BODY FLUID REGULATION DURING WATER DEPRIVATION: ROLE OF SOLUTE BALANCE IN OSMOREGULATION

A Thesis Submitted to the College

of Graduate Studies and Research

in Partial Fulfillment of the Requirements

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in the Department of Physiology

University of Saskatchewan

Saskatoon

by

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by

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Body fluid regulation during water deprivation: role of solute balance in osmoregulation.

Volume and composition of the body fluid compartments are kept within narrow limits. This is done by changes in intake and excretion of water and salt. I found that changes in food intake and salt excretion play an important role in body fluid regulation during water deprivation and investigated the mechanisms of these changes.

Urine volume changed little in rats deprived of water for 10 hours. The main osmoregulatory response during water deprivation was a loss of solute from the body. Food intake fell by 43%, which reduced the load of solute to the tissues and allowed absorption of water already in the gut. Water deprived rats also excreted more sodium, potassium and chloride. Thus, although rats lost 8% of their body water during 10 hours of water deprivation, plasma tonicity rose by only 2%.

On rehydration, when no food was present rats rapidly reduced excretion of sodium, potassium and chloride. Rats allowed water and food drank more, and excreted more solute. These changes contribute to restoration of the body fluid compartments.

Brain infusions caused changes in electrolyte excretion that were similar to those seen during water deprivation and rehydration. Electrolyte excretion increased during infusion (1 mL/min for 2 h) of cerebrospinal fluid (CSF) with 300 mM NaCl in the lateral ventricle. Infusion of low-sodium CSF reduced electrolyte excretion in water deprived rats, but had little effect in rats that were not water deprived. The time course of the changes after rehydration and brain infusions was the same, and the same solutes were involved. This suggests the

mechanisms are similar. Low-sodium CSF made isotonic with mannitol had the same effect as hypotonic low-sodium CSF. The sensor probably monitors brain interstitial fluid, not CSF.

Food intake fell within 1 hour of water deprivation. Meals were smaller, but meal frequency did not change. Dehydration anorexia is caused by a sensor located in the gut, portal circulation or liver, because infusion of water in the stomach, jejunum or cecum (10 mL/6 h) restored food intake in rats not allowed to drink, but intravenous infusions had no effect. Intravenous infusions did not alter urinary water loss and did not alter food intake in rats allowed to drink.

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ABSTRACT

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LIST OF ABBREVIATIONS

ADH	antidiuretic hormone
Ang II	angiotensin II
ANP	atrial natriuretic peptide
CSF	cerebrospinal fluid
ECF	extracellular fluid
GFR	glomerular filtration rate
ICF	intracellular fluid
i.c.v.	intracereboventricular
i.d.	inner diameter
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
$[Na^+]$	sodium concentration
o.d.	outer diameter
OVLT	organum vasculosum of the lamina terminalis
RBF	renal blood flow
s.c.	subcutaneous

1 INTRODUCTION: BODY FLUID REGULATION

Volume and composition of the body fluid compartments are usually kept within narrow limits. Many mechanisms are involved, and although much is known about these mechanisms, much is left to be learned. A good test of our understanding of body fluid regulation is to see if we can explain how an organism deals with commonly encountered challenges to body fluid homeostasis. We could test how an organism deals with a salty meal for example, or with a period of water deprivation. In this thesis I used short periods of water deprivation to investigate the importance of several mechanisms involved in body fluid regulation.

During water deprivation, water is lost from the body, and this causes changes in several body water compartments. Changes in volume of water in the cells, and changes in blood volume stimulate many mechanisms that help restore the size of these compartments. These mechanisms are the subject of this introduction. It has long been known that changes in thirst and water excretion play a major role in regulation of intracellular volume and blood volume. Changes in sodium excretion are generally recognized to be important in regulation of blood volume, and changes in salt intake play an obvious role under some circumstances as well. The surprise is that changes in salt intake and excretion play a role not only in blood volume regulation, but in osmoregulation as well. Even more surprising is that they are by far the most important osmoregulatory mechanisms during water deprivation, at least in the rat. About the

mechanisms much remains unknown. This area - how changes in solute intake and excretion contribute to osmoregulation - is the topic of my research.

I will start this introduction with very basic information about body fluid compartments and how water flows between them. Then I will review in detail mechanisms that regulate exchange of water and solute between the body and its environment. These include thirst and ADH release, as well as changes in salt intake and excretion. Because many of my experiments investigate the sensors involved in such responses I will emphasize the signals that are sensed and the receptors that monitor them.

1.1 Fluid compartments

Most of the body consists of water. In rats like those used in this study about 65% of the body weight is water (Altman and Dittmer 1973). About 39% of the body weight (60% of the body water) is intracellular fluid (ICF). About 24% of the body weight (Barratt and Walser 1969) is extracellular fluid (ECF). Most of the extracellular fluid is found between cells: about 20% of the body is interstitial fluid. Plasma volume in rats is about 4% of the body weight (Kutscher 1971). Gut fluid, ocular fluid, CSF, and synovial and pleural fluids are transcellular fluids: there is a barrier between them and the rest of the ECF. Especially gut water is important. The amount varies with the amount of food in the gastro-intestinal tract (Lepkovski et al. 1957). For example, in rats used for the experiments of section 3.2 gut water was about 4% of the body weight, but it may be less at the end of the light period, which is the start of the rat's active period.

Large changes in intracellular volume are harmful, especially to the brain (Arieff and Guisado 1976), which can not expand because it is enclosed by the skull. Increased cell volume can cause brain edema. Reductions in cell volume can cause tearing of cerebral blood vessels and damage myelin (Verbalis and Martinez 1991). Smaller changes in cell volume can induce confusion, paralysis, coma and seizures (Andrew et al. 1989). The usual cause of such changes in cell volume is a rapid change in blood tonicity. If, however, changes in blood tonicity are slow to develop, cells will alter their solute content to reduce changes in cell size (see Macknight et al. 1992 for review). Therefore symptoms can be absent when hyponatremia develops slowly, even if plasma [Na^+] falls below 100 mM. Large reductions in extracellular volume compromise blood circulation and tissue perfusion, and can lead to shock and death.

Therefore it is not surprising that there are mechanisms that guard the size and composition of the body fluid compartments. To understand how volume of the fluid compartments is regulated, we need to consider not only exchange of water between the body and the environment, but also how water in the body moves between compartments.

1.2 Movement of fluid between compartments

Movement of fluid between compartments depends on the characteristics of the barriers between the compartments. The barrier between intracellular and extracellular fluid is the cell membrane. It is permeable to water, but its permeability to most solutes is low. Net water transport between intracellular and interstitial compartments occurs when the chemical potential of water outside the cell differs from that inside. Chemical potential for water depends on hydrostatic pressure and on water activity. Water activity in turn depends on solute concentration (see Dawson 1992), so that water will flow when

total solute concentration or osmolality inside the cell differs from that outside. For example, injection of a concentrated NaCl solution in a vein will increase interstitial sodium concentration, which causes water to move from the cell into the interstitial fluid until the total solute concentration inside the cell equals that outside. Eating salty food has the same effect: it increases osmolality of the interstitial fluid, which causes water to move out of the cells. In contrast, intravenous infusions of urea will not reduce cell volume because urea crosses the cell membrane, increasing solute concentration within the cell as well as outside.

The barrier between interstitial fluid and plasma is the vascular endothelium of the capillaries. It is permeable to water and small solutes but its permeability to large proteins such as albumin is low. Water activity in capillaries is lower than in interstitial fluid because protein concentration in the interstitial fluid is lower. Net water transport through the vascular epithelium depends on differences in protein osmotic pressures and hydrostatic pressures in the capillary and in the interstitial fluid (see Guyton 1986, chapter 30, for review). At the start of the capillary, protein osmotic pressure inside the vessel is higher than outside, but blood pressure is so high that water will flow out of the vessel. Protein osmotic pressure increases and blood pressure falls toward the end of the capillary, favouring entry of water. Normally total flow of water out of the capillary is slightly larger than inward, and the difference returns to the blood through the lymphatics. Therefore, interference with blood pressure or lymphatic drainage, or changes in protein concentrations in blood or interstitial compartment will alter interstitial fluid volume. For example, reduced blood pressure in capillaries favours entry of water into the blood from the interstitial compartment. Increased vascular permeability to

proteins will increase interstitial fluid volume, because it increases protein osmotic pressure in the interstitial fluid and reduces protein osmotic pressure in the vasculature.

The gut is a separate compartment as well. Because there is so much water in the gut (in rats up to 25 mL, see section 4) and because the amount varies so much (Kutscher 1966), gut fluid deserves extra attention. The barrier between gut contents and interstitial fluid is the tight junctions that join the cells of the intestinal epithelium (see Madara 1991 for review). The epithelium is rather leaky, so most transport is through the tight junctions rather than through the cell. Still, permeability of the gastrointestinal tract to most solutes is fairly low. Because permeability of the gut epithelium to water is high, tonicity in the intestine is similar to that of the rest of the body. (Tonicity of the stomach content and first part of the duodenum can differ markedly from that of the rest of the body, especially after a meal.) Unlike other compartments, major changes in the amount of solute in the gut are common. Solute is ingested with food, and is absorbed from the gut between meals. These changes are accompanied by changes in gut water. After a meal water rapidly enters the gut from the extracellular compartment (Lepkovsky et al. 1957), and this can reduce blood volume (Blair-West and Brook, 1969). After a drink, water will move rapidly from the gut to the rest of the body, reducing plasma tonicity (Yang and Houpt 1994).

The brain is a relatively small compartment, even more so in rats than in man. Fluid shifts from the brain have little direct effect on water content in the rest of the body. However, as will be explained later in this introduction, sensors located in the brain are important in osmoregulatory responses. Therefore it is useful to review how tonicity in the rest of the body affects the brain. The brain, except for the circumventricular organs, has a blood-brain barrier (see Bradbury 1979, Fenstermacher 1984 for reviews).

It consists of the tight junctions of the endothelial cells that line the blood vessels. The barrier is permeable to water and fat-soluble solutes, but its permeability to most other solutes is very low. It does however have carriers that facilitate transport of glucose, amino acids, and several other substances. Brain ECF differs a bit from that of ECF of the rest of the body. It consists of cerebrospinal fluid (CSF), the fluid in the brain ventricles, that is secreted by the choroid plexus, and of interstitial fluid, part of which may be secreted by brain tissue (see Bradbury 1979). Interstitial fluid and CSF mix; the cell layer separating them is permeable to large molecules (Bradbury 1979). We can selectively dehydrate the brain, but not the rest of the body by infusing urea or glycerol in the circulation, because these solutes enter the cell, but do not easily enter the brain. Infusion of hypertonic fluid into the brain ventricles will dehydrate brain cells as well: it causes movement of water from brain cells into CSF. Such infusions are frequently used to investigate the location and characteristics of the brain sensors involved in body fluid regulation.

1.3 Water balance: ins and outs of water

In the remainder of this introduction the exchange of water and salt between the body and its environment is reviewed.

Water is gained by drinking, by eating (most foods contain some water), and by oxidation of food. Water is lost from the body as urine, with feces, and from the lungs and the skin. (Rat feces contain about 70% water, see Fyhn 1979). Rats do not sweat, but will cover their fur with saliva to loose heat when environmental temperature exceeds 32°C (Hainesworth 1967).

Turnover of water is relatively rapid, especially in small animals such as rats. Therefore changes in water intake and loss can have rapid and large effects on the amount of water in the body. For example, a rat that weighs 300 gram would contain about 200 mL water. Normal water intake is about 30 mL/day (Fyhn 1979). If water were withheld for 2 days, and water losses would not change, these rats would loose about 30% of their body water. If these rats would not adjust salt intake and salt excretion, solute concentration in the rat's body would increase by 30%. Such an increase would be lethal. Clearly the mechanisms that help keep water volume and solute concentration in the body constant are essential for life.

Water intake and excretion are regulated by changes in thirst and ADH secretion. The mechanisms that control drinking and water excretion help guard cell volume, blood volume and blood pressure. These mechanisms have been reviewed recently by Fitzsimons (1992), Robertson and Berl (1991), Ramsay and Thrasher (1990), and Bourque et al. (1994).

1.3.1 Thirst and ADH in regulation of cell volume

Extracellular tonicity is closely guarded: increases of 1-2% will stimulate thirst (Fitzsimons 1963) and the secretion of antidiuretic hormone (ADH) (Zerbe and Robertson 1983). The receptors involved in these responses are thought to be cells that are located in the hypothalamus, but outside the blood-brain barrier, and that are sensitive to changes in their volume (Fitzsimons 1992). This widely held theory agrees with many experimental results. Because there is little controversy in this area, I will only show how this hypothesis fits with a few crucial findings rather than review the field exhaustively.

Intravenous infusions of hypertonic saline that reduce cell volume cause drinking and secretion of antidiuretic hormone (ADH). For example, Gilman (1937) found that intravenous infusion of concentrated NaCl caused drinking, while infusion of equally hyperosmotic solutions of urea had little effect. Gilman noticed that infusion of hypertonic saline diluted the blood with water from the cells, but urea infusions did not dilute the blood, and he concluded that thirst depends on the water content of the cells. The receptors are located in the brain, because hypertonic intracarotid infusions that do not alter systemic osmolality cause drinking (Wood et al. 1977). They lie outside the blood-brain barrier because intravenous infusions with urea or glucose have little or no effect on thirst, even though such infusions increase CSF [Na^+] and osmolality (McKinley et al. 1978, Wood et al. 1977). However, the fact that urea infusions often cause some drinking (Holmes and Gregersen 1950, Olsson 1972, McKinley et al. 1978) and ADH secretion (Eriksson et al. 1971, Zerbe and Robertson 1983) indicates that sensors behind the blood-brain barrier may also contribute to normal drinking and ADH secretion.

A few brain areas lack a blood brain barrier, among them the organum vasculosum of the lamina terminalis (OVLT). After intravenous infusion of hypertonic saline (Oldfield et al. 1994) and during water deprivation, the OVLT produces the FOS protein (McKinley et al. 1994), which is thought to be an indicator for activity. Many of the neurons that become active during water deprivation project to the supraoptic nucleus (McKinley et al. 1994), suggesting these may be the osmoreceptors that control the secretion of ADH.

In most brain areas, transport of glucose into the cell does not depend on insulin. Vokes et al. (1987) found that intravenous infusion of glucose stimulates ADH release

in insulin deplete diabetics, but not in insulin replete diabetics, which suggests that the osmoreceptors are in a brain area where glucose transport is insulin dependent.

Although it has been claimed that in goats sodium concentration, and not osmolality is sensed (see Anderson et al. 1984), osmoreceptors located outside the blood-brain barrier may account for drinking in goats as well. Intracarotid infusion of hypertonic urea or glycerol caused little drinking in goats (Olsson 1972). Such infusions dehydrate the brain (see Bradbury 1979), but not cells in the rest of the body, indicating the sensor lies outside the blood-brain barrier. Infusion of sodium or fructose in the carotid artery caused drinking (Olsson 1972), indicating an osmoreceptor is involved. Glucose and galactose caused no drinking (Olsson 1972). These solutes cross the cell membrane. Thus, unless goats have a very unusual blood-brain barrier, an osmoreceptor outside the blood-brain barrier can account for their drinking. Also, infusion of sucrose and fructose in the carotid artery reduced water diuresis in goats, and infusion of hypertonic urea, glucose, galactose or glycerol had little or no effect (Eriksson et al. 1971). This suggests that ADH secretion in goats depends on an osmoreceptor outside the blood-brain barrier as well. As discussed below, we need a sodium sensor to explain the changes in thirst and ADH caused by infusions of hypertonic solutes in the brain. The fact that intracarotid infusion of urea, glucose or glycerol did not stimulate thirst or alter urine flow suggests that, under normal conditions, these sodium sensors play a minor role, at most.

The receptors have not been isolated. Many hypothalamic neurons alter their firing rate when local tonicity changes (Sibbald et al. 1988, Vivas et al. 1990, Bourque et al. 1994), but it seems doubtful that all these neurons are osmoreceptors that contribute to thirst and ADH secretion under normal conditions. For example, they may not be

outside the blood-brain barrier, or not sensitive enough, sensitive to [Na^+] instead of tonicity, or they may be involved in other osmoregulatory responses, such as dehydration natriuresis. For example, magnocellular neurons in the supra-optic nucleus are sensitive to small changes in osmolality (Oliet and Bourque 1994), but are inside the blood-brain barrier. How cell volume alters neuronal firing is not known, but stretch-sensitive ion channels may play a role (see Oliet and Bourque 1994 for review). Activation of such channels reduces membrane potential. How signals from the brain receptors ultimately cause drinking is as yet not clear. New histochemical methods that detect proteins such as FOS that are expressed in active neurons could help clarify the brain pathways involved.

Jewell and Verney (1957) infused hypertonic solutions into carotid arteries and subsequently tied vessels that supplied blood to different parts of the brain. They concluded that osmoreceptors that stimulate ADH secretion are located in the hypothalamus. The receptors involved in thirst sometimes are assumed to be the same as those regulating ADH secretion. Although this idea agrees with the finding that their specificity for solutes is the same (compare Eriksson et al. 1971 and Olsson 1972, see also McKinley et al. 1978, Thrasher et al. 1980, Zerbe and Robertson 1983), direct evidence is not available, as the receptors have not been isolated.

Infusion of high- or low-sodium CSF into the brain alter thirst and ADH secretion as well. The problem with these infusions is that they often cause changes in brain tonicity that are much larger than those seen after treatments that stimulate drinking, such as eating, or water deprivation. Therefore their effects may be caused by non-specific stimulation of brain tissue. For example, McKinley et al. (1978) concluded that an osmoreceptor, not a sodium sensor, accounted for drinking in sheep after intracarotid

infusions, because infusion of sucrose caused thirst, but urea did not. Both sodium and osmoreceptors may be needed to account for the effect of brain infusions. Infusion of sucrose in the brain ventricles stimulated drinking, suggesting an osmoreceptor is involved (McKinley et al. 1978). Infusion of sodium is more effective, indicating a sodium receptor plays a role as well. In conclusion, it is possible that brain infusions exert their effect on drinking through a pathway not involved in normal drinking. As a last note, it appears that the effect of brain infusions is species dependent. Infusions with normal cerebrospinal fluid to which sucrose is added stimulate drinking in dogs (Thrasher et al. 1980) but not in goats (see Andersson et al. 1984 for review).

1.3.2 Thirst and ADH in regulation of blood volume and pressure

Changes in blood volume and blood pressure cause changes in thirst and ADH secretion that help restore the normal state. Thus, many experimental treatments that reduce blood volume will cause drinking. For example, cholera and other causes of diarrhoea, hemorrhage, dietary sodium depletion, and injections of hyperoncotic colloids in the interstitial space reduce blood volume and stimulate thirst (see Fitzsimons 1979 for review). Procedures that reduce central venous filling, such as ligation of the inferior vena cava stimulate thirst as well (see Fitzsimons 1979). A nice method to alter central venous filling is to alter the pressure on the lower part of the body, for example by immersing it in water, or changing the air pressure around it. Kass et al. (1980) found that squirrel monkeys drank less when pressure on the lower part of their body increased. Treatments that cause hypotension, such as administration of vasodilating drugs stimulate drinking as well (see Evered 1992). Increases in blood pressure can inhibit drinking.

For example, rats drink more after injections of Angiotensin II (Ang II) when the increase in blood pressure caused by such injections is prevented (Evered et al. 1988).

The mechanisms that guard blood volume and pressure are not very sensitive: reductions in blood volume or pressure smaller than 5 or 10% will not stimulate drinking (Fitzsimons 1961, Evered 1990) or alter ADH secretion (Robertson and Berl 1991). Note that drinking does not do much to increase blood volume unless sodium is taken as well, because the osmotic gradient that develops will force most of the ingested water into the cells.

The mechanisms that control blood volume are often difficult to separate experimentally from those that control blood pressure because changes in blood volume often are accompanied by changes in blood pressure. For example, caval ligation not only reduces central venous pressure, but arterial pressure as well. In addition the mechanisms that are involved in blood volume and pressure regulation overlap, at least anatomically.

The receptors that stimulate thirst when blood volume or pressure are reduced are thought to be nerve endings in the walls of blood vessels that are sensitive to stretch (Fitzsimons 1992). Receptors located in the large veins and atria, as well as arterial baroreceptors in the carotid sinus and in aortic arch are thought to contribute. Signals from these receptors travel through the vagus and glossopharyngeal nerves to the brain stem, and from there presumably to the hypothalamic areas that regulate thirst and ADH secretion. Stimulation of stretch receptors at the junction of vena cava and atrium with venous balloons reduces water intake and increases urine flow (Kaufman 1984). Distension of a junction of the pulmonary vein and the atrium has the same effects (Moore-Gillon and Fitzsimons 1982). Signals from stretch receptors are thought to

tonically inhibit thirst, because blocking vagal transmission stimulates spontaneous drinking (Fitzsimons and Moore-Gillon 1980) as well as drinking after osmotic stimulation (Sobocinska 1969).

The effect of removal of the signals from these receptors is unclear. In dogs, combined denervation of the heart, aortic arch, and carotid sinus blocks drinking to obstruction of the vena cava (Quillen et al. 1990). On the other hand, such signals may have little effect on drinking. Denervation of aortic arch and carotid sinus did not reduce the drinking caused by subcutaneous injections of hyperoncotic colloid (Rettig and Johnson 1986). Such injections cause blood volume to fall because water is drawn into the interstitial space. Signals from aortic arch and carotid sinus also were not necessary for the drinking caused by the vasodilator isoproterenol, which reduces blood pressure (Rettig and Johnson 1986).

There is a second pathway that stimulates thirst after hypotension. Hypotension stimulates the secretion of renin. The direct cause for this renin release is not clear, but the renal baroreceptor and changes in the flow of NaCl through the macula densa may play a role, as well as changes in renal nerve activity. Renin cleaves angiotensin I from angiotensinogen. Angiotensin I subsequently is converted to angiotensin II (Ang II). Circulating Ang II does not cross the blood-brain barrier, but acts on receptors in two circumventricular organs of the brain, the subfornical organ and the organum vasculosum of the lamina terminalis, to cause thirst and salt appetite (Johnson and Gross 1991, Fitzsimons 1992). Ang II infusions that cause plasma Ang II concentration to rise to about 200 fmol/mL cause drinking in rats (Mann et al. 1987). The levels that are seen after caval ligation, injection with hyperoncotic colloid, or 48 hours of water deprivation are higher (Mann et al. 1987), indicating Ang II may contribute to drinking after these

treatments. In agreement with this idea, drinking caused by injection of colloids (Mann et al. 1988) or caval obstruction (Fitzsimons and Moore-Gillon 1980) was reduced after intravenous injection of the Ang II receptor blocker, saralasin. Circulating Ang II also appears to mediate thirst after injection of the vasodilating drug diazoxide. Drinking after injection of diazoxide can be blocked completely with the converting enzyme inhibitor captopril, and Ang II infusions restore drinking (Evered 1992).

The role of Ang II in thirst after hypotension and hypovolemia however is controversial (see Evered 1992). Several treatments that cause hypovolemia, such as injection of colloids under the skin, do cause drinking in rats with no kidneys (Fitzsimons 1961, Stricker et al. 1979), indicating circulating Ang II plays little role in these types of thirst. Also, Quillen et al. (1990) blocked drinking to caval obstruction by denervating the heart and carotid sinus. This procedure did not alter the increase in plasma [Ang II] normally seen after caval obstruction, suggesting Ang II is not the cause of thirst in this experiment. However, they did not test if their treatments interfered with drinking to other stimuli. The animals may have been too sick to drink.

Hypotension and hypovolemia also stimulate release of ADH (see Robertson and Berl 1991 for review). ADH not only reduces renal water loss, it also causes vasoconstriction, which helps maintain blood pressure and tissue perfusion. In general, hypovolemic and hypotensive stimuli that cause thirst also increase the secretion of ADH, and the receptors involved in thirst and ADH release are probably the same. For example, cardiac and arterial baroreceptors mediate ADH release during hemorrhage (Quail et al. 1987). Ventricular receptors may be involved in this response, because it can be blocked by ventricular denervation, but not by denervation of the carotid sinus and aortic arch (Wang et al. 1988).

1.4 Salt balance: ins and outs of salt

Sodium and potassium determine the size of the extra- and intracellular compartments. In a sense, sodium is the backbone of the extracellular fluid. It is the main extracellular cation. Loss of sodium from the body will reduce ECF volume because usually thirst and ADH secretion keep sodium concentration of the blood constant. When sodium is retained ECF volume increases. Because sodium is such an important factor for ECF volume, it is not surprising that the mechanisms that determine sodium excretion have received much attention.

Most potassium is found within the cells, and large losses of potassium likely will reduce ICF volume. Note however that changes in potassium balance are not the only way to alter ICF volume but that other intracellular solutes also play a role in cell volume regulation.

The amount of sodium and potassium in the body obviously depends on salt intake and excretion. Intake of salt normally depends on the salt content of the food. Intake of salty fluids may play an important role as well under some conditions. The mechanisms that are involved are discussed in sections 1.4.1 and 1.4.2. Commercially available rat food normally is rather salty (see for example Table 2.1), therefore lab rats eat every day a substantial amount of sodium and potassium, compared with the total amount of these solutes in the body. Because turnover of salt is so fast, small changes in the mechanisms that regulate salt intake and excretion can have large effects on the amount of salt in the body. Rats loose salt almost exclusively with urine. Faecal losses of sodium and potassium are negligible in healthy rats (Möhring and Möhring 1972). Mechanisms involved in regulation of urinary salt loss are discussed in sections 1.4.3 and 1.4.4.

1.4.1 Food intake

Sodium intake depends on sodium content of the food. Food intake normally depends on caloric needs, but there is evidence that animals, when given the choice, select sodium-rich food when they are in a sodium-deficient state (see Denton 1982, Bertino and Tordoff 1988). Because almost all diets contain enough potassium, potassium deficiency is rare. Currently there is no evidence that intake of potassium increases in potassium deficient animals.

When food is ingested, solutes enter the body that have to be excreted later. Therefore it is not surprising that when water intake is restricted, animals reduce food intake (Adolph 1947, McFarland 1964, Thrasher et al. 1984, Langhans, Scharrer and Meyer 1991, Engell 1988). This would reduce the amount of solute to be excreted, thus reducing water loss. It also aids osmoregulation: tonicity of the body fluids increases only little or not at all when no food is available during water deprivation (Gutman and Krausz 1968, Kutscher 1971, Houpt and Yang 1995). Rehydration stimulates food intake (Deaux et al. 1970, Kakolewski and Deaux 1970, Deaux and Kakolewski 1971, Hsiao and Trankina 1969).

Little is known about the mechanisms that are involved in the reduction in food intake during water deprivation. Potential mechanisms include dry mouth, signals from the gut, brain osmoreceptors, blood volume receptors, and behavioral coupling of eating and drinking.

Dehydration causes a dry mouth, and this could make eating difficult. Saliva flow falls during water deprivation (Winsor 1930, Adolph and associates 1947). When saliva production is blocked by surgical removal of the salivary glands or ligation of their ducts, rats will reduce food intake (Vance 1965), presumably because swallowing is

difficult with a dry mouth. Anecdotal evidence indicates that swallowing is impossible during severe water deprivation because of lack of saliva (see Wolf 1958, chapter 7). Consistent with this idea is that the inhibition of feeding during water deprivation appears to depend on the texture of the food. Jacobs (1964) claimed that food intake in rats that receive only water for 22 h per day, and only food for the remaining 2 h, depends on texture of the food, but did not report the composition of the diets used. Corey et al. (1977) claimed that rats preferred carbohydrates over protein during water deprivation because a carbohydrate diet is easier to swallow, but did not test this idea.

Duodenal osmoreceptors may play a role in dehydration anorexia. When no drinking water is available, large changes in duodenal osmolality occur after eating (Houpt 1991). It is well known that signals from these receptors inhibit gastric emptying when tonicity in the duodenum is high (Barker et al. 1974), and that the brain plays a role in this response (Mayer 1994). These receptors may have a role in food intake as well, because hypertonic infusions into the duodenum reduce food intake in pigs (Houpt et al. 1979). Intravenous control infusions had no effect. Hypertonic infusions in the rat stomach reduce food intake as well (Schwartzbaum and Ward 1958), but these infusions were rather large: they increased salt content of the body by 10% or more. In ruminants tonicity of ruminal contents may play a role in food intake (Ternouth 1967, Ternouth and Beattie 1971, Bergen 1972).

Hepatic osmoreceptors such as those involved in ADH release (Haberich 1968, Adachi et al. 1976, Choi-Kwon et al. 1990) could play a role. Such sensors may play a role in drinking because hypotonic infusions in the portal vein reduced drinking after water deprivation, but control infusions into the jugular vein did not (Kobashi and Adachi 1992). Hepatic sensors may play a role in food intake when peripheral glucose

metabolism is blocked (see Friedman 1991 for review). But the role of the liver in dehydration anorexia is unknown.

I.p. injections with hypertonic saline or hyperoncotic colloid reduce spontaneous food intake (Gutman and Kraus 1968) and food intake after food deprivation (Hsiao 1970). This suggests that the sensors involved in thirst and ADH secretion could also mediate the reduction in food during water deprivation. The experimental treatments may have made the rats sick, because they probably caused rather large depletions of intracellular and extracellular compartments. However, small changes in body fluid tonicity may have an effect on food intake as well. Novin et al. (1966) found that intravenous infusion of only 5 mL water stimulated food intake in thirsty rats. Rats ate little after infusion of 5 mL 0.15 M NaCl. Other treatments that cause thirst may inhibit feeding as well. For example, injections of Ang II in the brain reduce feeding (Rolls and McFarland 1973), but allowing rats to drink after such injections restored food intake.

