2,14,15}

The lower lactate in the dextran continuous group and the albumin groups is in agreement with the larger PV at the end of the experiments, which may be interpreted as improved microcirculation. We cannot explain why this agreement was not seen for the dextran bolus group. The infused fluids contain no potassium, and therefore, the higher potassium

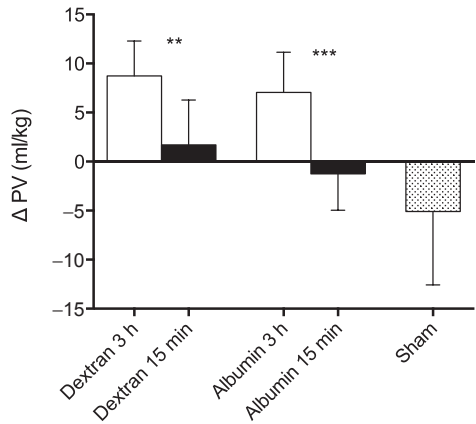


Fig. 3. Change in plasma volume. Comparison of change in plasma volume from the start of infusion (PV₂) to the end of the experiment (PV₃) for the continuous (3 h) groups and the bolus (15 min) groups. There was a significant difference between the continuous (3 h) groups and the bolus (15 min) groups for both fluids analysed. There was also significant difference between both dextran groups and the continuous albumin group, and the sham group (P < 0.05). Student's t-test for unpaired observations was used for the statistical analyses (**P < 0.01; ***P < 0.001).

concentrations at the end of the experiments in all groups are probably caused by sepsis/SIRS-induced cell destruction.

In the present study, the mortality rate was much greater than in our previous study on the rat,¹⁶ except for the dextran group. It appears that guinea pigs are more sensitive to the caecal ligation and incision procedure than the rat, resulting in a more aggressive systemic response. We cannot tell whether the lower mortality rate in the dextran group was a beneficial effect of dextran, or if the higher mortality rate in the albumin group was caused by negative effects of albumin. We cannot exclude that the use of human albumin might cause negative immunological reactions in guinea pigs.

One limitation in the present study is that the experiments were performed on the guinea pig and therefore cannot be directly transferred to man. Further, there may be some variations in the degree of sepsis and thus in degree of increase in microvascular permeability between animals, even if the surgical technique to induce sepsis was carefully standardised. Also, this study is performed in a standardised animal model of the early stage of severe sepsis, which does not reflect the case mix and variability of sepsis in clinical practice.

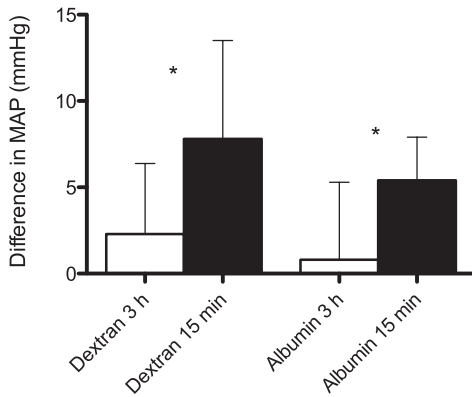


Fig. 4. Difference in blood pressure. Mean of mean arterial blood pressure (MAP) during a 30-min period just after the start of infusion subtracted with that 30 min just before the start of infusion for the continuous (3 h) and the bolus (15 min) groups for the 2 solutions analysed. There was a significantly higher MAP in the bolus group than in the continuous groups for both fluids analysed. Student's t-test for unpaired observations was used for the statistical analyses (* $P < 0.05$).

In conclusion, the present study in a guinea pig sepsis model showed that the PV-expanding effect of 6% dextran 70 is greater 3 h after the start of infusion when given with a slow infusion rate. It also confirms our previous results in a rat sepsis model that the PV-expanding effect of a fixed volume of 5% albumin is greater when given with a slow infusion rate.

Acknowledgements

This study was supported by the Swedish Research Council, Stockholm, Sweden (11581), and Region Skåne (ALF 18401). We thank Helene Axelberg for skilled technical assistance.

Conflicts of interest: The authors have no conflicts of interest.

