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NEUROENDOCRINE FACTORS MEDIATING FLUID ELECTROLYTE HOMEOSTASIS DURING CHRONIC METABOLIC ALKALOSIS IN THE DOG

BY

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ABSTRACT

Hypochloremic metabolic alkalosis was induced in 7 dogs with a combination of Cl^--free diet and furosemide injections, and was subsequently maintained for 8 weeks by feeding Cl^--free diet. Alkalotic dogs demonstrated hypokalemia, hyponatremia, plasma hypoosmolality and polyuria with impaired urine concentrating ability. Alkalotic dogs were also extracellular fluid volume (ECFV)-contracted as indicated by the body weight (BWT) loss and a 10-fold plasma renin activity (PRA) increase.

The plasma arginine vasopressin (pAVP) was not different in unstressed normohydrated or dehydrated alkalotic or non-alkalotic dogs. Non-suppressed pAVP during alkalosis indicated that the urine concentrating impairment was independent of pAVP and that it was of nephrogenic origin. Further, the quantitative relationship between Posm and pAVP was significantly altered in alkalosis. The osmotic threshold for AVP release was approximately 10 mOsm/Kg.H_2O lower in alkalotic dogs compared to controls, while the slope of pAVP/Posm relationship increased nearly two-fold (from 1.185 $pg.ml^{-1}/mOsm.Kg^{-1}$ control to 2.487 $pg.ml^{-1}/mOsm.Kg^{-1}$).

The polydipsia observed in alkalotic dogs appeared to be due to the chronically increased circulating angiotensin II (AII) levels, as indicated by a 10-fold increase in PRA. The alkalotic dogs failed to drink in response to AII infusions (250 ng min^{-1} for 15 min, i.v.) which may be related to pre-occupancy of AII receptors with elevated circulating endogenous AII.

Although water intake (ml/KgBWT) induced by a 2 h i.v. infusion of 1500 mOsm Na_2SO_4 (0.09 ml/Kg BWT/min) was not significantly different in alkalosis, the rate of drinking (ml water/Posm) was significantly lowered (.297 ml/min in alkalosis from .420 ml/min in the control).

Considered together these results suggest that the set-points for osmotic release of AVP was lowered and the sensitivity of AVP release was increased in chronic metabolic alkalosis. The rate of drinking in alkalosis is reduced. The urine concentrating impairment is not due to inappropriate release of AVP and rather is of nephrogenic origin.

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INTRODUCTION

Maintenance of homeostasis depends upon the integrated actions of physiological and behavioral mechanisms. So called "motivated behaviors" (Mogenson, 1977) such as thermoregulation, feeding, and drinking have evolved in order to help preserve homeostasis in conjunction with physiological regulators. In case of fluid and electrolyte homeostasis, a combination of renal, endocrine and drinking behavioral mechanisms all make important contributions. In situations of fluid-electrolyte imbalance such as in pregnancy, the luteal phase of menstrual cycle, certain bronchiogenic tumors, circulatory and renal disorders, both physiological and behavioral mechanisms are altered.

The present study attempts to elucidate how neuroendocrine and thirst mechanisms are altered in metabolic alkalosis, an acid-base disorder that also presents with fluid-electrolyte disturbances.

1.1 Fluid electrolyte Homeostasis

Fluid-electrolyte homeostasis contributes to the hemodynamics of the circulatory system. A variety of physiological mechanisms are involved in the regulation of extracellular fluid volume (ECFV) and composition. At the capillary level, the Starling forces promote fluid exchange in accordance with changes in hydrostatic pressure, colloid osmotic pressure, and lymphatic drainage. Controls intrinsic to the circulation include the adjustment of cardiac output to venous return, the autoregulation of blood flow in peripheral tissues, and the direct effects of arterial pressure on glomerular filtration and urinary output. As well, there are cardiovascular reflexes involving the autonomic nervous system, adrenal medullary secretions and other

vasomotor controls. Finally, a strong endocrine influence is exerted on both renal function and drinking behavior by the renin-angiotensin-aldosterone (RAA) system and by arginine vasopressin (AVP), especially in response to internal needs for sodium and water.

The total body water (TBW about 60% of body weight in males) is distributed between the intracellular (60% of TBW) and extracellular (40% of TBW) spaces. Approximately 20% of the ECFV is confined to the intravascular spaces (the plasma water). Osmotic forces are important in determining the distribution of water between these compartments. Each compartment has one major solute which, because of its restricted trans-membrane mobility, acts to hold water within that compartment. Therefore, Na^+ salts (extracellularly), K^+ salts (intracellularly), and proteins (intravascularly) help to maintain the volumes of the extracellular, intracellular and intravascular spaces. Since the cell membranes are permeable to water, the extracellular and intracellular fluids are in osmotic equilibrium.

Despite wide variations in dietary intake, the volumes and composition of the body fluids are maintained within an extremely narrow range. Variations in urinary excretion are effective means by which the body maintains water and electrolyte balance. However, while urinary excretion can effectively eliminate excesses of substances, deficits can be corrected only by increased intake. When relying exclusively on renal systems, animals suffer from an accompanying loss of electrolytes and negative fluid and electrolyte balances are realized. On the other hand, the behavioral adjustments to fluid deficits are rapid and precise.

1.2 Regulation of Fluid Intake

Of the three main sources of fluid intake, ie. drinking, water contained in food, and water generated by oxidation of carbohydrates, fats and proteins, the latter two are relatively constant. On the other hand, the amount of water ingested by drinking can vary depending upon the tonicity of body fluids, states of cellular and extracellular hydration and volume. Their relative contributions to fluid-electrolyte homeostasis are described below in more detail.

i) Osmotic stimuli for thirst. The mechanisms underlying cellular-dehydration-stimulated thirst were established by Gilman (1937) who demonstrated that the dogs drank twice as much water following infusions of hypertonic NaCl, which dehydrated cells, than following equiosmotic urea, which did not. Holmes et al. (1950) confirmed that the drinking activity was initiated only by solution excluded from the cell interior and not by changes in serum Na^+ and Cl^- levels. The evidence for a quantitative relationship between cellular dehydration and water intake was provided by Fitzsimons (1961). He noticed that the volume of water ingested by bilaterally nephrectomized rats given injections of hypertonic solution was precisely that which was needed to restore body fluid tonicity.

Wolf (1950) was able to determine experimentally that a 1-2% decrease in the volume of the intracellular fluid induced by slow i.v. infusion of hypertonic NaCl initiated drinking in dog and man. These results were confirmed in nephrectomized and intact rats by Fitzsimons (1963). The mechanisms controlling urinary water loss via AVP from the neurohypophysis (Verney, 1947) and those regulating water intake operate synergistically in most conditions to maintain cellular fluid volume.

ii) Extracellular-dehydration-induced thirst. There are multiple mechanisms for maintaining extracellular volume and composition. These include regulation of fluid exchange at the capillary and, controls intrinsic to the circulatory system, such as the adjustment of cardiac output to venous return, the direct effect of arterial pressure on glomerular filtration, cardiovascular reflexes involving the autonomic nervous system, and other vasomotor controls (Fitzsimons, 1972). However, an actual body fluid deficit can only be corrected by the ingestion of water. A decrease in ECF volume, either naturally-occurring or experimentally-induced, is known to stimulate thirst (Fitzsimons, 1972). Experimentally, effective dehydration of ECF can be produced by injecting a hypertonic colloid solution (polyethylene glycol, PG) into the peritoneal cavity (Fitzsimons, 1961). The colloid draws fluid from the plasma by a Starling mechanism without exerting a concurrent effect on body fluid osmolality. A functional hypovolemia induced by physically impeding the circulation to the renal arteries, leads to increased plasma renin activity (PRA) and subsequently to drinking (Fitzsimons, 1972). Baroreceptors, in large vessels entering the heart and in the walls of the atria, send impulses carried in the vagus nerves that exert an inhibitory effect on thirst neurons. In addition to direct nervous activity from baroreceptors, there are hormonal responses to ECF hypovolemia. The regulatory hormonal system of interest to this study, the renin-angiotensin system, is discussed in the next section.

From the evidence cited above, it is clear that water intake is important for maintaining body fluid homeostasis and that drinking is initiated as a regulatory response to reduction of either the cellular

or extracellular compartments. Furthermore, cellular dehydration and extracellular volume loss are each separately competent stimuli for thirst, but have an additive effect on drinking when activated together (Epstein, 1982).

1.3 Renin-angiotensin System

The RAA system plays a major role in blood pressure homeostasis and in the regulation of fluid and electrolytes.

i) Generation of renin and angiotensin

The kidney juxtaglomerular (JG) cells in the renal afferent arteriole are the major source of circulating renin, although brain, arteriolar smooth muscle, uterine smooth muscle, placental tissue, submandibular glands, and certain tumors have been shown to produce renin-like activity (Oparil et al., 1974). Renin is released in response to hypochloremia (Abboud et al., 1979), hypovolemia (Davis et al., 1976) and hyponatremia (Fray, J.C., 1980). Active renin is an acid protease that cleaves angiotensinogen at the leucine-valine bond in human angiotensinogen, and at the leucine-leucine bond in angiotensinogen of other mammalian species, to form angiotensin I (AI) (Tewksbury et al., 1976). AI, a decapeptide, is converted into AII, an octapeptide, by the action of a converting enzyme called dipeptidylcarboxypeptidase, found in the vascular endothelium of the lung, kidney and other organs (Oparil et al., 1974). The half-life of AII is short, 30 seconds, due to rapid degradation by angiotensinases in the blood. AIII, a heptapeptide product of AII metabolism, plays an important role in fluid-electrolyte balance (Mutter et al., 1984) which is described below.

ii) Physiological effects of angiotensin

AII is the major active component of the renin-angiotensin system and has a variety of physiological actions. Its action is mediated by receptors on vascular smooth muscle to promote vasoconstriction. AII interacts with the sympathetic nervous system, both centrally by increasing efferent nerve activity to the periphery, and peripherally by increasing release of catecholamines from the adrenal medulla and by enhancing release and blocking reuptake of norepinephrine from peripheral sympathetic neurons (Peach, 1977). The central pressor effect of AII is partly mediated through the area postrema of the caudal medulla which lies outside the blood-brain barrier. Low Na^+ salt intake decreases the pressor response of AII and high Na^+ salt diet increases this response (Brunner et al., 1972).

AII is a potent dipsogen and may play an important role in ECF volume homeostasis (Kucharczyk and Mogenson 1975; 1977). The dipsogenic action of AII is mediated via AII receptors present in the preoptic area (POA) (Epstein, Fitzsimons and Rolls, 1970), the subfornical organ (SFO) (Simpson and Rottenberg, 1973), and the organum vasculosum of the lamina terminalis (OVLT) (Schrager and Johnson, 1980). It appears that the preoptic region is the principal receptor site for AII of cerebral origin (Kucharczyk and Mogenson, 1977), whereas the SFO mediates drinking induced by increased peripheral AII (Kucharczyk, Assaf and Mogenson, 1976).

AII has also been shown to stimulate AVP release when administered systemically (Malvin, 1971; Bonjour et al., 1970; Haack et al., 1978) and centrally by direct injection of AII into the SFO (Simpson et al., 1979). These interactions are described in detail in section 1.6 below.

AI, along with AII, is capable of stimulating catecholamine production by the adrenal medulla, and may facilitate release of norepinephrine from peripheral sympathetic neurons (Peach, 1977).

An important physiological effect of AII and AIII is the stimulation of aldosterone production in the adrenal zona glomerulosa. This action contributes to the changes in ECF volume and Na^+ and K^+ homeostasis. Aldosterone binds to a cytoplasmic receptor and the complex migrates to the nucleus where it initiates an increase in nRNA synthesis at the level of transcription of DNA. The induced RNA stimulates protein synthesis at the ribosomal level. The function of the aldosterone-induced protein or proteins is a topic of current debate. One hypothesis holds that the protein increases the passive permeability of the cell to Na^+ from the tubular lumen (permease hypothesis); another holds that the protein increases the oxidation of substrate to provide ATP (metabolic hypothesis); and a third holds that the protein acts directly to increase the activity of the sodium pump (Na^+ pump hypothesis). In any case, the effect is increased active transport of Na^+ from the tubular lumen to the interstitium and hence to the blood-stream. Low Na^+ and elevated K^+ levels are also potent stimuli for aldosterone production (Marver and Kokko, 1983). The studies of Hanley and Kokko (1978) suggest chronic mineralocorticoid treatment of rabbit stimulates net chloride flux out of the cortical collecting tubule. Thus these studies show that mineralocorticoids do participate in overall volume homeostasis via effects on the cortical collecting tubule salt reabsorption.

iii) Regulation of renin-angiotensin system

The renin-angiotensin system is regulated at its activation stage via the release of renin, as well as at the level of AII receptors and AII destruction by enzymes present in the blood. The renin substrate and the converting enzymes are normally present in sufficient amounts so that their concentrations are not physiologically rate-limiting for the formation of AII. Therefore, a relatively small change in concentration of PRA is usally the major determinant of the final concentration of circulating AII. AII suppresses renin secretion by a direct effect on the JG cells (Keeton et al., 1980), representing a means of 'feedback regulation'. Renin release in response to Na^+ transport into the macula densa (Opara-Stitzer and Martinez-Maldonado, 1976) may be inhibited by potassium entry into these cells (Linas, 1981). Renin has also been known to inhibit its own release (Davis, 1976).

Vasopressin inhibits the secretion of renin in a variety of species including the rat (Henderson et al., 1978), dog (Malayan et al., 1980), and human (Khokhar et al., 1976). Mechanisms by which AVP inhibits renin release may include reflex response mediated via the renal nerves, by virtue of its antidiuretic activity, by alterations in the renal handling of Na^+, or by its direct action on the JG cells. Further studies are required to establish the relative importance of these different mechanisms in the inhibition of renin secretion by vasopressin (Gregory and Reid, 1984).

1.4. Role of Arginine Vasopressin (AVP)

AVP plays an important role in maintaining fluid-electrolyte homeostasis in the body. Water is eliminated from the body via the urine, stool and evaporation from the skin and respiratory tract. The

evaporative losses are relatively constant in contrast to urinary water excretion, which is highly variable, being largely dependent upon the presence or absence of arginine vasopressin (AVP).

AVP is a neuropeptide with a molecular weight of 1084 daltons and the following amino acid composition: H-Cys-Tyr-Phe-Glu-(NH_2)-Asp-(NH_2)-Cys-Pro-Arg-Gly-NH_2 (Walter et al., 1967). AVP is synthesized in the supraoptic (SON) and paraventricular (PVN) nuclei and transferred to the infundibular process of the neurohypophysis by a fast axonal transport mechanism. Histochemical studies by Shute et al. (1966) and electrical stimulatory studies by Brooks et al. (1966) have provided evidence for hypothalamo-neurohypophysial transport of AVP release.

By virtue of its principal physiological effect of promoting renal conservation of water, AVP helps maintain the osmotic pressure and the volume of ECF and blood. Thus AVP plays an important role in fluid-electrolyte homeostasis.

Circulating AVP (pAVP) allows water reabsorption to occur at the distal tubule and the collecting duct of the nephron. The medullary thick ascending limb of Henle in rats also responds to AVP by increasing NaCl reabsorption (Hall et al., 1980; Herbert et al., 1981). AVP, by increasing the permeability of collecting ducts to urea maintains the hypertonicity of the medulla which, along with the countercurrent multiplication system, is responsible for urine concentration and dilution mechanisms (Burg et al., 1973; Kokko et al., 1972; Rocha et al., 1973).

1.5 Control of Vasopressin Secretion

i) Osmotic stimuli

It has been demonstrated that an increase of 1-2% in body fluid osmolality can effectively stimulate AVP release (Robertson et al., 1976). Thus at plasma osmolalities below a certain minimum or threshold value, pAVP is uniformly suppressed to below 1 pg/ml, a concentration low enough to permit the development of maximum urinary dilution. The marked rise in free-water excretion that normally results from urinary dilution serves to prevent further expansion and dilution of body fluids. Thus, provided renal function and solute excretion are normal, the osmotic threshold for AVP release normally determines the lowest level to which plasma osmolality and sodium can be depressed. Changes in the osmolality (number of osmotically active particles per kilogram of solvent) of the body fluids are sensed by central and peripheral osmoreceptors. The anatomical location of these receptors and their role in the osmoregulation of AVP are discussed next.

a) Central osmoreceptors

The antidiuresis resulting from injections of various hyperosmolar solutions into the carotid artery of conscious dogs undergoing a transient water diuresis led Verney (1947) to formulate the osmoreceptor hypothesis. Since the antidiuresis produced by these intracarotid injections could be mimicked by the injections of posterior pituitary extract, he suggested that the osmoreceptor influenced the release of an antidiuretic hormone from the posterior pituitary gland. Subsequent to ligation of the right internal carotid artery, injections of hypertonic solutions into this artery failed to produce an antidiuresis, thereby suggesting that the osmoreceptors are located somewhere along the

internal carotid artery. Later studies by Jewell and Verney (1957) localized this effect to the anterior hypothalamus. More recent studies have established the presence of osmoreceptors in the subfornical organ, the organum vasculum of the lamina terminalis (Weinjan, 1975), and the supraoptic and paraventricular nuclei (Sachs, 1967; Schrier et al., 1979).

b) Peripheral osmoreceptors.

There is some evidence that liver is capable of responding to the osmolality of the portal blood in the rat (Habreich, 1971) and guinea pig (Niijima, 1969). However, the results of more recent studies do not support hepatic osmoreception (Glasby and Ramsay, 1974; Liard et al., 1984). Thus, the evidence to date for a physiological role of extracerebral osmoreceptors has been inconclusive.

ii) Volumetric regulation

pAVP is also influenced by volume stimuli. Volumetric control of AVP secretion is modulated by sensory elements in the cardiovascular system, with afferent pathways passing primarily via the carotid sinus nerves, the aortic nerves, and the vagi. It has been well established that atrial stretch receptors with vagal afferents can, when stimulated, inhibit the secretion of AVP in anesthetized (DeTorrente et al. 1969) and conscious animals (Zehr et al., 1969; Zucker et al., 1979). In its simplest form the role of the left atrial volume receptor is as follows: an increase in left atrial pressure excites receptors and their rate of firing increases; the impulses are transmitted via the vagus to the diencephalon where they effect a decrease in the release of AVP. Conversely, a decrease in the rate of firing occurs at low left atrial pressures and more AVP is secreted. These changes in AVP secretion

following atrial distension and relaxation are rather quick: 2 and 4 min, respectively (Ledsome et al., 1983).

While plasma osmolality, blood volume and atrial pressure are known to influence AVP secretion, the relative importance and interactions of these different controllers are poorly understood. Dunn et al. (1973) reported that in rat a 14% decrease of estimated blood volume could enhance the osmotic sensitivity for AVP release. Similarly, Robertson and Athar (1976) reported that the osmotic threshold for vasopressin release in human was altered by changes in central venous volume induced by either postural changes or 3% saline infusion. Quillen et al. (1983) have demonstrated a significant modulation of the osmoregulation of pAVP by changes in blood volume induced by left atrial pressure. As a result of this modulation, pAVP is regulated so that contraction or expansion of the blood volume is more expeditiously corrected than would occur if osmoreceptors alone regulated pAVP. Wade et. al., (1983) investigated the contribution of hypovolemia and hyperosmolality to the rise of pAVP in dogs and concluded that the increase in tonicity plays a greater role in the elevation of pAVP than does the reduction in the volume.