Rats usually drink immediately before, during or shortly after a meal (Fitzsimons and LeMagnen 1969, Kissileff 1969). This is thought to be a learned response because meal associated drinking changes only slowly when the diet is changed. Fitzsimons and LeMagnen (1969) gave rats a novel high-protein diet that normally causes high water intake. At first rats drank much between meals, but after a few days much of the drinking became associated with meals. Interfering with this link between eating and drinking could alter food intake during water deprivation.

1.4.2 Salt appetite

Salt appetite currently is the subject of relatively vigorous research, and has been for 30 years (for review see Fitzsimons 1992, Stricker and Verbalis 1990, Denton 1982).

Research shows that intake of solutions of concentrated saline, that would be aversive to animals normally, increases in sodium depleted animals. Thus, we see salt appetite after adrenalectomy, after administration of diuretics such as furosemide, or after intraperitoneal dialysis with isotonic glucose (this causes accumulation of sodium in the peritoneal cavity). Treatments that cause or mimic hypovolemia, such as hemorrhage, ligation of the inferior vena cava, and injection of colloids such as polyethylene glycol under the skin also stimulate intake of fluids that contain sodium.

However, sodium intake is often much larger than the deficit, and the excess is then excreted (Jalowiec and Stricker 1973). A second complication is the delay (usually several hours) that is often seen between induction of the deficit and initiation of the response. Third, drinking of sodium containing fluids is not limited to sodium depleted animals, but sodium replete animals drink saline solutions as well, especially if it is not very concentrated (see Epstein 1991 for review). The amount of salt ingested this way strongly depends on the concentration of the saline. Therefore the salt is taken apparently because it tastes good, not because it is needed in the body. This 'need-free' sodium intake increases after experience with sodium depletion (Epstein 1991). Because need-free sodium intake does not contribute to body fluid regulation it does not need to concern us here. Instead I'll focus on the role of salt appetite in body fluid homeostasis.

The direct stimulus for need induced sodium appetite is not known. There is evidence that reduced blood volume as well as hyponatremia contribute, but hyperkalemia, altered taste, Ang II, and aldosterone may play a role as well.

Sodium depletion almost invariably is accompanied by hypovolemia. The reason is that sodium is found almost exclusively in the ECF. Loss of sodium is accompanied by loss of ECF volume, because sodium concentration of the ECF is kept constant by

changes in thirst and ADH release. Stretch receptors similar to those involved in hypovolemic thirst may be involved in sodium appetite, because stimulation of these receptors with a balloon inhibits salt appetite caused by injection of colloids (colloids were injected in dialysis sacs placed in the peritoneal cavity, the injections cause hypovolemia because extracellular fluid is drawn in the sacs, Kaufman 1986). Information from high-pressure receptors may play a role as well, because combined denervation of carotid sinus and aortic arch reduced the salt appetite normally seen after administration of the diuretic furosemide (Thunhorst et al. 1994).

As pointed out before, hypovolemia and hypotension stimulate the release of renin, leading to Ang II production. Ang II does not cross the blood-brain barrier, but instead acts on receptors in the vascular organ of the lamina terminalis (Fitts and Masson 1990) or subfornical organ (Weisinger et al. 1990), areas that lack this barrier. Thunhorst and Fitts (1994) suggest that circulating Ang II mediates the salt appetite that is seen after administration of the diuretic furosemide. A small i.v. dose of captopril that prevented conversion of circulating Ang I to Ang II completely blocked the salt appetite. Without a doubt brain angiotensin plays an important role in salt appetite, but the dose of captopril used probably did not block production of Ang II in the brain. Injection of Ang I, the inactive Ang II precursor, in the brain caused drinking and increased blood pressure, just as seen after injection of Ang II in the brain.

Angiotensin II also stimulates secretion of aldosterone, which can act on brain receptors to stimulate salt appetite (see Epstein 1991 for review). In the brain, Ang II and aldosterone act synergistically to enhance salt appetite.

Sodium deficiency may reduce blood [Na^+] and osmolality, because it stimulates thirst and ADH secretion. Because these responses develop much faster than salt appetite

(Stricker and Verbalis 1990), animals may be hyponatremic when sodium intake starts. By itself, hypotonicity does not cause salt appetite, because water loading an animal does not stimulate salt intake. But salt appetite can be reduced with hypertonic subcutaneous injections in sodium depleted rats (Fitzsimons and Wirth 1976, Blackburn et al. 1993). Infusions with hypertonic saline strongly reduce salt intake in sodium deficient sheep (Muller et al. 1983), but whether this is caused by hypertonicity or by increased blood volume is unclear.

Changes in CSF [Na^+] may play a role in salt appetite. In sodium deficient sheep, reduction of CSF [Na^+] causes a rapid increase in salt intake, and increased CSF [Na^+] inhibits it (Weisinger et al. 1982). Reducing CSF [Na^+] also stimulates salt appetite in sodium replete sheep (Weisinger et al. 1982). Although this seems to indicate that hypotonicity affects salt appetite by a mechanism similar to thirst and ADH secretion, the neural mechanisms may be different. Large brain lesions that abolish drinking after hypertonic injections did not alter sodium appetite in sheep (Weisinger et al. 1993). The lesions included the vascular organ of the lamina terminalis and the median preoptic nucleus, and usually some surrounding tissue. In rats CSF [Na^+] does not play a role, because brain infusions that alter CSF [Na^+] do not influence salt appetite (Epstein et al. 1984). Such species differences are not entirely surprising because herbivores like sheep often eat low sodium diets (Denton 1982). Sodium deficiency could be common in such animals. Dietary sodium intake of omnivores and carnivores is much higher.

1.4.3 Salt excretion in osmoregulation

In many species excretion of sodium, potassium and chloride increases during water deprivation, or increases compared with food-matched, but water replete controls (Luke

1973, McKinley et al. 1983b, Zucker, Gleason, and Schneider 1982, Ben Goumi et al. 1993). On rehydration, electrolyte excretion is reduced. These changes play an important role in osmoregulation: when this response is blocked with brain lesions, the body becomes very hypertonic during water deprivation (McKinley et al. 1983c).

It is not clear whether water deprivation stimulates salt excretion in humans as well. Black, McCance and Young (1944) measured electrolyte balance during water deprivation and rehydration, while food intake was kept constant. Excretion of potassium increased during water deprivation, but sodium excretion was variable. Both sodium and potassium were retained on rehydration. Wiley and Wiley (1933) found that water deprivation caused a negative sodium and potassium balance in a person whose food intake was kept constant. Unfortunately the number of subjects was small in both studies (two and one). More conclusive data are not available. Sodium excretion fell after rehydration after 24 h of water deprivation in an experiment from Rolls et al. (1980), but their data lack information on solute excretion in the water deprived state.

In addition to these changes during water deprivation, osmoregulatory changes in salt excretion may play a role in other situations as well. They may play a role after eating a meal. McKinley et al. (1994) found that feeding in sheep was followed by a pronounced natriuresis. The post-prandial natriuresis was completely blocked by infusion of low-sodium CSF into the brain ventricles. As explained later, dehydration natriuresis is thought to be mediated by a brain sensor.

The system involved in dehydration natriuresis may play a role in mineralocorticoid escape (Pennington and McKinley 1993). Mineralocorticoids reduce sodium excretion, but the effect is not maintained during prolonged administration. During this 'escape', plasma [Na^+] was elevated. Infusion of low-sodium CSF in the brain of sheep

undergoing this 'escape' reduced sodium excretion, suggesting the mechanism for dehydration natriuresis was activated.

The mechanism for dehydration natriuresis is not fully known. In general, it is clear that increased sodium concentration is sensed in the brain, causing the release of a hormone that acts on the kidney to increase electrolyte excretion. Location of the receptor, identity of the hormone and mode of action on the kidney are unknown.

1.4.3.1 Role of the brain. There is little doubt that the brain is involved in the changes in salt excretion during water deprivation. Damage to the anterior wall of the third ventricle prevent dehydration natriuresis, resulting in gross hyponatremia during water deprivation (McKinley et al. 1983c). There are several nuclei in this area. The organum vasculosum of the lamina terminalis (OVLT) and the ventral part of the median pre-optic nucleus may be involved in the response, because damage to these areas strongly reduces natriuresis caused by a small intravenous infusion with hypertonic saline (McKinley et al. 1992). Damage to the supra-optic nucleus has no effect (McKinley et al. 1992). The effect of these lesions is fairly specific, because they did not prevent natriuresis after infusion of isotonic saline. (For review of natriuresis after infusion of isotonic saline see de Wardener, 1985).

The sensor that is involved in dehydration natriuresis is located in the brain. Intracarotid infusion of hypertonic saline increases sodium excretion more than similar infusions in the jugular vein (Olsson 1972, Blaine et al. 1975). Intracarotid infusions that increased brain [Na^+] by 2.3 mM, but did not alter [Na^+] in the rest of the body increased sodium excretion more than isotonic control infusions (Emmeluth et al. 1992). However, the possibility that other mechanisms were involved in the natriuresis in these

studies has not been ruled out completely. In these studies, it is possible for example that higher blood pressure contributes to the higher sodium excretion. Also ADH release may have contributed, because ADH infusions can stimulate sodium excretion (Cowley et al. 1984, Park et al. 1985). The infusions used in the studies by Blaine et al., Emmeluth et al. and Olsson would be expected to increase blood volume.

The type of sensor, and its location with respect to the blood-brain barrier is not clear. It may be a sodium sensor or a cell volume sensor, inside or outside the blood-brain barrier. If the sensor is a sodium sensor, it must be located inside the blood-brain barrier, because Blaine et al. (1975) found that infusions with hypertonic sucrose or fructose in the carotid artery increased sodium excretion. Infusion of the same solution into the jugular vein had little effect.

Brain [Na^+] can also be changed by infusion of hypertonic or hypotonic solutions into the brain. The urinary changes after such infusions are very similar to those during water deprivation and rehydration. Therefore brain infusions have often been used in attempts to elucidate the mechanism of dehydration natriuresis. Andersson et al. (1967) found that hypertonic injections in the third ventricle are natriuretic. The area most sensitive to such infusions is the anterio-dorsal part (Cox et al. 1987). As noted before, brain lesions that destroy tissue lining the anterior wall of the third ventricle block dehydration natriuresis. Bealer (1982) showed that similar lesions also block natriuresis after infusion of hypertonic saline in the brain ventricles. Infusion of low-sodium CSF reduces sodium excretion during water deprivation (Leksell et al. 1982), but has not much effect in water replete animals (Mouw et al. 1980). Infusion of low-sodium CSF in the brain also prevented natriuresis during intravenous infusions with hypertonic saline (Olsson 1973). Unfortunately, brain infusions locally cause changes outside the

physiological range. Because membrane potential depends on electrolyte concentration inside and outside the cell such infusions could alter activity of cells not normally influenced by water deprivation.

Under normal circumstances changes in extracellular osmolality will usually be accompanied by changes in [Na^+], because sodium is the main cation in the ECF. For experimental purposes however, it can be useful to make a distinction. With few exceptions, investigators found that the receptor involved in changes in sodium excretion after brain infusions is sensitive to changes in [Na^+], not tonicity (see McKinley 1992, Leksell et al., 1981, 1982). However, Osborne et al. (1989) concluded that in rats the receptors that are involved in this response are sensitive to changes in brain osmolality, because i.c.v infusions that contained 150 mM sodium and 0.7 M sucrose or mannitol increased sodium excretion. These infusions will increase CSF osmolality but reduce its [Na^+] because they cause flow of water from brain tissue into the CSF.

1.4.3.2 Between brain and kidney. It seems likely that dehydration natriuresis is mediated by a hormone. First, renal denervation neither prevents natriuresis and kaliuresis during water deprivation, nor salt retention after rehydration (Park et al. 1989). Also it does not prevent natriuresis after hypertonic brain infusions (Beasley et al. 1983).

Second, it seems unlikely that the increase in sodium excretion is caused by increased blood pressure. Short periods of water deprivation do not change blood pressure (Woods and Johnson 1983, Schwartz and Reid 1983). Surprisingly, several reports indicate that blood pressure may rise slightly after longer periods of water deprivation (Rockhold et al. 1984, Januszewicz et al. 1986, Kadekaro et al. 1992). Dehydration natriuresis however is seen most easily during the early phase of water deprivation. I.v. and i.c.v

infusions with hypertonic saline often increase blood pressure (Bealer 1982, Osborne 1989, Emmeluth et al. 1992), but natriuresis is also seen in experiments in which blood pressure does not rise (Mouw et al. 1979, Beasley et al. 1983). Note however that in Mouw's experiment animals were anaesthetized and that very large brain infusions were given.

The classical hormones that regulate sodium excretion do not appear to play a role in dehydration natriuresis. Water deprivation increases plasma renin and Ang II concentrations (di Nicolantonio and Mendelson, 1986, Metzler et al., 1986), which would not promote sodium excretion. Plasma Ang II increases on rehydration (di Nicolantonio and Mendelson, 1986) but the increase is not necessary for the rapid fall in electrolyte excretion. We found that blocking Ang II production with a high dose of captopril (100 mg/kg s.c., 30 min before water was returned) does not prevent retention of sodium, potassium and chloride after rehydration in rats (Schoorlemmer and Evered, unpublished). Reduced sodium excretion after infusion of low-sodium CSF in water deprived sheep did not depend on Ang II production either (Leksell et al. 1982).

Changes in aldosterone concentrations are not the cause of dehydration natriuresis. Aldosterone levels usually increase or do not change during water deprivation (Zucker, Gleason and Schneider 1982, Thrasher et al. 1984, Metzler et al. 1986), making a role for this hormone unlikely. Adrenalectomy does not prevent the changes in sodium excretion during water deprivation and rehydration (McKinley, Evered and Mathai, 1993). However, aldosterone may play a role under some conditions. First, sodium deficient dogs increased sodium excretion during water deprivation, and preventing the fall in aldosterone concentration in this experiment prevented the natriuresis (Merrill, Skelton and Cowley, 1986). Natriuresis in this experiment was small however, and urine

was collected only once a day. Second, increased sodium excretion after hypertonic i.v. infusion was accompanied by reduced plasma aldosterone levels, and the natriuresis could be blocked by pretreatment with deoxycorticosterone acetate (Childers and Schneider 1981). However, this treatment reduced sodium excretion after a hypotonic load as well. In general it seems unlikely that changes in aldosterone can account for the rapid urinary changes seen during brain infusions with hypertonic saline or after rehydration of dehydrated animals. Also aldosterone by itself can not account for increases in both sodium and potassium at the same time.

Infusion of ADH, in amounts that result in ADH levels in the blood that are similar to those seen during water deprivation increase sodium excretion (Cowley et al. 1984, Park et al. 1985). However, such infusions also increase blood pressure, which would stimulate sodium excretion. Usually blood pressure does not increase during water deprivation. It seems likely that ADH does not mediate dehydration natriuresis, as hypophysectomy does not prevent it (Park et al. 1985). Natriuresis is also seen after brain infusion with hypertonic saline in Brattleboro rats that lack ADH (Beasley, Malvin and Mouw 1983).

Oxytocin stimulates sodium excretion (Verbalis et al. 1991, Conrad et al. 1986). However, in sheep (Thornton et al. 1987) and man (Williams et al. 1985) plasma oxytocin levels do not increase during water deprivation. (But note that it is not sure that dehydration natriuresis occurs in humans). In rats, oxytocin secretion is very sensitive to changes in plasma tonicity and may contribute to dehydration natriuresis. Infusion of oxytocin stimulates sodium excretion in rats loaded with hypertonic saline (Forsling et al. 1994). Huang et al. (1995) showed that in anaesthetized rats the natriuresis caused

by intravenous infusion of hypertonic saline is reduced after injection of an oxytocin receptor antagonist.

Atrial natriuretic peptide (ANP) stimulates sodium excretion. However, plasma ANP concentration falls during water deprivation, and rapidly increases on rehydration (Januszewicz et al., 1986). Therefore, a role for ANP is very unlikely. Also ANP is not essential for natriuresis during hypertonic intravenous infusions, because infusions that increased sodium excretion did not alter plasma ANP concentration (Emmeluth et al. 1990).

Infusions of high-sodium CSF into the brain may trigger the release of an inhibitor of sodium-potassium ATPase (Jandhyala and Ansari 1986). It is not likely that this accounts for the natriuresis seen during brain infusions, because sodium pump inhibitors, such as ouabain, are natriuretic only in supraphysiological concentrations (Yates and McDoughall 1993).

Hypertonic brain infusions may release an inhibitor of prostaglandin synthesis (Diez et al. 1984). Prostaglandins can change sodium excretion (Kirshenbaum and Serros 1980), but whether they play a role in the natriuresis seen during brain infusion and water deprivation remains unclear.

In conclusion, it appears that dehydration is mediated by a hormone, but not by any of the classical hormones known to regulate sodium excretion.

1.4.3.3 In the kidney. Water deprivation does not have strong effects on glomerular filtration rate (GFR) and renal blood flow (RBF). Zucker et al. (1982) saw no changes in creatinine clearance during four days of water deprivation and after rehydration in dogs (food intake in this experiment did not change). This does not rule

out the possibility that the changes in solute excretion are caused by changes in GFR: given the large amount of solute filtered by the kidney, even small changes in GFR may influence solute excretion significantly.

Hypertonic intravenous infusions increased GFR and RBF by 30% (McKinley et al., 1992), but increased blood volume and reduced plasma protein concentrations may have contributed to these increases (no isotonic control infusions were used in this study). Hypertonic brain infusions can increase GFR in rats (Beasley et al. 1983). In this experiment solute retention after the end of the infusion was accompanied by a fall in GFR. Whether these changes in GFR account for the changes in solute excretion is not known. Reducing i.c.v $[Na^+]$ in water deprived sheep sharply reduced sodium excretion, but did not change GFR and RBF (Leksell et al. 1982, McKinley et al. 1983c).

Infusions of Na-free CSF sharply reduced lithium clearance in sheep undergoing mineralocorticoid escape (Pennington and McKinley 1990). Lithium clearance has been used as a marker for reabsorption of sodium in proximal tubule and loop of Henle. As explained earlier in this section, it seems likely that the mechanism that increases sodium excretion during water deprivation is the same as the one that is responsible for escape from mineralocorticoid administration. This suggests that during water deprivation proximal reabsorption in the kidney is reduced. Reduced proximal absorption would lead to increased excretion of many solutes. Therefore reduced proximal absorption would be compatible with the finding that water deprivation increases excretion of sodium as well as potassium. The effect of water deprivation on lithium clearance has not been investigated, unfortunately. Also note that the use of lithium clearance as a marker for proximal reabsorption has been disputed (see Navar and Schafer 1987).

1.4.4 Salt excretion in regulation of blood volume and pressure

Changes in sodium excretion play a role in blood pressure regulation. When blood pressure increases, renal sodium loss increases, which causes ECF volume, and blood volume, to fall. The result is that venous return to the heart falls, reducing cardiac output, which leads to a fall in blood pressure (Guyton 1986 chapter 22, Guyton 1990). The relation between blood pressure and sodium excretion is seen even in isolated kidneys.

There are many other mechanisms that influence sodium excretion and guard blood volume and pressure. Stimulation of stretch sensors in the atria and ventricles, carotid and renal baroreceptors, and sensors in the liver stimulate sodium excretion. These sensors monitor venous filling, blood pressure, and renal perfusion (see Miller et al. 1996 for review).

Sensors monitoring venous and pulmonary filling are stretch receptors in the walls of atria and large veins. These receptors are thought to be involved for example in the natriuresis (and diuresis) that occur during immersion of the body in water. This procedure causes an increase in central venous blood volume, because blood and interstitial fluid from the legs is forced into the central veins. The role these receptors play in restoration of long-term changes may be limited because they adapt (see Miller et al. 1996). Stimulation of these receptors reduces renal nerve activity. The procedure also stimulates ANP secretion. Whether these changes account for the natriuresis is still under debate.

Atrial natriuretic peptide (ANP) is a hormone that is released from the atria of the heart whenever atrial volume is increased, for example during water immersion, or after intravenous infusion of saline. ANP release also is influenced by acetylcholine,

norepinephrine and hormones like ADH or glucocorticoids. Whether it plays a significant role in sodium excretion is still controversial (see Blaine 1990 and Goetz 1990). It is clear that infusion of ANP stimulates sodium excretion, although the natriuresis that is seen after doses in the physiological range is small. But the natriuresis that is seen after atrial stretch may be mediated by cardiac nerves instead of ANP, because Goetz et al. (see Goetz 1990) found that cardiac denervation abolished the natriuresis after stretch of the left atrium, although it did not prevent ANP secretion after atrial stretch. There doesn't seem to be a role for ANP in long-term regulation of sodium excretion either, because plasma ANP concentrations often do not increase when dietary salt intake increases.

1.5 Objectives

As explained in the introduction, much is known about the mechanisms that are involved in body fluid regulation. These mechanisms are thought to act together to keep the size and composition of the body fluids constant. However there are not many examples of research where the concerted action of these mechanisms has been investigated. The importance of such research is that it shows the relative importance of the mechanisms about which we know so much already. Sometimes such research also reveals important mechanisms that have been overlooked, or have not received the attention they merit.

A good setting to study regulatory mechanisms involved in body fluid regulation is water deprivation. Lack of drinking water is among the most common threats to the constancy of the body fluids. Even short periods of water deprivation may cause

significant deficits, especially in small animals such as rats, where turnover of water is high. Water deprivation causes the activation of many homeostatic mechanisms.

The objective of this thesis was to investigate how changes in water and solute intake and excretion influence the body fluids during water deprivation and rehydration. The specific objectives were as follows:

1) To measure the changes in water intake, food intake, and water and solute excretion during a short period of water deprivation, and to quantify the importance of these changes for body fluid regulation (section 3).
2) To measure the changes in water intake, food intake, and water and solute excretion when water is returned after a period of water deprivation, and investigate their effect on the body fluids (section 4 and 5).
3) To compare the changes in solute excretion seen during water deprivation and rehydration with those that occur after infusion of solutions into the CSF that alter CSF [Na^+] or tonicity (section 6).
4) To investigate the contribution of oropharyngeal signals to the eating that occurs after rehydration (section 7).
5) To investigate the mechanisms that cause the changes in food intake during water deprivation, in particular the location of the sensor that mediates the feeding inhibition (section 8).

2 MATERIALS AND METHODS

This section describes materials and methods common to most of the experiments. Experimental designs and details unique to particular experiments are given with those experiments.

2.1 Animals and maintenance

Male Long-Evans rats, bought from Charles River Canada, St-Constant, Que., Canada, or first-generation offspring of these were used. At the time of the experiments they weighed 250-600 grams. The rats were housed individually in plastic "shoebox" cages with wood shavings, or in metabolism cages (diameter 25 cm, 18 cm high, Nalgene 650-0350, Nalge Company, Rochester, NY). They were kept in an environmental chamber. Temperature was controlled at 21 $\pm$ 1°C. Humidity was not controlled, but was usually low (less than 20%). Lights were on from 07:00 to 19:00 h. In experiments where data were collected during the dark period, a red bulb (40 W) was always burning. Food and tap water were available except where indicated. Purina lab chow (#5001, Purina Mills, St. Louis, Mo.) was used in the experiments in sections 3 (except 3.1), 4, 5, and 8.2. Because the university switched suppliers, Prolab RMH3000 (Agway, Syracuse, NY) was used in the other experiments. For composition of these diets see Table 2.1. All procedures were approved by the Animal Care

Table 2.1 Composition of the diets used.

	Agway RMH3000	Purina 5001
protein (%)	22.5	23.4
fat (%)	5.5	4.5
carbohydrate (%)	52	49
calcium (mmol/kg)	242	250
potassium (mmol/kg)	244	282
sodium (mmol/kg)	191	174
magnesium (mmol/kg)	86	24
iron (mmol/kg)	5	4
chloride (mmol/kg)	42	158
phosphorus (mmol/kg)	275	197

Data based on manufacturer's analysis.

Committee at the University of Saskatchewan and were in accordance with the principles of the Canadian Council of Animal Care.

2.2 Test cages

In some experiments, rats were held in restraining cages for sample collection. These were made from wire mesh formed into a half-cylinder (diameter 9 cm, length 20 cm) with slotted openings in the bottom and the top for access to cannulas. The restraining cages were large enough to permit rats to turn around but prevented them from interfering with the cannulas. Before the start of the experiments rats were adapted to these cages at least 6 times. During the experiments rats appeared to be quietly resting in these cages. Water-deprived rats drink as readily in restraining cages as they do in their home cages (Adolph et al. 1954).

Some experiments were done in unrestrained rats. Rats were placed in wooden cages 15 cm wide, 20 cm deep, and 35 cm high, with a clear acrylic front and a wire mesh bottom. A 1 cm wide slot was cut almost the length of the bottom for access to the bladder cannula. Other cannulas exited through the top of the cage.

Some experiments were done in metabolism cages (see section 2.1). Rats were placed in them a few days before measurements started.

2.3 Cannula construction, surgical techniques and sample collection

Rats were anaesthetized with Equithesin (Gandal 1969, 3 mL/kg body weight i.p.). Buprenorphine-hydrochloride, 0.1 mg i.m., or 0.015 mg, s.c. (Temgesic, Reckit & Colman Pharm., Hull, England) was given for analgesia with abdominal surgery. Instruments were sterilized with boiling water. Sterile drapes were used during

abdominal surgery. Cannulas were sterilized by soaking them for an hour in a mixture of iodine and 70% ethanol.

Experiments were started 10 days after surgery, when rats had regained pre-operative body weight.

2.3.1 Venous cannulas and blood sampling

Venous cannulas were made from silicon tubing (10 cm long, i.d. 0.6 mm, o.d. 1.2 mm, no 602-155, Dow Corning, Midland, Michigan) joined to polyurethane tubing (15 cm long, i.d. 0.5 mm, o.d. 1.0 mm, MRE 040, Braintree Scientific, Braintree MA).

The silicon end of the venous cannula was inserted 8 cm into the femoral vein. This advances the cannula tip into the vena cava, close to the heart. The other end of the cannula was tunnelled under the skin and connected to an elbow made of 23 g stainless steel tubing. The elbow was mounted with acrylic cement on a pedestal made from polypropylene mesh (about 2 cm diameter, mesh diameter 0.5 mm, Small Parts, Miami Lakes, FL). The mesh was implanted under the skin between the shoulder blades. The elbow protruded through the skin and could be connected to sampling tubing. The cannula was filled with heparinized saline (10 U/Ml), and closed with a cap. These cannulas are maintenance free.

Before experiments the elbow was connected to polyethylene tubing (i.d. 0.6 mm, o.d. 1.0 mm, PE-50, Clay Adams, Parsippany NJ). To take a sample, the saline was removed from the cannula, and 0.35 mL blood was withdrawn into a heparinized 1 mL syringe. The same volume of isotonic saline was returned to reduce changes in blood volume. The cannula tip was then filled with heparinized saline, with a small air bubble behind it to separate the next sample from the saline that filled the rest of the cannula.

2.3.2 Brain cannulas and brain infusions

Brain cannulas were stainless steel tubes, 13 mm long, i.d. 0.3 mm, o.d. 0.7 mm (Small Parts). The cannula was inserted through a small hole drilled through the skull. It was located so that the tip was just above the left lateral ventricle, 3.5 mm below the dura, 1.0 mm lateral from midline, and 0.5 mm anterior of bregma (skull level). The cannula was anchored to stainless steel skull screws with acrylic cement. It was plugged with a stylet the same length as the cannula.

To make infusions, the stylet was replaced by an injector that was 0.5 mm longer than the cannula. It was connected with PE-50 tubing to a Hamilton 'gastight' syringe that was driven by a syringe pump. A cannula was assumed to be located in the ventricle if, on completion of all experiments, the rat drank at least 4 mL in 15 min after an i.c.v. injection of Ang II (10 ng in 1μL).

2.3.3 Bladder cannulas and urine collection

I developed a bladder cannula that has several unique advantages. First, it has a very small residual volume. Therefore it can be used to collect urine frequently even during water deprivation, when urine flow is low. (With other types of cannulas, usually rats are force fed a large amount of water, or saline is infused i.v., to ensure high urine flow during the collection period.) Second the cannula can be used for a long time. This is useful because rats often eat little during the first few days after surgery, and this influences solute excretion. Third, the cannula can be used in unrestrained rats, which would help reduce stress-related urinary changes.