References

- Haskell A, Nadel ER, Stachenfeld NS, Nagashima K, Mack GW. Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 1997; 83: 407–13.
- Huxley VH, Scallan J. Lymphatic fluid: exchange mechanisms and regulation. *J Physiol* 2011; 589: 2935–43.
- Fleck A, Raines G, Hawker F, Trotter J, Wallace PJ, Ledingham IM, Calman KC. Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985; 1: 781–4.
- Groeneveld AB, Teule GJ, Bronsveld W, van den Bos GC, Thijs LG. Increased systemic microvascular albumin flux in septic shock. *Intensive Care Med* 1987; 13: 140–2.
- Koller ME, Reed RK. Increased negativity of interstitial fluid pressure in rat trachea in dextran anaphylaxis. *J Appl Physiol* 1992; 72: 53–7.
- Wiig H, Rubin K, Reed RK. New and active role of the interstitium in control of interstitial fluid pressure: potential therapeutic consequences. *Acta Anaesthesiol Scand* 2003; 47: 111–21.
- Nolan J. Fluid replacement. *Br Med Bull* 1999; 55: 821–43.
- Marx G. Fluid therapy in sepsis with capillary leakage. *Eur J Anaesthesiol* 2003; 20: 429–42.
- Rippe B, Haraldsson B. Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 1994; 74: 163–219.
- Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. *Am J Physiol* 1986; 250: H706–10.
- Parving HP, Gyntelberg F. Transcapillary escape rate of albumin and plasma volume in essential hypertension. *Circ Res* 1973; 32: 643–51.
- Dubniks M, Persson J, Grande PO. Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 2007; 33: 2192–8.
- Nygren A, Redfors B, Thoren A, Ricksten SE. Norepinephrine causes a pressure-dependent plasma volume decrease in clinical vasodilatory shock. *Acta Anaesthesiol Scand* 2010; 54: 814–20.
- McKay MK, Huxley VH. ANP increases capillary permeability to protein independent of perfusate protein composition. *Am J Physiol* 1995; 268: H1139–48.
- Woodard GE, Rosado JA. Natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol* 2008; 268: 59–93.
- Bark BP, Persson J, Grande PO. Importance of the infusion rate for the plasma expanding effect of 5% albumin, 6% HES 130/0.4, 4% gelatin, and 0.9% NaCl in the septic rat. *Crit Care Med* 2013; 41: 857–66.
- Voorhees AB, Baker HJ, Pulaski EJ. Reactions of albino rats to injections of dextran. *Proc Soc Exp Biol Med* 1951; 76: 254–6.
- Dubniks M, Persson J, Grande PO. Comparison of the plasma volume-expanding effects of 6% dextran 70, 5% albumin, and 6% HES 130/0.4 after hemorrhage in the guinea pig. *J Trauma* 2009; 67: 1200–4.
- Zdolsek HJ, Vegfors M, Lindahl TL, Tornquist T, Bortnik P, Hahn RG. Hydroxyethyl starches and dextran during hip replacement surgery: effects on blood volume and coagulation. *Acta Anaesthesiol Scand* 2011; 55: 677–85.
- Karanko MS. Effects of three colloid solutions on plasma volume and hemodynamics after coronary bypass surgery. *Crit Care Med* 1987; 15: 1015–22.
- Persson J, Grande PO. Plasma volume expansion and transcapillary fluid exchange in skeletal muscle of albumin, dextran, gelatin, hydroxyethyl starch, and saline after trauma in the cat. *Crit Care Med* 2006; 34: 2456–62.
- Reinhart K, Perner A, Sprung CL, Jaeschke R, Schortgen F, Johan Groeneveld AB, Beale R, Hartog CS. Consensus statement of the ESICM task force on colloid volume therapy in critically ill patients. *Intensive Care Med* 2012; 38: 368–83.
- Scheiermann P, Hoegl S, Revermann M, Ahluwalia D, Zander J, Boost KA, Nguyen T, Zwissler B, Muhl H, Hofstetter C. Cecal ligation and incision: an acute onset model of severe sepsis in rats. *J Surg Res* 2009; 151: 132–7.
- Margaron MP, Soni NC. Plasma volume measurement in septic patients using an albumin dilution technique: comparison with the standard radio-labelled albumin method. *Intensive Care Med* 2005; 31: 289–95.

B. P. Bark and P.-O. Grände

25. Hall JE. Nervous regulation of the circulation and rapid control of arterial pressure, In: Hall JE ed. Guyton and Hall: Textbook of medical physiology, 12th edn. Philadelphia: Elsevier, 2011: 201–11.

Address:
Björn Bark
Department of Anaesthesia and Intensive Care
Lund University Hospital
SE-221 85 Lund
Sweden
e-mail: bjorn.bark@med.lu.se

Paper III

PLASMA VOLUME EXPANSION BY 0.9% NaCl DURING SEPSIS/SYSTEMIC INFLAMMATORY RESPONSE SYNDROME, AFTER HEMORRHAGE, AND DURING A NORMAL STATE

Björn P. Bark,* Carl M. Öberg,[†] and Per-Olof Grände*

Departments of *Anesthesia and Intensive Care and [†]Nephrology, Lund University and Lund University Hospital, Lund, Sweden

Received 6 Mar 2013; first review completed 25 Mar 2013; accepted in final form 23 Apr 2013

ABSTRACT—Objective: The objective of this study was to determine the degree of plasma volume expansion by 0.9% NaCl in relation to the infused volume, in sepsis/systemic inflammatory response syndrome (SIRS), after a standardized hemorrhage, and in a normal condition. **Design:** Prospective, randomized animal study. **Setting:** The study was performed at a university hospital laboratory. **Subjects:** Thirty anesthetized adult male rats were included in the study. **Interventions:** The study was performed in three groups: a sepsis/SIRS group (the S group), in which sepsis/SIRS was induced by cecal ligation and incision; a hemorrhage group (the H group), in which the rats were left without intervention for 4 h and bled 8 mL/kg thereafter; and a group that was left without intervention (the N group). Then, 4 h after baseline, all three groups were given an infusion of 0.9% NaCl (32 mL/kg) for 15 min. Baseline was defined as the time point when the surgical preparation was finished. **Measurements and Main Results:** Plasma volumes were measured using ¹²⁵I-albumin dilution technique at baseline, after 4 h, and 20 min after the end of infusion. The plasma volume-expanding effect 20 min after end of infusion was 0.6% ± 2.9% in the S group, 20% ± 6.4% in the H group, and 12% ± 11% in the N group, compared with just before start of infusion. **Conclusions:** The present study in rats showed that the plasma volume-expanding effect after an infusion of 0.9% NaCl was smaller in a septic/SIRS state than after hemorrhage and in a normal state. This indicates that the plasma volume-expanding effect of a crystalloid is dependent on pathophysiologic changes in sepsis/SIRS.

