Homeostatic Blood States Theory

Audrius Andrijauskas

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Audrius Andrijauskas

HOMEOSTATIC BLOOD STATES THEORY

Doctoral dissertation
Biomedical sciences, medicine (07 B)

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DELIMITATIONS

- Time of the study: March 2003 through May 2006 at Vilnius University, Vilnius, Lithuania.
- This is a theoretical study. Chapters of 'Clinical Physiology' in the 'Review of Literature' also serve as background for the new theory proposed in this study.
- No experiments on human beings or animals were performed. The author's pilot clinical investigations (published and unpublished materials) are presented only in part.

The philosophy

We need to learn the body talk and read the message written in the blood.

Scientific consultant:

Prof. Dr. Juozas Ivaskevicius

(Vilnius University, Biomedical Sciences, Medicine 07 B).

ABBREVIATIONS

- BV – Circulating blood volume
- PV – Circulating plasma volume
- PVE – Plasma volume expansion
- PVEE - Plasma volume expansion efficacy
- RCM - Red cell mass (volume)
- IBV – Normal/ideal circulating blood volume (calculated by conventional formulas)
- IPV - Normal or ideal circulating plasma volume (calculated by new formulas)
- Hb - Blood hemoglobin concentration
- Hct – Blood hematocrit (packed cell volume)
- tBV – Circulating red cell mass specific homeostatic target blood volume
- tPV - Homeostatic target state specific plasma volume
- tRCM – Homeostatic target state specific red cell mass (volume)
- tOsm – Homeostatic target state specific plasma osmolality
- tMCV - Homeostatic target state specific mean cell volume
- tMCHC - Homeostatic target state specific mean cell hemoglobin concentration
- MCH – Mean cell hemoglobin content (non-specific to target states)
- PRBC - Packed red blood cells (collected for transfusion purposes)
- HHL - Homeostatic hematocrit limits
- UHL – Upper homeostatic hematocrit limit (lowest physiologically critical value)
- LHL – Lower homeostatic hematocrit limit (highest physiologically critical value)
- MTD - Maximal target deviation
- k or Constant k – New unit of measure for blood and plasma volume, etc.
- MSD – Maximal safe deviation
- RL - Radiating Line (the MCHC value specific graphical projection)
- VLT-test – Volume loading test (for verifying target plasma hydration)
- ICF - Intracellular fluid
- ECF - Extracellular fluid
- NS – Normal saline (0,9% solution of NaCl in water for intravenous infusion)
- HES - Hydroxyethylstarch preparations for intravenous infusion
- mE - Maximal expansion (maximal safe or isoosmotic plasma dilution)
- mD - maximal depletion (maximal safe or isoosmotic plasma dehydration)
- TPL – Tissue priority levels (homeostatic perfusion priority levels)
- HPT – High and superior homeostatic priority tissues
- RPT – Regular homeostatic priority tissues
- LPT – Low homeostatic priority tissues
- TPF - Target tissue perfusion focused vasomotor tone
- iTPF – Ideal target tissue perfusion focused vasomotor tone
- TPFi - Target tissue perfusion focused increased vasomotor tone
- TPFd - Target tissue perfusion focused decreased vasomotor tone
- PVP – Preset volume potential
- POP – Preset osmotic potential
- EQP – Equilibration pause (steady state without any intravenous infusion)
- VLT-test – Volume loading test (clinical verification of target states)
- TVL – Test volume load (isotonic crystalloid solution volume for VLT-test)
- ODC – Osmotic deviation center (vertical MCH projection in Osmonomogram©)
- SDL – Safe deviation line (MSD-mE-mD projections in Devi-safe© nomogram)

DEFINITIONS

- *Ideal Total Match* (ITM) *hematocrit* is the unique Hct value, where both - blood and plasma - maintain normal or ideal volume (IBV and IPV) as homeostatic target. It is the countdown Hct value in the new mathematical model (HBS trends).
- *Homeostatic target state or target state* is the homeostatically maintained target combination of circulating red cell mass specific homeostatic target values - tHct, tBV, tPV, tMCV, tMCHC and tOsm. Case specific homeostatic target plasma hydration and osmolality are the major conditions for the maintenance of tBV.
- *Homeostatic Hematocrit Limits* (HHL) are physiologically critical Hct values: the new method argues the lowest Hct-13.3% (UHL) and highest Hct-60.0% (LHL).
- *Maximal target deviation* (MTD) is the sum absolute blood and plasma volume deviation from normal values applicable to target states.
- *Constant k or k* is the sum of absolute tBV and tPV deviations from IBV and IPV at critically low and high Hct values (HHL). It is expressed as MTD to IBV ratio (new method argues $k \sim 0.3 \cdot IBV$).
- *Maximal safe deviations* (MSD) are target Hct specific limits of maximal safe or isoosmotic plasma hydration origin deviations from target. These limits are reached when either BV or PV reaches maximal deviation 0.5k in respect to IBV or IPV.
- *Maximal safe plasma dilution* (mE) is the target state specific maximal isoosmotic plasma volume expansion from target state. The MSD consistent state.
- *Maximal safe plasma dehydration* (mD) is target state specific maximal isoosmotic plasma volume decrease from target state. The MSD consistent state.
- *IBV-target plasma deviations* are plasma hydration origin deviations from target states in attempt to reach the normal (ideal) blood volume.
- *Target tissue perfusion focused* (TPF) or *normal* vasomotor tone is present under the control of intact sympathetic stimulation and homeostatic guidance.
- *Ideal target tissue perfusion focused* (iTPF) vasomotor tone is present only with ideal blood volume, which is maintained by target states only at ITM-tHct, but also may be reached in dilution origin deviation from target states with other tHct values.
- *Increased* (TPFi) or *decreased* (TPFd) *target tissue perfusion focused* vasomotor tone is meant in respect to ideal pattern (iTPF). It adjusts in maintaining homeostatic targets of tissue perfusion with the ever-changing blood volume.
- *Homeostatic stability patterns* (HSP) is predisposition to retain in or eliminate from circulation an additional load of isotonic non-colloid fluid.
- *Pre-set potentials* describe the HSP: osmotic and volemic predisposition to plasma dilution for maintaining adequate effective circulating volume and osmolality**.**
- *Pre-set volume potential* [$PVP^{-/0/+}$] describes predisposition of proper homeostatic blood state to isotonic plasma hydration solely for blood volume increase.
- *Pre-set osmotic potential* [$POP^{-/0/+}$] describes predisposition to isotonic plasma hydration solely for decreasing osmolality (dilution).
- *Volume Loading Test* (VLT-test) is for clinical verification of target states.
- *Test volume load* (TVL) is the volume of isotonic crystalloid solution that has a purpose to recover the target state in case of preexisting dehydration.
- *Maximal Functional Osmotic Deviations* (MFOD) - standard projections originating from MCH value specific points along the ODC projection in Osmonomogram©.
- *Radiating Line* (RL) is the MCHC value specific graphical projection in the HBS Graphics© model, which is the basic part of the HBS Nomogram©.