Figure 2.1 shows diagrams of this cannula. A hole (1 mm diam.) was drilled through a nylon set screw (#6-32, 1/4" long, SSN-632/4, Small Parts, Miami Lakes, FL), and

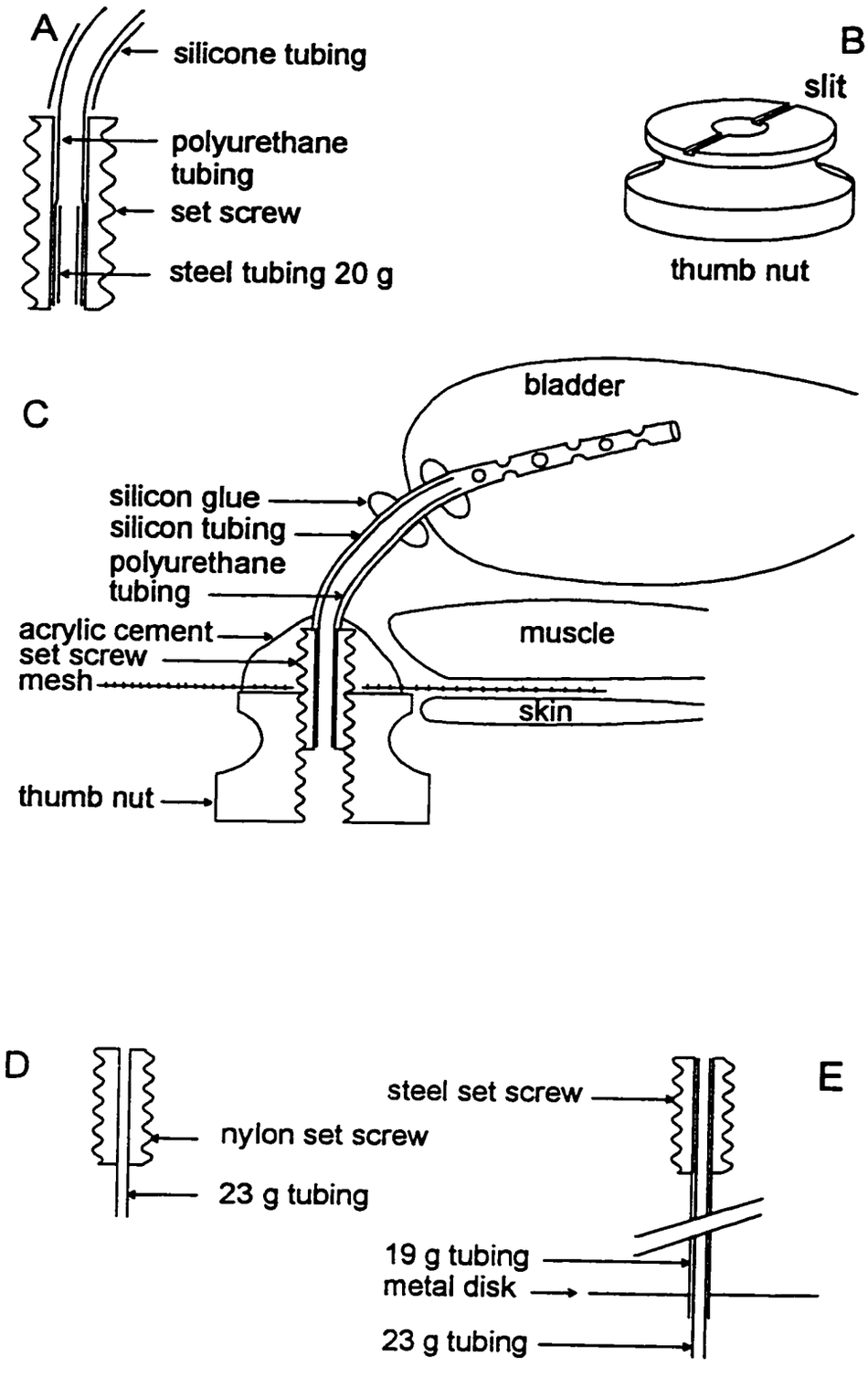

Figure 2.1. Bladder cannula construction. A. To fasten tubing in the set screw, one end of the polyurethane tubing was expanded by inserting a piece of hypodermic tubing. B. Thumb nut with slit. C. Diagram of completed cannula. D. Sampling adapter for restrained rats. E) Sampling adapter for unrestrained rats.

a piece of polyurethane tubing (length 15 mm, i.d. 0.5 mm, o.d. 1.0 mm, MRE 040, Braintree Scientific, Braintree MA) was led through the hole. To fasten the tubing in the screw, one end of the polyurethane tubing was expanded with a piece of stainless steel tubing (20 g, 3 mm long). The expanded end was pushed into the hole (Figure 2.1A). It should fit very tight. A piece of silicone tubing (length 25 mm, i.d. 0.6 mm, o.d. 1.2 mm, Dow Corning, Midland, MI) was soaked in ether and slipped over the polyurethane tubing. To facilitate bladder emptying, holes were punched through the silicone tubing with 20 g hypodermic tubing. Flanges were made with silicone glue (use glue with no mould inhibitor).

Then a slit was ground in a thumb nut (Figure 2.1B, TNB 632, Small Parts). The slit helps to anchor the parts together. To anchor the cannula to the body we used polypropylene mesh (3 cm diameter, mesh size 0.5 mm, Small Parts). To prevent fraying of the mesh, the edges were melted with a soldering iron. A small hole was melted in the middle. Set screw, mesh and thumb nut were anchored together with acrylic cement (Figure 2.1C). We coated the thumb nut with silicone glue to reduce corrosion of the nickel surface.

To implant the bladder cannula a small midline incision was made below the umbilicus. The bladder was pulled through the incision and a pursestring suture (about 3 mm diameter) of 5-0 silk was placed in the apex. A small hole was cut in the middle of the suture, the cannula was inserted, and the suture was tightened between the silicon collars. The abdominal wall and skin incision were closed with 3-0 silk with the polypropylene mesh between. The cannula was sealed with a stainless steel set screw. This screw should be inserted tightly to prevent urine leakage.

When the cannula was not used, it was closed with a stainless steel set screw (SSX-632/4, Small Parts). To collect urine from restrained rats the set screw was removed and the attachment shown in Figure 2.1D was connected to the cannula. It was a nylon set screw with a hole drilled through it, in which a piece of hypodermic tubing (23 g, 15 mm long) was glued. The attachment of Figure 2.1E was used to collect urine in unrestrained rats. It is a stainless steel set screw soldered to 7 cm 23 g stainless steel tubing. It was reinforced with 19 g tubing. At the distal end a disc (25 mm diameter) was fastened, which prevented the rats from pulling the cannula end up through the cage bottom. The sampling attachment was connected to 20 cm of PE 50 drainage tubing. Urine was collected by gravity drainage into pre-weighed tubes. The drainage tubing was disconnected after each sample and emptied to reduce cannula residual volume.

To reduce formation of bladder stones, once or twice a week 0.5 ml sterile Suby's solution (citric acid 154 mM, Na_2CO_3 41 mM, MgO 94 mM in water; Olin 1987) was injected into the bladder and withdrawn 1 h later.

Residual volume of the cannulas was measured as follows. The bladder was emptied, 1 mL marker solution (10 mM LiCl) was injected through the cannula and immediately the bladder was allowed to drain again. Then a 20 min urine collection was made. Residual volume was calculated as amount of marker in the 20 min urine sample divided by the concentration of the marker in the solution drained immediately after injection. Two weeks after surgery residual volume was 6.7 $\pm$ 0.8 μL (n=12, triplicates in four rats). Two months after surgery it was 14.2 $\pm$ 1.6μL (n=9, triplicates in 3 rats). These values were obtained with the sampling attachment for restrained rats. Residual volume would be about 10 μL larger with the attachment for unrestrained rats.

2.3.4 Gastric cannulas and gastric infusions

For gastric infusions I used several types of cannulas. Cannulas used in section 8 were made from a 3 cm piece of flanged polyethylene tubing (i.d. 1.2 mm, o.d. 1.7 mm, PE 190, Clay Adams) joined to polyurethane tubing (i.d. 0.5 mm, o.d. 1.0 mm, MRE 040, Braintree Scientific) with silastic tubing (i.d. 0.6 mm, o.d. 1.2 mm, # 605-105, Dow Corning). The cannulas used in section 7.2 were made from silastic tubing (about 20 cm long, i.d 1.6 mm, o.d. 3.2 mm, #602-285, Dow Corning) with a small flange made of silicone glue at the end. The flanged end was inserted into the muscular part of the stomach, in the greater curvature, and tied in place with a purse-string suture. The other end was tunnelled under the skin to the area between the shoulder blades and exteriorized similar to the venous cannulas described in section 2.3.1.

The cannulas were flushed once a week with water to prevent blocking. For infusions the cannula was connected to infusion tubing. It was connected to a syringe that was driven by a syringe pump. In experiments with long lasting infusions, the tubing was protected with a metal spring (about 40 cm long), and connected to the syringe through a swivel joint (see Figure 2.2).

2.3.5 Intestinal cannulas and infusions

For infusions in the intestine polyurethane tubing (o.d. 1.0 mm, i.d 0.5 mm, about 20 cm long) was used. The last few cm was covered with silastic tubing, and two flanges, 3 mm diameter, about 2 mm apart, were made at the end with silicone glue. The flanged end was inserted in the intestine and the intestinal wall was tied between the flanges with a pursestring ligature. The cannula was anchored to the abdominal muscle wall, and tunnelled under the skin to the back. The same cannulas were used for

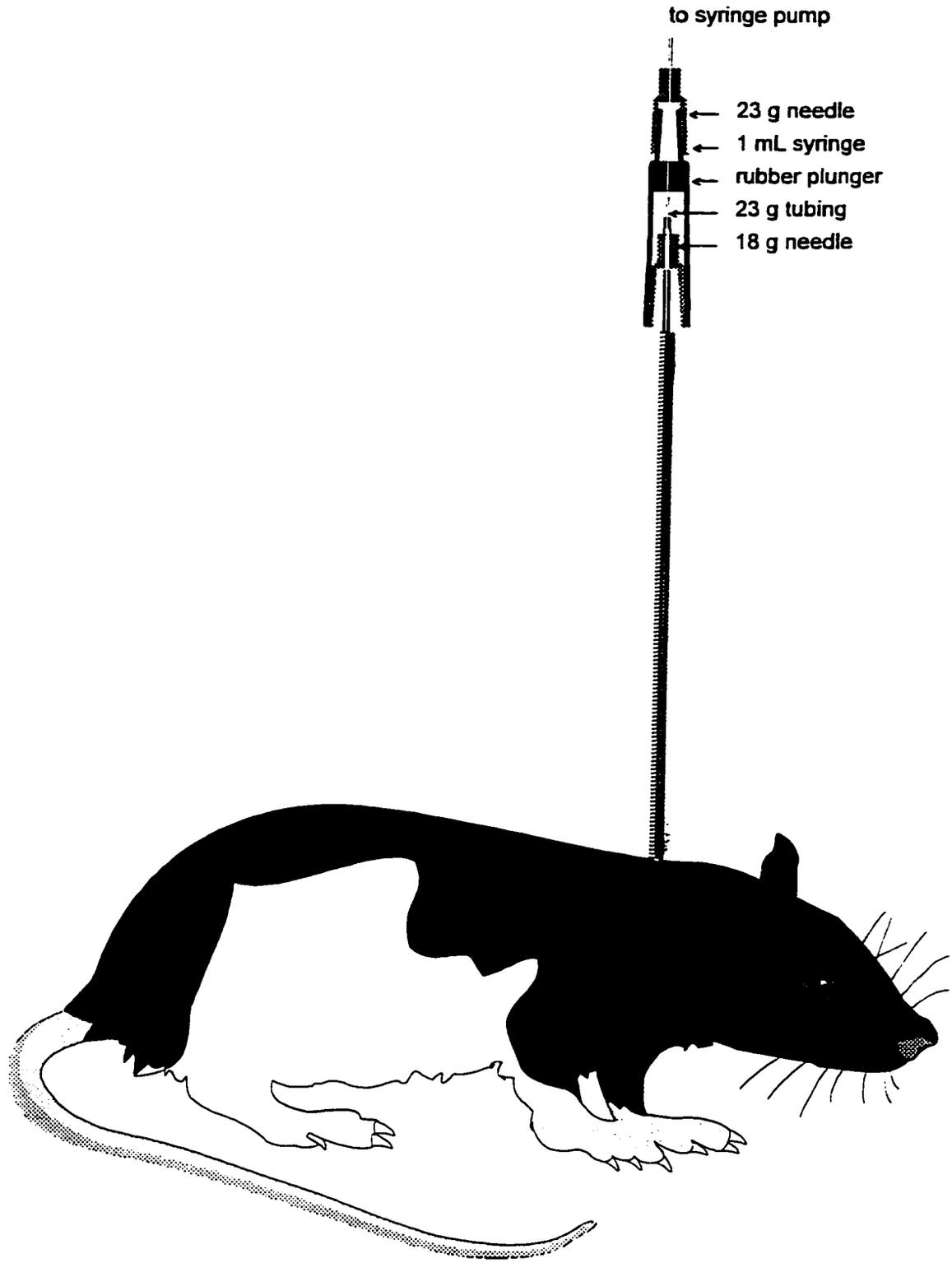

Figure 2.2 Setup for longterm infusions. Swivel joint allowed rat freedom to turn around. Tubing leading from the swivel joint to the rat was protected with a metal spring.

infusion into the cecum. Exteriorizing the cannula, cannula maintenance and infusion procedure were the same as for gastric cannulas (see section 2.3.4).

2.3.6 Gastric fistulas

Gastric fistulas have been described before (Young et al. 1974). They are stainless steel tubes that can be closed with a screw. Figure 2.3 shows a diagram. They were implanted with a purse-string suture in the muscular part of the stomach, in the greater curvature. A small ring of polypropylene mesh (about 2 cm diameter, mesh size 0.25 mm, Small Parts), was placed against the outer wall of the stomach, and another (mesh size 0.5 mm) between the skin and the abdominal muscle layer. The connective tissue that forms around the mesh anchors the cannula to the stomach and body wall. Between experiments the cannulas were closed with a steel screw.

Before each experiment the rat's stomach was flushed with warm water to remove food that may block the fistulas. Water drained easily from these fistulas: in a pilot experiment I recovered 26.5 $\pm$ 2.9 mL of fluid from the fistulas after rats drank 27.1 $\pm$ 2.8 mL in 15 min (n=12).

2.4 Blood and urine analysis

Blood samples were transferred from the syringes to heparinized (0.2 U) microcentrifuge tubes and to two heparinized microhematocrit capillaries (50 μL). Two 20 μL blood samples were removed for hemoglobin assay, which was done immediately. The sample was centrifuged for 5 min at 12700 x *g*, and plasma was removed. The hematocrit capillaries were centrifuged for 5 min at 13500 x *g* and hematocrit was measured on a Damon/IEC microhematocrit reader. Plasma from one of the hematocrit

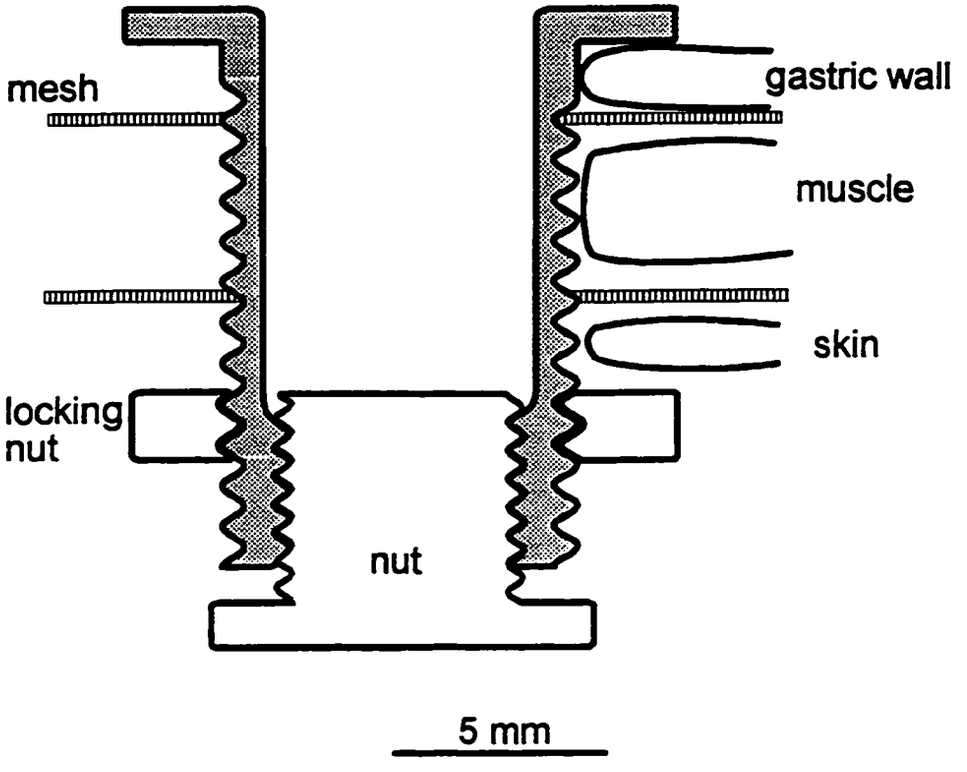

Figure 2.3 Diagram of gastric fistula.

tubes was used to measure protein concentration (refractive index, SPR-T2, Atago Co., Japan). Plasma from the other tube was added to the rest of the sample. The samples were kept at 4°C until osmolality was measured (within 8 hours). Plasma used to determine osmolality was returned to the rest of the sample, and the sample was stored at -20°C until further analysis.

Osmolality was measured as freezing point depression (Osmette model 5004, Precision Systems, Natick, Mass). Sodium, potassium and lithium concentrations were measured with a flame emission spectrophotometer (model 943, Instrumentation Laboratory, Lexington, Mass.) and chloride by coulometry (model CMT 10, Radiometer, Copenhagen, Denmark). Hemoglobin, urea and glucose were measured colorimetrically with commercially available kits from Sigma (Diagnostics kit 525, 640 and 510, Drabkin's reagent, urease-Berthelot reaction, and glucose oxidase method, respectively, Sigma Chemical Co., St. Louis, Mo.). Urine volume was estimated from weight, without correcting for specific gravity.

2.5 Data analysis

Data are presented as means $\pm$ SE. To analyze the data they were log-transformed when large means were accompanied by large standard deviations. Then one or two way ANOVA was used, with or without repeated measures, depending on experimental design. Significant ANOVAs were followed by post-hoc tests. Dunnett's t-test was used to compare treatments or time points to a control, and Tukey's HSD test or Bonferroni's t test to compare all treatments to the others. Differences between means were considered significant if the probability that they occurred by chance was < 0.05.

3 WATER AND SOLUTE BALANCE DURING 10 HOURS OF WATER DEPRIVATION

Lack of drinking water is among the most common threats to the constancy of the body fluids. As explained in the introduction the amount of water in the body is usually kept within very narrow limits. Especially in small animals such as rats turnover of water is rapid, and even short periods of water deprivation will reduce body fluid volume significantly. To survive periods of water deprivation, adaptations are made that reduce the need for water. This set of experiments examined what the adaptations are, how effective they are, and what their effect is on the body fluids.

3.1 Water and solute balance during water deprivation

3.1.1 Introduction Water deprivation stimulates several homeostatic responses. Much has been written about the conservation of water through changes in urine volume and osmolality but these are often small during the early phase of dehydration (Zucker et al. 1982, Thrasher et al. 1984, McKinley et al. 1983c). On the other hand, changes in solute balance may play a more important role than generally appreciated. Animals reduce food intake and increase electrolyte excretion during water deprivation (Wiley and

Wiley 1933, Luke 1973, Zucker et al. 1982, McKinley et al. 1983c, Thrasher et al. 1984, Weisinger et al. 1985). Net solute loss not only helps maintain isotonicity but also influences the distribution of the deficit between fluid compartments.

I measured changes in water and solute balance occurring during water deprivation. To separate the contribution of changes in intake from the other responses, food-matched controls were studied as well.

3.1.2 Methods Nine rats (491±7 gram) were housed in metabolism cages. The rats were water deprived for 10 hours, starting at 24:00 h and ending at 10:00 the next morning. This period included the last 7 hours of the dark period, when water and food intake are normally high (Johnson and Johnson 1990). I measured food and water intake and the change in body weight during this period, and collected all feces and urine. The data were compared with control data obtained the day before, when rats had access to water.

I also measured intake and excretion in these rats when water was available during the 10 hour test period, but food intake was matched to that during water deprivation. At the start of the food matching period the food hopper was emptied, and then each hour I simply added the amount of food the rat ate during the same hour when water deprived. Most rations were eaten before the end of the next hour, so food intake was matched very closely to food intake during water deprivation (see Figure 3.2). The food matching experiment was done 3 days after the water deprivation experiment.

3.1.3 Results Food intake fell during water deprivation (Figure 3.1). Also shown is food intake during food matching. During food matching, feeding closely matched

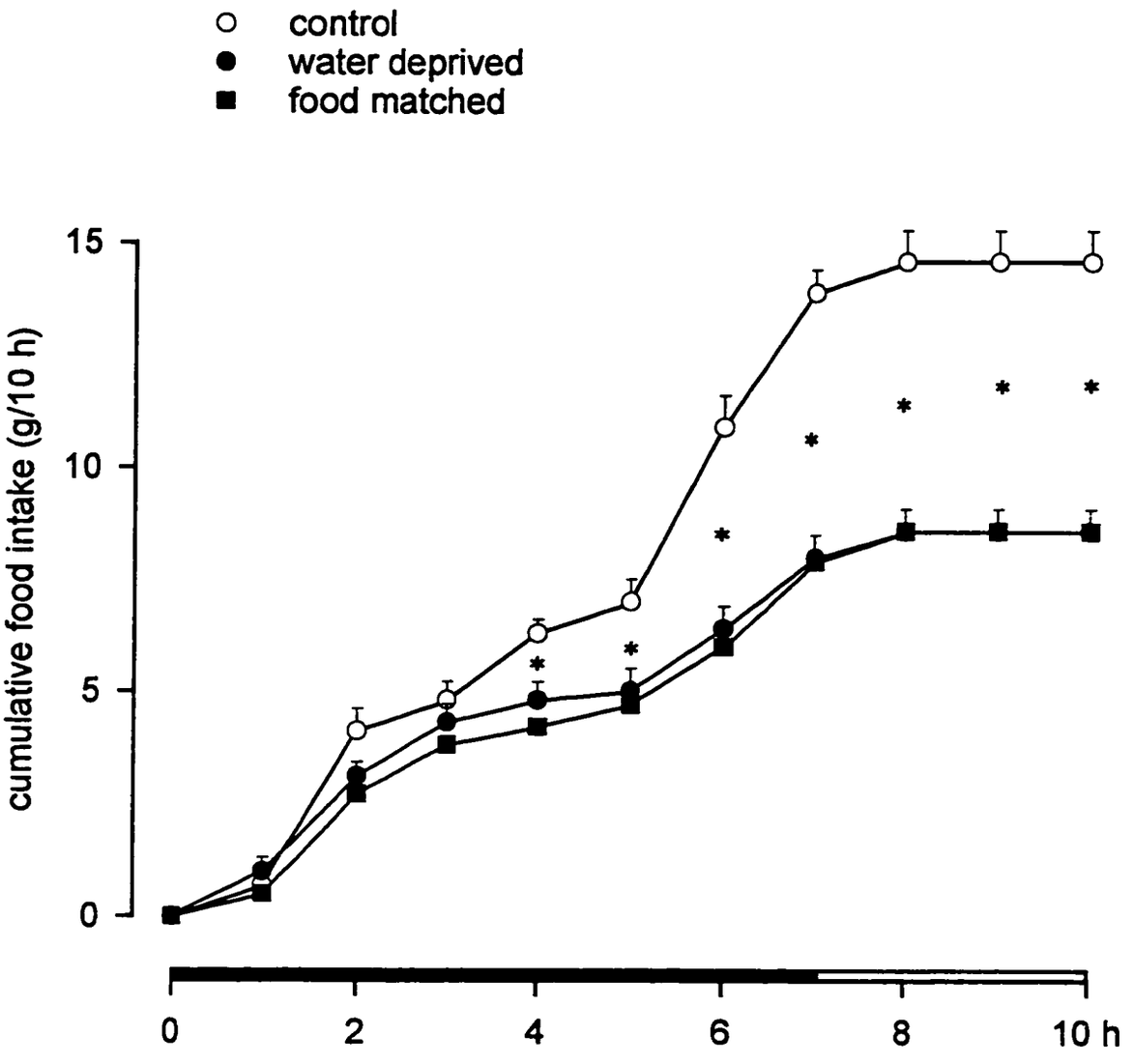

Figure 3.1 Cumulative food intake in rats that either had free access to water and food (control), or were water deprived for 10 hours. Food intake in food matched rats is also shown. During food matching each rat was allowed to eat the amount it took during water deprivation. * indicates difference between water deprived and control ($p < 0.05$, Tukey's HSD test, $n=9$).

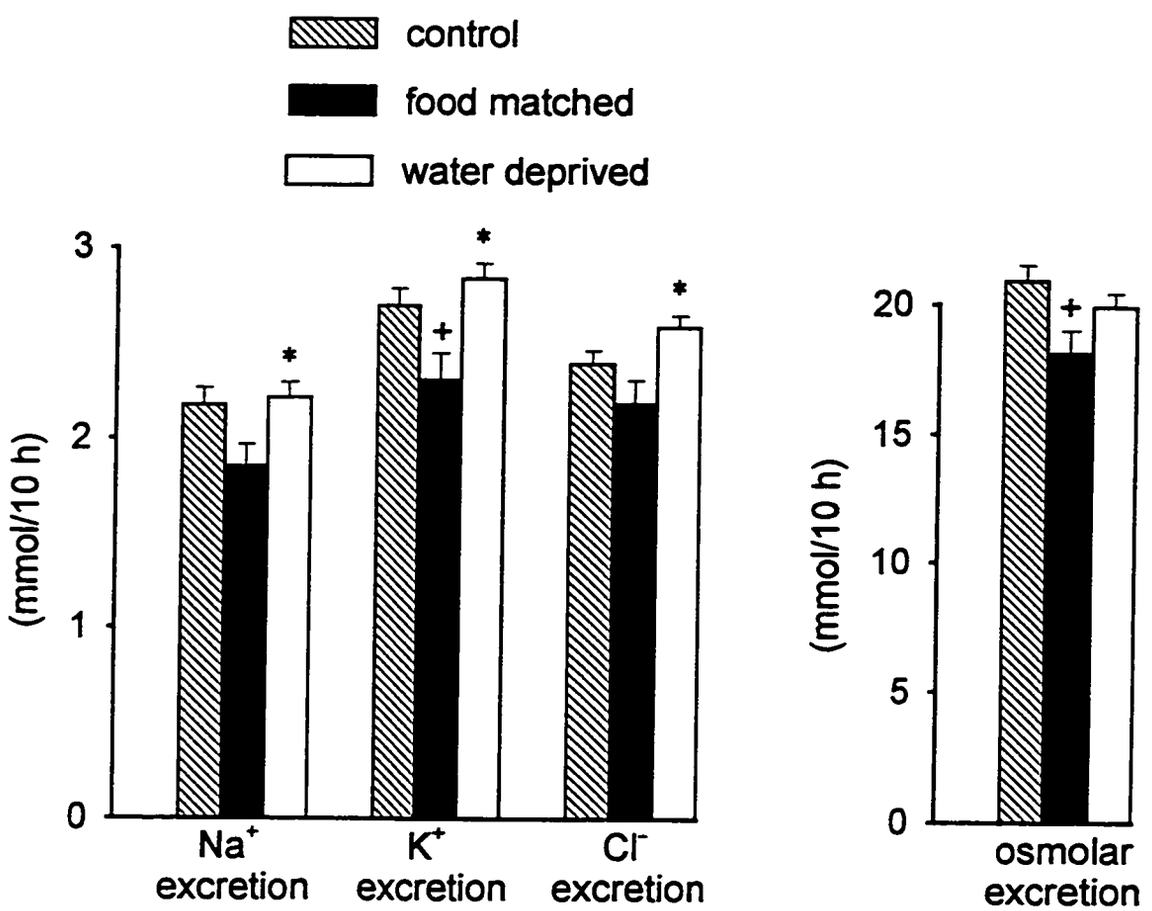

Figure 3.2 Urinary excretion of sodium, potassium and chloride in rats that either had free access to water and food (control), were water deprived, or had food intake restricted to the amount eaten during water deprivation. + indicates difference from control ($p<0.05$, Tukey's HSD test, $n=9$); * indicates difference between water deprived and food matched.

feeding during water deprivation.

Urinary excretion of Na^+, K^+ and Cl^- did not significantly increase during water deprivation, compared with freely feeding controls. However, it is better to compare water deprived rats with food-matched controls, because they had the same salt intake. Water deprived rats excreted more Na^+ (0.36 mmol), K^+ (0.53 mmol) and Cl^- (0.40 mmol) than food matched controls (Figure 3.2).

Water intake and urinary water loss are shown in Figure 3.3. Changes in urine flow during water deprivation were small. Compared to food matched controls, water deprived rats saved only 1.8 $\pm$ 0.7 mL by reducing urinary water loss, equivalent to 0.6% of the estimated body water, assuming 65% of the body weight is water. The result of the changes in intake and excretion was a net water loss of 22 $\pm$ 1 mL, or about 7% of the body water. Urine concentration increased during water deprivation, but remained well below the maximum value reported for rats (Schmidt-Nielsen 1964). Rats drank little during food matching.