KEYWORDS—Plasma volume expander, plasma volume, crystalloid, hypovolemia, hypervolemia, sepsis, SIRS, normovolemia, hemorrhage

INTRODUCTION

As the capillary membrane is freely permeable to a crystalloid solution, the plasma volume (PV)-expanding effect is traditionally believed to be determined by the ratio between the volume of plasma and the total extracellular volume of the body, a ratio thought to be in the range 20% to 25% (1–3). Substitution of a reduced PV with crystalloids would therefore require a four to five times larger volume infused than the PV expansion needed to achieve normovolemia (2, 4–6). The use of crystalloids will therefore mean accumulation of interstitial fluid (7, 8). Whether the PV-expanding effect of a crystalloid is affected by pathophysiologic changes in systemic inflammation (i.e., microvascular permeability) is still unclear (9).

A recent study from our group on the septic rat did not support the hypothesis that 20% to 25% of the infused crystalloid is distributed intravascularly in the inflammatory state, as the PV-expanding effect of 0.9% NaCl (48 mL/kg) was insignificant 3 h after the infusion (10). The results could not be explained by an increase in urinary production. The poor PV-expanding effect may partly be explained by a lower PV and a larger interstitial volume in sepsis/systemic inflammatory response syndrome (SIRS), because of increased transcapillary leakage of plasma, meaning that the ratio between PV and extracellular volume is

smaller than that in the normal condition. However, it is unreasonable to believe that this mechanism alone would explain the unexpectedly poor PV-expanding effect of the infusion. It may be that other pathophysiologic changes in sepsis/SIRS also influence the PV-expanding effect for a crystalloid. The importance of the pathophysiologic changes in systemic inflammation for the loss of PV during the infusion of 0.9% NaCl could be investigated by comparing the PV expansion during a sepsis/SIRS condition with that after an acute hemorrhage, which is characterized by hypovolemia with preserved microvascular permeability. The rather long period of 3 h from start of infusion up to evaluation of PV, used in the previous study, may have contributed to the poor result. Therefore, to evaluate the initial PV-expanding effect of 0.9% NaCl, PV should be analyzed as soon as it has been fully distributed to the whole extracellular space.

Based on these considerations, we investigated the hypothesis that the degree of PV expansion of an infusion of a fixed volume of 0.9% NaCl 20 min after end of the infusion would be dependent on pathophysiologic changes in sepsis/SIRS. We also investigated the PV-expanding effect in normal permeability from both a hypovolemic and a normovolemic state. The analyses were performed during sepsis/SIRS, at normal permeability after a standardized hemorrhage, and at normal permeability under normovolemia.

MATERIALS AND METHODS

Anesthesia and setup

The study was approved by the Ethical Committee for Animal Research, Lund University, Sweden (application M180-10), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats (n = 30) weighing

Address reprint requests to Björn P. Bark, MD, Department of Anesthesia and Intensive Care, Lund University Hospital, SE-221 85 Lund, Sweden. E-mail: bjorn.bark@med.lu.se.

This study was supported by the Swedish Research Council, Stockholm, Sweden (11581), and Region Skåne (ALF 18401).

There are no potential conflicts of interest.

DOI: 10.1097/SHK.0b013e3182986a62

Copyright © 2013 by the Shock Society

356 ± 16 g (mean ± SD) were used. The animals were anesthetized in a covered glass container with a continuous supply of 5% isoflurane in air (Forene® 100%; Abbot Scandinavia AB, Solna, Sweden). After induction of anesthesia, they were removed from the container, and anesthesia was maintained with 1.5% to 1.8% isoflurane in air, first using a mask until tracheostomy was completed. Ventilation was performed in a volume-controlled mode by a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy) using a positive end-expiratory pressure of 4 cmH₂O. End-tidal P_{CO}₂ was monitored continuously and kept between 4.9 and 5.5 kPa (Capstar-100; CWE, Ardmore, Pa). Body temperature was measured rectally and kept at 37.1°C to 37.3°C via a feedback-controlled heating pad. The left femoral artery was cannulated to record arterial blood pressure and to obtain blood samples for analysis of arterial blood gases, electrolytes, lactate, hematocrit (Hct) (I-STAT; Abbot Point of Care Inc, Abbot Park, Ill), and PVs. The left femoral vein was cannulated and used for infusions and kept open with a continuous infusion of saline at 0.2 μL/min. The right internal jugular vein was cannulated and used for injection of ¹²⁵I-albumin to measure PVs. After the experiments, the animals were killed by intravenous injection of potassium chloride.

Experimental procedure

We used a well-established sepsis/SIRS model in the rat (10–12). A longitudinal midline skin incision over the abdominal wall was performed with diathermia, followed by laparotomy by incision along the linea alba. The cecum was ligated just below the ileocecal valve, and an incision of 1 cm was made to extrude fecal material into the abdominal cavity, thereby inducing sepsis/SIRS. The abdominal wall and the skin were then closed with clips. There was no accidental bleeding during and after the surgical procedure.

