DEVELOPMENT OF CHRONIC SEVERE HYPOVOLEMIC HYPONATREMIA BY CHRONIC HEMORRHAGE AND ITS INFLUENCE ON RENAL CONCENTRATING ABILITY

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PREFACE

The observations and studies presented herein provide some basic information and a model by which more information can be generated. Severe hypovolemic hyponatremia can be caused by chronic hemorrhage concomitant with sodium restriction. During severe hypovolemic hyponatremia renal concentrating ability is impaired, renal medullary osmolality is markedly decreased, and ADH release appears to be submaximal. This model is the first of chronic severe hypovolemic hyponatremia in the euadrenal animal. It should facilitate further investigation of the interaction of osmotic and nonosmotic factors which influence ADH release, thirst, and urine concentration.

A deviated format is used to maintain the integrity of Chapters II, III, and IV which were prepared for publication in referred scientific journals. The deviations include the following changes in format. Each of these chapters contain an abstract. Tables are numbered by chapter and the list of tables reflects this change. Footnotes indicating instrumentation or methodology are contained parenthetically in the text. To avoid redundancy the bibliography has been placed in its normal position.

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

Starling, in 1909, stated, "the kidney presents in the highest degree the 'phenomenon of sensibility', the power of reacting to various stimuli in a direction which is appropriate for the survival of the organism; a power of adaption which almost gives one the idea that its component parts must be endowed with intelligence." (Starling, 1909; Reineck et al, 1985). The ability of the kidney to respond appropriately to many different complex situations is almost casually accepted. The mechanisms by which the kidney's response is regulated, both intrarenally and extrarenally have been intensely investigated during the last half century. Countercurrent systems, both active and passive, have been hypothesized and supported, renal medullary hyperosmolality has been verified, and osmotic and nonosmotic factors have been shown to interact in control of urine dilution and concentration.

The Countercurrent System

Kuhn and Ryffel (1942) hypothesized that the loop of Henle functioned as a countercurrent "multiplier." They theorized that a small transverse difference in osmotic pressures was augmented by countercurrent flow, resulting in

a large axial difference in osmotic pressures between cortex and papilla. They developed a model to test their hypothesis. Its basic structure consisted of three adjacent channels (channels 1, 2, and 3). Channels 1 and 2 were joined at one end. Initially the contents of all three channels were isosmotic and consisted of a 0.1M sucrose solution in channels one and two and a 0.1M phenol solution in channel three. A cupric ferrocyanate membrane, permeable to water but not to sucrose or phenol, separated channels one and two. A rubber membrane, permeable to phenol but not to water or sucrose, separated channels two and three. Phenol from channel three permeated the rubber membrane to enter channel two. The entry of phenol into channel two raised the osmotic pressure of its contents, causing the withdrawal of water from channel one, which increased the concentration of sucrose in channel one until, at steady state, the osmotic pressures became equal on both sides of the cupric ferrocyanate membrane. Continuity of flow brought the now more concentrated sucrose solution from channel one to channel two, where the entry of phenol further raised its osmotic pressure. In its course through channel two, the flow rate of the sucrose/phenol solution increased as more water was added from channel one. The osmotic pressure of the solution in channel two, although always higher than that in channel one at any given point, progressively decreased from the hairpin turn. Channels one and two were intended to represent a loop of Henle. Channel

three was not meant to be representative of any structure in the kidney; it was only to supply energy.

Wirz and coworkers (1951) using cryoscopy of renal tissue from antidiuretic rats showed that the osmolalities of the cortex and plasma were identical, but the osmolality of the renal medulla increased progressively from the corticomedullary junction to the papillary tip. These findings verified one of the predictions from Kuhn's hypothesis, i.e., that the medulla and its contents were hypertonic. Hargitay and Kuhn, in that same year, revised and expanded version of their original model (Hargitay and Kuhn, 1951). In this model the active driving force was hydrostatic pressure rather than phenol solution, and the membrane was permeable to water but not to solute. With the countercurrent system partially verified by Wirz's findings, intensive investigation began on the mechanism(s) by which renal countercurrent exchange and multiplication might occur. Because hydrostatic pressures in excess of those found *in vivo* were required to produce the measured medullary osmolalities (Wirz and Kuhn, 1951), an alternative source of energy (active transport of salt from the ascending limb to the descending limb) was proposed by Wirz (1953 and 1956). This concept was incorporated into a mathematical model by Kuhn and Ramel (1959). In this model, the solution in the descending limb was concentrated by solute addition rather than by water removal. Although many

other models were subsequently developed, none were generally accepted.

In 1972, because of failure to find evidence for active solute transport in the thin limb of Henle, Stephenson proposed two new models (Stephenson, 1972) which took into account the observation, originally made by Gamble and his coworkers (1934), that urea administration enhances maximum urinary concentration in protein-deprived animals. Stephenson suggested that the basic unit responsible for concentration consists of a vascular space (central core) surrounded by the descending and ascending limbs of the loop of Henle and a collecting duct. The vascular space (Vasa recta plus interstitium) is closed at the tip of the papilla and is in communication with the systemic circulation by its opened end at the cortical medullary junction. The permeability of the vasa recta to small solutes is assumed to be so large that vasa recta and interstitial space merge into a single fluid filled space. Because passive transport of a single solute out of the ascending limb was thought to be insufficient to account for the cortical medullary osmotic gradient found *in vivo*, Stephenson considered two modes of operation, one driven by the active transport of a single solute and the other involving the passive transport of two solids, sodium chloride and urea. In 1978, Stephenson confirmed his suspicion that passive transport of a single solute out of the ascending limb was insufficient to account for the corticomedullary osmotic gradient found

in vivo (Stephenson, 1978). Stephenson hypothesized that the two models operated together. Model one was of active solute transport. Three chambers, a, b, and c, contained an equiosmolar solution of salt. Membrane one (a-b) was permeable to water but not to salt, and membrane two (b-c) actively transported salt but was impermeable to water. Transport of salt from c to b raised the osmotic pressure of b, and this in turn extracted water from a. At equilibrium, chambers a and b contained a concentrated solution of salt and chamber c a dilute solution of salt. Model two was one of solute mixing. In this case, chambers a and b were filled with an equimolar solution of urea and chamber c with an equiosmolar salt solution. Membrane (a-b) was permeable to water but not to urea and membrane (b-c), although permeable to salt, did not actively transport salt. Salt diffused across membrane two from chamber c until its concentrations were equal on both sides of the membrane. The resulting increased osmotic pressure in b extracted water from a until the osmotic pressure equalized on both sides of membrane one. At equilibrium, chamber a contained a concentrated solution of urea, chamber b, a mixed solution of salt and urea, and chamber c, a dilute solution of salt. Both models resulted in a larger volume in chamber b.

Kokko and Rector, also in 1972, independently hypothesized a specific example of Stephenson's general model (Kokko and Rector, 1972). This model involved countercurrent multiplication in the inner medulla without

active transport. The critical assumption of the Kokko-Rector model is that fluid is concentrated in the descending limb almost entirely by water extraction. This assumption has not been verified experimentally *in vivo*. An alternative model devised by Bonventrie and Lechene (1980) assumes substantial solute addition to the descending-limb fluid. The Kokko-Rector and the Bonventrie-Lechene models are the current working models. However, both fail to satisfy one or more of the requirements of a functional countercurrent system (Jamison and Robertson, 1979; Jamison, 1983).

Antidiuretic Hormone (ADH)

Biochemistry, Synthesis, and Secretion of Vasopressin

Vasopressin has both vasoactive and antidiuretic properties. When referring to its antiduretic properties this hormone is generally called ADH. The antidiuretic hormone of humans and most other mammals is arginine vasopressin (Du Vigneaud, 1956). It is a nonapeptide (9 amino acids) containing an intrachain disulfide bridge and a tripeptide tail on which the terminal carboxyl group is amidated. In pigs and other members of the suborder Suina, arginine, at the number 8 position, is substituted for lysine, yielding lysine vasopressin (Sawyer, 1967). Substitution of isoleucine for phenylalanine at position three and leucine for arginine at position 8 yields

oxytocin, which is a strong stimulator of smooth muscle contraction in mammary glands and the uterus but has little antidiuretic effect (Sawyer, 1967).

ADH is produced by the neurohypophysis (Scharrer and Scharrer, 1954; Haymaker, 1969). Brownstein, Russell, and Gainer (1980) demonstrated that vasopressin was synthesized by way of a peptide precursor or prohormone with a molecular mass of approximately 20,000 Daltons. This concept was supported by Land, and his colleagues (1982). This precursor is called propressophysin. Breslow (1974) had previously demonstrated that it is synthesized in cell bodies of the supraoptic nuclei, packaged in secretory granules, and cleaved progressively as it moves down the axon to yield neurophysin and vasopressin. In the nerve terminals, vasopressin and neurophysin are stored as insoluble complexes in the secretory granules. When released into the systemic circulation, vasopressin and neurophysin disassociate rapidly and completely because of the resultant dilution and increase in pH. Sachs (1969) demonstrated that biosynthesis of vasopressin is accelerated by dehydration and hypovolemia, stimuli which result in increased secretion of vasopressin. However, this compensatory response develops slowly and may never completely match the increased rate of release. As a consequence, neurohypophyseal stores of vasopressin may be severely depleted by a chronic secretory stimulus such as water deprivation as demonstrated by Moses and Miller

(1970). Zerbe, Miller, and Robertson (1980) demonstrated that secretion of vasopressin and its associated neurophysin occurred by a calcium-dependent exocytic process similar to that described for neurosecretory systems. According to this concept, secretion is triggered by propagation along the axon of an electrical impulse that causes depolarization of the cell membrane, an influx of calcium, fusion of secretory granules with the cell membrane, and extrusion of their contents. This concept is supported by the observation that vasopressin and its neurophysin are released simultaneously by many stimuli (Zimmerman and Robinson, 1976).

Biological Function

Vasopressin receptors are localized in arterial vessel walls (probably also in the capillaries), in the distal tubules of the kidney, and in the hypothalamus (Sandow and Kong, 1978). Activation of the receptors involves the formation of a ternary complex between the hormone, the binding site of the receptor, and magnesium ions. Receptor binding results in activation of an adenylcyclase system which requires the presence of calcium and magnesium ions. Response to receptor stimulation is also dependent upon the presence of arachidonic acid metabolites (Sandow and Kong, 1978). Normal levels of the adenylcyclase associated with the ADH receptors is dependent on normal plasma ADH concentrations (Dousa et al., 1975). Activation of ADH

receptors is responsible for resorption of water in the collecting tubules and ducts and, hence, concentration of urine. Antidiuretic hormone stimulated water movement is indirect and passive and is mediated by a change in water permeability of nephron segments (Herbert and Thomas, 1985).

These changes have been measured by three techniques: *in vivo* micropuncture, *in vivo* microcatheterization, and *in vitro* perfusion (Herbert and Thomas, 1985). Wirz, (1956) and Gottschalk and Mylle (1959), using a free-flow micropuncture technique, demonstrated that distal tubular fluid approached isotonicity during antidiuresis but remained dilute in water diuresis. In contrast, Clapp and Robinson (1966) and Berliner, and Bennett (1967) demonstrated that in the dog and monkey, respectively, distal tubular fluid remained dilute even during hydropenia. Sonnenberg (1974) used microcatheterization *in vivo* to show that in the hydropenic state the collecting duct fluid osmolality increased progressively from the outer medulla to the papillary tip. Morgan and his coworkers (1968) used microperfusion techniques to study segments of the collecting duct *in situ* and also *in vitro* in medullary slices. They demonstrated that ADH increased diffusional water permeability and osmotic water permeability in the collecting ducts (Morgan et al., 1968; Morgan and Berliner, 1968). These actions have been demonstrated to be associated with an ADH-sensitive adenylate cyclase (adenylcyclase) (Morel et al., 1976), which is dependent

upon arachidonic acid metabolism with production of prostaglandin (PG) E and PGF_2 (Sandow and Kong, 1978). Several investigators have demonstrated that the ADH-mediated increase in cortical collecting tubule permeability is highly selective (Grantham and Burg, 1966; Grantham and Orloff, 1968; Schafer and Andreoli, 1972a and 1972b). ADH increases both the diffusional and osmotic water permeabilities, but permeability to small hydrophilic nonelectrolytes having molecular dimensions similar to that of water remains slow. This characteristic has also been demonstrated in synthetic lipid bilayers (Finkelstein, 1976; Cass and Finkelstein, 1967). Thus, the antidiuretic response involves a profound increase in the water permeability of collecting duct epithelium, which can discriminate between water and hydrophilic solute. Ganote and colleagues (1968) and Grantham and coworkers (1969) demonstrated that the final site of ADH action on water and solute permeability is at the apical (luminal) epithelial surface. These investigators used transmission electron micrographs of tubules fixed at various stages of ADH response to show that in the presence of lumen-to-bath osmotic flow, there were increases in the apex-to-base cell height, intracellular vacuolation, and distention of lateral intercellular spaces. These observations indicated that the apical surfaces of ADH-responsive epithelia are the rate-limiting barriers for water flow and that these surfaces respond to ADH by increasing water permeability. They,

however, did not indicate whether the hormone altered the water permeability of apical plasma membranes or of junctional complexes. Recently, Kirk and coworkers have confirmed these earlier morphologic observations on living cells of the rabbit cortical collecting tubule using computer-assisted analysis of *in vitro* perfused tubules (Kirk et al., 1982). In these studies, the temporal sequence of events after adding ADH consisted of an early 30% increase in cell volume followed by the formation of large intracellular vacuoles. These events were produced only in the presence of luminal hypotonicity, i.e., during lumen-to-bath net volume flux, suggesting that the bulk of transepithelial water flow is transcellular rather than paracellular. Additional evidence against a significant component of paracellular water transport in the rabbit cortical collecting tubule was provided by Schafer and coworkers (1974). They compared ADH-independent osmotic water permeability and urea permeability for situations in which the permeability properties of junctional complexes either were unmodified, i.e., the control circumstances, or were altered by exposure to luminal hypertonicity.

On the other hand, some investigators (Ussing and Windhagger, 1964; Lindley et al., 1964; Ussing, 1966; DiBona and Avon, 1973) have demonstrated that in electrically tight epithelia, such as the toad bladder and frog skin, hypertonicity of the apical solution induces marked decreases in transepithelial electrical resistance and an

increase in junctional complex permeability to hydrophilic solutes. Associated with these changes are marked alterations, which may represent junctional complex opening, in the structure of junctional complexes. These changes have been observed by differential interference contrast microscopy *in vitro* (Di Bona, 1978) and by transmission electron microscopy of fixed specimens (Ussing and Windhager, 1964; Erlig and Martinez-Palomo, 1972). However, Schafer's studies (1974) indicated that in the rabbit cortical collecting tubule, bath-to-lumen volume flow produced by luminal hypertonicity was inadequate to account for the magnitude of ADH-dependent lumen-to-bath osmosis, even when luminal hypertonicity had increased the junctional complex urea permeability, i.e., had opened junctional complexes. These observations indicate that water flow through junctional complexes, even when the latter have relatively high urea permeabilities, is inadequate to account for the rate of lumen-to-bath (antidiuretic) water flow in the presence of ADH, when junctional complexes are rather impermeable to urea. Thus, these studies verified that lumen-to-bath water flow proceeds primarily through apical plasma membranes, rather than through junctional complexes, and that water permeability change induced by ADH occurs in the apical membranes of the collecting duct.