3.1.4 Discussion Rats reduced food intake during water deprivation, as many species do (rats, Adolph 1947; camels, Ben Goumi et al. 1993: humans, Engell 1988; doves, McFarland 1964; rabbits, McKinley et al. 1983b). The reduction of food intake sharply reduced the need for water: food restricted rats drank only half as much as food replete controls. Many species reduce water intake during food deprivation (rats Verplanck and Hayes 1953, dogs Cizek 1959), which suggests that they benefit from a reduction in food intake during water deprivation. In some species the change in water intake may underestimate how effective a reduction in food intake is. Rabbits for example reduce food intake during water deprivation (McKinley et al 1983c) but develop polydipsia

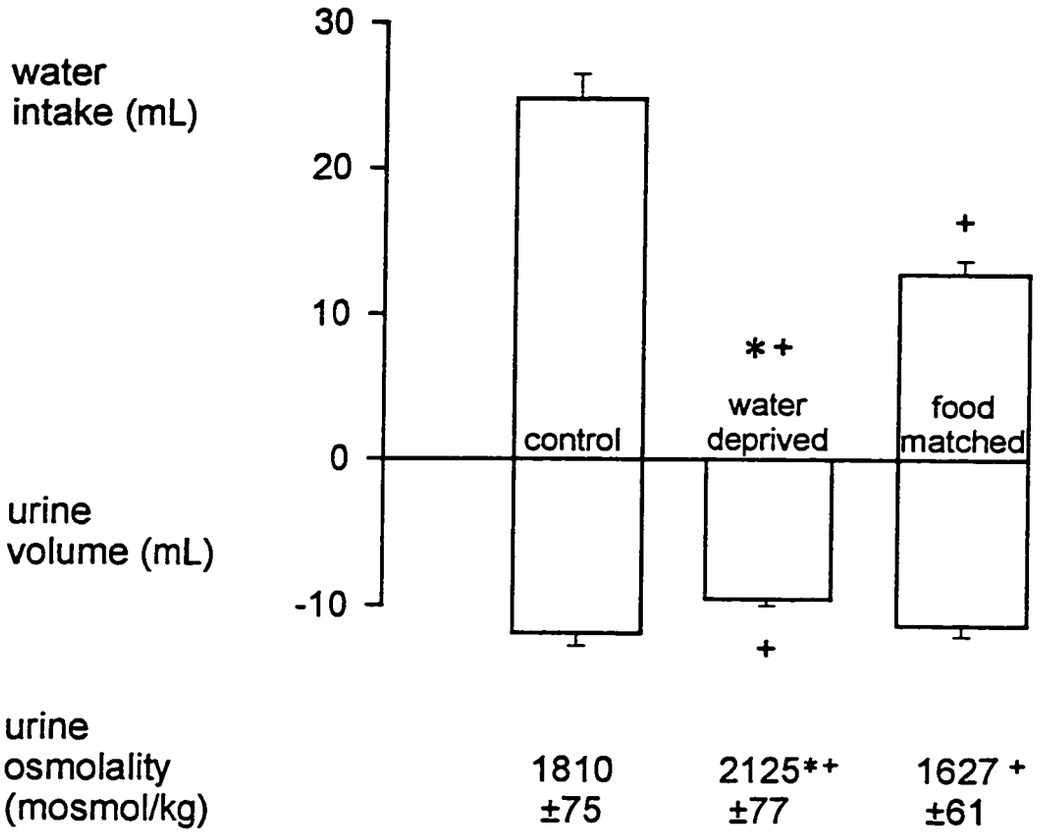

Figure 3.3 Water intake and urine volume and osmolality in rats that either had free access to water and food (control), were water deprived for 10 hours, or had food intake matched to the amount eaten during water deprivation. + indicates difference from control; * indicates difference between water deprived and food matched ($p<0.05$, Tukey's HSD test, n=9).

during food deprivation (Huang 1954).

Water deprived rats excreted more electrolytes than food matched controls. This would help prevent hypertonicity of the body fluids. The differences in electrolyte excretion appear rather small, but they are not negligible. The difference in sodium excretion between water deprived and food matched rats was 0.36 mmol, equivalent to about 2% of the sodium in the ECF (assuming ECF volume was 24% of the body weight, and ECF [Na^+] was 140 mM). The difference in potassium excretion, 0.53 mmol, would be about 2% of the ICF K^+ content (assuming ICF volume was 40% of body weight and ICF [K^+] was 140 mM, see Guyton, 1986). Increased in salt excretion in the rat during water deprivation has been shown before (Luke 1973, McKinley 1983b), although food matching in those studies was not as close. Excretion of urea, the main urinary solute, in water deprived rats does not differ from food-matched controls (Schoorlemmer and Evered 1993). Thus it is not surprising that osmolar excretion during water deprivation and food matching were not significantly different.

Water deprived rats reduced urinary water loss, and excreted a more concentrated urine, but the difference with food-matched controls was small (see Woods and Johnston 1983 for similar results). Water deprived rats excreted their urinary solutes in 9.5 $\pm$ 0.5 mL, while food matched rats would have needed 12.4 $\pm$ 0.5 mL to excrete the same amount of solute. The difference, 2.9 mL on average, is less than 1% of the body water in these rats. The conclusion is that changes in salt balance play a more important role than changes in urinary water loss. This will be discussed in more detail in section 3.4.1.

As indicated in the introduction, much of the water lost from the rat's body is lost as evaporation from skin and lungs (about 50%, see also Fyhn 1979). Does evaporative

Table 3.1 Calculation of insensible weight loss during 10 hours under control, water deprived, or food matched conditions.

	control	water deprived	food matched
weight change (g)	2.7±1.2	-19.6±0.7°*	-10.0±1.0*
water intake (mL)	24.8±1.7	0.0	12.8±0.9*
food intake (g)	14.6±0.7	8.6±0.5*	8.6±0.5*
faecal weight (g)	7.4±0.4	6.7±0.5	7.2±0.3
urine volume (mL)	11.9±0.9	9.5±0.5°*	11.3±0.8
insensible weight loss (g)	17.4±0.8	12.0±0.9*	12.9±0.6*

Data from section 3.1. N=9.

* indicates difference from free feeding controls, Tukey's HSD test, $p<0.05$.

° indicates difference between water deprived and food matched, $p<0.05$.

water loss change during water deprivation? I have not directly measured evaporative water loss. From the data however, we can calculate total insensible weight loss. It is equal to (food intake + water intake - urine volume - fecal weight - change in body weight). These data are shown in Table 3.1. Compared to free feeding controls, water deprivation reduced insensible weight loss by 5 g. Food restriction had the same effect. Insensible weight loss equals evaporative water loss + CO_2 production - O_2 consumption. I did not measure O_2 consumption and CO_2 production but the following calculation shows that it is not likely that changes in O_2 consumption and CO_2 production account for the change in insensible weight loss.

O_2 consumption and CO_2 production can be calculated as follows. To burn 1 gram of carbohydrate, fat or protein, respectively, 1.18, 2.88, and 1.37 gram O_2 are needed (see Appendix 3 in McLean and Tobin, 1987), and 1.63, 2.81 and 1.52 gram CO_2 are produced. When rats were allowed to drink, they ate 14.6 g food during the test period, which contained 7.60 g carbohydrates, 0.80 g fat, and 3.29 g protein (see Table 2.1). If we assume all this food was burned, these rats would consume 15.8 g O_2 and produce 19.6 g CO_2. Gas exchange would account for 19.6 - 15.8 = 3.8 g of the insensible weight loss under control conditions. (If only part of the food was burned, and the rest stored as fat or glycogen, the difference between O_2 consumption and CO_2 production would be even smaller.) Evaporative water loss would be 17.4 - 3.8 = 13.6 mL.

Water deprived rats ate only 8.6 g. By burning this food rats would consume O_2 and produce CO_2, causing a net weight loss of 8.6 / 14.6 * 3.8 = 2.3 g. Burning this amount of food would produce 124 kJ (manufacturer's analysis). Water deprived rats may need more energy than provided by the food, because behavioral activity changes little during short periods of water deprivation (Bolles 1965). To produce the same

energy as water replete rats did, water deprived rats would need an extra 86 kJ (equivalent to 6 g food). Rats could break down 2.2 g body fat to produce 86 kJ (McLean and Tobin 1987, Appendix 3), consuming 6.3 g O_2 in the process, and producing 6.2 g CO_2. Gas exchanges caused by burning fat obviously contribute little to insensible weight loss. Rats could break down 4.9 g glycogen, consuming 5.8 g O_2 and producing 8 g CO_2. Burning glycogen causes an insensible weight loss of 2.2 g. In conclusion, during water deprivation the insensible weight loss due to gas exchange would probably be between 2.1 and 4.4 g, depending on the amount and composition of the fuel burned. Evaporative water loss would be between 7.6 and 9.8 mL, (compared to 13.6 mL in rats allowed to drink).

Although these estimates of O_2 consumption and CO_2 production are probably not very accurate, the calculations do suggest that the contribution of gas exchange to the observed insensible weight losses is small. More importantly, *changes* in gas exchange probably do not account for the reduction in insensible loss during water deprivation, although the calculations suggest such changes may contribute.

The fact that insensible weight loss fell during food restriction, just as it did during water deprivation, suggests that the reduction of evaporative water loss is probably caused by a reduced food intake. It may be beneficial to reduce evaporative water loss during food deprivation because that saves energy (evaporation of 1 mL water requires 2.4 kJ). Haines and Shield (1971) found mice reduced evaporative water loss after a few weeks of water restriction (see also Fyhn 1979 for review). Water loss from the skin fell in their experiment but respiratory water loss did not change. However the mechanism for reduced evaporative water loss in their experiment differs from mine: it was not caused by a reduction of food intake. Food intake in their experiment did not change.

3.2 Dehydration anorexia reduces water and solute content of the gut

3.2.1 Introduction As shown in section 3.1, rats eat less during water deprivation, which reduces their need for water. There are two ways in which the gut can play a role in the reduction of the need for water. First, the gut is a large fluid compartment. Changes in its water content can have significant effects on the rest of the body. Second, a reduction in food intake reduces the amount of salt to be excreted. The amount of waste salt depends on the rate of salt absorption from the gut. A nice example of the importance of gut water and salt in body fluid regulation is given by Darlington et al. (1995). They found that after hemorrhage, rats were able to restore blood volume with water from the gut. Rats previously food deprived were not able to restore blood volume because they had only little water in the gut.

To investigate if changes in gut contents help reduce the need for water during water deprivation I measured gut water and salt content after water deprivation, and compared it with gut content in water and food replete controls and with food matched controls.

3.2.2 Methods Rats (504 $\pm$ 15 g) were housed in metabolism cages. Gut content was measured in rats killed at midnight (n=5), or at 10:00 in the morning, after 10 hours of water deprivation (n=5). It was also measured at 10:00 h in rats that had free access to water and food (n=4) and in rats (n=5) that had access to water, but had food intake matched to the amount eaten by the water deprived rats. Food matched rats hourly received the amount of food eaten by the water deprived rat that they were paired to. As in section 3.1, the rations were usually consumed within one hour.

To measure gut contents, rats were killed with an overdose of ether. The content of the gastrointestinal tract (from stomach to colon, inclusive) was removed, frozen, and lyophilized. Gut water content was calculated from the weight change during lyophilizing. The solid matter was digested for 3 days with 4 mL concentrated nitric acid and diluted to 40 mL with distilled water for determination of sodium and potassium.

As in section 3.1, I also measured urinary changes, changes in body weight, and food and water intake in these rats.

3.2.3 Results Effects of the treatments on gut contents are shown in Figure 3.4. Wet and dry weight, and water and solute content were similar in control groups killed at the beginning and end of the experimental period. Water deprived rats had less water, solute and dry matter in the gut. Gut contents in food matched controls were similar to that in water deprived rats. The fraction of water in the gut contents was higher in food matched controls, but the difference with the other groups was small. Water and food intake and solute excretion were similar to the values measured in section 3.1 (see Table 3.2), but most of the urinary changes failed to reach significance.

3.2.4 Discussion Water deprivation reduced the amount of water and solute in the gut, as has been demonstrated before by Kutscher (1966). Identical changes were seen in food matched rats. This indicates that the change in gut content seen after water deprivation is caused by a reduction in food intake.

The data show two ways by which lowered food intake reduces the need for water. First, during food matching the amount of gut water fell. The explanation is

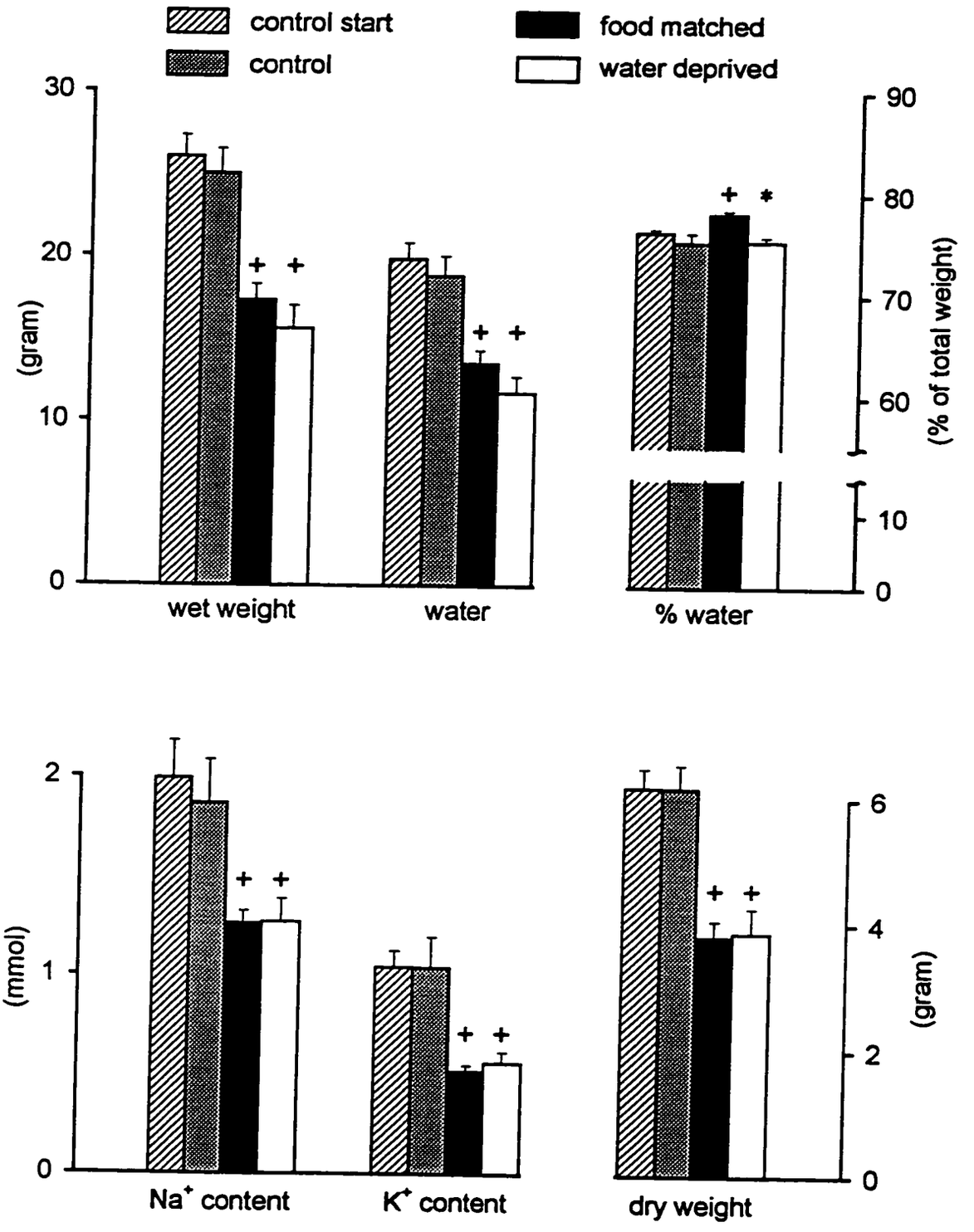

Figure 3.4 Effect of water deprivation and food restriction on gut content. Gut content was measured in rats that had free access to water and food (control, n=4), were water deprived for 10 hours (n=5), or had food intake restricted to the amount eaten during water deprivation (n=5). Gut contents before deprivation is shown also (control start, n=5). + indicates difference from control ($p<0.05$, Tukey's HSD test). * indicates difference between water deprived and food matched.

Table 3.2 Intakes, excretion, and weight changes during 10 hours under control, water deprived, or food matched conditions.

	control	water deprived	food matched
weight change (g)	2.7±1.2	-25.0±1.1*°	-9.9±2.0*
water intake (mL)	24.5±1.8	—	13.9±1.4*
food intake (g)	14.9±0.6	8.8±1.8*	8.5±0.4*
faecal weight (g)	7.8±0.9	6.9±0.6	7.8±0.9
urine volume (mL)	11.0±1.1	10.2±0.9	11.3±1.5
insensible weight loss (g)	17.9±1.4	15.8±2.1	13.1±0.7
urine osmolality (mosmol/kg)	2044±152	1955±102	1672±100
sodium excretion (mmol)	2.65±0.14	2.89±0.21°	2.15±0.16
potassium excretion (mmol)	3.63±0.30	3.43±0.33	3.06±0.15
chloride excretion (mmol)	3.28±0.25	3.28±0.29	2.56±0.15
osmolar excretion (mosmol)	22.2±1.6	20.0±2.1	18.1±1.1

Data from experiment 3.2. Control n=4, water deprived n=5, food matched n=5.
* indicates difference from free feeding controls, Tukey's HSD test, $p<0.05$.
° indicates difference between water deprived and food matched, $p<0.05$.

straightforward. As little food entered the gut during food restriction, and absorption of solute already in the gut continued, the amount of solute in the gut fell. An osmotic gradient formed that promoted movement of water from the gut to the rest of the body. The amount of salt in the gut depends on dietary salt intake, on salt secreted in the gut with digestive juices, and on the rate of absorption of salt from the gut. Obviously dietary salt intake was reduced, but it seems likely that the secretion of digestive juices was reduced as well, because feeding stimulates release of saliva, gastric acid (Debas 1987) and intestinal secretions (Flemström 1987).

Secondly, reduced food intake reduced the solute load to the tissues, as the following calculation shows. Food matched rats ate 1.1 mmol sodium and 1.9 mmol potassium less than controls (6.4 g food containing sodium 167 mmol/kg and potassium 297 mmol/kg). Gut sodium and potassium contents were lower in food matched rats, about 0.6 and 0.5 mmol compared with controls. Therefore, the reductions in sodium and potassium load to the tissues would be about 0.5 and 1.4 mmol, respectively. Less solute means less water is needed to dilute it to isotonicity and subsequently to excrete it. (Fecal content normally contributes little to solute balance and was not included; Möhring and Möhring, 1972).

3.3 Effect of water deprivation on blood solutes

3.3.1 Introduction Water deprivation causes changes in the size of the body fluid compartments. Water deprivation also alters food intake and salt excretion, causing changes in salt balance. Changes in solute balance influence the distribution of the water

in the fluid compartments. For example, since almost all sodium is found in the ECF, net loss of sodium will cause water to flow from ECF into the cells. What is the effect of the changes in water and solute on the body fluid compartments? I measured the effect of water deprivation on plasma osmolality and [Na^+], and on indicators of changes in blood volume.

3.3.2 Methods Blood samples were taken after 10 h water deprivation (starting at 24:00 h) from 21 rats (383 $\pm$ 13 g) with venous and bladder cannulas, and compared with samples taken at the same time the previous day.

3.3.3 Results Changes in blood solutes after water deprivation are shown in Figure 3.5. There were significant increases in the indicators of changes in blood volume. Water deprivation increased hematocrit by 5.2 $\pm$ 0.8% and plasma protein concentration by 8.3 $\pm$ 1.0% (both $p<0.001$, $n=21$). Hemoglobin changes were measured in 14 of these rats. Hemoglobin changed from 14.3 $\pm$ 0.3 to 15.1 $\pm$ 0.3 g/dL ($p<0.001$), an increase of 5.6 $\pm$ 1.0%. Hematocrit in these 14 rats increased by 5.9 $\pm$ 1.1%.

Changes in blood tonicity were small. Plasma osmolality did not change significantly (0.3 $\pm$ 0.5%) and plasma [Na^+] increased only 1.7 $\pm$ 0.5% ($p<0.001$, $n=21$).

Water deprivation also reduced plasma [K^+] from 4.7 $\pm$ 0.07 to 4.3 $\pm$ 0.05 mmol/L ($p<0.001$, $n=21$). Plasma [urea] did not change significantly (from 7.5 $\pm$ 0.3 to 7.8 $\pm$ 0.3 mmol/L, $n=14$), and neither did plasma [Cl^-] (from 101.8 $\pm$ 1.4 to 104.1 $\pm$ 1.5, $n=7$).

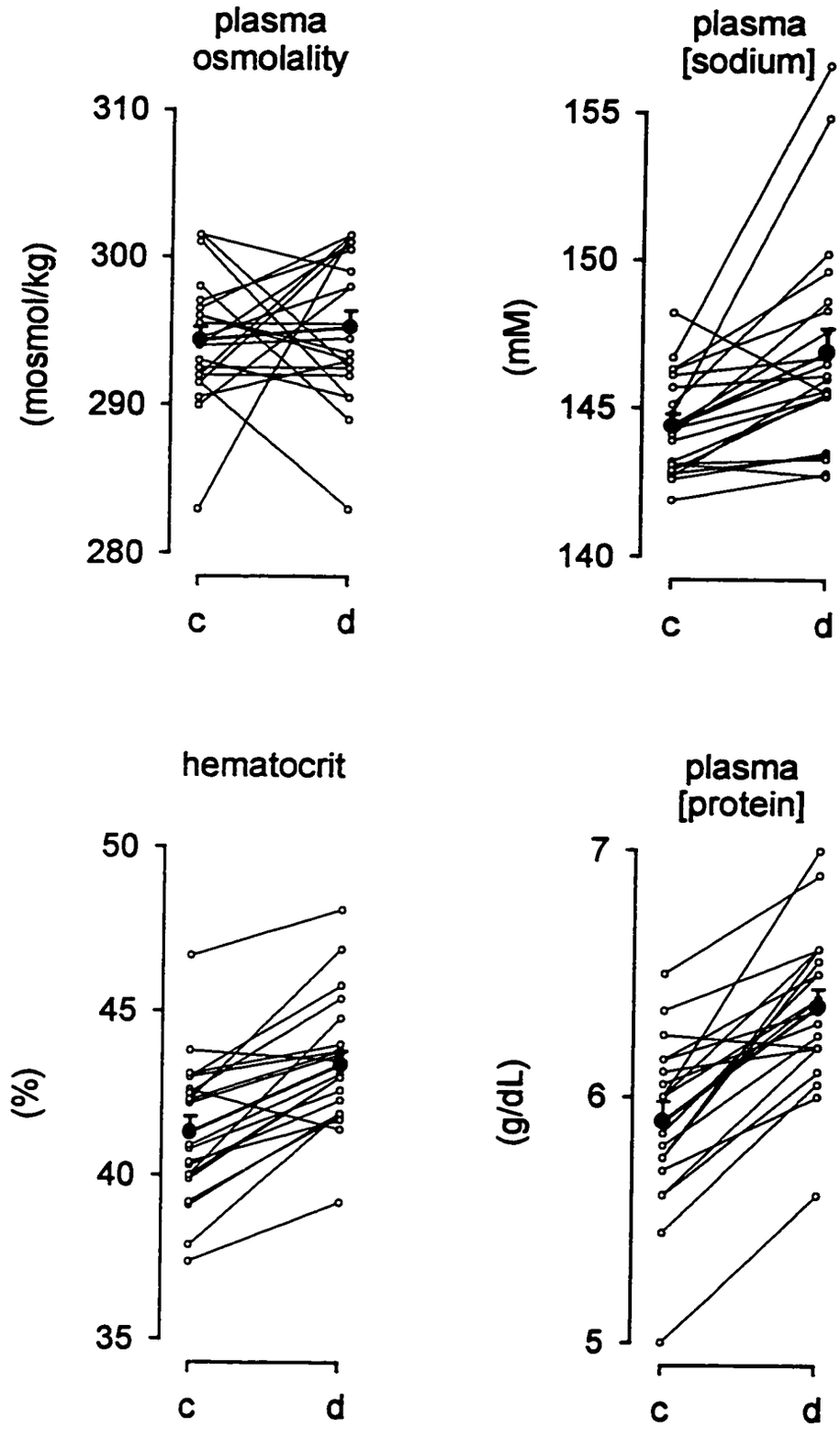

Figure 3.5 Blood solutes of individual rats under control (c) conditions and after 10 hours of water deprivation (d). Thick line indicates mean of all 21 rats.

3.3.4 Discussion During water deprivation, hematocrit, blood hemoglobin and plasma protein all increased, indicating blood volume fell. Using the formulas given in the Appendix we can estimate the changes in blood volume in the 14 experiments in which both hematocrit, plasma protein and hemoglobin were measured. The change in hematocrit suggests blood volume fell by 6.7 ± 0.9% (equation 1 in the Appendix). The change estimated from hemoglobin is similar (6.5 ± 0.9%, equation 2 in the Appendix). Changes in the size of the red cells therefore did not seem to influence the results. Plasma protein data suggest that blood volume fell by 4.6 ± 1.1% (equation 3 of the Appendix). Changes in blood volume calculated from changes in hematocrit correlated strongly with those calculated from hemoglobin ($r=0.77$, $n=14$, $p<0.01$), but not with those calculated from plasma protein ($r=-0.25$, $n=21$, not significant). As described in the Appendix, when the experimental period is long, estimates of changes in blood volume based on hematocrit or hemoglobin appear more reliable. Therefore the data suggest blood volume fell by 6-7% during water deprivation.

The changes in plasma osmolality and $[Na^+]$ were much smaller. Other researchers also found only small changes in plasma osmolality and sodium concentration during short or moderate periods of water deprivation (Kutscher 1966, Hatton 1971, Hall and Blass 1975, Ramsay et al. 1977). Clearly tonicity is a well guarded variable during water deprivation.

Increased plasma tonicity causes cellular dehydration, because water moves out of the cells. Note however, that changes in plasma tonicity are not an accurate indicator for intracellular volume if the amount of intracellular solute changes. As section 3.1 shows, potassium is lost from the body during water deprivation, probably from the intracellular fluid. Therefore the reduction of intracellular fluid volume likely is larger than the 1-2%

that would be expected from the increase in plasma tonicity and sodium concentration. The next section (3.4) will give a more accurate estimate of the changes in intracellular fluid volume.

3.4 General discussion

The relatively brief deprivation period chosen here is probably not an uncommon event in the natural environment. Water was withdrawn at midnight to include 7 h of the dark period when water and food intake are normally high. Deprivation ended 3 h into the light period, when basal levels of intake are normally low (Johnson and Johnson, 1990). Even over such short periods the need for water is considerable: the rats in section 3.1, when allowed to drink, took between 19 mL and 34 mL during this period. This corresponds with 5-10% of the total water content of these rats, assuming 65% of the rat's body is water. Assuming solute balance and water losses do not change, this would increase body fluid tonicity by 5-10%. Such large increases in plasma tonicity are seen only after several days of water deprivation (Kutscher 1971, Hatton 1971). The changes in plasma tonicity that were measured after 11 hours of water deprivation were much smaller, about 1 or 2%.

3.4.1 Role of changes in salt and water quantified. Several responses contribute to osmoregulation during water deprivation. Their relative contribution to osmoregulation is estimated below.

During water deprivation food intake fell. The reduction of food intake during water deprivation reduced the need for water: the food matched rats from section 3.1 reduced water intake by 11.9 mL or 48%, and the rats from section 3.2 by 10.6 mL or 43%. Rats were able to absorb water from the gut when they reduced food intake, and also absorbed less solute (section 3.2). Reduced food intake may also reduce evaporative water loss (section 3.1).

Water deprived rats achieve some savings beside those resulting from reduced food intake. To estimate these savings, we should compare water deprived rats with food matched controls.

Compared to food matched controls, water deprived rats increased the excretion of osmotically active solutes. How much does this contribute to osmoregulation? Rats in section 3.1 increased excretion of sodium by 0.36 mmol and potassium by 0.53 mmol during water deprivation, compared to food matching. It would take 6.4 mL of fluid to dilute this amount to the concentrations found in the body (sodium 140 mM, potassium 140 mM). However these rats needed only a very small volume of water to excrete this amount of solute: about 2.1 mL ($[Na^+]$ and $[K^+]$ in the urine were 221 and 284 mM on average, respectively). Thus increased salt excretion caused a saving equivalent with 4.3 mL water. (Rats from section 3.2 would need 7.9 mL to dilute the solute, but only 2.6 mL to excrete it).

Compared to food matched controls, water deprived rats increased urine concentration. The water deprived rats from section 3.1 saved 2.6 mL by excreting the amount of solutes that was excreted by the food matched controls in a smaller volume (urine osmolality was 1641 mosmol/kg in food matched rats, but 2117 mosmol/kg during water deprivation). Similarly, the rats from section 3.2 saved 1.6 mL.

Table 3.3 Relative importance of changes in food intake, salt intake and water excretion to osmoregulation during water deprivation.

	section 3.1	section 3.2
NEEDED		
normal water intake (mL)	24.8±1.5 (100%)	24.5 (100%)
SAVING BY		
reduced food intake (mL)	11.9±1.6 (48%)	10.6 (43%)
increased salt excretion (mL)	4.3±1.2 (17%)	6.4 (26%)
increased urine concentration (mL)	2.6±0.5 (10%)	1.6 (7%)
ESTIMATED DEFICIT (mL)	5.9±1.7 (24%)	5.9 (24%)
estimated increase of plasma osmolality (%)	1.8±0.5%	1.8%

During 10 h of water deprivation rats reduced food intake, increased salt excretion, and produced a more concentrated urine. These responses reduced the amount of water needed to maintain plasma tonicity from about 25 mL (the normal water intake during the test period) to 6 mL. A 6 mL deficit would cause an increase in plasma tonicity of about 2% in these rats, which is close to the value found in section 3.3. Note that changes in food intake and salt excretion played a much more important role in osmoregulation than reduced urinary water loss did. (See section 3.4 for more information on the calculations used).

These data are summarized in Table 3.3. The reduction in food intake reduced the need for water by almost 50%, increased excretion of Na^+, K^+ and Cl^- contributed another 20%, and increased urinary concentration contributed about 10%. Clearly the main osmoregulatory response to water deprivation is a loss of electrolytes, and reduction of urinary water loss is of very minor importance. As a last remark, note that the expected rise in plasma osmolality after accounting for the changes in water and solute loss, would be about 2%, which is close to the increase measured in section 3.3. Plasma $[Na^+]$ increased 1.7% in the rats in section 3.3, and plasma osmolality increased 0.3%.

That changes in food intake can play an important role in osmoregulation is also clear from work from Houpt and Yang (1995). When these authors water deprived pigs, plasma tonicity increased, as expected. In pigs deprived of food as well as water, plasma osmolality did not rise at all. Similar results were found in rats by Kutscher (1971). That changes in salt excretion play an important role in osmoregulation is suggested by a paper by McKinley et al. (1983c). The authors blocked dehydration natriuresis in sheep with a lesion in the hypothalamus. When these sheep were water deprived for 2 to 3 days, plasma $[Na^+]$ rose to extremely high levels, while unlesioned controls developed only a moderate hypernatremia. Because the lesions did not alter food intake or urinary water loss the authors concluded that the hypernatremia was caused by reduced salt excretion.

