The Department of Anaesthesia and Intensive Care
Södersjukhuset
KAROLINSKA INSTITUTET
STOCKHOLM, SWEDEN

The Effect of Anaesthesia and Adrenergic Therapy on the Distribution and Elimination of a Crystalloid Solution Studied by Volume Kinetic Analysis

Carl-Arne Ewaldsson

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Intravenous fluid therapy is a mandatory measure during anaesthesia and surgery. It is sometimes combined with adrenergic therapy to maintain haemodynamic stability. It is of great importance to know how the body handles the distribution of fluid in these circumstances in order to minimize the risk of fluid overload. The objective of this thesis was to examine intravenous fluid handling by studying changes in cardiovascular parameters and by using a volume kinetic method to analyze fluid volumes in the body.

Methods: In Paper I we studied whether anaesthesia and surgery affect the sensitivity of the β-2 adrenergic receptor in vivo. 10 patients and 10 volunteers were given an intravenous infusion of epinephrine (50 ng/kg/min). Both cardiovascular and biochemical changes were measured for which the ratios of the areas under the curve were calculated.

In Paper II an animal model was used to evaluate how different adrenergic stimuli affect the distribution and elimination of crystalloid fluid bolus. The impact of three different drugs (dopamine 50 µg/kg/min, isoprenaline 0.1 µg/kg/min and phenylephrine 3 µg/kg/min) on the relationship between plasma dilution and haemodynamics was evaluated. The plasma dilution (an index of volume expansion) was studied using volume kinetic analysis. Paper III studied the initial effect of spinal and general anaesthesia on the distribution and elimination of crystalloid fluid loads. The volume kinetic model was fitted to data from a total of 20 patients who received 20 ml/kg BW of Ringer’s acetate iv. The haemodynamic changes were also recorded. In Paper IV three different intravenous fluid regimens (a bolus of 5ml/kg BW of Ringer’s acetate, 2 ml/kg BW of dextran I and a continuous infusion of Ringer’s acetate 15 ml/kg BW over 40 min) were given during the induction of spinal anaesthesia to prevent arterial hypotension. A total of 75 patients were studied using haemodynamic measurements and volume kinetic analysis. The anaesthetic agent isoflurane has earlier been shown in an animal model to promote extravascular accumulation of crystalloid fluid and, in Paper V, thirty patients undergoing thyroid surgery were randomly anaesthetized with isoflurane or propofol (controls) respectively, to evaluate whether isoflurane also promotes extravascular accumulation in a human model given 25 ml/kg BW of Ringer’s acetate. The volume kinetic model was again used to analyse the distribution and elimination of fluid.

Results: The response to epinephrine (Paper I) measured as the AUC (area under the curve) of P-cAMP divided by the AUC for P-epinephrine, was more pronounced in the patient group than among the controls ($p<0.02$). This was reflected in greater hypokalaemic and hyperglycaemic responses ($p<0.0004$). All results indicate an increased adrenergic response during the first hour of abdominal surgery. All adrenergic drugs (Paper II) changed the baseline of the haemodynamic parameters. Alpha stimulus (phenylephrine) promoted renal excretion of fluid at the expense of fluid distribution to the periphery ($p<0.05$ vs. controls) while beta stimulus (isoprenaline) had the opposite effect. Normal saline caused an increase in atrial, arterial pressures and in cardiac output. These increases showed a linear correlation with the plasma dilution, which was strong for both the phenylephrine and control groups.

The volume kinetics in Paper III showed that the induction of anaesthesia resulted in similar changes in both groups. The elimination of fluid was significantly reduced ($p<0.003$) and the distribution of fluid from a central fluid space to a peripheral one was halved ($p<0.01$). Both types of anaesthesia decreased the mean arterial pressure significantly, and general
anaesthesia to a higher degree than spinal anaesthesia ($p<0.05$). A computer simulation of the obtained kinetic data suggested that a small i.v. fluid load given immediately following the induction of spinal anaesthesia could be more effective in preventing hypotension and this was confirmed in 5 additional patients. In Paper IV there were no differences between the groups and the mean arterial pressure decreased by approximately 26%. The height of the block was the only factor that correlated with the drop in blood pressure. Patient discomfort (nausea, sweating) was more common in the dextran 1 and control groups. Volume kinetic analysis showed that the bolus regimens diluted plasma by 10% and, in the control group, by almost 20%. The dilution-time curve shows no apparent elimination during the bolus experiments, but the patients still had a diuresis. Fluid must therefore have been recruited from the periphery. Plasma dilution in Paper V increased to 30% during the infusion and then remained half as high throughout the experiment. Urinary excretion during the experiment amounted to only 11% of infused volume. The amount of water loss through extracellular retention and evaporation was equal in both groups and amounted to 2.0-2.2 ml/min.

**Conclusion:** Abdominal surgery under general anaesthesia for one hour does not cause desensitization of adrenergic receptors. Anaesthesia causes an accumulation of infused fluid in a central compartment by reducing the tendency for distribution to a peripheral compartment. Urinary excretion is markedly reduced. Both these facts contribute to a prolonged plasma dilution by crystalloid solutions. By adding an adrenergic drug, the distribution and elimination of such a fluid can be changed. Alpha stimuli cause a centralization of fluid and promote diuresis, while beta stimuli have the opposite effect. Isoflurane does not cause a greater extravascular accumulation of fluid than propofol.
2 LIST OF PUBLICATIONS


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III. Ewaldsson C-A, Hahn RG. Volume kinetics of Ringer’s solution during induction of spinal and general anaesthesia. British Journal of Anaesthesia 2001; 87: 406-14


V. Ewaldsson C-A, Hahn RG. Kinetics and extravascular retention of acetated Ringer’s solution during isoflurane or propofol anesthesia for thyroid surgery. Anesthesiology 2005;103:1-10
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>B-Hb</td>
<td>Blood haemoglobin</td>
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<tr>
<td>Bpm</td>
<td>Beats per minute</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>CI</td>
<td>Clearance (used in pharmacokinetics)</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic-adenosin mono phosphate</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>DF</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>EPK</td>
<td>Number of red blood cells per ml</td>
</tr>
<tr>
<td>ECF</td>
<td>Extra cellular fluid</td>
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<tr>
<td>Epinephrine</td>
<td>Adrenaline</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>Iso</td>
<td>Isoprenaline</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>S-K⁺</td>
<td>Serum potassium</td>
</tr>
<tr>
<td>kₑ</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>kᵳ</td>
<td>Distribution rate constant</td>
</tr>
<tr>
<td>kᵦ</td>
<td>Basal diuresis + evaporation</td>
</tr>
<tr>
<td>kᵲ</td>
<td>Infusion rate constant</td>
</tr>
<tr>
<td>kD</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
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<tr>
<td>l</td>
<td>Litres</td>
</tr>
<tr>
<td>LAP</td>
<td>Left atrial pressure</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitres</td>
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<tr>
<td>mmol</td>
<td>Millimoles</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>-------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>MODS</td>
<td>Multiple organ dysfunction syndrome</td>
</tr>
<tr>
<td>MSQ</td>
<td>Mean square error</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
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<tr>
<td>NaCl</td>
<td>Sodium chloride, saline</td>
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<tr>
<td>PAP</td>
<td>Pulmonary artery pressure</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>RAP</td>
<td>Right atrial pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SSQ</td>
<td>Sum of squared errors</td>
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<tr>
<td>TBW</td>
<td>Total body water</td>
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<tr>
<td>t₁/₂</td>
<td>Half-life</td>
</tr>
<tr>
<td>v, v₁, v₂</td>
<td>Expanded body fluid volumes</td>
</tr>
<tr>
<td>V, V₁, V₂</td>
<td>Not expanded body fluid volumes</td>
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5 INTRODUCTION

5.1 ANAESTHESIA

Throughout history, surgical procedures such as amputations, trepanations, cataract operations and sectiones altae have been performed by using herbal extracts, hypnosis, trance, neural pressure and alcohol intoxication as the anaesthetic method.

Around 1540, Paracelsus, a Swiss physician and alchemist, prepared a substance called Aether by Frobenius (diethyl ether) and noticed that animals fell asleep and awakened later without harm. The use of anaesthesia to facilitate surgical procedures is a relatively new science. It started in the USA during the 1840’s by using ether or nitrous oxide for dental procedures. The 16th of October 1846 is the official birth of anaesthesia when William Morton administered ether narcosis for a surgical procedure (Fig.1).

During the following decades the major developments of anaesthesia took place in England and the USA. In England, John Snow, an expert on infectious diseases and the father of epidemiology, developed inhalation techniques, different types of masks and breathing systems. In 1884 the first department of anaesthesia was established in London by Sir Fredric Hewitt, who also wrote the first textbook.

Ralph Guedel in the USA published findings regarding the depth of inhalation anaesthesia at the beginning of the 20th century. Ether was the leading inhalational agent for about 100 years despite the risk of explosion. The search for non-explosive agents continued and, in 1954, Suckling presented halothane, a fluorinated hydrocarbon substance that was to become the most used inhalational agent for a long time. Due to its hepatotoxic effect, the search for other agents continued, and enflurane, isoflurane, sevoflurane and desflurane have all been developed during the past decades [1].
In 1872 the Frenchman Cyprién-Oré is said to have developed intravenous anaesthesia by injecting chloral hydrate in a human. The next step was the introduction of short acting barbiturates during the first half of the 20th century. Lundy in the USA introduced Pentothal into clinical practice in 1934, and it is still being used. The next major step was the introduction of propofol in the 1980s.

Lundy coined the phrase “balanced anaesthesia” to describe the use of an i.v. agent combined with a potent opioid and a muscle relaxant. Curare was the first muscle relaxant to be used in the 1940s and necessitated the use of tracheal intubation to control pulmonary ventilation. Curare was later replaced by synthetic muscle relaxants, such as succinylcholine, pancuronium etc. The first description of oral intubation is from 1878 by MacEwan, a surgeon in Glasgow. O’Dwyer in the USA developed a sealing tube which made artificial ventilation possible.