Hypovolemia is known to stimulate drinking and secretion of AVP and activation of the renin-angiotensin-aldosterone system (Davis et al., 1976; Share 1974). Furthermore, these responses are physiologically appropriate in that they lead to increased water intake and retention of extracellular fluid and solutes, and thus provide the means for restoration of ECF volume.

Another nonosmotic stimulus, nausea, has also been discovered recently. Unlike the other two stimuli, however, it seems to be extremely potent and usually increases pAVP to levels 10 to 1000 times

those required to produce maximum antidiuresis (Rowe et al., 1979).

1.6 Interaction of AVP and Angiotensin II in Fluid-Electrolyte Homeostasis

The importance of the integrated activities of drinking, AVP secretion and the renin-angiotensin system in the total body water and extracellular osmolality is well recognized (Cowley, 1975; Fitzsimons, 1979; Kucharczyk, 1984). AVP and the renin-angiotensin system interact in two major ways. Angiotensin II, the physiologically active component of the renin-angiotensin system, acts centrally to stimulate the release of AVP. AVP, in turn, acts on the kidney to inhibit the secretion of renin (Reid et al., 1983). Angiotensin II, when injected into the cerebral ventricles in dogs and rats (Share, 1979; Keil et al., 1975) stimulates AVP release as determined by increased AVP in CSF. Angiotensin II has been reported to increase pAVP following i.v. injections in dogs (Bonjour and Malvin, 1970; Ramsay et al., 1978), in rats (Knepel and Meyer, 1980) and in the human (Uhlich et al., 1975; Padfield and Morton, 1977), or after intracarotid injection in dogs (Reid et al., 1982), however, in all cases the doses required are large and the responses small. The likely sites of action of angiotensin-II in effecting AVP release include the subfornical organ (Simpson et al., 1979; Mangiapane et al., 1982), the organum vasculosum of the lamina terminalis (Bealer et al., 1979), and the supraoptic nucleus itself (Simmonet et al., 1979). The physiological significance of angiotensin II-induced AVP release is still not clear. Observations indicate that the renin-angiotensin system plays little or no role in control of AVP secretion during hemorrhage, postural changes, sodium depletion or caval ligation. It may play a role during dehydration and in some forms of

hypertension, but further studies are required (Reid et al., 1983).

1.7 Chronic Disturbances of Fluid-Electrolyte Homeostasis

i) Hemodynamic influences on AVP secretion. The hemodynamic effects were discovered many years ago by Verney and have since been shown to be mediated largely, if not totally, by pressure-sensitive receptors located in the left atrium and large arteries of chest and neck. Therefore, it is important to understand both the functional properties of the baroregulatory system and the way it interacts with the osmoregulation of vasopressin.

Robertson and Athar (1976) demonstrated that acute hemodynamic stimuli appear to act by producing modest upward or downward adjustments in the set-point of the osmoregulatory system. As a consequence of these shifts, the amount of AVP secreted in response to a given plasma osmolality change, is increased or decreased by an amount proportional to the magnitude of the disturbance in blood volume or pressure. However, the secretion of AVP still remains fully responsive to osmotic influences. Consequently, if plasma osmolality falls, AVP secretion can still be suppressed to levels that permit the development of a maximum water diuresis. The only difference is that the protective limit is lower when blood pressure or blood volume is reduced, and is slightly higher when blood pressure or volume is increased. Therefore the only effect of intermittent hemodynamic stimuli in healthy adults is to widen slightly the allowable limits for fluctuations in plasma osmolality.

Although AVP secretion is regulated principally by Posm, the responsiveness of this mechanism may be significantly altered by modest changes in blood volume (Dunn et al., 1973). Intraperiotoneal injections of polyethylene glycol in the rat, which decreased blood

volume without altering osmolality, also increased plasma AVP but this response followed an exponential pattern and did not become significant until volume had decreased by 8% or more. At these levels of acute state of hypovolemia, the osmoregulatory system continued to function but showed a lower threshold and increased sensitivity to osmotic stimulation.

Recent studies have shown that larger, more sustained alterations in blood volume, bring about adaptive changes in the low-and high-pressure baroreceptor control for AVP release. Quillen et al. (1984) have demonstrated that in anephric dogs maintained at chronically low, normal and high volume states, the slope of Posm-pAVP relationships were not significantly different. This was in marked contrast to the findings of Quillen et al. (1983), in which acute volume changes were demonstrated to modulate the normal osmotic control of pAVP. The absence of differences in the chronic hypo-, normo- and hyper-volemic state Posm-pAVP relationship in anephric dogs suggested that low- and high-pressure baroreceptor control has adapted so as not to influence AVP secretion as has been shown to occur acutely (Quillen et al., 1983).

ii) Pregnancy and osmoregulation

It is important to note that a downward resetting of the osmostat can also occur in the absence of hypovolemia or hypotension. One fascinating example is in pregnancy. Recently Durr et al. (1981) showed that the fall in plasma osmolality and sodium concentration during pregnancy in rats is associated with a downward resetting of the osmostat for both thirst and vasopressin secretion.

It has been demonstrated that during pregnancy in rats plasma volume increases up to 74% (Barron et al., 1984) yet the rats secrete

AVP in response to fractional decreases in blood volume in a manner similar to virgin animals, although this occurs in the former at a markedly increased intravascular volume. This indicates that the relationship between total blood volume and AVP secretion is altered during gestation, such that the expanded intravascular volume that accompanies the gravid state is recognized as normal. These results complement the previous results of Dürr et al. (1981) who demonstrated a resetting of the osmotic threshold for AVP release during pregnancy. Similar alterations of osmoregulation have been reported in human pregnancy (Davison et al., 1984).

iii) Hypochloremic metabolic alkalosis and osmoregulation

Hypochloremic metabolic alkalosis is primarily a disturbance of acid-base homeostasis and has several metabolic consequences. These include increased pH, HCO_3, pCO_2, and decreased plasma Cl^-, Na^+, K^+ and osmolality. Extracellular fluid volume is contracted which gives rise to PRA and plasma AII (Nascimento and Calcagno, 1981). Polydipsia (Sztorc, 1984), polyuria and impaired urine concentrating ability are also observed (Cogan and Liu, 1983; Kassiren and Schwartz, 1966; Schwartz and Cohen, 1978).

More than thirty years ago evidence was obtained in the rat that chloride depletion may interfere with normal water conservation (Cooke et al., 1952). Since then, numerous studies have evaluated the concentrating ability of the rat and dog using nitrate or bicarbonate administration. For example, nitrate infusion in the dog leads to a reduction in free-water clearance acutely (Luke et al., 1977; Wallin et al., 1973) and in a more chronic preparation, nitrate-induced-chloride depletion metabolic alkalosis has been associated with a concentrating

impairment (Van Ypersele de Strihou, 1965). Luke et al. (1977) found increased urine flow rate, natriuresis and diminished free-water reabsorption in acutely chloride-depleted rats which were not altered by administration of synthetic AVP. The results of more recent studies (Luke et al., 1978; Galla et al., 1981) suggest that selective chloride depletion alkalosis is associated with impaired solute removal by the thick ascending limb which leads to the development of a concentrating defect. Gutsche et al. (1984) have recently obtained evidence in perfused nephrons that nitrate or bicarbonate substitution for chloride leads to impaired solute removal by the thick ascending limb in vivo. Thus, decreased NaCl reabsorption by the thick ascending loop impairs renal diluting and concentrating ability.

The results of the above-cited studies, along with the observation of polydipsia and hypoosmolality, indicates that metabolic alkalosis is also a disturbance of fluid-electrolyte homeostasis. Although attempts have been made to elucidate mechanisms underlying the urine concentrating defects in metabolic alkalosis prior to our studies (Sztorc, 1984), factors such as changes in water intake and mechanisms of AVP release have not been examined.

1.8 Objectives of the Present Study

This research project had four objectives:

1. To maintain a state of stable chronic metabolic alkalosis in dogs, initially induced by furosemide and Cl^--free diet;

2. To determine the effects of chronic metabolic alkalosis on daily water intake, and on the responsiveness to osmotic and extracellular stimuli of drinking;

3. To examine the role of AVP in the urine concentrating defect in alkalosis, and,

4. Finally, to determine the mechanism underlying persistant hyponatremia in metabolic alkalosis.

2. METHODS

2.1 Animals

The experiments were carried out on 2 male and 5 female adult mongrel dogs that were fully conditioned and weighed between 9.0 and 18.0 kg. The dogs were housed in standard dog metabolic cages in a temperature- and humidity-controlled room with lights on from 06.00 - 18.00 h. The animals were allowed to go outdoors into a special animal containment facility for 20-30 min at the same time each morning. During these exercise periods the dogs were watched closely to avoid ingestion of NaCl or other salt deposits left on the floor or the fencing enclosing the area. The metabolic cages were washed daily with detergent and water and dried before the animals were returned.

2.2 Diets

For a period of time which averaged 2 weeks, the dogs were fed 30 g/kg body weight per day of Purina Dog Meal (Ralston Purina Canada Inc., Mississauga, Ont.). Distilled water was available to the animals *ad libitum*. Following completion of the preliminary measurements (see 2.4 below), the regular lab diet was replaced by a special chloride-free synthetic diet at a daily ration of 30 g/kg body weight (Van Ypersele de Strihou et al., 1969). The percent composition of the Cl^--free diet is shown in Table 2.1 below:

Table 2.1. Composition of dog diet*

	g/Kg Cl-free diet	g % dryweight
Caseine	137.5	30.6
Dextrin	137.5	30.6
Dextrose	75.5	16.7
Fat (corn oil)	68.7	15.3
Agar	13.7	3.0
Polyvitamin mixture Vitadol (Orangeville, Ont.)	0.8	0.2
**Salt mixture	16.4	3.6
Distilled water	550 L	-

**Salt mixture composed of:

	% w/w
Mg Acetate	17.64
$ZnSO_4$	.08
$CaCO_3$	62.7
$MnSO_4 \cdot H_2O$	0.8
$CuSo_4 \cdot 5H_2O$	0.06
$CaHPO_4$	12.96
Fe citrate $\cdot 5H_2O$	5.75

3.5 mEq/kg BWT Na^+ and 2.5 mEq/kg BWT K^+ were added to the diet as sulfates.

*All chemicals except vitamin mixture were purchased from Sigma Chemical Co., St. Louis, MO., U.S.A.

The diet was prepared in small amounts of approximately 5 Kg. This avoided frequent handling and thus chloride contamination of the diet. The specific procedures used were as follows: Agar was added to a freshly washed and distilled-water-rinsed 6L stainless steel pot. Distilled water was added and the pot was placed on a hot plate. The solution was heated and stirred with a magnetic stirrer until the solution became quite viscous. At this point salt solution and corn oil were added. After 10 minutes of heating, all of the other ingredients, except the vitamin mixture, were added. The mixture was stirred continuously until the temperature was near boiling. The vitamin mix, which was pre-dissolved in corn oil, was then added. Stirring was continued for another 10 minutes after which the mixture was poured into 5 cm deep plastic dishes and allowed to solidify. These dishes were then stored and refrigerated at 4°C in the laboratory. The finished diet had a semi-solid consistency. The dogs were offered this diet once a day at the same time in the morning. In all cases the entire daily dietary allotment was promptly eaten. Occasionally, following the completion of experiments, the dogs were given small amounts of Cl^--free dried diet as a reward.

The distilled water available for drinking was held in 5 L polyethylene bottles connected by tubing to a leak-proof nozzle located within easy reach of the animal in the metabolic cage. The reservoirs were refilled to the rim at the same time each day, and the volume added was recorded as the daily intakes (± 1 ml).

Urine osmolality was measured in small (2 ml) samples collected in a washed, distilled-water-rinsed and dried 20 cm diameter x 10 cm deep stainless-steel dish at desired intervals (see below).

Body weights were recorded daily on a large animal balance (Toledo Scale Co., Model 2081, Windsor, Canada).

2.3 Procedure for Induction of Alkalosis

Following a control period lasting a minimum of 15 days, dogs were fed Cl^--free diet for at least 5 days. The study can be divided into the following three periods.

Period I: Furosemide injections. The animals received an injection of furosemide (4-chlor-N-furfuryl-5-sulfamoyl-anthranilic acid, 20 mg i.m. per day, Lasix, Hoechst Canada Inc., Montreal) for 4 or 5 consecutive days until arterial pH and plasma HCO_3 values were significantly elevated compared to controls.

Period II: Post-furosemide. Observations were continued for an additional 15 days without further injections of furosemide until the acid-base status of the animal had stabilized at a level (ie. pH > 7.48) at which animals can be classified as alkalotic.

Period III: Testing period. All urine-concentrating tests, water-restriction tests and infusion tests (see below) were carried out at this time.

2.4 Blood Sampling and Processing.

(i) Acid-base evaluation: Dogs were trained to lie on their sides on a table. Their front legs were then tied together with a cotton strap, one end of which was fastened to the table. One hind leg, the

one on the resting side, was also tied with a cotton strap and fastened to the table. The femoral artery was located by palpation in the upper thigh and a small puncture insertion was made with a 20-23 gauge needle attached to a 3-5 ml heparinized glass syringe. The syringe quickly filled with blood and was then withdrawn and the needle stoppered quickly. A cotton swab was promptly placed over the puncture point and pressed firmly for at least 3 minutes in order to avoid a hematoma. The blood sample was quickly taken to the lab for acid-base analysis, the blood was analyzed for pH and tCO_2 within 5 minutes of its arrival in the laboratory. The total elapsed time between the sample collection and the determination of the acid-base status of the animal was less than 10 minutes.

(ii) Venipunctures: Blood samples were taken from either the cephalic or saphenous vein with the dogs standing quietly in a modified Pavlov stand, to which they were accustomed. For arginine vasopressin (AVP), plasma renin activity (PRA) and angiotensin I (AI) analysis, blood was collected into pre-chilled EDTA tubes kept on an ice bath. After collection the tubes were gently mixed and centrifuged at 760 G for 15 min at 4°C. Hemolysis-free plasma was then collected in aliquots of 1 ml and was frozen (at -20°C) immediately. For analysis of osmolality and electrolytes the blood was collected in heparinized vacutainer tubes and samples were spun down at 4°C, and hemolysis-free fresh plasma was used for pOsm determinations, where as an aliquote of the same plasma was frozen to be utilized for electrolyte determinations later on.

2.5 Minor Surgical Procedures

The dogs were brought to the experimental room at least 30 minutes prior to the start of infusion tests and placed in the Pavlov stand with drinking water available ad libitum. This procedure was repeated several times in order to ensure that the dogs were familiar with the experimental situation. The cephalic vein was cannulated with an 18 gauge in-dwelling catheter (Monoject, St. Louis, MO.). Upon confirmation of the proper insertion of the butterfly into the vessel (determined by the blood flow), the catheter was secured in this position by wrapping a piece of masking tape around the leg. The catheter was then attached to a 1.0 meter-long piece of polyethylene tubing (PE-190; I.D. 1.19 mm., Clay Adams Becton, Dickinson Co., Parsippany, N.J., U.S.A.) via a custom-made adaptor. The other end of the polyethylene tubing was attached to the infusion pump. At this point the saphenous vein in the contralateral hind limb was punctured with a 21 gauge butterfly (winged infusion set, Jermo Corp, Tokyo, Japan); the pre-infusion blood sample and subsequent timed blood samples were collected from this site. On certain days, pre-infusion samples were drawn from the site of infusion before the catheter was hooked up to the infusion pump.

2.6 Infusion Tests

About 15-20 minutes after the minor surgery described in 2.5 above a pre-infusion blood sample was drawn from a contralateral saphenous vein using a butterfly. Two types of infusion tests were carried out: (a) Na_2SO_4 infusion - (1) above Na_2SO_4 infusion with no access to water; 0.75 M Na_2SO_4 (1500 mOsm) was infused for 120 minutes at a rate of 0.09 ml/kg.min and blood samples were drawn at 20 minute intervals for

pAVP, PRA/AI and Posm and were processed as described in 2.4(ii).
(2) Na_2SO_4 (1500 mOsm) was infused as in (1) but with access to water and blood samples were drawn for the Posm determinations. The time of onset of drinking and the cumulative water intake were recorded.
(3) 300 mOsm and 281 mOsm Na_2SO_4 infusions were also carried out in the control and alkalotic periods, respectively as described in (1) above.
(b) Angiotensin II infusion. Angiotensin II was infused at 250 ng/min for 15 minutes; the latency period till the drinking commenced and the cumulative water intake were recorded. Blood samples were drawn at 2-minute intervals for determination of pAVP and PRA/AI.

All infusion tests described in (a) and (b) above were carried out in the control and alkalotic period. A 48 hr rest period was allowed between any two infusion tests.

The infusion pump (Sage Instruments Model 341, Cambridge, Mass., U.S.A.) was pre-calibrated by dispensing the test solution into a 25 ml graduated cylinders for timed intervals of 5 minutes. Then by dividing the volume by the time, infusion rates were calculated for four speeds of the infusion pump. For a 30 ml plastic syringe (Plastipak, Becton-Dickinson, Co., Rutherford, N.J., U.S.A.), speed settings 6, 7, 8 and 9 were tested. To double-check the calibration, a fixed volume of 10 ml was dispensed and time was recorded at speeds 6,7,8 and 9. Then by using the same formula (volume/time) the infusion rate was calculated. Mean values of infusion rates obtained by both methods were then plotted on a linear graph paper as pump speed versus infusion rate. The best-fitting straight line was drawn and the desired infusion rate was matched to the pump speed. The speed setting on the infusion pump was adjusted to 0.09 ml/kg.min according to the individual dog's body

weight. Solutions were infused for 120 minutes and the blood samples were drawn at 20-minute intervals during the infusion period.

2.7 Infusion Solutions

Angiotensin II [(Ile^5)-Angiotensin II, Sigma Chemical Co., St. Louis, Mo.], Na_2SO_4 and NaCl solutions were used. AII and Na_2SO_4 solutions were freshly prepared on the day of the infusion experiments. Isotonic Na_2SO_4 (0.15 M) was prepared by adding 21.30 g of Na_2SO_4 to 1 liter of distilled water. This solution was then sterilized for 20 minutes at 121°C and stored at 4°C for later use. A 0.75 M Na_2SO_4 solution was prepared by dissolving 10.6545 g of the salt in 100 ml sterile distilled water. A stock solution of 250 ng/ml AII was prepared by dissolving precisely 1 mg of AII powder in 40 ml of sterile 0.15 M Na_2SO_4. On the day of the experiment, 1 ml of stock AII was added to 99 ml of 0.15 M Na_2SO_4 to achieve a final concentration of 250 ng ml^{-1} of AII. A recovery (rest) period of 48 hrs was allowed between infusion experiments in individual dogs.