3.4.2 Estimate of changes of the body fluid compartments. During water deprivation water is lost from the body. Where does it come from? The following part of the discussion estimates the effect of water deprivation on the size of the different body fluid compartments.

If we account for changes in food intake and faecal loss, change in body weight estimates the water deficit in water-deprived animals. I compared water-deprived rats with food-matched controls drinking freely, since their food intakes, faecal losses and gut contents were similar. The difference in weight loss averaged 10 g in the 490 g rats of experiment 3.1 and 15 g in the 500 g rats of experiment 3.2, 2 and 3% of body weight, respectively. Note that reductions in gut water are not included in this estimate of the water deficit.

Solute loss affects the distribution of fluids between intracellular and extracellular compartments. Since sodium is lost mainly from the extracellular fluid, increasing sodium loss increases the proportion of the water deficit lost from this compartment (see Rose 1984). We can calculate the change in ECF volume as follows. Assume that pre-deprivation ECF volume of the rats from section 3.2 was about 121 mL (24% of body weight, see Barratt and Walser 1969). If plasma [Na^+] was 145 mM, ECF sodium content in these rats would be 17.5 mmol. During water deprivation these rats lost 0.7 mmol sodium, which would reduce ECF sodium content to 16.7 mmol. (Water deprived rats excreted about 0.7 mmol more sodium than food matched controls. Alternatively, we can compare sodium balances in free-feeding controls and water deprived rats, taking gut changes in account; the difference was also 0.7 mmol.) If we assume ECF [Na^+] increased to 148 mmol/L as in section 3.3, ECF volume after water deprivation would be 16.7/0.148 = 114 mL. This suggests ECF volume fell by 7 mL. This is about 45% of the total 15 mL fluid deficit in these rats.

Similar results are obtained for the rats of section 3.1 (dehydrated rats lost 0.4 mmol sodium more than food-matched controls, giving a calculated ECF deficit of about 4.8 mL, 4% of ECF volume, and 48% of the total 10 mL deficit). These estimates of the

ECF deficits are close to the blood volume depletion estimated from the changes in hematocrit and hemoglobin concentration (about 6.5%, see section 3.3).

Thus, it appears from these calculations and measurements that nearly half (45%) of the total water deficit in 10 h water deprived rats comes from the ECF, depleting this compartment about 6%. The rest (55% of the total deficit) must be from the intracellular fluid (ICF). Since ICF volume is larger than that of ECF, the relative depletion would be smaller. From the size of the deficit observed here and assuming ICF is about 40% of body weight, we can estimate that water deprivation reduced ICF volume by 4% in the rats of section 3.2, and by 3% in the rats used in section 3.1.

In summary, reduced food intake and increased salt excretion play an important role in osmoregulation during water deprivation. In contrast, reduction of urinary water loss plays only a very minor role, at least during the short deprivation period used in this study (see Table 3.3). Water deprivation reduced both ICF and ECF volume, but the relative depletion of the ECF is larger.

4 WATER AND SOLUTE BALANCE DURING REHYDRATION WHEN FOOD IS NOT AVAILABLE.

4.1 Introduction Section 3 showed that during water deprivation both water and electrolytes are lost from the body. This section (and section 5) shows how the deficits are restored. I measured water balance, urinary changes and changes in body fluid composition when water was returned to water deprived rats. The results show that changes in solutes play an important role in osmoregulation during rehydration as well as during water deprivation.

4.2 Methods Ten rats (body weight 353 ± 14 g) with permanent bladder and venous cannulas were water deprived for 11 hours, starting at 24:00. The deprivation period included the last 7 hours of the dark period. The next morning, rats were transferred to restraining cages (section 2.2), where water (but not food) became available. Water intake was measured. Urine samples were collected from 1 hour before water was returned until the end of the experiment, five hours later. The urine samples were compared with control samples taken the day before deprivation in the same rats and with samples from another 6 rats (330 ± 8 g) that had been water deprived but were not allowed to drink (or eat) during the sampling period. Blood samples (0.35 mL each)

were taken from venous cannulas in 8 of the rats. Blood samples were compared with samples collected the day before, when water was available.

4.3 Results Rats drank immediately when water was provided. Cumulative water intake is shown in Figure 4.1. Rats drank about 60% of their total 4 h intake in the first 10 min. Because during the remainder of the experiment urine volume was similar to water intake, water balance, calculated as intake minus urine volume, at the end of the experiment was similar to that 10 min after drinking.

Urine flow after water was returned was variable between rats, but was related to the amount of water drunk. There were significant correlations between water intake during the first 10 min and urine volume over the 4 hour period after drinking started ($r=0.84$, $n=10$, $p<0.005$) and between total water intake and urine volume over the 4 h period ($r=0.72$, $n=10$, $p<0.05$). As a result, at the end of the experiment there was little difference in water balance between rats that drank much or those that drank less.

Changes in blood solutes during rehydration are shown in Figure 4.2. Plasma osmolality and $[Na^+]$ fell within 20 min after drinking began. By 1 h both were well below the pre-deprivation values recorded the previous day. Plasma chloride concentration fell gradually (from 106.2 ± 2.4 to 100.9 ± 2.7 at the end of the experiment) but the change was not significant.

Hematocrit and plasma protein fell after drinking. As explained in the Appendix, changes in hematocrit and plasma protein can be used to estimate changes in blood volume. I used formulas 1 and 3 from the Appendix to calculate the estimates shown in Figure 4.2. Blood volume fell during water deprivation. Protein changes indicate blood volume increased after drinking. At the end of the test period, blood volume estimated

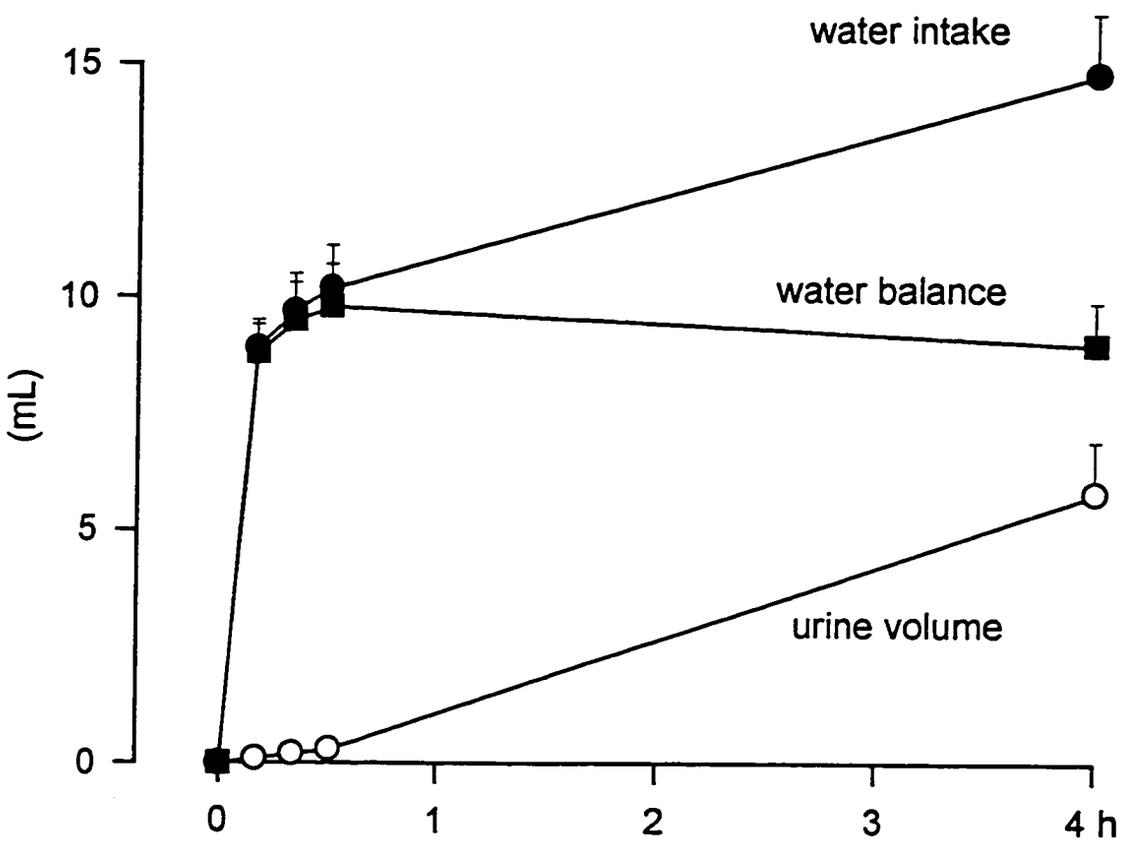

Figure 4.1 Cumulative water intake, cumulative urine volume and water balance (calculated as water intake - urine volume) in rats offered water (but no food) after 11 hours of water deprivation. n=10.

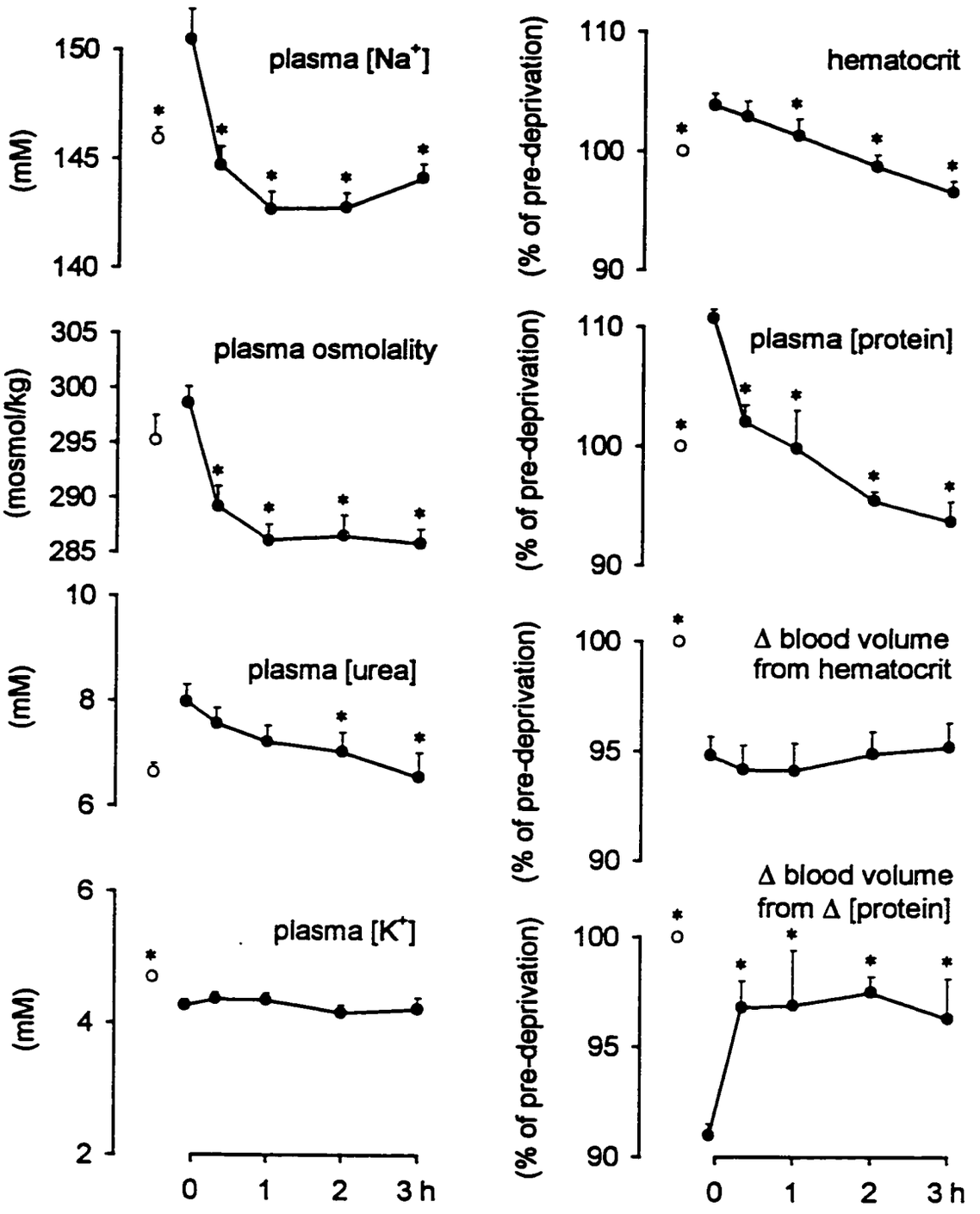

Figure 4.2 Plasma solutes and estimated blood volume after rehydration. Open symbols indicate pre-deprivation values. Water was returned at t=0 hours. * indicates difference from water deprived value at t = 0 h, just before drinking water was returned ($p < 0.05$, Dunnet's t test, n=8).

from protein changes did not differ significantly from pre-deprivation level. Hematocrit changes in contrast suggest drinking did not influence blood volume. Blood volume, estimated from hematocrit changes remained lower than before water deprivation. Hematocrit changes may underestimate blood volume changes after rehydration because osmolality fell. This would cause swelling of the red cells.

Figure 4.3 shows excretion rates and urine osmolality before water deprivation, after water deprivation and after water was returned. After 11 hours of water deprivation urine flow was lower and urine osmolality had increased (both $p<0.01$, t test, $n=16$).

In water deprived rats not permitted to drink, urine flow and osmolality remained stable throughout the five hour observation period. In these rats solute excretion rates fell during the first hour, but remained stable afterward. This control group tended to excrete a more dilute urine, and their urine flow tended to be higher than in the experimental group, but the difference was not significant.

Drinking caused rapid and large changes in water and solute excretion. Excretion of Na^+, K^+ and Cl^- fell within 20-40 min. Electrolyte excretion continued to fall to very low levels. Excretion of Na^+ and Cl^- fell by over 90%, and K^+ excretion fell by 75%. Urea excretion after rehydration did not differ from that of controls that received no water.

4.4 Discussion When water was returned, rats began drinking immediately. After the first 10 min water intake balanced urinary water loss and net water gain remained fairly stable. Net water gain at the end of the 4 h drinking period was 9 ± 1 mL. Water drunk after water deprivation is absorbed rapidly. Hall and Blass (1975) sacrificed rats 30 min after the drinking period started, and found that only 40% of the 10 mL

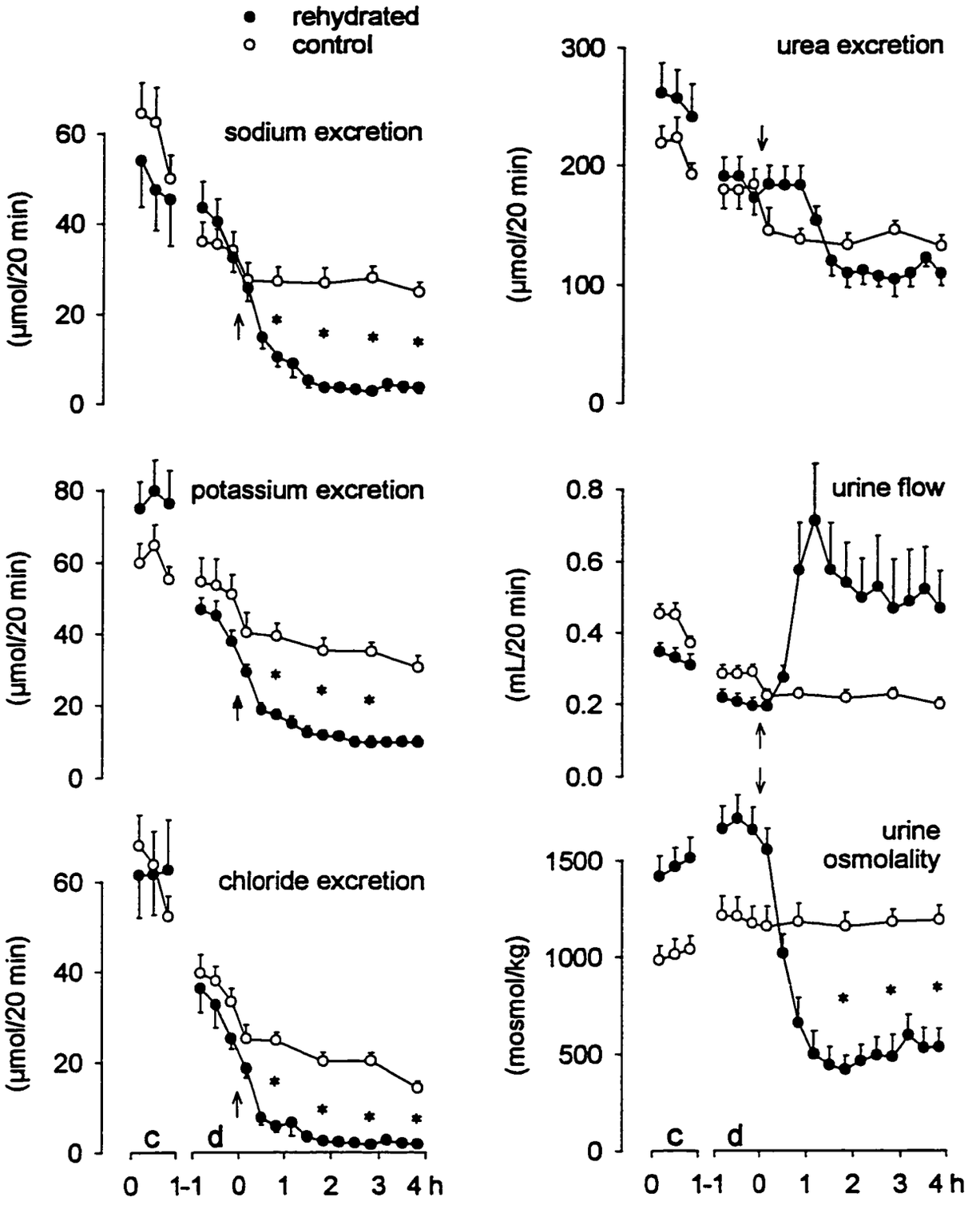

Figure 4.3 Water and solute excretion before and after 11 hours of water deprivation and after rehydration. c indicates pre-deprivation value, d is water deprived. At t = 0 h (arrow) drinking water was returned to one group, but control rats were not allowed to drink. Food was not available during the sampling period. * indicates difference from controls not allowed to drink ($p < 0.05$, Bonferroni t test, rehydrated n=10, control n=6).

drunk remained in the GI tract. Because water absorption is rapid we can calculate the expected changes in osmolality caused by the net water gain. The rats contained about 230 mL water (65% of the body weight), and adding 9 mL would decrease osmolality and $[Na^+]$ of the body fluids by almost 4%. This is close to the change observed in Figure 4.2. It caused plasma osmolality and $[Na^+]$ to fall below pre-deprivation values. Others have also observed that rats drink sufficient water to cause hypotonicity after water deprivation (Hall and Blass, 1975).

Did drinking restore the fluid deficits? Since no food matched controls were used in this experiment there is no good estimate of the water deficit in these rats. Normal water intake (26 ± 1 mL) and urine volume (13 ± 1 mL) during the 10 hour deprivation period in these rats was similar to the rats used in sections 3.1 and 3.2 (see Table 3.1 and 3.2). Food intake in these rats fell by 54% during water deprivation, which is more than in sections 3.1 and 3.2. It seems reasonable to assume the water deficit was similar or slightly less than in sections 3.1 and 3.2 (10 and 15 mL, respectively). The net water gain was probably close to the deficit.

Since rats ingested no solute with the water, the water gained would be distributed between ICF and ECF according to their relative volumes (Rose 1984). ECF volume is about half the volume of the ICF, hence 1/3 of the gained water, about 3 mL, would remain in the ECF, and 6 mL would enter the cells. Assuming that, as in section 3, water deficit was 10-15 mL, and 55% of this deficit was ICF, suggests ICF deficit after water deprivation was 5.5-8.3 mL, and ECF deficit 4.5-6.7 mL. This suggests ICF volume was almost or completely restored after drinking, but a small ECF deficit may have remained. This is confirmed by the tendency of blood volume to remain low after drinking (see also section 5 and Hall and Blass 1975).

Given these estimates about the fluid loss during water deprivation, and how drinking helps restore the deficits, the finding that rats overdilute the body fluids after drinking is not surprising. Drinking almost completely restored the fluid deficits. To restore fluid deficits, rats must drink enough to cause overdilution of the body fluids, because solute is lost during water deprivation. Also, because during water deprivation, relative loss of sodium from ECF was larger than relative loss of potassium from the ICF, it may be difficult to restore both fluid compartments at the same time. To restore both ICF and ECF volume, the ratio of intracellular to extracellular solute has to be restored.

There was a large reduction in Na^+, K^+ and Cl^- excretion after rats began drinking. Total excretion of sodium after drinking started was 0.09 ± 0.01 mmol, while rats that were not allowed to drink excreted 0.28 ± 0.03 mmol sodium during the same period. The savings, 0.19 mmol, is about 1.5% of the total sodium content of the ECF in these rats (assuming ECF volume was 24% of body weight, and ECF $[Na^+]$ was 140 mM, ECF sodium content would be 12.7 mmol). Potassium excretion was reduced by 0.20 mmol. Solute retention after rehydration has been observed before (Hall and Blass 1975, Rolls et al. 1980, McKinley et al. 1983b, Thrasher et al. 1984, Weisinger et al. 1985). Electrolyte retention began within 20-40 min and continued for the duration of the experiment. This is faster than the change observed by Hall and Blass (1975), but since their rats did not have bladder cannulas, the time course could not be determined with as much precision. Retention of Na^+, Cl^- and K^+ is appropriate for an animal that has lost these electrolytes during dehydration. Weisinger et al. (1985) have shown that rats also increase intake of NaCl solutions after water deprivation and possibly increase intake of KCl as well.

In conclusion, these findings confirm the importance of changes in solute balance to fluid regulation. After water deprivation rats drank enough to restore their fluid deficit. Because the solute deficit incurred during water deprivation was not restored, drinking caused plasma hypotonicity and hyponatremia. Water deprivation reduces especially ECF volume, but much of the ingested water does not remain in the ECF, but enters the cells instead. To restore both body fluid volume and tonicity, and both ICF and ECF volume, solutes would be needed as well as water. In the experiment solutes could not be restored, because food was not available. However, the renal changes seen on rehydration, i.e. solute retention, would help to prevent further losses.

5 WATER AND SOLUTE BALANCE DURING REHYDRATION WHEN FOOD IS AVAILABLE.

5.1 Introduction In section 4, water balance, urinary changes and body fluid changes were measured when water was returned after water deprivation. Rehydration caused salt retention, which would contribute to restoration of salt balance and osmoregulation. Normally, water deprived rats given water eat shortly after drinking. This is appropriate, since they reduce food intake during water deprivation. With the food they ingest salt as well. This section investigates drinking, urinary and body fluid changes when water as well as food are returned after water deprivation. The results show that intake of food alters the renal responses to rehydration, and that these changes contribute to the restoration of the internal environment.

5.2 Methods Experiments in this section were done on 7 rats (398 $\pm$ 24 g) with bladder and venous cannulas. They were water deprived for 11 hours, starting at 24:00. The next morning they were transferred to restraining cages where either water or water and food became available. All rats received both treatments in mixed order. Water and food intake was measured and blood samples (0.35 mL each) and urine were collected.

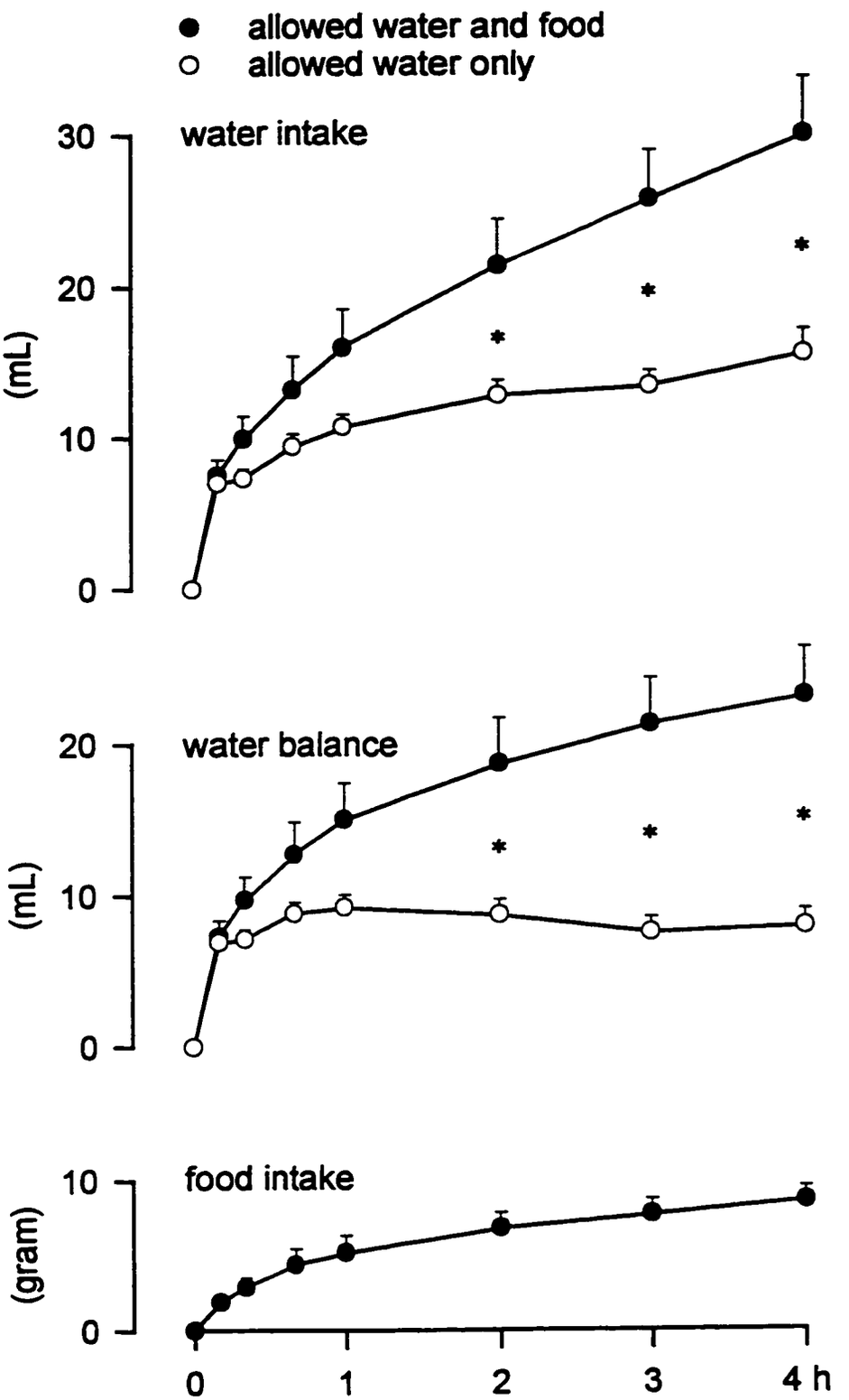

Figure 5.1 Cumulative water intake, water balance (calculated as intake - urinary loss) and food intake in rats allowed to eat and drink after 11 hours of water deprivation. * indicates difference between rats allowed to eat and drink and rats allowed water, but no food ($p<0.05$, t-test, $n=7$).

5.3 Results Water intake and water balance are shown in Figure 5.1. Changes in rats not allowed to eat were similar to those seen in section 4. Rats allowed to eat and drink drank more, and retained more water. Total food intake was 8.6 ± 0.9 g, 60% of which was eaten during the first hour.

Urinary changes in rats not allowed to eat were similar to those seen in section 4. Rehydration rapidly reduced excretion of Na^+, K^+ and Cl^- and caused a diuresis, but had little effect on urea excretion (Figure 5.2). Rats allowed to eat and drink excreted much more sodium, potassium, chloride, urea, and osmoles than did controls. Urine osmolality and concentrations of Na^+, K^+, Cl^- and urea were higher when food was available (all $p<0.001$). Differences in urine flow were not significant.

Changes in blood solutes in rats allowed water but not food were similar to those in section 4. Plasma [Na^+] and osmolality fell within 20 min (Figure 5.3, $p<0.05$ and $p<0.01$), and continued to fall below pre-deprivation values (both $p<0.01$). Unlike section 4, plasma [K^+] increased slightly after drinking ($p<0.01$). Urea concentration tended to fall after drinking, but the change was not significant.

Eating prevented plasma hypotonicity on rehydration but did not prevent hyponatremia ($p<0.01$). Changes in hemoglobin, hematocrit, and plasma protein were similar to those seen in rats not allowed to eat. Plasma [K^+] was higher when food was eaten. Differences in plasma [urea] were not significant.