Plasma volume

Plasma volume was measured with a well-established technique, which has been shown previously to give reliable and reproducible results (10, 13–16). Plasma volume was determined by measuring the radioactivity in 100 μL of plasma taken 5 min after an intravenous injection of human ¹²⁵I-albumin (0.5 mL) with a known amount of activity. The increase in radioactivity was calculated by subtracting the activity in a blood sample taken just before the injection from that taken 5 min after the injection, thereby allowing adjustment for any remaining radioactivity from previous measurements. To calculate the amount of radioactivity given, the remaining activity in the emptied vial, syringe, and needle used was measured and subtracted from the total activity in the prepared dose. As will be discussed, sources of error are small with the design of the technique used (see Discussion). Free iodine was measured regularly following precipitation with 10% trichloroacetic acid, and it was found to be 1.5% ± 0.6% in the prepared samples. Radioactivity was measured with a gamma counter (Wizard 1480; LKB-Wallace, Turku, Finland).

Protocol

The study involved three different study groups: a septic group (the S group, n = 10), a hemorrhage group (the H group, n = 10), and a normovolemic group (the N group, n = 10). The time scale for each group is shown in Figure 1. Plasma volume was measured at baseline (PV₁) in all groups (just after anesthesia and surgical preparation).

Four hours after induction of sepsis in the S group (i.e., 4 h after the end of the surgical procedure), a time period previously shown to be sufficient for systemic inflammation and plasma leakage to develop (10), a second PV measurement was performed (PV₂) followed by a 15-min infusion of 0.9% NaCl (32 mL/kg, Natriumklorid; Fresenius Kabi, 9 mg/mL). After another 20 min, a length of time that would allow total distribution of the infused NaCl to the whole extracellular volume (14, 17), we performed a final PV measurement (PV₃).

In the H group, the rats were left undisturbed for 4 h, and then a second PV measurement (PV₂) was performed. Then the rats were bled (8 mL/kg) via the femoral artery cannula (bleeding time <2 min). Immediately after bleeding, a 15-min infusion of 0.9% NaCl (32 mL/kg) was given. After another 20 min, we performed the final PV measurement (PV₃).

In the N group, the rats were left undisturbed for 4 h, and then a second PV measurement (PV₂) was performed. Then a 15-min infusion of 0.9% NaCl (32 mL/kg) was given to the normovolemic rats. After another 20 min, the final PV measurement was performed (PV₃).

The infused volume of 0.9% NaCl was chosen, as our previous study (10) showed that the PV loss in this rat model after 3 h was about 6 mL/kg, which, extrapolated to 4 h, would be around 8 mL/kg. To substitute this plasma loss, a four times larger volume of 0.9% NaCl (32 mL/kg) was chosen (2, 4–6). Accordingly, the animals in the H group were bled 8 mL/kg.

Blood samples for measurement of arterial pH, P_{CO}₂, P_{aO}₂, Hct, lactate, sodium, and potassium were taken just before measurement of the PV (Fig. 1). Urine was collected in a glass vial placed at the external meatus of the urethra throughout the whole experiment, and the bladder was emptied by external compression at the end of the experiment.

In Results, changes in PV caused by the taking of blood samples for PV measurements and blood gases, and by the infused volume of ¹²⁵I-albumin, have been taken into account. To illustrate the PV-expanding effect in relation to the given volume, the difference between PV₃ and PV₂ was divided by the infused volume of 0.9% NaCl (32 mL/kg).

Statistical analysis

Statistical comparisons between the groups regarding urine production and PV-expanding effect of the infusion (Fig. 3) were performed with 2-tailed Student *t* test for unpaired observations. Analyses of differences in physiological

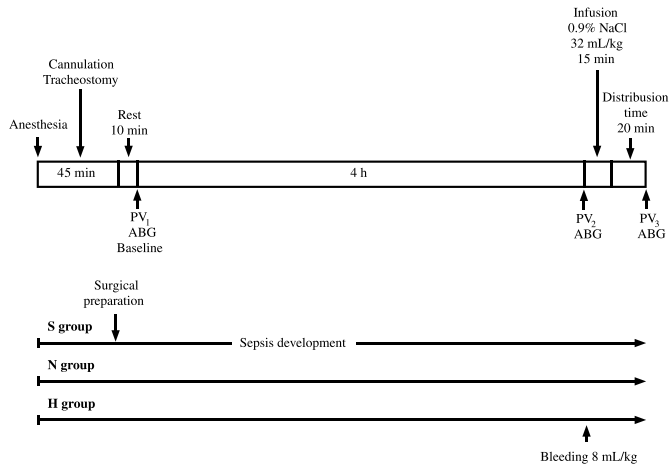


FIG. 1. Time scale of the experimental protocol for the sepsis group (S group), the normovolemic group (N group), and the hemorrhage group (H group). PV₁, PV at baseline; PV₂, PV 4 h after surgical preparation, just before the start of infusion; PV₃, PV at the end of the experiment; ABG, arterial blood sample for analysis of blood gases, Hct, and electrolytes.