In 1806 Sertüner isolated morphine from opium. Morphine was difficult to use due to its long-lasting effect, but it was followed by several synthetic analgetic substances such as pethidin, fentanyl, alfentanil, sufentanil, remifentanil, each being more potent and more short-acting than the previous one [1].

The development of anaesthetic practice necessitated the use of separate recovery departments to care for the postoperative patient. Intensive care units were invented during a polio epidemic in Europe during the 1950s [2]. Initially, polio patients with respiratory failure were put into an “iron lung”, but during this epidemic there was a shortage of iron lungs. Instead, patients were tracheotomized and ventilated by positive pressure ventilation [3]. Intensive care departments continued to develop and handle patients with barbiturate intoxication, and from this time highly specialized wards for the treatment of patients with failure of vital functions have emerged.

### 5.2  INTRAVENOUS TREATMENT

In spite of an only rudimentary knowledge of the circulatory system, an attempt at intravenous treatment of a disease was made when blood from three youngsters were given to an anaemic Pope at the end of the 15th century, but it was all in vain as the pope succumbed to his disease [4]. In an animal experiment by Richard Lower in 1667, blood was successfully transfused from one animal to another. Further experimentation was taking place with transfusions from animals to humans, often with fatal results.

Harvey’s studies of the circulation published in 1638 described the circulatory system as two simultaneous parallel working pumps. Thus, he was the first to describe the arterial and
venous sides of the circulation [5]. The discovery of the connections between arteries and veins, the capillaries, was made half a century later by Malpighi [6]. Harvey’s discoveries led Christopher Wren and Daniel Johann Major in 1656 to begin experimenting with intravenous administration of drugs [7]. The first books on intravenous infusions in humans was published by D.J. Major in Germany in 1664 (Chirurgia Infusoria). The instruments used (Fig. 2) were made of animal bladders and quills from birds. In 1853 a French surgeon, C. Pravaz, invented a small graded syringe that made it possible to give exact amounts as an i.v. injection.

In 1818 a woman dying of a postpartum haemorrhage was resuscitated by James Blundell by means of a blood transfusion from her husband. Dr Blundell continued to treat patients with transfusions with a mortality of 50% [8]. A few years later during a cholera epidemic in Great Britain and Ireland an Irish physician, William O’Shaughnessy, noted that the blood of cholera victims was thick and black. He thereby deduced that it was due to a lack of water, dehydration. Together with some colleagues, he performed intravenous infusions of saline solutions to resuscitate cholera patients. Due to the lack of sterility of the solutions and the low tonicity, there was a high degree of complications and this i.v. practice fell into disrepute among contemporary physicians. During this period physicians in the USA experimented with i.v. infused milk, which was later replaced by saline solutions due to severe side effects [9].

Towards the end of the nineteenth century, Sidney Ringer, a cardiovascular physiologist,
examined solutions with different levels of electrolytes during *in vitro* experiments on beating hearts. He discovered that a certain composition of electrolytes made the heart beat for a longer period of time, and this was the birth of Ringer solutions. Lactated or acetated Ringer solution is still the main solution used for volume support and as a rehydrating agent.

During the first half of the 20th century the discovery of blood groups, cross-matching and blood storage possibilities led to safer transfusions. Fractionation of collected blood into separate parts led to the use of concentrated albumin as a colloid solution.

During the major wars of the last century (World War 1, 2, Korea and Vietnam), the methods for resuscitating casualties were improved [10]. Crystalloid solutions were widely used and together with better transportation and a quicker surgical treatment, this lead to reduced mortality rates. Furthermore, the high frequency of urban violence and motor vehicle accidents necessitated the development of standardized trauma care (ATLS) and specialized trauma centers in the USA [11].

### 5.3 FLUID BALANCE

Intravenous infusion of fluids is an integral part of the perioperative management employed to avoid dehydration, maintain an effective circulating blood volume and prevent inadequate tissue perfusion. The choice of i.v. fluid must be guided rationally by an understanding of human physiology, the chemical properties of the fluid and how surgery and anaesthesia affect fluid handling [12].

![Fig. 3](image)

Schematic drawing of fluid compartments in the body. Approximate fluid volumes for a body weight of 75 kg. The volumes of distribution for different i.v. fluids are indicated.
The total body water (TBW) is about 60% (45 l in 75 kg) of the total body weight [13, 14]. Two-thirds of this is intracellular water (30 l) and the remaining third is extracellular water [15, 16]. The extracellular compartment is further divided into intravascular and extravascular compartments. The total intravascular volume makes up about 7% of BW and the plasma water amounts to approximately 3 l. Plasma is a water solution of inorganic ions and proteins (Fig. 3). The endothelium divides the intravascular compartment from the interstitial compartment. The cell membrane separates the interstitium from the intracellular compartment, but the cell membranes are freely permeable to water.

The cells must be in osmotic and solute balance with the surrounding interstitial space to maintain normal cellular function. Intracellular and extracellular fluid compartments have very different solute concentrations owing to an active transport mechanism across the cell membrane [17]. Potassium is actively transported into the cells and sodium out of the cells. Disturbances in solute concentration are quickly corrected when water diffuses from a compartment with lower solute concentration to a compartment with a higher one and thus balances the concentration differences. Only substances that cannot freely cross membranes or substances that require active transport exert this driving force for the water.

The concentration of solutes in the body is called osmolality, which is the inverse of the concentration of water. Tonicity is a term that describes the effect of a fluid on the volume of cells. A fluid that is hypertonic dehydrates the cells and a hypotonic solution causes cellular swelling when water either moves out of or into the cell.

The normal regulatory mechanisms for water balance in the body are dependent on the tonicity of the body fluids. Maintenance of tonicity in the extracellular fluid is primarily regulated by hormones and thirst. An increase in tonicity activates osmo receptors in the hypothalamus which in turn activate the pituitary gland. Increased secretion of antidiuretic hormone (ADH) causes the kidneys to reabsorb more water from the tubules. Hypertonicity also leads to thirst and an increased water intake. Hypotonicity causes the opposite reactions, which lead to an increase in the renal elimination of water [18].

The amount of sodium is the most important determinant of the volume of the extravascular compartment. Stretch receptors in the atria of the heart respond to the varying wall-tension of the cardiac atria due to changes in intravascular volume. Hypovolaemia is a powerful stimulus for activation of the compensatory mechanisms [19]. Increased secretion of ADH is followed by thirst and renal water reabsorption.
Hypovolaemia also triggers neurohumoral responses by activating the sympathetic nervous system. The release of catecholamines increases the rate and contractility of the heart and divert the blood flow from peripheral organs to more vital organs. Extravascular fluid is mobilized through changes in precapillary sphincter contraction, which favours water entry into the bloodstream by reducing the hydrostatic pressure in the capillaries [20]. The plasma concentration of glucose is raised due to catecholamine stimulation and thus adds a hyperosmolar mobilization of water from the interstitial compartment. The Starling equation describes the movement of fluid across the capillary endothelium [21].

\[ J_v = [(P_c - P_i) - \sigma (\pi_c - \pi_i)] \]

\( J_v \) is the fluid flux and \( P \) indicates hydrostatic pressure in the capillaries and the interstitium. \( \pi \) indicates oncotic pressures in the capillaries and the interstitium and \( \sigma \) is the reflection coefficient, a mathematical expression of the permeability of a membrane to any substance. In the healthy state the net fluid flux is from the capillaries to the interstitium and is removed from there by the lymphatic system [22-24].

The surgical condition can result in an altered fluid distribution. Patients can be dehydrated because of a decreased intake of water and electrolytes (fasting, nausea, anorexia etc.) or increased losses due to vomiting, diarrhea, ileus or high temperature [25].

All anaesthetic techniques cause vasodilatation and thus a relative hypovolemia and they also have a negative inotropic effect with a reduction of the cardiac output. Fluid shifts between compartments may also reduce the circulating blood volume, with losses into the interstitium due to increased permeability, losses into the lumen of the gut, etc [26, 27].

An increased capillary permeability is the result of the inflammatory process caused by sepsis, anaphylactic reactions, trauma/shock and major surgical procedures [28]. Colloid molecules leave the capillaries through the endothelium and thereby reduce the colloid volume-effect in the plasma water and increase the oncotic pressure in the interstitium. These changes increase the fluid flux into the interstitial space and favour oedema. Inflammation can also increase fluid losses into spaces that normally should not contain any significant amount of fluid, such as the pleural and abdominal cavities. This fluid originates from the interstitial fluid, but has to be replaced from other compartments. These fluid losses have been termed “third space losses” since the 1960s [29].
I.v. solutions used clinically can be divided into crystalloid and colloid solutions. Crystalloid solutions consist of small inorganic ions dissolved in water. Solutions may be hypo-, iso- or hypertonic with respect to plasma. Isotonic saline 0.9% or balanced solutions (acetated or lactated Ringer solution) are the most commonly used ones. The risk in using normal saline-based solutions is the development of hyperchloraemic metabolic acidosis due to the high chloride load. Glucose solutions are either isotonic (5%) or hypertonic (10-50%). The glucose content in 5% glucose will be rapidly metabolized and the remaining water will be distributed through the body to treat simple dehydration. Hypertonic glucose solutions are used mainly for nutrition.

Colloid solutions are divided into synthetic or natural ones [30]. Colloids can be synthesised from a variety of sources. Gelatins are hydrolysed from bovine collagen, dextran is biosynthesized from sucrose, and starches (HES) are derived from maize. These synthetic colloids are heterogeneous and the plasma volume effect is therefore diverse. The duration of the volume effect depends on molecule size and the rate of molecule loss from the circulation, where gelatins have a short plasma half-life and dextran and HES have a longer half-life. Albumin solution is derived from human plasma and is more expensive than the synthetic colloids.

