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OSMOREGULATION IN UNCONTROLLED DIABETES MELLITUS

by

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Abstract

In this thesis we studied the influence of osmotic loading on vasopressin secretion and water intake in experimentally-induced diabetes mellitus, in the insulin deprived state as well as when treated with insulin, in order to investigate whether the osmotic drive for vasopressin release and thirst is altered in the diabetic state. Four dogs were used for the experiments to be reported. They were infused with hypertonic sodium sulfate to investigate the influence of osmotic loading on water intake and vasopressin secretion in the control, insulin-treated diabetic and diabetic conditions.

Forty eight hours of insulin depletion did not produce a change in the basal plasma vasopressin levels, even though there was a significant increase in plasma osmolality.

In addition, forty eight hours of insulin depletion did not alter the sensitivity of the osmoreceptors controlling vasopressin release and thirst.

The effect of the diabetic condition on the osmotic threshold is subject to interpretation of the data. If glucose is considered an osmotically effective solute in the diabetic state, there is an upward resetting of the osmostat for vasopressin release and thirst, and a downward or leftward shift of the osmostat when glucose is not considered to be effective osmotically.

The results of the present study provide evidence that the osmotic sensitivity of vasopressin release and thirst is not affected by the presence

or absence of insulin. However, whether there is a true resetting of the osmostat for vasopressin release and thirst in the diabetic state depends on the assumption mode concerning glucose permeability.

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Table of Contents

List of Figures

LIST OF TABLES

1.0 Introduction

It has been shown more than 40 years ago that thirst and vasopressin secretion are sensitive to plasma sodium levels, but are unaffected by changes in plasma glucose or other solutes to which the putative brain receptors are freely permeable (Verney, 1947). Recent work indicates, however, that plasma glucose becomes an effective stimulus in the absence of insulin (Robertson ,1987). The infusion of glucose to healthy individuals or insulin-treated diabetic patients fails to stimulate vasopressin secretion or thirst despite plasma hyperosmolality (Thompson et al., 1986, Zerbe et al., 1983). In insulin-deficient diabetics, on the other hand, hyperglycemia increases the concentration of vasopressin in the plasma (Vokes et al., 1987). Robertson (1987) has proposed that in the presence of insulin, hyperglycemia does not stimulate brain osmoreceptors because extracellular glucose is taken up too rapidly to permit the establishment of a significant transcellular osmotic gradient. In contrast, when insulin is lacking, glucose uptake by osmoreceptors is reduced, leading to an increased transcellular osmotic gradient which stimulates the osmoreceptors by dehydration.

To determine whether the osmoregulation of vasopressin and thirst is altered in the diabetic state, we studied the influence of osmotic loading on vasopressin secretion and water intake in experimentally-induced insulin dependent diabetes mellitus in the insulin deprived diabetic state as well as when treated with insulin.

Accordingly, in the following sections I discuss the regulation of fluid-electrolyte balance by vasopressin and the thirst mechanism in the

healthy human body followed by a section on fluid-electrolyte disturbances in insulin dependent diabetes mellitus.

1.1 Regulation of Body volume and Isotonicity

More than half of the weight of the human body consists of fluid, of which two thirds are located in the intracellular compartment and one third in the extracellular compartment. Potassium and its anions constitute the predominant electrolytes inside the cell and sodium and its anions are the main solutes of the extracellular fluid; the extracellular components are plasma and interstitial water. The solute concentration or osmolality of the body water is identical in all compartments, due to the fact that water diffuses freely across cell membranes in response to an osmotic gradient, the latter being created by a change in the water or the osmotically effective solute content in one of the compartments. Sodium and its anions can generate osmotic pressure and therefore contribute to the effective plasma osmolality. Plasma osmolality is maintained within a very narrow range. Changes in plasma osmolality are counteracted by rapidly increasing or decreasing total body water. Water is excreted via the urine and stool and by evaporation from skin and lungs. In healthy people water and electrolyte homeostasis depends mainly on the antidiuretic hormone, arginine vasopressin, acting upon water excretion by the kidney, on the renin-angiotensin-aldosterone system, acting on renal sodium excretion, and on the thirst mechanism by its effect on fluid intake. Insulin-deficient diabetes mellitus is characterized by thirst, polydipsia, polyuria and elevated plasma osmolality.

1.1.2 Role of Vasopressin

a) osmotic drive

Arginine vasopressin (AVP) is a polypeptide hormone (du Vigneaud, 1956) with a molecular weight of 1099 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 and paraventricular nuclei of the hypothalamus (Sachs, 1963; 1967; 1969) and stored and released by the posterior pituitary; it produces its antidiuretic action by rendering the distal and collecting tubules of the nephron more permeable to water, which leads to increased water reabsorption. 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). In the presence of vasopressin, water is reabsorbed resulting in a decrease of plasma osmolality, whereas in the absence of vasopressin water is excreted as dilute urine with a consequent rise of plasma osmolality.

The regulation of vasopressin secretion by osmosensitive neurons or "osmoreceptors", located in the anterior hypothalamus (Jewell and Verney,1957), has been demonstrated by Verney (1947) in indirect studies in conscious hydrated dogs. Subsequent studies have used radioimmunoassay to measure plasma AVP in healthy adults (Robertson et al.,1973; Robertson and Athar 1976), laboratory rats (Dunn et al, 1973) and other mammals (Robertson et al., 1976).

In every case, vasopressin secretion responded to a change in plasma osmolality of less than 2 %. The osmoreceptors can detect extremely small changes in the plasma osmolality and can convert this information into neural impulses that result in vasopressin secretion or, if appropriate, can inhibit vasopressin release in order to restore isotonicity.

Robertson and his associates applied linear regression analysis to relate the values of plasma osmolality, urine osmolality and plasma vasopressin, obtained from a group of healthy subjects in varying states of hydration. They found a significant correlation between plasma vasopressin and plasma osmolality as well as between plasma vasopressin and urine osmolality (Robertson et al.,1973; Robertson et al., 1973). They defined the osmotic threshold for vasopressin release as the plasma osmolality at the abscissal intercept of the regression line. The slope of the line is taken as a measure of the sensitivity of the osmoregulatory system.

According to Robertson's data the sensitivity of the mechanism is such that a change in plasma osmolality of only 1% is sufficient to increase or decrease plasma vasopressin by an average of 1 pg/ml, an amount large enough to produce marked changes in urinary concentration.

Other analytical models have been employed to describe the relationship between plasma vasopressin and plasma osmolality. Weitzman and Fisher (1977) found that their results were better fitted by a curvilinear or exponential (log-linear) function. They challenged the concept of an osmotic threshold for AVP release. Still other researchers felt that the data of Weitzman and Fisher could equally well be fitted by a parabolic curve (Moses et al., 1978). Using the standard deviations of observations around the line to test the goodness of the fit they conclude that the exponential model is not superior to the threshold model. The linear model still provides the simplest way to assess osmoregulatory function and clinical disorders

of salt and water balance (Robertson et al., 1976; Baylis and Robertson, 1980). Several important aspects of the osmoregulatory system have to be taken into account when interpreting its response to osmotic stimuli.

First, there is a great variability in the threshold and/or sensitivity of the osmoreceptor among individuals. In healthy adults the threshold may range from 276 to 291 mOsmol/kg (Robertson et al., 1976; Beardwell, 1971; Fressinaud et al., 1974) and the slope or sensitivity from 0.15 to 0.98. In rats (Dunn et al., 1973), monkeys (Hayward et al., 1976) and dogs (Robertson et al., 1977) the threshold values are significantly higher, ranging from 285 to 292 mOsmol/kg, consistent with the higher plasma osmolality and sodium concentration observed in these three mammals. Second, the vasopressin response to osmotic stimulation becomes rate dependent whenever the plasma osmolality increases more than 2%/h (Athar and Robertson, 1974). Last, the nature of the osmotic stimulus may affect the osmoreceptor. Hypertonic saline and hypertonic mannitol are equally potent stimuli for vasopressin secretion (Verney, 1947; Athar and Robertson, 1974; Robertson et al., 1977; Zerbe and Robertson, 1983). On the other hand, hypertonic urea (Verney, 1947; Zerbe and Robertson, 1983) and hypertonic glucose are osmotically ineffective vis a vis the osmoreceptor, even though they increase the plasma osmolality. Glucose actually suppresses ADH release (Zerbe and Robertson, 1983; Robertson et al., 1977). High levels of glucose and urea, often seen in patients with diabetes mellitus, will therefore distort the usual relationship between plasma osmolality and vasopressin. In that case plasma vasopressin must be assessed in relation to effective or corrected osmolality; the latter is obtained by omitting the contribution of glucose and urea. However caution must be exercised since the permeability of certain solutes may be altered under certain conditions. Vokes and Robertson (1987) found that during insulin deficiency the osmoreceptor becomes sensitive to stimulation by hyperglycemia.

In the latter case glucose thus contributes to the effective osmolality. This point will be treated in greater detail in the discussion.

b) non-osmotic

Several non-osmotic stimuli have been shown to influence vasopressin secretion. Amongst these are the renin-angiotensin-aldosterone system (references, see below), hypoglycemia (Baylis et al., 1978; Baylis and Heath, 1977), changes in blood volume and blood pressure (Robertson and Athar, 1976; Dunn et al., 1973; Robertson et al., 1974), nausea (Rowe et al., 1976) and aging (Helderman et al., 1975).

The renin- angiotensin-aldosterone system (renin, angiotensin I,II,III and aldosterone) plays a major role in blood pressure homeostasis and in the regulation of fluid and electrolyte homeostasis. The system is vasoconstrictor and antinatriuretic acting in concert with other homeostatic mechanisms to maintain constant arterial pressure over a wide range of physical activity and sodium intake.

Angiotensin II is the major physiologically active component of the renin-angiotensin system. Renin is synthesized in the juxtaglomerular (JG) cells of the renal afferent artiole, and released in response to hypochloremia (Abboud et al., 1979), indirectly to hypovolemia (Davis and Freeman, 1976), hyponatremia (Fray, 1980) and sympathetic stimulation. The active renin, an acid protease, acts on one of the plasma proteins, angiotensinogen (renin substrate), to split away the decapeptide angiotensin I. Angiotensin I in turn is converted into the octapeptide angiotensin II by the converting enzyme dipeptidylcarboxy-peptidase, found in the vascular endothelium of the lung, kidney and other organs (Oparil and Haber, 1974). The half-life of circulating AII is short, about 30 seconds, due to rapid

degradation by angiotensinases in blood. Angiotensin II (A II) has several physiological effects important to fluid-electrolyte balance. It can act on the central nervous system to increase blood pressure, stimulate drinking (Kucharczyk and Mogenson, 1975; Kucharczyk and Mogenson, 1977) and increase efferent sympathetic nerve activity to the periphery (Peach, 1977). It can act on the adrenal cortex (zona glomerulosa) to stimulate aldosterone production (McDonald and Schrier, 1976), which consequently will increase Na^+ reabsorption from the distal tubular lumen, and on the adrenal medulla to increase the release of catecholamines (Peach, 1977). Its stimulatory effect on vasopressin release was initially suggested by Bonjour and coworkers. A II infusion, either intravenously or into the carotid artery, increased bioassayable titers of plasma vasopressin in their dogs (Bonjour and Malvin, 1970; Mouw et al., 1971). This view was supported by Ramsay and colleagues (Ramsay et al., 1978). Other investigators did not observe such increases in plasma vasopressin concentration (Cowley et al., 1981; Hammer et al., 1980) or reported mostly small responses to large doses of angiotensin II, which increased plasma angiotensin II concentration above the normal physiological range. This has been observed in dogs (Reid et al., 1982), rats (Knepel and Meyer, 1980) as well as man (Uhlich et al., 1975; Morton et al., 1977; Philips et al., 1985). On the contrary, the stimulation of vasopressin release by intracerebroventricular angiotensin II is a well established fact (Share, 1979; Keil et al., 1975; Sterling et al., 1980). The possible sites of action of angiotensin II are the subfornical organ (Simpson et al., 1979; Mangiapane et al., 1982), the organum vasculosum of the lamina terminales (Bealer et al., 1979) and the supraoptic nucleus (Simmonet et al., 1979). The physiological significance of angiotensin II-induced AVP release is not clear.

The hemodynamic influences on vasopressin secretion are mediated by pressure-sensitive receptors, located in the left atrium and large arteries and neck

(Share, 1961; Share, 1961; Brennan et al., 1971; Schrier et al., 1977; Fater et al., 1982; Schultz et al., 1982), and do not appear to interfere with either the stimulatory or inhibitory effects of blood osmolality, but merely alter the relationship between vasopressin and osmolality. The relationship between blood volume or blood pressure and vasopressin secretion follows an exponential pattern. Small changes in blood volume or blood pressure have minimal effects on vasopressin secretion in contrast to the extraordinary sensitivity of the osmoregulatory system. Hypovolemia and/or hypotension of 15%, produced in rats, was found to shift the osmotic threshold to the left (Dunn et al., 1973). A similar shift was observed in man in the upright posture, which reduces the central or effective volume by 10 to 15% (Robertson and Athar, 1976). Hypervolemia and/or hypertension seem to produce changes in osmoreceptor function opposite to those produced by hypovolemia (Moses and Miller, 1971; Moses et al., 1967; Robertson and Athar, 1976). This resetting of the vasopressin osmoreceptor leads to an appropiate change in ECF volume an /or pressure, but at the expense of the tonicity of body fluids. This point will be treated in more detail in the discussion. Downward resetting of the osmostat has also been observed in other situations. It has been found to occur during gestation in the rat (Durr et al.,1981) and in the human (Davison et al.,1984), during the luteal phase of the human menstrual cycle (Lindheimer et al., 1991; Spruce et al., 1985) and during chronic chloride depletion metabolic alkalosis (Peterson et al., 1988).