Hence, it has been demonstrated that ADH, via mechanisms which require arachidonic acid metabolism, increases water resorption in the collecting tubules and

ducts of all animals and in the distal convoluted tubules of some animals. The increased water resorption under the influence of ADH is apparently transcellular and is specific, i.e., movement of solids similar in size to water is not increased when movement of water is increased.

Osmotic Regulation of ADH Release

Verney (1947) delivered his classic Croonian Lecture entitled "The antidiuretic hormone and the factors which determine its release" to the Royal College of Physicians in London. This treatise and Verney's experimental work provided the basis of knowledge for the intensive investigation of ADH release during the subsequent years. Verney, using conscious, trained dogs, undergoing a water diuresis, demonstrated that the rapid injection of hypertonic saline over a 10 second period into a carotid artery was associated with a rapid fall in urine flow. This antidiuresis could be produced by similar intracarotid injection of nonsodium-containing solutions leading Verney to postulate the presence of osmoreceptors. The antidiuresis produced by intracarotid injections could be mimicked by the injection of posterior pituitary extract. When the right internal carotid artery was ligated no antidiuretic effect was stimulated by injection of hypertonic solutions into the right carotid artery. These findings led Verney to propose that osmoreceptors existed along the circulation of the internal carotid artery and

that stimulation of these osmoreceptors resulted in release of an antidiuretic substance (ADH) from the posterior pituitary gland. Later studies by Jewell and Verney (1957) localized this effect to the anterior hypothalamus.

For the next two decades considerable information was accrued concerning the anatomy and chemistry of the neurohypothesis but the concepts of osmoregulation of ADH were not advanced to any great extent beyond those proposed by Verney. However, with the advent of sensitive radioimmunoassays for arginine vasopressin, additional information further extending and refining Verney's original concepts is being produced. Using radioimmunoassay for ADH, Robertson and his coworkers (1974) demonstrated a close correlation between plasma ADH levels and plasma osmolality in patients in various stages of hydration. These authors defined the osmotic threshold for ADH release as the point of intercept on the horizontal axis (280 mosmol/kg water in the average human) and the sensitivity of the osmoreceptor as the slope of the linear regression line of plasma ADH concentration graphed against plasma osmolality. Weitzman and Fisher (1977) challenged the use of linear regression analysis to evaluate the functional properties of the osmoreceptors. These investigators felt that the results they produced in conscious sheep demonstrated an exponential rather than a linear relationship. Rodbard and Munson (1978) questioned Weitzman and Fisher's analysis. They

concluded, upon reviewing Weitzman and Fisher's results that the results were compatible with either an exponential or threshold model (Rodbard and Munson, 1978). The question of linearity is currently unresolved.

Robertson and colleagues (1976) demonstrated a very close relationship between urine osmolality and radioimmunoassay titers of plasma ADH in individuals with various states of hydration. These authors detailed the exquisite sensitivity and gain of the osmoreceptor -ADH- renal reflex. Their studies demonstrated that a normally hydrated man having a plasma osmolality of 287 mosmol/kg water, a plasma ADH level of 2 pg/ml and a urine osmolality of 500 mosmol/kg water will have a drop in plasma osmolality of 1% (2.8 mosmol/kg water), a plasma ADH decrease to one pg/ml, and his urine osmolality will diminish to 250 mosmol/kg water if his total body water is increased by only 1%. It is only necessary to increase total body water by 2% to suppress plasma ADH maximally (less than 0.25 pg/ml) and maximally dilute urine (osmolality less than 100 mosmol/kg of water). In the opposite direction, it was demonstrated that a 2% decrease in total body water will increase plasma osmolality by 2% (5.6 mosmol/kg water), plasma ADH will rise from 2 to 4 pg/ml, and urine will be maximally concentrated (greater than 1,000 mosmol/kg water). Thus a 1-mosmol rise in plasma osmalality would be expected to increase plasma ADH by 0.38 pg/ml and urinary osmolality by 100 mosmol/kg water (Robertson et al., 1976).

Studies by Dunn and colleagues (1973); Robertson, Athar, and Shelton (1977); and Hayward and coworkers (1976) performed in the rat, dog, and monkey, respectively, demonstrated a significant variation in the average osmotic threshold (285 to 292 mosmol/kg water) for these species compared to the average osmotic threshold for man (280 mosmol/kg water) (Beardwell, 1971; Fressinaud, 1974; Robertson, 1974). These studies also demonstrated that there is significant variation in osmotic thresholds between individuals of a species but that a single individual's osmotic threshold remains very constant throughout life.

The affect of different osmotic agents on ADH release was studied by Athar and Robertson (1974) and by Robertson and colleagues (1977). These studies demonstrated that only effective osmotic agents, i.e., those substances which do not readily pass cytoplasmic membranes, stimulate ADH release (Athar et al., 1974; Robertson et al., 1977). Urea infusion and glucose infusion did not result in significant ADH release; whereas, sodium infusion and mannitol infusion did. Robertson and coworkers proposed that an increase in plasma osmolalilty results in an osmotic gradient between the osmoreceptors' cytoplasm and the plasma (Robertson et al., 1977). If the osmoreceptors' cytoplasmic membranes are permeable to the substance eliciting the increase in plasma osmolality, i.e, the substance is not an effective osmotic agent, the substance crosses the osmoreceptors' cytoplasmic membranes into the osmoreceptors cytoplasm. This increases

the osmorecptors' cytoplasmic osmolality and decreases the plasma's osmolality dissipating the osmotic gradient. If the osmoreceptors' cytoplasmic membranes are impermeable to the substance eliciting the increase in plasma osmolality, i.e., the substance is an effective osmotic agent, the substance can not cross the osmoreceptors' cytoplasmic membranes and an osmotic gradient is created. Water is pulled out of the osmoreceptors' cytoplasm into the plasma until the osmoreceptors' cytoplasmic osmolality and the plasma's osmolalilty equilibrate. This results in loss of water from and subsequent shrinkage of the osmoreceptor. The volume change in the osmoreceptors stimulate ADH release. On the other hand, decreases in effective plasma osmolality have the opposite effect and the resultant swelling of the osmoreceptors inhibits ADH release (Robertson, 1977; Schrier et al., 1979).

A sodium receptor mechanism was proposed by Anderson (1971) in 1971, and investigated by Olsson and coworkers in a series of experiments (Erickson et al., 1971; Olsson, 1972; Olsson, 1973; Olsson et al., 1976). These authors based their assumption, that sodium receptors were present within the blood-brain barrier, on the observation that urea and glucose do not stimulate ADH release. The observation that hypertonic mannitol solutions elicit ADH release while causing decreases in sodium concentration contradict the sodium receptor theory and recognition that urea and glucose are not effective osmotic agents has obviated the need for

other receptor mechanisms. Consequently, the osmoreceptor concept has become generally accepted (McKinley et al., 1978; Rodbard and Munson, 1978; Dunn et al., 1973; Robertson, 1985; Robertson, 1981; Quillen and Crowley, 1983; Robertson et al., 1977; Kannan and Yagi, 1978; Robertson, 1980; Robertson and Athar, 1976).

Nonosmotic Regulation of ADH Release

Although the major focus of Verney's Croonian Lecture dealt with osmotic control of ADH, he and his colleagues also performed studies which examined the effect of "emotional stress" on urine flow in dogs experiencing water diuresis (Verney, 1947; O'Connor and Verney, 1942). Electrical stimulation of the flanks of dogs produced only a small and transient antidiuresis but was accompanied by a large and consistent increase in blood pressure. O'Connor, and Verney (1942) demonstrated that bilateral sectioning of the splenic nerves and denervation of the kidneys and adrenal glands resulted in a much stronger and more sustained antidiuresis in response to electrical stimulation of the dog's flanks. These authors also demonstrated that intravenous injections of norepinephrine impaired renal concentrating ability in response to antidiuretic stimuli. Several years after Verneys studies, several investigators demonstrated that intravenous norepinephrine causes water diuresis in both man (Baldwin et al., 1963; Smythe et al., 1952) and animals (Liberman et al., 1970; Klein et al.,

1971). Studies by Schrier and coworkers (1975) performed on the dog, rat, and man demonstrated that the effect of intravenous norepinephrine, in causing water diuresis, was dependent on an intact source of endogenous vasopressin (Schrier et al., 1975). Acutely hypophysectomized dogs, Brattleboro rats (hereditary cranial diabetes insipidus), and human patients with central diabetes insipidus receiving a constant infusion of vasopressin failed to demonstrate a diuretic response to a dose of noriepinephrine which increased water excretion in intact individuals. These studies supported Verney's interpretation that sympathetic stimulation interferes with "the chain of chemical reactions initiated in the central nervous system by the electrical stimulus and ending in release of posterior pituitary antidiuretic substance" (Verney, 1947).

Verney suggested that intracarotid injection of norepinephrine might be much more effective in causing a water diuresis than intravenous norepinephrine. Berl and his coworkers (1974a) performed experiments in dogs using an intracarotid dose of norepinephrine which was estimated to equal the amount reaching the cerebral circulation during the administration of intravenous norepinephrine. The intravenous, but not the intracarotid, norepinephrine caused an increase in solute free water excretion. This finding suggested that the effect of norepinephrine in suppressing ADH release was not direct but rather involved an extracerebral reflex mechanism. Berl, in these studies, also

demonstrated that inspite of an intact pituitary source of ADH release, intravenous norepinephrine failed to cause a water diuresis in baroreceptor-denervated animals (Berl et al., 1974a). Liberman, Klein, and Kleeman (1970) had shown that the effect of intravenous norepinephrine on increasing water excretion was abolished by alpha-adrenergic blockade. The combination of these two studies indicated that alpha-adrenergic stimulation primarily alters solute-free water excretion by suppressing ADH release via a baroreceptor mechanism.

Since alpha-adrenergic and beta adrenergic stimulation often oppose each other in various tissues, the effect of beta adrenergic stimulation on water excretion was examined in the rat, dog and man (McDonald et al., 1977; Schrier et al., 1972a; and Berl et al., 1974b). In these studies beta-adrenergic stimulation with isoproterenol caused antidiuresis in intact rats, dogs, and humans. This effect was not demonstratable in states of ADH deficiency in any of these species. These results and studies by Berl and his coworkers (1974c) showing the failure of intracarotid isoproterenol to induce antiduresis demonstrated a baroreceptor-mediated effect on ADH release similar to, but in the opposite direction, of alpha-adrenergic stimulation. Berl and his colleagues (1974c) performed experiments in which the low- (atrial) and high- (carotid sinus) pressure baroreceptors were sequentially denervated. A primary importance of the high-pressure baroreceptors was implicated

in the reflex stimulation of ADH release, since bilateral carotid sinus denervation, but not bilateral cervical vagotomy, abolished the antidiuretic response to beta-adrenergic stimulation. This series of experiments indicated that alpha- and beta-adrenergic stimulation alter, in oppposite directions, renal water excretion by modulating ADH release via baroreceptor pathways (Schrier et al., 1975).

Secretion of vasopressin is also affected by changes in blood volume and/or pressure (Robertson, 1977a and 1977b; Share, 1969; Buggy and Johnson, 1977). These hemodynamic influences are mediated largely, if not exclusively, by neurogenic afferents that arise in pressure-sensitive receptors in the cardiac atria, aortic arch, and carotid sinuses and travel via the vagal and glossopharyngeal nerves to primary synapses in the nuclei tractus solitarii in the brainstem (Share, 1969; Brennan et al., 1971; Wang et al., 1984). Studies by Blessing, Sved, and Reis (1983); Schrier, Berl, and Harbottle (1972b); and Thames and Schmidt (1979) indicate that the input from these pathways is predominantly negative or inhibitory under basal normovolemic, normotensive conditions, because their elimination results in an acute rise in vasopressin secretion. In healthy adult humans, monkeys, and rats, acutely lowering blood pressure by any of several methods increases plasma vasopressin by an amount that is roughly proportional to the degree of hypotension achieved

(Robertson, 1977a and 1977b; Fumox et al., 1978; Robertson, 1981). This stimulus-response relation follows a distinctly exponential pattern. Small decreases in blood pressure of the order of 5 to 10% usually have little effect on plasma vasopressin concentration, whereas decreases in blood pressure of 20 to 30% result in ADH levels many times that required to produce maximal diuresis. The influence of chronic hemodynamic alterations on vasopressin release have been studied only in patients with chronic hypovolemia (Robertson and Aycinena, 1982) or hypervolemia (Ganguly and Robertson, 1980) due to excess and deficient secretion of aldosterone, respectively.

Interaction of Osmotic and Nonosmotic Factors in Regulation of ADH Release

Numerous studies, including those of Robertson and Athar (1976); Robertson, Athar, and Shelton (1977); Dunn, et al. (1973); Quillen and Crowley (1983); and Wang et al. (1984) have demonstrated that changes in blood volume or pressure large enough to affect vasopressin secretion do not interfere with osmoregulation of the hormone. Instead, they appear to act by shifting the set of the system (osmoreceptor threshold) in such a way as to increase or decrease the effect of a given osmotic stimulus on vasopressin release. Thus, in the presence of a hemodynamic stimulus, plasma vasopressin continues to respond appropriately to small changes in plasma osmolality. Hence,

vasopressin release can still be fully supressed if plasma osmolality falls below the lowered threshold. The authors of these studies conclude that the retention of the threshold function insures that the level of free water retention and hyperosmolality can be regulated and limited, even in the presence of significant hypovolemia or hypotension (Robertson and Athar, 1976; Robertson et al., 1977; Dunn et al., 1973; Quillen and Crowley, 1983; Wang et al., 1984). These investigators' findings also support studies by Kannan and Yagi (1978) and Robertson, Athar, and Shelton (1977) which indicate that the osmoregulatory and baroregulatory systems, though different in location and function, ultimately converge and act on the same population of neurosecretory neurons. The site of this integration is unknown but is proposed to involve one or more interneurons linking the osmoreceptor and neurosecretory cells (Robertson, 1980).

Research Problem and Approach

Evaluation of renal concentrating ability is one of the most common and useful mechanisms of separating prerenal azotemia from renal azotemia. Studies by Bradford (1899) and by Hayman and colleagues (1939) demonstrated that blood urea nitrogen levels begin to increase when 2/3 or more of the functional renal mass is lost, but renal concentrating ability persists until 75% or more of the functional renal mass is lost. Diagnostic application of these studies

allow separation of prerenal from renal causes of azotemia (Hardy and Osborne, 1979). Demonstration of the inability to concentrate urine, in the absence of diabetes insipidus, in response to dehydration, hypotension, or in the presence of azotemia has historically been considered diagnostic of renal disease (Hardy and Osborne, 1979; Finco, 1980; Osborne and Polzin, 1983a and 1983b; Lage, 1984; Bichet et al., 1985; Coles, 1986).