2.8 Urine-Concentrating Tests

After the daily urine output volume, body weight and water intake had been recorded, a venous blood sample was collected for AVP, AII and osmolality determination. Dogs were then water-deprived for a period of 24 hrs. An overnight urine sample was collected, and its volume was measured and saved for urine osmolality determination; another blood sample was withdrawn for AVP, AII and osmolality analysis before returning food and water to the dogs. All urine samples were collected under mineral oil and stored at 4°C. Osmolality analysis were done within 48 hrs of urine collection.

2.9 Water-Loading Tests

Dogs were allowed to have free access to food and water. The urine initially voided on the morning after the feeding was discarded. Dogs were offered 500 mls of 2% milk-water solution of about 2 mOsm/Kg.H_2O in a normal feeding pan. Usually this milk-water was ingested quickly. Blood samples for Posm were drawn before offering the drink and afterwards, at 30-minute intervals for 120 mins. Urine samples were collected in the metabolic cage collecting receptacles for Usom at every 30-minute intervals for 120 minutes. Percent of water load excreted (WLE) was calculated by the following formula: WLE =

$$\frac{\text{Total Urine volume}}{\text{volume ingested}} \times 100$$

2.10 Analyses

(i) Na^+ and K^+ were analyzed by flame photometer (Instrumentation Laboratory Inc., Lexington, Mass. U.S.A., Model IL343 and 443) using a lithium internal standard. Plasma chloride concentration was determined on a Chloride Titrator (Radiometer, Copenhagen, NV, Denmark, CMT 10 Chloride Titrator) using a coulometric principle. In coulometric analysis the sample concentration is determined from the equivalent amount of total electrical charge consumed during titration, i.e., the current-time integral. The sample concentration (miliequivalent/litre) is proportional to the elapsed time of the titration.

(ii) Blood pH. Heparinized whole blood pH was measured on the acid-base analyzer (Radiometer Type PHM72, Copenhagen N.V. - Denmark).

(iii) Total CO_2. Total CO_2 of heparinized plasma was analyzed on a 965 Carbon Dioxide Analyzer (Corning Medical and Scientific, Medfield,

MA. U.S.A.) CO_2 of plasma is released through reaction with lactic acid and then released. CO_2 concentration in mmoles/L was measured by means of a thermal conductivity detector.

(iv) Osmolality. Osmolality measurements in plasma and urine samples were determined by freezing-point depression (μ Osmette model 5004; Precision Science). The total concentration of all dissolved particles in solution, including ionic and non-ionic substances, causes a proportional lowering of freezing point which is measured by the osmometer.

(v) Packed cell volume and plasma proteins. Micro-capillary hematocrit tubes were filled with heparinized blood samples and spun in an IEC micro-capillary centrifuge Model MB. PCV was determined on an IEC micro-capillary reader Model CR (IEC, Needham Hts., Mass).

The plasma in the hematocrit tube was used for protein analysis (Model 10406 Proteinometer, American Optical, Buffalo, N.Y.). The proteinometer utilizes the principle of refraction to determine protein concentration of plasma.

2.11 Hormonal Analyses

(i) AVP radioimmunoassay. AVP concentrations were determined by Dr. D. Bichet, Sacre Coeur Hospital, Montreal. Blood samples collected in chilled EDTA tubes were centrifuged at 4°C at 760 G for 20 min and plasma was collected for the AVP determination. AVP was extracted by a modification of the acetone method described by Robertson et al. (1973) and Durr et al. (1981). Briefly, 1 ml of thawed sample is mixed with 2 ml of cold acetone and centrifuged. The supernatant is then mixed with 5 ml of cold petroleum ether and recentrifuged. Tubes are then frozen to -80°C and the top phase (liquid) is discarded. The bottom phase is

then thawed and evaporated to dryness at room temperature under a stream of cold air (Concevator sample concentrator, E-C-Apparatus Corp. St. Petersburg, Florida 33709). The dry residue is reconstituted in 750 µl of a 0.1 % bovine albumin solution (Miles, P.O. Box 2000, Alkhart, Indiana, 46515) also containing 0.1% sodium azide (pH is adjusted to 7.2 with Tris-Base).

The assay buffer was 0.1 M sodium phosphate at pH 7.6 and contained 0.3% (wt/vol) NaCl, 0.1 g/100 g bovine serum albumin (Miles) and 0.1 g/100 g of sodium azide. Standard curves were prepared with purified AVP (Batch No. BAA 525, 1 mg = 450 IU, Ferring Pharmaceuticals, Box 30561, S-200 62, Malmo, Sweden) in quantities that ranged from 0.05 to 10 pg per assay tube. 200 l of standards in buffer or 200 l of reconstituted plasma extract, and 200 l of antiserum in buffer were incubated in triplicate for the standards and in duplicate for the unknown, for 2 days at 4°C; then 100 l of tracer (200 to 800 cpm) was added and incubation was carried on for an additional 3 days. Free and bound fractions were then separated by a dextran-charcoal method.

Extracts of plasma from four patients at Sacre Coeur Hospital with complete central diabetes insipidus failed to displace tracer and were used regularly as controls.

The tracer used was vasopressin-8-arginine [^{125}I] -monoiodinated (Amersham) with a high specific activity (1820 to 2200 Ci/mM). Non-specific binding was always 3%.

The antiserum (As-2849) used at a final dilution of 1/2.5 x 10^6 was generously provided by J. Durr and M. Lindheimer (Dept. Obstetrics, Gynecology and Medicine, University of Chicago, Illinois 60637). The cross reactivity of this antiserum was less than 8% for

lysine-vasopressin and less than 4% for arginine-vasotocin. Sensitivity of the assay using this antiserum in our laboratory was always 0.1 pg/assay tube and the 50% displacement was 1.2 pg/tube. Cold vasopressin was added to the plasma of patients with central diabetes insipidus and the mean recovery was 102 ± 4%. Intraassay coefficient of variation for AVP plasma values between 2 and 5 pg/ml was 5 to 13%. Mean interassay coefficient of variation for plasma vasopressin values between 0.5 and 18 pg/ml was 20% (0.2 ± 0.12, mean ± SD). Characteristics of this antiserum and radioimmunoassay have been described previously (Bichet et al., 1984).

(ii) Measurements of plasma renin activity (PRA).

Blood samples were collected in chilled EDTA tubes, centrifuged at 4°C at 760 G for 20 min and kept at -20°C until assay. Incubation for generation of angiotensin I was carried out at pH 6.0 for 2 hours with 8-hydroxyquinoleine. The antiserum used was highly specific. Tracer was ^{125}I-angiotensin I from New England Nuclear (Boston, Mass). Standard curves were prepared with synthetic angiotensin I (Sigma, St. Louis, Mo) in quantities that ranged from 10 to 500 pg per assay tube. Plasma renin activity was measured as ng of angiotensin I per ml of plasma per hour of incubation (Stockigt et al., 1971).

2.12 Renal Histology

At the end of the experimental period, dogs were sacrificed and the kidneys were preserved in 10% buffered-formalin solution. Several parts of the cortex and the medulla of both kidneys of each dog were fixed and wax sections were cut. These sections were stained with HPS, Van Kossa and Alzarin "S" stains (Sarkar et al., 1973). The sections were examined under light microscope by Dr. Blair Carpenter at the Children's Hospital of Eastern Ontario.

2.13 Statistical Analyses

All data were analyzed on a Wang 600 series Computer (Wang Laboratories Inc. Tewksbury Mass. U.S.A.). Quantitative results are expressed throughout as Means ± Standard error of the mean (S.E.M.). Paired t-test was used when only two related groups were compared and a value with $p < 0.05$ was considered significant.

Analysis of the quantitive relationship between Posm-pAVP and Posm-ml(water were determined by linear regression for several experiments in each animal and for pooled data for three animals during the control and alkalotic conditions. Significance of the differences between the slopes was determined by ANOVA, and a value with p 0.05 was considered statistically significant.

3. RESULTS

3.1 Characteristics of chronic metabolic alkalosis induced by dietary Cl^- deprivation and furosemide.

Alkalosis developed gradually after the combined Cl-free diet and furosemide treatment, and was maintained for several weeks in the dogs kept on Cl^--free diet. The maintenance of stable alkalotic state was determined by blood pH elevation as presented in Fig. 3.1 Longitudinal profiles of plasma electrolytes are also presented in the same diagram. A comparison of pH and electrolytes in alkalosis and pre-alkalosis are shown in Table 3.1 (Each animal served as its own control).

Blood pH and plasma HCO_3 were significantly elevated in alkalosis (7.41 ± .004 to 7.51 ± .005, and 21.2 ± .19 to 31.7 ± .74 mmol/L, respectively), whereas plasma Cl^-, Na^+, and K^+ were decreased significantly (114 ± .6 to 91 ± 2.0, 152 ± 1.2 to 140 ± .9, 4.2 ± .10 to 2.8 ± .15 mEq/L, respectively) in all 7 dogs.

Alkalotic dogs showed a significant loss of body weight (14.9 ± .91 control to 12.6 ± 1.0 kg alkalosis), elevation of hematocrit and plasma protein (42 ± 1.3% vs 52 ± 1.1% and 5.9 ± .08 vs 7.0 ± .06 g/dl, respectively) (Fig. 3.2 & Table 3.2).

Daily water intakes and urine volumes were measured in four dogs. Fig. 3.3 indicates that there was a significant increase in daily water intake (76 ± 6.7 to 117 ± 10.2 ml/kg) and urine volume (20 ± 2.9 to 70 ± 11.0 ml/kg) A gradual decrease in plasma osmolality was observed in alkalotic dogs (Fig. 3.4A). Pooled data of Posm during 1-6 weeks (289 ± 2.4 mOsm/Kg.H_2O) and 6-8 weeks (282 ± 1.0 mOsm/Kg.H_2O) were both

Fig. 3.1 Blood pH and plasma electrolyte concentrations of 7 dogs in the control (non-alkalotic) and stabilized alkalotic period. The arrow represents the start of 4-5 injections of 20 mg/d i.m. furosemide. Each point represents mean ± S.E.M. of weekly determinations.

FIGURE 3.1

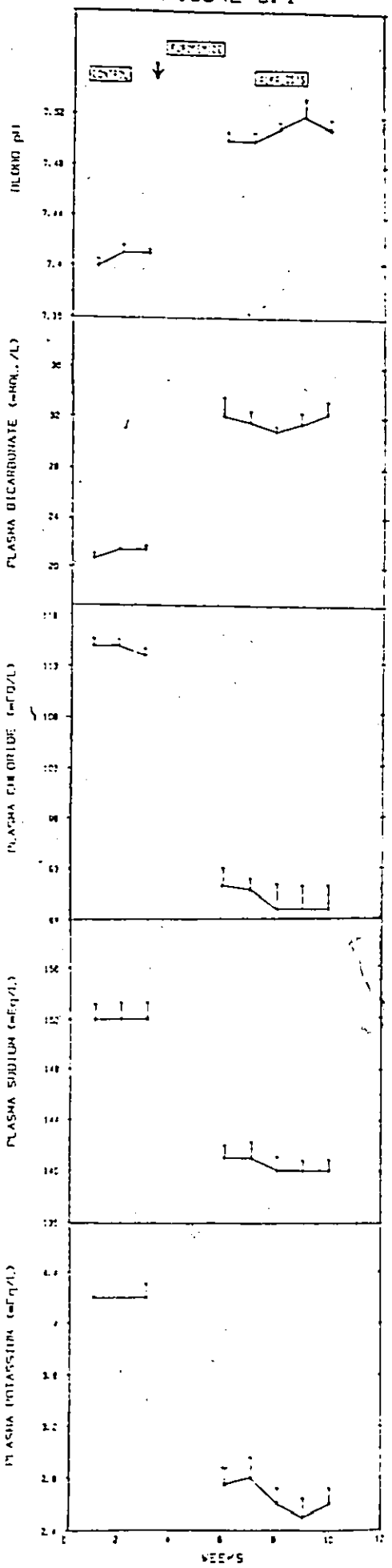

BLOOD pH
PLASMA BICARBONATE (mMOL./L)
PLASMA CHLORIDE (mEQ/L)
PLASMA SODIUM (mEq/L)
PLASMA POTASSIUM (mEq/L)
WEEKS

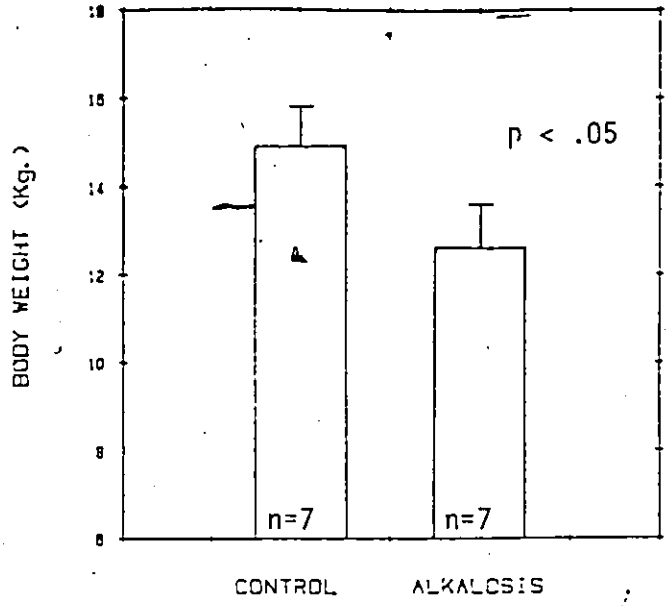
TABLE 3.1: Blood pH, plasma HCO_3, plasma Cl^-, plasma Na^+, and plasma K^+ before and during alkalosis (n =7).

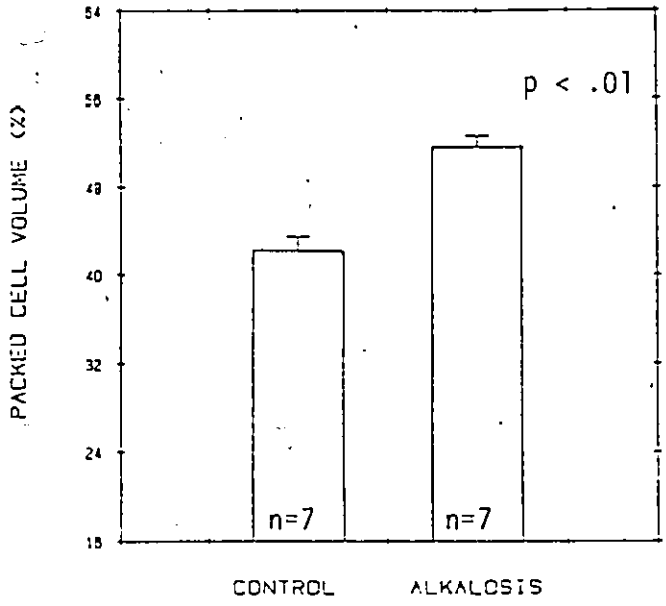
	pH		HCO_3 (mMol/L)		**CL^- (mEq/L)		**NA^+ (mEq/L)		**K^+ (mEq/L)	
DOG	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS
'B'*	7.41 ±.003	7.51 1.011	21.4 ±.45	32.6 ±.72	113 ±.9	89 ±1.0	156 ±.6	138 ±.5	4.5 ±.03	2.3 ±.07
'E'*	7.39 ±.013	7.49 ±.002	21.9 ±.32	32.6 ±.71	114 ±.6	95 ±.6	154 ±.3	143 ±.3	4.5 ±.06	2.2 ±.07
'F'*	7.41 ±.003	7.53 ±.015	21.6 ±.20	32.9 ±.91	113 ±.7	85 ±1.6	154 ±.6	143 ±1.0	4.4 ±.03	2.8 ±.10
'H'*	7.40 ±.001	7.52 ±.008	21.1 ±.48	33.9 ±1.10	116 ±.6	85 ±.8	147 ±.6	137 ±.5	4.0 1.03	3.0 ±.15
'M'*	7.41 ±.006	7.51 ±.004	20.8 ±.50	29.7 ±.12	112 ±.6	89 ±.3	149 ±.6	139 ±.2	4.0 ±.03	3.1 ±.10
'T'*	7.40 ±.009	7.51 ±.005	20.9 ±.19	32.5 ±2.15	116 ±.7	93 ±.7	151 ±.3	139 ±.6	4.0 ±.03	3.2 ±.25
'W'*	7.42 ±.006	7.50 ±.005	20.4 ±.60	28.4 ±.59	115 ±.7	99 ±1.2	151 ±.6	142 ±1.1	3.9 ±.03	3.0 ±.10
MEAN**	7.41 ±.004	7.51 ±.005	21.2 ±.19	31.7 ±.74	114 ±.6	91 ±2.0	152 ±1.2	140 ±.9	4.2 ±.10	2.8 ±.15
PAIRED T-TEST t =	19.7466		17.4081		12.2465		10.2321		5.6700	
P <	.001		.001		.001		.001		.01	

*Mean ± S.E.M. of multiple determinations of each variable during control and stable period.

**Cl^-, Na^+, K^+ values may be falsely high, since these were determined on plasma samples stored frozen for several weeks in polyethylene tubes that may have allowed some dehydration to take place.

Fig. 3.2 Body weight (BWT) packed cell volume (PCV) and plasma protein (PP) in control and alkalotic dogs. Data are means ± S.E.M. Weekly averages of BWT, PCV and PP measured every second day were pooled for the control and alkalotic periods. The means were statistically compared by paired t-test. Significant losses in BWT occurred in alkalosis ($t = 3.4272$, $p < .05$, $n = 7$), and increases in PCV ($t = 5.0531$, $p < .01$, $n = 7$) and PP ($t = 12.3858$, $p < .001$, $n = 7$) were also observed.