1.1.2 Role of Thirst

a) osmotic

For a long time it was believed that dryness of the mouth and throat region was the principal factor determining the presence of thirst. In the nineteenth century several investigators showed that the sensation of thirst arises from a lack of body water, since the restoration of the body fluids by the intravascular route caused complete relief of thirst. Moistening of the mouth and the throat without restoring the body water did not relieve thirst (Bernard, 1856). The attempts of Mayer (1900) and Wettendorf (1901) to define the immediate stimulus underlying thirst, at the turn of the century, eventually led to the cellular dehydration theory. They found that the osmotic pressure of the blood increased in water deprived dogs and decreased to normal when the animals were allowed to drink. The observation that there were only very small changes in osmotic pressure the first few days of water deprivation, led Wettendorf to believe that water is withdrawn from all the cells and thirst originates in the tissues themselves. In 1937 Gilman provided proof that cell dehydration rather than an increase in osmolality per se was the stimulus for thirst. He observed that after intravenous hypertonic saline, a substance which causes fluid to leave cells, dogs drank more than twice as much than when they were given an osmotically equivalent amount of hypertonic urea, a substance which penetrates cells easily, although extracellular osmotic pressure increased to a comparable degree. Subsequent studies by other investigators, using different hypertonic solutions, confirmed this observation (Holmes and Gregersen, 1950; Fitzsimons, 1971; Szczepanska-Sadowska and Kozlowski, 1975; Wood et al., 1977).

The evidence that thirst like ADH release (Verney, 1947) is regulated by cerebral osmoreceptors, sensitive to small changes in receptor volume, was provided by Andersson and colleagues (1955). They showed that small amounts of hypertonic saline or electrical stimuli, introduced directly into the hypothalamus, caused excessive drinking as well as antidiuresis and milk ejection in water-replete goats. The finding that when some areas are stimulated polydipsia ensues without antidiuresis suggested that the osmoreceptors controlling thirst might be physically separate from those controlling ADH secretion (Andersson, 1952;1953; Andersson and McCann, 1955). Later studies support the concept of separate sites (Andersson et al., 1967; Peck and Blass, 1975; Brody and Johnson, 1980). The basal forebrain (Kucharczyk and Mogenson, 1976; Malmo and Mundl, 1975; Hubbard et al., 1985) and the region of the anterioventral third ventricle including the subfornical organ and organum vasculosum (Andersson et al., 1975; Buggy and Johnson, 1977; Trasher et al., 1980) have been suggested as possible locations for osmoreceptors mediating drinking to osmotic drive. The osmoregulation of thirst closely resembles that of AVP secretion. Thirst is stimulated at a certain level of plasma osmolality, the "threshold" or "setpoint", below which drinking is absent, and above which drinking increases in direct proportion with plasma osmolality. Earlier studies suggested that the thirst mechanism comes into play only when plasma vasopressin level is not sufficient to maintain a normal osmolality of plasma in spite of its maximal antidiuretic effect (Wolf, 1950; Baylis and Robertson, 1980). More recent work (Rollin et al.,1989) indicates, however, that thirst may be activated at lower plasma osmolality levels than vasopressin release under some circumstances.

b) non-osmotic

Thirst is also elicited by nonosmotic stimuli. Extracellular dehydration or hypovolemia produced by hemorrhage or polyethylene glycol, induced drinking (Abdelaal et al., 1974). Hemodynamic stimulation also resets the osmotic threshold for thirst, as it does for vasopressin (see discussion on p.8.). There is evidence that drinking to non-osmotic stimuli is mediated neurally by the volume receptors controlling vasopressin release (Fitzsimons and Moore-Gillon, 1980) and hormonally by the renin-angiotensin system. Angiotensin II has been shown to be a potent dipsogen in the rat (Fitzsimons, 1972; 1978).

1.2 Water Metabolism in Insulin Dependent Diabetes Mellitus

Polyuria and polydipsia, the result of the concentrating defect seen in patients with uncontrolled diabetes mellitus, has generally been attributed to the inability of the renal tubules to reabsorb the overload of glucose (Brodsky et al., 1950; Hays et al., 1976). Based on the observation that the infusion of an hypertonic solution of glucose in healthy subjects caused a fall in plasma vasopressin and urine osmolality (Zerbe et al.,1977), the same authors later (1979) suggested that low levels of vasopressin in insulin-deficient diabetics might contribute to the water diuresis. Glucose uptake by the neurons is believed to be independent of insulin and is taken up too rapidly to permit the establishment of a significant transcellular osmotic gradient. The slight fall in vasopressin is possibly caused by the reduction of the plasma sodium. The opposite was in fact true; most of the patients with uncontrolled diabetes had highly elevated plasma vasopressin levels (Zerbe et al., 1979; Walsh et al., 1979). Hyperosmolality, hypovolemia and nausea, factors accompanying uncontrolled diabetes, are all

stimuli for vasopressin release, but several observations indicate that they cannot fully account for the present hypervasopressinemia (Robertson, 1977). Hyperglycemia, largely responsible for the hyperosmolality might be an effective stimulus for vasopressin release and thirst in insulin-deficient diabetics, assuming that the glucose uptake by osmoreceptor neurons is insulin dependent (Van Houten, 1979). Glucose is not an effective stimulus for the osmoreceptor in healthy adults or insulin-treated diabetic patients (Zerbe et al., 1983; Thompson et al.,1988). The observations of increased thirst and plasma vasopressin levels during 2-Deoxy-D glucose induced central nervous system glucoprivation (Thompson et al., 1981) and of increased plasma vasopressin levels during insulin induced hypoglycemia (Baylis et al., 1981), also producing intracellular neuroglycopenia, in healthy humans support the hypothesis of glucose as an effective stimulus of the osmoreceptor in insulin-deficient diabetics (Van Houten et al., 1979). Vasopressin itself might contribute to the hyperglycemia. Vasopressin infusions have been shown to stimulate glycogen phosphorylase activity to break down glycogen and raise plasma glucose concentrations in dogs (Bergen et al., 1960), rodents (Ma and Hems, 1975; Hems et al., 1975) and in man (Spruce et al., 1985). Recent studies by Zerbe and colleagues on a group of uncomplicated, controlled insulin-dependent diabetic patients with a significantly higher glucose level than the controls (the usual morning dose of insulin was given after the experiment), revealed an abnormality in the osmoregulation of vasopressin release during a hypertonic saline infusion. The abnormality was a shift of the osmotic threshold to a lower level of plasma sodium or effective osmolality[1].

[1] Effective osmolality is defined as plasma osmolality - plasma glucose (mmol/L) - plasma urea (mmol/L) (Zerbe et al., 1985)

There was no significant difference between controls and diabetic subjects when the vasopressin levels were correlated with uncorrected plasma osmolality. Similar studies were done by Thompson et al. (1989) on a group of insulin dependent diabetic patients in the euglycemic and hyperglycemic state. They found lowered plasma sodium thresholds for both thirst appreciation and plasma vasopressin concentrations during the hyperglycemic study. There were no differences in the osmotic thresholds for thirst or vasopressin release when plasma osmolality was corrected for the increase in glucose in the hyperglycemic study (corrected plasma osmolality= plasma osmolality in the hyperglycemic state - (blood glucose in the hyperglycemic state -blood glucose in the euglycemic state)). The cause and the consequences of the osmoregulatory abnormality remain to be settled and form the major objective of the present study.

1.3 Chemically Induced Diabetes.

In 1943 Dunn et al. discovered that alloxan produced diabetes mellitus in rabbits by destroying, more or less specifically, the beta-cells. Since then this drug has been used for the experimental induction of diabetes.

a) Effect of Alloxan on Plasma Glucose Concentration

An intravenous injection of a diabetogenic dose of alloxan produces hyperglycemia between 1 an 4 h after the injection followed by a transitory hypoglycemic period between 6 and 12 hours and ending in a permanent hyperglycemia 12 to 24 h postinjection (Cooperstein and Watkins , 1981).

The initial hyperglycemia might be caused partly by an inhibition of insulin secretion (Dixit et al., 1962) and partly by an increased release of epinephrine, which stimulates hepatic glucose production (Boquist and Lorentzon, 1980). The period of hypoglycemia is caused by the permanent destruction of the beta cells, resulting in an transient increase of insulin in the plasma (Lundquist and Rerup, 1967).

b) Effect of Alloxan on the Pancreas

Light microscopic studies show that the alpha-cells of the islets of Langerhans survive diabetogenic or even lethal doses of alloxan, though their morphology changes to swolien and rounded cells, showing increased granulation (Dunn et al., 1944). The beta-cells, however, are permanently destroyed. 10 to 60 minutes after the treatment the cytoplasm becomes vacuolated, the nuclei are pycnotic and the cells have shrunk (Lazarus et al., 1962). After 3h the beta-cells are detached from one another. Disintegration of the nuclear membrane is observed after 5h (Cooperstein and Watkins, 1981). Within 24h the islets are made up mostly of alpha-cells (Lukens,1948).

c) Effect of Alloxan on the Kidney

Kidney lesions produced by alloxan are most conspicuous during the first four days after the injection. The glomeruli are usually unaffected, but the luminal side of the convoluted tubules are often the site of vacuolization, necrosis and desquamation. These lesions usually disappear with time. In rats these lesions occur when the dose is greater than 40 mg/kg (Lukens,1948).

d) Effect of Alloxan on the liver

In dogs a very large dose of alloxan will cause hepatic lesions such as central lobular necrosis, fatty infiltration and in some cases jaundice. Lethal doses of alloxan for dogs were found to range from 125-200 mg/kg, nephropatic doses from 75-100 mg/kg and diabetogenic doses from 50-75 mg/kg. Our dogs were given 65 mg/kg of alloxan, a relatively low diabetogenic dose (Lussier et al., 1986). In the rat a low diabetogenic dose will produce focal necrosis of peripheral lobules (Lukens et al.,1948). However, caution must be taken in interpreting these results since diabetes in itself causes hepatic changes such as fatty infiltration.

1.4 The Objective of the Present Study

1. The purpose of the present study is to determine whether osmotic drive for vasopressin release and thirst is altered in experimentally-induced insulin dependent diabetes mellitus.

2.0 Methods

2.1 Animals

Experiments were carried out on 4 mongrel dogs of either sex, weighing 18-26 kg, and trained to stand quietly in a Pavlov harness for up to 3 h during the course of the experiment. The animals were housed in a temperature and humidity controlled room with lights on between 6:00 and 19:00 hours, and were maintained on commercial dog food (42% protein, 10% fat, 38 % carbohydrate and 9% fiber on a dry weight basis) with ad libitum access to tap water.

2.2 Experimental Design

To determine whether short-term depletion (48 h) of insulin influences the osmoregulation of plasma vasopressin (PAVP) and thirst, the dogs were tested with an i.v. infusion of 0.5 M Na_2SO_4 (1500 mOsm) at the rate of 0.09 ml/kg bw/min for 100 minutes in each of 3 metabolic conditions: control; insulin-treated diabetic; diabetic. Plasma insulin depletion was produced by discontinuing PZI injections at least 48 h before loading. During this period the dogs were allowed free access to water but were food deprived for 20 h. Following the osmotic loading experiments PZI injections were immediately resumed to restore normoglycemia. The effect of osmotic loading on vasopressin release and water intake was tested in separate experiments using the same dogs. In the experiments on osmoregulation of water intake the dogs had free access to drinking water during the infusion of Na_2SO_4. Blood samples (10 ml) were taken at t=-5 and 0 and every 20 min after the start of infusion. The time of onset of drinking and cumulative 20 min interval intakes of water were recorded. An additional blood sample (10ml) was taken at the time of

the first bout of drinking in order to measure plasma AVP, plasma osmolality and plasma Na concentration (PNa). The total volume of blood withdrawn was 80 ml, while the volume of hypertonic Na_2SO_4 infused varied from 162 to 235 ml depending on the weight of the animal. The same procedures were used to study the osmoregulation of AVP secretion, except that water was not available to the animals during the infusion of Na_2SO_4.

Water intake and AVP secretion were tested in separate experiments in each metabolic state over a period of 8-10 weeks, with no more than 2 infusions of Na_2SO_4 made in any week.