One condition which consistently results in dehydration and hypotension is hypovolemic hyponatremia (Narins et al., 1982; Knapp, 1984). Hypovolemic hyponatremia occurs when there is a negative sodium balance, i.e., there is a net sodium loss from the body. During hypovolemic hyponatremia the extracellular fluid volume (ECF) is decreased and signs of dehydration and hypotension are present (Perkin and Levin, 1980; Narins et al., 1982; Baesl and Buckley, 1983; Knapp, 1984; Bichet et al., 1985). When hypovolemic hyponatremia is severe azotemia may also be present (Narins et al., 1982). Urine concentration is commonly used to separate hypovolemic hyponatremia due to renal or adrenal disease from hypovolemic hyponatremia due to other conditions (Perkin and Levin, 1980; Narins et al., 1982; Baesl and Buckley, 1983; Knapp, 1984; Osborne and Polzin, 1983a and b; Finco, 1980; Coles, 1986). Animals which are hypovolemic and hyponatremic due to renal or adrenal disease are not expected to excrete appropriately concentrated urine, whereas hyponatremic hypovolemic animals without

renal or adrenal disease are expected to excrete maximally concentrated urine (Narins et al., 1982; Perkins and Levin, 1980; Baesl and Buckley, 1983; Knapp, 1984; Osborne and Polzin, 1983b; Finco, 1980). However, several hypovolemic hyponatremic patients of the Boren Veterinary Medical Teaching Hospital were observed to excrete unconcentrated urine in the absence of renal and adrenal disease. Also, there are several published reports of humans (Kingston, 1973; Thomas and Morgan, 1979; Miller et al., 1980) and animals (Di Bartola et al., 1985; Neatherly, 1981) excreting unconcentrated urine in the absence of renal or adrenal disease. The significance of these findings were not discussed in the reports.

No studies or experimental models of chronic severe hypovolemic hyponatremia in euadrenal animals were found during an exhaustive literature review. To study the influence of chronic severe hypovolemic hyponatremia on the renal concentrating ability in euadrenal animals, a model will be developed by chronic blood removal from sodium restricted dogs. This model will be used to study the influence of chronic severe hypovolemic hyponatremia on renal concentrating ability by measuring and comparing renal medullary osmolalilty, urine concentration, and responsiveness to exogenous vasopressin during chronic severe hypovolemic hyponatremia and during normovolemic normonatremia. This model should facilitate other studies on chronic severe hypovolemic hyponatremia. The

experimental studies should generate the initial information demonstrating the affect of chronic severe hypovolemic hyponatremia on renal concentrating ability. They should, also, indicate the status of medullary osmolality and plasma ADH concentrations during chronic severe hypovolemic hyponatremia.

CHAPTER II

RENAL CONCENTRATING ABILITY IN DEHYDRATED HYPONATREMIC DOGS

Abstract

Evaluation of free water resorption via measurement of urine concentration is a commonly used aid in evaluating renal tubular function. Failure to concentrate urine during severe dehydration and/or azotemia is highly suggestive of renal disease. Eleven hyponatremic dogs were unable to concentrate urine during periods of severe dehydration and azotemia. Normonatremia was established in 8 of the dogs and renal concentrating ability returned in these dogs. Six dogs either died or were humanely killed and no renal lesions were found during postmortem examination. Two dogs had hypoadrenocorticism, which has been documented as a cause of hyponatremia and impaired renal concentrating ability. Two dogs had gastrointestinal disease, which has been documented as a cause of hyponatremia but not impairment of renal concentrating ability. All of the dogs without hypoadrenocorticism had clinical and clinicopathologic indications of blood loss, which has not previously been documented as a cause of hyponatremia or impairment of renal concentrating ability.

Hyponatremia (< 120 mEq/L) was created in an experimental dog, maintained on a low sodium diet, by chronic blood removal. While hyponatremic, the experimental dog became azotemic and severely dehydrated and his renal concentrating ability was impaired. Water deprivation exacerbated the dog's azotemia but did not elicit a significant increase in its urine concentration.

It was concluded that hyponatremia can be caused by hemorrhage and, irrespective of the cause, hyponatremia impairs renal concentrating ability.

Introduction

Resorption of free water is one of the primary functions of the kidney. Water resorption is regulated by plasma osmolality and blood pressure and results in excretion of concentrated urine. Evaluation of this function is a common and useful means of detecting renal tubular dysfunction (Knapp, 1984; Osborne, 1983a and b).

During periods of hypotension and/or increased plasma osmolality, the rate of antidiuretic hormone (ADH) release from the posterior pituitary is increased (Robertson, 1985). As the plasma ADH concentration increases ADH binding to the ADH receptors of renal tubular epithelial cells increases resulting in increased free water resorption. The increased free water resorption returns water to the plasma increasing plasma volume and, therefore, blood pressure, diluting the particles in the plasma hence decreasing plasma

osmolality, and concentrating the particles in the fluid remaining in the renal tubules causing concentrated urine to be excreted (Robertson, 1985). For this process to occur, the osmoreceptors and baroreceptors must respond to changes in plasma osmolality and blood pressure, ADH must be produced and released, ADH receptors on renal tubular epithelial cells must be present in appropriate numbers and must function properly, renal tubules must be present in adequate numbers, and there must be an adequate corticomedullary osmolality gradient (Robertson, 1985). A defect in any of these functions results in excretion of urine of inappropriately low concentration (Bichet et al., 1985).

Urine concentration is usually determined by measuring urine specific gravity (USG) or urine osmolality. Dogs with normal ability to concentrate urine should excrete appropriately concentrated urine, i.e., urine with a specific gravity equal to or greater than 1.030, in response to dehydration and/or hypotension (Osborne and Polzin, 1983a and 1983b; Finco, 1980). Excretion of urine of inappropriately low concentration, i.e., urine with a specific gravity below 1.030, while under the influence of dehydration, is indicative of renal disease if diabetes insipidus, hypoadrenocorticism, excessive glucocorticoid levels, and diuretic therapy have been excluded (Osborne and Polzin, 1983a and 1983b; Finco 1980; Maddison, 1984).

Several patients seen at the Boren Veterinary Medicine Teaching Hospital (BVMTH) appear to be exceptions to the diagnostic dogma described above. These patients were severely dehydrated, hypotensive, and azotemic, and excreted urine of inappropriately low concentration resulting in a diagnosis of renal disease. These patients were all severely hyponatremic during the period that they excreted urine of inappropriately low concentration. When normonatremia was established they excreted appropriately concentrated urine. This observation indicates that the inability to appropriately concentrate urine during dehydration and apparent low blood pressure in these patients may have been due to severe hyponatremia instead of renal disease or diabetes insipidus.

To evaluate the effect of severe hyponatremia on the kidneys' ability to reabsorb free water and hence concentrate urine in response to dehydration and low blood pressure a model for hyponatremia with concurrent dehydration was developed. A water deprivation test and blood pressure measurements were performed on the experimental subject during severe hyponatremia, dehydration, and azotemia.

Materials and Methods

Clinical Cases

The 11 cases described herein were seen at the BVMTH during the period of June 1981 through December 1985 (Table

I). Upon admittance to the BVMTH and daily thereafter, each patient received a complete physical examination.

Initial Presentation. Patients 1 through 9 were admitted for illnesses other than renal disease but were believed to subsequently develop renal disease. The initial medical problems in patients 1 through 6, patient 7, and patients 8 and 9 were intervertebral disc prolapse, trauma (dog fight), and hemorrhagic enteritis, respectively. Patients 10 and 11 were admitted to the BVMTH for renal disease but were subsequently demonstrated to have hypoadrenocorticism. All patients were anorectic for at least four days before hyponatremia was recognized.

Treatment. The patients with intervertebral disc prolapse had received short acting steroids before referral to the BVMTH. Steroid administration was discontinued at least 24 hours before the prehyponatremia blood and urine samples were collected, and steriods were not subsequently administered. None of the other patients received steroids. All patients received fluid therapy consisting of isotonic saline, isotonic glucose, and/or balanced electrolyte solutions. Nadir of hyponatremia samples (Table II and III) were collected before fluid and electrolyte therapy was began. Antibiotic therapy varied among patients but none of the antibiotics used are known to interfere with renal concentrating ability. Neither diuretics nor diuretic solutions were used in any of the patients.

TABLE I

PATIENT SIGNALMENT AND PERTINENT CASE DATA

Patient No.	Species	Sex	Age (yrs)	Initial medical problem	Outcome
1	canine	M	5	I.V.D. prolapse*	euthanized
2	canine	M	11	I.V.D. prolapse	recovered
3	canine	M	5	I.V.D. prolapse	recovered
4	canine	M	5	I.V.D. prolapse	euthanized
5	canine	F	3	I.V.D. prolapse	euthanized
6	canine	M	8	I.V.D. prolapse	recovered
7	canine	M	1	trauma	recovered
8	canine	F	0.75	hemorrhagic enteritis	died
9	canine	F	6	hemorrhagic enteritis	died
10	canine	M	4	renal failure	recovered
11	canine	F	4	renal failure	died

*Intervertebral disc prolapse

TABLE II

INDIVIDUAL VALUES AND RANGES FOR SELECTED PARAMETERS DURING THE THREE CLINICAL PERIODS

	Prehyponatremia				Nadir of hyponatremia				Posthyponatremia			
	Serum				Serum				Serum			
Patient No.	SUN (mg/dl)	Na+ (mEq/L)	K+ (mEq/L)	Urine Sp.Gr.	SUN (mg/dl)	Na+ (mEq/L)	K+ (mEq/L)	Urine Sp.Gr.	SUN (mg/dl)	Na+ (mEq/L)	K+ (mEq/L)	Urine Sp.Gr.
1	12	144	4.1	1.041	80	112	5.2	1.014	12	145	4.0	1.048
2	10	150	4.0	1.034	55	117	5.9	1.016	16	142	4.1	1.042
3	16	153	4.4	1.062	118	103	6.1	1.010	18	145	4.7	1.055
4	14	148	3.9	1.032	67	117	5.7	1.018	12	148	4.2	1.039
5	10	148	4.2	1.056	85	112	5.8	1.013	16	146	4.6	1.037
6	16	148	4.2	1.058	82	114	5.7	1.015	17	148	4.0	1.040
7	--	---	---	---	75	115	6.7	1.016	8	147	4.2	1.051
8	--	---	---	---	88	105	6.0	1.011	--	---	---	---
9	--	---	---	---	51	110	5.4	1.013	--	---	---	---
10	--	---	---	---	115	120	7.9	1.014	17	139	5.0	1.035
11	--	---	---	---	146	115	10.5	1.014	--	---	---	---
Range												
High	17	153	4.4	1.062	146	120	10.5	1.018	18	148	5.0	1.055
Low	10	144	3.9	1.032	51	103	5.2	1.010	8	139	4.0	1.035

TABLE III

INDIVIDUAL VALUES AND RANGES FOR SELECTED HEMATOLOGIC PARAMETERS DURING THE THREE CLINICAL PERIODS

Patient No.	Prehyponatremia		Nadir of hyponatremia		Posthyponatremia	
	PCV(%)	TPP(g/dl)	PCV(%)	TPP(g/dl)	PCV(%)	TPP(g/dl)
1	51	7.3	21	6.6	25	7.2
2	44	6.4	28	4.5	20	5.8
3	56	6.6	10	4.0	30	7.0
4	47	6.8	22	5.2	25	6.5
5	44	7.1	23	4.9	21	5.9
6	53	7.9	30	6.0	22	7.0
7	--	---	20	4.1	23	6.2
8	--	---	35	4.2	--	---
9	--	---	32	5.0	--	---
10	--	---	52	6.0	40	6.1
11	--	---	47	5.6	--	---
Range	44-56	6.4-7.9	10-52	4.0-6.6	20-40	5.8-7.2

Hemilaminectomies were performed on all of the patients with prolapsed intervertebral discs. Patients 2, 3, and 6 recovered neurologic function postsurgically, but patients 1, 4, and 5 did not and were subsequently euthanized. Patient 7 had numerous acute cutaneous lacerations which were surgically closed. Patients 8, 9, and 11 died before normonatremia was established.

Clinicopathologic and Pathologic Analyses. Although clinicopathologic analyses varied among the patients, serum urea nitrogen (SUN) and serum electrolytes were assayed and a hemogram and urinalysis were performed on every patient upon admittance and periodically thereafter. A complete urinalysis with USG, a hemogram, and a serum chemistry profile consisting of at least a serum sodium, potassium, and SUN were performed on all patients in which normonatremia was established. The feces of patients 1, 2, 3, 5, and 6 were chemically analyzed for blood.

Complete postmortem and histologic examinations were performed on all patients who died or were euthanized.

Experimental Model

The subject for the experimental model was a 50 lb young adult, intact male, mixed breed dog. Abnormalities were not identified on physical, urologic, hematologic, and serum chemistry examinations. The dog was administered routine vaccinations and anthelmintics and allowed 21 days for physiologic adaption.

After the adaption period, 2 baseline hemograms, serum chemistry profiles (SUN, serum alanine transaminase (SALT), serum alkaline phosphatase (SAP), glucose, phosphorus, creatinine, albumen, osmolality, sodium, potassium, chloride, and calcium), urine chemistry profiles (urine urea nitrogen (UUN), sodium, potassium, osmolality, phosphorus, chloride and creatinine), urinalyses, mean arterial pressure (MAP) measurements, and water deprivation tests were performed. These procedures were repeated when the subject was severely hyponatremic and again when normonatremia was reestablished. Hemograms, urinalyses, serum chemistry profiles, and urine chemistry profiles were performed daily throughout the experiment.

Production of Hyponatremia. After baseline values were established the subject was placed on a low sodium diet (Hills H/D^{TM} Diet, Hills Pet Products, Topeka, Ks.) and each day about 350 ml of blood (about 15% of the subject's original blood volume) was removed by jugular phlebotomy, until the plasma sodium concentration was less than 120 mEq/L. While the subject was severely hyponatremic a hemogram, serum chemistry profile, urine chemistry profile, urinalysis with specific gravity, blood pressure measurement, and water deprivation test were performed.

When these procedures were completed, the subject was placed on a conventional canine maintenance diet (Promix, Ellisson Feed and Seed, Norman, Ok.). When the subject's serum sodium concentration returned to the normal range for

adult dogs, a hemogram, serum chemistry profile, urine chemistry profile, urinalysis with specific gravity, and MAP measurement was performed.

Analytical Procedures

Serum and urine chemistries, enzymes, and electrolytes were assayed using established laboratory procedures: UUN and BUN by measuring the ammonia released when the serum was treated with a urease reagent (BUN analyzer 2, Beckman Instruments, Inc., Fullerton, Ca.); creatinine by a modified Jaffe reaction (Creatinine analyzer 2, Beckman); glucose by measuring the oxygen released when the serum was treated with a glucose oxidase reagent (glucose analyzer 2, Beckman); sodium and potassium by flame photometry (Klina Flame^{R} model 652210, Beckman); inorganic phosphorus by a kinetic procedure using a modification of the Tauasky and Shorr reagent (Beckman); calcium by a modification of the Baginski method (Calcium Analyser, Corning Scientific Instruments, Medfield, Mass.); chloride by chloridometry (Chloridometer, Nuclear of Chicago, Ft. Lee, New Jersey); SALT and SAP by kinetic procedures (ALT Liquid-STAT^{TM} and AP Liquid-STAT^{TM}, Beckman); albumen by a bromocresol green procedure (Albumen Spect Tru^{R} BCG, Lancer, St. Louis, Mo.); and osmolality by vapor pressure osmometry (Vapor pressure osmometer model 5100 B, Wescor Inc., Logan, Utah). Urinalyses were performed by established laboratory procedures utilizing reagent impregnated strips (N-

multistix^{R} 5429K, Ames Co., Miles Labs., Elkhart, In.) read by automated instrumentation (Ames Clini-Tek^{R}, Ames) for semiquanitative chemical analysis. Hemograms were performed using an automated cell counter (Coulter model S^{R}, Coulter Electronics, Inc., Hialeah, Fl.) for determination of hematocrits (HCTs), white blood cell (WBC) counts, red blood cell (RBC) counts, and RBC indeces. Refractometry was used for estimation of TPP concentration. Differential WBC counts were performed on Wright's stained blood smears. Fecal blood assays were performed using the quaiac procedure with a vegetable peroxidase inhibitor (Coloscreen/VPI^{TM}, Helena Labs., Beaumont, Tx.)