BODY WEIGHT (Kg.)
p < .05
n=7
n=7
CONTROL
ALKALOSIS


PACKED CELL VOLUME (%)
p < .01
n=7
n=7
CONTROL
ALKALOSIS

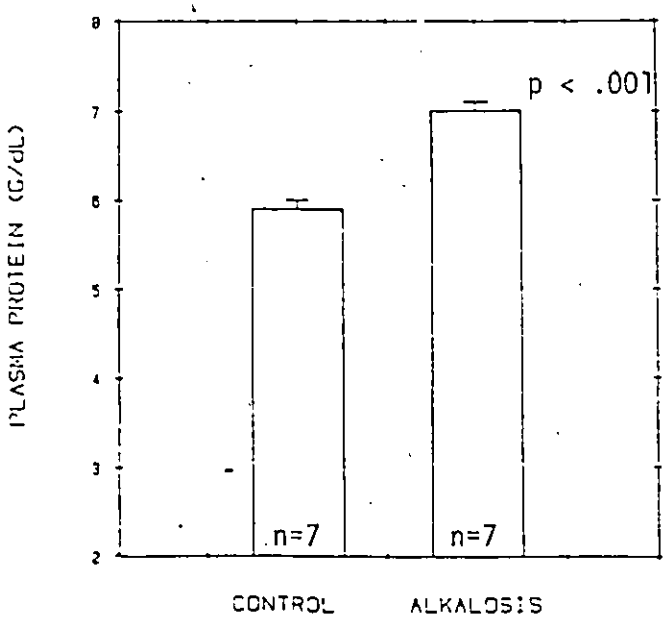

PLASMA PROTEIN (G/dL)
p < .001
n=7
n=7
CONTROL
ALKALOSIS

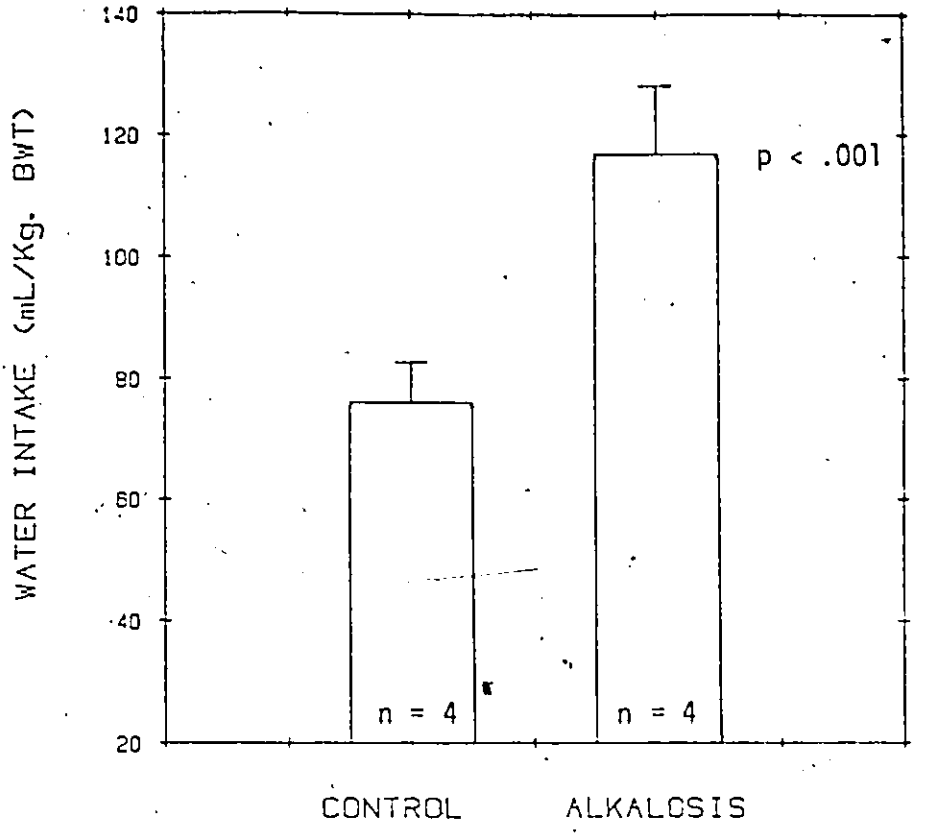
Table 3.2: Body weights, hematocrits, and plasma proteins of dogs during the control and alkalotic periods (n = 4).

DOG	BODY WEIGHT (Kg)		HEMATOCRIT %		PLASMA PROTEIN (g/dL)	
	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS	CONTROL	ALKALOSIS
'B' *	15.2 ±.12	13.2 ±.18	44 ±.3	47 ±.5	5.7 ±.06	6.7 ±.07
'E' *	15.8 ±.12	13.2 ±.17	45 ±.3	53 ±.3	6.1 ±.03	7.0 ±.05
'F' *	15.0 ±.01	13.9 ±.06	40 ±.3	55 ±.7	6.0 ±.03	7.2 ±.10
'H' *	15.5 ±.07	13.9 ±.10	37 ±.2	51 ±.4	6.0 ±.09	7.1 ±.05
'M' *	18.2 ±.06	16.6 ±.11	42 ±.3	49 ±.6	6.0 ±.03	7.0 ±.06
'T' *	10.2 ±.06	9.2 ±.01	41 ±.3	55 ±.6	5.6 ±.03	7.1 ±.06
'W' *	14.1 ±.07	13.1 ±.05	48 ±.3	52 ±.3	6.2 ±.10	7.0 ±.05
MEAN	14.9 ±.91	12.6 ±1.0	42 ±1.3	52 ±1.1	5.9 ±.08	7.0 ±.06
PAIRED T-Test t =	3.4272		5.0531		12.3858	
p <	.05		.01		.001	

*Mean ± S.E.M. of multiple determinations of each variable during control and stable alkalotic period in individual dogs.

Fig. 3.3 Daily normalized water intakes (WI) and urine volumes (UV) of dogs during the control period and in alkalosis. Weekly averages were pooled for the control and the alkalotic period. The mean WI in the control period (76 ± 6.7 ml/kg) and alkalotic period (117 ± 10.2 ml/kg) and the mean UV in the control period (20 ± 2.9 ml/kg) and in alkalosis (70 ± 11.0 ml/kg) were statistically compared by paired t-test. Significant polydipsia, WI = ($t = 6.8576$, $p < .001$, $n = 4$)) and polyuria, UV = ($t = 3.8047$, $p < .05$, $n = 4$) were observed in alkalosis.

FIGURE 3.3


WATER INTAKE (mL/Kg. BWT)
140
120
100
80
60
40
20
p < .001
n = 4
n = 4
CONTROL
ALKALOSIS

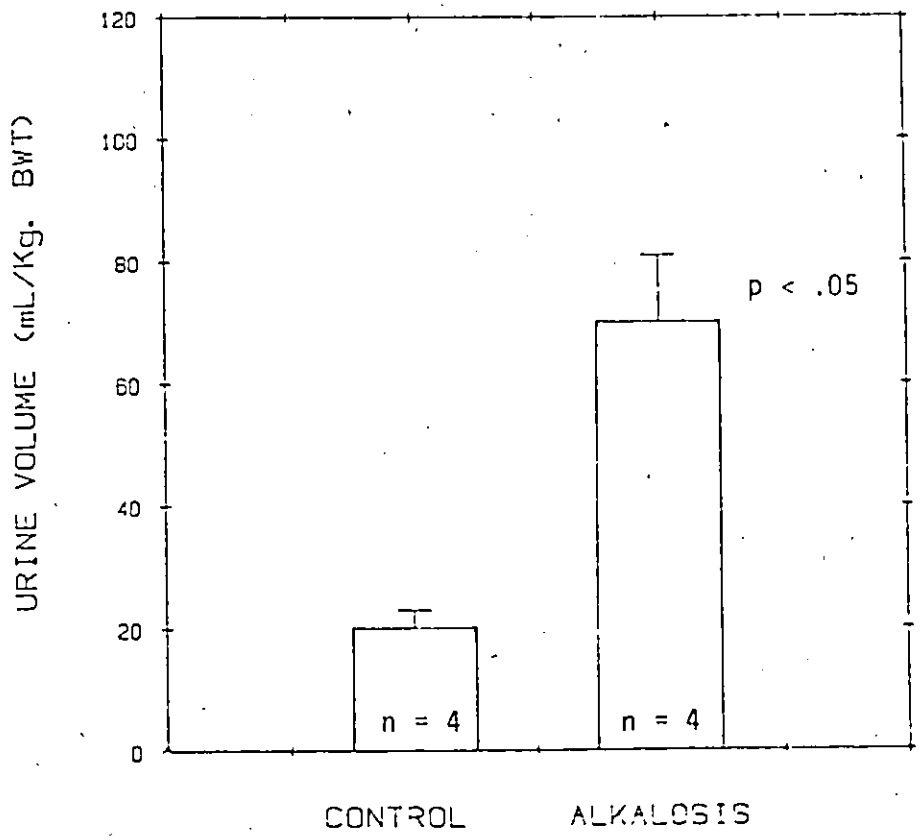

URINE VOLUME (mL/Kg. BWT)
120
100
80
60
40
20
0
p < .05
n = 4
n = 4
CONTROL
ALKALOSIS

Fig. 3.4 Plasma osmolalities (Posm) of control and alkalotic dogs. Fig. 3.4(A) shows a gradual decrease in Posm (weekly average + S.E.M.) over the alkalotic period. In Fig. 3.4(B), the mean Posm in the control period (300 ± .4 mOsm/kg·H_2O) was compared to the mean Posm during 1-6 weeks (289 ± 2.4 mOsm/Kg·H_2O) and 6-8 weeks (282 ± 1.0 mOsm/Kg·H_2O) in alkalosis, with a paired t-test. During metabolic alkalosis the dogs were hypoosmotic [$t = 4.0466$, $p < .05$, $n = 4$ (during 1-6 weeks) and $t = 14.6000$, $p < .001$, $n = 4$ (during 6-8 weeks].

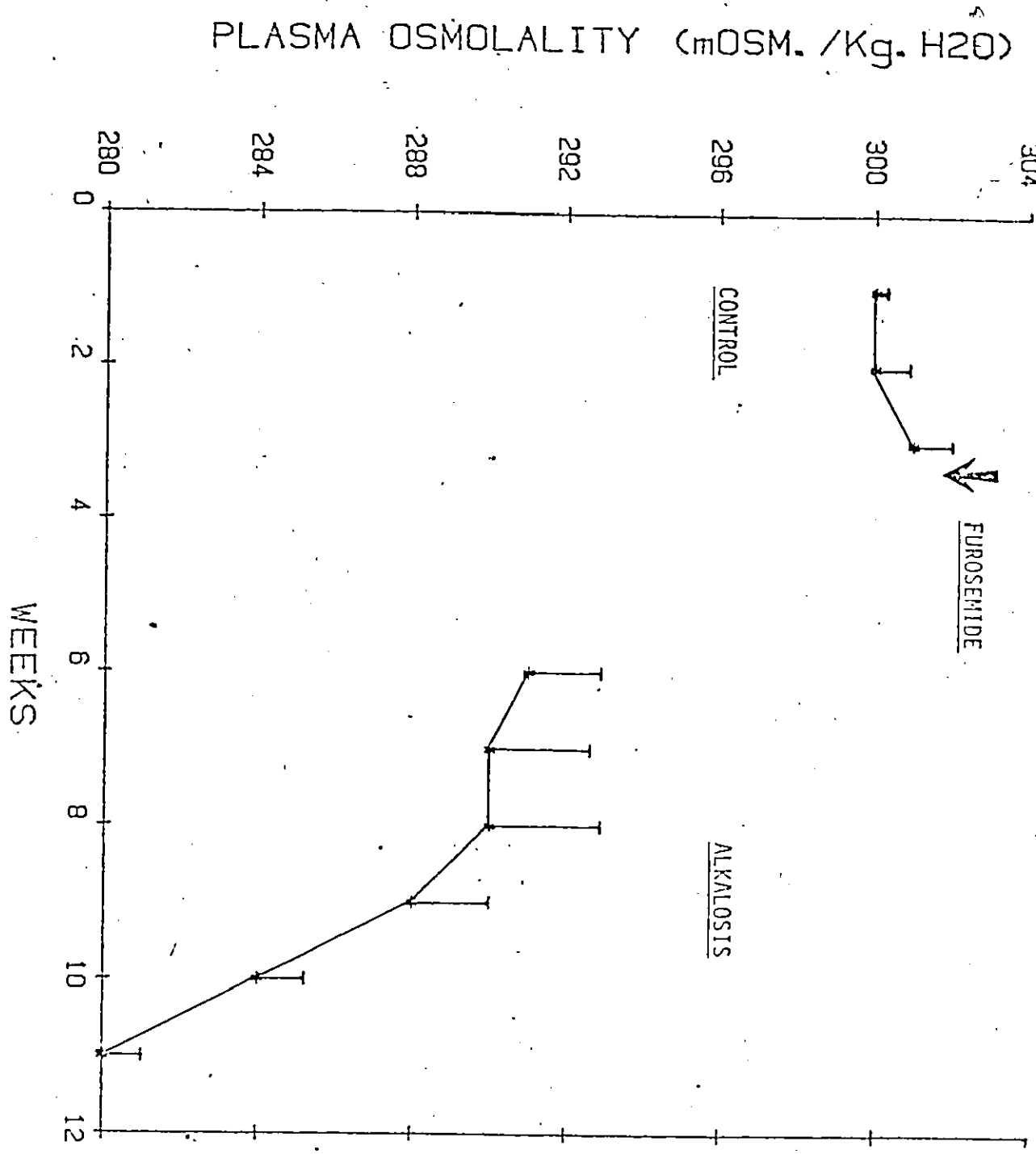

PLASMA OSMOLALITY (mOSM./Kg. H2O)
280
284
288
292
296
300
304
0
2
4
6
8
10
12
WEEKS
CONTROL
FUROSEMIDE
ALKALOSIS

FIGURE 3.4 (A)

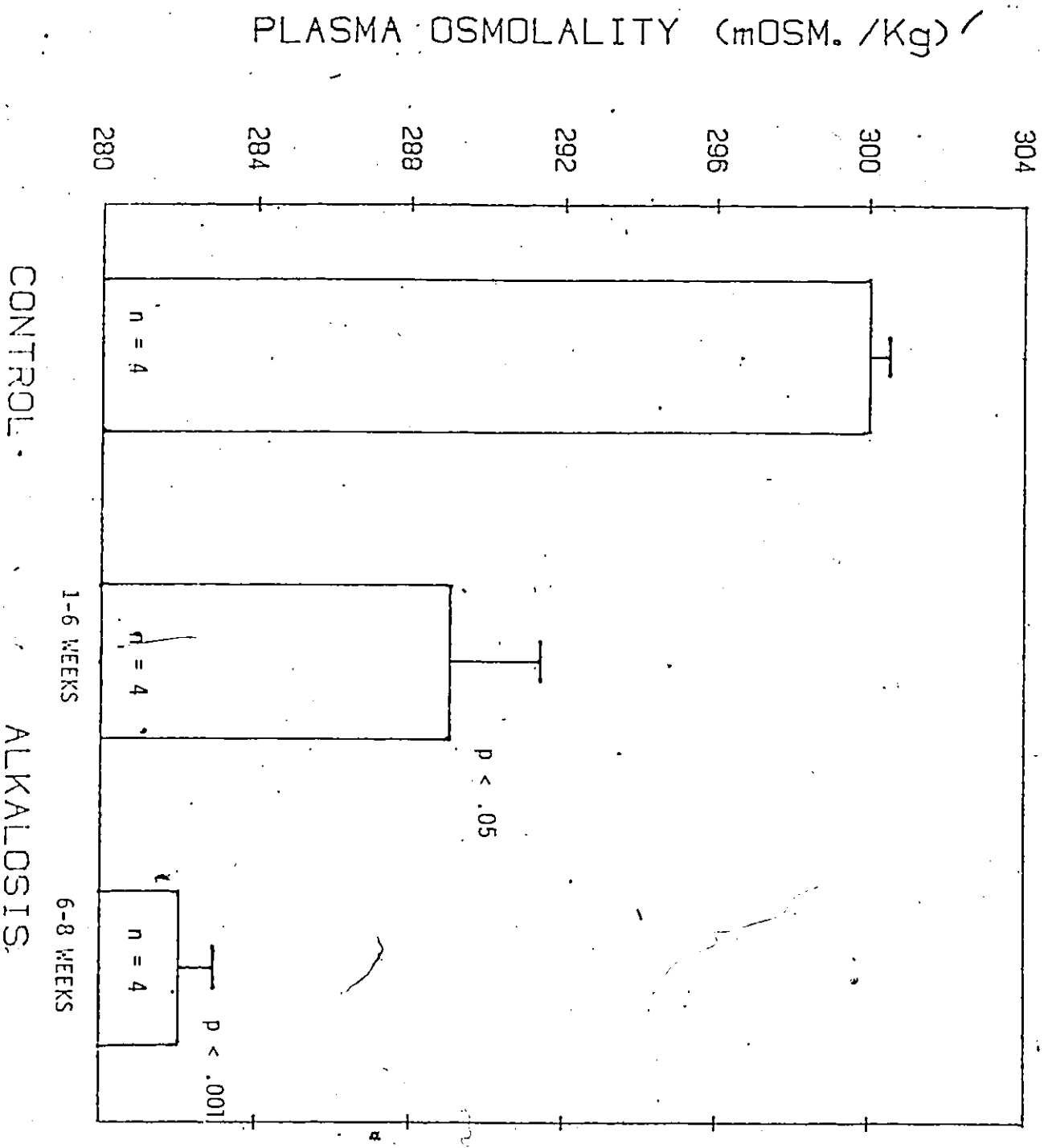

PLASMA OSMOLALITY (mOSM./Kg)
280
284
288
292
296
300
304
CONTROL
ALKALOSIS
1-6 WEEKS
6-8 WEEKS
n = 4
n = 4
n = 4
p < .05
p < .001

FIGURE 3.4 (B)

significantly different from the control (300 ± .4 mOsm/Kg.H_2O) shown in Fig. 3.4B.

Plasma AVP was measured in 3 dogs. Ambient AVP levels in alkalosis were similar to those found in the control conditions in the same animals (Fig. 3.5). A 10-fold increase in PRA was observed in alkalosis (from 1.11 ± .128 to 11.57 ± 1.882 ng/ml/h) (Fig. 3.6) As described previously, ECF contraction and low plasma chloride (91 mEq/L) are known to be potent stimuli for PRA increases.

3.2 Impaired urine-concentrating ability.

Dogs were water-deprived for 24 h and their pOsm, Max Uosm, plasma AVP and PRA were measured. As shown in Fig. 3.7 there was a 30% decrease in urine-concentrating ability in 6 dogs during alkalosis (Max. Uosm 1559 ± 75.7 in alkalosis vs 2296 ± 92.6 mOsm/Kg.H_2O in control conditions). This decrease in Max Uosm is statistically significant. Posm elevations during dehydration in the control (300 ± .4 to 312 ± 3.3 mOsm/Kg.H_2O) and the alkalotic state (290 ± 1.6 to 301 ± 2.9 mOsm/Kg.H_2O) were of similar magnitude (Table 3.3).

A 24 h dehydration induced an increase of similar magnitude in plasma AVP during control and alkalosis periods (Fig. 3.8). The observation that the increase in pAVP following dehydration in alkalosis was of the same magnitude as found in the control period (Table 3.4) indicated that dehydration was equipotent stimulus for AVP release in the non-alkalotic and alkalotic periods in the same animal. A significant increase in PRA was observed after 24 h water-deprivation in both alkalotic and control dogs (Fig. 3.9). However, since ambient PRA levels in alkalosis were already 10-fold higher, the proportional increase following dehydration was less than in control dogs

Fig. 3.5 Plasma arginine vasopressin (pAVP) concentrations in control and alkalotic dogs. Weekly averages were pooled for the control and alkalotic periods. The mean pAVP in the control period (1.48 ± .193 pg/ml) and alkalotic period (2.02 ± 1.233 pg/ml) were not significantly different ($t = .4892$, $p < .05$, $n = 3$) (paired t-test).