5.4 PHARMACOKINETIC AND VOLUME KINETIC ANALYSIS

A basic knowledge of pharmacokinetic principles is important to understand the mathematical model used in this Thesis. Pharmacokinetics is a mathematical approach describing how the body handles a drug. The concentration of the drug, \( C \), is the main input variable. The body is described as consisting of one or more fictive compartments with permeable walls through which the drug can be transported at velocities which can be estimated.

The apparent volume of distribution \( (V_d) \) is a factor in determining the concentration of a drug:

\[
V_d = \frac{Q}{C}
\]

\( V_d \) = the volume of distribution, \( Q \) = the amount of drug given, \( C \) = the concentration of the drug.

The volume of distribution is not equivalent to any defined anatomical compartment. \( V_d \) is a fictive quantity as it indicates the volume of distribution before
any of the administered drug is eliminated. The calculation of $V_d$ has to be performed indirectly by extrapolation of measurements of the plasma concentration over time.

Drugs with a small $V_d$ remain in the plasma volume due to a high degree of protein binding. Drugs that have a high lipid solubility will easily pass across cell membranes and give a lower plasma concentration and thus a larger $V_d$ [31].

Elimination of drugs from the body is mostly dependent on renal or hepato biliary mechanisms. Elimination of a drug in the simplest model, i.e. the one-compartment model, and can be described as concentration-dependent. The higher the concentration, the faster the elimination. This is called a first-order elimination, which governs the elimination of most drugs. For some drugs, the elimination rate is limited by the maximal capacity of the elimination process and not by the concentration of the drug. At drug concentrations exceeding the maximum capacity of an enzyme, the elimination rate is constant and is said to be by zero-order. An example of zero-order elimination is the degradation of ethanol by the alcohol dehydrogenase in the hepatocytes.

The concentration-time curve (Fig. 4) for a drug that is eliminated by a first-order process is an exponential function that is described mathematically by the simple wash-out exponential function.

$$C_t = C_0 e^{-kt}$$

($C_0$ is the concentration at time zero, $C_t$ at time $t$ and $e$ is the base for the natural logarithmic system and $k$ is the rate constant and $t$ is the time).

The natural logarithm of both sides gives a new equation that describes a straight line.

$$\ln C_t = \ln C_0 - kt$$

Where the rate-constant $k$ is the same as the total clearance divided by the volume of distribution:

$$k = Cl / V_d$$

The elimination rate constant $k$ is the same as the slope of the semi logarithmic concentration-time curve for a drug [31].
The half-life \( t_{1/2} \) is a common designation to describe the elimination of a drug and, for a *first-order* elimination the half-life remains constant. Using the above equations:

\[
t_{1/2} = \frac{\ln 2}{k} = 0.693 \frac{k}{Cl} = 0.693 \frac{V_d}{Cl}
\]

The model most used to describe how the body handles a drug is the open two-compartment model. In this model it takes some time for the drug to distribute throughout \( V_d (V_1 + V_2 \) in Fig. 5)

In this model a drug is administered \((k_a)\) into a central compartment \((V_1)\) that consists of plasma and the well-perfused organs and thus gives rise to a central concentration \((C_1)\). From the central compartment, the drug is further distributed \((k_{12})\) to a peripheral compartment \((V_2)\) consisting of less well-perfused organs. Distribution to the periphery \((k_{12})\) continues until equilibrium has been reached. Elimination \((k_e)\) takes place from the central compartment and as the concentration here decreases redistribution \((k_{21})\) from the periphery to the central compartment increases [31].

In this model the concentration-time plot is governed by a fast phase \((\alpha)\) and a slower phase \((\beta)\). In the open two-compartment model data cannot be fitted to a single exponential curve but instead the curve can be described by the sum of two exponentials (Fig. 6).

The change in concentration in the central compartment can be described as

\[
dC_1/dt = -(k_{12}+k_e) + k_{21}C_2
\]

and accordingly the change in concentration for the peripheral compartment as

\[
dC_2/dt = k_{12}C_1 - k_{21}C_2
\]

These differential equations form the basis for the analysis of the two-compartment model. Curves with a continuous decrease over time can be described as the sum of two exponentials.
\[ C_t = Ae^{-\alpha t} - Be^{-\beta t} \]

A and B are the extrapolated drug concentrations at time zero. Alpha (\(\alpha\)) is the slope for the initial fast distribution phase and beta (\(\beta\)) is the slope for the slower elimination phase.

If pharmacokinetic principles are applied to the effect of infused intravenous fluids we arrive at a way of describing the distribution and elimination of fluids, the **volume kinetic analysis**.

This has been validated by several studies [32-37] [38] and theses [39-41]. This method takes into account the dynamics of the fluid, including a continuous elimination. Volume kinetic analysis is not dependent on the administration of a dye or a radioactive tracer, or the time required for equilibration of the dye to measure the effect of a given i.v. fluid.

In volume kinetics the disposition of an infused fluid is indicated as a dilution of the plasma as compared to drug concentrations in pharmacokinetic analysis. Serial measurements of the haemoglobin concentration and the number of red blood cells per millilitre are used as markers of dilution. The curves below (Fig. 7) show a typical pattern for distribution and elimination of an i.v. fluid at the same time. These dilution curves over time describe the initial intravenous distribution of fluid in a central body fluid space, the elimination from the same fluid space (one-volume model), and also the distribution to and subsequent dilution of a peripheral body fluid space (two-volume model).
Volume kinetic analysis is a mathematical tool used to describe the whole body distribution and elimination of an intravenous fluid infusion. A schematic model and some definitions are presented below (Fig. 8).

**Fig. 7**
Example of a dilution-time curve after an i.v. infusion. Dilution of a central and a peripheral body fluid space is indicated.

**Fig. 8**
The upper panel shows the one-volume model and the lower one the two-volume model.

- $k_i$ = rate of infusion
- $V$ = baseline volume, $V_1$ = central fluid space, $V_2$ = peripheral fluid space
- $v$ = expanded volume, $v_1$ = expanded central volume, $v_2$ = expanded peripheral volume
- $k_p$ = dilution-dependent elimination rate constant
- $k_b$ = basal elimination
- $k_t$ = dilution-dependent distribution rate constant
- $v/V$ = dilution of $V$
In the upper part of this schematic model, a fluid that is administered by i.v. infusion at a rate $k_i$ expands a body fluid space to a volume ($v$), which the body strives to maintain at a target (baseline) volume ($V$). The dilution in this fluid space is thus given as $(v-V)/V$.

Fluid leaves the space at a controlled rate proportional by a constant ($k_r$) to the deviation of $V$ and also at a fixed basal rate ($k_b$). This basal elimination represents *perspiratio insensibilis* and baseline diuresis and is usually fixed at a low rate, 0.4 - 0.8 ml/min. The dilution-dependent elimination ($k_r$) is governed only by the excess fluid in the system. The dilution-dependent elimination is therefore equal to $k_r \cdot (v-V)/V$ ml/min. The following differential equation describes the one-volume model.

$$\frac{dv}{dt} = k_i - k_b - k_r \cdot (v-V)/V$$

The dilution of the plasma in the cubital vein is used to measure the water load as Ringer solution remains outside the erythrocytes. Since the sampled plasma is a part of $V$, we obtain the following dilution at time $t$:

$$\frac{v(t)-V}{V} = \frac{\text{baseline Hb}}{\text{Hb}(t)} - 1$$

1 - baseline haematocrit

A correction for the loss of erythrocytes during blood sampling was made based on a preoperative blood volume estimated from the patient’s height and body weight according to this formula by Nadler [42].

**Blood volume (l) = 0.03219 \cdot \text{weight (kg)} + 0.3669 \cdot \text{height}^3 \cdot \text{(m)} + 0.6041**

In the two-volume model (bottom part of Fig. 8), a central fluid space ($V_1$) communicates with a remote fluid space ($V_2$). The rate of volume equilibration between the expandable fluid spaces is proportional to the relative difference in deviation from the target values ($V_1$ and $V_2$) by a constant ($k_t$). Elimination from the 2-volume system is from $V_1$ via a basal ($k_b$) and a dilution-dependent elimination ($k_r$). Differential equations governing the two-volume model are given below.
\[
\frac{dv_1}{dt} = k_i - k_b - k_r \left( \frac{v_1(t) - V_1}{V_1} \right) - k_o \left[ \frac{(v_1(t) - V_1)}{V_1} - \frac{(v_2(t) - V_2)}{V_2} \right]
\]

\[
\frac{dv_2}{dt} = k_i \left( \frac{v_1(t) - V_1}{V_1} \right) - k_o \left( \frac{v_2(t) - V_2}{V_2} \right)
\]

Data on fluid infusion, dilution and fluid elimination during the experiment are programmed into a computer and, to obtain the best curve-fit through the use of a mathematical software (Mat Lab version 5.2, Math Works Inc., Notich, MA, USA). Estimates and the standard deviations of the unknown parameters \((V_1, V_2, k_r, k_t)\) in the fluid space models were obtained by a computer search using a non-linear least squares regression curve fitting method (modified Gauss-Newton method) [43]. The iterations stopped when the parameters changed less than 0.1% for each iteration.

The model calculates the residual sum of squares, which is used to discriminate between the two models using an F-test [31]. The results were reported as a two-volume model only if the F-test indicated that it was statistically justified to fit a biexponential curve instead of a mono-exponential one to the data [44].

\[
F = \left[ \frac{SSQ \text{ 1-vol} - SSQ \text{ 2-vol}}{SSQ \text{ 2-vol}} \right] \cdot \left[ \frac{(df \text{ 2-vol} / (df \text{ 1-vol} - df \text{ 2-vol}))}{SSQ \text{ 2-vol}} \right]
\]

\(SSQ\) is the sum of squared errors for the difference between the dilution of the sampled plasma and the optimal curve fit according to the one-volume and the two-volume model, respectively. Degrees of freedom, \(df\), is the number of data points used in the fitting minus the number of parameters fitted. The calculated \(F\) is compared with the critical value for significance in a standard statistical table.