Dehydration was estimated by evaluation of changes in skin pliability and resiliency (skin turgor) as compared to previous measurements on the same animal and other normal and dehydrated animals. Skin of several anatomical locations, inclusive of at least the dorsal lumbar area and the eyebrow, was elevated as high as possible, without causing pain to the dog, and released. The rate at which the skin returned to its normal position and configuration was timed. No change from previous evaluations or from findings in normal animals was classified as "0" change, an increase of less than one second was classified as slight (1+) dehydration, an increase of 1 to 2 seconds was classified as moderate (2+) dehydration, and an increase of greater than 2 seconds was classified as severe (3+) dehydration. If evaluations differed between locations at

the same testing period the more severe classification was used.

Water deprivation tests were performed by withholding all liquids for 48 hours, regardless of the subjects hydration status, SUN, SC, USG, or urine osmolality. A dry diet of appropriate sodium content was provided ad libitum. Serum and urine chemistry and electrolyte profiles, urinalyses with USG, and hemograms were performed at the start and end of water deprivation.

MAP was measured by an oscillometric method (ASDI Sentry^{R} 400, Automated Screening Devices, Inc., Costa Mesa, Ca.) which takes as the MAP the lowest cuff pressure which results in the maximum amplitude of pressure pulsation in the cuff.

Results

Patient Cases

The species, sex, age, initial medical problem, and outcome of the case for all patients are presented in Table I. Five of the 6 surgery patients (patients 1,2,3,5, and 6) had melena, indicating upper gastrointestinal hemorrhage. Chemical analysis for blood was positive in all 5. Observations of the feces of patient 4 were not reported in the clinical record. The final diagnoses for patients 8 and 9 were intestinal parvovirus infection and hemorrhagic enteritis of unknown etiology, respectively.

Hypoadrenocorticism was diagnosed in patients 10 and 11. Patient 10 had basal and post ACTH cortisol values (both < 1 ug/dl) diagnostic of hypoadrenocorticism and responded excellently to therapy consisting of sodium containing fluids and mineralocorticoid and glucocorticoid supplementation. Patient 11 died before hormone assays could be performed and before appropriate therapy could be instituted. At necropsy, renal lesions were not found but there was severe bilateral adrenal cortical necrosis.

Serum sodium, SUN, serum potassium, USG, HCT, and total plasma protein values are presented in Table II and III for the prehyponatremic, nadir of hyponatremic, and posthyponatremic periods. Prehyponatremia values are not available for patients 7 through 11 because these patients were at their nadir of hyponatremia when they were first examined. Patients 8 and 9 died before normonatremia was established, therefore, no posthyponatremia values are available.

At the nadir of hyponatremia all patients appeared depressed and weak. Skin turgor tests revealed severe dehydration and capillary refill time was greater than 3 seconds indicating hypotension.

Renal lesions were not identified in any of the patients which died. Adrenal lesions were not found in any patients except patient 11.

Experimental Model

Serum and urine sodium, potassium, urea nitrogen, creatinine, and osmolality; USG; HCT; total plasma protein; and mean arterial blood pressure values changed substantially as hyponatremia developed (Table IV). The other variables measured in the urinalyses, hemograms, and serum and urine chemistry profiles did not change significantly. Water deprivation exacerbated the azotemia but did not elicit an increase in the USG (Table IV).

The experimental subject's MAP dropped daily from 97 mm Hg before hemorrhage to 68 mm Hg on day 9 of hemorrhage. On day 10 it was undetectable. It remained undetectable until the fourth day after hemorrhage was discontinued, when it was 72 mm Hg. The subject had become severely dehydrated by day 12 of hemorrhage and remained so until two days after hemorrhage was discontinued.

Discussion

Evaluation of urine concentration in dehydrated and/or azotemic animals is a valuable aid in differentiating prerenal azotemia from renal azotemia (Osborne and Polzin, 1983a and 1983b; Finco, 1980; Coles, 1986). Animals which do not appropriately concentrate their urine during periods of dehydration and/or azotemia have historically been considered to have diabetes insipidus or renal disease (Osborne and Polzin, 1983a and 1983b; Finco, 1980; Coles, 1986). When an animal is suspected of having renal

TABLE IV

SELECTED PARAMETERS DURING PRETREATMENT AND TREATMENT PERIODS IN THE EXPERIMENTAL DOG

	Normals[a]	Prehyponatremia	Hyponatremia H_2O deprivation Start	Hyponatremia H_2O deprivation End	Posthyponatremia H_2O deprivation Start	Posthyponatremia H_2O deprivation End
Serum						
Na+ (mEq/L)	141.0-154.0	150.0	119.0	120.0	148.0	150.0
K+ (mEq/L)	3.7- 5.6	4.9	5.7	6.5	4.0	3.8
UN (mg/dl)	10.0- 28.0	16.0	38.0	68.0	14.0	18.0
Creatinine (mg/dl)	0.5- 2.0	0.6	1.2	2.5	0.5	0.6
Osmolality (mosm/L)		303	265	278	300	305
Urine						
Na+ (mEq/L)	V[b]	405.0	undetectable[c]	undetectable	218.0	515.0
K+ (mEq/L)	V	295.0	18.0	48.0	154.0	311.0
UN (mg/dl)[e]	V	2560.0	550.0	510.0	1280.0	2810.0
Creatinine (mg/dl)	V	240.0	78.0	98.0	120.0	262.0
Osmolarity (mosm/L)	V	2140.0	523.0	510.0	1350.0	2348.0
SG[f]	V	1.054	1.017	1.017	1.040	1.063
PCV (%)	35.0- 54.0	42.0	16.0	19.0	13.0	16.0
TPP (g/dl)	6.0- 7.5	6.8	7.0	7.5	5.8	6.3
MAP (mm Hg)	NVNE[d]	97.0	undetectable	undetectable	85.0	80.0

[a]Normal values for the BVMTH.
[b]V = Variable.
[c]The procedure is sensitive to 3 mEq/L.
[d]No normal values established.
[e]UN = Urea Nitrogen.
[f]SG = Specific Gravity.

disease or diabetes insipidus but is not azotemic or dehydrated, a water deprivation test is often performed (Finco, 1980; Hardy, 1982; Coles, 1986). This procedure deprives the subject of water until the subject concentrates his urine or he becomes azotemic and/or dehydrated (indicated by a loss of 5% of the subject's body weight since the start of the procedure). The subject's USG is measured periodically throughout the procedure. If the subject's USG becomes appropriately elevated, equal to or greater than 1.030, the procedure is ended and the subject is considered to have adequate ability to concentrate urine. If the subject becomes dehydrated and/or azotemic without producing urine with an appropriately elevated specific gravity the test is ended and the subject is considered to have decreased ability to concentrate urine (Finco, 1980; Hardy, 1982; Coles, 1986), ie., renal disease or diabetes insipidus.

Diabetes insipidus may occur for several reasons and is usually categorized as cranial (hypothalamic or hypophyseal) diabetes insipidus and nephrogenic diabetes insipidus (Capen, 1983; Bovee, 1984). Cranial diabetes insipidus is due to dysfunction of ADH synthesis, storage, and/or release mechanisms (Capen, 1983; Bovee 1984), whereas nephrogenic diabetes insipidus is caused by failure of target cells of the renal tubules to respond to ADH (Capen, 1983; Bovee, 1984).

Renal disease causes decreased urine concentrating ability due to a deficiency of functional renal mass. When the functional renal mass falls below 1/3 of the original renal mass the kidney's ability to adequately concentrate urine becomes decreased (Bradford, 1899; Hayman, 1939; Hardy, 1979; Finco, 1980). The kidney's ability to adequately remove urea from the plasma does not become impaired until the functional renal mass is 1/4 or less of the original renal mass (Bradford, 1899; Hayman, 1939; Hardy, 1979; Finco, 1980). Diagnostic application of this information allows at least three conclusions. First, a patient which is azotemic due to renal disease will not be able to appropriately concentrate his urine. Second, a patient which is azotemic but has an appropriately concentrated urine has pre-renal azotemia. Third, when azotemia is induced by dehydration or hypotension, if the USG is not appropriately elevated the patient has decreased ability to concentrate urine.

All patients presented in this study produced urine of inappropriately low concentration while they were severely dehydrated and azotemic (Table II). This resulted in the patients being diagnosed as having renal disease. Patients 1 through 6 had laboratory data (Table II) previous to their dehydrated and azotemic state which suggested they did not have renal disease upon initial presentation. Surgical procedures were performed on all of these patients. It was speculated that these patients had developed renal disease

subsequent to surgery. Evaluation of the serum electrolyte values (Table II) revealed that all patients were severely hyponatremic during their dehydrated and azotemic period. When normonatremia was established in patients 1 through 7 and patient 10, random urine samples from each of these animals had USG values sufficiently high to indicate that they could adequately concentrate urine. This demonstrated that the loss of renal concentrating ability was not due to renal disease or diabetes insipidus. The absence of gross and histologic renal lesions in the 6 patients who died and the recovery of renal concentrating ability by the patients in which normonatremia was established indicates that severe hyponatremia, not renal disease, prevented these patients from appropriately concentrating urine in response to severe dehydration and hypotension.

This hypothesis is supported by the experimental study which demonstrated that, during periods of severe hyponatremia, the subject did not appropriately concentrate urine even though he was severely dehydrated, hypotensive, and azotemic (Table IV). Water deprivation during the hyponatremic period exacerbated the azotemia and dehydration but did not elicit a significant change in the subject's urine concentration. When normonatremia was reestablished the ability to concentrate urine was regained. Severe hyponatremia appears, therefore, to interfere with the normal physiologic processes which produce concentrated urine in response to dehydration and hypotension.

Although steroid administration can impair renal concentrating ability, it is unlikely that the steroids administered to patients 1 through 6 were responsible for the decreased renal concentrating ability in these patients. Any affect which the steroids had on the renal concentrating ability should have been present within eight hours after steroid administration (Aronson, 1985). In the present study, the USGs were 1.032 or greater for prehyponatremia urine samples collected more than 24 hours after steroid administration had ceased and while the patients were well hydrated (Table III). These values are far above those of urine samples collected 6 or more days later (Table III) when the patients were severely dehydrated, hypotensive, azotemic and at the nadir of their hyponatremia.

One mechanism by which severe hyponatremia could result in production of urine of inappropriately low concentration during periods of dehydration and low blood pressure is by its effect on plasma osmolality. Since sodium, under normal circumstances, accounts for virtually all of the osmotically active solute in the extracellular fluid, control of plasma sodium concentration is synonymous with control of effective plasma osmolality (Leaf, 1962; Narins et al., 1982). Hypothalamic osmoreceptors and extracranial baroreceptors interact in the regulation of ADH release. Therefore, ADH release might have been markedly decreased in our patients due to severely decreased effective plasma osmolalities. Studies in rat, dog, and man indicate that

plasma osmolality initiates ADH release. Whereas, blood pressure and/or volume controls the osmoreceptor threshold at which plasma osmolality initiates ADH release and influences the rate of ADH release (Robertson, 1985; Robertson et al., 1976). A 10% decrease in blood pressure and/or volume results in a 3 to 5 milliosmol drop in the plasma osmolality at which ADH release is initiated. Whereas, a 10% increase in blood pressure and/or volume results in about a 3 to 5 milliosmol elevation of the plasma osmolality at which ADH release is initiated (Robertson, 1985; Robertson et al., 1976). The relationship of plasma osmolality and blood pressure in the regulation of ADH release has not been studied in animals with severe hypotension and extremely low effective plasma osmolality. If the relationship demonstrated in the previous studies in animals with mild to moderate hypotension (Robertson, 1985; Robertson et al., 1976) exists during severe hypotension, the marked decrease in effective plasma osmolality due to severe hyponatremia may have contributed to the failure of the patients reported herein to appropriately concentrate their urine in response to severe dehydration and hypotension.

Another mechanism by which hyponatremia may interfere with the ability of the kidneys to concentrate urine is by preventing the production and maintenance of an adequate corticomedullary osmolality gradient. Sodium and its attendant anions, such as chloride, make up about 50% of the

particles in the renal medullary interstitium (Pitts, 1974; Roy and Jamison, 1985). Establishment and maintenance of the renal corticomedullary osmolality gradient is dependent upon the chloride pump in the thick ascending limb (Roy and Jamison, 1985). Concommitant with severe hyponatremia there is hypochloremia. The ability of the chloride pump to function properly may have been impaired during the patients' severe hyponatremia and hypochloremia. These factors may have contributed to the excretion of urine of inappropriately low concentration during severe dehydration, hypotension, and hyponatremia.

Patients with hypoadrenocorticism excrete urine of inappropriately low concentration when they are hyponatremic, severely dehydrated, and hypotensive (Willard et al., 1982). It has been proposed that the hypernatriuresis which occurs in hypoadrenocorticism causes a solute diuresis and renal medullary washout resulting in decreased renal concentrating ability (Osborne and Polzin, 1983b). It is unlikely that this is the cause of the failure of patients 1 through 9 to produce appropriately concentrated urine during their periods of severe dehydration, low blood pressure and hyponatremia. These patients all concentrated urine when normonatremia was established and those which were examined post mortem did not have adrenal lesions. The absence of detectable amounts of sodium in the urine of the experimental subject rules out renal medullary washout due to solute diuresis caused by

hypernatriuresis in that animal. The mechanisms which prevented the patients without hypoadrenocorticism and the experimental subject from producing appropriately concentrated urine during dehydration, hypotension, and hyponatremia are probably involved in preventing animals with hypoadrenocorticism from producing appropriately concentrated urine during dehydration, hypotension, and hyponatremia.

Hypovolemic hyponatremia has been reported to develop subsequent to numerous conditions including hypoadrenocorticism, diarrhea, diuretics, and renal disease (Narins et al., 1982). Hypoadrenocorticism explains the development of hyponatremia in patients 10 and 11 and gastrointestinal losses may have contributed to the development of hyponatremia in patients 8 and 9. The other patients, however, did not demonstrate clinical signs consistent with any of the previously reported causes of hypovolemic hyponatremia other than renal disease.

Evidence of blood loss was present in all patients except those with hypoadrenocorticism. Patient 7 did not have clinical signs of gastrointestinal disease, but had both clinical and clinicopathologic signs of blood loss. Patients 1 through 6 and patients 8 and 9 also had clinicopathologic evidence of blood loss. As shown in Table III, patients 1 through 5 and patients 7, 8 and 9 had low HCT and/or low total plasma protein values at the nadir of their hyponatremia. Patient 6, who had only a slight

decrease in his HCT at the nadir of his hyponatremia, had a significant decrease in his HCT when normonatremia was reestablished. This suggests that at the nadir of his hyponatremia he had significant blood loss which was obscured by hemoconcentration. Patients 1,2,3,5,6,7, and 8 had clinical evidence of blood loss.

Because extensive evidence of blood loss was present in such a large number of the patients it was hypothesized that blood loss may have contributed to the development of hyponatremia in some patients. An extensive literature search failed to yielded any reports of hyponatremia developing subsequent to hemorrhage; however, this hypothesis was supported by the experimental study. Hyponatremia was created in the experimental subject by hemorrhage concommitant with a low sodium diet, confirming that blood loss without appropriate dietary intake of sodium can result in severe hyponatremia. Since patients 1 through 9 were all anorectic for more than 4 days before the nadir of their hyponatremia, it is plausible that hemorrhage concomitant with decreased sodium intake caused hyponatremia.