TABLE 1. Physiological data

	Na ⁺ , mmol/L	K ⁺ , mmol/L	Hct, %	Lac, mmol/L	pH	Paco ₂ , kPa	PaO ₂ , kPa
S group (n = 10)							
Baseline	136 ± 2	4.8 ± 0.4	42 ± 2	2.5 ± 0.3	7.49 ± 0.03	4.8 ± 0.4	11.3 ± 1.5
4 h	133 ± 2*	6.2 ± 0.5 [†]	50 ± 3*	2.4 ± 0.3	7.44 ± 0.02 [‡]	4.5 ± 0.4	10.8 ± 0.9
20 min After infusion	135 ± 2*	6.1 ± 0.7 [†]	48 ± 4*	1.9 ± 0.7	7.42 ± 0.03 [§]	4.6 ± 0.8	10.9 ± 0.6
H group (n = 10)							
Baseline	136 ± 1	4.7 ± 0.3	43 ± 2	2.1 ± 0.5	7.52 ± 0.03	4.7 ± 0.4	11.6 ± 0.8
4 h	136 ± 1	4.9 ± 0.5	43 ± 2	2.0 ± 0.4	7.49 ± 0.02	4.4 ± 0.4	11.6 ± 1.0
20 min After infusion	138 ± 2	4.8 ± 0.5	35 ± 3	1.5 ± 0.5	7.47 ± 0.02	4.2 ± 0.2	11.8 ± 1.2
N group (n = 10)							
Baseline	136 ± 2	4.8 ± 0.3	43 ± 2	2.3 ± 0.6	7.50 ± 0.05	4.9 ± 0.3	11.0 ± 1.0
4 h	136 ± 2	4.9 ± 0.5	42 ± 3	1.9 ± 0.4	7.48 ± 0.03	4.7 ± 0.5	10.9 ± 1.0
20 min After infusion	138 ± 2	4.5 ± 0.5	39 ± 4	1.5 ± 0.6	7.44 ± 0.02	4.6 ± 0.3	11.0 ± 0.9

Data (mean ± SD) for sodium (Na⁺), potassium (K⁺), Hct, lactate (Lac), pH, Paco₂, and PaO₂.

**P* < 0.001 compared with the H and N groups at the same time point.

[†]*P* < 0.01 compared with the H and N groups at the same time point.

[‡]*P* < 0.05 compared with H and N groups at the same time point.

[§]*P* < 0.05 compared with the H group at the same time point.

^{||}*P* < 0.05 compared with the N group at the same time point.

parameters (Tables 1 and 2) and differences in PVs (Fig. 2) were performed with a 2-way analysis of variance for repeated measures followed by Bonferroni post hoc test. *P* < 0.05 was considered significant. All the data were normally distributed. The results are presented as mean ± SD. GraphPad Prism software version 5.0c for Mac OS was used (GraphPad Software, San Diego, Calif).

RESULTS

Physiological data

Data for pH, PaO₂, Paco₂, lactate (Lac), sodium (Na⁺), potassium (K⁺), and Hct—derived from arterial blood samples taken at baseline, after 4 h, and at the end of the experiment—are summarized in Table 1.

There were no significant differences in any of the parameters analyzed at baseline. PaO₂, Paco₂, and lactate were not significantly different between groups at any time point. pH was lower in the S group than in the N and H groups at 4 h (*P* < 0.05) and lower in the S group than in the H group at the end of the experiment (*P* < 0.05). Sodium was lower in the S group than in the N and H groups at 4 h and at the end of the experiment (*P* < 0.01). Potassium was higher in the S group than in the N and H groups at 4 h and at the end of the experiment (*P* < 0.001). Hematocrit was higher in the S group than in the N and H groups at 4 h and at the end of the experiment (*P* < 0.001). The H group had lower Hct than the N group at the end of the experiment (*P* < 0.01).

TABLE 2. Blood pressure

	Baseline	4 h After surgical prep	20 min After infusion
S group (n = 10)	97 ± 22	90 ± 12	97 ± 15
N group (n = 10)	83 ± 19	82 ± 10	96 ± 12
H group (n = 10)	84 ± 17	83 ± 15	78 ± 25*

Data (mean ± SD) for mean arterial blood pressure (in mmHg) at baseline, 4 h after the surgical preparation, and 20 min after the infusion.

**P* < 0.05 compared with the N and S groups at the same time point.

Data for mean arterial blood pressure at baseline, just before the start of infusion 4 h later, and at the end of the experiment are given in Table 2. There were no differences in mean arterial blood pressure between groups at baseline and at 4 h. After the infusion, there was significantly lower blood pressure in the H group than in the S and N groups (*P* < 0.05).