A table below gives a comparison and translation of pharmacokinetic variables to volume-kinetic variables (Fig. 9) [35].
Surgical trauma elicits an inflammatory reaction in response to the injury. The main purpose of the inflammatory reaction is to restore tissue function. Minor trauma is usually followed by an inflammation of limited duration. In contrast, a major surgical trauma can initiate an overwhelming inflammatory response that can threaten the survival of the patient by causing multiple organ failure (MOF) if appropriate countermeasures are not taken.

The systemic inflammatory response can be divided into a primary pro-inflammatory reaction (SIRS, systemic inflammatory response syndrome) that is followed by an anti-inflammatory reaction (CARS, counter anti-inflammatory response syndrome) to prevent an exaggerated pro-inflammatory reaction. If the SIRS is not thwarted, the risk of organ injury is increased, while a prolonged CARS can result in prolonged immunosuppression.

After a trauma the central nervous system (CNS) receives its input from both neural and circulatory pathways. An afferent signal from an injury site passes through the sensory part of the vagus nerve to the CNS and elicits a local response by secretion
of acetylcholine at the injury site which causes an attenuation of the inflammation. Circulating inflammatory mediators, such as TNF-α (tumor necrosis factor), can pass into the CNS and cause a release into the bloodstream of such hormones as ACTH. The adrenal glands are thus activated and raises the levels of glucocorticoids and adrenaline. Noradrenaline is released from an activated sympathetic nervous system. The sympathetic system is further activated by changes in the intravascular volume status caused by bleeding [28].

The release of adrenaline and noradrenaline are increased approximately 4-fold following injury and thereby cause a hypermetabolic state. These catecholamines are maintained at an elevated level for about 24-48 hours. Noradrenaline is the major transmitter substance released from postganglionic sympathetic nerve-endings and adrenaline is a hormone released from the adrenal medulla. Both adrenaline and noradrenaline are amino-acid hormones. Both catecholamines are synthesized from tyrosine. Blaschko described the steps of the synthesis as early as 1939, tyrosine - DOPA - dopamine - noradrenaline and, finally, adrenaline.

Noradrenaline is stored in granules in the postganglionic sympathetic fibres, while adrenaline is stored in chromaffin granules in the adrenal medulla. The synthesis of adrenaline is dependent on a normal level of cortisol which is transported from the cortex of the adrenal gland to the medulla and induces synthesis of an enzyme. The effect of released catecholamines is terminated either by cellular uptake, enzymatic degradation or dilution of the chemical in the synaptic space [45].

Catecholamines have a wide variety of effects depending on the dose, which catecholamine is used and which organ is stimulated. Ahlquist proposed in 1948 that there was more than one adrenergic receptor depending on the pattern of reactions to stimulation by adrenaline and noradrenaline, which later studies have confirmed. Ahlquist divided these receptors into alpha and beta receptors and nowadays there are at least seven alpha- and three different beta-adrenergic receptors [46]. All receptors are coupled to a G-protein that generates the second messengers that bind to an intracellular protein kinase. Phosphorylation of cellular proteins gives rise to various intracellular effects, such as the opening and closing of ion channels.

Repeated stimulation of adrenergic receptors causes a progressive diminution of the adrenergic response and this is called desensitization or tachaphylaxis. This could be due to a down regulation of the sensitivity of the receptors, a reduced number of receptors or an uncoupling of the G-protein from the receptor. This mechanism can limit the use of synthetic catecholamines during anaesthesia and intensive care [47-49].
6 AIMS OF THIS THESIS

Paper I: To determine if abdominal surgery under general anaesthesia for one hour is sufficient to cause desensitization of adrenoreceptors.

Paper II: To determine if inotropic/vasoactive drugs influence the distribution and elimination of a crystalloid infusion in an animal model.

Paper III: To explore if induction of general or spinal anaesthesia changes how the body handles an intravenous load of crystalloid fluid.

Paper IV: To see if a properly timed crystalloid fluid injection can prevent hypotension during the induction of spinal anaesthesia.

Paper V: To investigate whether either isoflurane or propofol anaesthesia causes extravascular fluid accumulation.

7 ETHICAL CONSIDERATIONS

Ethical permits: Paper I 364/95
Paper II American permit
Paper III 378/96
Paper IV 349/00 + 350/00
Paper V 269/02

Humans were studied in Papers I, III, IV and V. All protocols were approved by the Ethics Committee of Southern Stockholm, Sweden, and all the patients and volunteers gave their informed consent. In Paper II six chronically instrumented adult female Merino sheep were studied. In this protocol, approval was given by the appropriate Animal Care and Use Committee and adhered to the NIH Guidelines for Care and Use of Laboratory Animals. The experiment was carried out at the University of Texas, Medical Branch at Galveston, TX, USA.

In Paper I patients and volunteers were subjected to an infusion of adrenaline. This stress hormone increases the heart rate, exerts several metabolic effects and has the potential to raise the arterial pressure. Extreme elevations of the heart rate and the blood pressure could cause harm to a person with a limited cardiovascular reserve capacity. In this study there were no inappropriate reactions among the subjects.

In Paper II all animals were subjected to an operation under general anaesthesia for splenectomy together with insertion of intravascular catheters. Postoperatively all
animals received analgesics to reduce pain. During the study all animals received infusions of catecholamines that considerably, but transiently, increased the blood pressure and the heart rate. The animals became tachycardic but no adverse reactions were noted. In Papers I, III, IV and V an extra i.v. cannula for the purpose of blood sampling was inserted into the patients while the volunteers received two cannulae. Apart from the limited pain during insertion, no harmful effects were noted.

In Papers III and V a bladder catheter was inserted under topical anaesthesia prior to the experiment. Usually this would have taken place after induction of the spinal or general anaesthesia. Despite the topical local anaesthetic, some patients may have experienced discomfort during this procedure.

8 MATERIAL AND METHODS

8.1 SUBJECTS:

In Paper I, 10 healthy patients with a mean age of 45 (range 34-56) scheduled for elective abdominal surgery under general anaesthesia were studied and compared to 10 healthy volunteers with a mean age of 31 (range 23-44).

In Paper II 6 female Merino sheep were studied.

In Paper III 20 patients with a mean age of 67 (range 33-93) scheduled for elective surgery under general or spinal anaesthesia were studied. After completion of the main study in Paper III, an additional 5 patients with a mean age of 71 (range 55-85), were also studied.

In Paper IV a total of 75 patients, with a mean age of 68 (range 32-83), ASA groups I and II, scheduled to undergo surgery under spinal anaesthesia were studied. Prior to the main study 8 volunteers, mean age 34 (range 24-50) were also enrolled.

In Paper V 30 patients, ASA groups I and II, scheduled for elective surgery of the thyroid or parathyroid glands were studied.

8.2 MONITORING

In Papers I, III, IV and V, all subjects were monitored non-invasively using standard anaesthesia monitors. The parameters monitored included systolic, diastolic and mean arterial blood pressures. Heart rate, pulse oximetry, end-tidal carbon dioxide
concentration and the percentage of anaesthetic gas in the inspired and expired air were also monitored. In Paper II all animals were monitored invasively. The monitored parameters were the mean arterial, right atrial, left atrial and mean pulmonary artery blood pressures. Cardiac output was measured via a pulmonary artery catheter. In all the Papers urinary excretion was monitored via an indwelling bladder catheter in both patients and animals. Human volunteers voided in sampling buckets and their urinary volumes were measured.

8.3 BLOOD CHEMISTRY

In Papers I, III, IV and V blood chemistry was analysed based on serial blood sampling from an i.v. cannula inserted into a cubital vein of the arm not used for infusions. In Paper II blood samples were obtained from an arterial vascular catheter. For every series, the first blood samples (3 ml) were drawn in duplicate and the mean value was used as baseline in the calculations. Before each blood collection, a discard volume of 3 ml was drawn to preclude admixture of blood from the previous sampling. This volume was returned to the subject after each blood sampling. The cannula was rinsed with 2 ml of 0.9% saline after each sampling to prevent clotting and to replace the amount of withdrawn plasma.

In Papers III, IV and V the haemoglobin concentration, the number of red blood cells and the volume of red blood cells were measured by a colorimetric method at 546 nm and a helium neon laser light dispersion method by the hospital laboratory using a Technicon H2 (Tarrytown, NY, USA). In Paper II the haemoglobin concentration was measured on a 482 CO-Oximeter (Instrumentation Laboratory System, Lexington, MA, USA) at the University of Texas, Medical Branch at Galveston.

Serum glucose and potassium in Paper I and serum albumin and sodium in Paper V were analysed by routine methods at the Clinical Chemistry Laboratory of Stockholm Söder Hospital.

Insulin concentrations in Paper I were measured using a RIA method (RIA 100, Pharmacia, Uppsala, Sweden) and, in the same paper, high pressure liquid chromatography was used for plasma-adrenaline and ion-pair reversed chromatography for plasma cAMP. These last two analyses were performed at Huddinge Hospital by a fellow researcher and all of the other analyses by the laboratory at Stockholm Söder Hospital.
Fluids used:
a) Ringer’s acetate in Papers III, IV and V.
b) Saline 0.9% in Papers I and II.
c) Promiten in Paper IV.

Ringer’s acetate (Pharmacia, Uppsala, Sweden) ionic content in mmol/l:
Na⁺ 130, K⁺ 4, Ca²⁺ 2, Mg²⁺ 1, Cl⁻ 110 and acetate 30.

Normal saline 0.9%, ionic content: Na⁺ 154 mmol/l, Cl⁻ 154 mmol/l.

Promiten (MEDA AB, Solna, Sweden) Dextran 1, 150 mg/ml.