2.3 Procedures for Chemical Induction and Maintenance of Diabetes

Diabetes was produced by an intravenous injection of a 13% solution of alloxan monohydrate in a 0.1 M acetate buffer at pH 4.4 at a dose of 65 mg/kg (0.5 ml/kg). The dogs were fasted 18 to 20 hours before the injection. A polyethylene catheter (Clay Adams PE 190) was introduced into the inferior vena cava via a saphenous vein. The animal was allowed to rest in a Pavlov stand for 30 minutes before the injection (Lussier et al., 1986). Once diabetes was established, the dogs were maintained on protamine zinc beef and pork insulin (PZI, Connaught Labs, Toronto, Ontario, Canada). The daily dose was adjusted to prevent or minimize glucosuria. Urine glucose concentration was estimated using clinitest tablets (Ames). From time to time the plasma glucose level was determined before the daily feeding.

2.4 Cannulation

All dogs used were in the post-absorptive state following an 18 to 20 hour fast. An 18 gauge (Monoject, St. Louis, MO) indwelling polyethylene catheter (PE 190 Clay Adams, Boston, Ma) was introduced into the cephalic vein for the infusion of the hypertonic Na_2SO_4 solution and the blood was sampled from the saphenous vein by a 21 gauge butterfly (winged infusion set, Termo Corp., Tokyo).

2.5 Blood sampling

Blood samples were taken from the saphenous vein. Blood for the determination of glucose was collected in pre-cooled polyethylene centrifuge tubes containing dry heparin. The tubes were kept in crushed ice until centrifugation. For arginine vasopressin (AVP) and plasma renin activity (PRA) blood was collected into pre-chilled EDTA tubes kept on an ice bath. After collection the tubes were gently mixed and centrifuged at 750 G for 20 min at 4°C. Plasma was then collected in aliquots of 1 ml and frozen (at -20°C) immediately.

For analysis of plasma osmolality, electrolytes and BUN the blood was collected in heparinized vacutainer tubes and samples were spun down at 4°C and assayed the same day.

2.6 Chemicals

Alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Both crystalline insulin and PZI were supplied by Connaught Laboratories Ltd. (Willowdale, Ontario, Canada). Na_2SO_4 was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.7 Analytical Methods

2.7.1 Concentration of Plasma Glucose (mmol/L)

The concentration of glucose was determined from a sample of 0.01 ml using a Beckman Glucose Analyzer II (Beckman Instruments Inc. Fullerton, CA). The principle is as follows: the enzyme glucose oxidase catalyzes the breakdown of β-D-glucose to gluconic acid and peroxide in the presence of oxygen. The oxygen consumption, as monitored by an oxygen electrode is directly proportional to the amount of glucose (Kardish et al., 1968).

2.7.2 Determination of Plasma Electrolytes $Na^{\pm}$, $K^{\pm}$, Cl^{-} (mEq/l)

Na^{+} and K^{+} were analyzed by a Corning 480 flame photometer (Corning Medical and Scientific Corning Glass Works, Medfield, Massachussetts, USA) using a lithium internal standard. Alkali metals after excitation by a flame emit light at a discrete frequency which can be isolated by an optical filter. The emission is proportional to the number of atoms excited, i.e. the concentration of the sample. Plasma chloride concentration was determined on a 925 Chloride Analyzer (Corning Limited, Halstead, Essex, England). The 925 measures the amount of silver ions necessary to precipitate all the chloride ions in the sample.

2.7.3 Determination of Plasma Osmolality (mOsmol/kg/H_2O)

Osmolality measurements were determined by freezing-point depression (m Osmette model 5004; Precision Science).
The freezing point of the solution is a measure of the solution's concentration.

2.7.4 Determination of Blood pH and HCO_3^- (mEq/L)

Plasma pH and HCO_3^- were determined by a pH/blood gas analyzer (Instrumentation Laboratories Model 813 Lexington, Ma, USA).

2.7.5 Determination of Blood Urea Nitrogen (BUN) (mmol/L)

The concentration of BUN was determined from a sample of 0.01 ml using a Beckman BUN Analyzer II (Beckman Instruments Inc., Fullerton, CA). The principle is as follows: The enzyme urease catalyzes the breakdown of urea to ammonium and carbonate ions in the presence of water. The rate of increase in conductivity, sensed by the electrode, is proportional to the BUN concentration of the sample.

2.8 Hormonal Analyses by Radioimmunoassay

2.8.1 Concentration of AVP in the Plasma

AVP concentrations were measured by radioimmunoassay (Dr. Daniel Bichet, Sacre Coeur Hospital, Montreal; Bichet et al., 1986). 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 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 thawed and evaporated to dryness at room temperature under a stream of cold air (Concevator sample concentrator, E.C.

Apparatus Corp., St. Petersburg, Florida, USA). The dry residue is reconstituted in 750µl of a 0.1% bovine albumin solution (Miles, P.O. Box 2000, Alkhart, Indiana, 46515, USA) also containing 0.1% sodium azide (pH is adjusted to 7.2 with Triz-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, 1 mg = 450 IU, Ferring Pharmaceuticals, Box 30561, S-20062, Malino, Sweden) in quantities that ranged from 0.05 to 10 pg per assay tube. 200 µl of standards in buffer or 200 µl of reconstituted plasma extract, and 200 µl of antiserum in buffer were incubated in triplicate for the standards and duplicate for the unknown, for 2 days at 4°C, then 100µl 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. The tracer used was vasopressin-8-arginine [^{125}I]-moniodinated with a high specific activity (1820 to 2200 Ci/mM). Non-specific binding was always < 3%.

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

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). 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 Dr. Bichet's 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%. Intra-assay 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.,1986).

2.8.2 Plasma Renin Activity

Incubation of the samples 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). PRA determinations were carried out by Dr. D. Bichet.

2.9 Statistical Analyses.

Quantitative data for the 3 metabolic conditions were compared by analyses of variance followed by Dunnett's tests (within group comparisons) or by Newman-Keuls tests (between group comparisons). Regression analysis was used to examine the relationship between effective estimated osmolality and PAVP, as well as between cumulative water intake and effective estimated osmolality. The differences in the relationships were analyzed by comparing the means of the slopes and the osmotic thresholds of the individual regression lines of each group with analysis of variance followed by Bonferoni's method. Data are reported as means ± SEM.

3.0 Results

3.1 Basal Situation

In the insulin-treated dogs normal values were found for plasma osmolality, sodium, vasopressin, glucose, potassium, bicarbonate,and urea as well as hematocrit, body weight and plasma renin activity (see table 1). A 48 hour insulin depletion resulted in an increase in plasma osmolality ($p < 0.01$). This change was due largely to an increase in plasma glucose ($p < 0.01$). Plasma sodium ($p < 0.01$) decreased due to the hydroosmotic effects of the hyperglycemia (Katz,1973), whereas potassium increased ($p < 0.01$). There was no indication of volume contraction as reflected by normal hematocrit, body weight and plasma renin activity levels. Ketonuria and ketonemia was not measured in this study, but bicarbonate levels did not change (see table 1).

3.2 Vasopressin Responses to Osmotic stimulation

To determine whether the osmotic drive for vasopressin release was altered in the diabetic condition, the effect of a hypertonic Na_2SO_4 infusion was compared in the control, insulin-treated diabetic and diabetic (48 hour insulin depletion) states. A 0.5 molar solution of Na_2SO_4 was infused at the rate of 0.09 ml/min/kg bw for 100 minutes, during which period the animals did not have access to water. The infusion of hypertonic sodium sulfate produced a progressive increase in plasma sodium from 146 ± 0.6 to 160 ± 0.9 ($p < 0.01$) for the controls and insulin-treated diabetics and from 140 ± 0.7 to 153 ± 0.9 mEq/L

($p < 0.01$) for the diabetics (see figure 1) and in an increase in plasma osmolality from 300 ± 1.4 to 311. 3 ± 1.68 ($p < 0.01$) for the controls, from 296.5 ± 1.29 to 307± 2.2 ($p < 0.01$) for the insulin-treated diabetics and from 308.8 ± 2.31 to 320.9± 2.06 mosm/kg H_2O ($p < 0.01$) for the diabetics (see figure 2). This response was accompanied by an increase in plasma vasopressin from 0.9 ± 0.2 to 7.7 ± 1.49 ($p < 0.01$) for the controls, from 1.1 ± 0.25 to 7.0 ± 1.29 ($p < 0.01$) for the insulin-treated diabetics and from 0.9 ± 0.16 to 7.0± 0.98 pg/ml ($p < 0.01$) for the diabetics (see figure 3). At the same time plasma potassium decreased slightly, whereas glucose and urea did not change.

3.2.1 Relation of plasma vasopressin to effective osmolality

The responsiveness of vasopressin secretion to the osmotic stimulation was expressed as a linear function of the effective osmolality.
In the controls and the insulin-treated diabetics the effective osmolality was calculated in two different ways. The effective osmolality was calculated by omitting [glucose] (mmol) and [urea] (mmol) from the measured plasma osmolality (effective osmoles). In addition it was estimated by taking two times the sodium values (2[Na]) (effective estimated osmolality)(see discussion). However in the diabetics, since the osmotic contribution of glucose to the effective osmolality is uncertain in insulin-deficient diabetics, the relationship between vasopressin and effective osmolality in this group was also analyzed by retaining the glucose in the effective osmolality (plasma osmolality - [urea] (effective osmoles) and 2[Na] +[Glu] (effective estimated osmolality)).
The differences in the relationship of plasma vasopressin to effective osmolality were analyzed by comparing the means of the slopes (measure of the sensitivity of the osmoreceptors) and the osmotic thresholds of the individual

regression lines of each group. The threshold value for AVP release predicted by this linear model is defined as the value of POSM when PAVP is 0.5 pg/ml, which is the lowest detectable level. When the osmotic threshold was compared, the values obtained in the diabetic condition were significantly higher than the control and insulin-treated condition when glucose was considered as an effective osmol and significantly lower when it was not included (see table 2 and figures 4, and 5). Similar results were obtained for the effective osmoles and effective estimated osmolality. Hyperglycemia reduces plasma sodium by osmotically extracting water from the insulin sensitive cells. Numerous observations in insulin-deficient diabetic patients have led to the estimation of a decrease of 1.6 mEq/L of sodium for every 5.5 mmol excess glucose (Katz.1973). In the diabetic condition, vasopressin was also correlated with the corrected 2[Na] (see table 2 and figure 4). No significant differences with controls and insulin-treated diabetics were seen in this approach. Regarding the sensitivity of the osmoregulatory system to osmotic drive there were no differences in the slopes between the three groups. This was very clear when vasopressin was correlated with the amount of Na_2SO_4 administered per minute (see table 3 and figure 6).

3.3 Drinking Response to Osmotic Stimulation

In order to assess whether the osmotic drive for thirst was altered in the diabetic state, the effect of a hypertonic Na_2SO_4 infusion was compared in the control, insulin-treated diabetic and diabetic (48 hour insulin depletion) states. A 0.5 molar solution of Na_2SO_4 was infused at the rate of 0.09 ml/min/kg bw for 100 minutes, during which period the animals had free access to water. The amount of water ingested at any moment during the infusion was recorded and

expressed in ml of water/kg of body weight. Water intake increased significantly with increasing osmotic load, in all conditions. The water intake of the animals in the diabetic state was significantly higher than in the insulin-treated diabetic state at 80 and 100 minutes of the infusion. The higher water intake of the diabetics did not reach a statistically significant difference with the normal controls (see figure 7).

3.4 Relation of thirst to effective osmolality

The responsiveness of the thirst mechanism to osmotic stimulation was expressed as a linear function of the estimated osmolality. The results followed the pattern of vasopressin. When glucose was added to the effective estimated osmolality (2[Na] + [Glu]) in the diabetic condition, the threshold was shifted to the right and a shift to the left was observed when glucose was omitted ([2Na]). Again when vasopressin was correlated with the corrected 2[Na], no differences were seen (see table 4 and figure 8). There is indication of an increased sensitivity of the osmoregulatory system in the diabetic group as shown by an increase in the slope, but no statistically significant difference was reached. The same was observed when the cumulative water intake was correlated with the amount of Na_2SO_4 administered per minute (see table 3 and figure 9).

TABLE 1. Basal plasma electrolyte, hormone, glucose and urea levels, Hct and body weight in each of 3 metabolic conditions.

	PNa	PK	POsm[2]	PAVP	PRA[1]	PGLu	Purea	Hct	HCO_3^-	weight
	mEq /L	mEq/L		pg/ml		mmol/L		%	mEq/L	kg
Control	146 ±0.3	4.4 ±0.06	301 ±0.9	0.8 ±0.12	0.8 ±0.06	5.5 ±0.06	6.3 ±0.42	49 ±0.8	20.6 ±0.38	22 ± 0.5
Insulin-treated Diabetic	147 ±0.4	4.4 ±0.10	296 ±1.1	0.9 ±0.14	0.9 ±0.09	4 ±0.06	5 ±0.4	48 ±0.7	19.1 ±0.36	22 ±0.6
Diabetic	141+* ±0 6	4.9+* ±0.04	308+* ±1.5	1.1 ±0.70	1.2 ±0.16	20+* ±1.2	6.5 ±0.66	48 ±1.5	18.9 ±0.47	21 ±0.6

*P<0.01 compared to control

+P<0.01 compared to insulin-treated diabetic

[1] PRA = plasma renin activity, measured as ng of angiotensin I per ml of plasma per hour of incubation

[2] POSM = plasma osmolality expressed in mOsm/kg H20

Figure 1. Increase in plasma sodium in 4 dogs during a hypertonic sodium sulfate infusion in the control, insulin-treated and diabetic condition. Results are based on 2 tests per animal and are expressed as the mean ± SEM. Plasma sodium increased significantly over the time of the infusion in the three conditions ($p < 0.01$). *$p < 0.01$ compared to normal controls and insulin-treated diabetics.