Plasma volume

Plasma volume at baseline (PV₁), after 4 h (PV₂), and at the end of the experiment 35 min later (PV₃) is shown in Figure 2. There were no significant differences between groups at baseline. Plasma volume in the S group was lower than that in the

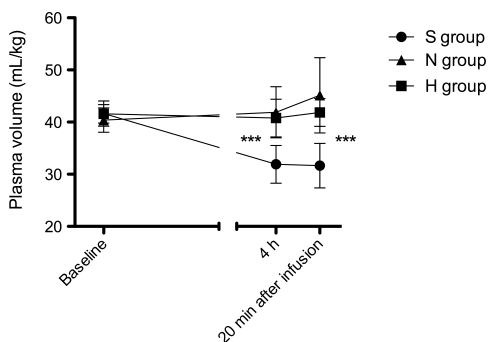


FIG. 2. Plasma volumes for the sepsis group (S group), the normovolemic group (N group), and the hemorrhage group (H group) at baseline, after 4 h, and at end of the experiment. There were significant differences between the S group and the N group, and between the S group and the H group at 4 h and at 20 min after the infusion (*P* < 0.001). Data are mean ± SD. Two-way analysis of variance for repeated measures with Bonferroni post hoc test was used for the statistical analyses (*P* < 0.001). Note that the animals in the H group have been bled 8 mL/kg after the PV measurement at 4 h, explaining the illusory small plasma volume expanding effect in the H group 20 min after infusion.

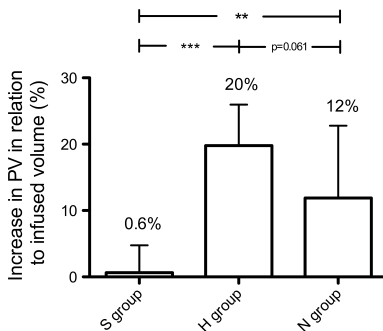


FIG. 3. Increase in PV in relation to the infused volume for the sepsis group (S group), the normovolemic group (N group), and the hemorrhage group (H group). There were significant differences between the S group and the N group, and the S group and the H group. Data are mean \pm SD. Two-tailed Student *t* test for unpaired observations was used for the statistical analyses (** $P < 0.01$, *** $P < 0.001$).

N and H groups after 4 h (PV_2) and at the end of the experiment (PV_3). There was no significant difference in PV between the N and H groups for PV_1 , PV_2 , and PV_3 . In the H group, PV_2 was measured before hemorrhage.

The increases in PV in relation to the infused volume of 0.9% NaCl (32 mL/kg) were $0.6\% \pm 2.9\%$ in the S group, $20\% \pm 6.4\%$ in the H group, and $12\% \pm 11\%$ in the N group (Fig. 3). There was a significant difference between the S and H groups ($P < 0.001$) and between the S and N groups ($P < 0.01$), whereas the difference between the H and N groups did not reach the stipulated significance level ($P = 0.061$).

Urine production

Urine production during the whole experiment was 4.6 ± 1.0 mL/kg in the S group, 4.7 ± 1.4 mL/kg in the H group, and 5.9 ± 2.1 mL/kg in the N group. There were no significant differences in urine production between the groups.

DISCUSSION

The purpose of the present study was to evaluate the initial PV-expanding effect of 0.9% NaCl in normal and in increased microvascular permeability. The analyses were performed during sepsis/SIRS, after hemorrhage, and from a normovolemic condition. The study showed that 20 min after the end of infusion, the PV-expanding effect of 0.9% NaCl (32 mL/kg) was 0.6% of the infused volume in sepsis/SIRS, 20% of the infused volume after hemorrhage, and 12% of the infused volume under normovolemia. The changes in PVs for the three groups were reflected in corresponding changes in Hct, in the sense that there were higher Hct values in the S group than in the N and H groups at 4 h after baseline and at the end of the experiment. However, the lower Hct in the H group than in the N group at the end of the experiment does not correlate to any difference in PV but reflects the loss of red blood cells after hemorrhage.

125 I-albumin dilution is a well-established technique for calculation of PV, but has some potential limitations. Free iodine in the tracer injected is distributed to the whole extracellular space

and will result in overestimation of the calculated PV, which however, must have had only a minor influence on the results of this study, as the proportion of free iodine was small ($1.5\% \pm 0.6\%$). There might also have been overestimation of PV because of transcapillary escape of radioactive albumin during the 5-min period between injection of the tracer and collection of the blood sample, especially in the S group with increased permeability (10, 13, 18). This overestimation would have been small, considering the short period (5 min) between the injection of 125 I-albumin and measurement. Five minutes have previously been shown to be sufficient for complete mixing of the tracer in plasma (13, 14). Remaining radioactivity in the syringe, the vial, and the needle used was subtracted from the radioactivity initially calculated and did not contribute to any error in the PV measurement.

The results from the present study of a small PV-expanding effect of 0.9% NaCl in the S group are in agreement with a previous study from our group (10), in which we analyzed the PV-expanding effect 3 h after the start of infusion, when 0.9% NaCl (48 mL/kg) was given at a fast or slow infusion rate. In that study, irrespective of infusion rate, the PVs did not even differ from the control group that was not given any infusion of fluid. In the present study, the PV-expanding effect was analyzed 20 min after the end of infusion, as this period of time has been shown previously to be sufficient for completion of the distribution of the infused volume to the whole of the extracellular space (14, 17). Given the traditional view that crystalloids are evenly distributed in the whole of the extracellular volume, irrespective of capillary permeability, one might expect that 20% to 25% of the infused volume should stay intravascularly. Our results of a PV-expanding effect of only about 1% of the given volume during sepsis/SIRS largely differ from this traditional view. Below, we have tried to give reasonable physiological explanations for these results.

The ratio between PV and extracellular volume is smaller in sepsis/SIRS than under normal conditions, which may contribute to the small PV-expanding effect of 0.9% NaCl in the present study.