It appears that severe sodium loss without sodium replenishment, whether due to hypoadrenocorticism, gastrointestinal disease, or hemorrhage, can cause severe hyponatremia. Severe hyponatremia, regardless of the cause, prevents animals from appropriately concentrating their urine in response to dehydration and hypotension.

Conclusion

The 11 patients reported herein all excreted urine of inappropriately low concentration while they were severely dehydrated, hypotensive, azotemic, and hyponatremic. This resulted in the diagnosis of renal disease. A similar condition was produced in an experimental subject by chronic hemorrhage concomitant with a low sodium diet. Recovery of the ability to concentrate urine when normonatremia was established and/or lack of any gross or histologic renal lesions makes diabetes insipidus and/or renal disease implausible causes of the inability to concentrate urine in these patients and the experimental subject. These findings indicate that severe hyponatremia, regardless of the cause, prevents excretion of appropriately concentrated urine in response to dehydration and hypotension. Hence, in severely hyponatremic animals, the diagnosis of renal disease should not be based solely on the inability to concentrate urine during azotemia, dehydration, hypotension or in response to water deprivation.

The patients and experimental subject with evidence of hemorrhage all developed hyponatremia. Blood loss has not previously been documented as a cause of hyponatremia.

Further studies are needed to determine the mechanisms which contribute to the effect of severe hyponatremia on the kidneys' ability to concentrate urine during periods of dehydration and hypotension.

CHAPTER III

A CANINE MODEL OF CHRONIC SEVERE HYPOVOLEMIC HYPONATREMIA

Abstract

Hypovolemic hyponatremia occurs when there is a decrease in body sodium and water with the decrease in sodium being greater than the decrease in water. Osmotic and nonosmotic factors regulating antidiuretic hormone (ADH) release and thirst act to control blood volume, blood pressure and plasma osmolality and, in so doing, affect the body sodium and water content. Studies of mild to moderate alterations of hemodynamic and/or osmotic changes indicate that plasma osmolality initiates the release of ADH, whereas blood pressure and/or volume changes alter the osmoreceptor threshold at which ADH release is initiated. No studies of hypovolemic hyponatremia have been performed in euadrenal animals. To facilitate the study of hypovolemic hyponatremia in euadrenal animals, a canine model was developed. Twelve dogs were hemorrhaged daily, while being maintained on a sodium restricted diet. Packed cell volumes (PCVs), total plasma protein (TPP) concentrations, and serum sodium concentrations were measured daily and blood volume measurements were performed before hemorrhage and during

severe hyponatremia (serum sodium concentration < 120 mEq/L).

The dogs became severely hyponatremic and hypovolemic after 10 to 16 days of hemorrhage. The development of hyponatremia by this method indicates that at severely decreased blood volumes and/or presures the nonosmotic factors affecting ADH release and/or thirst become dominant over the osmotic factors affecting ADH release and/or thirst. This model, therefore, should facilitate the study of the integration of osmotic and nonosmotic factors influencing ADH release and/or thirst during hypovolemic hyponatremia in euadrenal dogs.

Introduction

Water is the major component (55-60% of body weight) of the mammalian body (Robertson, 1985). The body water and its solutes provide the critical environment necessary for normal physiologic processes and, therefore, are maintained within narrow limits (Shrier et al., 1979; Narins et al., 1982; Robertson, 1985). Plasma osmolality and plasma sodium concentration, in health, are restricted to fluctuations of less than 2% (Shrier et al., 1979; Narins et al., 1982; Robertson, 1985). Body water content is regulated by body sodium content which is strongly influenced by the plasma osmolality (Narins et al., 1982). Because sodium accounts for virtually all the osmotically active solutes in the plasma, control of plasma sodium

concentration is synonymous with control of effective plasma osmolality (Narins et al., 1982). This control is mediated by the triarchy of thirst, antidiuretic hormone (ADH) release, and renal concentrating and diluting mechanisms which act in concert to modify the volume of water in which particles are dispersed (Narins et al., 1982).

Release of ADH is regulated by both osmotic and nonosmotic factors. Osmotic regulation is mediated via hypothalamic osmoreceptors, whereas nonosmotic regulation is mediated via reflex mechanisms (carotid sinus high pressure baroreceptors and left atrial volume receptors or low pressure baroreceptors) and humoral (renin-angiotensin) mechanisms (Bonjour and Malvin, 1970; Schrier et al., 1979; Goldsmith et al., 1985; Claybough and Share, 1973; Wietzman et al., 1978). The integration of these controlling mechanisms is complex and has been studied extensively in the acute situation (Schrier et al., 1979; Robertson et al., 1985) However, the influence of chronic hemodynamic alterations and/or osmotic alterations on vasopressin release and/or renal concentrating and diluting ability is incompletely defined (Robertson, 1985).

Verney's (1947) classic studies on the influence of plasma osmotic changes on urine flow pioneered the study of ADH, its influence on renal concentrating and diluting mechanisms, and the factors regulating the release of ADH. These and subsequent studies demonstrated that only solutes which do not readily cross membranes can stimulate the

osmoreceptors (Schrier et al., 1979; Athar and Robertson, 1974; Robertson et al., 1974b). The osmoreceptors are located in the hypothalamus in the proximity of the third ventricle (Thrasher, 1982; Thrasher et al., 1980a and 1980b). An increase in the plasma osmolality of 1% results in an increase of plasma ADH concentration of about 1 pg/ml, within the normal range of plasma osmolality. Antidiuretic Hormone release is biphasic (Robertson, 1985). And, small (<10%) blood pressure or volume alterations have little affect on ADH release, while large (>20%) blood pressure or volume alterations markedly affect the plasma osmolality at which plasma ADH concentrations are detectable (osmoreceptor threshold) and possibly affect the rate of ADH release (Robertson, 1985; Schrier et al., 1979). The effect of changes in plasma osmolality on ADH release and renal concentrating and diluting mechanisms have been studied using infusions of various hypertonic or hypotonic solutions. Studies on the effect of blood pressure and blood volume changes on ADH release and renal concentrating and diluting ability have altered blood volume and/or pressure by hemorrhage, postural changes, infusion of substances such as dextran and/or autologous blood, and administration of hypotensive or hypertensive drugs. These studies have been performed only in the acute situation (Robertson, 1985). Studies on the effect(s) of chronic hemodynamic disturbances on ADH release have been performed in patients with chronic hypovolemia (Robertons and

Aycinena, 1982) and patients with chronic hypervolemia (Ganguly and Robertson, 1980) due to hypoaldosteronism and hyperaldosteronism, respectively. In these studies, changes in ADH release were similar to those produced by acute, experimentally induced, changes in blood volume. Studies of chronic hypovolemic hyponatremia in euadrenal animals have not been documented.

Several patients seen at the Boren Veterinary Teaching Hospital became hypovolemic due to chronic hemorrhage concomitant with restricted sodium intake. This observation resulted in the development of a model of severe chronic hypovolemic hyponatremia. The model, reported herein, should faciliate studies on the affect of severe chronic hypovolemic hyponatremia on the integration of osmotic and nonosmotic stimuli in euadrenal animals.

Materials and Methods

Experimental Subjects

The experimental subjects were 12 healthy young adult mixed breed, intact male, dogs. Abnormalities were not identified during a thorough physical examination, nor were any abnormalities present on hematologic, urologic, and serum chemistry and electrolyte evaluations. Each subject appropriately concentrated urine in response to water deprivation. The subjects were then vaccinated, dewormed, and allowed to physiologically adapt for 21 days.

Experimental Treatment

After the adaption period, 5 urologic, hematologic, and serum chemistry analyses; and 6 blood volume assays (one at the start and one at the end of each water deprivation test) were performed on each subject. When these prehemorrhage studies were completed, the dogs were placed on a restricted sodium diet (H/D™ Diet, Hills Pet Products, Inc., Topkea, Ks.) and each animal was hemorrhaged by jugular phletomy until one or more of the following signs were detected: cool extremities, pale mucous membranes, and increased capillary refill time (> 2 seconds). Initially, four dogs could not be properly handled with manual restraint alone. These dogs were administered acetylpromazine (Acepromazine maleate, Fort Dodge Laboratories, Fort Dodge, Iowa) intravenously (IV) about 5 minutes before the first three phlebotomies. They could be managed with manual restraint alone after the third phlebotomy. The packed cell volume (PCV), total plasma protein (TPP) concentration, and serum and urine sodium concentration were measured daily for each subject. Each subject was hemorrhaged on two separate occasions daily until its serum sodium concentration was below 120 mEq/L; at which time, two blood volume assays were performed two days apart. When the last blood volume assay was completed, 500 ml of 0.9% sodium chloride solution was administered IV and the subject was furnished a conventional canine maintenance diet (Promix, Ellison Feed and Seed Co., Norman, Ok.) ad

libitum. Intramuscular iron injections (Iron Dextran, Pfizer, New York, New York) were administered weekly to prevent iron depletion.

Analytical Procedures

PCVs and TPP concentrations were determined by microhematocrit centrifugation and refractometry, respectively. Serum and urine sodium concentrations were measured by flame photometry (Klina Flame^{R} Model 652210, Beckman Instruments, Inc., Fullerton, Ca.).

Plasma volumes were determined by a modification of the Evans blue dye dilution procedure described by Guyton (Guyton, 1981b). Samples were maintained in light free containers before assay and were assayed within 2 hours of collection. Centrifugation with serum filtration was used to separate the serum for assay from the RBC and other clot constituents. Blood volumes were determined by the following formula:

$$\text{Blood volume} = \frac{\text{plasma volume X 100}}{\text{100-PCV}}$$

The % of the body weight which was plasma and blood was calculated by the following formulae.

$$\%\ \text{body weight plasma} = \frac{\text{plasma in kg.*x100}}{\text{body wt in kg}}$$

$$\%\ \text{body weight blood} = \frac{\text{blood in kg.*x 100}}{\text{body wt in kg}}$$

*One ml of plasma or blood was assumed to equal one g.

The relative change in blood volume between the baseline period and hyponatremic period was calculated by the following formula:

$$**\Delta RBV = 1 - \frac{\text{\% body weight blood during hyponatremia}}{\text{\% body weight blood during baseline period}}$$

**Change in Relative blood volume.

Baseline and hyponatremia samples were treated as paired samples and a one-tailed t test was used to determine the statistical significance of the decreases in blood volumes and % body weight blood volumes (Steele and Torrie, 1980).

Dehydration was assessed by evaluation of changes in skin pliability and resiliency (skin turgor) as compared to previous measurements on the same animal and other normal and dehydrated animals. Skin of several anatomical locations, inclusive of at least the dorsal lumbar area and the eyebrow, was elevated as high as possible, without causing pain to the dog, and released. The rate at which the skin returned to its normal position and configuration was timed. No change from previous evaluations was classified as "0" change, an increase of less than one second was classified as slight (1+) dehydration, an increase greater than a second but less than 2 seconds was classified as moderate (2+) dehydration, and an increase of 2 seconds or more was classified as severe (3+) dehydration. If evaluation differed between locations at the same testing period, the more severe classification was used.

Results

It took an average of 12 days of hemorrhage for serum sodium to become, 120 mEq/l (Table I). It was necessary to remove an average of 2.05 blood volumes to produce hyponatremia (Table I). At the nadir of hyponatremia the subjects' PCVs and blood volumes were markedly decreased (Table I). Because body weights also decreased during hyponatremia, the relative blood volumes (% of the body weight which was blood) were calculated. These were also markedly decreased during hyponatremia (Table I).

Serum sodium and PCV decreased in proportion to the amountof blood removed (Figures 1 and 3). After an initial decline, TPP returned to normal levels (Fig. 2).

Urine sodium values decreased rapidly upon initiation of hemorrhage and were consistently less than 4 mEq/L after the second day of hemorrhage. All dogs developed signs of slight dehydration, moderate dehydration, and severe dehydration when serum sodium concentrations were about 135 mEq/L, 125 mEq/L, and 120 mEq/L, respectively.

Discussion

To reduce the serum sodium concentration below 120 mEq/L it was necessary to remove about 2 times the animal's initial blood volume (Table I). Hyponatremia (<120 mEq/L) developed in all dogs after 10 to 16 days of hemorrhage. The variability in time required to develop hyponatremia appears to have been related to the amount of hemorrhage

TABLE I

SELECTED BLOOD PARAMETERS, BODY WEIGHT, AND TOTAL HEMORRHAGE VALUES AND COMPARISONS BETWEEN BASELINE AND HYPONATREMIC PERIODS

	Baseline					Hemorrhage			Hyponatremia					Comparisons			
Dog #	PCV (%)	Na+ (mEq/L)	BV ABS[a] (ml)	BV %BW[b]	BODY WT.(kg)	Day	Amt[c] (ml)	AMT H /BV[d]	PCV (%)	Na+ (mEq/L)	BV ABS (ml)	BV %BW	BODY WT.(kg)	CHANGE BV[e](ml)	CHANGE BV%BW[f]	HBV /BBV[g]	HBV% /BBV%[h]
1	42	143	1335	8.70	15.34	10	2730	2.04	14	115	703	5.43	12.95	632	3.27	.5266	.8241
2	39	142	1529	7.96	19.20	11	3150	2.06	16	112	913	5.62	11.70	616	2.34	.5971	.7060
3	45	147	1736	9.92	17.50	13	3250	1.87	13	116	1012	7.01	14.43	724	2.91	.5829	.7067
4	47	146	1707	10.15	16.82	13	3150	1.85	15	114	948	6.78	13.98	759	3.37	.5554	.6680
5	44	143	1687	8.63	19.55	12	3690	2.19	18	117	1018	6.05	16.82	669	2.58	.6034	.7010
6	49	145	2076	8.30	20.11	13	4250	2.05	16	118	1119	5.56	20.11	957	2.74	.5390	.6699
7	46	147	1381	8.68	16.14	14	3090	2.24	18	116	756	5.45	13.86	625	3.23	.5474	.6279
8	51	148	2181	9.79	22.16	10	3690	1.69	17	114	1184	6.43	18.41	997	3.36	.5429	.6568
9	43	150	1559	9.77	15.34	16	4080	2.62	17	115	742	6.16	12.05	817	3.61	.4759	.6305
10	48	143	2955	11.21	26.36	12	5980	2.02	18	116	1658	7.26	22.84	1297	3.95	.5611	.6476
11	42	144	1064	7.80	13.30	10	2400	2.26	19	114	638	5.61	11.36	426	2.19	.5996	.7192
12	42	148	1997	8.45	23.64	11	3490	1.75	17	116	1164	5.63	20.68	833	2.82	.5829	.6663
MEAN	45	146	1767	9.11	18.80	12	3579	2.05	17	115	988	6.08	15.76	779[i]	3.03[j]	.5595	.6687
SD	3.49	6.45	493	1.034	3.82	1.83	921	0.26	2.92	2.35	280	0.65	3.90	226	0.53	0.04	0.03

[a]BVABS = Absolute Blood Volume.
[b]BV%BW = Percent of the animal's body weight which was estimated to be blood (relative blood volume).
[c]Amt = Total amount hemorrhaged.
[d]Amt H/BV = Total amount hemorrhaged divided by the animal's initial body volume.
[e]Change BV = The initial blood volume minus the hyponatremia blood volume.
[f]Change BV%BW = The initial BV%BW minus the hyponatremia BV%BW.
[g]HBV/BBV = Hyponatremia absolute blood volume divided by baseline absolute blood volume.
[h]HBV%/BBV% = Hyponatremia BV%BW divided by baseline BV%BW.
[i]The decrease in blood volume is significant at $P < 0.0005$.
[j]The decrease in relative blood volume is significant at $P < 0.0005$.