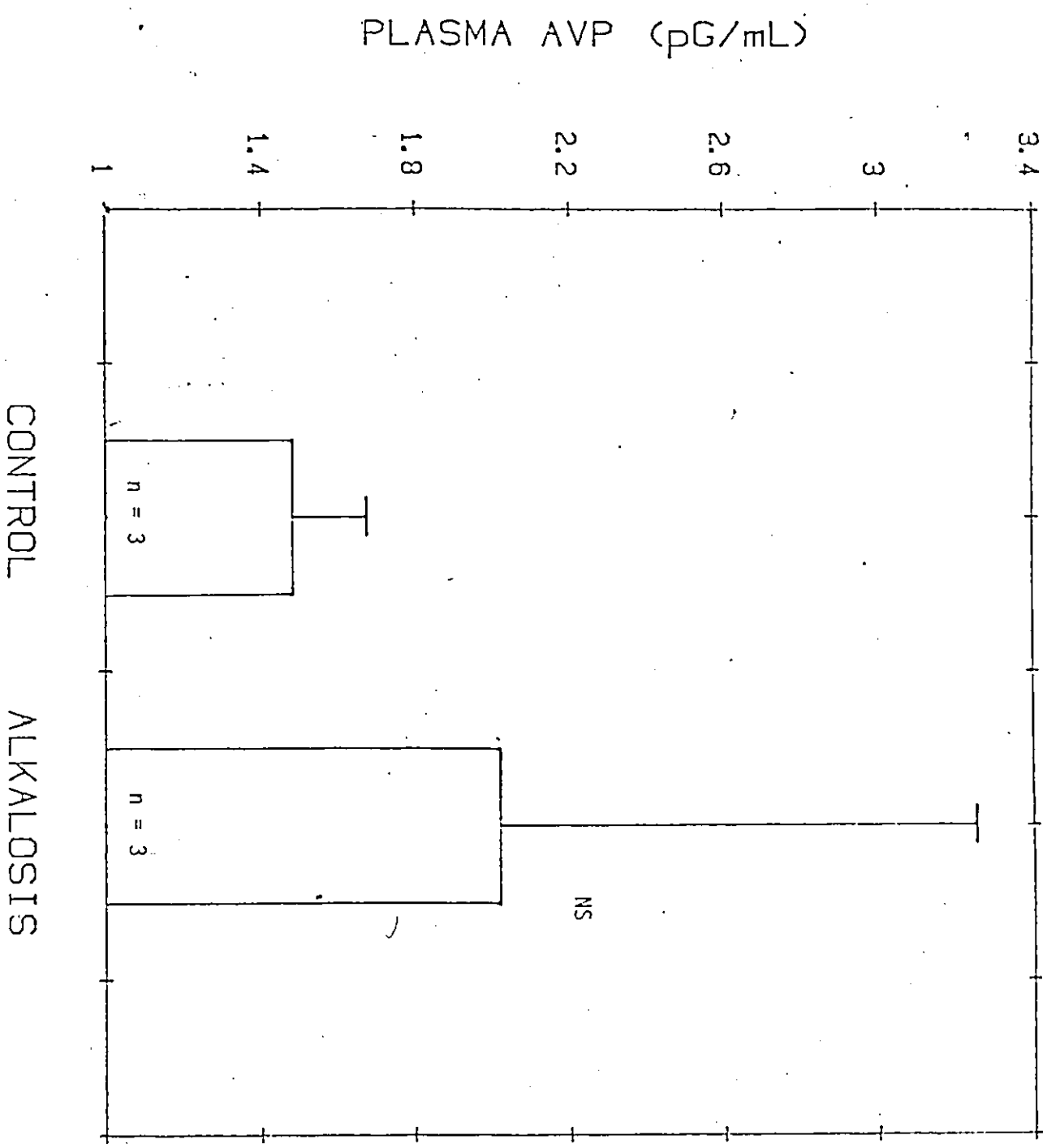


FIGURE 3.5

Fig. 3.6 Plasma renin activity (PRA) in control and alkalotic dogs. Weekly averages were pooled for the control and alkalotic period. The mean PRA in the control period (1.11 ± .128 ng/ml/n) and alkalotic period (11.57 ± 1.882 ng/ml/h) were statistically different ($t = 5.9186$, $p < .05$, $n = 3$) by paired t-test. Alkalotic dogs had an elevated resting PRA.

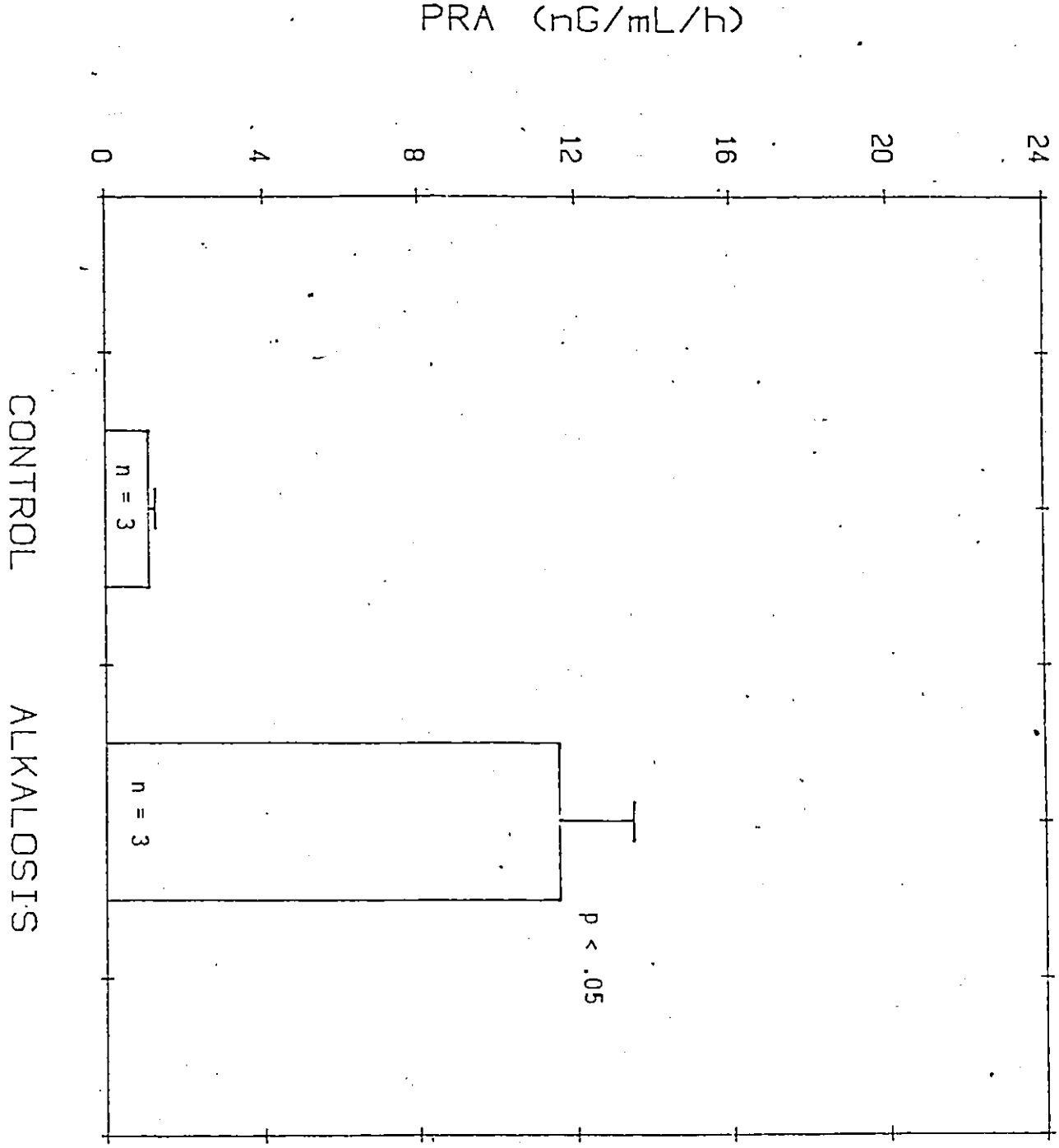


FIGURE 3.6

Fig. 3.7 Maximum urine concentrating ability (Max Uosm) in control and alkalotic dogs. The average of at least 2 measurements of Max Uosm following 24 h water deprivation for each dog was determined from pooled data from the control and alkalotic periods. The mean Max Uosm in the control period (2216 ± 91.4 mOsm/kg.H_2O) and the alkalotic period (1559 ± 75.7 mOsm/kg.H_2O) was compared statistically by paired t-test. Alkalotic dogs showed a significant decrease in urine concentrating ability ($t = 5.5540$, $p < .01$ $n = 6$).

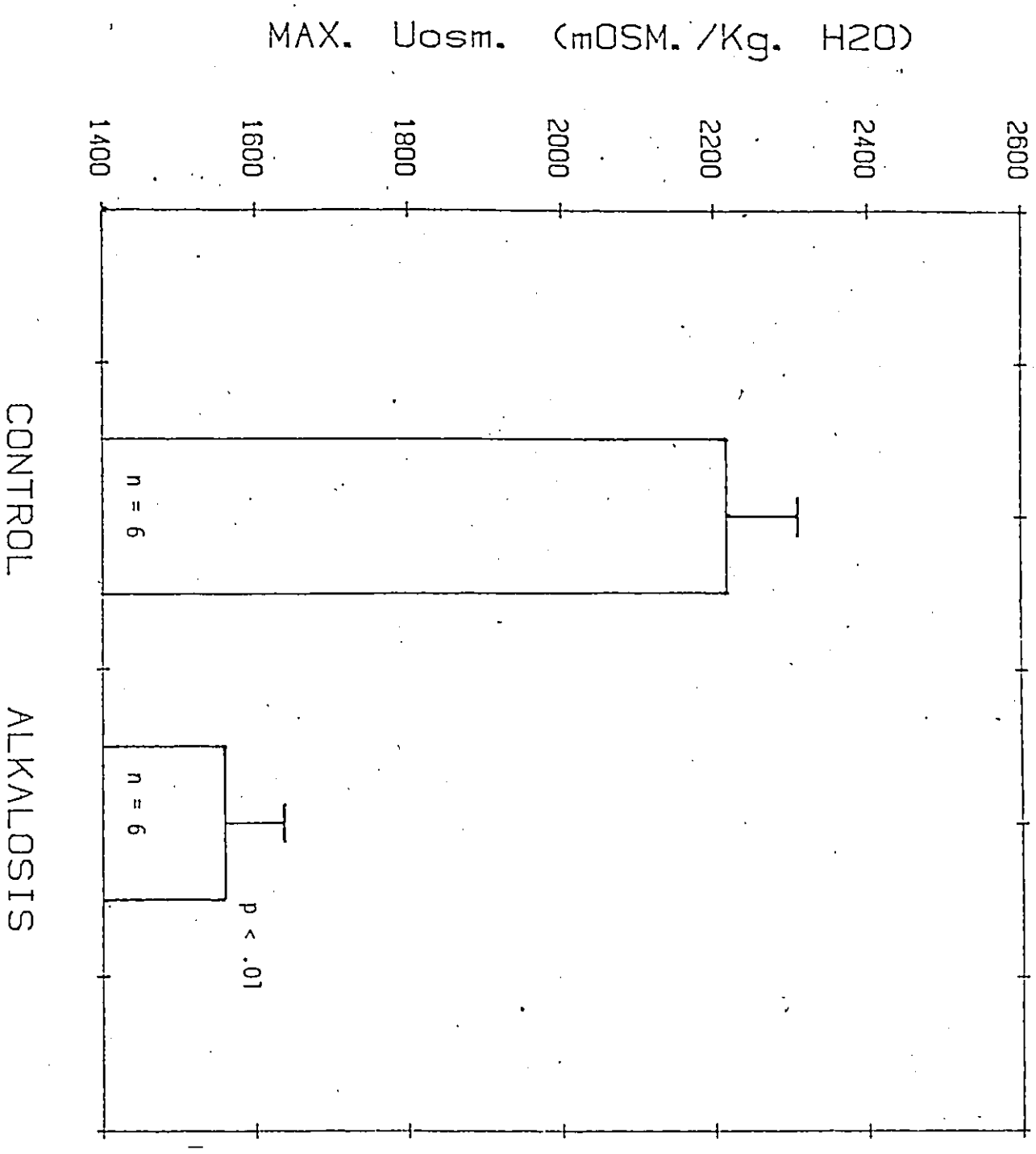

MAX. Uosm. (mOSM./Kg. H2O)
1400
1600
1800
2000
2200
2400
2600
n = 6
n = 6
p < .01
CONTROL
ALKALOSIS

FIGURE 3.7

Table 3.3: Increases in Posm during alkalosis and control (before alkalosis) in hydrated and dehydrated dogs. Posm values are presented as means ± S.E.M. (of 4 dogs). Each animal served as its own control.

	PLASMA OSMOLALITY ($mOsm/Kg.H_2O$)		
	Ambient	24 H dehydration	Difference
CONTROL	300 ± .4	312 ± 3.3	12
ALKALOSIS	289 ± 2.4	301 ± 2.9	12

Fig. 3.8 Plasma arginine vasopressin (pAVP) after 24 h water-deprivation in control and alkalotic dogs. The average of at least 2 measurements of pAVP for each dehydrated dog was determined for the control and alkalotic periods. The mean pAVP in the control period (11.06 ± .680 pg/ml) and the alkalotic period (8.74 ± 3.040 pg/ml) were not different ($t = .9032$, $p > .05$, $n = 3$) (paired t-test).

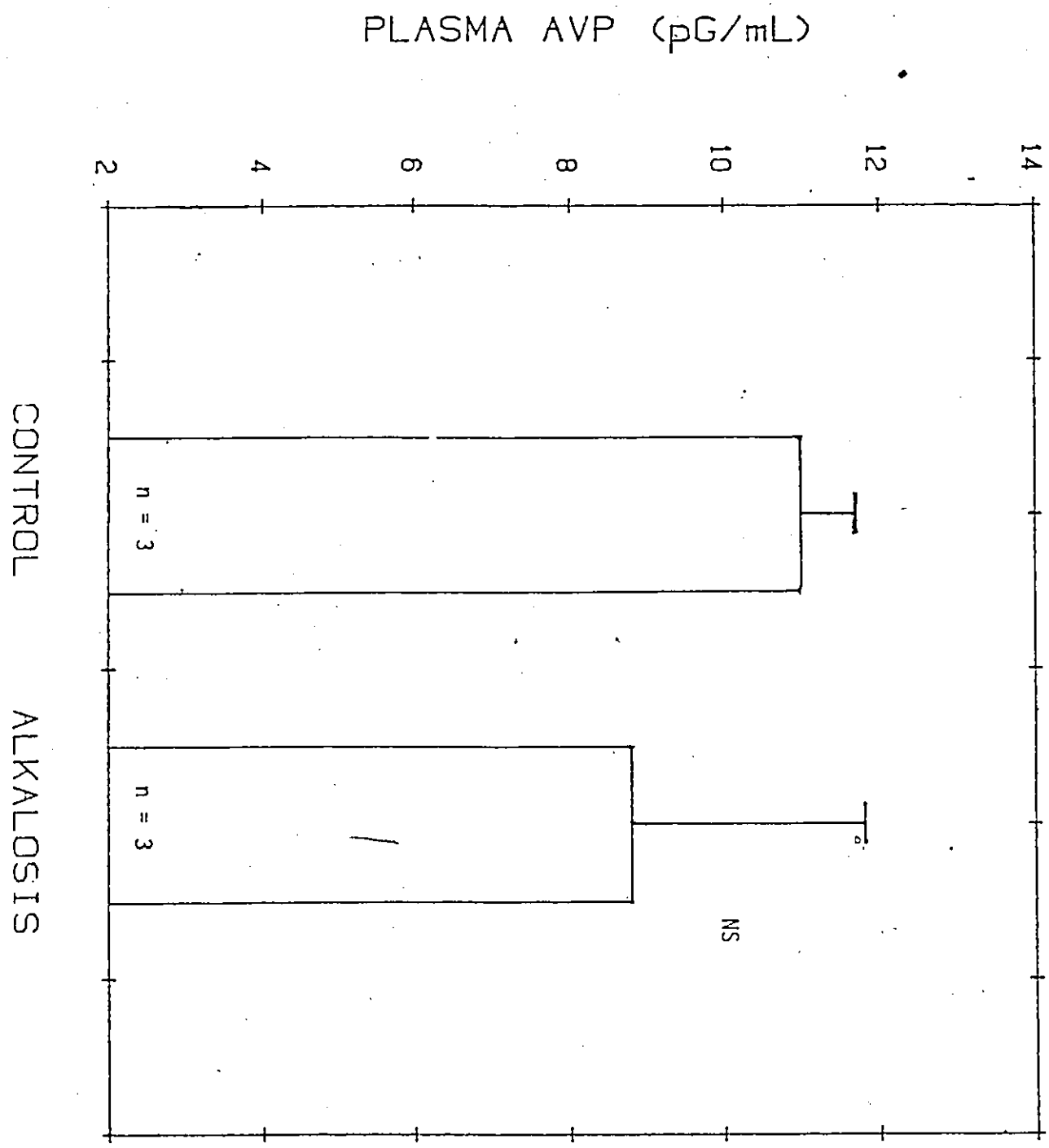


FIGURE 3.8

Table 3.4: Changes in plasma AVP during control (pre-alkalosis) and alkalosis periods in hydrated and dehydrated dogs. pAVP values are presented as means ± S.E.M. of 3 dogs. Each animal served as its own control.

	pAVP (pg/ml)	
	Ambient	24 H Dehydration
CONTROL	1.48 ± .193	11.06 ± .680
ALKALOSIS	2.02 ± 1.233	8.74 ± 3.040

Fig. 3.9 Plasma renin activity (PRA) after 24 h water deprivation in control and alkalotic dogs. At least 2 measurements of PRA were made for each dehdyrated dog in the control and alkalotic period. The mean of PRA in the control period (2.43 ± .651 ng/ml/h) and in the alkalotic period (12.56 ± .483 ng/ml/h) were compared statistically by paired t-test. Alkalotic dogs were found to have significantly elevated PRA ($t = 27.64$, $p < .001$, $n = 3$).