CONTENTS

1. BACKGROUND

Intravenous fluid and blood component resuscitation is an integral part of modern medicine practice in a variety of medical fields. Studies of the last decade have provided important clues for intravenous fluid handling and blood transfusions. Volume-deficit hypovolemia and fluid overload are both deleterious and associated with a significant increase in morbidity and mortality. However, blood volume (BV) is unknown in most clinical settings. Blood hemoglobin concentration (Hb) and hematocrit (Hct) are classic parameters used for guiding infusion and blood transfusion therapy. They are suitable for tracing dilution origin shifts in respect to baseline, but cannot be used for accurate clinical evaluation of plasma volume (PV) and red cell mass (RCM). Advanced shifts of plasma hydration induce osmolality changes, but threshold limits of isoosmotic plasma dilution are unknown. Therefore, it is hard to differentiate Hb and Hct changes resulting from mild or initial stage bleeding and similar changes induced by isoosmotic plasma dilution shifts. Therefore, relying on indirect classic signs of body hydration (1-2), tissue oxygenation and adequacy of effective circulating volume (3-7) is a common clinical practice.

Formulas for calculating normal blood and plasma volume are conventionally used in research and clinical practice. (8-11) However, the most accurate BV value is obtained by direct measurements (12-18), especially by simultaneous measuring of RCM and PV. (19) However, such methods have limited clinical applicability. Research studies showed that normal or ideal (as referred to by the new method) blood volume (IBV) and plasma volume (IPV) calculated by formulas for normal values do not always fit the values obtained by direct measurements. (20,21) Therefore conventional assumption of normal blood volume as homeostatic target regardless of circulating red cell mass, leads to significant errors in estimates of BV, PV, RCM and plasma dilution. Various mathematical formulas (10-22) are used to predict Hb and Hct changes resulting from blood withdrawal and plasma dilution during perioperative hemodilution that serves to reduce allogeneic transfusions. Their accuracy is questionable though. Probably the most accurate methods dynamically investigating plasma volume as part of central expandable fluid space are based on volume kinetic analysis and volume turnover kinetics. (23-24) They use routinely available clinical parameters - Hb and Hct

- as markers of plasma dilution. However, these methods have limited clinical applicability in the current stage of development.

Sophisticated monitoring of infusion therapy like measuring the adequacy of preload, level of systemic oxygen delivery, gastric intramucosal pHi and others depend on their on-site availability and induce inherent intervention risk. (25-41)

Despite significant advance in determining transfusion triggers (42-43), transfusion decision making remains controversial (44-53), choosing transfusion amount for proper Hct increase is still challenging as volume selection guidelines are very approximate (44) and subject to constant debate. Coagulation disorders resulting from massive bleeding, blood component resuscitation and plasma dilution induced by volume resuscitation measures are challenging and debated. (53) Resulting errors in clinical decision making are reported to be even fatal. (54) Inaccurate estimates of baseline BV and RCM along with missing limits of isoosmotic plasma hydration are major deficiencies of existing nomograms and guidelines for infusion and transfusion measures. Definition of the "ideal" intravascular fluid volume replacement strategy remains a critical problem (55,56) and ‘gold standard’ still missing.

Addressing issues and deficiencies of existing methods, the current study has developed a new theory describing blood volume homeostasis – Homeostatic Blood States Method (HBS Method), patent pending – USA. It proposed a new conceptual approach and possibly new solutions for the outlined problems in research and clinical practice. The new method proposed nomograms for monitoring of RCM specific homeostatic target blood volume (tBV) patterns and plasma hydration shifts. It also enables calculation of packed red blood cell (PRBC) transfusion volume for proper Hct and Hb increase targets accounting for plasma dilution. As an alternative to “piles of files”, the new method has also introduced a new way for systemized filing of blood test derived red blood parameters in patient’s medical records, making dynamic evaluation easier, more practical and time saving.