5.4 Discussion The results illustrate how water intake, solute intake and solute excretion interact to provide accurate regulation of body fluid tonicity. During water deprivation water and electrolytes are lost from the body. When only water was returned after water deprivation, rats saved electrolytes, preventing further losses. When food

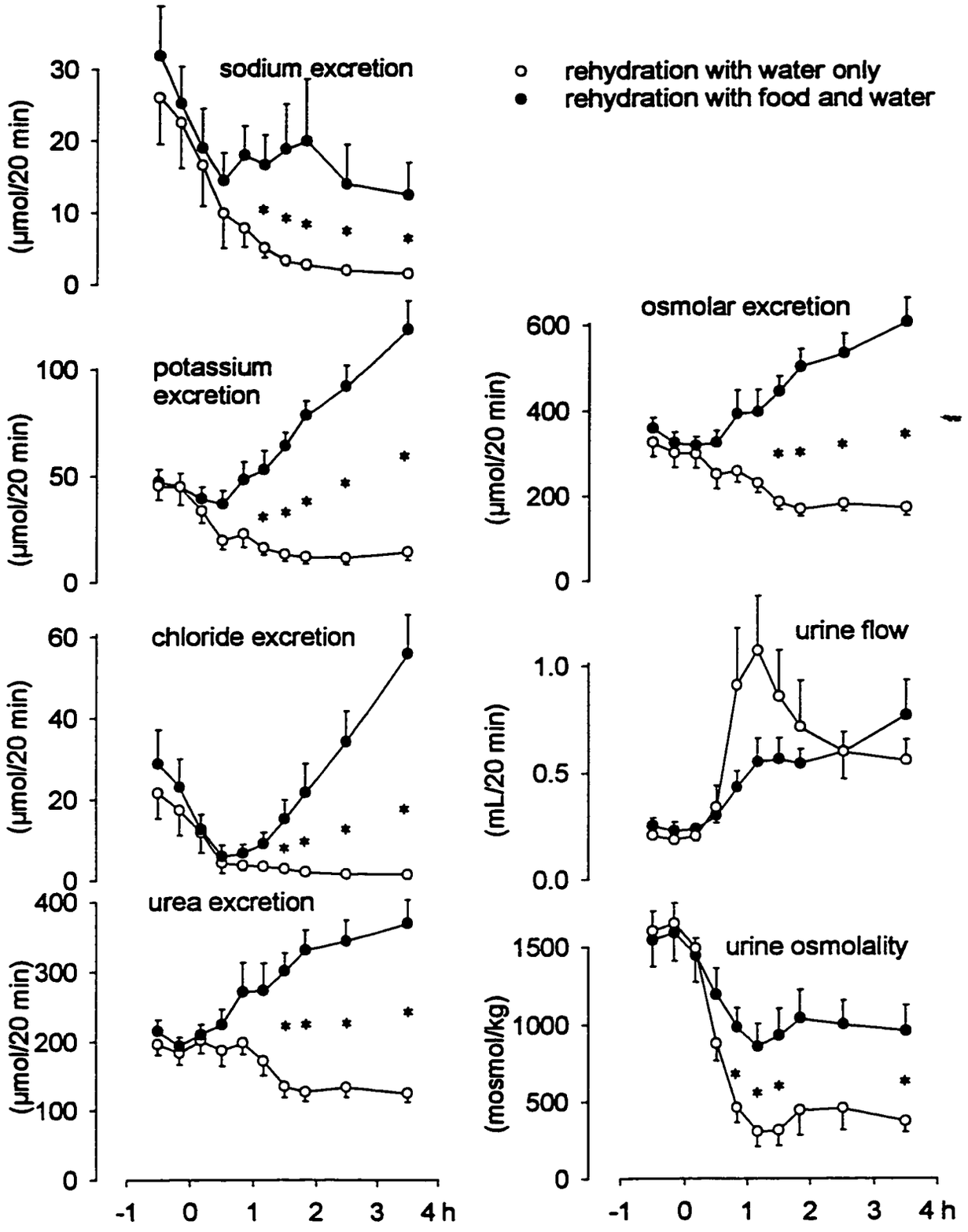

Figure 5.2 Water and solute excretion in rats allowed to eat and drink after 11 hours of water deprivation. At t=0 hours either water or food and water became available. * indicates difference between rats allowed to eat and drink and rats allowed water, but no food ($p<0.05$, Bonferroni t-test, n=7).

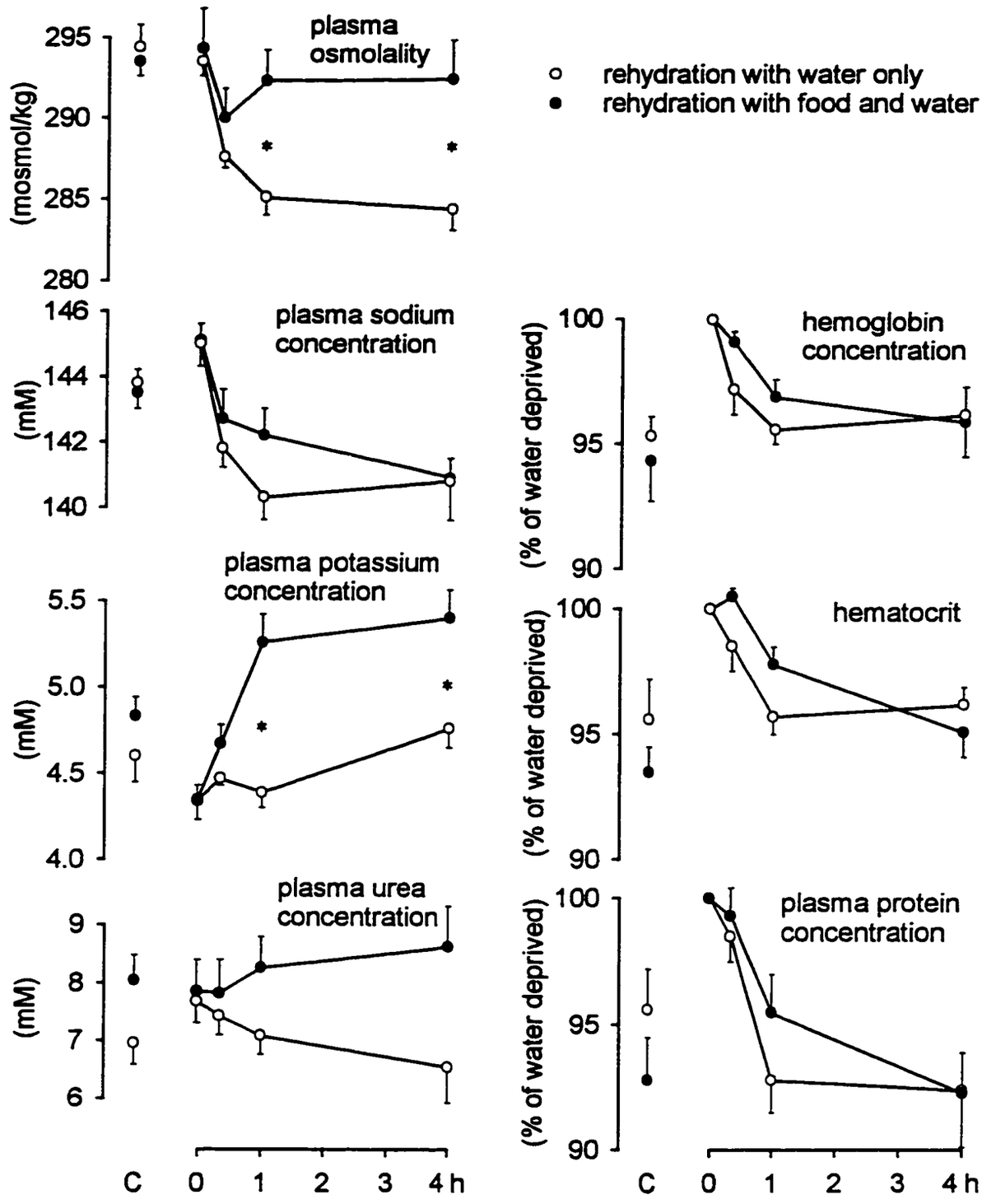

Figure 5.3 Blood solute changes in rats allowed to eat and drink after 11 hours of water deprivation. C indicates pre-deprivation control value. At t=0 either water or food and water became available. * indicates difference between rats allowed to eat and drink and time controls allowed water, but no food ($p<0.05$, Bonferroni t-test, n=7).

was available as well, rats ate, and also drank more. Salt was ingested, the need for solute retention was reduced, and more was excreted. Although salt intake, water intake and solute excretion were much higher in rats that ate, changes in the body fluids appear similar to those in rats not allowed to eat.

Changes in blood volume were similar in rats that ate and rats not allowed food. Rats not allowed to eat tended to be hypovolemic at the end of the experiment: blood volume was 2.7 $\pm$ 1.9% less than before water deprivation (estimated from changes in hemoglobin, equation 2 of the Appendix). Hypovolemia was 2.2 $\pm$ 1.3% in rats allowed water and food. It is difficult to compare ECF volume in rats that ate and rats that were not allowed to eat because the amount of sodium absorbed from the gut is not known.

What was the effect of eating on ICF volume? ICF volume depends on the amount of intracellular solute and on plasma tonicity. Whether eating influenced the amount of intracellular solute is not clear. Eating seems to have little effect on plasma tonicity. Plasma tonicity is determined mainly by the [Na^+], and plasma [Na^+] was similar in rats that ate and rats not allowed food. Osmolality of the blood was higher when food was available, but probably the solutes that contribute to the higher osmolality have little effect on tonicity. Plasma [urea] tended to be higher after eating, but because the cell membrane is permeable to urea it does not influence tonicity. Plasma [K^+] was higher in rats that ate but the difference was small: only 0.6 mM. Most of the difference in plasma tonicity may be caused by higher plasma [glucose] in rats allowed food. Plasma [glucose] increases after eating (Steffens 1969). Glucose has little effect on tonicity because it crosses the cell membrane.

Thus, although food intake increased the amount of solute in the body, it probably had little effect on size of the body fluid compartments. The reason is that water intake

was higher in rats allowed to eat, and they excreted more solutes. Several mechanisms may contribute to the increased drinking. Osmoreceptors in the brain may play a role, but osmoreceptors in the gut, liver, or portal vein may contribute to drinking as well (Kobashi and Adachi 1992). Also, eating causes release of histamine from the gut, which stimulates drinking (Kraly 1984).

Rats that ate excreted more sodium, potassium and chloride than rats not allowed to eat. The difference in sodium excretion was small, only 141 μmol/4 h. The difference in potassium excretion was larger: 767 μmol/4 h. Assuming ICF volume is 39% of the body weight, and ICF [K^+] is 140 mM, this would be 3% of the K^+ in the ICF. The stimulus for the urinary changes after eating is not clear. Correlations between solute excretion and plasma solute concentration were small and not significant (plasma [Na^+] with sodium excretion: r=0.48, plasma [K^+] with potassium excretion r=-0.13). Rats that excreted much potassium tended to excrete less sodium (r=-0.50). The correlation is not significant, but the trend is remarkable, because changes in the activity of the mechanism for dehydration natriuresis may have made the correlation less negative. (That mechanism changes sodium and potassium excretion in the same direction, see sections 4 and 6). Therefore the negative correlation coefficient suggests that aldosterone may play a role. However, it is unlikely that aldosterone is the only cause of the higher excretion after eating. Changes in excretion after aldosterone administration are slower to develop, because they depend on protein synthesis (Guyton 1986, chapter 77). Differences in potassium excretion were significant within 20 min.

In conclusion, when rats ate after water deprivation, they also drank more, and excreted more salt. Increased water intake and salt excretion would contribute to body fluid regulation.

6 ROLE OF BRAIN SODIUM CONCENTRATION IN DEHYDRATION NATRIURESIS

The previous sections showed that changes in salt excretion contribute to osmoregulation during water deprivation and after rehydration. This section investigates one of the possible mechanisms of these changes. As explained in the introduction there is much evidence that indicates that a brain sensor is involved. Dehydration natriuresis can be blocked with brain lesions (Bealer 1983, McKinley et al. 1983c). Brain infusions that alter CSF [Na^+] affect solute excretion (see introduction). Natriuresis caused by intravenous infusion of hypertonic saline can be blocked by infusing hyponatremic solutions into the brain (Olsson 1973, Chodobski and McKinley 1989). Whether the time course of the changes after brain infusion and water deprivation and rehydration are the same has never been measured. Also it is not known if the same solutes are involved. Therefore I infused solutions into a lateral brain ventricle that reduced or increased brain [Na^+], and measured changes in solute excretion. The results show that urinary changes after water deprivation, rehydration and brain infusions are similar in several ways, indicating the same mechanisms could be involved.

6.1 Brain infusions in water deprived rats

6.1.1 Introduction As shown in section 4, when rats drink after a period of water deprivation, electrolyte excretion rapidly falls to very low levels. If a brain sensor is involved, we would expect to see a similar fall in electrolyte excretion when during water deprivation brain [Na^+] is artificially lowered. To test this hypothesis I infused low-sodium CSF into a lateral brain ventricle, and measured urinary excretion. To test if the infusions acted on receptors outside the brain (after leaking out of the brain), plasma tonicity was measured also.

It is not clear what type of sensor causes the changes in electrolyte excretion after brain infusions. Most research suggests changes in [Na^+] or maybe [Cl^-] of the CSF are critical, and CSF tonicity is not (Mouw and Vander 1970, Olsson 1973, Andersson et al. 1976, Leksell et al. 1981, 1982, McKinley et al. 1983a). There is only one paper that tests the type of sensor in rats (Osborne et al. 1989), and it suggests an osmoreceptor is involved. In Osborne's experiments, infusion of normal CSF to which mannitol or sucrose was added stimulated excretion of sodium and potassium. (Mannitol and sucrose are sugars that do not easily cross the cell membrane.) Such infusions have many effects. They increase CSF tonicity, which will dehydrate brain cells close to the ventricles and increase solute concentration in these cells. The water withdrawn from the cells dilutes the CSF, reducing its [Na^+]. The effect on [Na^+] of brain interstitial fluid is unpredictable. Where the sugar diffuses into the interstitial fluid its [Na^+] will fall. In brain areas where the sugar does not penetrate the interstitial fluid may become hypernatremic. Thus Osborne's results could be compatible with a sensor that measures [Na^+] in the interstitial fluid adjacent to the ventricles. To test if a sodium sensor is

involved I used infusions that selectively alter CSF [Na^+] without affecting its tonicity. In contrast with Osborne's results my results show that a sodium sensor is involved in electrolyte excretion.

6.1.2 Methods For these experiments I used 18 rats that had a guide cannula implanted in a lateral brain ventricle. These rats also had cannulas in the bladder and in the femoral vein. At the start of the experiments the rats weighed 324±5 g.

Rats were water deprived and tested in wooden testing cages, 15 cm wide, 20 cm deep, and 35 cm high, that had a clear acrylic front and a wire mesh bottom. Water deprivation started at midnight. Food was available during water deprivation. The next morning food was removed, the cannulas were connected, and the rat was allowed to rest for 30-60 min. Urine sampling started at 11:00 h, and brain infusions started at 12:00 (noon). I took blood samples (0.35 mL each) before, during and after the infusion to measure the effect of the infusions on hematocrit and plasma [Na^+] and osmolality.

I infused either control CSF, hypotonic low-sodium CSF or isotonic low-sodium CSF in the lateral brain ventricle. Composition of the infusion fluids is shown in Table 6.1. Solutions were filtered (filter pore size 0.2 μm) to sterilize them and stored at 4°C. Before use, solutions were bubbled for 10 min with 5% CO_2, 95% air, and filtered again. The solutions were infused for two hours at a rate of 1 μL/min. In control experiments, infusion tubing was connected to the brain cannula but no infusion was given. Rats received the treatments in mixed order, with at least 4 days between experiments.

Table 6.1 Composition of solutions infused into the brain.

	$[Na^+]$ (mM)	$[Cl^-]$ (mM)	[mannitol] (mM)	osmolality (mosmol/kg)
hypotonic low-sodium CSF	25	7	0	55
isotonic low-sodium CSF	25	7	233	287
control CSF	150	132	0	287
high-sodium CSF	300	282	0	547

Infusion fluids also contained K^+ 3 mM, Ca^{2+} 1.2 mM, Mg^{2+} 1 mM, HCO_3^- 25 mM, and PO_4^- 0.5 mM. pH was 7.3-7.4 after bubbling for 10 min with 5% CO_2, 95% air.

Table 6.2. Solute excretion rate in the four conditions of experiment 6.1 during the 30 min before the start of the infusions.

	Na^+	K^+	Cl^-	urea	flow	osmolality
no infusion	0.6±0.1	1.6±0.1	0.6±0.1	7±1	10±1	1689±145
control CSF	0.8±0.1	1.9±0.2	0.7±0.1	8±1	14±2	1473±145
hypotonic low Na^+	1.1±0.2	2.2±0.2	1.2±0.2	10±2	14±1	1774±125
isotonic low Na^+	1.0±0.2	1.6±0.2	0.7±0.2	8±1	12±1	1757±134

Excretion rates of Na^+, K^+, Cl^-, and urea in μmol/min. Urine flow in μL/min. Osmolality in mosmol/kg. Urea excretion in rats about to receive hypotonic low-sodium CSF was higher than in rats that received no infusion (one way ANOVA, Tukey's HSD post-hoc test, all groups n = 6-7). Other differences were not significant.

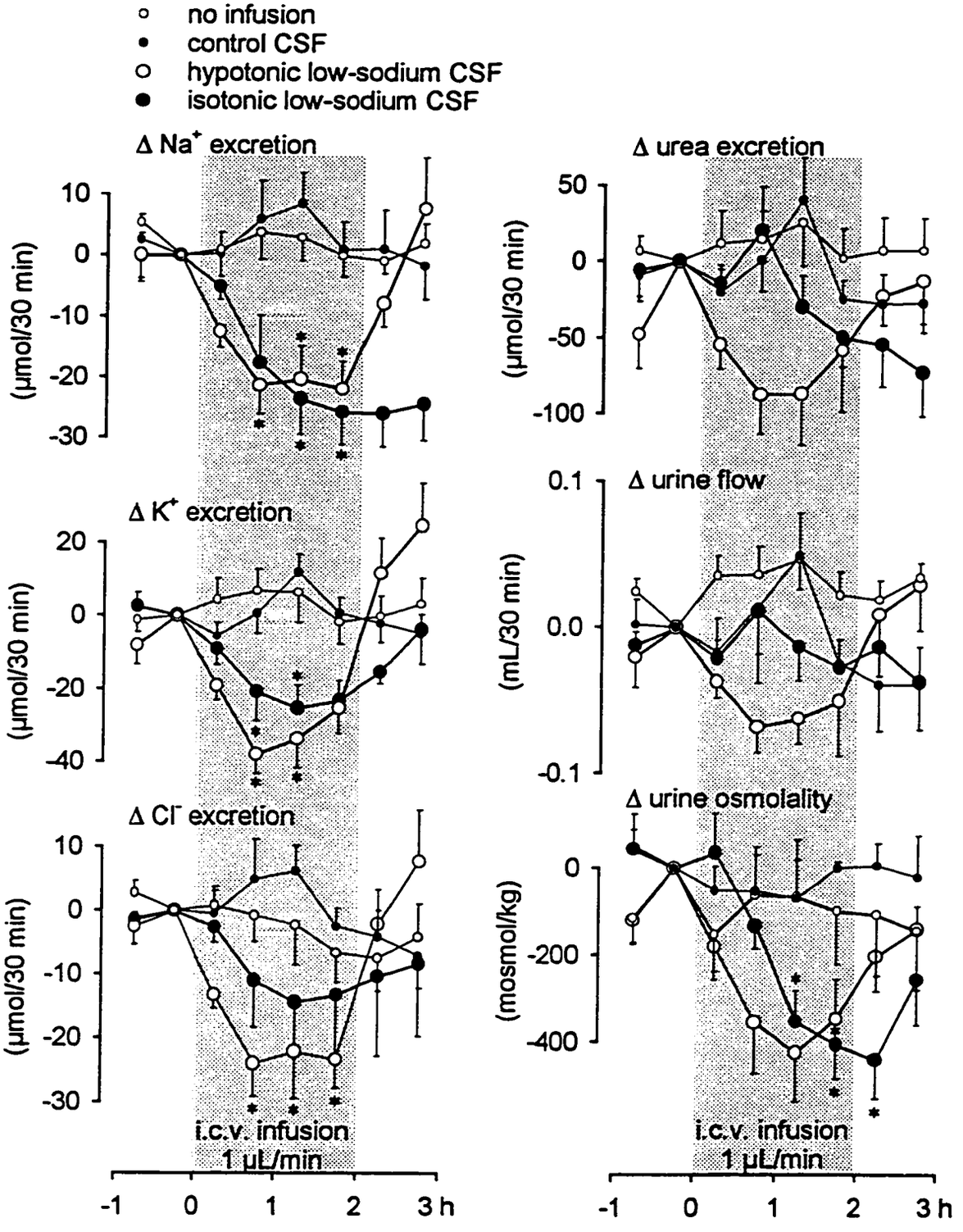

Figure 6.1 Changes in water and solute excretion during infusion of low-sodium CSF in the lateral brain ventricle of water deprived rats. Either hypotonic low-sodium CSF (n=7), isotonic low-sodium CSF (mannitol added, n=6) or normal CSF (n=7) was infused, starting at t=0 hours (shaded area). Controls (n=6) received no infusion. * indicates difference with controls infused with normal CSF ($p<0.05$, Bonferroni t-test).

6.1.3 Results Excretion rates before the start of the infusions are shown in Table 6.2. The changes caused by the infusions are shown in Figure 6.1. Excretion of water and solute did not change during control experiments where no infusion was given or normal CSF was infused. Solute excretion fell during infusion of hypotonic low-sodium CSF. Average excretion of sodium during the infusion period was 40 ± 10% of the pre-infusion value, and potassium and chloride excretion were 53 ± 8% and 38 ± 11% of the pre-infusion value. Urea excretion remained high during the infusion period (75 ± 8% of the pre-infusion value). Urine osmolality fell slightly, but urine remained hypertonic. Urine osmolality was lowest in urine collected between 60 and 90 min after the start of the infusion (1350 ± 138 mosmol/kg). Infusion of hypotonic low-sodium CSF had little effect on urine flow. The infusion clearly did not cause a diuresis. Excretion of Na^+, K^+ and Cl^- increased on stopping the infusion.

Isotonic low-sodium CSF had effects similar to hypotonic low-sodium CSF. In contrast with hypotonic low-sodium CSF, excretion of sodium and potassium remained low after the end of the infusion (both $p < 0.05$ compared with hypotonic low-sodium CSF, t test). Differences in chloride excretion were not significant.

The effect of the infusions on plasma solutes was similar in all groups (Figure 6.2). Hematocrit fell in all groups because with each sample red blood cells were removed. Osmolality tended to fall during the experiments, but $[Na^+]$ did not change. Chloride concentration slightly increased (from 104.5 ± 0.3 to 105.7 ± 0.5 mM, mean of all 26 experiments, $p<0.01$).

6.1.4 Discussion Infusion of hypotonic low-sodium CSF caused large and rapid changes in excretion of sodium, potassium and chloride that lasted for the duration of the

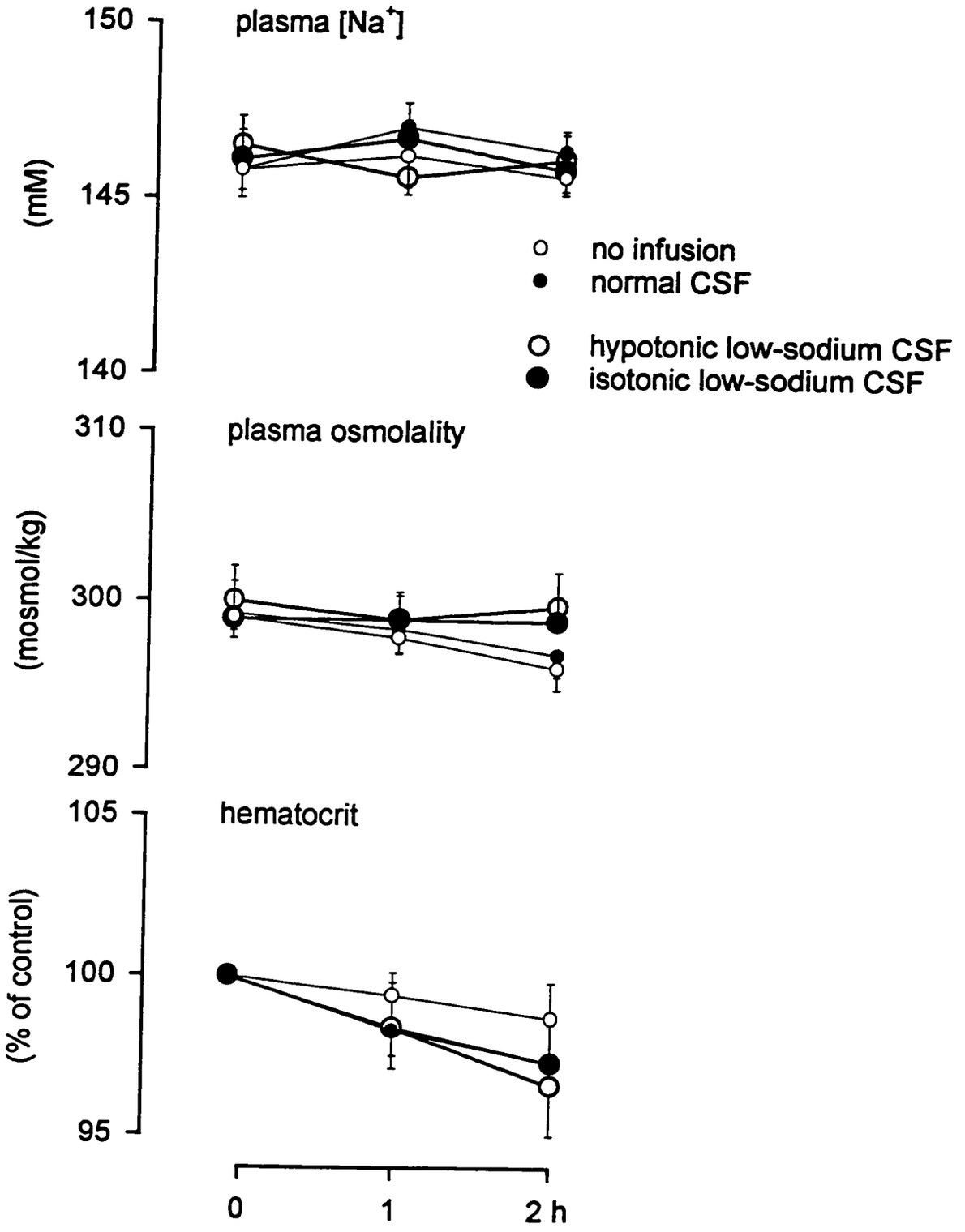

Figure 6.2 Plasma sodium and osmolality, and hematocrit during infusion of low-sodium CSF in the lateral brain ventricle of water deprived rats. All treatments affect the blood solutes in the same way. No infusion n=6, control CSF n=7, hypotonic low-sodium CSF n=7, isotonic low-sodium CSF n=6.

infusion (see also Leksell et al. 1982 and Beasley et al. 1983). On ending the infusions excretion rates rapidly returned to control values.

The urinary changes are not caused by a fall in plasma osmolality or [Na^+]. Plasma osmolality did not fall during infusion with low-sodium CSF and the treatments had no significant effects on plasma osmolality. It is not surprising that the changes in blood solute concentration are small during these short infusions, because neither the water infused in the brain nor the urinary changes would be expected to have much effect on body fluid tonicity or blood volume. The water infused in the brain, 120 μL, is less than 0.1% of the water in the body. Rats excreted less sodium (93 μmol) and chloride (91 μmol) during infusion with hypotonic low-sodium CSF, compared with control infusion. These savings would be about 1% of the total amount of sodium and chloride in the ECF. The 125 μmol of potassium saved during infusion of hypotonic CSF is about 0.7% of the ICF potassium (assuming ICF is about 40% of the body weight, and ICF [K^+] is 140 mM). Such changes are probably too small to measure. In conclusion, the infusions alter solute excretion by acting on the brain. This conclusion agrees with results found by many other researchers (see section 1.4.3).

The data show that the sensor is sensitive to changes in [Na^+] or [Cl^-]. Infusion of isotonic low sodium CSF (mannitol added) reduced salt excretion. Such infusions reduce CSF [Na^+], but do not change CSF osmolality (Leksell et al. 1981, 1982). Mannitol does not easily cross the cell membrane (Fenstermacher and Rapoport 1984) and is not easily metabolized. Infusions with isotonic mannitol are thought not to alter cell volume. Thus the reduction in salt excretion seen in water deprived rats during infusion of low-sodium CSF with mannitol is probably not caused by a change in cell volume, but by the low [Na^+] or [Cl^-]. Infusion of hyponatremic isotonic CSF reduces electrolyte excretion

in other species as well (Mouw and Vander 1970, Olsson 1973, Leksell et al. 1981, 1982, McKinley et al. 1983a). My results do not rule out that an osmoreceptor is also involved in salt excretion (Osborne et al. 1989), but demonstrate that its contribution to the urinary changes during brain infusion is minor, at most.

The sensor is probably inside the blood-brain barrier. A sodium sensor outside the blood brain barrier is not consistent with the finding that infusion of hypertonic sucrose into the carotid artery stimulates sodium excretion (Blaine et al. 1975). It is unlikely that infusion of isotonic fluid in the CSF increases tonicity outside the blood brain barrier.

Osborne et al. (1989) concluded that in rats an osmoreceptor is involved. They found that infusion of CSF with normal [Na^+] plus 0.7 M mannitol or sucrose increased salt excretion. Such infusions reduce CSF [Na^+]. Infusions with normal CSF made hypertonic with mannitol or sucrose reduce salt excretion in sheep (Leksell et al. 1981, McKinley et al. 1983a). The reason for the apparent discrepancy is not clear. It seems unlikely that the sensor for hypotonicity differs from the sensor for hypertonicity, because experiments in our lab (Bouzane et al. 1996) indicate that infusion of low-sodium hypertonic CSF in the lateral brain ventricle does not stimulate excretion of sodium and potassium in rats. Very recent experiments (bouzane and Evered, unpublished) show that excretion of sodium, potassium and chloride did not increase during a one hour infusion of normal CSF with mannitol or sucrose added.

In conclusion the results show that a sensor sensitive to changes in [Na^+] or [Cl^-] is involved in the urinary changes seen during brain infusions. This sensor is located inside the blood-brain barrier.

6.2 Brain infusions in normally hydrated rats

6.2.1 Introduction I also measured the effect of infusion of low-sodium CSF in rats that were not water deprived. I tested also the effect of infusions with high-sodium CSF in these rats.