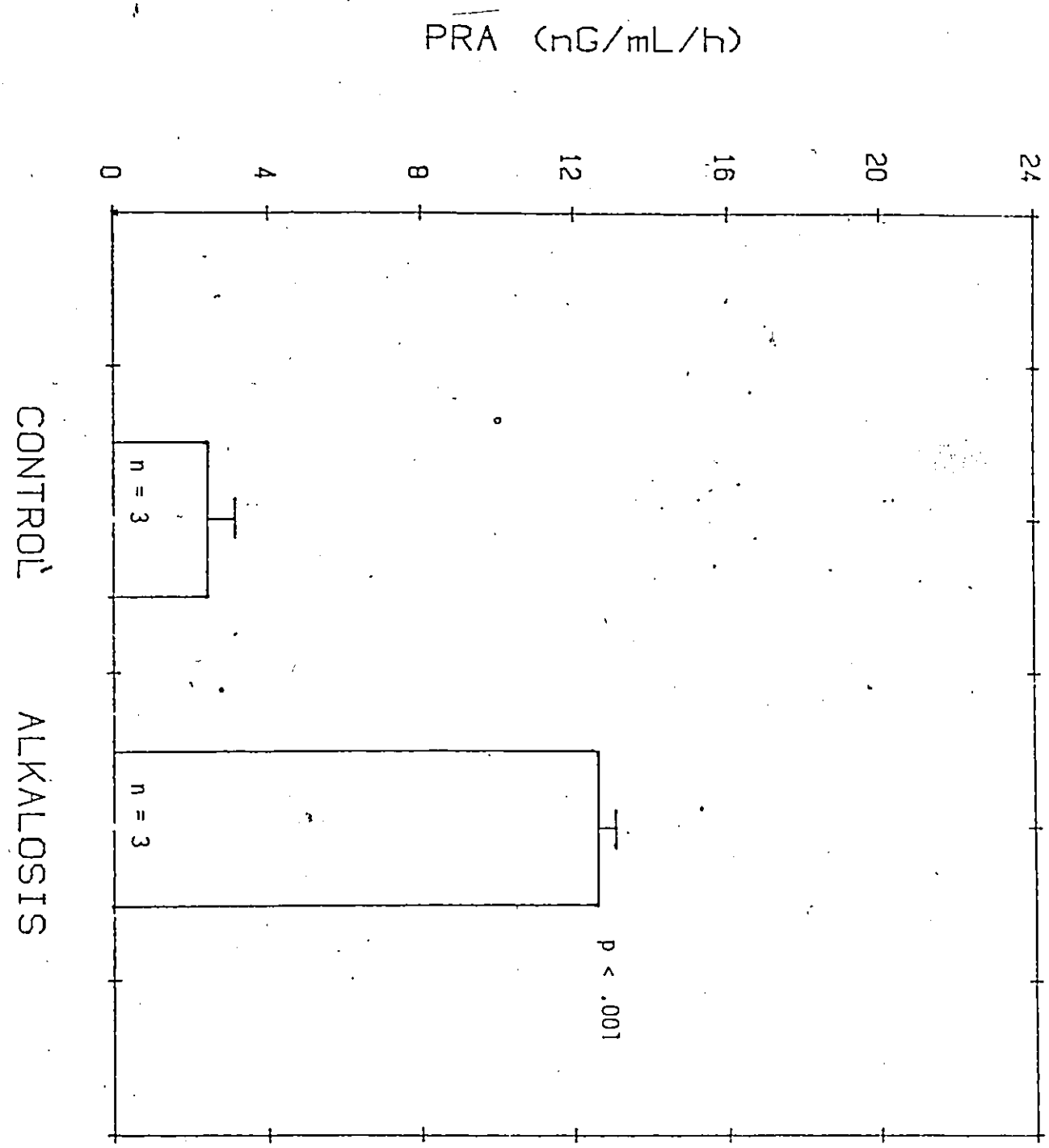


FIGURE 3.9

Table 3.5: Changes in PRA during control (non-alkalotic) and alkalotic periods in hydrated and dehydrated dogs. PRA values are presented as means ± S.E.M. of 3 dogs. Each dog served as its own control.

	PRA (ng/ml/h)	
	Ambient	24 H Dehydration
CONTROL	1.11 ± .128	2.43 ± .651
ALKALOSIS	11.57 ± 1.882	12.56 ± .483

(Table 3.5).

3.3 Excretion of a water-load.

Several water-loading tests were performed in one alkalotic dog and the results are summarized in Table 3.6. In the hydrated state, 77% of the water load was excreted within 1-2 hours of voluntary drinking of dilute milk-water (2-3 mOsm/kg.H_2O). Minimum Uosm was found to be 176 mOsm/Kg.H_2O after 30-60 minutes, and a drop of 1.0 mOsm in Posm was observed during the same period. After 60 minutes of water loading, Uosm and Posm started increasing.

3.4 Altered drinking response to Angiotensin II (AII).

Alkalotic dogs did not drink when they were infused i.v. with 250 ng/min AII, whereas the same dogs responded to the AII stimulus when they were not alkalotic.

3.5 Altered thirst response to osmotic stimulus in alkalosis.

The latency for drinking to a hyperosmotic Na_2SO_4 stimulus in alkalotic and control dogs was about the same (Fig. 3.10). Although the water drunk (ml/kg) over a 2 h period of Na_2SO_4 infusion (1500 mOsm Na_2SO_4 infused at 0.09 ml/kg/min, i.v.) was not significantly different in alkalosis when compared to the control dogs (35.2 ± 6.08 ml/kg alkalosis vs. 46.6 ± 4.45 ml/kg control, $p > .05$ $n = 3$) (fig. 3.12), the rate of drinking was significantly lower in alkalosis (.297 ml/min alkalosis vs. .420 ml/min control) (Fig. 3.11) determined by ANOVA. Therefore, the rate of drinking in response to a hyperosmotic stimulus was reduced in alkalosis. Control experiments of Na_2SO_4 infusion equiosmotic to ambient Posm (300 ± 1.0 mOsm during control and 281 ± 1.0 mOsm during alkalosis) did not induce drinking. This ruled out the possibility of non-specific drinking or drinking induced by excitement.

Table 3.6: Excretion of a water-load in one alkalotic dog.

#	*Volume consumed (ml)	**Urine excreted (ml)	Excretion %	Uosm Pre-test	Uosm 30-60 min	Uosm 60-120 min	Posm Pre-test	Posm 30 min	Posm 120 min
1	400	300	75	220	155	208	286	285	287
2	360	280	77.8	252	178	238	284	282	286
3	360	270	75	245	185	-	285	284	288
4	235	185	78.7	279	185	-	285	283	285
MEAN ±SEM	339 ±41.4	259 ±29.3	77 ±1.1	249 ±14.0	176 ±8.2	223 ±21.2	285 ±.5	284 ±.7	287 ±.7

*500 ml of a dilute solution of 2% milk and water of 2 ± 1.0 mOsm was presented to a fully hydrated dog.

**All urine samples were collected in a metabolic cage.

Fig. 3.10 Hyperosmotic sodium sulfate (1500 mOsm/kg, Na_2SO_4 at 0.09 ml/kg BWT/min, i.v.) induced water intake during the control and alkalosis periods. Results are averages of 2 experiments in each condition. Mean water intakes during osmotic loading in the control (46.6 ± 4.45 ml/kg. BWT) and alkalosis (35.2 ± 6.08 ml/kg. BWT) periods were compared statistically by paired t-test. The volumes of water ingested were similar ($t = 3.3282$, $p < .05$, $n = 3$).

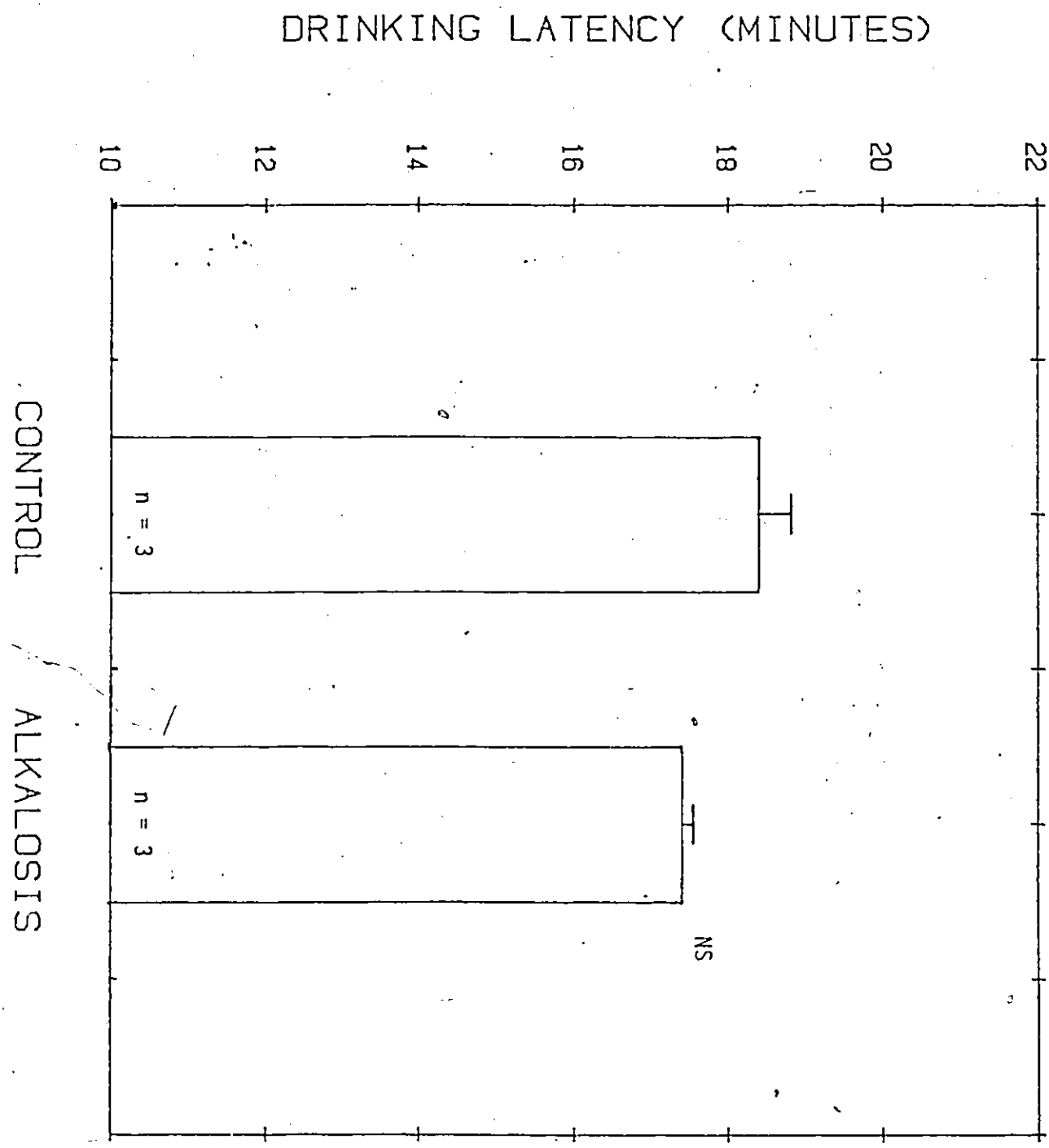

DRINKING LATENCY (MINUTES)
10
12
14
16
18
20
22
n = 3
n = 3
NS
CONTROL
ALKALOSIS

FIGURE 3.10

Fig. 3.11 Cumulative water intake versus time in 3 dogs subjected to osmotic loading (1500 mOsm·Na_2SO_4 at 0.09 ml/kg BWT/min, i.v.) during the control (thin line) and alkalosis (thick line) period. The slopes of regression lines during alkalosis and control were significantly different as determined by ANOVA. Regression equation for each period was: Control rate of drinking = .420 [time - 3.10], r = .936; Alkalosis rate of drinking = .297 [Time - 4.754], r = .799.

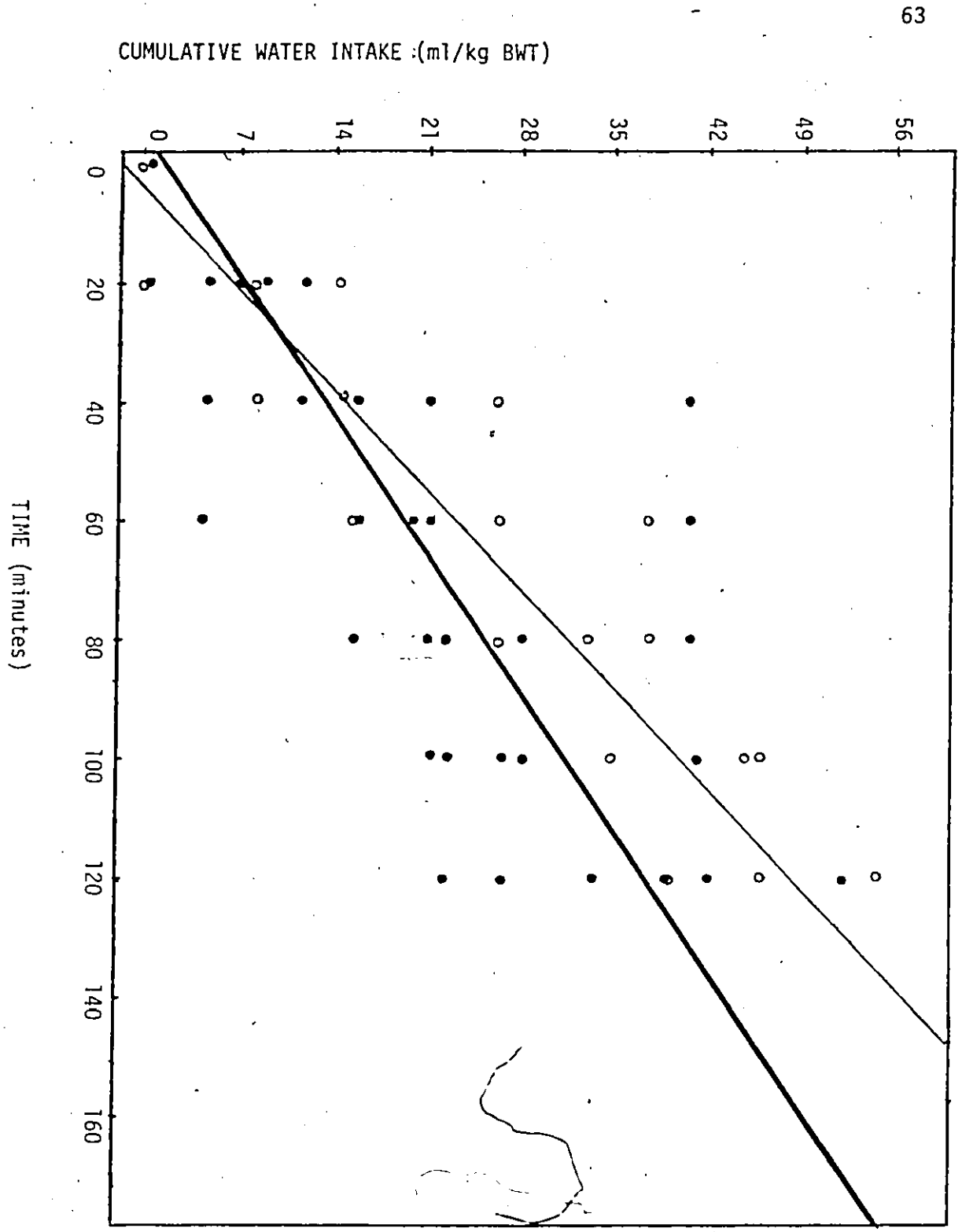

CUMULATIVE WATER INTAKE :(ml/kg BWT)
0
7
14
21
28
35
42
49
56
TIME (minutes)
0
20
40
60
80
100
120
140
160

FIGURE 3.11

Fig. 3.12 Hyperosmotic sodium sulfate (1500 mOsm/kg, Na_2SO_4 at 0.09 ml/kg BWT/min, i.v.) induced water intake during the control and alkalosis periods. Results are averages of 2 experiments in each condition. Mean water intakes during osmotic loading in the control (46.6 ± 4.45 ml/kg. BWT) and alkalosis (35.2 ± 6.08 ml/kg. BWT) periods were compared statistically by paired t-test. The volumes of water ingested were similar ($t = 3.3282$, $p < .05$, $n = 3$).

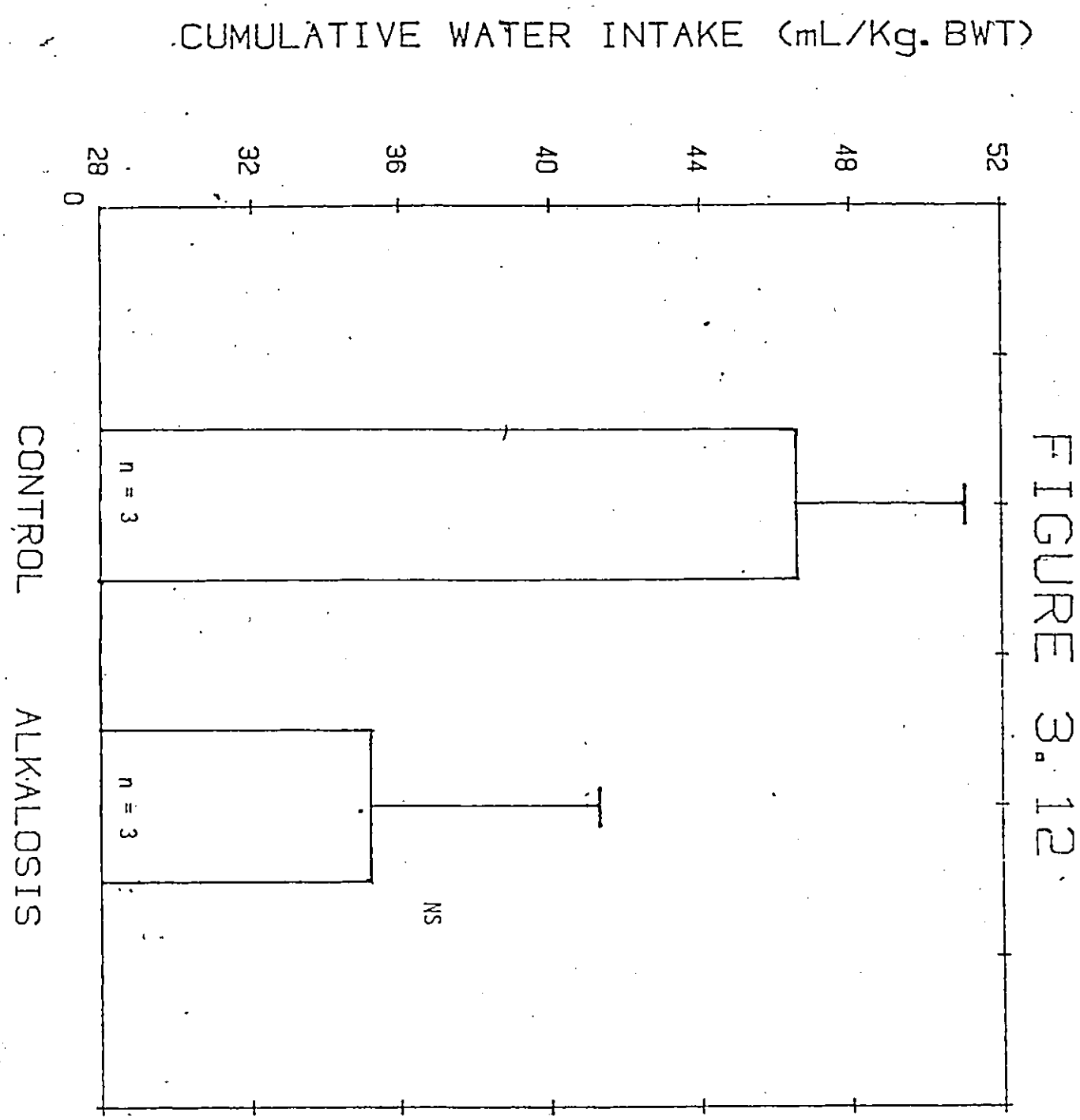

CUMULATIVE WATER INTAKE (mL/Kg. BWT)
0
28
32
36
40
44
48
52
CONTROL
n = 3
ALKALOSIS
n = 3
NS

FIGURE 3. 12

3.6 Altered sensitivity for osmotic release of AVP.

Although the osmotic threshold for AVP release was lowered by approximately 10 mOsm/kg in alkalosis compared to the control period, (287.6 mOsm/kg alkalosis vs. 297.7 mOsm/kg control)(Fig. 3.13). This difference was not statistically significant. However the sensitivity for AVP release in response to osmotic loading was increased nearly two-fold (2.487 pg/ml/mOsm alkalosis vs. 1.185 pg/ml/mOsm control), as determined by linear regression.