New method’s clinical applicability could be significantly increased if combined with noninvasive methods for blood hemoglobin concentration and hematocrit monitoring like continuous optoacoustic monitoring of hemoglobin concentration and hematocrit recently introduced by Esenaliev and his colleagues. (57,58)

Manuals of diagnostic and laboratory tests (59-62) suggest critically low hematocrit and hemoglobin concentration values that are higher than found acceptable for general surgery (63) and those in survival cases of Jehovah’s Witnesses patients.

(64-67) The proposed new method of investigating homeostatic hematocrit limits may be used for establishing more accurate critical limits.

The current study proposed new trends in research, possibly leading to better infusion and blood component transfusion practice, increased patient safety, decreased overall cost (68,69) and better outcomes of treatment. In general, the new method fits major requirements for "gold standard".

Decades of research resulted in numerous studies that cover the widest range of issues in the related fields. Acknowledging no need to repeat numerous published investigations, the current study has set the purpose to summarize and join into one physiological-mathematical model the major concepts of human physiology and pathophysiology, also clinical experience and evidence from research, putting them together with brand new hypotheses raised by the author. The new model is supposed to serve as background for clinically applicable new nomogram ready for verification trials. Therefore, current study is pure theoretical, referring to results of published materials, including author's papers in peer reviewed journals. (70-72)

1.1. PURPOSE

Addressing deficiencies of existing methods for guidance and evaluation of intravenous fluid and blood component resuscitation, the current study followed the purpose to establish the "gold standard" for guiding and evaluation of transfusion and infusion therapy by creating a new theory of blood volume homeostasis.

1.2. OBJECTIVES

Following the purpose of the study, objectives were set as follows:

1. Create the mathematical-physiological model of blood volume homeostasis.
2. Propose nomograms for measures of infusion therapy, also for blood loss evaluation and calculation of red cell transfusion volume.
3. Use blood hemoglobin concentration and hematocrit as nomographic parameters.
4. Propose the new guidelines for blood transfusion and infusion therapy.
5. Propose a method of early occult bleeding verification based on the dynamics of blood hemoglobin concentration and hematocrit.

2. NEW RELEVANCE TO THE CURRENT ART

In the current clinical environment RCM and PV are approximate estimates obtained by indirect parameters like Hct or Hb, but results are subject to the myriad of unverifiable errors. Meanwhile high precision methods require sophisticated modalities, induce inherent risk, cannot be frequently repeated or require steady state, etc. It makes them inappropriate or unavailable in most clinical settings.

Nomographic interpretation of serial conventional blood tests derived markers of plasma dilution (i.e., Hb and Hct) is a less invasive and risky, also much cheaper alternative. The sophisticated nomograms for evaluation of plasma volume as part of the central body fluid compartment are used by methods based on volume kinetics of infused intravenous solutions. However they have limited clinical applicability mainly due to steady state requirement. Volume turnover kinetics is a promising trend in advancement of volume kinetics by approaching clinical settings, but it is in the early stage of development.

The current study proposed a new method for nomographic interpretation of serial Hb and Hct tests for BV, PV and RCM estimates applicable to dynamically changing clinical settings. In contrast to conventional approach considering normal (ideal) blood volume as homeostatic target regardless of circulating RCM, the current study introduced a concept of circulating RCM specific homeostatic *target blood volume* (tBV). The definition of *homeostatic target state* as combination of RCM specific target values - tHct, tBV, tPV, tMCV, tMCHC and tOsm - is introduced for the first time. To some extent, the new definition of homeostatic target blood volume echoes with the definition of target volume described as baseline value in studies on volume kinetics, but they have not considered its RCM specificity. Therefore, the new definition is the extension of the target state, which is the most powerful concept of volume kinetic analysis applied to body fluid handling evaluation during and after intravenous infusions. The new method hypothesizes that tBV homeostatically accommodates to fit different RCM patterns in keeping the optimal balance of BV and PV, and consequently viscosity, deviations from ideal values. The RCM specific absolute BV deviation from ideal value is considered equal to corresponding absolute PV deviation, assuming ideal values – IBV and IPV - are met only once along Hct scale. That unique Hct value is referred to as *Ideal Total Match* (ITM). It serves as countdown point in the new

mathematical model – *HBS trends* – providing numeric values for the new nomogram – *HBS Nomogram*©. Case specific normal or homeostatic target plasma hydration and osmolality (tOsm) are considered as major conditions for the maintenance of tBV. The new method proposed definition of *Constant k* (k) as sum of absolute tBV and tPV deviations from IBV and IPV at critically low and high Hct values. That sum deviation applicable to target states is referred to as *maximal target deviation* (MTD). Critical Hct values are described in the new model of *Homeostatic Hematocrit Limits* (HHL). Constant k is the MTD to IBV ratio, where IBV is individual physical features – usually body weight, height or body surface - specific. Therefore Constant k serves as unit of measure for BV, PV and plasma volume expansion (PVE) in HBS Nomogram© making it applicable to any individual. Target Hct specific limits of maximal isoosmotic or *safe* plasma hydration origin deviations from target are also proposed for the first time. These values are derived from the new concept of safe limits being reached when either BV or PV reaches maximal deviation in respect to IBV or IPV. That deviation referred to as *maximal safe deviation* (MSD) is assumed being equal to 0.5k in this study. However, the study proposed a concept that allows investigating alternative settings of key elements, which are ITM and HHL, therefore offering trends for future investigations.