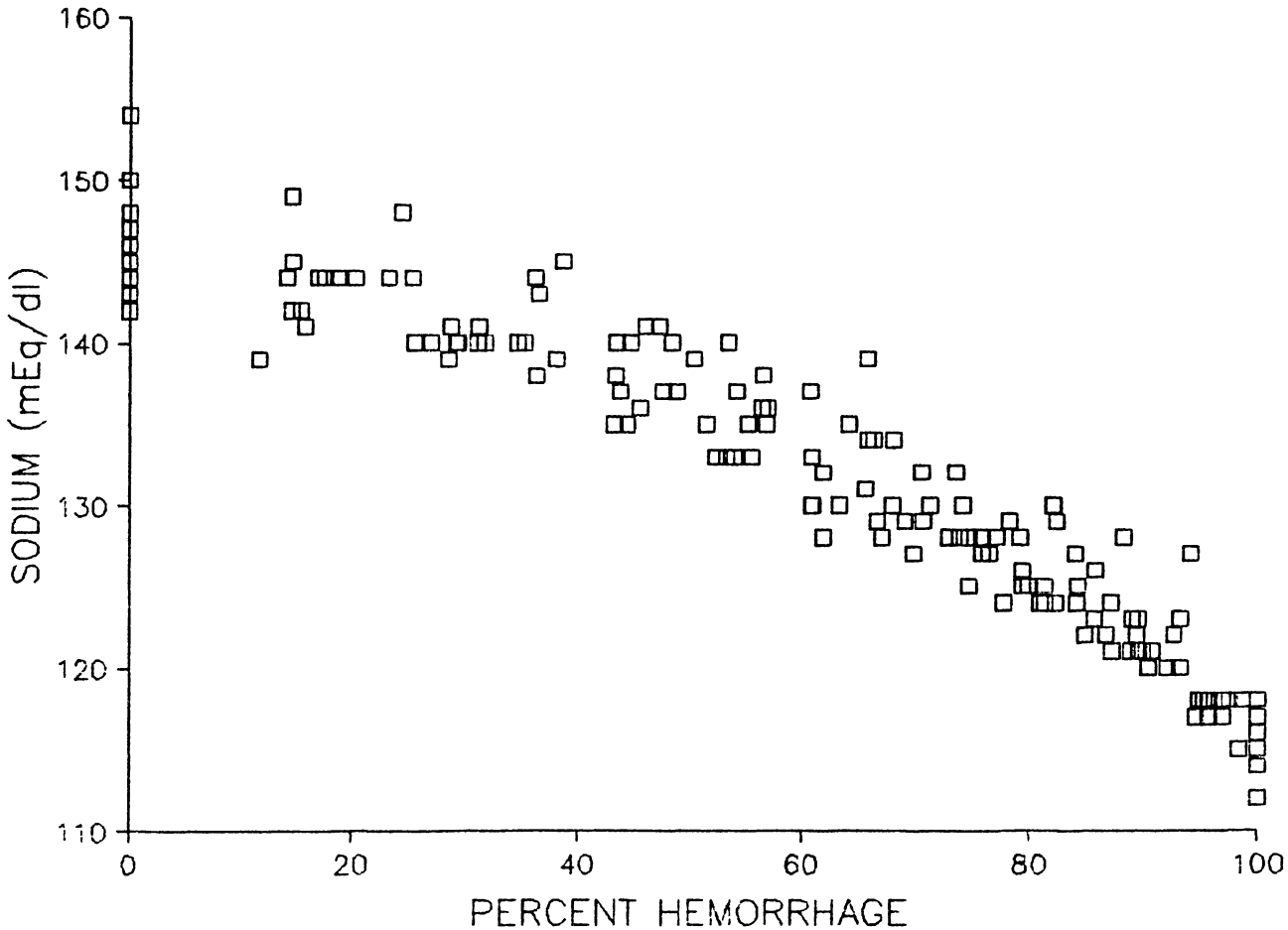

Figure 1. Serum sodium concentration versus percent hemorrhage for all dogs.

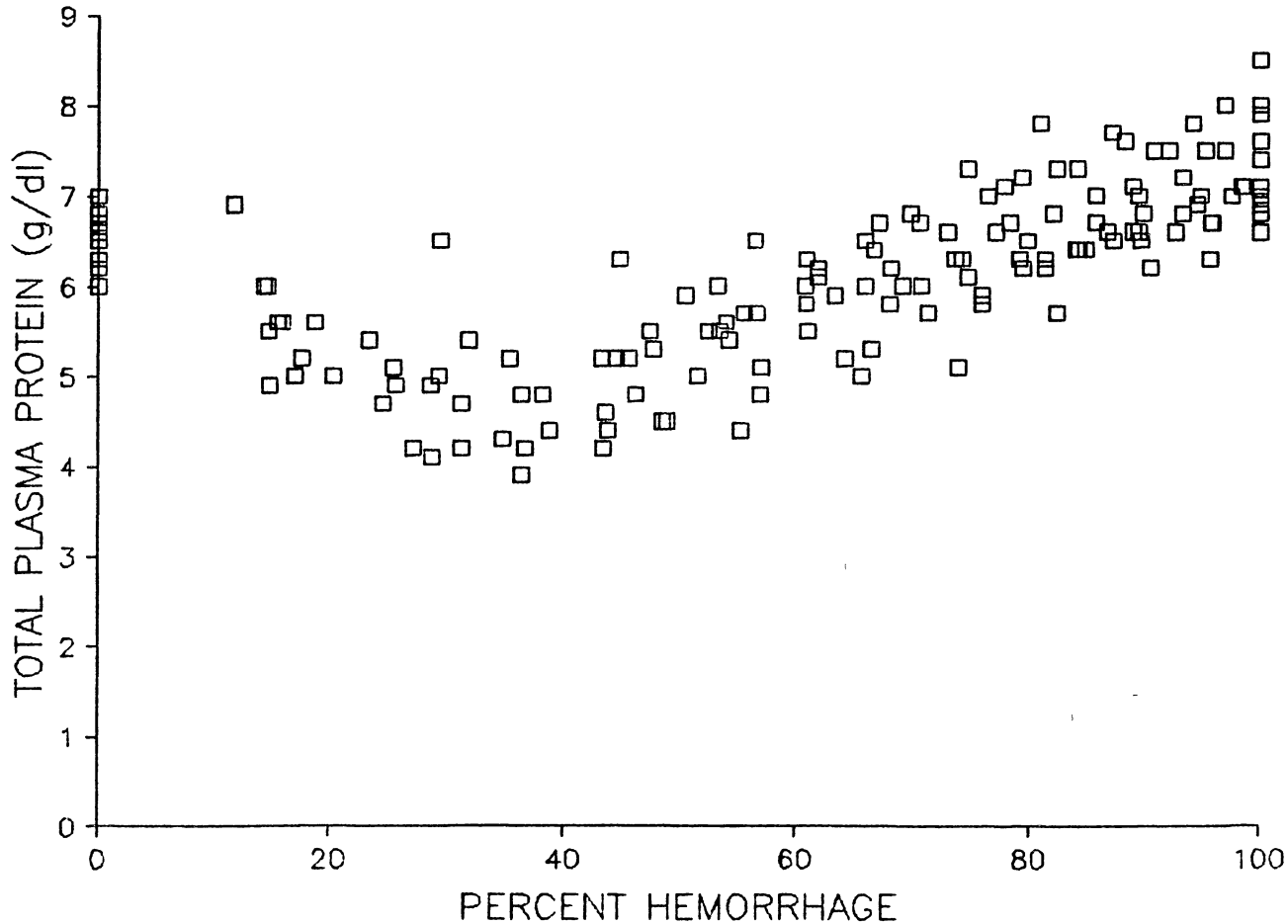

Figure 2. Total plasma protein concentration versus precent hemorrhage for all dogs.

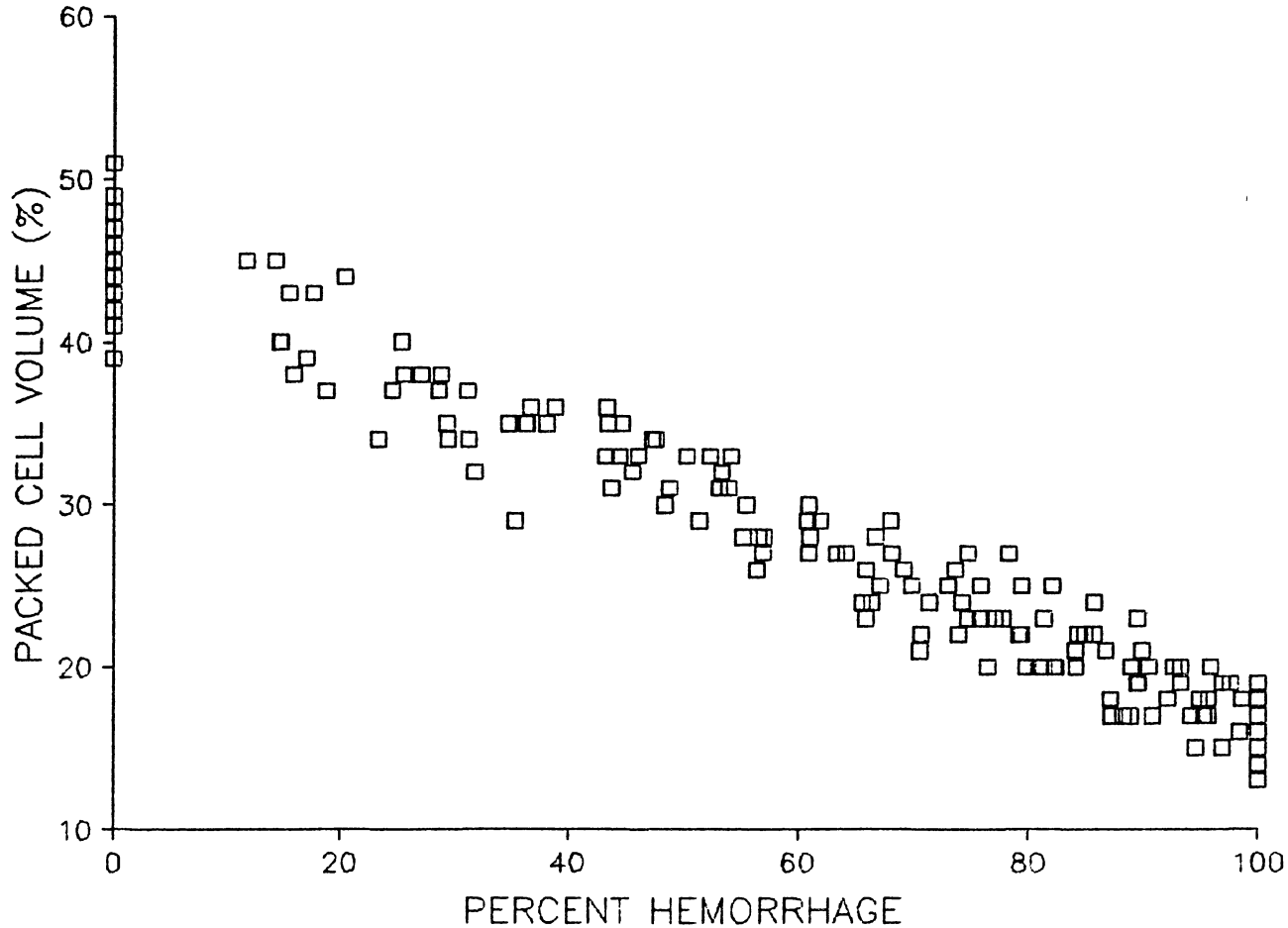

Figure 3. Packed cell volume versus percent hemorrhage for all dogs.

which could be achieved each day. This variation was due to difficulties in hemorrhaging certain animals, such as subject 9, and variation in sensitivity to hemorrhage, e.g., Dog 7, who required 14 days to develop hyponatremia, consistently demonstrated signs of shock (cool extremities, pale mucous membranes, and capillary refill time greater than 2 seconds) upon removal of only 50 to 100 ml of blood after the first 7 days of hemorrhage.

Total plasma protein concentrations and PCV values dropped during the first 3 to 4 days. The TPP concentration began to increase in all dogs by the fifth day, whereas the PCV continued to drop throughout the hemorrhage. The difference in the behavior of TPP concentrations and PCVs may be due to differences in the regenerative capacities of the plasma proteins and the erythron. Normal physiologic responses result in TPP concentrations returning to prehemorrhage levels within 5 days after hemorrhage, irrespective of the extent of hemorrhage (Schalm, 1975a and 1975b). Whereas, normal physiologic responses result in PCV values returning to prehemorrhage levels about 3 to 5 weeks after hemorrhage, regardless of the extent of hemorrhage (Coles, 1986a). During hyponatremia both TPP concentrations, which became elevated above prehemorrhage levels, and PCVs were probably deceptively high due to hemoconcentration, as indicated by signs of dehydration and by the decreased blood volumes (Table I).

Blood volumes decreased by about one half (Table I)

from the prehyponatremia values (mean = 1767 ml) to the nadir of hyponatremia values (mean = 988 ml). However, when weight loss, which occurred in all animals during the experiment, was accounted for by comparing the % body weight blood during the prehemorrahge period to the % body weight blood during hyponatremia (Table I), the relative decrease in blood volume was about one third. Numerous studies have demonstrated that acute blood loss of greater than 1/3 blood volume results in irreversible shock and subsequent death in the dog (Schalm, 1975a; Guyton, 1981a). Thus, it appears that physiologic mechanisms which function to maintain blood volume (i.e., ADH release with subsequent free water resorption, thirst, and sodium retention) have become dominant over those which control body solute concentration (i.e., osmoreceptors and sodium sensors for ADH release and thirst, respectively). Sodium retention is verified by the very low sodium content of the urine. The development of hyponatremia indicates that either ADH release has occurred in the absence of osmotic stimulus or thirst has occurred in the absence of sodium receptor stimulation. These results imply that the relationship between osmotic and nonosmotic regulation of ADH release and thirst may differ between acute and chronic hypovolemic hyponatremia in euadrenal animals. Studies of severe chronic hypovolemic hyponatremia in euadrenal animals are needed to better define the integration of osmotic and nonosmotic control (via thirst, ADH release, and

vasoactivity) of blood volume, blood pressure, and plasma osmolality. This model should provide a mechanism for these studies.

Conclusion

Hyponatremia (<120 mEq/L) can be produced by removal of about 2 times a dogs blood volume over a 10 to 16 day period. Variation in the rate at which hyponatremia develops is related to variation in the rate at which individual animals are hemorrhaged. Hyponatremia produced by hemorrhage results in relative blood volume decreases of about 1/3 and severe dehydration. Dogs can be maintained in this condition for several days. This model provides a mechanism to study the physiologic processes which regulate blood volume, blood pressure, and plasma osmolality to protect the individual from pathologic conditions such as hypovolemic shock.