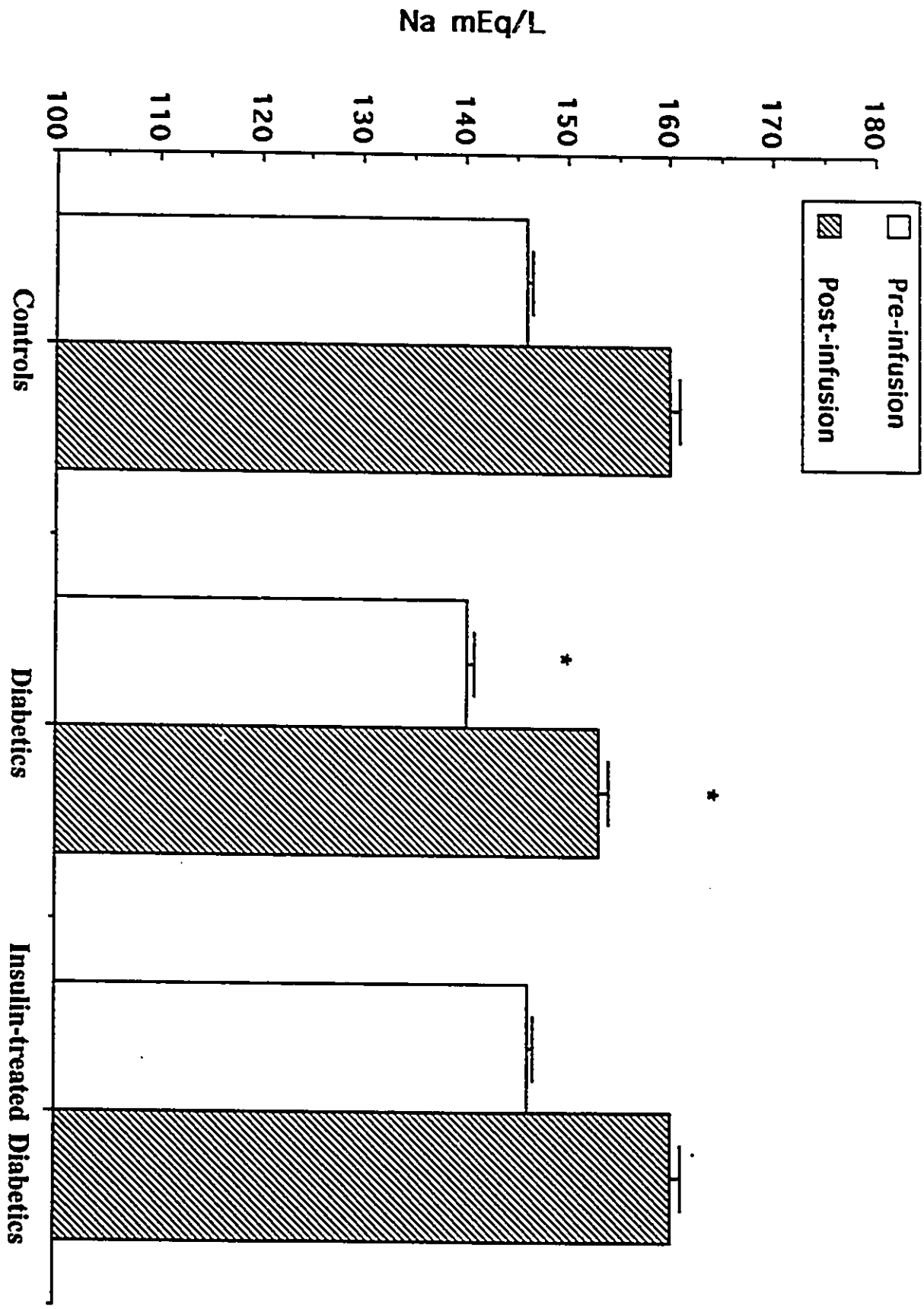

Na mEq/L
100
110
120
130
140
150
160
170
180
Pre-infusion
Post-infusion
Controls
Diabetics
Insulin-treated Diabetics
*
*

Figure 2. Increase in plasma osmolality in 4 dogs during a hypertonic sodium sulfate infusion in the control, insulin-treated diabetic and diabetic condition. Results are based on 2 tests per animal and are expressed as the mean ± SEM. Plasma osmolality increased significantly over time of the infusion in the three conditions ($p < 0.01$). *$p < 0.01$ compared to normal controls and insulin-treated diabetics.

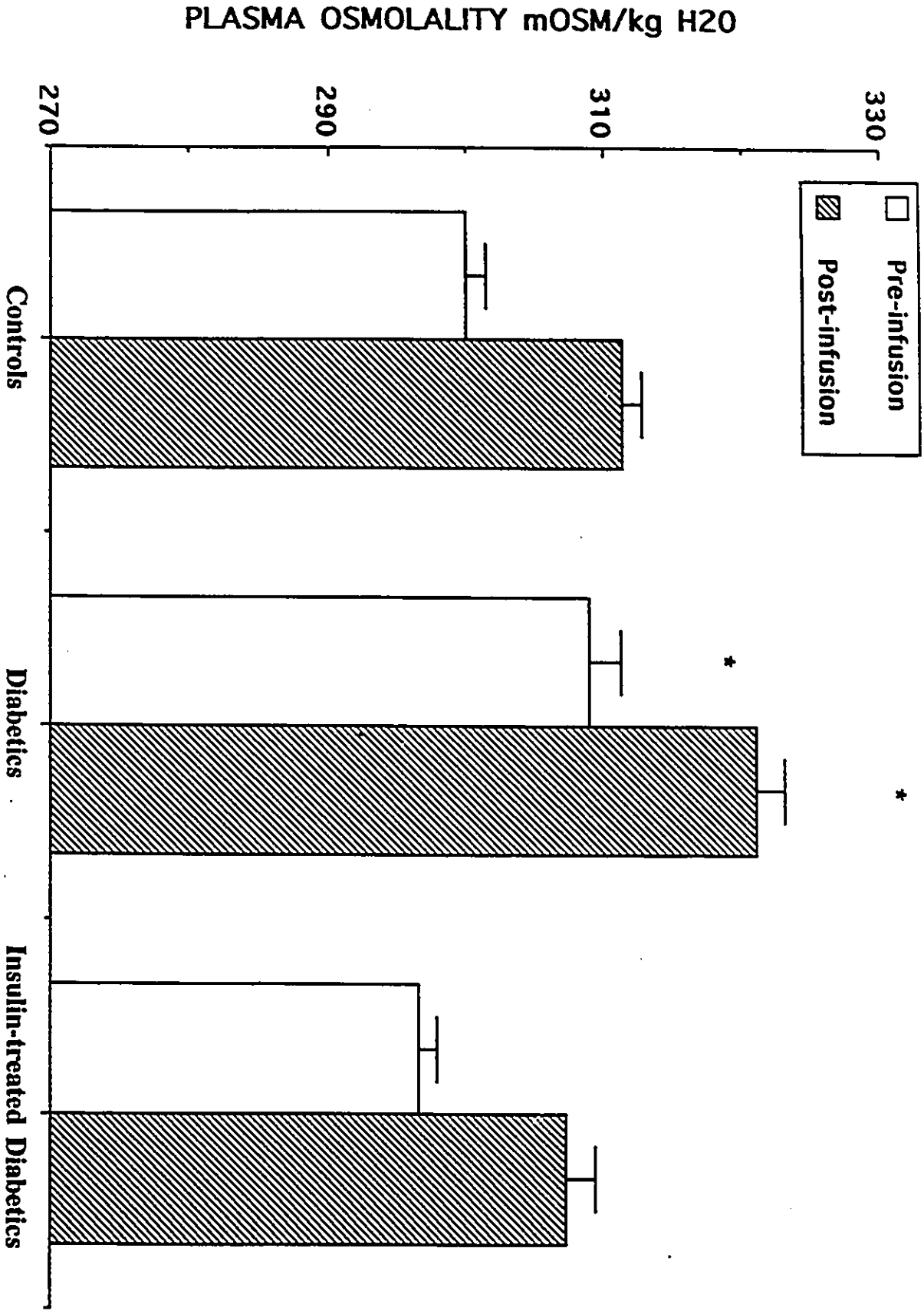

PLASMA OSMOLALITY mOSM/kg H20
270
290
310
330
Pre-infusion
Post-infusion
Controls
Diabetics
Insulin-treated Diabetics
*
*

Figure 3. Increase in plasma vasopressin in 4 dogs during a hypertonic sodium sulfate infusion in the control, insulin-treated diabetic and diabetic condition. Results are based on 2 tests per animal and are expressed as the mean ± SEM. Plasma vasopressin increased significantly over time of the infusion in the three conditions ($p < 0.01$). No significant differences were observed between the groups.

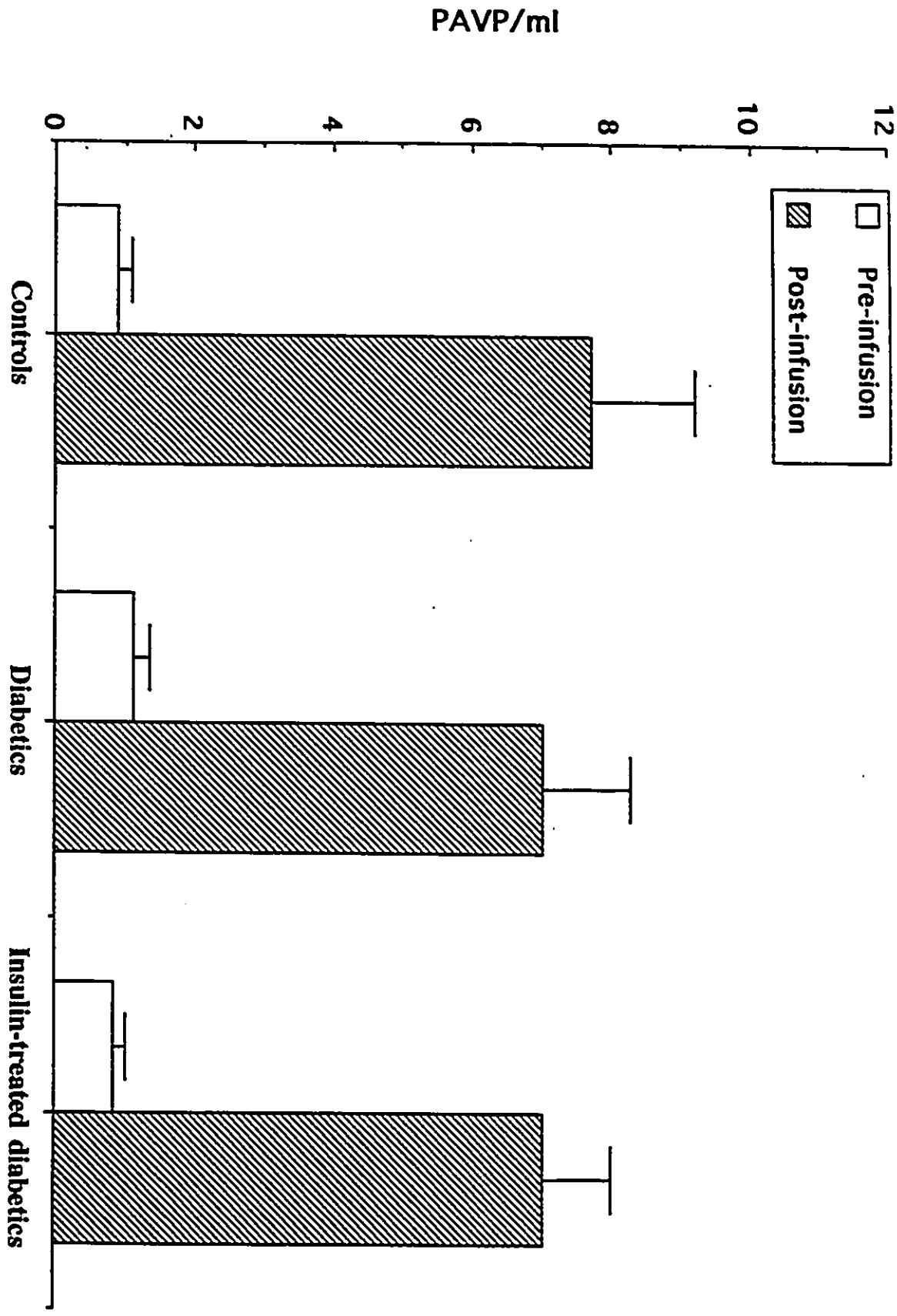

PAVP/ml
0
2
4
6
8
10
12
Pre-infusion
Post-infusion
Controls
Diabetics
Insulin-treated diabetics