Research studies of the last decade have provided important clues for evaluation and prediction of intravenous fluid handling. Current theories on volume kinetics of infused intravenous solutions describe osmotic inter-compartment fluid shifts on the basis of 1-2 or 3 Volumes of Fluid Spaces (VOFS) models. The current study is the first to consider RCM as independent intracellular fluid compartment, which takes part in osmotic plasma fluid equilibration. The study proposed a method of tracing plasma osmolality shifts by following RCM changes that are reflected by conventional blood test derived mean cell hemoglobin concentration (MCHC), which in turn is Hb to Hct ratio. The linear graphical relationship Hb to Hct ratio is well known to reflect the dynamics of plasma dilution, but inclusion of MCHC parameter has never been considered. This is the first study to propose the graphical triple factor - Hb/Hct/MCHC - relationship referred to as *HBS Graphics*© as part of the HBS Nomogram©. Safe (isoosmotic) plasma hydration shifts take part in the setting of unchanging tMCHC. Therefore, isoosmotic shifts can be graphically traced in HBS Nomogram© by shifts along one MCHC trend-projection or *radiating line* (RL). The RCM specific safe deviation limits – MSD - from target states are also specified in the HBS Nomogram©

enabling graphical monitoring in HBS Graphics© part. More specifically, safe deviation limits are displayed in *Devi-safe*© nomogram. The new nomograms have proposed a brand new approach to monitoring RCM, BV, PV and osmolality.

Body tissues and fluid compartments are known to maintain different compliance in fluid handling. Vasomotor tone has an important role in blood flow distribution during homeostatic accommodations to changes in blood volume. This study is the first to propose *tissue homeostatic priority stratification* in relation to perfusion patterns. Changes in tissue perfusion with corresponding changes in tissue fluid compliance are described as major factors deployed by homeostasis in maintaining target states and opposing deteriorating plasma hydration shifts. Lymphatics is described as independent expandable fluid compartment with an exceptional role in plasma oncotic state regulation and accommodations during advanced deteriorations affecting blood volume. The current study is the first to investigate compensatory oncotic accommodations that strive to preserve plasma viscosity in advanced deteriorations of plasma hydration.

The new method proposed an algorithm for evaluation of plasma hydration: target states with normal (target) plasma hydration or proper plasma dilution origin deviations from target states can be uncovered by *volume loading tests* (VLT). Target state's verification is of major importance in guiding infusion and blood transfusion measures.

Existing methods of predicting post-transfusion Hct increase do not account for the volume of PRBC transfusion. The influence of pre-existing plasma dilution is also unverified. This is the first study that proposed PRBC transfusion volume calculation for proper Hct increase accounting for plasma dilution.

The blood volume that has to be exchanged for crystalloids and/or colloids during acute normovolemic hemodilution (ANH) in order to reach a preset target Hb is usually predicted by *the Bourke and Smith formula* developed in 1974. This and many other formulas are reported to overestimate the 'true' exchangeable blood volume (EBV). It endangers patients, because target Hb is usually missed and normovolemic anemia appears to be more severe than intended. More accurate mathematical models are needed to determine the exchangeable blood volume in order to increase patients safety and reduce blood transfusions. The HBS Nomogram proposed a brand new approach to by introducing target states specific RCM and tBV values maintained during blood loss.

3. REVIEW OF LITERATURE

3.1. CLINICAL PHYSIOLOGY

3.1.1. ***Homeostasis*** (3)

The body carefully controls a seemingly endless list of vital parameters by means of homeostasis. Homeostasis acts similarly at the level of a single cell and systemic parameters that affect the whole body, i.e. blood volume and arterial pressure. The major mechanism responsible for homeostasis is the *negative-feedback.* A single feedback loop does not operate in isolation, but rather as part of a larger network of controls. Thus a complex interplay may exist among feedback loops within single cells, within a tissue, within an organ or organ system, or at the level of the whole body. There is a competition among feedback loops. It acts according to proper physiologic hierarchy explained by *homeostatic redundancy.* It means, the more vital a parameter is, the more systems that the body mobilizes to regulate it. If one system should fail, others are there to help maintain homeostasis. A well regulated parameter is generally in a steady state. That is, its value is constant because the body or the cell carefully matches actions that lower the parameter value with other actions that rise it. The net effect is that the vital parameter is held at a constant value. An important principle in physiology is that each cell plays a specialized role in the overall function of the body. In return, the body provides the "milieu interieur", in which the tissues and cells of the organism live, appropriate for the life of each cell. As part of the bargain, each cell or organ must respect the needs of the body as a whole and not run amok for its own greedy interests. For example (2), during exercise, the increased production of sweat is targeted to reduce body core temperature, what ultimately reduces blood volume. If the individual stops exercising, the blood volume, which is considered in the higher hierarchy level than core temperature, will inform the system to reduce the production of sweat. However it does not work, if exercise continues, because it would lead to heat stroke. It works because of the *adaptability* of an organism. Flexible feedback loops are at the root of many forms of physiological adaptation. Medicine takes its physicochemical principles from physiology. One malfunction (e.g., heart failure) can lead to a primary pathologic effect (e.g., a decrease in cardiac output) that in chain reaction style leads to a series of

secondary effects (e.g., fluid overload) that are the appropriate responses to physiological feedback loops.