CHAPTER IV

RENAL CONCENTRATING ABILITY IN THE DOG DURING EXPERIMENTAL CHRONIC HYPOVOLEMIC HYPONATREMIA

Abstract

Evaluation of renal concentrating ability is useful in separating nonrenal causes of hypovolemic hyponatremia from renal causes, i.e., renal disease and adrenal insufficiency. To evaluate renal concentrating ability, renal cortical and medullary osmolalities, and vasopressin responsiveness during hypovolemic hyponatremia, 5 dogs were made hypovolemic and hyponatremic on two separate occasions by chronic hemorrhage concomitant with a low sodium diet. Serum and urine chemistry profiles, hemograms, water deprivation tests, plasma and blood volume measurements, and vasopressin response tests were performed before hemorrhage, during both severe hypovolemic hyponatremic (sodium < 120 mEq/L), periods, and during both convalescent periods (vasopressin response tests were not performed during the convalescent periods). During the second hyponatremic period the left kidney was surgically removed during water

deprivation. The dogs were humanely killed during water deprivation in the second convalescent period and the right kidney was removed. Cortical and medullary osmolalities were measured on all kidneys.

During the hyponatremic periods renal concentrating ability was decreased in all dogs. Renal medullary osmolalities of the kidneys removed during hyponatremia were markedly lower than those removed during the normonatremic convalescent periods. Exogenous vasopressin elicited a small but significant increase in urine concentration during hyponatremia. It appears, therefore, that renal concentrating ability is decreased during hypovolemic hyponatremia and that this dysfunction is due, at least in part, to a decrease in renal medullary osmolality and submaximal plasma antidiuretic hormone concentration.

Introduction

Evaluation of renal concentrating ability is extremely useful in differentiating renal disease and adrenal insufficiency from other causes of hypovolemic hyponatremia (Narins et al., 1982; Baesl and Buckley, 1983; Osborne and Polzin, 1983a and 1983b; Knapp, 1984). Current diagnostic references state that renal disease and adrenal insufficiency result in excretion of urine which is not appropriately concentrated, whereas other causes of hypovolemic hyponatremia result in excretion of appropriately concentrated urine (Narins, 1982; Baesl and

Buckley, 1983; Osborne and Polzin, 1983a and 1983b; Knapp, 1984).

Several patients of the Boren Veterinary Medical Teaching Hospital excreted urine of inappropriately low concentration while severely dehydrated, hypotensive, and hyponatremic in the absence of renal disease and/or adrenal insufficiency (Tyler et al., 1986a). This observation and other reports of human (Kingston 1973; Thomas and Morgan, 1979; Miller et al., 1980) and veterinary (Neatherly, 1981; DiBartola et al., 1985) subjects excreting unconcentrated urine during periods of hypovolemic hyponatremia indicate that current diagnostic dogma is fallible.

Urine is concentrated, in the normal animal, by resorption of free water in the collecting tubules and collecting ducts under the influence of antidiuretic hormone (ADH) (Lage, 1984). Plasma ADH concentration is regulated by plasma osmolality, via hypothalamic osmoreceptors, and blood pressure/volume via humoral mechanisms (renin-angiotensin II) and reflex mechanisms (left atrial low pressure and carotid high pressure baroreceptors) (Schier et al., 1979). Osmoreceptors located in the proximity of the third ventricle apparently shrink, due to water passing from the osmoreceptors' cytoplasm to the plasma, in response to increases in plasma osmolality. This results in increased ADH release from the posterior pituitary gland (Schrier et al, 1979; Robertson, 1985). Decreases in plasma osmolality result in water passing from the plasma into the

osmoreceptors' cytoplasm, resulting in osmoreceptor swelling and inhibition of ADH release (Schrier et al., 1979). Blood pressure/volume changes alter the plasma osmolality at which plasma ADH concentration reaches detectable levels (osmoreceptor threshold) (Schrier et al., 1979) and, apparently, alter the rate at which ADH is released (Robertson, 1985). Therefore, in the normal animal, increases in plasma osmolality and/or decreases in blood pressure/volume result in increased ADH release, and subsequent increased free water resorption with increased urine concentration (Schrier et al., 1979; Robertson, 1985).

The reports of severely dehydrated, hypovolemic and hyponatremic human (Kingston, 1973; Thomas and Morgan, 1979; Miller et al., 1980) and veterinary (DiBartola et al., 1985; Neatherly, 1981) subjects excreting urine of inappropriately low concentration in the absence of renal disease and adrenal insufficiency indicate that renal concentrating ability may be impaired during hyponatremic hyponatremia. A recently developed model (Tyler et al., 1986b) of chronic hypovolemic hyponatremia was employed to determine if severe chronic hyponatremia impairs renal concentrating ability during dehyration and hypovolemia and, if so, to identify the renal concentraing mechanisms affected by severe chronic hyponatremia. Urine concentration during water deprivation and in response to exogenous ADH and renal cortical and medullary osmolalities were determined during chronic hypovolemic hyponatremia.

Materials and Methods

Experimental Subjects

The experimental subjects were 5 young adult, intact male, dogs weighing between 13 and 27 kg. Abnormalities were not detected in any of the dogs on physical, urologic, hematologic, and serum and urine chemistry examinations. Upon arrival, the dogs were administered routine vaccinations and anthelmintics then allowed to physiologically adapt for 21 days.

Experimental Treatment

To establish baseline values, when adaption was complete, serum and urine chemistry profiles (serum sodium, potassium, urea nitrogen (SUN), creatinine (SC), chloride, phosphorus, and osmolality and urine sodium, potassium, urea nitrogen (UUN), creatinine (UC), phosphorus, osmolality, specific gravity), packed cell volumes (PCVs), and total plasma protein concentrations (TPPs) were measured 7 times at intervals of two or more days on each dog while he had free access to water. Water deprivation tests were performed on each dog three times at weekly intervals. Plasma and blood volume assays, serum and urine chemistry profiles, PCVs, and TPP concentrations were performed at the beginning and end of all water deprivation tests. A single intravenous (IV) vasopressin response test was performed on each dog during the pretreatment period.

Intramuscular iron injections (Iron Daxtran, Pfizer, New York, New york) were given weekly throughout the experiment to prevent iron depletion.

When baseline values were established, each dog was placed on a low sodium diet (H/D^{TM} Diet, Hills Pet Products, Inc., Topeka, Ks.) and hemorrhaged by jugular phlebotomy until one or more of the following signs was detected: cool extremities, pale mucous membranes, and increased capillary refill time (greater than 2 seconds). Hemorrhage was repeated 2 or 3 times daily until the dog's serum sodium concentration was below 120 mEq/L. Serum sodium concentration, PCV, and TPP concentration were measured daily throughout the experiment.

When a dog's serum sodium concentration was below 120 mEq/L, a water deprivation test with attendant assays was performed on each dog. An IV vasopressin response test was performed at the end of water deprivation.

Upon completion of the studies each dog was administered 500 ml of a balanced electrolyte solution (Isolyte^{R}, American-McGaw, Irvine, Ca.) via the cephalic vein and given free access to a conventional canine maintenance diet (Promix, Ellisson Feed and Seed Co., Norman, Ok.). When a dog's serum sodium concentration had returned to within the normal range for adult dogs, a 48 hour water deprivation test and attendant assays were performed. Each dog was then allowed to convalesce until his PCV and TPP concentration returned to baseline ranges.

When convalescence was complete, a water deprivation test with attendant assays was performed.

Each dog was again placed on a low sodium diet, and again hemorrhaged as described above. When a dog's serum sodium concentration was again below 120 mEq/L, a water deprivation test and attendant assays were performed. After the water deprivation test was completed, an IV ADH response test was performed, and the dog was prepared for nephrectomy. The left kidney was surgically removed by sterile procedures using a dorsal flank approach. After the kidney was removed, the dog was allowed to recover from anesthesia, administered 500 ml of a balanced electrolyte solution via the cephalic vein, and given free access to a conventional canine maintenance diet. When the dog's serum sodium concentration returned to the normal range for adult dogs, a water deprivation test and attendant assays were performed and the dog was humanely killed (barbiturate overdose). The right kidney was immediately removed, and a thorough post mortem and histopathologic examination was performed.

Analytical Procedures and Clinical Evaluations

Serum and urine chemistries, enzymes, and electrolytes were assayed using established laboratory procedures: UUN and SUN by measuring (with an ammonia sensitive electrode) the ammonia released when the serum was treated with a

urease reagent (BUN analyzer, Beckman Instruments, Inc., Fullerton, Ca.); creatinine by a modified Jaffe reaction (Creatinine analyzer, Beckman); glucose by measuring (with an oxygen sensitive electrode) the oxygen released when the serum was treated with a glucose oxidase reagent (glucose analyzer, Beckman); sodium and potassium by flame photometry (Klina Flame^{R} model 652210, Beckman); inorganic phosphorus by a kinetic procedure using a modification of the Tauasky and Shorr reagent (Beckman); calcium by a modification of the Baginski method (Corning Calcium Titrator, Scientific Instruments, Medfield, Mass.); chloride by chloridometry; SALT and SAP by kinetic procedures (ALT Liquid-STAT^{TM} and AP Liquid-STAT^{TM}, Beckman); albumen by a bromocresol green procedure (Albumen Spect Tru^{R} BCG Lancer, St. Louis, Mo.); and osmolality by vapor pressure osmometry (Vapor pressure osmometer, Wescor, Inc., Logan, Utah). Urinalyses were performed by established laboratory procedures utilizing reagent impregnated paper strips (N-multistix^{TM} 5429K, Ames co., Elkhart, In.) read by automated instrumentation (Clin-Tek^{R}, Ames) for semiquanitative chemical analysis. Microscopic evaluation of urine sediment was performed by a medical technologist with any abnormalities reviewed by a clinic pathologist. Hemograms were performed using an automated cell counter (Coulter model S^{R}, Coulter Electronics, Inc., Hialeah, Fl.) for determination of hematocrits (HCTs) white blood cell (WBC) counts, red blood cell (RBC) counts and RBC indeces and refractometry for

estimation of TPP concentration. Differential WBC counts were performed by a medical technologist with any abnormalities reviewed by a clinical pathologist.

Within three minutes after surgical removal of the left kidney and post mortem removal of the right kidney the kidneys were bisected longitudinally. Complete cross sections (less than 1/4 inch thick) were placed in a 10% buffered formalin solution for histologic examination. And, 3 (about 0.25 g) samples of tissue were dissected from both the inner renal medulla and the outer renal cortex for osmolality measurement. Each tissue sample weighed about 0.25 g. These tissues were homogenized separately using mortar and pestle and the osmolality of each homogenate was measured three times by vapor pressure osmometry. The mean and standard error of the cortical and medullary samples were calculated. For the purpose of statistical analysis, the left and right kidneys were treated as paired samples and a one-tailed t test was used to determine the significance of the difference in their osmolalities (Steele and Torrie, 1980).

Dehydration was assessed by evaluation of changes in skin pliability and resiliency (skin turgor) as compared to previous measurements on the same animal. Skin of the dorsal lumbar area and the eyebrow was elevated as high as possible without causing pain to the dog, and released. The rate at which the skin returned to its normal position and configuration was timed. No change from previous

evaluations or from findings in normal animals was classified as "0" change, an increase of less than one second was classified as slight (1+) dehydration, an increase greater than a second but less than 2 seconds was classified as moderate (2+) dehydration, and an increase of 2 seconds or more was classified as severe (3+) dehydration. If evaluations differed between locations at the same testing period the more severe classification was used.

Water deprivation tests were performed by withholding all liquids for a period of 48 hours regardless of the subjects hydration status, SUN, SC, USG, or urine osmolality. The test was to be ended prematurely only if death appeared imminent. Serum and urine chemistry and electrolyte profiles, urinalyses with USG, and hemograms were performed at the start and end of water deprivation.

Plasma volumes were determined by a modification of the Evans blue dye dilution procedure described by Guyton (1981b). Samples were maintained in light free containers before assay and were assayed within 2 hours of collection. Centrifugation with serum filtration was used to separate the serum for assay from the RBC and other clot constitutents. Blood volumes were determined by the following formula:

$$\text{Blood volume} = \frac{\text{Plasma volume X 100}}{\text{100-PCV}}$$

The % of the body weight which was blood was calculated by the following formula:

$$\% \text{ body weight blood} = \frac{\text{blood in kg}^{*} \times 100}{\text{body wt in kg}}$$

*One ml of blood was assumed to equal one gram.

A modification of the IV vasopressin response test described by Hardy (1982) was used to evaluate the dogs' response to exogenous vasopressin during baseline and both hyponatremic periods. The initial urine samples during the baseline period were very concentrated. It was, therefore, necessary to water load the dogs during the baseline period to verify their responsiveness to the IV vasopressin response test. The dogs were water loaded by intravenous administration of 5% glucose until their urine specific gravities dropped below 1.020. It was not necessary to water load the dogs during the hyponatremic periods. When USG values were decreased, a 5% glucose solution containing 5 mU/ml of aqueous arginine vasopressin (Pitressin^{R}, Parke-Davis, Morris Plains, Mo.) was administered IV at a rate of 2.2 ml/kg/hour until three consecutive urine samples failed to have an increase in specific gravity. The pre and post vasopressin samples were treated as paired samples and a one-tailed t test was used to determine the statistical significance of the change in USG and urine osmolality (Steele and Torrie, 1980).

Results

Severe hypovolemic hyponatremia was produced twice in all dogs. Between 1.75 and 2.75 blood volumes were removed from each dog each time before his serum sodium concentration decreased to 120 mEq/L or less.

Urine sodium and chloride concentrations dropped markedly during the first 2 days of hemorrhage and were often undetectable (limits of detection were 3 mEq/L and 2 mEq/L for sodium and chloride, respectively) from the second day of hemorrhage until the end of the hypovolemic hyponatremic period. At that time, sodium chloride was provided via administration of IV balanced electrolyte solutions and/or provision of a conventional canine maintenance diet. Urine potassium, phosphorus, UUN, and UC concentration fluctuated erratically without any apparent relationship to the development of hypovolemic hyponatremia other than as urine concentration decreased their concentrations tended to decrease.

Serum urea nitrogen increased markedly in all subjects during severe hyponatremia, but decreased to values approaching baseline levels during early convalescence (Table I). Urine osmolalities and blood volumes decreased markedly during severe hyponatremia (Table I). Urine osmolalities returned to levels similar to those of the baseline period, but blood volumes remained slightly below baseline values (Table I). Serum osmolality changes during hyponatremia varied among the subjects (Table I).

TABLE I

INDIVIDUAL, MEAN, AND STANDARD DEVIATION VALUES FOR SELECTED SERUM AND URINE PARAMETERS AND BLOOD VOLUMES DURING THE THREE EXPERIMENTAL PERIODS

	Baseline					Hyponatremia					Early Convalescance				
Dog #	NA+ mEq/L	SUN mg/dl	S.OS[a] mosm/L	U.OS.[b] mosm/L	B.V.[c] ml	NA+ mEq/L	SUN mg/dl	S.OS mosm/L	U.OS. mosm/L	B.V. ml	NA+ mEq/L	SUN mg/dl	S.OS mosm/L	U.OS. mosm/L	B.V. ml
1a[d]	152	12	311	2030	2084	118	130	295	423	1061	150	28	308	1875	1829
2a	152	14	314	1874	1386	118	143	311	467	724	154	28	315	2212	1055
3a	149	12	302	1765	2187	117	155	310	440	1145	149	22	311	1785	1759
4a	152	14	309	1671	1444	107	140	280	380	699	148	18	309	2114	1140
5a	151	13	310	1707	1031	112	142	294	360	606	151	21	310	1812	771
1b[e]	150	12	315	2204	2055	118	151	308	421	1063	150	24	310	1860	1754
2b	143	20	311	1710	1267	118	157	309	498	686	149	28	309	1321	937
3b	146	11	308	2004	2010	119	129	280	660	1041	156	28	320	1959	1564
4b	152	20	316	2101	1422	110	150	280	372	645	152	25	310	1559	1169
5b	148	21	294	1790	955	117	138	305	398	564	149	19	306	1601	773
MEAN	150	15	309	1886	1584	115	144	297	442	823	151	24	311	1810	1275
SD	3.10	3.87	6.62	187.29	460.00	4.17	9.72	13.22	87.87	224.91	2.53	3.93	2.97	264.44	415.30

[a] S.OS = Serum Osmolality.
[b] U.OS = Urine Osmolality.
[c] B.V. = Blood Volume.
[d] a indicates the first experimental trial.
[e] b indicates the second experimental trial.