TABLE 2. Relationship of vasopressin to estimated plasma osmolality and effective osmoles during individual hypertonic sodium sulfate infusions in the 3 metabolic conditions.

	r±SE	Slope±SE	Threshold±SE	r±SE	Slope±SE	Threshold±SE
	Estimated Osmolality 2[Na]			Effective osmoles Posm-[Glu]-[urea]		
Control (n=8)	0.83±0.038	0.2±0.04	293±1.8	0.85±0.039	0.5±0.08	288±1.8
Insulin-treated Diabetic (n=8)	0.91±0.022	0.2±0.03	293±2.3	0.90±0.017	0.6±0.09	284±1.1
		2[Na] ± [Glu]			Posm ± [Glu]-[urea]	
Diabetics -[Glu]	0.91±0.014	0.2±0.03	280±1.7*+	0.92±0.011	0.5±0.05	282±1.1*
Diabetics +[Glu]	0.92±0.010	0.2±0.03	300±2.1*+	0.93±0.010	0.5±0.05	302±2.1*+
		Corrected 2[Na][1]				
Diabetics (n=7)	0.92±0.012	0.2±0.03	289±1.6			

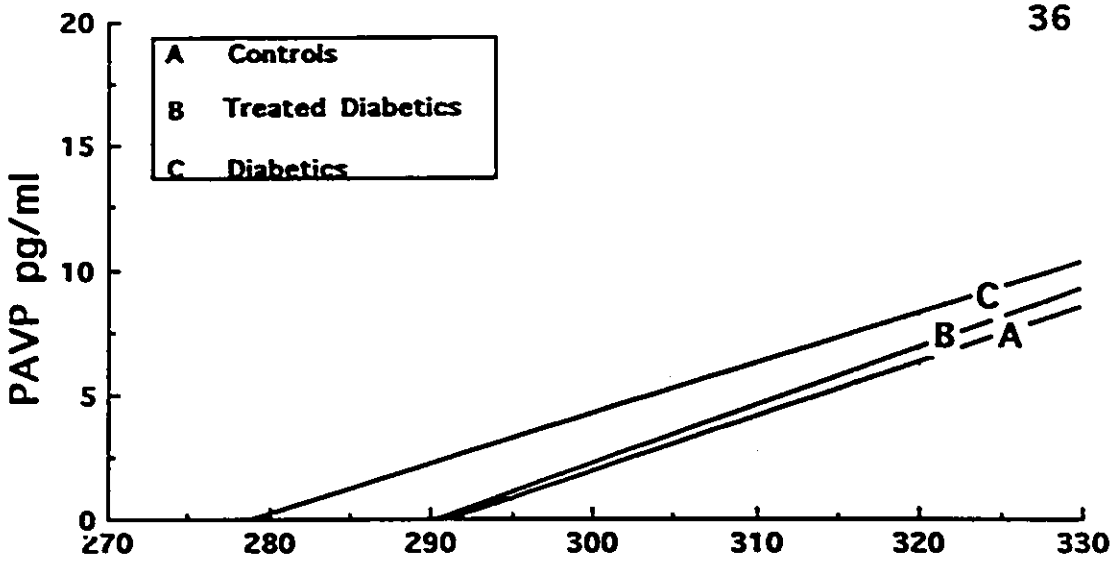
* Designates significant ($p < 0.01$) difference from controls

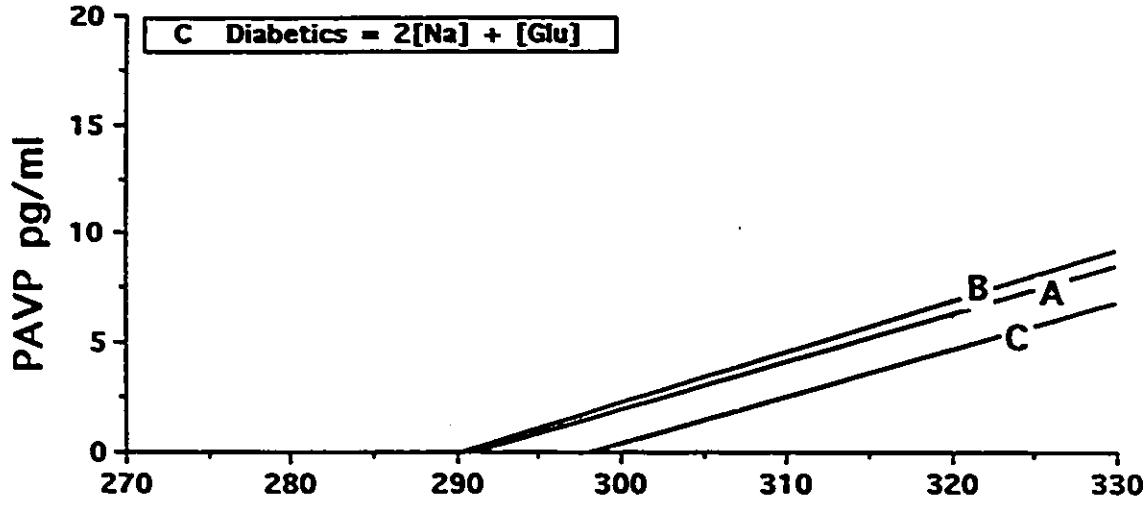
\+ Designates significant ($p < 0.01$) difference from insulin-treated diabetics

[1] 2[Na] corrected for presence of glucose; [Na] was increased 1.6 mEq for every 5.5 mmol glucose

n = number of tests

Figure 4. Relationship of plasma vasopressin to estimated plasma osmolality, in 4 dogs during a hypertonic sodium sulfate infusion with continuous access to water in the control, insulin-tretated diabetic and diabetic condition. Estimated osmolality was defined as 2[Na]. In the diabetics, plasma vasopressin was also correlated with 2[Na] + [Glu] and with 2[Na] corrected for the presence of glucose. [Na] was increased 1.6 mEq for every 5.5 mmol glucose. The osmotic threshold in the diabetic state was significantly higher when glucose was considered as an effective osmol and lower when not. There was no difference from the normal controls and insulin-treated diabetics when [Na] was corrected for the presence of glucose. There were no differences among the slopes. For more details on the regression analysis see table 4.


A Controls
B Treated Diabetics
C Diabetics
PAVP pg/ml
20
15
10
5
0
270
280
290
300
310
320
330
C
B
A


C Diabetics = 2[Na] + [Glu]
PAVP pg/ml
20
15
10
5
0
270
280
290
300
310
320
330
B
A
C

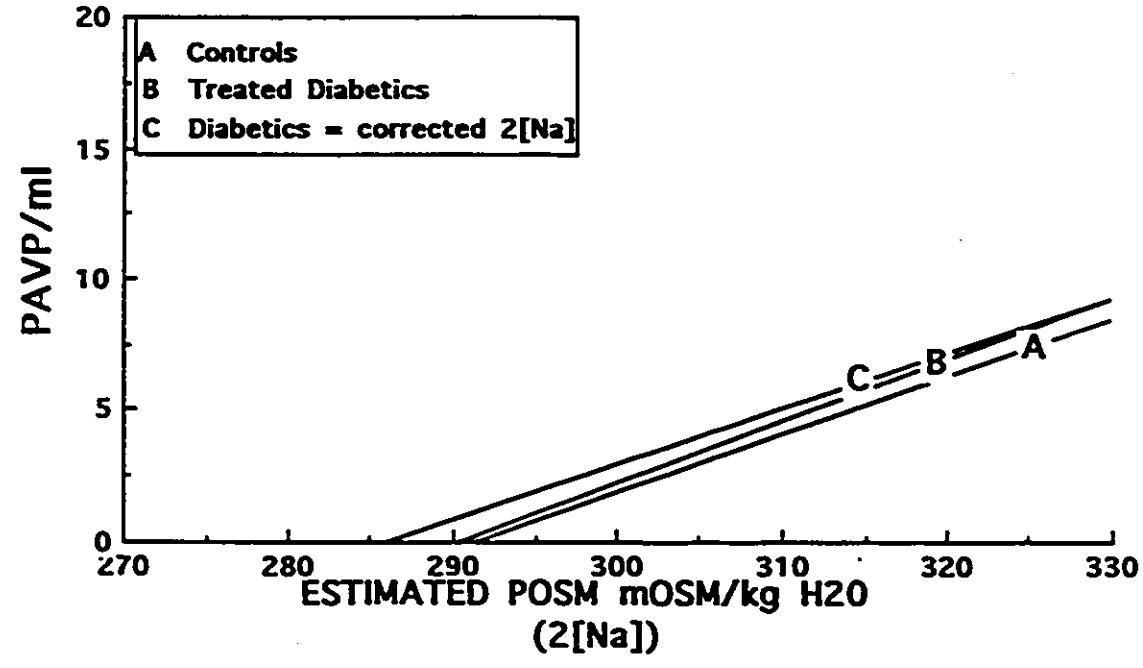
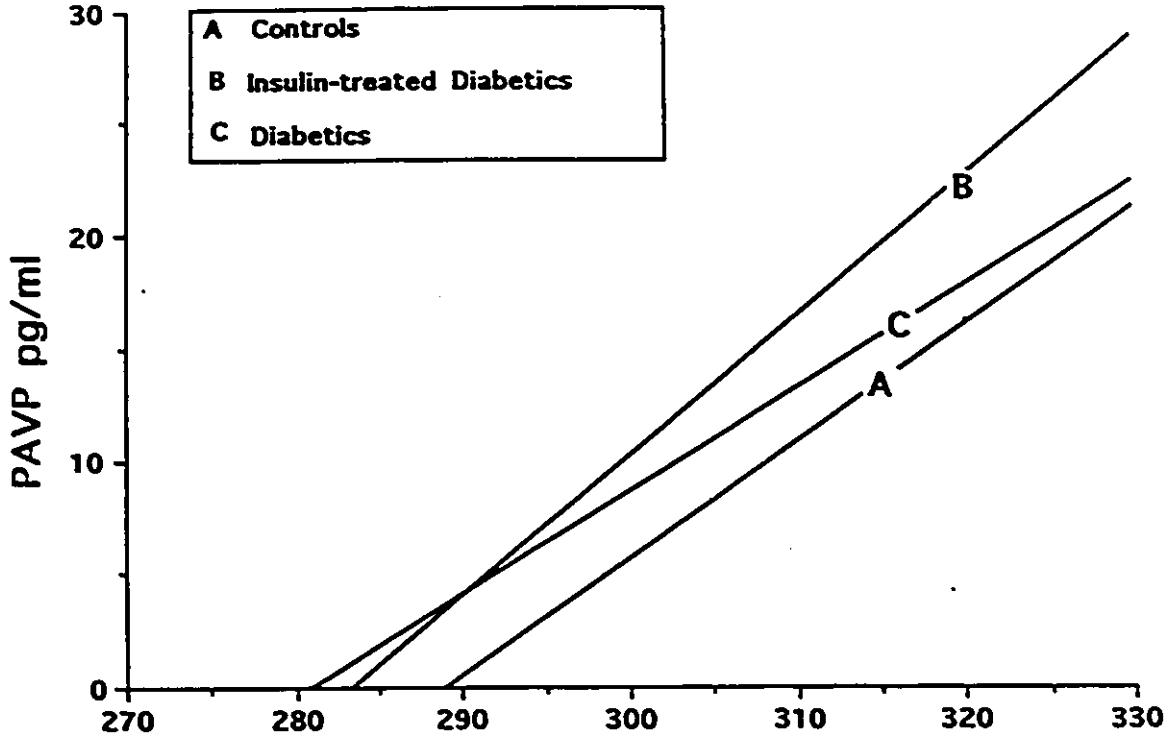

A Controls
B Treated Diabetics
C Diabetics = corrected 2[Na]
PAVP/ml
20
15
10
5
0
270
280
290
300
310
320
330
C
B
A
ESTIMATED POSM mOSM/kg H20
(2[Na])

Figure 5. Relationship of plasma vasopressin to the effective osmoles, in 4 dogs during a hypertonic sodium sulfate infusion in the control, insulin-treated diabetic and diabetic condition. Effective osmoles were calculated as Posm - [Glu] - [urea]. In the diabetics, plasma vasopressin was also correlated with Posm - [urea]. The osmotic threshold in the diabetic state was significantly higher when glucose was considered as an effective osmol and lower when not. The lower osmotic threshold did not reach significance when compared with the insulin-treated diabetics. There were no differences among the slopes. For more details on the regression analysis see table 2.