3.1.2. ***Body fluid compartments*** (8,73)

Body cells live in a carefully regulated fluid environment. The fluid inside the cells, the intracellular fluid (ICF), occupies what is called the intracellular compartment, and the fluid outside the cells, the extracellular fluid (ECF), occupies the extracellular compartment. The barriers that separate these two compartments are the cell membranes. For life to be sustained, the homeostasis rigorously maintains the volume and composition of both compartments. Total body water (TBW) is the sum of the IVF and ECF volumes. It is approximately 60% of the total body weight in a young adult human male, approximately 50% in female and 65% to 75% in an infant. The fraction of total body weight contributed by water is not constant for all individuals under all conditions. For example, variability of the amount of adipose tissue can influence the fraction. Referring to the anatomy of the body fluid compartments, the prototypic 70 kg male has approximately 42 liters of TBW. Of these 42 liters, approximately 60% is ICF and 40% - ECF. The ECF is composed of blood plasma, interstitial fluid and transcellular fluid. Total body water is distributed among plasma, interstitial, intracellular and transcellular fluids. Of the approximately 17 liters of ECF, the only approximately 20% is contained within the cardiac chambers and blood vessels, that is, within the intravascular compartment. The total volume of this intravascular compartment is the blood volume, approximately 5.5 liters. The extracellular 3.0 liters of the blood volume is plasma volume. The balance, approximately 2.5 liters, consists of the cellular elements of blood: erythrocytes, leukocytes and platelets. Plasma is a watery solution of electrolytes, plasma proteins, carbohydrates and lipids. Many proteins are involved in blood coagulation through a “coagulation cascade”. Serum is the fluid part of plasma that does not contain fibrinogen and other coagulation factors. The proteins and, to much lesser extent, the lipids in plasma ordinary occupy approximately 7% of the total plasma volume. About 75% of the ECF is located outside the intravascular compartment, where it bathes the nonblood cells of the body. Within this interstitial fluid there are two smaller compartments that communicate *only slowly* with the bulk of the interstitial fluid: dense connective tissue, such as cartilage and tendons, and bone matrix. The barriers that separate the intravascular and interstitial compartments are the walls of capillaries. Water and solutes can move between the interstitium and plasma by crossing capillary walls, and between the interstitium and

cytoplasm by crossing cell membranes. Finally, approximately 5% of ECF is trapped within spaces that are completely surrounded by epithelial cells. This transcellular fluid includes the synovial fluid within joints and the cerebrospinal fluid surrounding the brain and spinal cord. It does not include contents of the gastrointestinal tract or urinary bladder.

3.1.3. ***Solute composition of key fluid compartments*** (8,74,75)

Not only do the various body fluid compartments have very different volumes, they also have radically different compositions. The ICF is rich in K^+, whereas the ECF is rich in Na^+ and Cl^-. The plasma and interstitial fluid have very similar composition as far as small solutes are concerned. For most cells, it is the composition of the interstitial fluid enveloping the cells that is the relevant parameter. The major difference between plasma and interstitial fluid is the absence of plasma proteins from the interstitium. The plasma proteins, which cannot equilibrate across the walls of most capillaries, are responsible for the usually slight difference in small-solute concentrations between plasma and interstitial fluid. Plasma proteins affect solute distribution because of the volume they occupy and the electrical charge they carry.

3.1.4. ***Osmolality*** (8,76)

All body fluids have approximately the same osmolality, and each fluid has equal numbers of positive and negative charges. Despite the differences in solute composition among the intracellular, interstitial and plasma compartments, they all have approximately the same osmolality. Osmolality describes the total concentration of all particles that are free in solutions. Thus, glucose contributes one particle, whereas fully dissociated NaCl contributes two. Particles bound to macromolecules do not contribute at all to osmolality. In all body compartments, humans have an osmolality – expressed as the number of osmotically active particles per kilogram of water – of approximately 290 mosmoles/kg water (290 mOsm). Moreover, even though the protein concentration – measured in terms of grams per liter – may be high, the high molecular weight of the average protein concentration – measured in terms of moles per liter – is very low. Thus, proteins actually contribute only slightly to the total number of osmotically active particles (~1 mOsm).Summing the total concentration of all the solutes in the cells and interstitial fluid (including metabolites) The total solute concentration of the intracellular compartment is than that of the interstitium. Because the flow of water across cell membranes is governed by differences in osmolality across the membrane,

and because the net flow is normally zero, intracellular and extracellular osmolality must be the same.

3.1.5. ***Electroneutrality*** (8)

All solutions respect the principle of bulk electroneutrality: the number of positive charges in the overall solution must be the same as the number of negative charges. The excess positive charge is balanced by the negative charge on intracellular macromolecules (e.g., proteins), as well as smaller anions such as organic phosphates. There is a similar difference between major cations and anions in plasma, where it is often referred to as the anion gap. The clinical definition of anion gap is:

$$\text{Anion gap}_{\text{plasma}} = [Na^+]_{\text{plasma}} - ([Cl^-]_{\text{plasma}} + [HCO3^-]_{\text{plasma}})$$

Note that plasma $[K^+]$ is ignored. The anion gap, usually 9-14 meq/liters, is the difference between ignored anions and ignored cations. Among the ignored anions are anionic proteins, as well as small anionic metabolites. The gap increases in various pathological conditions, like type 1 diabetes. The differences in ionic composition between the ICF and ECF compartments are extremely important for normal functioning of the body. Disturbances may be life-threatening, e.g. disorders of extracellular $[Na^+]$ Cause abnormal extracellular osmolality, with water being shifted into or out of brain cells; if uncorrected, such disorders lead to seizures, coma and death. This example emphasizes the necessity of understanding the processes that control the volume and composition of the body fluid compartments. These processes are the one that move water and solutes between the compartments and between the body and the outside world.