According to the 2-pore theory of transcapillary fluid exchange, the capillary membrane contains small pores that are permeable only to water and small solutes, and the much less abundant large pores, which are also permeable to proteins (19). Proteins are normally lost to the interstitial space through the large pores, mainly by convection. This means that the part of a crystalloid infusion that passes through the large pores will increase the loss of proteins when the infused fluid is distributed from the intravascular space to the interstitial space (20, 21). The loss of proteins by this mechanism will be aggravated in sepsis, because the number of large pores is increased (22), and a relatively greater proportion of the volume infused will pass through the large pores.

An increased loss of proteins to the interstitium through the large pores during sepsis means a reduction in the oncotic transcapillary gradient, resulting in a decrease in the normal absorbing oncotic force (23). The dilution of plasma proteins caused by the crystalloid infusion will also reduce the transcapillary oncotic gradient, further increasing the loss of plasma fluid to the interstitium.

Transcapillary leakage also depends on the hydrostatic pressure. As previously shown (10), a high rate of infusion of 0.9% NaCl would result in a transient increase in arterial and hydrostatic capillary pressure, which would lead to a transient increase in the loss of plasma fluid to the interstitium (15, 23–25). Also, in sepsis, the interstitial pressure may be reduced as a result of the systemic inflammation, which would further increase the PV loss (26).

Each of the mechanisms presented above will lead to increased loss of PV to the interstitium, and all of them together could contribute to the small PV expansion in the S group.

The lower pH in the S group at 4 h and at the end of the experiment is to be expected in a sepsis/SIRS situation. The higher potassium concentrations observed in the S group at 4 h and at the end of the experiment are compatible with a lower pH (Table 1) and sepsis/SIRS-induced cell destruction. The decrease in sodium concentration in the S group during the first 4 h (Table 1) may be secondary to the increase in potassium concentration to maintain electroneutrality.

It is reasonable to believe that microvascular permeability is normal after an acute hemorrhage, and especially in this experiment, in which the hemorrhage was relatively small and the time of hypovolemia short (3–5 min). In contrast to the S group, the PV expansion of 20% of the infused volume of 0.9% NaCl in the H group was closer to the traditional view of the PV-expanding effect of a crystalloid (1–6).

The normal compensatory reabsorption of interstitial fluid after hemorrhage may contribute to the calculated PV expansion of the crystalloid (27). However, the absorbed volumes must be small, as the time from start of hemorrhage until the infusion of 0.9% NaCl has substituted the bled volume (8 mL/kg) is less than 5 min.

Even though there was a normalized PV in the H group, mean arterial blood pressure was lower in this group at the end of the experiment than in the N and S groups (Table 2). This can be explained by the lower blood volume caused by the loss of red blood cells during the hemorrhage and by the lower blood viscosity.

In the N group, the PV-expanding effect was 12% of the infused volume, which was lower than that in the H group ($P = 0.061$). This difference occurred despite the fact that microvascular permeability was normal in both groups. As the infusion in the N group was given from a normovolemic state, it created a hypervolemic situation. The induced hypervolemia should result in an increase in hydrostatic capillary pressure and reduced transcapillary oncotic gradient, resulting in increased transcapillary leakage of fluid to the interstitium (28). Another mechanism could be hypervolemia-induced release of atrial natriuretic peptide and brain natriuretic peptide, resulting in an increased production of urine and an increase in microvascular permeability with subsequent transcapillary leakage of plasma fluid (29, 30).

In the present study, a strict protocol was used to reduce limitations. There may have been some variations in the degree of sepsis between the animals in the S group, even though the surgical technique to induce sepsis was carefully standardized. Also, the experiments were performed on the rat, and the results cannot be directly transferred to man. Furthermore, the

vasodilatory effect of isoflurane might have contributed to an increase in transcapillary leakage in all groups, an effect possibly more pronounced in the S group because of higher capillary permeability. It has also been suggested that isoflurane may contribute to edema formation and tissue damage in sepsis/SIRS, which may have aggravated the PV loss in the S group (31).

In summary, the present study in the rat showed that the PV-expanding effect 20 min after the end of an infusion of 0.9% NaCl differed significantly between sepsis/SIRS and the normal state, and after a short period of hemorrhagic hypovolemia. The PV-expanding effect after hemorrhage was 20%, whereas it was lower (12%) when given to rats in a normal state. The much smaller PV expansion in sepsis/SIRS shows that the PV-expanding effect of a crystalloid is highly dependent on pathophysiologic changes in systemic inflammation. The present study indicates that the use of crystalloids as PV expanders in systemic inflammatory conditions may be much less effective than previously believed.

ACKNOWLEDGMENTS

The authors thank Helene Axelberg for skilled technical assistance.