A Controls
B Insulin-treated Diabetics
C Diabetics
PAVP pg/ml
30
20
10
0
270
280
290
300
310
320
330
B
C
A

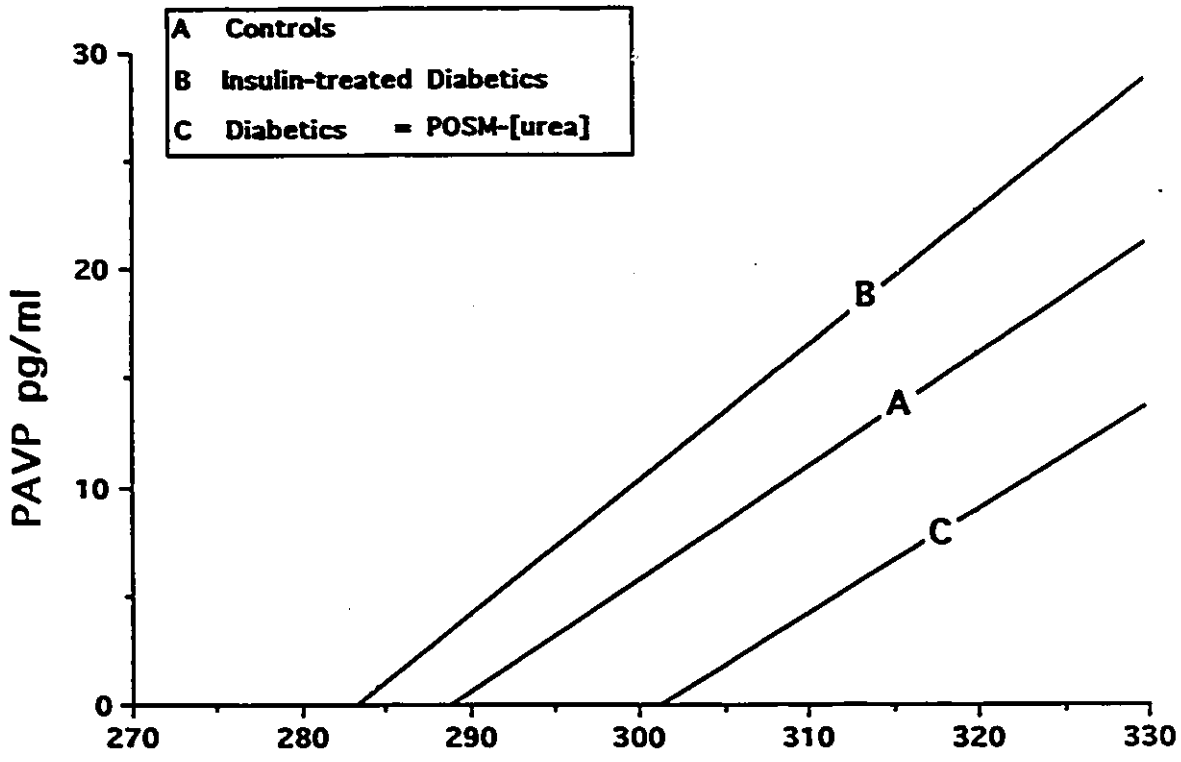

A Controls
B Insulin-treated Diabetics
C Diabetics = POSM-[urea]
PAVP pg/ml
30
20
10
0
270
280
290
300
310
320
330
B
A
C
EFFECTIVE OSMOLES mOSM/kg H20
(POSM-[Glu]-[urea])

TABLE 3. Relationship of vasopressin and cumulative water intake to the amount of sodium sulfate administered.

	r±SE	Slope±SE	r±SE	Slope±SE
	Vasopressin		Thirst	
Control (n=8)	0.89±0.031	0.5±0.09	0.95±0.012	1.3±0.15
Insulin-treated Diabetic (n=8)	0.90±0.033	0.5±0.07	0.94±0.014	0.95±0.07
Diabetics (n=7)	0.95±0.001	0.5±0.06	0.94±0.023	1.7 ±0.29

n = number of tests

Figure 6. Relationship of plasma vasopressin to the amount of Na_2SO_4 administered per minute (1500 mOsm Na_2SO_4 at 0.09 ml/kg bw/min, i.v.) in the control, insulin-treated diabetic and diabetic condition. There were no differences in the slopes among the three groups. For more details on the regression analysis see table 3.

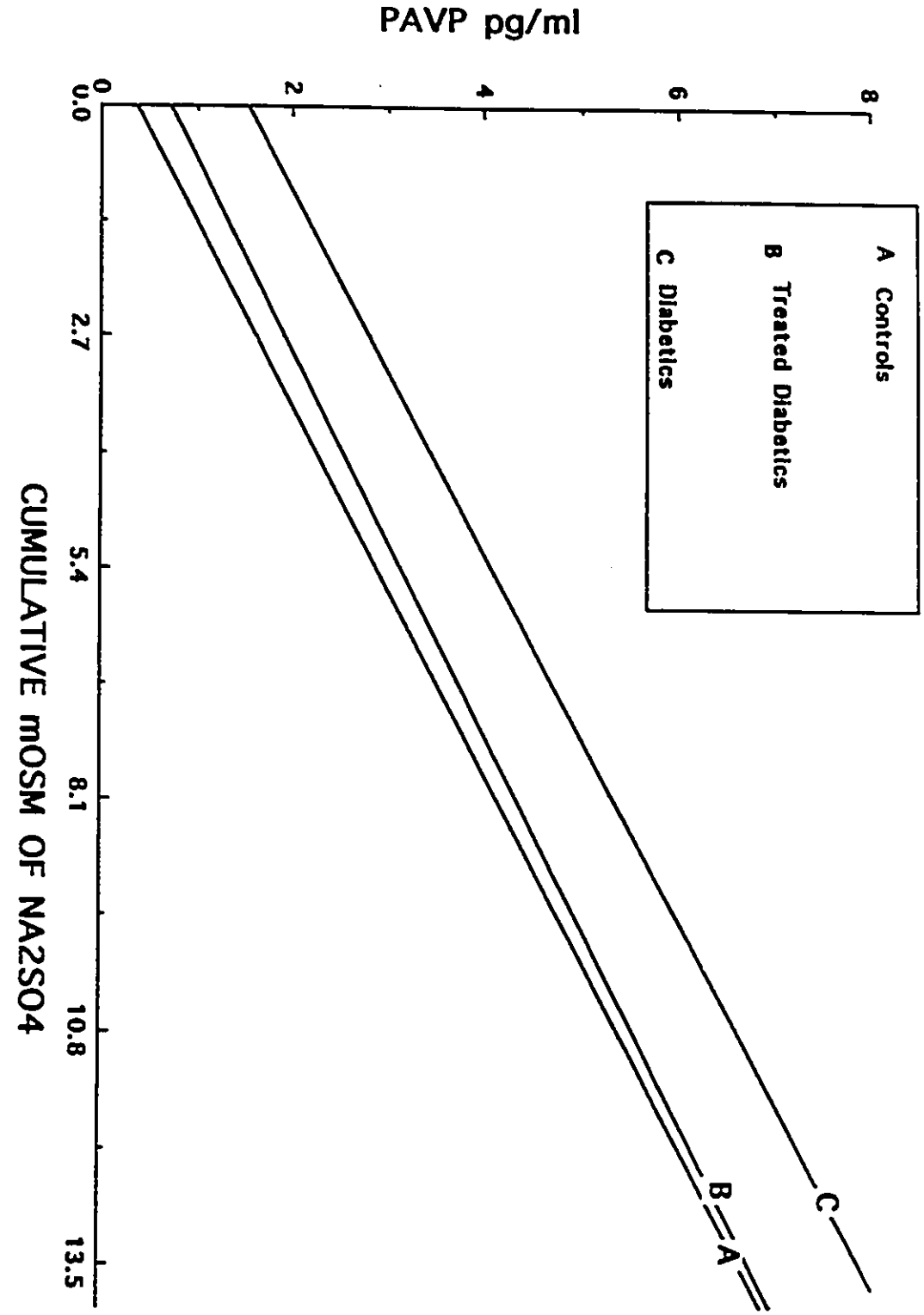

PAVP pg/ml
0
2
4
6
8
A Controls
B Treated Diabetics
C Diabetics
C
B
A
0.0
2.7
5.4
8.1
10.8
13.5
CUMULATIVE mOSM OF NA2SO4

Figure 7. Increase in cumulative water intake in 4 dogs, during a hypertonic sodium sulfate infusion, with continuous access to water, in the control, insulin-treated diabetic and diabetic condition. Results are based on 2 tests per animal and are expressed as the mean ± SEM. Cumulative water intake increased significantly over the time of the infusion in the three conditions ($p < 0.01$). *$p < 0.05$ compared to insulin-treated diabetic.

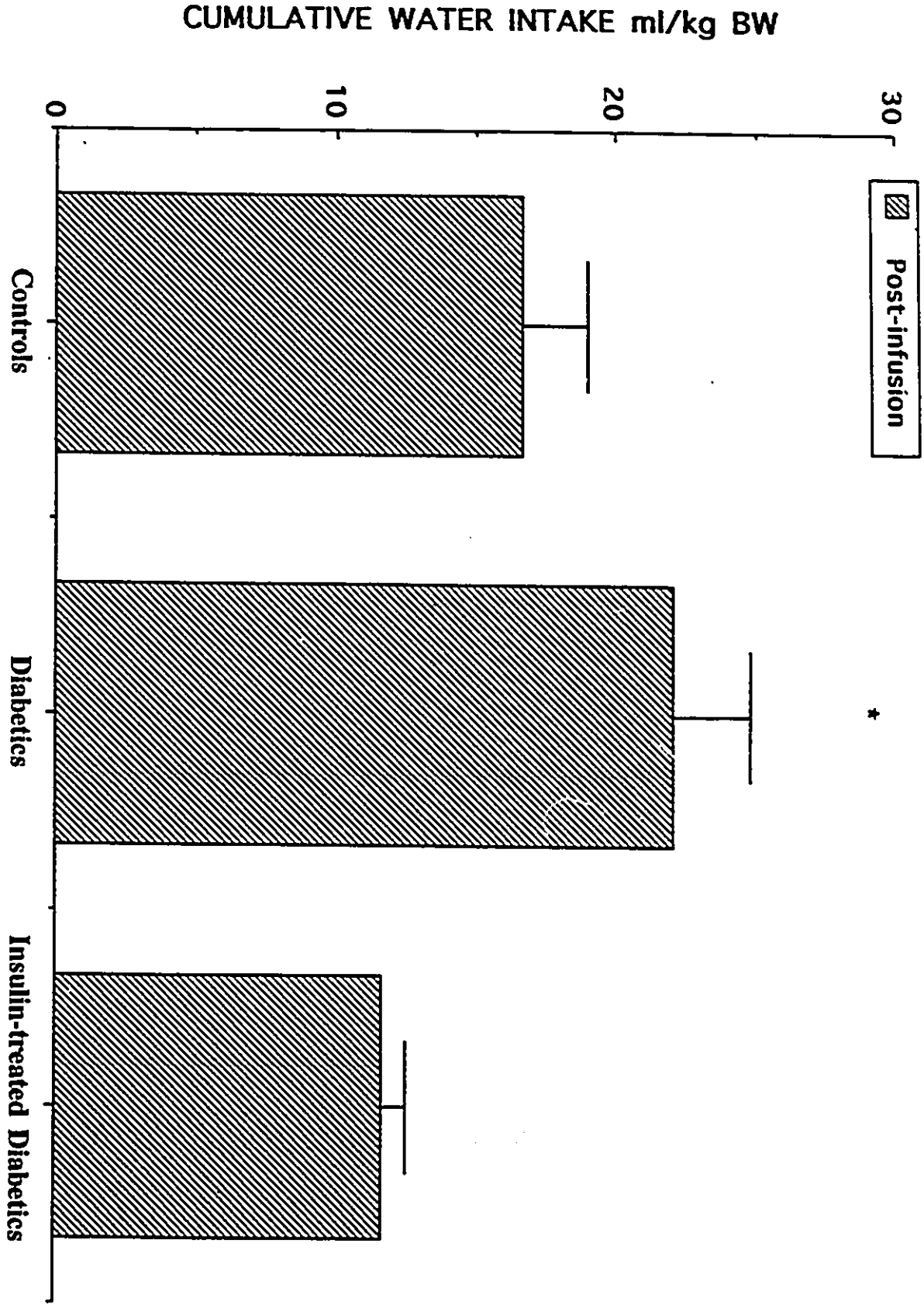

CUMULATIVE WATER INTAKE ml/kg BW
0
10
20
30
Post-infusion
Controls
Diabetics
Insulin-treated Diabetics
*

Figure 8. Relationship of cumulative water intake to estimated plasma osmolality, in 4 dogs during a hypertonic sodium sulfate infusion with continuous access to water in the control, insulin-treated diabetic and diabetic condition. Estimated osmolality was defined as 2[Na]. In the diabetics, cumulative water intake was also correlated with 2[Na] + [Glu] and with 2[Na] corrected for the presence of glucose. [Na] was increased 1.6 mEq for every 5.5 mmol glucose. The osmotic threshold in the diabetic state was significantly higher when glucose was considered as an effective osmol and lower when not. There was no difference from the normal controls and insulin-treated diabetics when [Na] was corrected for the presence of glucose. There were no significant differences among the slopes. For more details on the regression analysis see table 4.