3.7 Osmoregulation in alkalosis.

Changes in Posm during 1500 mOsm Na_2SO_4 infusion in alkalotic dogs were similar to the non-alkalotic dogs when the dogs were allowed to drink during infusion (Fig. 3.14) or when the dogs were not allowed to drink (Fig. 3.15). Thus it appeared that alkalotic dogs were capable of osmoregulating their Posm.

3.8 Renal histology

The HPS, van Kossa and Alzarin "S" stains showed scattered calcium deposits in the lumen of the tubules at the cortico-medullary junction as well as within the collecting tubules of the medulla in one of the dogs. In the other two dogs the calcium deposits were mainly in the lower medulla. The calcium deposits, in all the kidneys of the three dogs, were mild in quantity (1+); thus the kidneys of these chronically hypochloremic, alkalotic dogs were not calcinotic to any significant degree.

Fig. 3.15 Relationship of plasma vasopressin (pAVP) to plasma osmolality (Posm) in 3 dogs subjected to i.v. infusion of 1500 mOsm Na_2SO_4 at 0.09 ml/kg BWT/min during the control (thin line) and the alkalotic (thick line) periods. The slope of the pAVP/mOsm·kg relationship for alkalosis was significantly increased (1.185 $pg \cdot ml^{-1}/mOsm \cdot Kg^{-1}$ control vs. 2.487 $pg \cdot ml^{-1}/mOsm\text{-}Kg^{-1}$) determined by linear regression analysis. Regression equation for each period was: control AVP release = 1.1846 [Posm - 297.7], r = .615; Alkalosis AVP release = 2.487 [Posm - 287.6], r = .744.

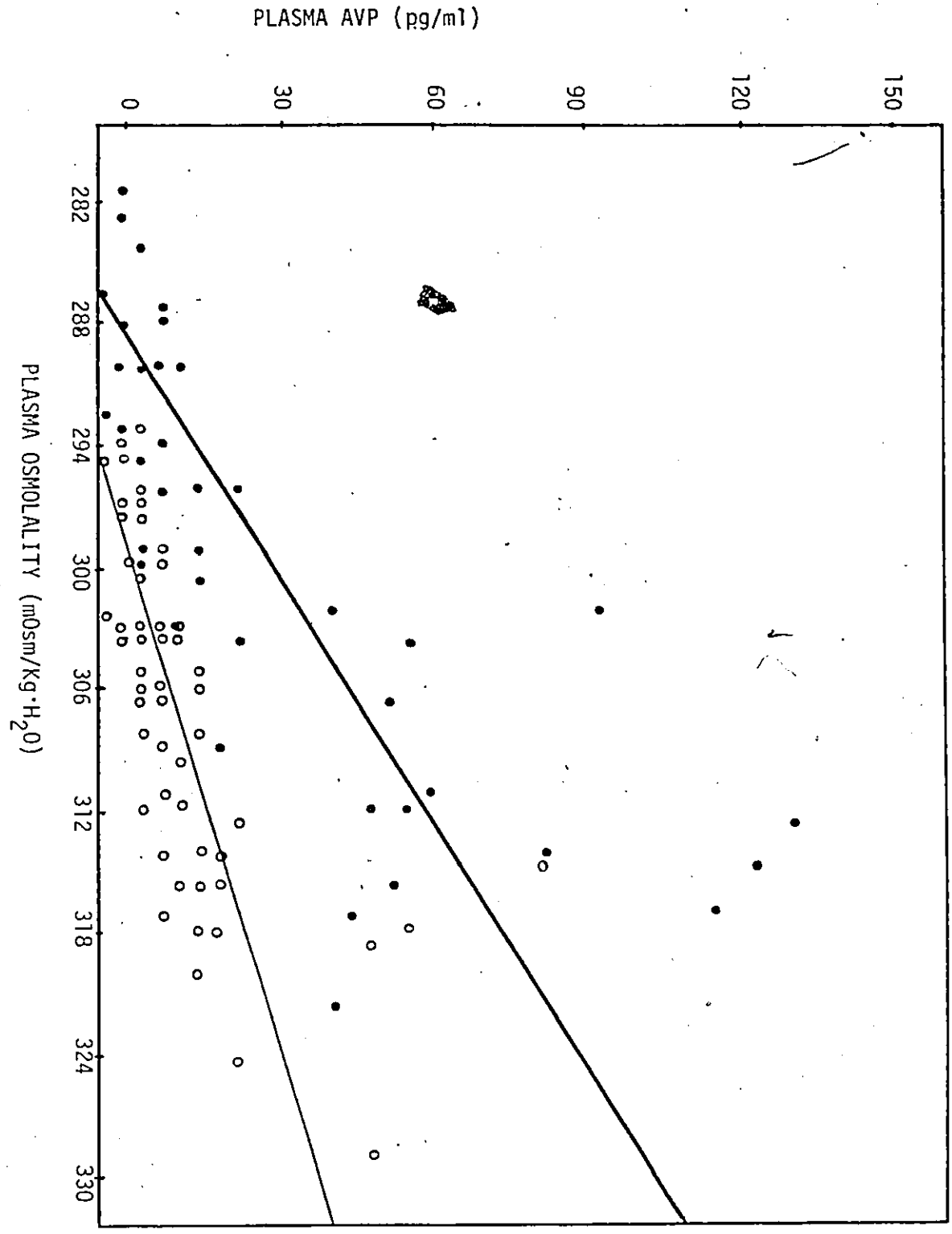

PLASMA AVP (pg/ml)
0
30
60
90
120
150
PLASMA OSMOLALITY (mOsm/Kg·H2O)
282
288
294
300
306
312
318
324
330

FIGURE 3.13

Fig. 3.14 Changes in Posm during osmoregulation by drinking in 3 dogs subjected to osmotic loading (1500 mOsm Na_2SO_4 at 0.09 ml/kg BWT/min, i.v.) in the control (thin line) and alkalotic (thick line) periods. Each experiment was repeated twice. The slopes of regression lines during alkalosis and the control period were not different ($t = .7680$, $p > 0.05$, $n = 42$) when compared by paired t-test. Regression equations for each period were: control Posm = .025 [Time - 12112], $r = .600$, Alkalosis Posm = .066 [Time - 4290.9], $r = .962$.

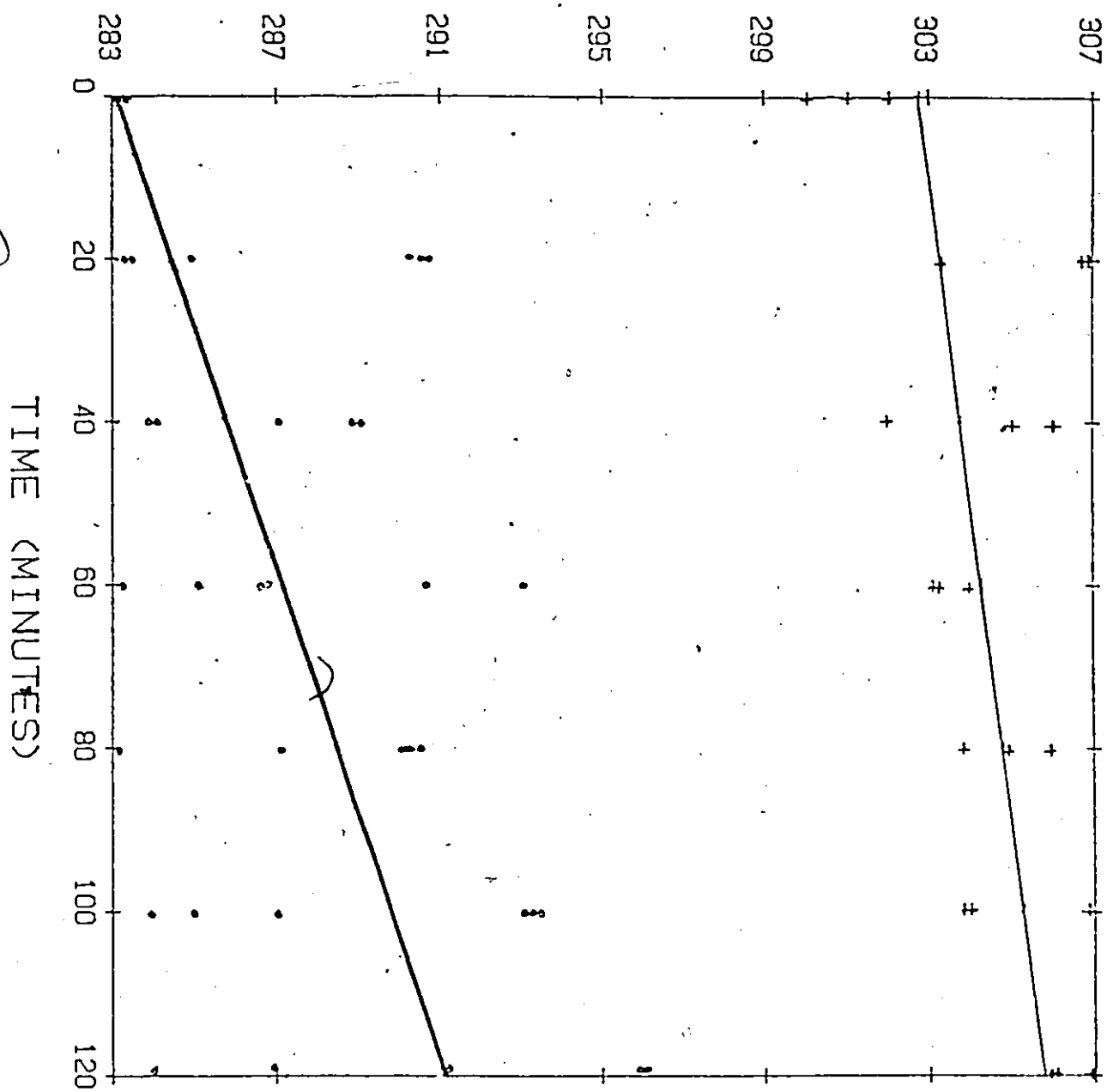

PLASMA OSMOLALITY (mOSM./Kg. H2O)
283
287
291
295
299
303
307
0
20
40
60
80
100
120
TIME (MINUTES)

FIGURE 3.14

Fig. 3.15 Posm during osmoregulation by AVP release in 3 dogs subjected to osmotic loading (1500 mOsm. Na_2SO_4 at 0.09 ml/kg. BWT/min, i.v.) in the control (thin line) and alkalotic (thick line) period. Each experiment was repeated twice. The slopes of regression lines during alkalosis and the control period were not different ($t = .4296$, $p < 0.05$, $n = 42$) when compared by paired t-test. During alkalosis dogs regulated Posm similarly to the control period. Regression equations for each period were: control Posm = .166 [Time + 1800], $r = .986$; Alkalosis Posm = .189 [Time + 1528.7], $r = .990$.

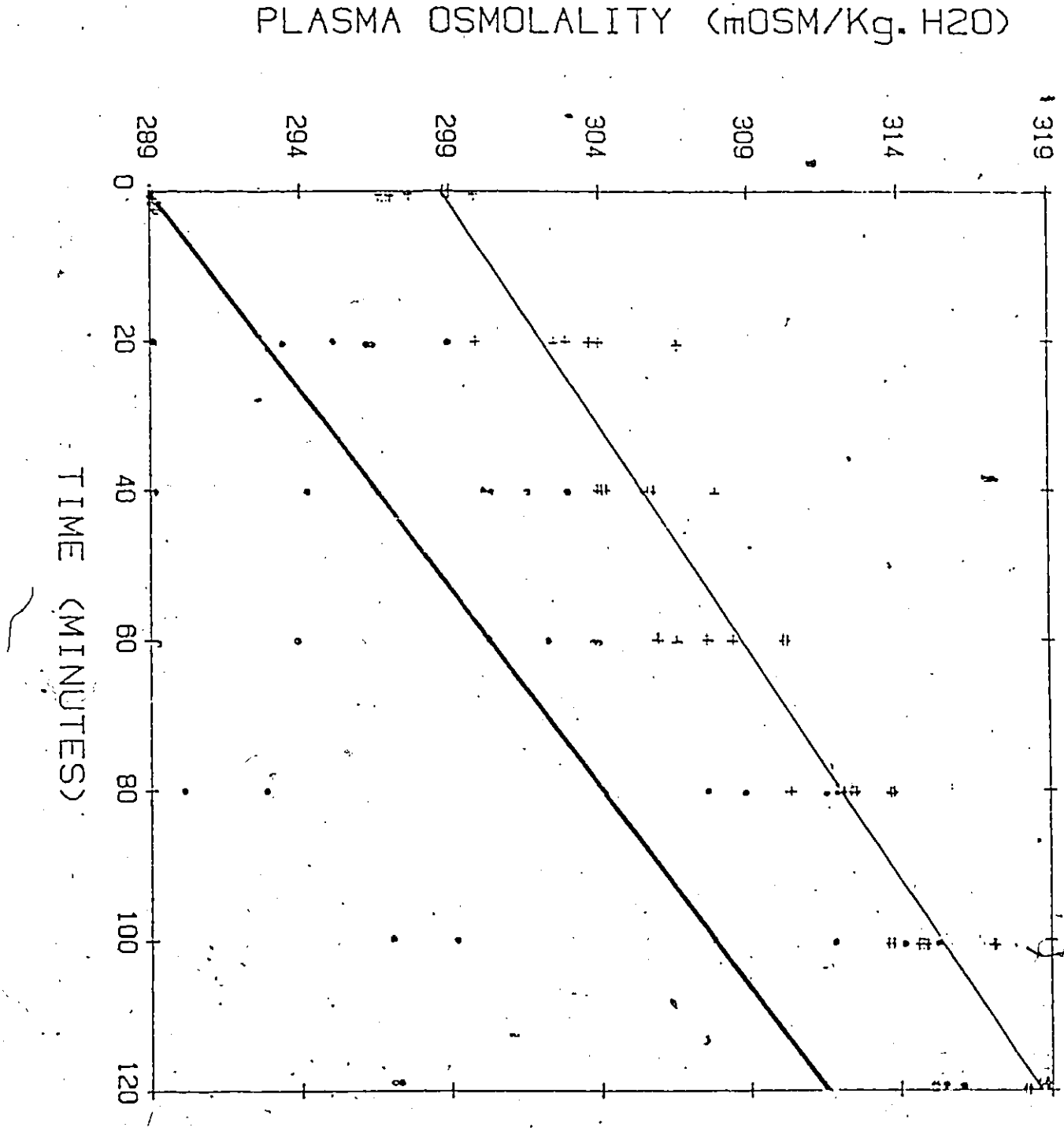

PLASMA OSMOLALITY (mOSM/Kg. H2O)
289
294
299
304
309
314
319
0
20
40
60
80
100
120
TIME (MINUTES)

FIGURE 3. 15

Fig. 3.16 Changes in plasma osmolality (Posm) during osmotic loading (1500 mOsm Na_2SO_4 at 0.09 ml/kg BWT/min, i.v.) in 3 dogs in the control and alkalotic periods. In one set of experiments, Posm was increased by hypertonic Na_2SO_4 infusion with drinking water available (W) in the control (thin solid line) and alkalosis (thick solid line) periods. In another set of experiments, the dogs were given the same osmotic load without drinking water available (NW) (control = thin broken line; alkalosis = thick broken line). All experiments were repeated twice. In each set of experiments the slopes of the regression lines for control and alkalosis periods were not significantly different. However, the regression lines for alkalosis Na_2SO_4 + W and alkalosis Na_2SO_4 + NW had significantly different slopes ($t = 2.67$, $p < .01$, $n = 42$). Regression line equations were: Control + W, Posm = .025 (Time + 12112), $r = .600$; Alkalosis + W, Posm = .066 (Time - 4291), $r = .962$; for control [Na_2SO_4 + NW] and alkalosis [Na_2SO_4 + NW], see figure legend for Fig. 3-15.