6.2.2 Methods I used 8 rats that weighed 337 ± 10 g. They had cannulas in the bladder and a lateral brain ventricle. Drinking water was always available during the experiment but food was withheld during the sampling period. Rats received infusions (1 μL/min for 2 hours) in the lateral ventricle with low-sodium CSF, control CSF, or isotonic low-sodium CSF. For composition of these solutions see Table 6.1. I also infused high-sodium CSF. With the high-sodium CSF infusions, drinking water was removed as soon as the rat started to drink (usually within 5 min).

6.2.3 Results Table 6.3 shows excretion rates before the start of the infusion. Changes caused by the infusions are shown in Figure 6.3 and 6.4. As in the previous section, infusions with normal CSF did not alter water and solute excretion.

Solute excretion increased manyfold during infusion of high-sodium CSF. Average excretion of sodium during the infusion period was 830 ± 228% of the pre-infusion value, and potassium and chloride excretion were 180 ± 26% and 572 ± 164% of the pre-infusion value, respectively (Figure 6.3). The total amount of sodium lost (0.55 mmol) was about 4% of the total amount of sodium in the ECF (assuming ECF was 24% of body weight, and ECF [Na^+] was 140 mM). Urine volume more than doubled (increased 169 ± 38%). Urine osmolality fell slightly during infusion. After the

Table 6.3. Solute excretion rate in the four conditions of experiment 6.2 during the 30 min before the start of the infusions.

	Na^+	K^+	Cl^-	osmoles	flow	osmolality
control CSF	1.1±0.2	2.7±0.3	1.4±0.4	19±1	25±3	1246±143
hypotonic low Na^+	1.0±0.3	2.9±0.8	1.6±0.4	18±3	22±4	1318±191
isotonic low Na^+	1.0±0.2	2.6±0.4	1.4±0.3	19±1	27±5	1179±187
high Na^+	0.9±0.2	2.9±0.4	1.6±0.5	19±2	18±2	1685±232

Excretion rates of Na^+, K^+ and Cl^- in μmol/min. Osmolar excretion in μmol/ min. Urine flow in μL/min. Osmolality in mosmol/kg. Differences were not significant (one way ANOVA, all groups n = 5-6).

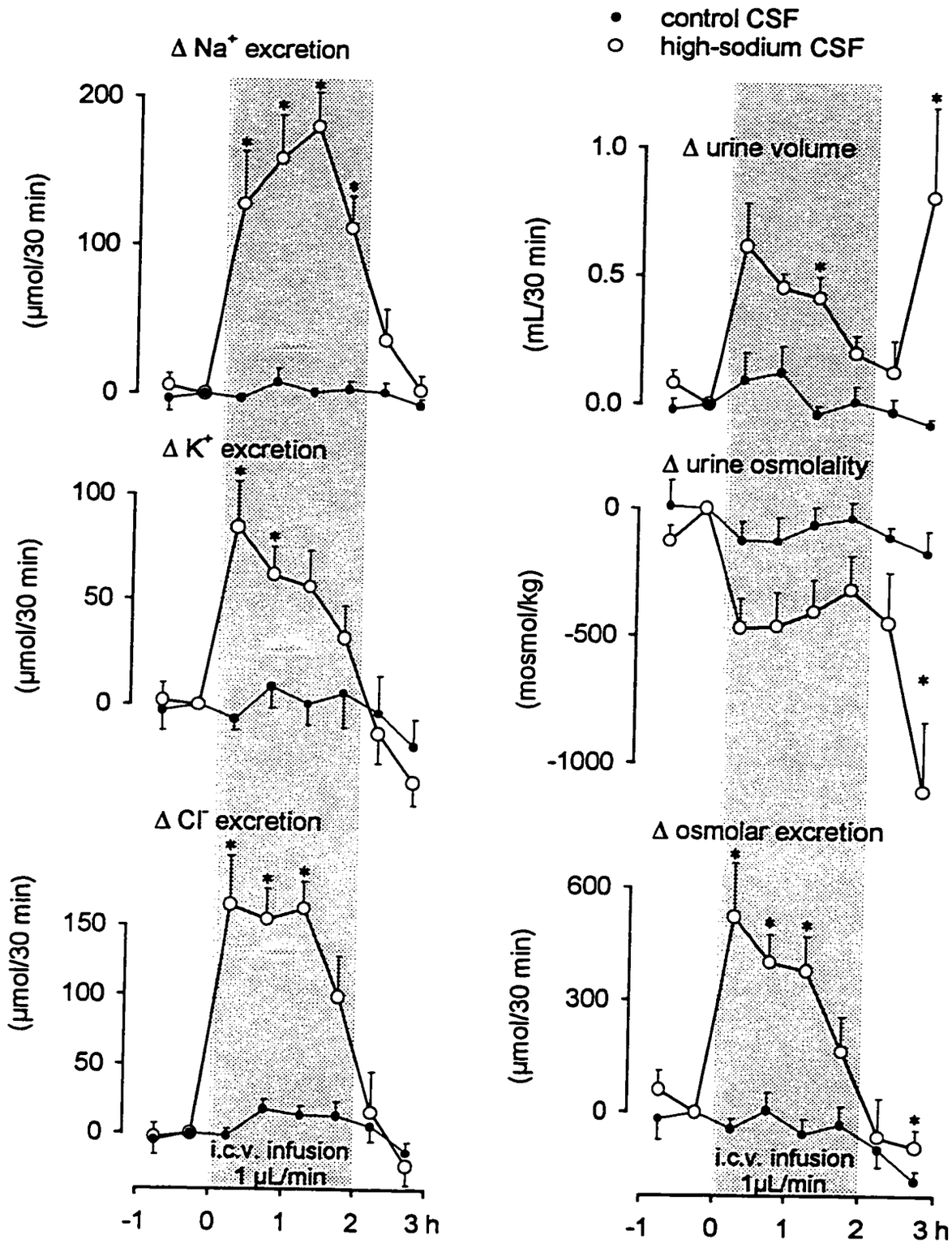

Figure 6.3 Changes in water and solute excretion during infusion of high-sodium CSF in the lateral brain ventricle of normally hydrated rats. * indicates difference with controls infused with normal CSF ($p < 0.05$, Bonferroni t-test, controls n=6, high-sodium CSF n=5).

infusion ended, salt excretion returned to control values and a water diuresis ensued.

Infusion of hypotonic low-sodium CSF did not have large effects in fluid replete rats (see Figure 6.4). During infusion, average sodium excretion was 86 ± 26% of the pre-infusion value, and potassium and chloride were 77 ± 7% and 78 ± 17% of the pre-infusion value, respectively. Sodium excretion rose after the end of the infusion ($p<0.05$). Urine flow and urine osmolality did not change significantly.

Compared with control infusions, isotonic low-sodium CSF reduced chloride excretion ($p<0.05$). There was also a trend to more sodium retention during the infusion period ($p<0.1$). Urine flow increased and urine osmolality fell to low values during infusion of isotonic low-sodium CSF (both $p<0.01$).

6.2.4 Discussion Infusion of high-sodium CSF caused a large and rapid increase in solute excretion, as has been shown before (Beasley et al. 1983). Infusion of low sodium CSF in rats that were not water deprived had little effect on solute excretion. Similar small changes in hydrated animals were found by Mouw et al. 1980, Cox et al. 1987, Chodobski and McKinley 1989, and Osborne et al. 1989. Changes were larger in water deprived rats. For example, in water deprived rats, potassium excretion during the two hour infusion period was 37 ± 8 μmol/30 min, compared to 66 ± 6 μmol/30 min before the infusion started, a change of 47 ± 8%. In water replete rats potassium excretion fell from 87 ± 23 to 65 ± 18 μmol/30 min, a fall of 23 ± 7%. Relative changes in potassium excretion were larger in water deprived rats ($p<0.05$). Relative changes in chloride excretion were larger too ($p<0.05$), but the difference in sodium excretion was not significant ($p=0.08$). (Sodium excretion fell from 33 ± 6 to 14 ± 4 μmol/30 min in water deprived rats, and from 29 ± 9 to 20 ± 6 μmol/30 min in water replete rats,

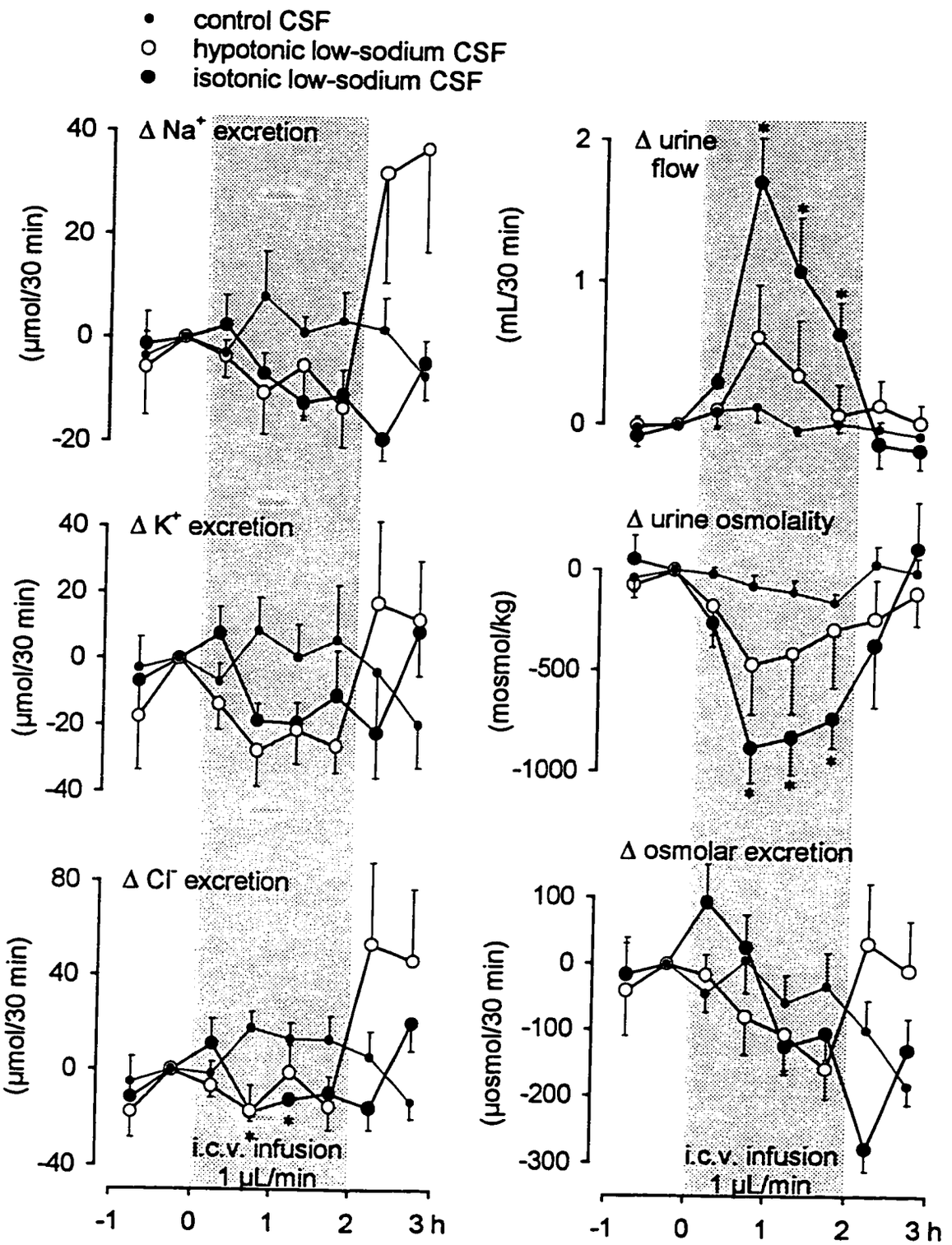

Figure 6.4 Changes in water and solute excretion during infusion of low-sodium CSF in the lateral brain ventricle of normally hydrated rats. Control CSF n=6, hypotonic low-sodium CSF n=6, isotonic low-sodium CSF n=5. * indicates difference with controls infused with normal CSF ($p<0.05$, Bonferroni t test).

changes of 60 ± 10 and 14 ± 26%, respectively). This suggests that the infusions act on a mechanism that is active during water deprivation, but less active in normally hydrated animals (McKinley et al. 1983a).

6.3 Effect of water deprivation and rehydration on CSF [Na^+]

6.3.1 Introduction Brain infusions that alter CSF [Na^+] cause changes in salt excretion that are similar to those seen during water deprivation and rehydration. This suggests that a brain sensor is involved in the renal changes seen during water deprivation and rehydration. In this section the effect of water deprivation and rehydration on CSF [Na^+] is measured.

Anaesthetized rats were used in this experiment, because pilot experiments showed that it was difficult to reliably collect small CSF samples in unanesthetized rats. Instead of allowing rats to drink, I infused water into the stomach. Infusion volume and rate were chosen to mimic drinking after water deprivation.

6.3.2 Methods Water replete (n=6, body weight 397±25 g) and water deprived rats (n=6, body weight 379±29 g) were used. Water deprivation started at midnight and lasted for 12 h. Rats were anaesthetized with Innovar Vet (MTC Pharmaceuticals, Cambridge, Ont., 0.1 mL i.m.).

CSF was collected through a hypodermic needle (0.3 mm o.d.) inserted through dura mater and foramen magna into the cisterna magna of the brain. CSF was withdrawn with a syringe mounted in a syringe pump. Rate of CSF collection was 1.3 μL/min. For

blood sampling a cannula in the femoral vein was used. Also, in water deprived rats a gastric tube was inserted through the mouth and tied in place with a ligature around the oesophagus just below the diaphragm. Water was infused for 12 min at a rate of 1 mL/min.

6.3.3 Results Water deprivation tended to increase $[Na^+]$ in CSF and in plasma, but the difference was not significant (see Figure 6.5). Plasma osmolality increased from 314.5 ± 2.0 to 322.0 ± 1.8 mosmol/kg ($p<0.05$).

Infusion of water in the stomach rapidly reduced $[Na^+]$ in CSF and in plasma. The change was faster in plasma, but the magnitude was similar. CSF $[Na^+]$ fell by 5.7 mM, plasma $[Na^+]$ fell by 5.2 mM. Plasma $[K^+]$ fell slightly after infusion of water (by 0.13 mM, $p<0.01$).

CSF $[Na^+]$ in this experiment was higher than expected (150 mM is normal, see Altman and Dittmer 1974), plasma osmolality was high, and plasma $[Na^+]$ was a bit low. This suggests that brain and cells were dehydrated by extracellular solutes. To test this I measured plasma [glucose] at the end of the experiment in five rats. It varied between 11 mM (slightly elevated) and 34 mM (very high).

6.3.4 Discussion Changes in plasma $[Na^+]$ during water deprivation and after rehydration were similar to those seen in sections 3 and 4. Although anaesthesia or high glucose levels may have influenced plasma $[Na^+]$, the changes in plasma $[Na^+]$ were as expected.

Changes in CSF $[Na^+]$ during water deprivation and rehydration paralleled plasma $[Na^+]$, but CSF $[Na^+]$ seemed to change slightly slower. Similar results were found by

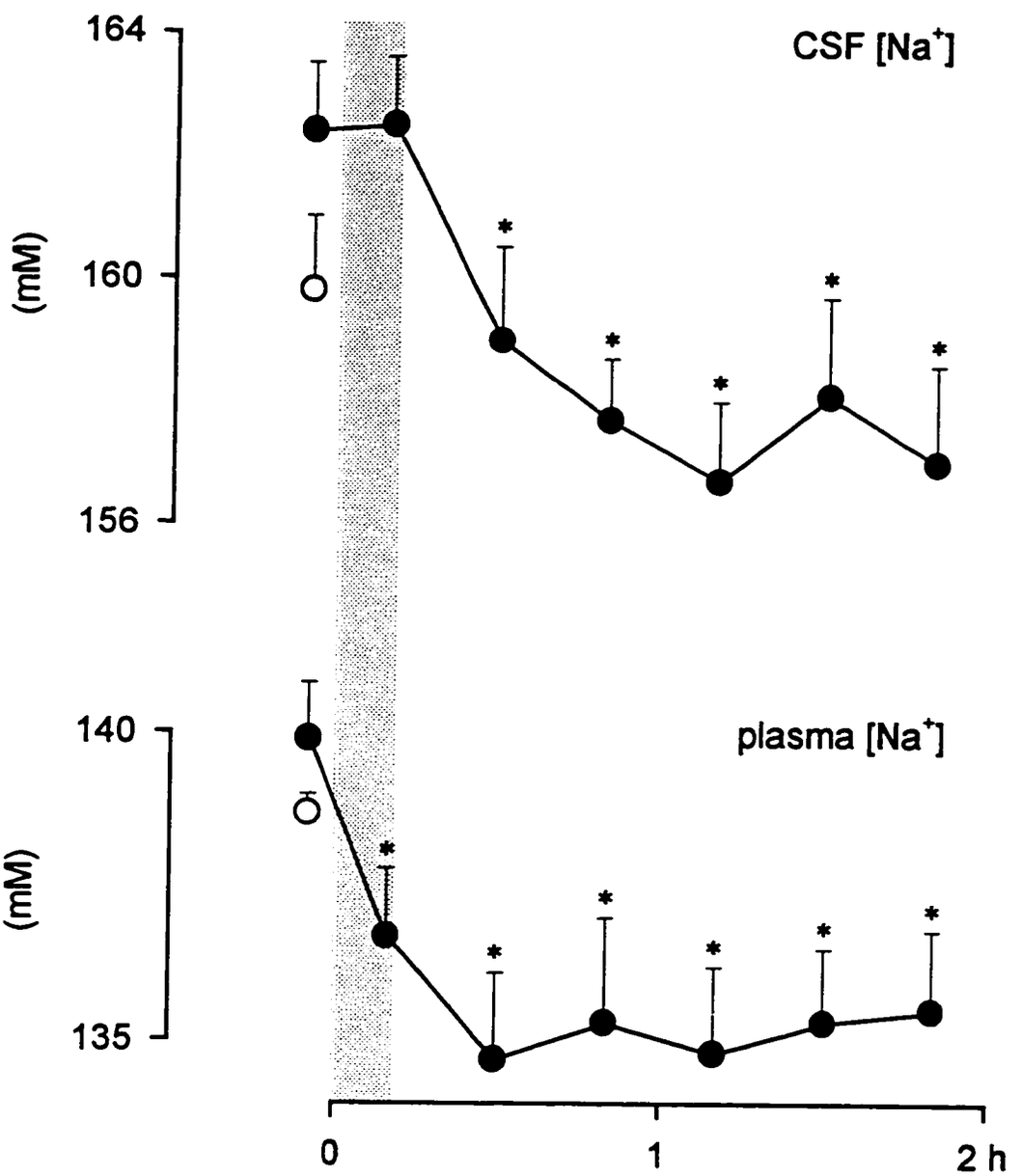

Figure 6.5 Top: Changes in CSF [Na^+] after infusion of 12 mL water into the stomach of water deprived rats. Shaded bar indicates infusion period. Open circle indicates [Na^+] in water replete controls. Bottom: plasma [Na^+] in the same experiment. * indicates difference from water deprived value ($p<0.05$, Dunnet's t-test, $n=6$).

Nose et al. (1992). These authors infused water in the stomach of water deprived, anaesthetized rats. They used sodium electrodes to measure plasma and CSF [Na^+] during the infusion and a short period (20 min) after the infusion ended. They found that changes in CSF [Na^+] are slightly smaller, and delayed by about 5 min compared to those in plasma [Na^+]. These results are consistent with my results.

6.4 Effect of brain infusions on CSF [Na^+]

6.4.1 Introduction To measure the changes in CSF [Na^+] caused by the brain infusions I infused low-sodium CSF in the lateral brain ventricle, and at the same time slowly collected CSF from either the cisterna magna or the third ventricle. The results show that the infusions cause large changes in CSF [Na^+], much larger than seen during water deprivation and rehydration.

6.4.2 Methods The effect of brain infusions on CSF composition in the cisterna magna was measured in 4 rats that weighed 329 $\pm$ 11 g. They were anaesthetized with Equithesin (3 mL/kg i.p.), followed by urethane (1.2 g/kg i.p.). A cannula (o.d. 0.7 mm) was placed in a lateral ventricle for infusion of CSF. Another cannula (needle, o.d. 0.3 mm) was inserted through the dura mater and foramen magna into the cisterna magna for CSF sampling.

Hypotonic low-sodium CSF (see Table 6.1 for composition) was infused first (1 μL/min for 2 hours). After at least 2 hours with no infusion, isotonic low-sodium CSF was infused. CSF was withdrawn at 0.3μL/min.

Changes in CSF composition in the third ventricle were measured in a separate group of rats (body weight 363 ± 25 g, n=6). This experiment was identical to the previous one, with the following exceptions. First, Innovar (0.1 mL s.c.) was used for anaesthesia. Second, samples were taken from the third ventricle (8 mm below the dura, 2 mm caudal from bregma, skull level). The sampling cannula, a sharp hypodermic needle (0.3 mm o.d.), was lowered through the sagittal sinus. This did not cause bleeding.

6.4.3 Results Infusion of both low-sodium CSF and isotonic low-sodium CSF reduced $[Na^+]$ in the third ventricle (Figure 6.6). Isotonic low-sodium CSF caused larger changes ($p<0.01$). After the end of the infusions CSF $[Na^+]$ returned to control level.

Low-sodium infusion did not alter CSF $[Na^+]$ in the cisterna magna, but isotonic low-sodium CSF did. $[Na^+]$ remained low after the infusion stopped.

6.4.4 Discussion The rate of withdrawal of CSF (0.3 μL/min) was slow relative to the normal rate of CSF production in rats (2-3μL/min, see Vogh et al. 1987, Go 1988). It is not likely that the sampling changed normal CSF flow. (Normal flow of CSF is from the lateral ventricles, through third and fourth, to the cisterna magna.)

The infusions caused large changes in CSF $[Na^+]$, especially in the third ventricle, which is close to where the sensor for dehydration natriuresis is thought to be (McKinley et al. 1983c, 1992, Bealer et al. 1983). Isotonic low-sodium CSF caused larger changes than hypotonic low-sodium CSF. The difference is probably caused by flow of water from the CSF into brain tissue and blood after hypotonic infusions. This would reduce the fall in CSF $[Na^+]$.

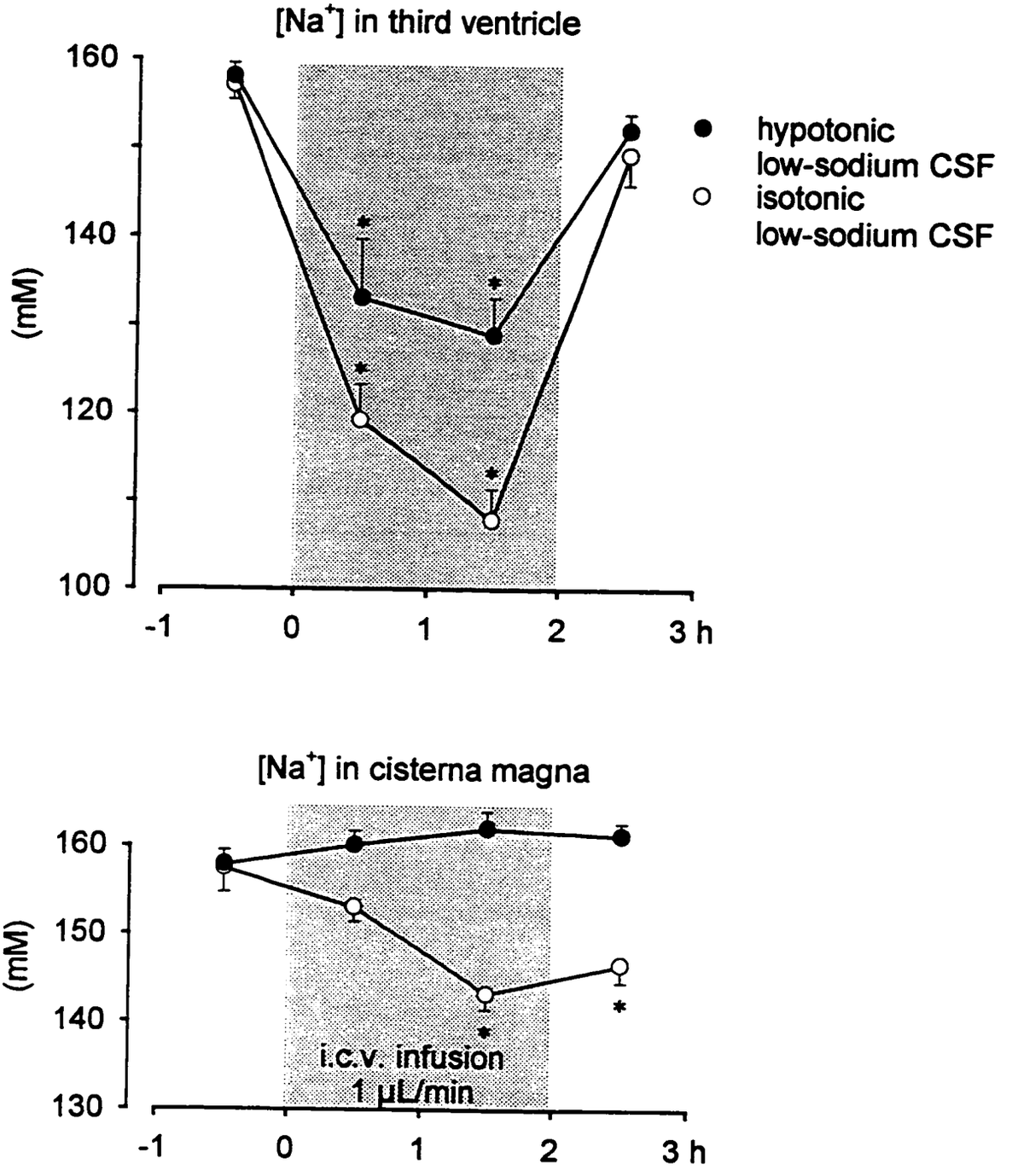

Figure 6.6 Effect of infusion of low-sodium CSF (1 μL/min) into the lateral brain ventricle on sodium concentration in the third ventricle (top graph, isotonic low-sodium CSF n=4, hypotonic low-sodium CSF n=5) and in the cisterna magna (bottom graph, n=3). * indicates difference from pre-infusion value ($p<0.05$, Dunnet's t-test).

After the infusion stopped, [Na^+] in the third ventricle rapidly returned to control levels. Probably much of the CSF is produced in the lateral ventricles because most of the choroid plexus tissue is there (see Bradbury 1979). Given the high flow through the third ventricle and its small volume, turnover of the fluid in the ventricle could be rapid. Changes in the cisterna magna were much smaller. During infusion of hypotonic low-sodium CSF, [Na^+] in the cisterna magna did not change significantly, probably because much of the water in the infusion left the CSF while traversing third and fourth ventricle. Changes during infusion of isotonic CSF were small, probably because the volume of the cisterna magna is quite large, and the mannitol was diluted by the fluid in the cisterna magna.

The changes in CSF [Na^+] in sections 6.1 and 6.2 may have been smaller than those measured here because anaesthetized rats were used. Anaesthetics often reduce cerebral blood flow (Edvinsson et al. 1993) and formation of CSF (Artru 1983, 1988). Low blood flow would reduce the removal of water from hypotonic CSF. Reduced CSF production would impair washout of mannitol. It seems safe however to conclude that the brain infusions that were used in sections 6.1 and 6.2 caused changes in [Na^+] in the third ventricle that are outside the physiological range, and much larger than those seen after water deprivation and after drinking. Changes in other brain areas, especially those far away from the ventricles, may be smaller.

In conclusion, the data are consistent with a sensor close to the third ventricle, that causes urinary changes during water deprivation, rehydration and brain infusions. However, it is possible that the renal changes after brain infusions are caused by a non-specific effect on brain tissue.

6.5 General discussion

6.5.1 Urinary changes during brain infusions. As explained in section 6.1, the sensor involved in changes in salt excretion during brain infusions is a sodium (or chloride) sensor, not an osmoreceptor, because isotonic infusions that lowered CSF [Na^+] reduced salt excretion.

The sensor is probably within the blood-brain barrier. First, a sodium sensor outside the blood-brain barrier is not compatible with results from Blaine et al. (1975), who found that infusion of hypertonic sucrose into the carotid artery stimulated sodium excretion. Therefore it is unlikely that the infusion fluid acts by diffusing to areas outside the blood-brain barrier. Second, isotonic infusions do not cause osmotic movement of water that could alter [Na^+] outside the blood brain barrier.

The sensor is not in the cisterna magna, because infusions with hypotonic CSF that reduced salt excretion did not reduce [Na^+] in the CSF of the cisterna magna (Figure 6.6). This agrees with results of other authors (McKinley et al. 1983c, 1992, Bealer 1983) who concluded that the lamina terminalis, a structure that forming the anterior part of the third ventricle, is involved.

The results suggest that the sensor is sensitive to changes in the brain interstitial fluid rather than the CSF. After the end of isotonic low-sodium infusions, solute excretion remained low (Figure 6.1), although [Na^+] in the third ventricle returned to control values (Figure 6.6). Although [Na^+] in the CSF returned to normal, it seems likely that [Na^+] in the brain tissue remained low. Restoration of interstitial [Na^+] depends on diffusion of mannitol out of the brain tissue, which may be a slow process. Therefore a sensor in the brain tissue could account for the long lasting inhibition of salt excretion

after isotonic infusions stopped. In contrast, restoration of interstitial [Na^+] after a hypotonic low-sodium infusion is a rapid process, because it depends on water transport caused by an osmotic gradient. Thus, the rapid urinary changes seen after the end of hypotonic low-sodium infusions are compatible with a sensor in the brain tissue.