The average of the three osmolality measurements of the three outer renal cortex samples and three inner medulla samples from each kidney of each of the dogs are presented in Table II. The mean medullary osmolality was significantly ($P < 0.0005$) reduced during hyponatremia, but the mean cortical osmolality was not ($P > 0.05$).

Results of the IV vasopressin response tests during the baseline and both hyponatremic periods are given in Table III. There was a significant ($P < 0.0005$) increase in USG following IV vasopressin.

No remarkable lesions were noted during post mortem and histopathologic examination of the kidneys, adrenals, and cadavers.

Discussion

Hypovolemic hyponatremia was successfully produced twice in all of the dogs (Table I). During the periods of hypvolemic hyponatremia, the dogs' renal concentrating ability was impaired (Table I) and glomerular filtration rate was decreased, indicated by increased SUN concentrations (Table I). Water deprivation exacerbated the azotemia but did not elicit a significant increase in urine concentration. Transient renal or adrenal damage is highly unlikely because all dogs excreted concentrated urine in response to water deprivation only a week after their hypovolemic hyponatremic period (Table I) and no recognizable gross or histologic lesions were present in the

TABLE II

INDIVIDUAL AVERAGES AND GROUP MEANS AND STANDARD DEVIATIONS FOR RENAL OSMOLALITIES AND THE CHANGE IN RENAL OSMOLALITIES

	Cortex			Medulla		
Dog No.	R[a]	L[b]	Δ[c]	R	L	Δ
1	313	306	7	1216	451	765
2	323	311	12	1180	358	822
3	338	295	43	1236	334	902
4	324	322	2	1080	336	744
5	311	296	25	1376	414	962
Means	321	306	17.8[e]	1218	372	839[f]
SD	10.76	15.84	16.48	106.98	60.29	92

[a]R = Right kidney removed after normonatremia was reestablished.
[b]L = Left kidney removed during severe hyponatremia.
[c]Δ = Right kidney osmolality - left kidney osmolality.
[d]Osmolality in mosm/L.
[e]Not significant at $P < 0.05$.
[f]Significant at $P < 0.0005$.

TABLE III

INDIVIDUAL MEAN AND STANDARD DEVIATION VALUES FOR USG AND URINE OSMOLALITY AND THEIR CHANGE IN RESPONSE TO IV VASOPRESSION DURING THE BASELINE AND HYPONATREMIC PERIODS

	Baseline					
	Pre		Post		Change	
Dog No.	USG*	U.OS.†	USG	U.OS.	USG	U.OS.
1	1.001	110	1.027	890	0.026	780
2	1.001	121	1.036	1390	0.035	1169
3	1.002	148	1.026	810	0.024	662
4	1.001	114	1.031	1298	0.03	1184
5	1.001	92	1.022	720	0.021	628
Mean	1.0012	117	1.0284	1022	0.0272‡	885‡
SD	0.00045	20.37	0.00532	302.15	0.0054	272.42
	Hyponatremia					
1a	1.014	423	1.016	523	0.002	100
1b	1.014	421	1.015	463	0.001	42
2a	1.014	428	1.018	601	0.004	173
2b	1.013	438	1.017	516	0.004	78
3a	1.015	480	1.019	620	0.004	140
3b	1.017	648	1.019	766	0.002	118
4a	1.013	355	1.015	481	0.002	126
4b	1.014	358	1.015	372	0.001	14
5a	1.012	340	1.015	453	0.003	113
5b	1.013	398	1.015	493	0.002	95
Mean	1.0139	428.9	1.0164	528.8	0.0027‡	99.9‡
SD	0.0137	88.28	0.0017	109.66	0.0013	46.3883

*USG = Urine Specific Gravity
†U.OS. = Urine Osmolality in mosm/L
‡Significant at $P < 0.0005$

kidney removed during the second hypovolemic hyponatremic period. It appears, therefore, that the impaired renal concentrating ability during hypovolemic hyponatremia was due to hyponatremia.

Although current diagnostic dogma indicates that hypovolemic hyponatremic patients without renal disease or adrenal insufficiency are expected to excrete concentrated urine (Perkins and Levin, 1980; Narins, 1982; Baesl and Buckley, 1983; Knapp, 1984), there are reports of human (Kingston, 1973; Thomas and Morgan, 1979; Miller et al. 1980) and veterinary (DiBartola et al., 1985; Neatherly, 1981) subjects without renal disease or adrenal insufficiency that excreted either isosthenuric urine or urine of inappropriately low concentration during hypovolemic hyponatremia. The subjects of these reports were severely dehydrated and mildly to severely azotemic. The significance of the low urine specific gravity values were discussed in only one of the reports (Neatherly, 1981). In that report (a study of hypochloremia in cattle), it was stated that low USG values may indicate renal disease but no renal lesions were found in the cattle which died and renal function was normal in the cattle which recovered from the electrolyte imbalances. Those reports and the results reported herein dictate that use of urine concentration to differentiate renal disease and adrenal insufficiency from other causes of hypovolemic hyponatremia must be reconsidered.

The decreased renal medullary osmolality, during hypovolemic hyponatremia (Table II), surely contributed to the impairment of renal concentrating ability. Sodium and its attendant anions account for about 50% of the solute in the renal medullary interstitium (Pitts, 1974; Roy and Jamison, 1985). It is likely that the decreased medullary osmolality during the hyponatremic periods is due to decreased medullary salt concentration. The medullary salt concentration may have become decreased by several mechanisms. Decreased sodium and chloride concentration in the glomerular filtrate coupled with slow fluid flow in the proximal tubule may have increased the amount of sodium and chloride resorbed in the proximal tubule, decreasing the sodium concentration in the thick ascending limb, and increasing the concentration gradient against which chloride must be pumped to establish and maintain medullary hyperosmolality. Severe hyponatremia may have caused increased diffusion from the medullary interstitium into the vasa recta resulting in medullary washout. Solute diuresis, which has been proposed as a mechanism for decreased medullary hyperosmolality in adrenal insufficiency (Osborne, 1983b), is untenable in this model, because urine sodium levels were extremely low during hyponatremia. It is likely that multiple processes contributed to the decreased renal medullary osmolality during hypovolemic hyponatremia and that the mechanisms involved in this model participate in

decreasing the medullary osmolality during adrenal insufficiency.

Measurement of plasma ADH concentration was prohibited by funding limitations. Therefore, response to exogenous vasopressin was used to determine if plasma ADH concentration was maximal. Intravenous administration of exogenous vasopressin elicited a small but significant increase in urine concentration (Table III). Although this increase was significantly ($P < 0.0005$) less than the increase elicited during the baseline period (possibly due to the lower medullary osmolality during the hyponatremic periods), it indicates that plasma ADH concentration was submaximal during the hypovolemic hyponatremic period. This may be due to the strong influence of sodium concentration on the effective plasma osmolality (Narins, 1982), i.e., the concentration of solutes which do not readily cross cell membranes and, therefore, can cause an osmotic effect (cells to shrink or swell). Increases in effective plasma osmolality initiates ADH release (Robertson, 1976; Schrier, 1979; Robertson, 1985), but plasma osmolality increases due to increases in substances which are not effective osmotic agents, i.e., urea and glucose, do not (Robertson, 1976; Schrier, 1979; Robertson, 1985). Although total serum osmolality varied from slightly decreased to increased during severe hyponatremia (Table I), the effective plasma osmolality was markedly decreased, because a large portion of the plasma solute was urea (Table I). Since decreases

in effective plasma osmolality results in osmoreceptor swelling and inhibition of ADH release (Robertson et al., 1976), ADH release may have been inhibited during the hypovolemic hyponatremic periods.

The decrease in blood volume during the hypovolemic hyponatremic periods might be expected to accommodate for the decrease in plasma osmolality, resulting in maximal ADH release inspite of an extremely low plasma osmolality (Osborne and Polzin, 1983b). Changes in blood volume and blood pressure have similar affects on the ADH release (Robertson, 1976; Schrier, 1979; Robertson, 1985). Blood volume and/or pressure changes alter the effective plasma osmolality (osmoreceptor set point or threshold) at which ADH release is initiated (Robertson, 1976; Schrier, 1979; Robertson, 1985). A 10% decrease in the blood volume and/or pressure results in a 3 to 5 mosmol decrease of the plasma osmolality at which ADH release is initiated (Robertson, 1976; Schrier, 1979; Robertson, 1985). Whereas, a 10% increase in blood volume and/or pressure results in a 3 to 5 mosmol increase in the plasma osmolality at which ADH release is initiated. Blood pressure and/or volume changes may also alter the rate at which ADH is released in response to changes in effective plasma osmolality (osmoreceptor sensitivity) (Robertson, 1976; Robertson, 1985). Studies to determine if marked hypovolemia and/or hypotension can decrease the osmoreceptor threshold sufficiently to allow ADH release during severe hyponatremia have not been

performed. However, if the results produced in studies of the effect of acute moderate hemodynamic changes (Robertson, 1985) are extrapolated to the chronic severe condition, the marked drop in effective plasma osmolality (indicated by serum sodium concentrations less than 120 mEq/L) was too great for the hemodynamic changes to decrease the osmoreceptor threshold sufficiently to allow ADH release.

Although hyposthenuria would be expected if ADH release was totally inhibited during the hypovolemic hyponatremic periods, urine can be concentrated during severe dehydration in the absence of ADH (Gellai, 1979). Therefore, ADH release may have been inhibited during the hypovolemic hyponatremic periods even though urine osmolalities during this period were above plasma osmolalities. Urine concentration increased slightly but significantly ($P < 0.0005$) in response to intravenously administered exogenous vasopressin (Table III). This may have been the maximum response possible (due to the low medullary osmolality). Measurement of plasma ADH concentrations in euadrenal animals during chronic hypovolemic hyponatremia is needed to better define the integration of osmotic and nonosmotic factors in the regulation of ADH release.

Conclusion

Hemorrhage concomitant with low sodium intake results in hypovolemic hyponatremia. When hypovolemic hyponatremia

is severe, dogs become dehydrated, hypotensive, and azotemic and cannot appropriately concentrate their urine. The decrease in renal medullary osmolality which occurred during severe hyponatremia surely contributed to the impairment of the renal concentrating ability. Inhibition of ADH release due to extremely low effective plasma osmolality during chronic severe hypovolemic hyponatremia was suggested by a small but significant increase in urine concentration in response to exogenous vasopressin.

CHAPTER V

SUMMARY AND CONCLUSION

Several severely hyponatremic patients of the Boren Veterinary Medical Teaching Hospital, with clinical and/or clinicopathologic evidence of hemorrhage, failed to excrete appropriately concentrated urine during severe dehydration, hypotension, and azotemia in the absence of adrenal disease. In accordance with current diagnostic dogma, renal disease was diagnosed in these patients (Hardy and Osborne, 1979; Finco, 1980; Osborne and Polzin, 1983a and 1983b; Perkin and Levin, 1980; Narins et al., 1982; Baesl and Buckley, 1983; Knapp, 1984; Lage, 1984; Coles, 1986). Normonatremia was established in some patients. These patients excreted appropriately concentrated urine during normonatremia. No renal lesions were found during complete (gross and histologic) post mortem examination of the animals which died or were humanely killed. These observations suggested that severe hyponatremia was caused by hemorrhage and that severe hyponatremia impaired the patients' renal concentrating ability. A pilot study using a normal, intact male, dog supported these hypotheses. To further test these hypotheses and to determine what mechanism(s) might be

involved, a model was developed and employed in an experimental study.

To develop a model twelve dogs were hemorrhaged daily, while being maintained on a sodium restricted diet. Packed cell volumes, total plasma protein concentrations, and serum sodium concentrations were measured daily and blood volume measurements were performed before hemorrhage and during severe hyponatremia (serum sodium concentration <120 mEq/L). The dogs became severely hyponatremic and hypovolemic after 10 to 16 days of hemorrhage. The development of hyponatremia by this method indicates that at severely decreased blood volumes and/or pressures the nonosmotic factors affecting ADH release and/or thirst become dominant over the osmotic factors affecting ADH release and/or thirst. This model is a useful tool for the study of chronic severe hypovolemic hyponatremia in euadrenal animals. It was subsequently used to study renal concentrating ability, renal cortical and medullary osmolalities, and responsiveness to exogenous vasopressin during chronic severe hypovolemic hyponatremia.

To evaluate renal concentrating ability, renal cortical and medullary osmolalities, and vasopressin responsiveness during hypovolemic hyponatremia, five dogs were made hypovolemic and hyponatremic on two separate occasions by chronic hemorrhage concomitant with a low sodium diet. Serum and urine chemistry profiles, hemograms, water deprivation tests, plasma and blood volume measurements, and

vasopressin response tests were performed before hemorrhage, during both severe hypovolemic hyponatremic (sodium < 120 mEq/L) periods, and during both convalescent periods (vasopressin response tests were not performed during the convalescent period). During the second hyponatremic period the left kidney was surgically removed during water deprivation. The dogs were humanely killed during water deprivation in the second convalescent period and the right kidney was removed. Cortical and medullary osmolalities were measured on all kidneys. During both hyponatremic periods, renal concentrating ability was decreased in all dogs. Renal medullary osmolalities of the kidneys removed during hyponatremia were significantly ($P < 0.0005$) lower than those removed during the normonatremic convalescent periods. Exogenous vasopressin elicited a small but significant ($P < 0.0005$) increase in urine concentration. It appears, therefore, that renal concentrating ability is decreased during hypovolemic hyponatremia and that this dysfunction is due, at least in part, to a decrease in renal medullary osmolality and, apparently, submaximal plasma ADH concentration.

The observations and studies presented herein provide some basic information and a model by which more information can be generated. Severe hypovolemic hyponatremia can be caused by chronic hemorrhage concomitant with sodium restriction. During severe hypovolemic hyponatremia renal concentrating ability is impaired, renal medullary

osmolality is markedly decreased, and ADH release appears to be submaximal. This model should facilitate further investigation of the interaction of osmotic and nonosmotic factors which influence ADH release, thirst, and urine concentration. Studies measuring plasma ADH concentration at serum sodium concentrations between 110 mEq/L and 150 mEq/L while the relative blood volume is held constant at 70% of the original blood volume will allow the ADH-osmoreceptor threshold and sensitivity to be determined during severe hypovolemia. Also, linear regression analysis of the relationship between serum sodium plus potassium concentrations and plasma ADH concentration should establish the ADH-osmoreceptor threshold for and sensitivity to the effective plasma osmolality. This will obviate the effect of substances which are not effective osmotic agents, i.e., urea and glucose, in regressional analysis. It will also allow serum sodium plus potassium concentration (values which are more readily available than plasma or serum osmolality in the clinical situation) to be used to predict when urine should be concentrated in clinical situations. These studies and others are needed to better define the integration of osmotic and nonosmotic factors in the control (via regulation of ADH release, thirst, and sodium retention) of plasma osmolality, blood volume, blood pressure, and body water content.

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VITA

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Doctor of Philosophy

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