All fluids were at room temperature when administered. In Papers II, III and V the Ringer solution was given via an infusion pump (Flo-Gard 6201, Baxter-Healthcare, Deerfield, IL, USA). In Paper I the rate of infusion was adjusted manually and, in Paper IV, fluid boluses of Ringer’s acetate and dextran 1 was given as manual injections over three minutes with the aid of a stopwatch.

8.4 STATISTICS

Data are reported as the mean and SD in Papers I, II, IV and V and as the mean and SEM in Paper III. Skewed data were presented as median and interquartile range. Differences between groups and treatments were analysed by one-way or repeated measures analysis of variance (ANOVA) or the Mann-Whitney test, as appropriate.

Correlations between parameters were tested by linear regression analysis in Papers I, II, III and V. Multiple regression analysis was employed in Papers I, IV and V. In Paper I the linear trapezoidal method was used to calculate area under the curve for measurements that were performed frequently.

In Paper IV differences in incidence were tested by contingency tables, and covariance analysis was used to identify factors of importance to the incidence of hypotension.

In Paper V the chi-square test was used to study incidence data. The Wilcoxon matched-pair test was used for pair-wise and the Mann-Whitney test for nonpair-wise comparisons.

The level of significance was set at $p < 0.05$ for all Papers.
8.5 AUC

Frequently measured serial data in Papers I and V were studied by calculating the area under the curve (AUC). The linear trapezoidal method was used for these calculations (Fig. 10). AUC is the area between the x-axis and a line that joins the measurements.

\[
AUC = \sum [(x_n - x_{n-1}) \cdot \frac{(y_n + y_{n+1})}{2}]
\]

AUC is calculated manually by multiplying the base of each segment on the x-axis by the average of the two vertical heights. By making the bases smaller and smaller the curve will become increasingly continuous and AUC can be calculated as the integral for the curve. In this Thesis the calculations of AUC were performed by computer.

9 SPECIFIC STUDY CONDITIONS

9.1 PAPER I

In this first study 10 patients (Table 1) were given an i.v. infusion of adrenaline (50 ng/kg BW/min) for 30 min. The experiment started after the skin incision. The same infusion was also given to 10 healthy volunteers (mean age 31).

<table>
<thead>
<tr>
<th>Types of surgery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colectomy</td>
<td>2</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>4</td>
</tr>
<tr>
<td>Ovarian cysts</td>
<td>3</td>
</tr>
<tr>
<td>Whipple procedure</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1
Anaesthesia was induced by i.v. injections of thiopentothal and fentanyl and the endotracheal intubation was facilitated by a muscle relaxant. Inhalation of isoflurane was employed to maintain an adequate depth of anaesthesia. During surgery and the experiment normal saline was given as an i.v. infusion (mean 4.9 ml/kg/h).

Measurements were taken every 5 min during the entire study. Insulin and cortisol were measured before, during and after the experiment. Specimens for measurements of the plasma concentrations of adrenaline and cAMP were placed on ice and centrifuged within 30 min and then stored at -70 °C until analysed.

Adrenaline and cAMP were analyzed by different types of chromatography by a fellow researcher and the other blood analyses were performed at the laboratory in the hospital.

9.2 PAPER II

In an animal model we examined how the plasma volume expansion produced by i.v. infusion of normal saline was affected by concomitant adrenergic therapy. The experiments were carried out at the University of Texas Medical Branch at Galveston. Six adult female Merino sheep were splenectomized and catheterized under general anaesthesia. Catheters were inserted in the femoral and pulmonary arteries of each animal, another catheter was placed in the left atrium and one in the bladder. All sheep were allowed to recover for one week. The animals underwent four experiments in a random order separated by at least two days. They received either a continuous intravenous infusion of 50 µg/kg/min of dopamine, 0.1 µg/kg/min of isoprenaline or 3 µg/kg/min of phenylephrine. On the fourth occasion, no catecholamine was administered. After 30 min of adjustment to the effects of the adrenergic drug, plasma dilution was induced by infusing 24 ml/kg of 0.9% NaCl over 20 min. The catecholamine infusion was maintained for the whole study period of 180 min. Serial arterial blood samples were drawn for measurements of haemoglobin and the haematocrit, and the urinary excretion was measured.

9.3 PAPER III

Twenty patients aged 33-93 (mean 67) years and scheduled for elective surgery requiring either spinal or general anaesthesia were studied (Table 2). The choice of anaesthetic method was made on clinical grounds. The ratio between males and females was 5/5 and 4/6 respectively.
Table 2

<table>
<thead>
<tr>
<th>Type of anaesthesia</th>
<th>Type of surgery</th>
<th>No of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal</td>
<td>Inguinal hernia repair</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Peripheral vascular</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>surgery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous varices</td>
<td>2</td>
</tr>
<tr>
<td>General</td>
<td>Cholecystectomy</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Aortic aneurysm</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Colonic resection</td>
<td>5</td>
</tr>
</tbody>
</table>

All patients were premedicated with an intramuscular injection of morphine before entering the operating theatre. An indwelling bladder catheter was inserted under topical anaesthesia. After 15 min an intravenous fluid challenge was started with 20 ml/kg BW of Ringer’s acetate given at a constant rate via an infusion pump over 60 min.

Spinal or general anaesthesia was induced 20 min after starting the infusion. For the induction of spinal anaesthesia, the patient was turned to a lateral position and 2-3 ml of isobar bupivacaine, 5 mg/ml, was injected intrathecally. General anaesthesia was induced by i.v. injection of thiopentothal 5 mg/kg BW and fentanyl 0.15 mg. Endotracheal intubation was facilitated by the injection of 0.5 mg/kg BW of rocuronium. Sevoflurane, 1-3%, was used to maintain an adequate depth of anaesthesia. A venous blood sample was drawn every 3 min during the study period of 60 min. The urinary excretion was measured through the bladder catheter. Surgery did not commence until after the whole study period.

9.4  PAPER IV

The patients were taken to the operating theatre after fasting overnight. Spinal anaesthesia was induced in the left lateral decubitus position using a 25G Whitacre needle. The subarachnoid space was punctured in the L3-4 or the L2-3 lumbar interspaces. Plain bupivacaine 2.4-3.4 ml was injected as required to achieve adequate surgical anaesthesia. The upper level of analgesia was evaluated by loss of pin-prick sensation 15 min later, when any decrease in arterial pressure would be expected to have been fully developed.
Before the induction, the patients were randomly allocated (using sealed envelopes) to one of three fluid treatments:

- Treatment group 1: “Bolus injection” of 5 ml kg\(^{-1}\) of Ringer’s acetate over 3 min starting immediately after an injection of bupivacaine.
- Treatment group 2: “Bolus injection” of 2 ml kg\(^{-1}\) of dextran 1 (molecular weight 1 kD, Promiten, Pharmalink, Stockholm, Sweden) over 3 min.
- Control group (infusion): Ringer infusion, which meant that 15 ml kg\(^{-1}\) of Ringer’s acetate, was administered at a constant rate from 20 min before to 20 min after the injection of bupivacaine.

Haemodynamic parameters were recorded every minute during the first 10 min after the injection of bupivacaine, and then every 2 min for another 20 min. During the constant-rate infusions, measurements were also done every 3 min during the initial volume loading before the induction.

**Hypotension** was defined as a decrease in systolic arterial pressure by more than 30% from baseline, which was the mean of two measurements obtained immediately before the induction. A **cardiovascular side effect** was recorded if the patient reported discomfort consisting of sweating combined with nausea or near-fainting during the hypotensive event, which was then treated with 5 mg of i.v. ephedrine. A heart rate of less than 50 bpm was considered to indicate bradycardia and, combined with patient discomfort, was also considered to be a cardiovascular side effect. The latter event was treated with an i.v. injection of 0.5 mg of atropine.

### 9.5 PAPER V

The patients were randomized, via sealed envelopes, to receive one of two methods of anaesthesia, isoflurane (n=15) or propofol (n=15), both supplemented with fentanyl and rocuronium. One patient in the propofol group was excluded due to excessive surgical blood loss (1,700 ml).

All patients entered the operating theatre at about 8.00 a.m. Induction of anaesthesia was accomplished by intravenous injection of 2 mg/kg of propofol and 100-150 \(\mu\)g of fentanyl. Endotracheal intubation was facilitated by an injection of 0.5-0.6 mg/kg of rocuronium. Nitrous oxide or positive end-expiratory pressure was not used.
In half of the patients, isoflurane was used to maintain anaesthesia. The other half received an intravenous infusion of propofol. Analgesia was ensured by boluses of fentanyl. To increase anaesthetic depth, a bolus injection of fentanyl was the primary intervention rather than an adjustment of the isoflurane or propofol dosage.

All patients were ventilated by positive pressure ventilation through a low-flow semi-closed anaesthesia circuit. Heart rate and non-invasive mean arterial pressure were displayed on an anaesthesia monitor. Ventilation of the patients was adjusted to maintain normocapnia and a haemoglobin saturation of >96%. The flow of fresh gas in both groups was 4 l/min during induction, and for the first 5 min of anaesthesia and was then reduced to 0.8 l/min. An intravenous bolus of 5 mg ephedrine was given if the mean arterial blood pressure decreased to below 55% of baseline.

Starting 10 min after endotracheal intubation, 25 ml/kg of Ringer’s acetate solution was administered intravenously over 30 min. No other fluids were infused during the experiment. The study period was 150 min. In the event that the surgical procedure was terminated before this, the anaesthesia was nevertheless continued to fulfill the protocol.

Two venous blood samples were collected every 5 min during the first 60 min and every 10 min during the following 90 min. An indwelling catheter was inserted into the bladder immediately after the induction, but before the intravenous infusion was started. The urine already in the bladder was discarded and urine was thereafter measured and collected every 15 min. The sodium concentration and the osmolality were measured in the total urine volume.