REFERENCES

- Berne RM, Levy MN (eds.): *Physiology*. St Louis, MO: Mosby-Year Book, pp. 754–758, 1993.
- Nolan J: Fluid replacement. *Br Med Bull* 55:821–843, 1999
- Guyton AC, Hall JE: *Textbook of Medical Physiology*. Philadelphia, PA: Elsevier, pp. 285–301, 2011.
- Lamke LO, Liljedahl SO: Plasma volume changes after infusion of various plasma expanders. *Resuscitation* 5:93–102, 1976
- Reid F, Lobo DN, Williams RN, Rowlands BJ, Allison SP: (Ab)normal saline and physiological Hartmann's solution: a randomized double-blind crossover study. *Clin Sci* 104:17–24, 2003.
- Grathwohl KW, Bruns BJ, LeBrun CJ, Ohno AK, Dillard TA, Cushner HM: Does hemodilution exist? Effects of saline infusion on hematologic parameters in euvoletic subjects. *South Med J* 89:51–55, 1996.
- Rackow EC, Falk JL, Fein IA, Siegel JS, Packman MI, Haupt MT, Kaufman BS, Putnam D: Fluid resuscitation in circulatory shock: a comparison of the cardiorespiratory effects of albumin, hetastarch, and saline solutions in patients with hypovolemic and septic shock. *Crit Care Med* 11:839–850, 1983.
- Falk JL, Rackow EC, Weil MH: Colloid and crystalloid fluid resuscitation. *Acute Care* 10:59–94, 1983.
- Marx G: Fluid therapy in sepsis with capillary leakage. *Eur J Anaesthesiol* 20:429–442, 2003.
- Bark BP, Persson J, Grände PO: Importance of the infusion rate for the plasma expanding effect of 5% albumin, 6% HES 130/0.4, 4% gelatin and 0.9% NaCl in the septic rat. *Crit Care Med* 41:857–866, 2013.
- Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LW 3rd, Bland KI, Chaudry IH: Cecal ligation and puncture. *Shock* 24(Suppl 1):52–57, 2005.
- Scheiermann P, Hoegl S, Revermann M, Ahluwalia D, Zander J, Boost KA, Nguyen T, Zwissler B, Muhl H, Hofstetter C: Cecal ligation and incision: an acute onset model of severe sepsis in rats. *J Surg Res* 151:132–137, 2009.
- Margaron MP, Soni NC: Plasma volume measurement in septic patients using an albumin dilution technique: comparison with the standard radio-labelled albumin method. *Intensive Care Med* 31:289–295, 2005.
- Persson J, Grände PO: Plasma volume expansion and transcapillary fluid exchange in skeletal muscle of albumin, dextran, gelatin, hydroxyethyl starch, and saline after trauma in the rat. *Crit Care Med* 34:2456–2462, 2006.
- Dubniks M, Persson J, Grände PO: Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 33:2192–2198, 2007.
- Bansch P, Lundblad C, Grände PO, Bentzer P: A model for evaluating the effects of blunt skeletal muscle trauma on microvascular permeability and plasma volume in the rat. *Shock* 33:399–404, 2010.
- Hahn RG: Crystalloid fluids. In: Hahn RG (ed.). *Clinical Fluid Therapy in the Perioperative Setting*. Cambridge, UK: Cambridge University Press, pp. 1–10, 2011.
- Valeri CR, Cooper AG, Pivacek LE: Limitations of measuring blood volume with iodinated I 125 serum albumin. *Arch Intern Med* 132:534–538, 1973.
- Rippe B, Haraldsson B: Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 74:163–219, 1994.

20. Mullins RJ, Bell DR: Changes in interstitial volume and masses of albumin and IgG in rabbit skin and skeletal muscle after saline volume loading. *Circ Res* 51:305–313, 1982.
21. Gandhi RR, Bell DR: Influence of saline infusions on transvascular protein transport. *Am J Physiol* 262:H443–H450, 1992.
22. Smith L, Andreasson S, Thoren-Tolling K: Sepsis in sheep reduces pulmonary microvascular sieving capacity. *J Appl Physiol* 62:1422–1429, 1987.
23. Rippe B, Haraldsson B: Fluid and protein fluxes across small and large pores in the microvasculature. Application of two-pore equations. *Acta Physiol Scand* 131:411–428, 1987.
24. Parving HP, Gynzelberg F: Transcapillary escape rate of albumin and plasma volume in essential hypertension. *Circ Res* 32:643–651, 1973.
25. Nygren A, Redfors B, Thoren A, Ricksten SE: Norepinephrine causes a pressure-dependent plasma volume decrease in clinical vasodilatory shock. *Acta Anaesthesiol Scand* 54:814–820, 2010.
26. Koller ME, Reed RK: Increased negativity of interstitial fluid pressure in rat trachea in dextran anaphylaxis. *J Appl Physiol* 72:53–57, 1992.
27. Hillman J, Lundvall J: Hormonal and neurogenic adrenergic control of the fluid transfer from skeletal muscle to blood during hemorrhage. *Acta Physiol Scand* 112:271–280, 1981.
28. Dubniks M, Grande PO: Change in plasma volume from a state of hyper-, normo- or hypovolemia with or without noradrenalin infusion in the rat. *Microvasc Res* 76:75–79, 2008.
29. Woodard GE, Rosado JA: Natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol* 268:59–93, 2008.
30. McKay MK, Huxley VH: ANP increases capillary permeability to protein independent of perfusate protein composition. *Am J Physiol* 268:H1139–H1148, 1995.
31. Soehnlein O, Eriksson S, Hjelmqvist H, Andersson A, Morgelin M, Lindbom L, Rundgren M, Frithiof R: Anesthesia aggravates lung damage and precipitates hypotension in endotoxemic sheep. *Shock* 34:412–419, 2010.



Paper IV