3.1.6. ***Water transport and the regulation of cell volume*** (8,73-76)

Water transport is driven by osmotic and hydrostatic pressure differences across membranes. Transport of water across biologic membranes is always passive. No water pumps have ever been described. Plasma membranes of many types of cells (e.g., erythrocytes and the renal proximal tubule) have specialized water channels – the aquaporins (AQP2) – that serve as passive conduits of water transport. Water transport across the membrane is always linear, nonsaturable function of its net driving force. The direction of the net passive transport of an uncharged solute is always down its chemical potential difference, which depends on the difference in water concentration on the two sides of the membrane.

The second is the energy difference that results from the difference in hydrostatic pressure across the membrane. Thus, the relevant energy difference across the membrane is the sum of the chemical and pressure potential differences. Water is in equilibrium across the membrane when the net driving force for water transport is nil. At equilibrium the osmotic pressure difference is equal to the hydrostatic pressure difference. As plasma membranes are not rigid, the hydrostatic pressure difference across cell membrane is virtually always near zero and is therefore not a significant driving force for water transport.

Movement of water in and out of cells is driven by osmotic gradients only, that is, by difference in osmolality across the membrane. It is called *osmosis*. Increasing extracellular osmolality by adding an impermeant solute such as mannitol, leads to extracellular hyperosmolality and exerts an osmotic force that draws water out of cells. Many types of cells respond to shrinking by activating solute uptake to increase water content. This response is known as a *regulatory volume increase* (RVI). Cells oppose hypoosmotic swelling by means of activating solute-efflux pathways known as *regulatory volume decrease* (RVD).

3.1.7. ***Water exchange across the capillary wall*** (8)

The barrier separating plasma and interstitial compartments – the capillary wall – is freely permeable to solutes that are smaller than plasma proteins. Thus any difference in osmotic pressure as a result of these small solutes does not exert a driving force for water flow across that capillary. The situation is quite different for plasma proteins, which are too large to penetrate the capillary wall freely. As a result, the presence of a greater concentration of plasma proteins in the intravascular compartment than in the interstitial fluid set up a difference in osmotic pressure that tends to pull fluid back into the capillaries. This difference is called the *colloid osmotic pressure* or *oncotic pressure*. Water is in equilibrium across the capillary wall when the oncotic and hydrostatic pressure differences are equal. When the hydrostatic pressure difference exceeds the oncotic pressure difference, the resulting movement of water out of the capillary is called *ultrafiltration.* All net movements across the capillary wall are accompanied by the small solutes dissolved in this water at their ECF concentrations. That is the pathway taken by the water across the capillary wall is so large that small solutes are not sieved out. I few regard plasma osmolality of 290mOsm as being normal, solutions with values equal to290mOsm are *isosmolal*, above it - *hyperosmolal*, and those with osmolality below it – *hypo-osmolal*. The terms *isotonic, hypertonic and*

hypotonic are used comparing solutions separated by the well-defined membrane, e.g., cell membrane. *Tonicity* or *effective osmolality* is an ability of proper solution to induce cell volume shifts if added to ECF with different tonicity. It is highly dependable on solutes permeability. If the ECF osmolality is increased by impermeant solute such as mannitol, the resulting osmotic gradient across the cell membrane causes water to move out of the cell. Meanwhile changes in the concentration of highly permeant solution such as urea, have no effect on tonicity. Adding various combinations of NaCl and solute-free water to the ECF alters the volume and composition of the body fluid compartments.

3.1.8. ***Infusion of isotonic saline*** *(0.9% NaCl)* (8)

Isotonic saline (NS), which is a 0,9% solution of NaCl in water, has a *tonicity* of 290mOsm in the ECF. Adding this solution to ECF does not alter its osmolality, therefore no change occurs in the effective osmotic gradient across the cell membranes, and the added water neither moves in nor out of cells.

Infusion of isotonic (5%) glucose solution (8)

Infusing the glucose solution intravenously is equivalent, in the long run, to infusing pure water because the glucose is metabolized to CO_2 and water, with no solutes left behind in ECF. This added water dilutes the pre-existing solutes in ECF, therefore lowering ECF osmolality, creating an appropriate osmotic gradient that favors the entry of water from the EFC into the ICF until osmotic equilibrium is restored. The added water is distributed between the ECF and ECF according to the initial ICF/ECF ratio of 60% to 40%. Thus infusion of solute-free water is a relatively ineffective means of expanding the ECF, because more of the added water ends up intracellularly. The major effect was to dilute the osmolality of body fluids.

3.1.9. ***Ingestion of pure NaCl salt*** (8)

The two extremely important principles that govern fluid and electrolyte homeostasis are as following: 1). Adding or removing solute-free water mainly affects the osmolality of body fluids; 2). Adding or removing Na^+ mainly affects ECF volume. The ingested water-free NaCl amount equal to the similar infused as normal saline solution will be distributed rapidly throughout the ECF increasing its osmolality. The resulting hyperosmolality draws water out of cells into ECF until osmotic equilibrium is established. The total body Na^+ content is the major determinant of ECF volume.