10 RESULTS

Infusion of epinephrine (adrenaline) during abdominal surgery (Paper I) raised the plasma level of the substance in both the patient group and in the control group. The sum of endogenous and infused epinephrine reached the same maximum plasma level at the same time, but the AUC for epinephrine was slightly higher in the control group due to a shorter $t_1/2$ during surgery (14 min vs. 46 min, $p<0.001$). In contrast to the lower AUC for epinephrine in the patient group, there was a significantly greater increase in the second messenger, cAMP, and an increase in the effect parameter blood glucose ($p<0.02$). (Fig, 11)
When AUC ratios were calculated to differentiate between the effects of the first or second messenger the surgery group showed the greatest changes in blood parameters (serum K+, blood glucose). The haemodynamic effect of infused adrenaline in Paper I consisted in a lower mean arterial pressure (MAP) in the control group due to a lower diastolic blood pressure ($p<0.02$). In the surgery group the baseline heart rate was higher than in the controls ($p<0.02$) and remained so during the entire experiment.

In Paper II infusions of dopamine, isoprenaline and phenylephrine changed the haemodynamic baseline values for the sheep. Dopamine and isoprenaline increased the cardiac output significantly (4.6 l/min vs. 7.7 and 7.1 l/min respectively, $p<0.01$) through a marked increase in heart rate (96 bpm vs. 213 and 248 bpm, respectively, $p<0.001$). While phenylephrine raised the mean arterial pressure (90 mm Hg vs. 139 mm Hg, $p<0.001$), the heart rate was not significantly changed. The intravenous infusion of normal saline in the sheep increased all the measured haemodynamic...
parameters to a varying degree depending on which adrenergic drug was given. In the control group the most pronounced increases were in the right and left atrial pressures and in the cardiac output. When dopamine or isoprenaline was administered the increased plasma dilution was reflected in an increase of the cardiac output and the left atrial pressure while when phenylephrine was given, the atrial pressures responded to an increased dilution but the cardiac output remained more or less unchanged (Fig. 12).

Infusion of normal saline in the sheep diluted the plasma to a different degree depending on which adrenergic drug was used. (Fig.13)

The plasma dilution increased to about 25% in the control group during infusion and receded during the study period to approximately baseline. Isoprenaline caused a greater dilution of plasma (30%) that remained high (20%) during the entire study period. This was a reflection of a smaller central body fluid space (V) determined by volume kinetic analysis of the plasma dilution and a markedly reduced renal clearance (k_r) of normal saline. In contrast, there was a six-fold increase in the renal excretion of infused saline in the phenylephrine-treated animals. Infusion of normal saline increased plasma dilution by 25%, which declined rapidly after completion of the infusion. At the end of the experiment, there was a net transport of fluid from the periphery to the central fluid compartment (negative k_t).
This figure (Fig. 14) shows the dilution ratios between the controls and the different catecholamine. The upper panel shows the first 40 min of the experiment and the bottom panel the entire experiment. From this picture you can see the seven-fold difference in fluid retention between the isoprenaline and the phenylephrine-treated animals after three hours.

![Fig. 14](image)

Induction of spinal or general anaesthesia (Paper III) caused a drop in mean arterial pressure to 79% vs. 70% of baseline, respectively, and this difference was statistically significant ($p<0.004$). In both forms of anaesthesia, the dilution-dependent elimination rate constant ($k_e$) was reduced to about 25% of baseline ($p<0.003$) and averaged only 10 ml/min.

Statistical analysis of the plasma dilution showed that the one-volume model was appropriate in four patients in each group. Among these patients, induction of both forms of anaesthesia did not alter the size of $V$. Plasma dilution increased by 20% in the spinal and by 30% in the general anaesthesia group. This difference was due to a significantly smaller $V$ in the general group compared to the spinal group ($p<0.02$).

Among the six patients who fitted the two-volume model statistically, the distribution rate constant ($k_t$) was reduced by 50% and resulted in a rapid increase in dilution. Both the small $V$ and the reduced $k_t$ act to centralize the infused fluid and thus cause a rapid increase in the plasma dilution.

The picture below depicts the fluid volume-time curve of the two anaesthesia groups in a *computer simulation* using actual data from the volume kinetic analysis of the experiment (Fig. 15). The curves for both $V_1$ and $V_2$ are shown and the retained
fluid volume is the sum of both curves. The initial plasma dilution in the general group is more pronounced than in the spinal group. The amount of Ringer’s acetate given was about 1500 ml in each group and after 1500 minutes (25 hours) one third still remains in the body.

Computer simulations indicated that induction of spinal anaesthesia caused an increase in the intravascular volume of $V_1$ of approximately 200 ml. An i.v. bolus of Ringer’s acetate was given to five additional patients directly after induction of the spinal anaesthesia aimed at filling this increase in vascular volume and thus preventing a drop in blood pressure. There was no decrease of blood pressure, although the level of anaesthesia was higher.

This was further evaluated in Paper IV where 75 patients (38 males and 37 females, median age 73) where given spinal anaesthesia. The rapid decline of the blood pressure after the induction of anaesthesia was fully developed after 16 min. The drop in mean arterial pressure seemed to be exponential (Fig. 16).
When the anaesthetic block was lower than dermatome Th7 no hypotensive episode was noted but, for higher blocks, the incidence of hypotension was between 33% and 42%. There were no significant differences between the treatment groups regarding the development and degree of hypotension.

Fig. 17 below shows the lack of a relationship in individual patients between the magnitude of the drop in systolic blood pressure and how fast it occurs.

None of three treatment regimens (Ringer bolus, dextran-bolus and Ringer infusion) could prevent the drop in blood pressure. A covariance analysis showed that the only factor correlating with the hypotension was the height of the spinal block. Hypotension did not occur if the sensory block was lower than the 7th thoracic dermatome.

The two bolus fluid regimens diluted the plasma volume by about 10% and the dilution-time curve showed no elimination during the experiment although urine was
excreted. This could be explained by a mobilization of fluid from the periphery to the central body fluid compartment and it was shown as a negative distribution rate constant \((k_t)\). The preinduction infusion of Ringer’s acetate diluted plasma to 10% which continued to increase to 20% during the first 20 min of the experiment. In this group the induction of spinal anaesthesia decreased the distribution rate constant (325 ml/min vs. 55 ml/min), but the direction of the fluid flow remained from a central to a peripherally located fluid space (Fig. 18).

There were differences between the three treatment groups regarding the degree of patient discomfort (sweating, nausea and vertigo). Twenty percent of the patients in the dextran bolus group and 12% in the Ringer infusion group and none of the Ringer bolus patients experienced discomfort.

In Paper V the induction of both forms of anaesthesia caused a plasma dilution of 6.5%. The subsequent infusion of Ringer’s acetate solution diluted the plasma further to a maximum of about 30%, and the dilution remained elevated throughout the experiment as can be seen in the picture below. (Fig. 19) The plasma dilution did not differ between the two anaesthesia groups. For comparison, data from previous studies [50] have been included. Infusion of Ringer’s acetate in awake volunteers does not dilute the plasma to the same degree as in anaesthetized patients and the fluid volume is almost eliminated after 150 min. A pre-eclamptic woman given the same amount of i.v. infusion attains a lower degree of plasma dilution and eliminates all of the administered fluid within an hour [51].
The extravascular retention of Ringer’s acetate was calculated in two ways. Firstly, by letting the rate constant $k_b$ represent the sum of evaporation, surgical plasma loss and extravascular fluid retention and, secondly, by comparing the model-predicted fluid elimination and the actual measured urine volume. Using the first method, $k_b$ amounted to 2.0 and 2.2 ml/min in the isoflurane and propofol groups respectively. After subtracting evaporated and bled volumes, the extravascular retention was 160 ml or about 9% of the infused fluid. Using the second method, which was applicable in 21 patients, the extravascular retention was 270 ml or 15% of the infused fluid.

By calculating the sodium balance, it was possible to estimate the fluid shifts between the intracellular and the extracellular compartments. In the isoflurane group there was a small intracellular accumulation of fluid [7 ml, range (-57) – 64 ml] in contrast to the propofol group, where translocation of fluid from the intra- to the extracellular space occurred [89 ml, range (-21) – 197 ml, $p< 0.04$].

11 DISCUSSION

This Thesis is based on four clinical studies in humans and one experimental study in animals with the focus on how fluid therapy and adrenergic treatment are affected by routine anaesthetic practices. Classical haemodynamic parameters have been used together with a mathematical model (volume kinetic analysis) to describe the effects of fluid therapy.

Paper I evaluated the adrenergic responsiveness in vivo during abdominal surgery compared to non-surgical controls. The result was, a little unexpectedly, an increased responsiveness during the first 80 min of surgery. Adrenergic therapy can apparently be
conducted during abdominal surgery without fear of a diminished effect. This might not apply, however, to lengthy procedures.

This scenario has been studied many times, mostly by harvesting lymphocytes from surgical patients and measuring the cAMP response in vitro or by analysing receptor density [49, 52]. When the study started, the current view was that the function of the β2-receptor is rapidly depressed during the perioperative period due to surgical stress and effects of anaesthetic drugs, but nothing in our study indicates that the efficacy of adrenergic stimuli would decrease during surgery of medium duration.

Epinephrine is probably not an ideal agonist as it produces both alpha and beta-receptor effects but it is suitable in the sense that the plasma concentration during surgery includes a major portion of the endogenous adrenergic response. The dose was chosen to generate mainly β2-receptor effects [53, 54]. The use of epinephrine made it possible to distinguish between the receptor agonist pressure (AUC for epinephrine) and the physiological and biochemical responses. The agonist activates the receptor which in turn activates a G-protein that catalyses the production of the second messenger, cAMP. Cyclic-AMP that leaks from cells can be measured in the plasma. As the levels of epinephrine and cAMP are known to correlate well [55, 56], the cAMP response was used as a measure of a potential desensitization and the biochemical and haemodynamic responses were used as indicators of blockage of either the first [57] or the second (cAMP) messenger. By calculating the AUC for the time-effect profiles and the ratios between them we were able to ascertain how surgery affected the adrenergic system. The cAMP response among the patients was greater, but the AUC ratios for the biochemical response and cAMP were the same. This can be taken to indicate an increase in receptor sensitivity to epinephrine.