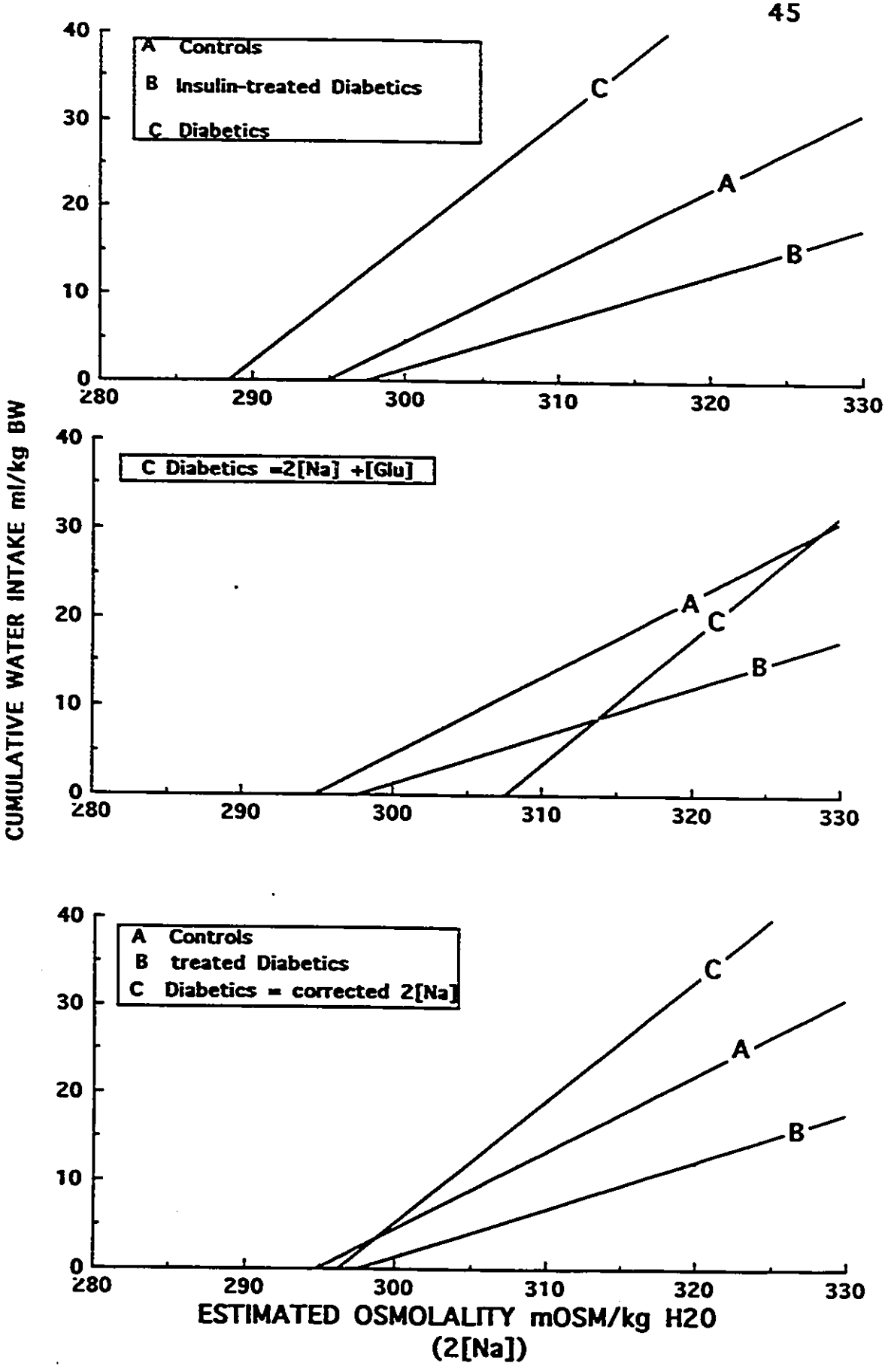

A Controls
B Insulin-treated Diabetics
C Diabetics
C Diabetics =2[Na] +[Glu]
A Controls
B treated Diabetics
C Diabetics = corrected 2[Na]
CUMULATIVE WATER INTAKE ml/kg BW
ESTIMATED OSMOLALITY mOSM/kg H20
(2[Na])
0
10
20
30
40
280
290
300
310
320
330

TABLE 4. Relationship of cumulative water intake to estimated plasma osmolality[1] during individual hypertonic sodium sulfate infusions in the 3 metabolic conditions.

	r±SE	Slope±SE	Threshold±SE
Control (n=8)	0.87±0.023	0.9±0.12	295±0.8
Insulin-treated Diabetic (n=8)	0.81±0.043	0.5±0.05	298±1.1
Diabetics (n=8)			
-glucose	0.86±0.050	1.4±0.42	286±1.3*
+glucose	0.86±0.050	1.4±0.39	306±1.0+
corrected 2[Na][2]	0.86±0.050	1.4±0.40	295±1.0

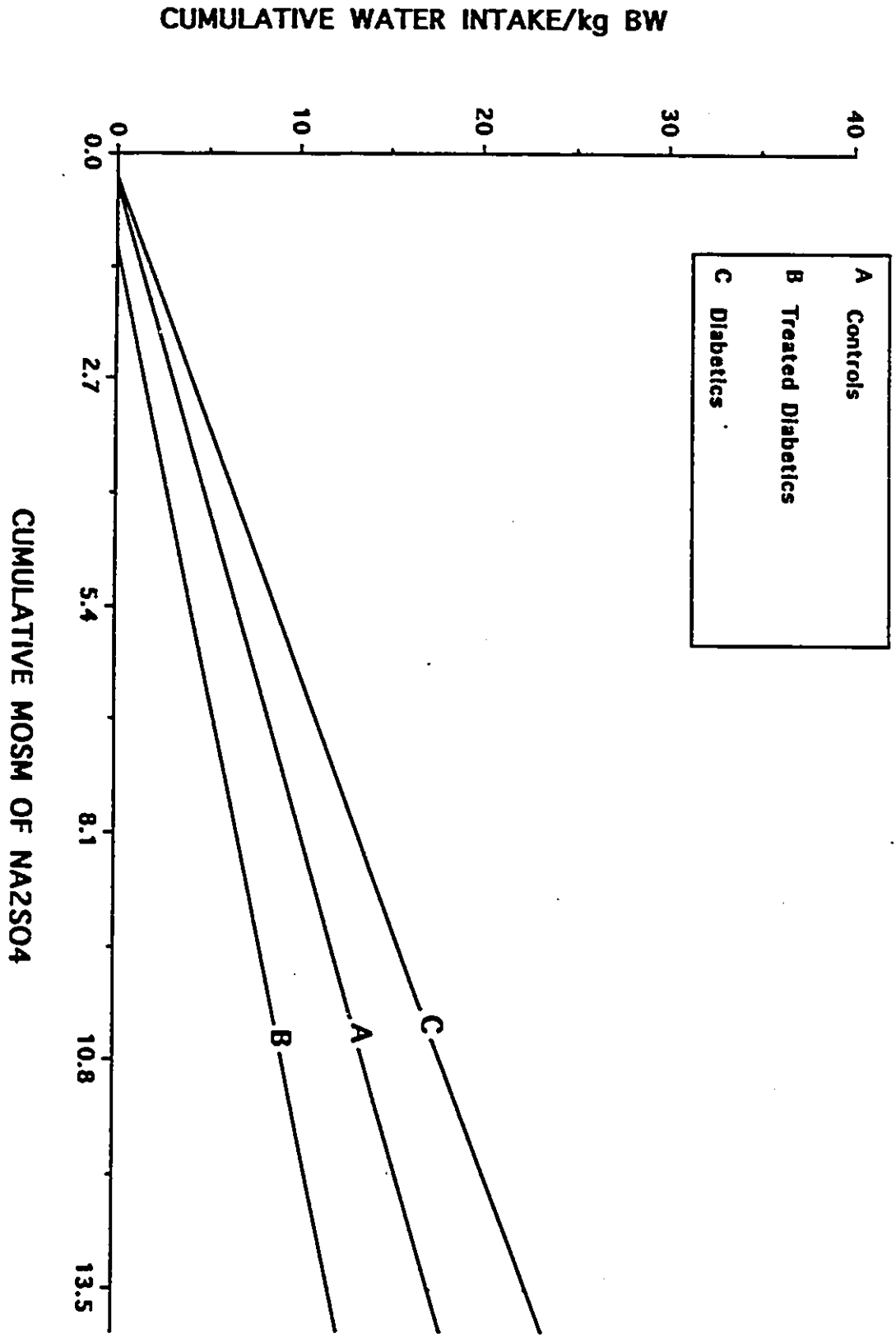
* Designates significant ($p < 0.01$) difference from controls and insulin-treated diabetics

[1] Estimated osmolality is defined as 2[Na]

[2] 2[Na] corrected for presence of glucose; [Na] was increased 1.6 mEq/L for every 5 mmol glucose

n= number of tests

Figure 9. Relationship of cumulative water intake to the amount of Na_2SO_4 administered per minute (1500 mOsm Na_2SO_4 at 0.09 ml/kg bw/min, i.v.) in the control, insulin-treated diabetic and diabetic condition. There were no significant differences in the slopes among the three groups. For more details on the regression analysis see table 3.


CUMULATIVE WATER INTAKE/kg BW
0
10
20
30
40
0.0
2.7
5.4
8.1
10.8
13.5
CUMULATIVE MOSM OF NA2SO4
A Controls
B Treated Diabetics
C Diabetics
A
B
C

5.0 Discussion

Uncontrolled diabetes mellitus is characterized by thirst, polydipsia, polyuria and elevated plasma osmolality. Therefore we addressed the following question in the thesis:

Is the osmotic drive for vasopressin release and thirst altered in the diabetic state ?

In order to determine whether there are any changes in the osmoregulation of vasopressin release in the diabetic state, dogs were osmotically stimulated with hypertonic sodium sulfate infusions in the control, insulin-treated diabetic and diabetic state. In another series of experiments, in order to assess the drinking behaviour in these three metabolic conditions, the dogs had free access to water during the hypertonic infusions. The major findings may be summarized as follows:

1. Forty eight hours of insulin depletion does not produce a change in the basal plasma vasopressin levels, even though there was a significant increase in plasma osmolality.

2. Forty eight hours of insulin depletion does not alter the sensitivity of the osmoreceptors controlling vasopressin release and thirst.

3. Regarding the effect on the osmotic threshold, the conclusion depends on the assumption mode concerning glucose permeability. If glucose is considered an osmotically effective solute in the diabetic state, there is an upward resetting of the osmostat and a downward resetting of the osmostat when glucose is not included in the effective osmolality.

4. The osmotic threshold for thirst, judged by the effective plasma osmolality at the time of the first drink, was significantly higher in the

diabetic state when glucose was considered osmotically effective and significantly lower when glucose was not taken into account.

Contrary to the findings of several other previous studies, insulin withdrawal did not raise the vasopressin levels in our animals. Although, as expected, it resulted in an increase in plasma osmolality and plasma potassium (Defronzo et al., 1978). The increase in potassium is due to efflux from cells (Defronzo et al., 1978). The increase in plasma osmolality was due largely to an increase in plasma glucose. This is reflected in our study by the very close linear relationship between PNa and Posm of the individual regression lines (mean of correlation coefficients equals 0.99) of the hyperglycemic group when Posm is corrected for the excess glucose.

Zerbe et al. (1979) previously demonstrated that insulin-dependent diabetic patients with glucose measurements greater than 17 mmol/L had markedly increased basal plasma vasopressin levels. Severe hypovolemia, nausea and vomiting could account, at least partly, for the hypervasopressinemia. Plasma vasopressin declined with insulin treatment, but remained inappropriately high in relation to the effective osmolality (defined as 2[Na] in that study). Hypovolemia was not present in our hyperglycemic group (glucose levels >17 mmol/L), as reflected by normal PRA and hematocrit values and might explain the discrepancy between the two studies. Although, more recent studies by Zerbe et al., (1985) also showed higher vasopressin levels in well controlled diabetic patients with no sign of severe hemodynamic or metabolic disturbances. A gradual increase in vasopressin levels was observed in patients during a 5 hour interruption of their intravenous insulin infusion (Vokes et al.,

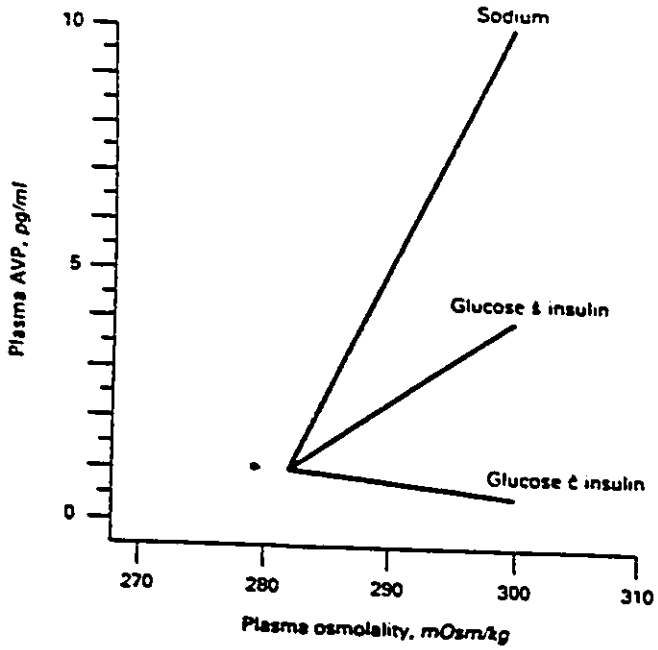
1987). The increase in vasopressin induced by insulin depletion was not due to loss of osmoregulation in these patients, since a hypertonic saline solution produced a progressive increase in vasopressin accompanied by an increase in plasma osmolality. However, there was an increase in sensitivity of the osmoreceptors as represented by a higher slope of the regression line relating vasopressin and plasma osmolality.