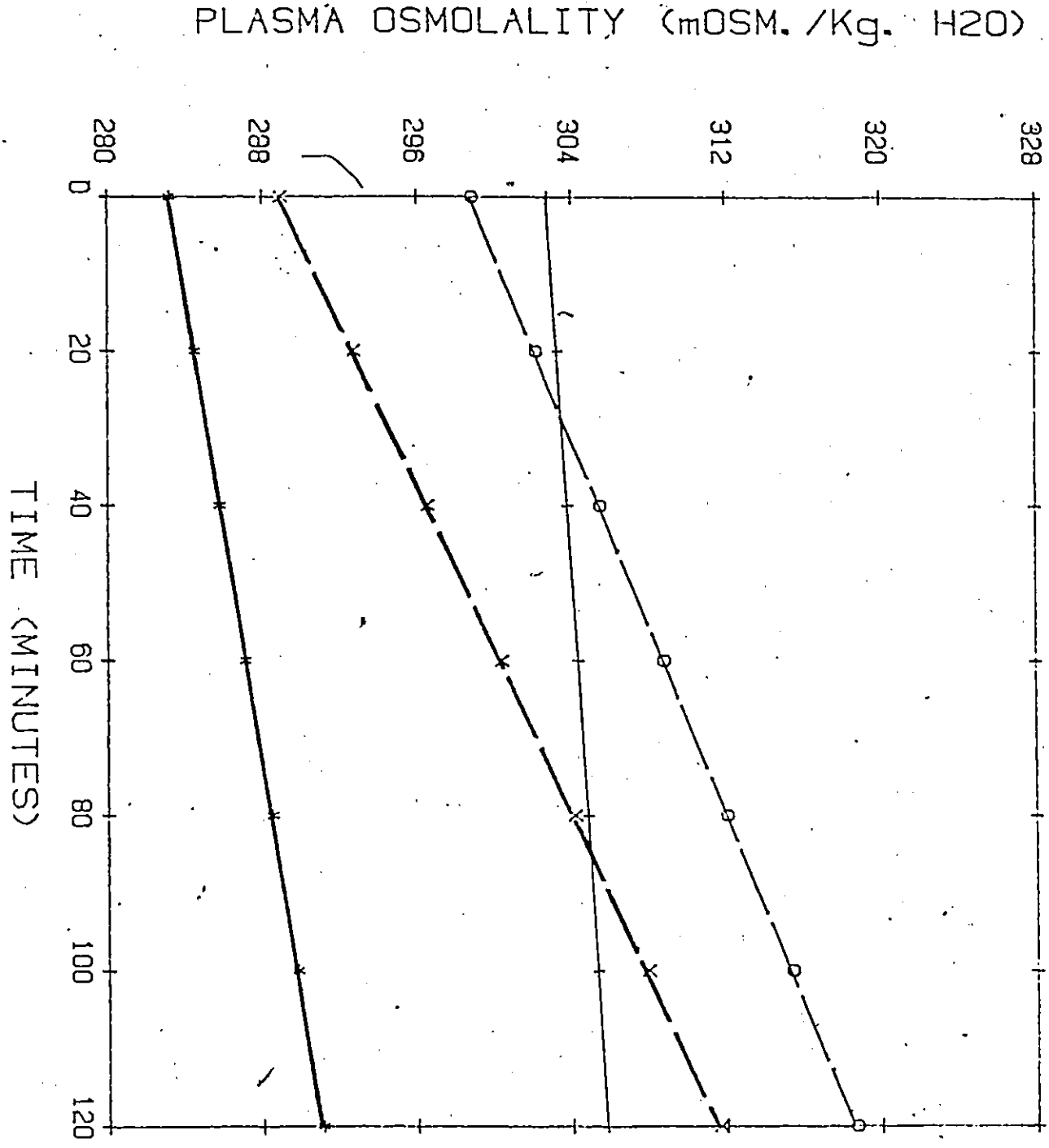

PLASMA OSMOLALITY (mOSM./Kg. H2O)
280
288
296
304
312
320
328
0
20
40
60
80
100
120
TIME (MINUTES)

FIGURE 3.16

4. DISCUSSION

Alkalosis has been successfully induced by vomiting and/or gastric drainage in the dog (Needle et al., 1964) and the rat (Cooke et al., 1952; Sztorc, 1984). Diuretic-induced metabolic alkalosis in dogs has been maintained by feeding Cl^--free diet (Van Ypersele de Strihou et al., 1969; Cohen, 1968). However, in previous studies the duration of the alkalotic period has been short (9-18 days). In this study, it was found that furosemide-induced metabolic alkalosis can be maintained by ingesting Cl^--free diet for up to 8 weeks in otherwise healthy dogs. Maintenance of metabolic alkalosis for a longer period was not the prime purpose of this study. However, it was imperative to maintain a stable chronic alkalosis in order to investigate its effects on water intake, urine-concentrating ability and the mechanisms of AVP release.

4.1 Characteristics of hypochloremic metabolic alkalosis

Diuretic-induced alkalosis was characterized by elevated blood pH and plasma HCO_3, hypochloremia, hyponatremia, hypokalemia, plasma hypo-osmolality, polydipsia, polyuria and a renal-concentrating impairment.

It has been shown that furosemide inhibits active transport of Cl^- in the thick ascending limb (Burg et al., 1973) and enhances hydrogen ion secretion, which leads to decreased Na^+ and Cl^- co-transport (Greger, 1981)

The combination of Cl^--free diet with furosemide injections induces a hypochloremic state (Van Ypersel de Strihou and Morales-Barria, 1969). In response to hypochloremia, renal absorption of bicarbonate increases and glomerular filtration rate decreases (Cogan et al., 1983). This inverse relation between hypochloremia and hyperbicarbonatemia is the

major contributor for maintaining elevated blood pH in metabolic alkalosis. The findings in this study of hypochloremia, hyperbicarbonatemia and elevated blood pH (Fig. 3.1, Table 3.1) are in complete agreement with that of earlier work.

Hyponatremia has been shown to have multiple etiology. It may ensue from restricted intake or excessive losses. It may be due to a dilutional effect by extra water retention by the body. Effective volume depletion predisposes towards the development of hyponatremia by reducing renal water excretion (Schrier and Bichet, 1981), and by K^+ loss (Laragh, 1954; Fishman et al., 1971). In order to investigate renal water retention in hyponatremic alkalotic dogs water-loading tests were performed in one dog. Water-load excretion (WLE) was found to be 77% which is considered within the normal range (normal range is WLE = 75% or more during 4 h after the ingestion of water) (de Wardener, 1985).

The hypokalemia, observed in the alkalotic dogs, may be a result of increased flow rate of tubular fluid. Increases in plasma concentration of HCO_3 or other nonchloride anions tend to promote increases in potassium excretion (Wright and Giebisch, 1985). Elevated pH, as well as a direct effect of increased plasma HCO_3 concentration, appear to contribute to the entry of K^+ into the cells (Farley and Alder, 1977). Hypokalemia is also brought about by hyperaldosteronism produced by diuretic-induced hypovolemia (Burg and Green, 1973; Kurtzman et al. 1973). Polyuria can also induce K^+ loss but only in the presence of a concurrent primary polydipsia (Hariprasad et al., 1980). Since all the etiological factors for hypokalemia described above were observed in the alkalotic dogs, it is very likely that some or all of them might be involved in the observed hypokalemic state.

It is important to note, however, that the finding of ECFV contraction is not universal in all types of metabolic alkalosis. The state of ECFV in alkalosis depends upon the means by which alkalosis is generated. For example, metabolic alkalosis brought about by an excessive mineralocorticoid secretion results in volume-expansion alkalosis, where as virtually all human and animal models of Cl^--responsive alkalosis have coexisting hypochloremia and reduction of effective blood volume (Jacobson and Seldin, 1983). Alkalosis may exist with virtually no change in ECFV in cases of surreptitious vomiting in which patients become highly skilled in their ability to conceal vomiting. They drink a lot of water which replaces the water-loss but electrolytes remain uncompensated (Wallace et al., 1968).

From the literature cited above it can be realized that loss of ECF does not necessarily lead to metabolic alkalosis. For example, in acute hemorrhage, loss of blood leads to an isotonic loss of ECFV with no concomitant elevation in blood pH, i.e. no alkalosis. As well, metabolic alkalosis and ECFV concentration do not always coexist.

Hypochloremic dogs were alkalotic and had contracted ECF volume. Although no direct measurements of ECF volume were made in the study, the decreased body weight, increased plasma protein and PCV (Fig. 3.2) and a 10-fold increase in PRA seen in the alkalotic dogs are all suggestive of ECF contraction. These results are consistent with other studies (Sztorc, 1984; Kassirer and Schwartz, 1966). However, body weight loss alone is too excessive to be considered as the sole indicator of ECFV loss. Water can also be lost by an increased metabolism of protein which may be occurring in alkalotic dogs.

The low plasma osmolality observed in alkalotic dogs (Fig. 3.4) can be explained as a result of the presence of hyponatremia (Fig. 3.1). Since more than 90% of the osmotic pressure of plasma and other body fluids is due to Na, the osmolality of the body fluids is virtually synonymous with the plasma Na^+. This effect can be discerned in previous studies (Bosch et al., 1977; Gulyassy et al., 1962; Kassirer and Schwartz, 1966) although surprisingly, it has not been commented upon in the context of studies related to metabolic alkalosis in the past. In our experiments the decrease in plasma osmolality was not due to polydipsia coupled with a renal impairment in free-water excretion, since it persisted even when the animals were pair-watered in a previous study (Sztorc, 1984). As the alkalosis progressed, Posm gradually decreased and in late alkalotic period reached a new low plateau of 282 ± 1.0. This gradual decline can be attributed to the gradual increasing renal imparement leading to an increased loss of electrolytes where as water and electrolyte intkaes remained unchanged, between early and late alkalosis.

It is possible that the following chain of events occurs in chronic chloride depletion which leads to development of hyponatremia. First, volume depletion caused by chloride (and Na^+) loss via the kidneys leads to non-osmotic release of AVP mediated by reduced inhibition from baroreceptors and/or volume receptors or stimulated by Angiotensin II. Second, the presence of AVP causes enhanced renal retention of free water which lead to the reduction in pOsm and the development of hyponatremia. In order for the osmolality of body fluids to decrease from 300 ± .4 mOsm/Kg.H_2O during the control condition to 289 ± 2.4 mOsm/Kg.H_2O after the development of metabolic alkalosis, approximately

340 ml of water would have to be retained. Since our animals had sustained approximately a 1.8 Kg body weight loss (although not all the loss was due to the loss of water and electrolytes), the increase in total body water mediated by AVP would clearly reduce plasma osmolality, but would be insufficient to correct the volume depletion. Since volume contraction still persists (85% of the deficit remains), AVP secretion continues at this lower value of pOsm as shown in our study of alkalotic animals. In addition we have demonstrated that the sensitivity of the osmotic release of AVP is also significantly enhanced in chronic metabolic alkalosis as discussed in 4.4 below.

4.2 Mechanisms for the increased water intake in alkalosis

Polydipsia developed in alkalotic dogs (Fig. 3.3) in the absence of an increase in solute intake and in the presence of a significant reduction in plasma osmolality. It is known that ECF contraction (Davis et al., 1976; Vander, 1967), hypochloremia (Abboud et al., 1977; Kirchner et al., 1978; Kotchen et al., 1976 and 1978) and hyponatremia (Fray, 1980; Merrill et al., 1973) are all potent stimuli for renin release. This leads to the activation of the peripheral renin-angiotensin system, which results in an increased circulating levels of AII. It seems likely that the primary polydipsia was due to increased plasma AII levels which were chronically elevated in chloride-depleted dogs (based on the observation of 10-fold elevated PRA levels, Fig. 3.6). Circulating AII is a potent dipsogen and plays an important role in ECF volume homeostasis (Fitzsimons et al. 1978; Kucharczyk and Mogenson, 1975). AIII, a product of AII metabolism, has also been shown to have some dipsogenic effects when given centrally in the pig (Mutter et al., 1984) and systemically in the gerbil (Wright et al., 1984).

AII infused at a rate of 250 ng/min (i.v.) failed, however, to induce drinking in alkalotic (chronically hypovolemic) dogs, whereas the same dogs responded to the same AII stimulus when they were not alkalotic (normovolemic). This finding might be explained on the basis of the extremely high endogenous AII levels, as observed in alkalotic dogs, so that AII receptors were already occupied. This assumption is consistent with the suggestion of Reid et al., (1978) that in a state of salt depletion, ECF is reduced and there is a high level of circulating endogenous AII which occupies the majority of its receptors in the circulation. Consequently, very few receptors are available to respond to the exogenous AII.

The osmotic stimulus for drinking remained intact in alkalosis (Fig. 3.10, Fig. 3.12). The dogs responded to hypertonic Na_2SO_4 at 0.09 ml/Kg BWT/min (i.v.) in both alkalosis and control periods. Lack of drinking response to Na_2SO_4 equosmotic to the ambient Posm ruled out the possibility of non-specific drinking or drinking due to excitement. However, in alkalosis the drinking (ml/min) in response to 1500 mOsm Na_2SO_4 infusion (i.v.) was considerably reduced (Fig. 3.11).

Altered thresholds for thirst and AVP release are observed in many volume related conditions, both physiological (pregnancy, luteal phase of menstruation; discussed in section 4.4) and pathologic (bronchogenic lung cancer).

Subnormal thresholds for both thirst and AVP have been reported in patients with bronchogenic carcinoma of the lung (Robertson, 1982). Lowering of the osmostat apparently was due to the chronic hypovolemia that results from obstruction of venous return by lung cancer (Robertson, 1982).

4.3 Impaired urine-concentrating ability

In the present study, alkalotic dogs demonstrated an impairment in the urine-concentrating ability (Fig. 3.7). Changes in renal hemodynamics, medullary solute concentration, and AVP release are several of the possibilites which can be postulated to mediate the urine- concentrating defect (UCD). It is well known that a UCD develops in states of hypochloremia (Luke et al., 1977; Abboud et al., 1979; and Galla et al., 1981), and hypokalemia (Eknoyan et al., 1970; Berl et al., 1977; Berl, 1980). Micropuncture studies have shown that Cl^--depletion leading to hypochloremia decreases net reabsorption of NaCl by the thick ascending limb of Henle (TAL) and K^+-depletion impairs NaCl reabsorption by the TAL (Galla et a., 1981; Gutsche et al., 1984). The reduction in plasma K^+ which occurred in the alkalotic dogs may well lead to an impairment in TAL NaCl reabsorption.

Another explanation for the failure to concentrate urine normally during electrolyte depletion is that it involves a possible defect in AVP release. However, resting AVP levels were found to be similar in alkalotic and non-alkalotic dogs (Fig. 3.5), and following 24 h dehydration pAVP increased in both periods (Fig. 3.8). These findings are in agreement with the work of Paller et al. (1983), who demonstrated that hypokalemic rats had pAVP similar to non-alkalemic rats following 24 h dehydration. Saikaley (1985) has also obtained similar results. This suggests that the UCD in alkalotic dogs is of nephrogenic origin rather than due to a deficient AVP release.

Finally, the renal structural changes, brought about by electrolyte depletion, should also be considered in UCD development. Tobak et al. (1976) have reported the K^+-depletion may be associated with

morphological changes in the TAL, and Levine et al. (1974) have drawn attention to the existence of nephrocalcinosis specifically associated with Cl^--depletion in the rat. However, microscopic histological examinations of the alkalotic dog kidneys did not reveal any gross lesions in either cortical or medullary regions.

4.4 Mechanism of AVP release in metabolic alkalosis

The osmotic threshold for AVP release in the alkalotic dogs was about 9 mOsm/kg lower than controls as evidenced by a statistically significant reduction in pOsm (measured repeatedly in each dog) after the induction of metabolic alkalosis. Reset of the osmostat was not evident from the osmotic looking experiments due to the small number of observation. There are several examples of reset of threshold for AVP release in different situations of fluid-electrolyte imbalance. Durr et al. (1982) have demonstrated that the osmotic threshold for AVP secretion decreases approximately 10 mOsm/kg during gestation in rats. Davison et al. (1984) have reported an approximately 6-8 mOsm/kg decrease in osmotic threshold for AVP release in pregnant women, whereas the osmotic threshold for thirst was lowered by approximately 10 mOsm/kg. Unlike the studies just cited, in the present study sensitivity for AVP release was increased two-fold (from 1.185 to 2.487 pgAVP/Posm/kg) in alkalotic dogs. Changes in the sensitivity for AVP release (ie. pAVP/Posm relationship) have been reported in several other states of volume and fluid-electrolyte imbalance. During the luteal phase of the menstrual cycle of human females, the threshold for AVP release and the slope of the pAVP/Posm relationship are reduced compared to in the follicular phase. The threshold for thirst is also reduced in the luteal phase (Spruce et al., 1985). Although not statistically

significant, a slight fall in the sensitivity of AVP release has also been reported by Davison et al. (1984) in early human pregnancy. According to these investigators, as a consequence of lower threshold for AVP release, at lower Posm, AVP secretion persists which reduces solute-free water clearance and causes plasma to become slightly hypotonic. This shift in the 'set of the osmostat' accounts for the lower basal plasma osmolality. The effect of the reduction in osmoreceptor sensitivity offsets the lowered threshold of AVP release, which is particularly apparent as plasma osmolality rises. In the present study, an increase was seen in the osmoreceptor sensitivity for AVP release despite of the trend toward lowering of threshold for AVP release.

Furthermore, the increase in osmoreceptor sensitivity for AVP release may be due to increased circulating AII found in the alkalotic animals. The importance of the integrated activities of drinking, AVP secretion and the renin-angiotensin system in the maintenance of total body water and extracellular osmolality has been well-recognized (Cowley, 1975; Fitzsimons, 1979). AII administered systemically has been shown to increase AVP secretion in dogs (Bonjour and Malvin, 1970; Ramsey et al., 1978), rats (Knepel and Meyer, 1980), and humans (Uhlich et al., 1975; Padfield and Morton, 1977). Intracranial injection of AII also stimulates AVP in the dog (Share, 1979) and the rat (Keil et al., 1975). It has been suggested that the site of action of AII on AVP release may include the subfornical organ (SFO) (Simpson et al., 1979; Mangiapane et al., 1982), the organum vasculosum of the lamina terminalis (OVLT) (Bealer et al., 1979) and the supraoptic nucleus (SON) (Simmonet et al., 1979). Neural connections between the SFO and the SON

and PVN have been demonstrated electrophysiologically (Ferguson and Renaud, 1984). It has also been shown that direct injection of AII into the SFO causes AVP release (Simpson et al., 1979). In view of the above cited evidence, it may be speculated that increased osmoreceptor sensitivity for AVP release in alkalotic dogs is brought about by an interaction of chronically elevated AII at the circumventricular organs.

Sladek et al. (1982) have reported that AII at physiological concentrations is able to effectively potentiate osmotic stimulation of AVP release from organ-cultured rat hypothalamo-neurohypophyseal (HNS) explants AVP release was significantly greater when two stimuli were presented together than when either was presented individually. The response of cultured HNS explants to simultaneous addition of subthreshold concentrations of AII and osmotic stimuli, provides support for synergism between AII and osmolality in the stimulation of AVP release. This observation is consistent with the synergistic effect of simultaneous intracerebroventricular application of AII and NaCl on single-unit electrical activity of paraventricular and supraoptic neurosecretory neurons (Akaishi et al., 1980), as well as the studies which suggest synergism between AII and osmolality in stimulating drinking and AVP release (Shimizu et al., 1973). Although the hyper-reninemic alkalotic dogs did not show a significant increase in ambient pAVP or in pAVP following a 24 h dehydration period, the sensitivity for AVP release in response to hypertonic Na_2SO_4 i.v. infusion was increased.

Osmoregulation by AVP is also altered by changes in blood volume. Barron et al. (1984) have demonstrated that during gestation in rats the relationship between total blood volume and pAVP is altered such that the expanded blood volume is recognized as normal. Osmoregulation, with water available, is mainly by drinking rather than by AVP secretion (Cowley et al., 1983). In the present study the regulation of Posm by drinking (Fig. 3.14) and by AVP release (Fig. 3.15) in alkalotic dogs was not altered when compared to the non-alkalotic responses.

5. CONCLUSIONS

1. A stable metabolic alkalosis was maintained in dogs for up to 8 weeks by a combination of diuretic administration and feeding Cl^--free diet.

2. The hypochloremic metabolic alkalosis was characterized by hyponatremia, hypokalemia, low plasma osmolality, polydipsia and polyuria. The dogs were ECFV-contracted and had markedly increased PRA.

3. The alkalotic dogs also had a significant impairment of urine-concentrating ability. However, the UCD was independent of the primary polydipsia. The defect was nephrogenic in origin and not a consequence of inappropriate release of AVP.

4. Finally, alkalotic dogs showed a disruption of normal thirst responses. Specifically, they did not respond to exogenous intravenous AII and they displayed a decreased rate of drinking in response to osmotic stimuli. The sensitivity of osmoreceptors for AVP release was increased, however, suggesting a lowered osmotic setpoint in alkalosis.

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