I have not seen the method of using the AUC to calculate the effect of the catecholamine in other papers. I hope it can be employed in other circumstances, as the method is easy to use. I believe that the AUC is a very good way to measure efficacy.

There were some differences between the study groups. First there was a tendency towards a higher basal heart rate among the patients which is most likely the result of increased preoperative stress and the stress of endotracheal intubation. Secondly, the patient group was older than the volunteers, but this demographic difference cannot explain the increased receptor responsiveness, as old age is known to attenuate the adrenergic response [58].

As serum potassium levels are easily affected by physical activity and the use of succinylcholine and tourniquets when samples are drawn, these confounders were
avoided [59]. The changes in serum potassium are due to an increased uptake in skeletal muscles after β2-receptor stimulation [53]. We believe that, despite the increased adrenergic response within an hour of abdominal surgery, there could have been a decreased response if surgery had been considerably prolonged. These findings and assumptions are consistent with published in vitro studies [60].

In Paper II we examined how infusions of adrenergic drugs can change the distribution and elimination of intravenous fluids in sheep. This is based on data presented elsewhere but, presented in a different way by Kramer and co-authors. Importantly, no kinetic analysis was included in their report [61].

The combined approach of studying the volume kinetic analysis and haemodynamic response to a crystalloid fluid load allowed a step-wise examination of different responses. The direct effects of adrenergic drugs [62] markedly changed the baseline haemodynamic parameters, as was expected, before any fluid was given [63]. Stimulation of β-adrenergic receptors (dopamine, isoprenaline) increased the cardiac output and the heart rate, while alpha-adrenergic stimulation (phenylephrine) increased the blood pressure and the systemic vascular resistance and thus diminished the increase in cardiac output. The indirect effects became apparent during the infusion of fluid as the adrenergic drugs influenced both the distribution and the elimination of the given fluid. When we plotted the haemodynamic parameters against the plasma dilution the indirect effects showed up as differences in the slope of the curves. It became even more evident when we plotted the changes in haemodynamic parameters against the plasma dilution.

A limiting factor in the interpretation of the direct effects is that the study was performed on sheep. When giving adrenergic drugs to intensive care patients the results could be different. A heart with coronary insufficiency may not be able to increase the heart rate without causing myocardial ischaemia and a reduction of the cardiac output, or a hypertrophic myocardium will not be able to relax sufficiently during diastole to allow an effective filling of the cardiac ventricles. Anaesthesia, intoxication or septicemia often has a negative inotropic effect and thus counteracts the effect of the given sympathomimetic drug. There could be inter-species differences in the response to adrenergic stimulation. Only one dose was given to the sheep, and the doses were higher than those normally administered to humans.

It is of great importance for the clinician to know how the plasma dilution and, indirectly, the plasma volume react if a sympathomimetic drug is given simultaneously with intravenous fluids as the adrenergic stimulation gives rise to indirect effects during
fluid administration. Stimulation of beta-receptors is a powerful tool for promoting a long-lasting volume effect of a given fluid bolus [64]. Additionally, alpha-stimulation can be used to treat a hypervolaemic patient by increasing the diuresis [65-67]. Alpha-adrenergic drugs are commonly used in the intensive care unit to treat arterial hypotension, but the increased diuresis is mostly attributed to a high blood pressure. Consequently, phenylephrine could possibly be used more frequently for this purpose, having mind that indiscriminate use of alpha-stimulation might be harmful, causing ischaemic damage due to vasoconstriction in internal organs [68].

It would be of great interest to study both healthy and sick humans for verification of these data. Resuscitation of patients almost always involves both intravenous fluids and different adrenergic stimuli. If these animal data are valid also for humans, the intensive care physician must take into account both the indirect and the interaction effects when evaluating the fluid response of a patient. This is a new view of the effects of adrenergic drugs and fluid and makes it important to evaluate them together to optimize treatment of a patient.

It is common practice to use fluid therapy during induction of anaesthesia to avert a drop in arterial blood pressure. Paper III describes how the body handles fluid during the onset of general and spinal anaesthesia. The overall results indicate that the body handles fluid in a similar way irrespective of the type of anaesthesia. The anaesthesia promoted fluid accumulation in the central fluid compartment \((V_1)\). This can be seen as a change in compliance between a central and a peripheral fluid compartment. The kinetic analysis demonstrated this as a reduction of the distribution rate constant \((k_d)\) to half of its original value. The central fluid compartment into which the fluid was infused was also quite small, about half of the plasma volume, in contrast to volunteer experiments where \(V_1\) is approximately the same as the plasma volume. This result is often seen during haemodynamic stress [69]. This small central fluid compartment might possibly reflect the plasma volume of the well perfused organs of the body rather than the complete plasma volume. The reduced tendency for fluid distribution and the small central volume indicate that during the onset of both types of anaesthesia the infused fluid remained in the central blood volume. This might possibly be regarded as an adaptation to anaesthesia since it counteracts the shifting of blood from the torso to the legs during the induction of anaesthesia [70, 71]. The retained fluid acts to prevent a drop in cardiac preload and a reduction of cardiac output.

A mathematical problem was that the size of \(V_2\) could not be reliably estimated, as this parameter is best obtained from post-infusion data which was lacking during this
experiment. To solve this curve-fitting problem we assumed a fixed sum of $V_1$ and $V_2$ as the solution, as have been demonstrated in previous studies [51].

Another general finding was that induction of anaesthesia caused a drastic reduction of the diuretic response to fluid administration. Only 5% of the given fluid was excreted during the hour the study lasted. This could be described as a more than ten-fold increase in the half-life of Ringer’s acetate, compared to volunteers [72]. In the kinetic analysis this appeared as a reduction of the elimination rate constant $k_c$. The overnight preoperative fasting and the preoperative stress reaction [73] might possibly contribute to this antidiuretic reaction. Such a reduced urinary elimination increases the risk of fluid overload during anaesthesia and subsequent cardiovascular complications.

Enteric lavage administered to the patients scheduled for colonic resection caused an unexpected dehydration which seems to have affected the results in Paper III. The patients presented with an increased heart rate at baseline and a more profound hypotensive reaction to the induction. The dehydration was also indicated in the kinetic analysis by a small $V$ before induction, which is consistent with hypovolemia [74].

By computer simulation, we estimated the increase in the size of the “vascular costume” caused by the induction of spinal anaesthesia to be about 200 ml. We hypothesized that a fluid bolus given directly after the induction could prevent hypotension, as $k_t$ would then be reduced and thus favour volume retention in the central compartment. The hypothesis was tested in an additional five patients and, to our delight, there was no hypotension although the anaesthesia was more widespread.

These findings were further evaluated in Paper IV. We studied 75 patients receiving spinal anaesthesia and three different fluid regimens intended to prevent arterial hypotension. In this study we compared two groups of patients who received intravenous fluid boluses directly after the induction of anaesthesia to a control group that received the fluid as an infusion. Contrary to the results of the pilot study in Paper III, we did not find any differences in the frequency and magnitude of the drop in blood pressure between the three groups. The only factor that influenced the hypotension was the extent of the spinal anaesthesia. No hypotensive event took place if the spread of analgesia was below the Th-7 dermatome. This emphasizes the importance of re-evaluating interesting results from a small study by conducting a larger one with a sufficient number of patients. A spinal block will not only affect sensory nerves but also nerves belonging to the sympathetic nervous system. This autonomous nervous system is constantly active and a local anaesthetic will inhibit the transmission of signals. The preganglionic sympathetic
fibres arise from cells located in the spinal cord, extending from the first thoracic to the second or third lumbar segment. Sympathetic fibres synapse in the paravertebral ganglia. There are 22 pairs of sympathetic ganglia that exert the sympathetic activity throughout the body via postganglionic fibres. Noradrenalin is the major transmitter substance in the sympathetic system [75]. The heart is innervated by the upper portion of the sympathetic system and therefore is not affected directly by a spinal block. The sympathetic innervation of blood vessels and muscles in the lower part of the body will be interrupted by spinal anaesthesia and thus the vessels will dilate and cause a drop in blood pressure. The higher the block, the more sympathetic nerves will be blocked and will thus have a greater effect on the cardiovascular system [1]. Most patients will have a drop in blood pressure, probably caused by a sympathetic blockade of the vasomotor fibers to blood vessels in the lower part of the body. When the spinal anaesthesia reaches higher, the sympathetic tone to the heart will also be affected and the reduction of beta-adrenergic stimuli will reduce the cardiac output through both negative inotrophy and chronotrophy. This is in accord with long-established knowledge [76, 77] and could be an explanation of the findings in Paper IV. Inter individual variability in adrenoceptor response among beta-2 receptors is known to occur [52] due to differences in genotype and could theoretically also affect the alpha-receptor response. Induction of spinal anaesthesia could also reveal a latent reduced intravascular fluid volume and cause hypotension.

The Ringer’s acetate regimens seemed to be better from the patient’s point of view as they were associated with a lower frequency of patient discomfort such as nausea, sweating and vertigo. No patient in the Ringer bolus group experienced discomfort, as compared to 12% in the Ringer infusion group and 20% among those who received the dextran bolus. Similar results have been reported by Mojica [78]. These results should prompt clinicians to use the method of giving a rapid fluid bolus at the time of induction. There are some practical difficulties as most volume pumps cannot deliver fluid at a sufficient rate, which would make it a manual task and thus more labour intensive. The technical evolution of pumps will probably make this easier in the near future. Long term rehydration appears to be of no benefit to either patients or staff. In a more recent study from Hong Kong on patients receiving spinal anaesthesia for Caesarean section the combination of a fluid bolus and an injection of a vasoactive drug was effective in preventing hypotension and discomfort [79].