In the present study the sensitivity of the osmoregulatory system was not changed in the diabetic state. A more recent study in diabetic patients by Thompson et al., (1989) showed results in total agreement with our data in dogs. The patients underwent a hypertonic saline infusion in either the euglycemic (4-5 mmol/L) or hyperglycemic (10-12 mmol) range (the glucose levels being maintained in these ranges by a variable rate of intravenous insulin infusion). Basal vasopressin levels were similar in the two different states. Also linear regression analysis revealed no change in the sensitivity of the osmoreceptors in the hyperglycemic group.

4.1 Mechanisms for an altered threshold for vasopressin release in uncontrolled diabetes mellitus

The osmoreceptors controlling arginine vasopressin release are sensitive to changes in the tonicity or effective osmolality of the extracellular compartment. The effective osmolality is a measure of the the concentration of solute molecules which because of their impermeability can cause water movement across the cell membrane. It has been shown for many years that vasopressin secretion is sensitive to changes in sodium and its anions but not to elevations in urea or glucose

in normal subjects. Urea and glucose enter the osmoreceptors too rapidly to establish an osmotic gradient. The principal determinants of the plasma osmolality are sodium and its anions, glucose and urea. Glucose does not stimulate thirst or vasopressin secretion in healthy humans (Zerbe and Robertson, 1983), dogs (Thrasher et al. 1982) or, in insulin-treated diabetic patients (Zerbe et al., 1985), when plasma osmolality is increased by the infusion of glucose. Therefore, it is a standard procedure to relate plasma vasopressin with the effective plasma osmolality, which is defined as: plasma osmality minus the [glucose] and [urea] in mM. However recent studies have demonstrated that the solute specificity of the osmoregulatory system is subject to change. Vokes et al.(1989) have shown that the stimulatory potency of plasma glucose varies markedly depending on the availibility of insulin. In their patients five hours after intravenous insulin was discontinued a 2 hour infusion of hypertonic dextrose raised the level of plasma AVP, despite a decrease in plasma sodium. When insulin was given the rise in plasma osmolality produced by the glucose was associated with a fall in vasopressin. However glucose remains considerably less potent than sodium in stimulating the osmoreceptors. This is clear from the slope of the regression lines relating plasma osmolality with sodium and glucose in the figure below taken from the review of Robertson,1987.



These findings suggest that the glucose permeability of the osmoreceptors in the insulin-deficient state is reduced to the extent that a transcellular osmotic gradient develops which causes dehydration and stimulates vasopressin secretion. There are other data that support this hypothesis. It has been known for many years that the uptake of gold thioglucose by the hypothalamus is insulin dependent (Liebelt and Perry, 1967). More recently it has been shown that plasma insulin binds avidly to several circumventricular organs (Van Houten et al., 1979), at least one of which probably contains the osmoreceptor cell population (Thrasher et al., 1982). Insulin also stimulates glucose uptake by other hypothalamic neurons (Oomura and Kita, 1981) and exerts considerable influence on the hypothalamic control of appetite (Woods and Porte,1983). The circumventricular organs lack an effective blood brain

barrier, leaving the osmoreceptors exposed to circulating insulin. Thus in the absence of appropriate circulating insulin levels, an osmotic gradient due to elevated blood glucose concentrations then stimulates vasopressin release. Earlier studies by Zerbe et al., (1985) were not in agreement with the findings of Vokes et al.,(1987). When the usual morning injection of insulin was delayed and plasma glucose concentration was allowed to rise above 10 mmol/L a hypertonic dextrose infusion in these patients did not raise the vasopressin levels. The discrepancy between the results of the studies of Zerbe and Vokes could be due to the difference in the severity of insulin deficiency of the diabetic patients.

Judging from their basal plasma glucose, the diabetics in the Zerbe study were only slightly insulin deficient at the time of the experiment. Thus the possibility that transport of glucose into the osmoreceptor is insulin dependent is not necessarily excluded. Insulin levels were not measured in our study, though again judging by the basal glucose levels which were similar to levels of Vokes' diabetic patients at the start of the hypertonic dextrose infusion, the hyperglycemic dogs were severely insulin deficient. This raises the question about the osmotic contribution of glucose to the plasma osmolality in the insulin-deficient diabetic state. Therefore in the present study, AVP was examined as a function of different expressions of plasma osmolality. When plasma AVP was correlated with 2 [Na], its normal determinant, a low Posm setpoint (as represented by the value Posm when PAVP is 0.5 pg/ml) of 280 mmol/l was observed in the diabetic state as compared to the values of 293 mmol/L in the control and insulin-treated diabetic state. Thus, during osmotic stimulation, higher plasma vasopressin levels were observed in the

hyperglycemic animals at Posm levels comparable to those of the control and insulin-treated diabetic animals.

Volume contraction could be responsible for the observed lowered osmotic threshold (Robertson,1987), but there was no change in the plasma renin activity or hematocrit in the diabetic state, nor did the animals suffer from any weight loss.

If glucose is osmotically active, stimulation by hyperglycemia could account for the altered relationship between the plasma vasopressin and effective estimated plasma osmolality. But when the osmotic contribution of glucose was included in the formula of effective estimated Posm in the diabetic animals and AVP was related with 2 [Na] +[Glu], an upward shift of the treshold to 300 mmol/l was observed. This could indicate that glucose is not as potent as sodium in stimulating the osmoreceptors. Considering glucose to be totally impermeable would lead to erroneous results.

Considerable volume expansion (Robertson, 1987) could account for this upward resetting of the osmostat, but as mentioned above the animals did not have a decrease in plasma renin activity or hematocrit, nor was their any weight gain. However in the latter formula the glucose was considered to be totally impermeable and according to the findings of Vokes and Robertson (see above) the stimulatory potency of glucose was less than that of sodium.

Another possibility is that glucose merely substitutes for the Na in the osmoregulation of AVP. Hyperglycemia osmotically induces shifts of water out of all insulin-sensitive targets and lowers the plasma sodium of an order of 1.6 meq/L Na per 5.5 mmol excess glucose (Katz, 1973). When PAVP is related with 2[Na] corrected for the presence of glucose in the

diabetic state, a threshold of 289 mmol/L is obtained, which approaches the threshold of 293mmol/L of the control and insulin-treated diabetic state. However one would have to know the degree of impermeability of glucose into the osmoreceptor cells to get a correct picture of the osmoregulation of vasopressin in the diabetic state. In a very recent study Durr et al.(1990) introduced a factor σ to account for the relative permeability of glucose in diabetic ketoacidotic patients. The recovery of ketoacidotic patients subjected to a fluid therapy and an insulin infusion, was observed for 12 hours. PNa , HCO_3^-, glucose and AVP were measured at 0, 6 and 12 hours. The investigator reasoned that decreasing permeability to glucose ($\sigma \rightarrow 1$) results in cell fasting and ketosis ($HCO_3^- \rightarrow 0$). Conversely improved permeability ($\sigma \rightarrow 0$) corrects the metabolic acidosis ($HCO_3^- \rightarrow 26$). An empirical formula for the relative osmoreceptor cell permeability σ to glucose was derived from plasma HCO_3^- as $\sigma = [26 - HCO_3^-] / 26$. Durr obtained the best fit when PAVP was related with $2[Na] + \sigma[Glu]$ and Na and Glu were considered as two independent variables. The osmotic threshold for vasopressin calculated from this equation was in the normal range. He suggested that the previous findings by Zerbe et al, (1985), Vokes et al. (1987) and Thompson et al. (1989) of a lowered Na threshold and an increased osmotic threshold in the diabetic state obtained by linear regression both represent an analytical artefact. However Durr did not have a stable starting point for his experiment, nor did he test the functioning of the osmoregulatory system with osmotic stimulation. He merely observed the improvement of hyperglycemia, hyponatremia and hypervasopressinemia with insulin treatment. In the studies by Zerbe, Vokes and Thompson and the present study, [glucose] did not vary during the osmotic stimulation. In the

present study no differences were found in the bicarbonate levels in the three metabolic conditions. According to Durr's reasoning this would be an indication of total permeability for glucose. In that case there would be a true downward resetting of the osmostat in the diabetic state. One could argue that a lowering of the osmostat is a physiological advantage to the osmoregulatory system in the diabetic state. Hyperglycemia lowers PNa by hydroosmotic effects and PNa falls in proportion to the elevation in blood glucose concentration. Lowering of the PNa concentration would usually inhibit vasopressin secretion, thus preventing the osmoregulatory response to the diuresis associated with hyperglycemia. The lowered threshold for vasopressin secretion enables osmoregulation to occur at lower PNa concentrations and presumably limit dehydration associated with poorly controlled diabetes mellitus.

However, the assumption by Durr of a direct relationship of glucose impermeability and ketosis, the severity of which is deducted from the bicarbonate levels, is debatable. In the study by Vokes and Robertson all subjects exhibited ketonuria and ketonemia after 5 hour insulin depletion, but the bicarbonate levels did not change. Also vasopressin interferes with the direct relationship; it decreases the rate of ketone production in the liver (Hems,1979). The vasopressin levels were very high in the ketoacidotic patients studied by Durr.

It would have been appropiate to subject our diabetic animals to a hypertomic dextrose infusion. However, the rate of penetration of glucose into the osmoreceptor and hence the potency of glucose as an osmotic stimulus would have remained uncertain. In vitro studies on the activity of hypothalamic structures bathing in a hypertonic glucose medium would be necessary.

4.2 Mechanisms for an altered thirst threshold in uncontrolled diabetes mellitus

The osmoregulation of thirst in the diabetic animals follows a similar pattern as the osmoregulation of vasopressin release. When cumulative water intake was correlated with the effective estimated osmolality, we observed a resetting of the osmotic thirst threshold to the left or the right for the diabetic state, depending on whether glucose was included in the effective estimated osmolality.

Again, no hypovolemia or hypervolemia was present in the hyperglycemic state, as is reflected by normal PRA and hematocrit values and a constant weight. Moreover, a decrease in blood volume of at least 8% (Robertson,1987) is necessary to stimulate thirst. Prolonged polyuria in excess of water intake is required to produce such deficits in blood volume. Although no statistical significant diffference was reached, there was indication of an increased sensitivity of the thirst osmoreceptors in the diabetic state, as shown by the slopes of the relationship between cumulative water intake and effective estimated osmolality and the amount of sodium sulfate administered per minute. This is strenghtened by the fact that the amount of water ingested at the end of the osmotic stimulation by the hyperglycemic animals was significantly greater than the ingested volume in the insulin-treated diabetic animals. This was only seen after 80 minutes of the hypertonic sodium sulfate infusion. The greater ingested volume in the hyperglycemic animals did not reach a significant difference when compared with the intact controls.

Thompson et al. (1988) were the first to establish a clear relationship between thirst and plasma osmolality in insulin-dependent diabetic patients. The quantitive measurements of thirst taken when stimulated by a hypertonic saline infusion, did not differ between the diabetic patients and healthy controls. A hypertonic dextrose infusion did not increase thirst in the diabetic patients. However normoglycemia was maintained during the course of the experiment, thus the latter observation does not exclude the possibility that glucose uptake by the osmoreceptors is insulin dependent and therefore becomes an effective osmotic stimulus during insulin deficiency. Later studies by Thompson et al., (1989) which consisted of hypertonic saline infusions in diabetic patients in the euglycemic state as well as in the hyperglycemic state, were mostly in agreement with our results. As was the case for vasopressin release (see above), linear regression analysis defined also a lowered plasma sodium threshold for thirst appreciation, but again no change in the sensitivity of the osmoreceptors. Our study differed from Thompsons' in that the hyperglycemic diabetic animals had ingested more water at the end of the infusion than when they were euglycemic. In Thompsons' study the increase in thirst appreciation was similar in the two studies and the patients drank similar quantities of water after the 2 hour hypertonic saline infusion despite a higher Posm in the hyperglycemic study. This discrepancy could be explained by a more severe insulin deficiency in our study, judging by the higher glucose levels, if indeed glucose becomes an effective osmotic stimulus. Another possible cause could be autonomic neuropathy, in which case the swallowing-mediated neuroendocrine reflex which abolishes thirst, might be defective.

5.0 Conclusions

It is certain from our study that the sensitivity of the osmoreceptors controlling vasopressin release to osmotic stimulation is not altered in the diabetic state. There is indication of an increased sensitivity of osmotic stimulation of thirst in the diabetic state, but the difference between the slopes of the regression lines did not reach significance. Whether there is a true resetting of the osmostat for vasopressin and thirst depends on the degree of the impermeability of glucose into the osmoreceptor. This can not be inferred from our study.

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