3.1.10. ***Homeostasis of ECF volume and osmolality*** (8,74-76)

Two separate but closely interrelated control systems regulate the volume and osmolality of the ECF (App.Fig.A-1). It is important to regulate ECF osmolality in order to maintain cell volume and function. The body regulates osmolality by monitoring and adjusting the total-body water content. It is also important to regulate ECF volume to maintain blood pressure, which is essential for adequate tissue perfusion and function. The body regulates ECF volume by monitoring and adjusting the total-body content of NaCl. These two homeostatic mechanisms use different sensors, different hormonal transducers and different effectors. They have one thing in common: some of their effectors, although different, are located in the kidney. In the case of ECF volume, the control system modulates the urinary excretion of Na^+; meanwhile in case of osmolality it modulates urinary excretion of water. The water content of the body is the main determinant of osmolality, because total-body osmolality is defined as total-body osmoles to total body water. Meanwhile, the ECF-volume control system can regulate the amount extracellular osmoles, but it has little effect on total-body osmoles. Total-body osmoles are largely a function of the intracellular milieu, because the intracellular compartment is large and its solute composition is highly regulated. Total-body osmoles do not change substantially except during growth or during certain disease states, such as diabetes mellitus (in which excess glucose increases total-body osmolality). The body can control osmolality only by controlling water independently from control of Na^+. Changes in body-water content lead to changes in osmolality, to which CNS is extremely sensitive. (73) Osmolality deviations of ± 15% lead to severe disturbances of CNS function. Thus, osmolality is critical. Two elements control water content, and thus whole-body osmolality: 1). The kidneys by controlling water excretion, and 2). Thirst mechanisms that control oral water intake. These two effectors are part of the negative feedback loops that begin with hypothalamus. An increase in osmolality stimulates separate osmoreceptors to secrete AVP to reduce water excretion and to trigger thirst for increase in water intake. As a result, the two complementary feedback loops stabilize osmolality and thus $[Na^+]$. In healthy individuals, plasma osmolality is approximately 290 mOsm. The threshold for AVP release is somewhat lower, approximately 280 mOsm. (73) Increasing the osmolality by only 1% above this level is sufficient to produce a detectable increase in plasma AVP, which rises steeply with further increases in osmolality. Thus, hyperosmolality leads to increased levels of AVP, which completes the feedback loop by instructing the kidneys to retain water. Although it is a change in plasma [NaCl] that is usually responsible for a change in

plasma osmolality, other solutes can do the same. For example, mannitol resembles NaCl in stimulating AVP release.

3.1.11. ***Homeostasis of effective circulating volume*** (74-76)

Changes in ECF volume are important because they are accompanied by proportional changes in plasma volume, which in turn affects the adequacy with which the circulatory system can perfuse vital organs with blood. The blood volume that is necessary to achieve adequate perfusion of key organs is referred to as the *effective circulating volume*. Because the body generally stabilizes osmolality, an increase in extracellular Na^+ content will increase ECF volume. The body regulates the effective circulating volume in a very special way: increase in effective circulating volume, which reflects an increase in ECF volume and total-body Na^+ content, stimulate the renal excretion of Na^+; in contrast, plasma Na^+ concentration does not regulate Na^+ excretion. It is because Na^+ concentration is not an indicator of ECF volume. In summary, when osmolality is kept constant, the Na^+ content determines ECF volume; when Na^+ content is kept constant, the total-body water content determines osmolality. Therefore, the body regulates osmolality very carefully. A small decrease in osmolality triggers osmoreceptors to diminish thirst and consequently solute-free water intake, and increase renal water elimination. However, in emergency states of very low ECF and effective circulating volume, a unique "crosstalk" occurs between the volume and osmolality control systems resulting in paradox preservation of Na^+, seeking water (increased thirst) and conserving it by concentrating the urine. During *exercise*, maintaining an adequate effective circulating volume can take precedence over maintaining cutaneous blood flow for temperature regulation (App.Fig.A-2). Effective circulating volume tends to fall during prolonged exercise (2), especially in the heat, for three reasons: 1). plasma water shifts from the intravascular to interstitial space mainly as a result of increased hydrostatic pressure in capillaries and raising osmolality within muscle cells, that way attracting water from the extracellular space, 2). loss of total-body water by evaporation of perspiration, 3). redistribution of blood flow by increasing perfusion of working muscles and the skin (in response to body heating). Perspiration is hypotonic in respect to plasma, therefore preserving extracellular NaCl and blood volume. If perspiration was isotonic, plasma osmolality would not transiently increase and no water would shift from ICF to ECF space, therefore leading to hypovolemic shock, just like in hemorrhage.

3.1.12. ***Integration of salt-water balance and effective circulating volume*** (74-76)

The maintenance of the ECF volume or Na^+ balance depends on signals that reflect the effective circulating volume. The ECF volume receptors are as follows:

1. Central vascular sensors

- Low-pressure (very important)

Cardiac atria

Pulmonary vasculature

- High-pressure (less important)

Carotid sinus

Aortic arch

Juxtaglomerular apparatus (renal afferent arteriole)

2. Sensors in the CNS (less important)

3. Sensors in the liver (less important)

Low and high-pressure baroreceptors in the circulation send afferent signals to the brain, which translates this "volume signal" into several responses that can affect ECF over either the short or the long term (App.Fig.A-3). The *short-term responses* occur over a period of seconds to minutes as the autonomic nervous system and humoral mechanisms modulate the heart and blood vessels to control blood pressure. The *long-term responses* occur over a period of hours to days as nervous, humoral and hemodynamic mechanisms modulate the kidney to control Na^+ excretion. Relatively small changes in Na^+ excretion lead to marked alterations in the ECF volume. Thus, precise and sensitive control mechanisms safeguard and regulate the body's content of Na^+. In the steady state, Na^+ intake via the gastrointestinal tract equals Na^+ output from renal and extrarenal pathways. The maintenance of osmolality, or water balance, depends on osmoreceptors in the hypothalamus that detect changes in the plasma osmolality. These receptors send signals to areas of the brain that 1). control thirst, thus regulating water intake, and 2). control the production of *arginine vasopressin* (AVP) – also known as *antidiuretic hormone* (ADH) – thus regulating water excretion by the kidneys. However, it is not the ECF volume as a whole, but the effective circulating volume, that regulates the entire feedback loop, of which water excretion is the end point. Only certain regions of the extracellular compartment are important for appropriate signaling. For an expansion in ECF volume to stimulate Na^+ excretion, the expansion must make itself evident in the part of the ECF compartment, where the ECF volume sensors are located. The thoracic blood vessels appear to be the site of greatest importance. The same ECF volume may be sensed differently by the receptors under the