In 20% of patients in each group, we evaluated the distribution and elimination of the given fluid by volume kinetic analysis. In the two bolus groups, the volume effect
of the given fluid was equally large but, in the infusion group, the volume effect was
doubled but the degree of hypotension was nonetheless the same. In all groups there
was a tendency to retain fluid in a central compartment \( V \). In accord with the results of
Paper III, the distribution rate constant, \( k_t \), was reduced, thus adding to the
centralization of the fluid. This constant took on a negative value in the two bolus
groups, which is consistent with fluid mobilization from the periphery to the central
fluid compartment. The fluid mobilization in the bolus groups is interesting as it is
probably an endogenous response to the haemodynamic stress of spinal anaesthesia. It
contributes to maintainance of the plasma volume despite vasodilatation due to
anaesthesia. Previous studies on patients receiving epidural anaesthesia without fluid
loading have not demonstrated this mobilization of fluid from the periphery [80-82].

Also in Paper IV we found that the elimination of fluid was decreased and only
10-36% of the infused fluid was excreted in the urine during the experiment, and
between 350 and 900 ml of Ringer’s acetate remained in the body after the experiment.
When the effect of the anaesthesia wears off and the vasomotor tone returns the risk of
oedema and cardiovascular complications is increased. In Paper V only 11% of
the infused fluid was excreted during the experiment, in contrast to volunteers who excrete
up to 75% of the infused crystalloid fluid within 3-4 hours [83]. This long-lasting
haemodilution by crystalloid infusion reduces the need for additional crystalloid
infusions during thyroid surgery.

Infusion of dextran 1 kD in volunteers, for the purpose of finding a dose range,
resulted in a forced diuresis. The dextran molecules are sufficiently small to be filtered
through the kidneys and thereby probably cause an osmotic diuresis. The volunteers
became fluid-depleted after the experiment, but this was not the case in the patient
group. The choice of dextran 1kD was made to study a colloid solution with
sufficiently short intravascular effect so that the plasma volume expansion would be
gone at the same time as the spinal anaesthesia to reduce the risks of postoperative
hypervolaemia. The use of dextran 1kD is not beneficial in these circumstances.
Another colloid solution might have yielded another result. Different colloids should be
tested in future work.

The use of vasoactive drugs (ephedrine, phenylephrine) seems to be a better
treatment than i.v. fluids for the hypotension caused by induction of spinal anaesthesia
in a normovolaemic patient.

In Paper V we studied the possible existence of “third spacing” of fluid. Third
spacing is a phrase that was coined in the 1960s [20] to describe an extravascular
retention of extracellular fluid in compartments that do not freely communicate with the extracellular compartment. Two studies in sheep examined whether isoflurane anaesthesia and/or mechanical ventilation had any influence on the distribution and elimination of an i.v. bolus of saline. The first study [84] showed, by applying both mass balance and volume kinetic calculations, that the combination of anaesthesia and mechanical ventilation reduced the urinary output and promoted peripheral accumulation of fluid. The second study [85] was a follow-up to differentiate between the effects of isoflurane and mechanical ventilation. It was demonstrated that isoflurane, but not mechanical ventilation, increased the interstitial fluid volume and decreased the urinary flow. Also in this study, both mass balance and volume kinetic methods were applied.

In Paper V our intention was to study humans during isoflurane anaesthesia and to use volume kinetic analysis to determine if extravascular retention of Ringer’s acetate occurred. Thyroid surgery was chosen as intraoperative blood losses are usually small. An equivalent group of patients who were anaesthetized with propofol and operated on served as a control group.

The distribution and elimination of infused Ringer’s acetate was calculated using volume kinetic analysis. These calculations accounted for the amount of fluid present in a central fluid compartment \( V_1 \) which freely communicates with a peripheral fluid space \( V_2 \). Fluid losses from this kinetic system that did not appear as measured urine were considered to be lost due to evaporation, bleeding and extravascular retention. Non-urinary losses can be measured or estimated. Intraoperative blood loss was measured and evaporation from the surgical wound was estimated to be 5-10 ml [86]. Evaporation from the respiratory tract was estimated to be insignificant due to the use of a low-flow closed anaesthesia circuit [87].

In both anaesthesia groups, the infused crystalloid solution expanded the plasma volume to the same degree and the inhibited urinary excretion delayed the elimination.

In Paper V, however, the use of isoflurane was not associated with any greater tendency towards extravascular retention than the use of propofol. Thus, sequestration of the given fluid within the body occurred in both anaesthesia groups, 2.0 ml/min in the isoflurane group and 2.2 ml/min in the propofol group. In the sheep study, isoflurane was shown to promote an extravascular fluid retention of 3-4 ml/min. Both types of anaesthesia decreased the blood pressure, but isoflurane to a higher degree. This contrasts with the results in sheep where the blood pressure remained stable or slightly elevated. A higher blood pressure could disturb the Starling
equilibrium. The hydrostatic pressure in the capillaries will increase and facilitate distribution of fluid from the intravascular to the extravascular space. The fluid distribution during infusion will be greater from $V_1$ to $V_2$ than in the opposite direction. The volume kinetic model does not take the hydrostatic pressures into account and the calculations can therefore yield a $k_b$ that is influenced by changes in hydrostatic pressure.

Several other factors may explain the differences in fluid distribution and elimination between humans and sheep. Only humans underwent surgery, and both types of anaesthesia included fentanyl and rocuronium, which the animals did not receive. Heart rate was also higher among the isoflurane anaesthetized patients, an effect isoflurane is known to elicit [88].

There were some methodological problems with the volume kinetic model when the last part of the dilution-time curve was close to horizontal and caused strong intercorrelations between $V_2$ and $k$. In this case, we could not let $k$ be determined by the urinary excretion as the model-predicted elimination comprises not only urine but also evaporation, the bled plasma and extravascular retention. When patients not showing a slope of the dilution-time curve were excluded, fluid losses were slightly greater but still smaller than for the sheep.

By measuring the plasma dilution over time and using albumin as the tracer and comparing these data to those obtained by using haemoglobin as a marker, we found that albumin must have been translocated from the interstitium to the plasma. This is usually consistent with capillary refill after haemorrhage [89] [90], but this was not the case in Paper V, as intraoperative blood losses were small. Arterial hypotension and/or anaesthesia per se could be a more likely cause.

The sodium dilution method was used to calculate whether any fluid shifts between the extracellular and intracellular spaces took place. This method is based on a mass balance calculation. As sodium is almost exclusively an extracellular ion, the number of ions and the amount of water in the ECF remain constant if no additions or losses occur. The data showed that no significant fluid shifts occurred during this study. The above-mentioned extravascular retention of fluid therefore could not have accumulated intracellularly. Also in Paper V, anaesthesia and surgery caused the infused crystalloid solution to be retained in the plasma volume and thus exert a long-standing volume effect, and the inhibited urinary excretion acted to enforce this almost colloid-like effect.
On comparing the time-dilution curves above (Fig. 13 from Paper II, Fig. 19 from Paper V) you will notice the similarities between the curves during adrenergic beta-stimulation (Iso) and during anaesthesia. In both situations there is a prolongation of the plasma dilution. Most anaesthetic agents are known to have a negative inotropic effect, but this is probably not caused by interaction with the beta-receptor. Also you can see the rapid decline in plasma dilution in the pre-eclamptic patient and during an alpha adrenergic (Phe) stimulation. The pre-eclamptic patient is known to be vasoconstricted both in the uterus and more generally throughout the body [91] causing arterial hypertension. The cause of pre-eclampsia is not fully understood but the similarities in the time-dilution curve indicate that the patients could be subject to alpha adrenergic stimuli. A word of caution is due when we interpret these findings as one study is an animal study and the other ones are human studies.

In the clinical setting many patients are taking beta or alpha adrenergic blockers and it would be interesting to study how plasma dilution is affected by these medications.

I see the need for further studies during anaesthesia and surgery to more fully explore the rate, amount and type of fluid to use for different types of patients.

12 CONCLUSIONS

This Thesis comprises five Papers in which the null hypothesis was proved to be wrong. One hour of abdominal surgery, in Paper I, did not cause desensitisation of beta-adrenergic receptors. On the contrary, there was an increase in receptor sensitivity
which was confirmed by calculating the ratios of the areas under the curves. Adrenergic stimulation did have a profound influence on both the distribution and elimination of an intravenous fluid infusion.

An animal model in Paper II showed that beta-adrenergic stimulation caused a greater plasma dilution and a tendency to distribute the given fluid, while alpha stimulation caused a rapid decline in plasma dilution by increasing urinary excretion.

Induction of both general and spinal anaesthesia, in Paper III, caused a centralization of the given fluid due to reductions of both the elimination and distribution rate constants thus causing a prolonged intravascular persistence and a prolonged plasma dilution.

Giving a rapid fluid bolus after the induction of spinal anaesthesia, in Paper IV, did not reduce the magnitude or the frequency of hypotension, but a crystalloid fluid bolus did reduce the incidence of patient discomfort.

In Paper V, I could not verify that anaesthesia based on the anaesthetic gas isoflurane caused a more pronounced extravascular accumulation of intravenously administered Ringer’s acetate compared to a propofol-based anaesthesia. Both types of anaesthetic procedures caused an increased plasma dilution which remained high during the whole operation.

Anaesthesia per se profoundly changes the way the body handles intravenous crystalloid fluids and causes a centralization of fluid together with a reduction of the urinary elimination. The effect of a given crystalloid fluid can be modified by simultaneous administration of adrenergic drugs, in which case at least in sheep, beta-stimulation delays and alpha-stimulation accelerates the elimination of fluid. These changes can be studied by applying the volume kinetic analysis method.
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