influence of such factors as gravity. Therefore the body recognizes the effective circulating volume as an index of changes in Na^+ content (App.Fig.A-4). The effective circulating volume is not something that can be identified anatomically. Rather, it is a functional blood volume that reflect the extension of tissue perfusion in specific regions, as evidenced by the fullness or pressure within their blood vessels. However, this relationship may be distorted in certain diseases. For example, in patients with congestive heart failure, nephrotic syndrome or liver cirrhosis, total ECF volume is grossly expanded, although the effective circulating volume is low, resulting in Na^+ retention (App.Fig.A-5).

3.1.13. ***Responses to decrease in effective circulating volume*** (74-76)

Low and high-pressure receptors sense decreases in effective circulating volume and use four parallel effector pathways to decrease renal Na^+ excretion. Sensors of the feedback loop that controls the effective circulating volume, are baroreceptors located in both high pressure and low pressure areas of the circulation. Although most are located within the vascular tree of the thorax, additional baroreceptors are present in the kidney, central nervous system and the liver. These sensors generate four distinct hormonal or neural signals. In the first pathway, a reduced effective circulating volume directly stimulates a hormonal effector pathway – the *renin-angiotensin-aldosterone* system. The second and third effector pathways are neural. Baroreceptors detect decreases in effective circulating volume and communicate these via afferent neurons to the medulla of the brain stem. Two types of efferent signals emerge from medulla, and act on the kidney. One signal increases activity of the sympathetic nervous system reducing renal blood flow, and thus decreasing Na^+ excretion. In the other effector pathway, the posterior pituitary increases its secretion of AVP, and thus conserves water. The latter mechanism becomes active only after large declines in effective circulating volume. The final pathway is hormonal. It results in decreased release of atrial natriuretic peptide (ANP), thus reducing Na^+ excretion. Large decreases in effective circulating volume and blood pressure not only stimulate the release of AVP, they also profoundly stimulate the sensation of thirst. In fact, hemorrhage id one of the most powerful stimuli of hypovolemic thirst. “Thirst among the wounded in the battlefield is legendary” (Fitzsimons). Therefore, three distinct stimuli – hyperosmolality, profound volume reduction and decreases in blood pressure – lead to the sensation of thirst. Low effective circulating volume and low blood pressure stimulate thirst centers in the hypothalamus

via the same pathways by which they stimulate AVP release. In addition to thirst, some of these hypothalamic areas are involved in stimulating the desire to ingest salt (Na^+).

3.1.14. ***Redundancy of response mechanisms*** (74-76)

An important feature of renal Na^+ excretion is the *two-way redundancy* of control mechanisms that react to decrease in effective circulating volume. First, several efferent pathways may act in synergy on a single effector within the kidney. For instance, both sympathetic and hemodynamic/physical factors often act on proximal tubules. Second, one efferent pathway may act on different effector sites – for example, regulating Na^+ reabsorbtion *directly* by modulating Na^+ transport by tubule cells and *indirectly* by modulating renal hemodynamic parameters. In particular, *angiotensin II* (ANG II) enhances Na^+ retention not only by stimulating Na-H exchange in tubule cells, but also by lowering renal plasma flow. Although a mere 1% increase in plasma osmolality stimulates AVP release by a detectable amount, the fairly large reductions in effective circulating volume (5-10%) are required to stimulate similar AVP release. However, once the rather high threshold for nonosmotic release of AVP is exceeded, AVP release rises steeply with further volume depletion. Thus, the effective circulating volume modifies the slope of relationship between plasma AVP levels and osmolality, as well as the osmotic threshold for AVP release. Therefore, during volume depletion, a low plasma osmolality that would normally inhibit the release of AVP allows its secretion to continue. Two clinical examples in which reduced effective circulating volume leads to increases in AVP are severe hemorrhagic and hypovolemic (i.e., in cholera) shock. In both cases, the water retention caused by AVP release accounts for the accompanying hyponatriemia. As already discusses above, appropriate renal response to decreased effective circulating volume is to retain Na^+ (i.e., normal saline). Why is that in response to shock, the body also retains free water? Compared with isotonic saline, free water is less effective as an expander of the ECF volume. Nevertheless, in times of profound need, the body uses free water retention to help expand extracellular (and plasma) volume. It tolerates some extra hypoosmolality of the body fluids as the price for blood volume resuscitation in striving to maintain optimal effective circulating volume. A clinical example in which reduced effective circulating volume can lead to an inappropriate increase in AVP levels is congestive heart failure. In this situation, the water retention may be so severe that the patient develops hyponatriemia and hypoosmolality. The threshold for AVP release also decreases in pregnancy, and under the influence of such factors as pain, nausea, and several drugs (e.g., morphine and high