The onset of the urinary changes after the start of isotonic low-sodium infusions was also slower than after isotonic low-sodium infusions (Figure 6.1), although the difference was not significant ($p=0.06$, t test). This supports a sensor in the brain tissue. It seems likely that changes in [Na^+] in the brain interstitial fluid are slower to develop during isotonic low-sodium infusions, because they depend on diffusion of mannitol into the brain tissue. In conclusion, an interstitial sensor can account for the delay in urinary changes after isotonic low-sodium infusions, and a CSF sensor can not.

A sodium (or chloride) sensor also accounts for the changes in water excretion. Infusion of isotonic low-sodium CSF caused a water diuresis in water replete rats (Figure 6.4), indicating a sodium sensor plays a role, that is probably located within the blood-brain barrier. Hypotonic low-sodium CSF had less effect, possibly because it had less effect on CSF [Na^+] (Figure 6.6). Although the infusion caused a water diuresis in water replete rats, it had no effect on urine flow in water deprived rats. This suggests that urine flow depends more on plasma tonicity than on CSF [Na^+]. There is strong evidence that water excretion depends mainly on osmoreceptors outside the blood-brain barrier (Robertson and Berl 1991), and my data are compatible with this idea. Brain infusions caused large changes in CSF [Na^+], and may affect the activity of neurons that are involved in secretion of antidiuretic hormone, but that are not involved in osmoreception during water deprivation.

6.5.2 Comparison of urinary changes during brain infusions and drinking.

As shown in the introduction, there is much evidence that brain infusions and water deprivation affect salt excretion through a common pathway. My results support this hypothesis. First, infusion of low-sodium CSF caused large changes in water deprived rats but had little effect in water replete rats (see also Mouw et al. 1980). Both relative and absolute changes were larger in water deprived rats. Sodium excretion in water deprived rats was 33 μmol/30 min before the infusion started, and was 14 μmol/30 min during infusion of low-sodium CSF, a fall of 58 $\pm$ 10%. The same infusions reduced sodium excretion in water replete rats from 26 to 20 μmol/30 min, a change of 16 $\pm$ 22%. McKinley et al. (1983a) therefore suggested that brain infusions influence a mechanism that is not normally active, but is activated during water deprivation.

Second, the same solutes were involved in the urinary responses to brain infusions and water deprivation. Brain infusions that reduced CSF [Na^+] reduced excretion of sodium, potassium and chloride, but had little effect on urea excretion (Figure 6.1). Similar changes were seen during rehydration (Figure 4.3). During infusion of hypertonic CSF, excretion of sodium, potassium and chloride increased (Figure 6.3). As shown in section 3, excretion of these ions increases during water deprivation. Urea excretion does not change during water deprivation, compared to food matched controls (Schoorlemmer and Evered 1993). I did not measure urea excretion during infusion of hypertonic CSF, but as Na^+, K^+ and Cl^- accounted for 95% of the increase in osmolar excretion, urea excretion probably did not change much.

Third, the time course of the urinary responses to brain infusions and drinking were similar. This is shown for sodium excretion in Figure 6.7. Sodium excretion fell after drinking, after starting an infusion with low-sodium CSF, and after stopping an infusion

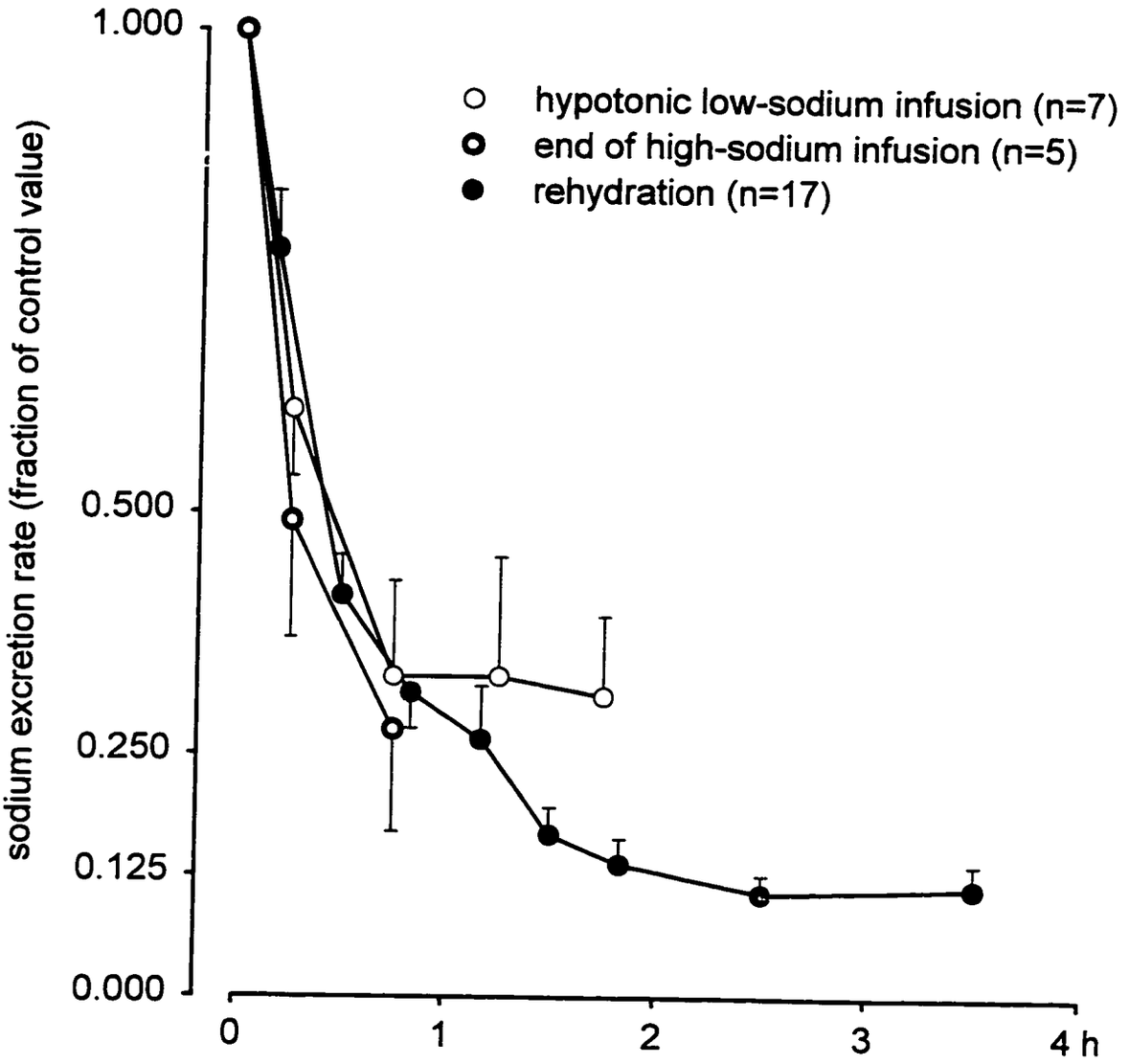

Figure 6.7 Comparison of urinary changes after rehydration and after brain infusions. At t=0 hours either rats were allowed to drink, a brain infusion with low-sodium CSF was started, or infusion with high-sodium CSF was stopped. Data from chapters 4, 5, 6.1 and 6.2.

with high-sodium CSF. The rate of change was similar in all cases, 50% in about 25 min, and 75% in slightly less than an hour. Urinary changes after drinking seemed slightly slower, which may have to do with the time it takes for the water to be absorbed from the stomach and to dilute the CSF. Drinking does cause a rapid reduction of CSF [Na^+], as is shown in Figure 6.5. A detailed analysis of the changes in CSF [Na^+] after infusion of water in the stomach was done by Nose et al. (1992). These authors infused slightly less water (2.5 mL/kg body weight in 10 min vs. 3.0 mL/kg in 12 min) and used a sodium electrode to measure [Na^+] in the lateral ventricle. They found that [Na^+] in the lateral ventricle fell by 1 mM 6 min after the start of the infusion. CSF [Na^+] fell by 2 mM and 5 mM 9 and 20 min after the start of the infusion.

In conclusion, the data support the hypothesis that brain infusions and water deprivation affect salt excretion through a common pathway. Whether the same sensor is involved remains to be seen. Brain infusions caused changes in CSF [Na^+] that were much larger than those seen during water deprivation. Thus it is possible that infusions influence cells not normally involved in osmosensitivity. It may be difficult to measure the sensitivity of the sensor that influences salt excretion during brain infusions, especially if the sensor monitors composition of the interstitial fluid of the brain.

7 MECHANISMS THAT STIMULATE FEEDING ON REHYDRATION

When water is returned after a period of water deprivation, rats will drink, and then usually start eating as well. Eating is an appropriate response, because it restores fuel reserves, and it also contributes to restoration of body salt content. In this section I investigated the mechanisms that are involved in the stimulation of feeding after rehydration. Specifically, I measured the role of oropharyngeal signals. I also measured the effect of rehydration with saline instead of water on food intake.

7.1 Effect of rehydration with saline on food intake

7.1.1 Introduction Ingestion of water has many effects: it wets the mouth, it expands the stomach, and it dilutes the body fluids. To see if the tonicity of the ingested fluids affect the stimulation of feeding on rehydration, water deprived rats were allowed to drink 0.15 M NaCl solution, and subsequent food intake was measured. Intake of NaCl solution did not stimulate feeding, suggesting taste and osmotic dilution play an important role in feeding after rehydration.

7.1.2 Methods Rats (n=10, 456 ± 6 g) were deprived of water, but not food, for 18 hours, from 17:00 until 11:00 the next morning. They were allowed to drink either water or 0.15 M NaCl solution for 15 min. Food was not available during the drinking period. Then fluids were removed, and food was returned. Intake during the next hour was measured. All rats received both treatments in mixed order. Period between experiments was 5 days.

7.1.3 Results Fluid intake was similar in both groups (rats drank 8.5 ± 0.9 mL saline, 9.9 ± 0.3 mL water), but food intake was much higher after rats drank water (Figure 7.1).

7.1.4 Discussion Water deprived rats ate little after drinking 0.15 M NaCl. The result is consistent with findings from Hsiao and Trankina (1969), who found that i.p. injection of water stimulates food intake in water deprived rats, but 0.9% NaCl has no effect. Apparently the taste of water, or dilution of the body fluids plays a critical role in eating after rehydration.

7.2 Contribution of pre-absorptive signals

7.2.1 Introduction Food intake and water intake typically stop long before the deficits that caused them are restored (see Ganong 1991). Signals from pre-absorptive sensors play an important role in these situations. Such signals also help prepare the body for the consequences of eating (for example by stimulating the secretion of gastric juices,

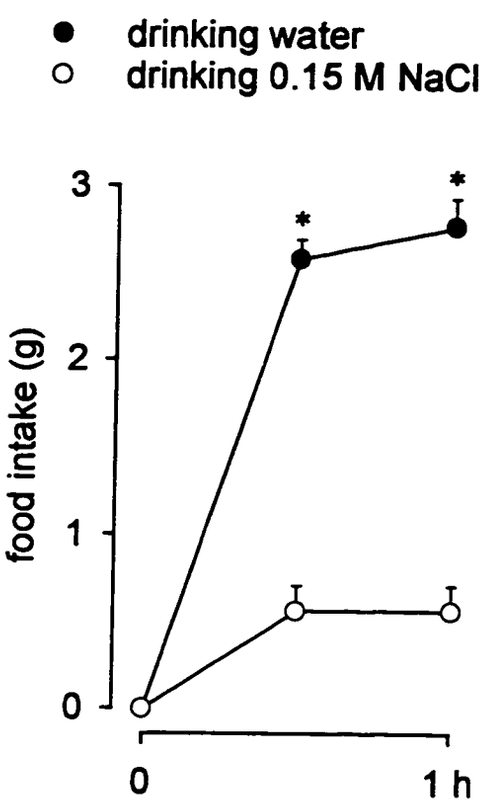

Figure 7.1 Drinking 150 mM NaCl did not stimulate feeding after water deprivation. Rats that had been water deprived for 18 hours drank either 0.15 M NaCl or water and then were allowed to eat. * indicates difference in food intake between drinking water and drinking saline ($p<0.05$, t test, $n=10$).

Debas 1987, and insulin, Strubbe and Steffens 1993). Similarly, drinking affects sensors located in the mouth and pharynx, resulting in a rapid fall in ADH secretion (Thrasher et al. 1981).

I investigated the contribution of pre-absorptive signals to the eating that occurs when water is returned after a period of water deprivation. It seems likely that pre-absorptive signals play a role, because food intake often starts soon after the initiation of drinking. Rats with gastric fistulas or gastric cannulas were used to selectively stimulate or bypass sensors in the mouth and pharynx. The results show that signals from both mouth and pharynx and from more distally located areas are involved in the stimulation of feeding after rehydration.

7.2.2 Methods To selectively bypass sensors located in the mouth and pharynx, rats (341 ± 5 g, n=9) with permanent gastric cannulas were used. They were water deprived for 18 hours, from 17:00 until 11:00 the next morning. Then either water was infused into the stomach (10 mL in 10 min), or the rats received a sham infusion, or they were allowed to drink for 10 min (water intake: 10.8 ± 0.4 mL). Then water was removed and food intake was measured during the next hour. All rats received all treatments in mixed order. Period between experiments was 3 days.

To selectively stimulate sensors located in the mouth and pharynx, rats (n=10, 456 ± 6 g) were used that had permanent gastric fistulas. These fistulas can be closed with a screw. When the fistulas are opened, water drains easily from them. In a pilot experiment, rats (n=12) were allowed to drink after overnight water deprivation, but the fistula was open during the drinking period. Rats drank 27.1 mL during the 15 min

drinking period, and I collected 26.5 mL fluid from the fistula during the drinking period.

The rats were water deprived for 18 h (from 17:00 to 11:00). Then the fistula was opened and flushed with warm water to remove all food from the stomach. Food tends to plug these fistulas. The rat was allowed to drink for 15 min, but the water escaped through the fistula. Food was not available during this period. The fistula was closed, and rats were allowed to eat (but not drink) for an hour. In the control experiment the fluid drank was retained, because the fistula was closed during the drinking period. All rats received both treatments in mixed order. Period between experiments was 5 days. I also measured food intake in these rats when they were not allowed to drink. This was done 3 days later. Fistulas were open during the 15 min waiting period.

The rats with gastric fistulas were also used to combine oropharyngeal and post-oropharyngeal signals. Rats were water deprived for 18 hours, fistulas were flushed to remove food from the stomach, and rats were allowed to drink for 15 min, but the fluid escaped through the open fistula. Fistulas were then closed, and a plastic feeding tube was inserted through the mouth into the stomach. Then 10 mL warm water was injected through the feeding tube over a period of about a minute. In the control experiment fistulas were closed during drinking, and the feeding tube was inserted in the stomach, but no fluid was injected. Then food intake was measured for one hour. Water was not available during the feeding period. All rats received both treatments in mixed order. Period between experiments was 3 days.

7.2.3 Results Infusion of water into the stomach stimulated food intake in water deprived rats, but drinking the same amount of water was more effective (Figure 7.2,

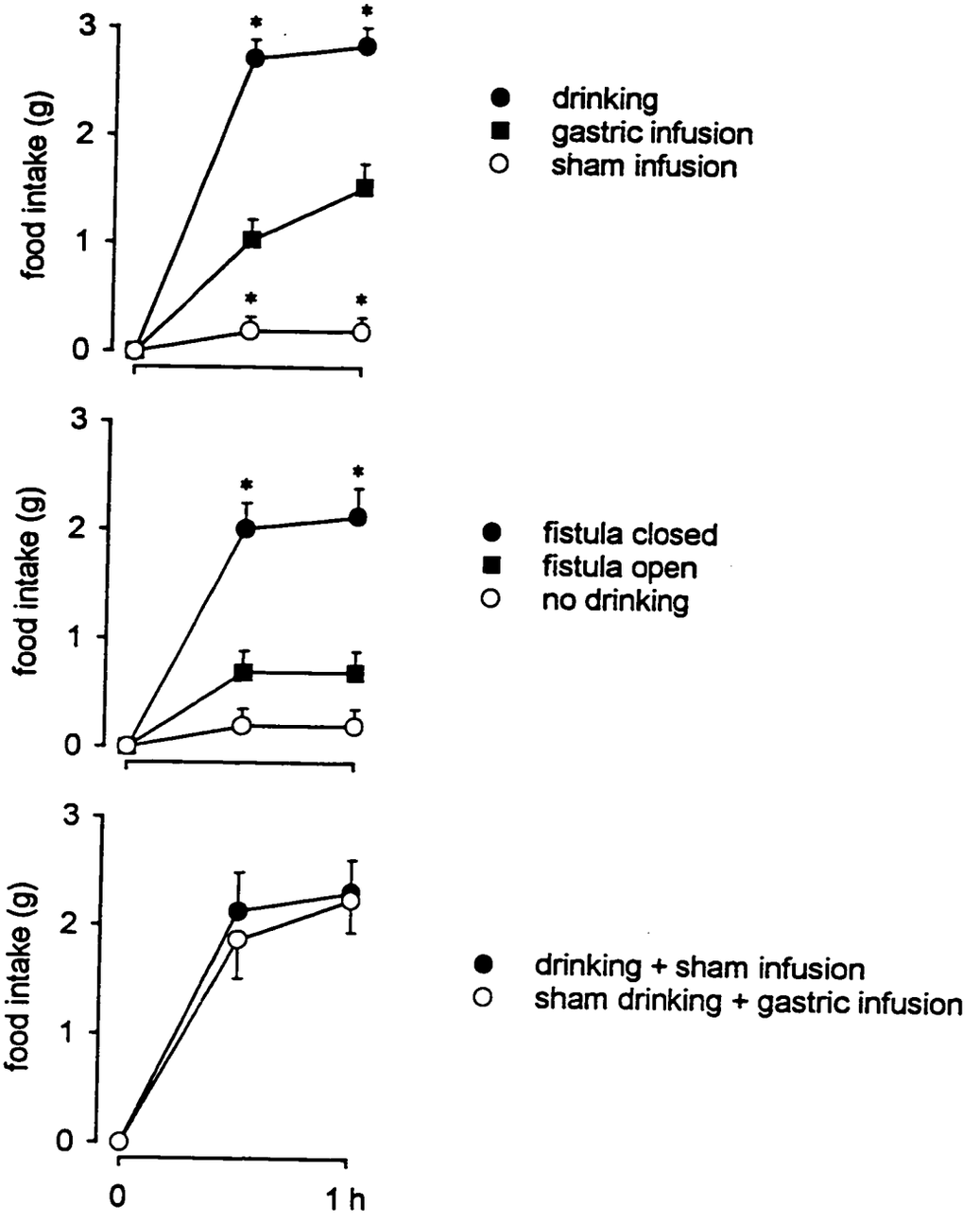

Figure 7.2 Pre-absorbtive signals contribute to eating after rehydration.
Top: infusion of water in the stomach stimulated food intake. Rats were water deprived overnight, then drank 10 mL water or received an infusion of 10 mL water in the stomach. * indicates difference from gastric infusion (Tukey's HSD test, $p<0.05$, $n=9$).
Middle: sham drinking (water drained through a gastric fistula) stimulated food intake after water deprivation, but drinking was more effective. Rats drank 10 mL with fistulas closed, 21 mL with fistulas open. * indicates difference from sham drinking. $n=10$.
Bottom: food intake was restored by sham drinking combined with gastric infusion. Controls drank 10 mL, followed by a sham injection through a gastric tube. The other group drank, but water escaped through a gastric fistula (water intake was 31 mL). Then the fistula was closed and 10 mL water was injected in the stomach. $n=10$.

top).

Rats ate little when the water drunk escaped through the fistula (Figure 7.2, middle), although almost all rats (9 out of 10) ate a bit. When these rats were not allowed to drink, only 2 rats ate. The difference was significant ($p<0.05$, Mann-Whitney U test). Note that rats drank more when the fistula was open than closed (21.1 $\pm$ 3.3 and 7.5 $\pm$ 0.6 mL).

Combining oropharyngeal and post-oropharyngeal stimuli restored food intake. Rats allowed to drink while the fistula was open, and receiving water in the stomach (the fistula was closed before the injection), ate just as much as rats allowed to drink normally (Figure 7.2, bottom). Note that rats drank much more when the fistula was open (30.6 $\pm$ 2.8 mL, compared with 9.8 $\pm$ 0.8 mL when the fistula was closed).

7.2.4 Discussion The results demonstrate that oropharyngeal signals contribute to feeding after water deprivation. First, selective stimulation of sensors in mouth and pharynx in rats with open gastric fistulas stimulated feeding. When these fistulas were opened (sham drinking) almost all the water drunk escaped through the fistula, so that post-gastric sensors were not stimulated. Eating after sham drinking must be explained by pre-absorptive signals. However, the role of signals from mouth and pharynx is not large: sham drinking rats did not eat much. The role of such signals may be even smaller than appears from this experiment, because sham drinking rats drank more. This may have exaggerated pre-absorptive signals in sham drinking rats.

Second, bypassing these sensors reduced food intake: infusion of water into the stomach did not cause much eating. The low food intake after gastric infusion is probably caused by the lack of oropharyngeal signals. It is possible that oropharyngeal

signals play an even more important role than appears, because the feeding after gastric infusions may have been caused by exaggerated post-absorptive signals. Even though the volume of the gastric infusion was similar to the volume drunk, the post-absorptive events may have been different in these two situations. For example, swallowing facilitates gastric relaxation (see Ganong 1991), which could alter gastric pressure or gastric emptying.

Intestinal tonicity or post-absorptive signals play a role as well, because rats ate after infusion of water in the stomach. With time, the difference in food intake between rats that drank and rats that received an infusion became smaller. It is possible that, if rats had been allowed more time to eat, food intake after gastric infusion would be similar to food intake after drinking. Thus the role of post-absorptive signals appears to increase with time, but it is not clear if they are sufficient to completely restore feeding after rehydration.

Combining oropharyngeal and post-oropharyngeal signals (sham drinking + gastric infusion) completely restored feeding. This suggests that the experimental approaches that were used (gastric infusions and sham drinking) had no harmful side effects. The result firmly supports the conclusion that ingested water acting on sensors in both the oropharyngeal area and in more distally located areas stimulates food intake after rehydration.

8 MECHANISM OF DEHYDRATION ANOREXIA

By far the most important osmoregulatory response in rats to short periods of water deprivation is a reduction of food intake (see section 3). Little is known about the time course of the feeding inhibition and the mechanism that is involved. The experiments in this section were designed to investigate these questions. I measured how rapidly food intake falls during water deprivation, and whether rats eat smaller meals or eat less frequently. I measured the effect on the body fluids of eating a normal sized meal, both in the presence and absence of drinking water. I also attempted to locate the sensor involved in the changes in food intake. This was done by infusing water in the stomach, in the intestine or in the vena cava throughout a period of water deprivation. Such infusions affect sensors located in different locations differently.

The results show that even very short periods of water deprivation reduce food intake. Meals were as frequent, but smaller. Eating caused rapid changes in plasma tonicity. The results also suggest that the sensor that causes the changes in food intake during water deprivation is located in the gastrointestinal tract, in the portal circulation or in the liver.

8.1 Meal pattern during water deprivation

8.1.1 Introduction In this experiment I measured the effect of water deprivation on meal pattern. The results show that food intake during water deprivation falls surprisingly rapidly, and that rats eat as often, but eat smaller meals.

8.1.2 Methods I measured eating pattern in 11 rats (466±8 g) that were kept in metabolism cages. Food intake was measured for 7.5 hours, starting at the beginning of the dark period (19:30). During this period the food hopper was removed from the cage every 15 min, weighed, and returned to the cage.

Drinking water was not available during the test period in six rats, but the other rats were allowed to drink. Two days later the experiment was repeated, but the treatments were reversed.

I also tested if the disturbance associated with removing the food hoppers every 15 min affected food intake. Food intake was measured in 8 rats (body weight 448±7 g) for 6 hours, starting at the onset of the dark period. During the test period rats were either water deprived, or had free access to drinking water. Food intake was measured either every 15 min, or only at t=0, 2, and 6 hours. All rats received all four treatments in mixed order. Period between experiments was 2 days.

8.1.3 Results Figure 8.1 shows the eating pattern in individual rats when drinking water was available or was removed. Clearly, rats did not eat continuously, but they ate discrete meals, as has been shown many times (Kissileff 1969, Oatley 1971). Most meals took less than 15 min. In some instances, rats ate during two consecutive 15 min

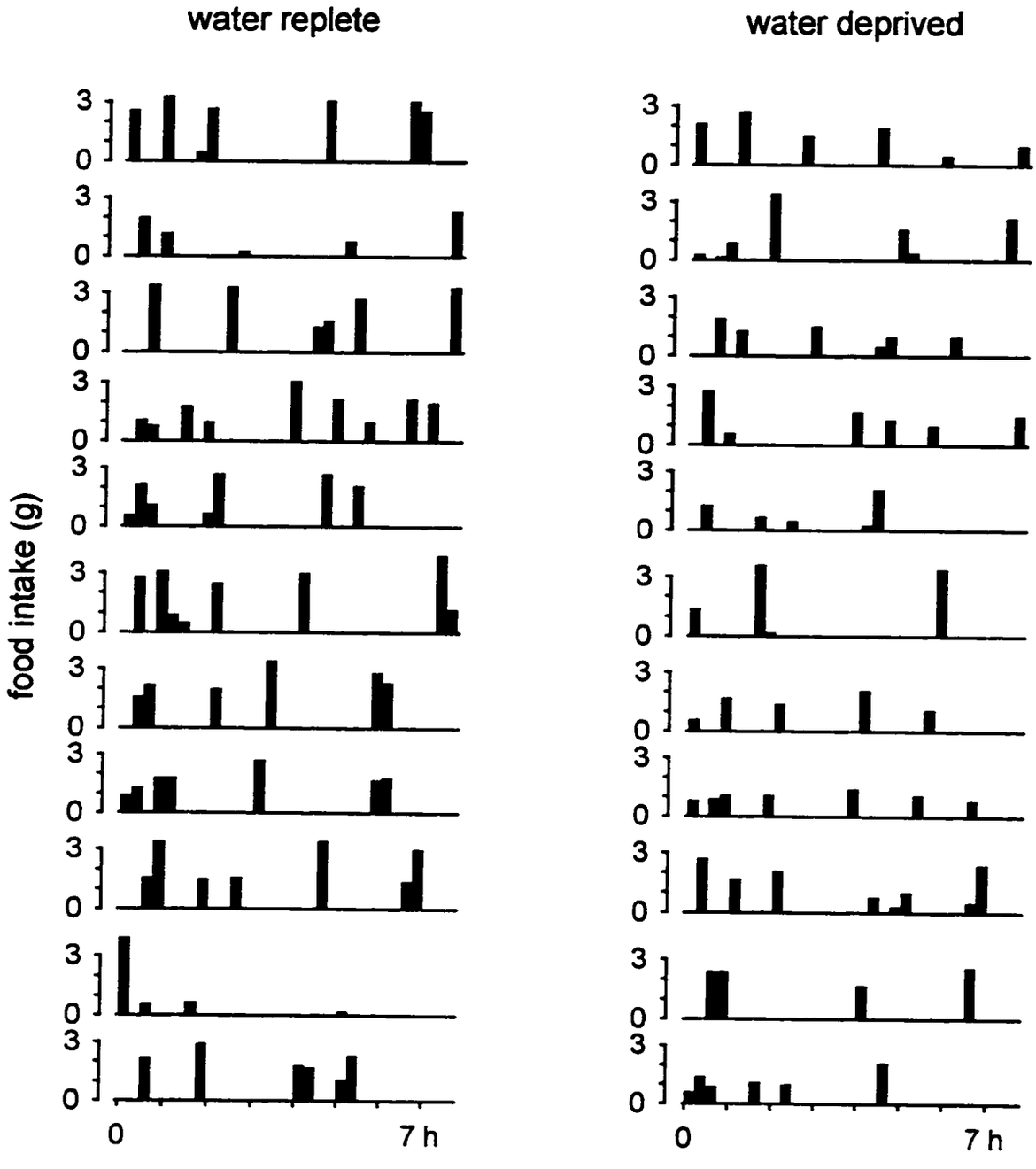

Figure 8.1 Feeding pattern in individual rats during water deprivation. Food intake was measured every 15 min when drinking water was available (left) and during water deprivation (right). Position of bars indicates time of meals, bar height indicates meal size.

periods. In the subsequent calculations, these cases are assumed to be single meals. A few times rats ate food during three consecutive 15 min periods. I counted these as two meals. Thus, the diagram at the bottom of Figure 8.1 shows a rat that ate four meals when water was available, and five meals during water deprivation.

The data are summarized in Figure 8.2. Food intake was reduced already after 45 min of water deprivation (1.85 vs. 2.81 g). Food intake fell by 38% during the 7.5 hour period of water deprivation. Water deprivation reduced meal size but did not change meal frequency. The first meal was bigger in rats that could drink (2.9 $\pm$ 0.3 vs. 2.1 $\pm$ 0.4 g, $p<0.05$, t test).

Disturbing the rats every 15 min for food intake measurement had little effect on food intake (Figure 8.3).

8.1.4 Discussion Water deprived rats reduced food intake within 45 min, after eating less than 2 gram food. Feeding inhibition was faster than that seen in section 3.1, but in section 3.1 food intake tended to be lower after 2 hours of water deprivation as well ($p=0.08$). The difference may be a consequence of the better experimental design (more rats, mixed treatment order), or food intake may be more sensitive to water deprivation at the start of the active period. For example, there is little food in the stomach at the start of the dark period, so changes in gut tonicity may be larger. While there are methods that avoid the disturbance caused by taking away the food hoppers every 15 min for weighing, the measurement procedure does not seem to have large effects on food intake: rats ate similar amounts whether disturbed or not.

Rats eat just as often, but eat smaller meals during short periods of water deprivation. This agrees with data from Bolles (1961), who found that rats eat smaller meals when

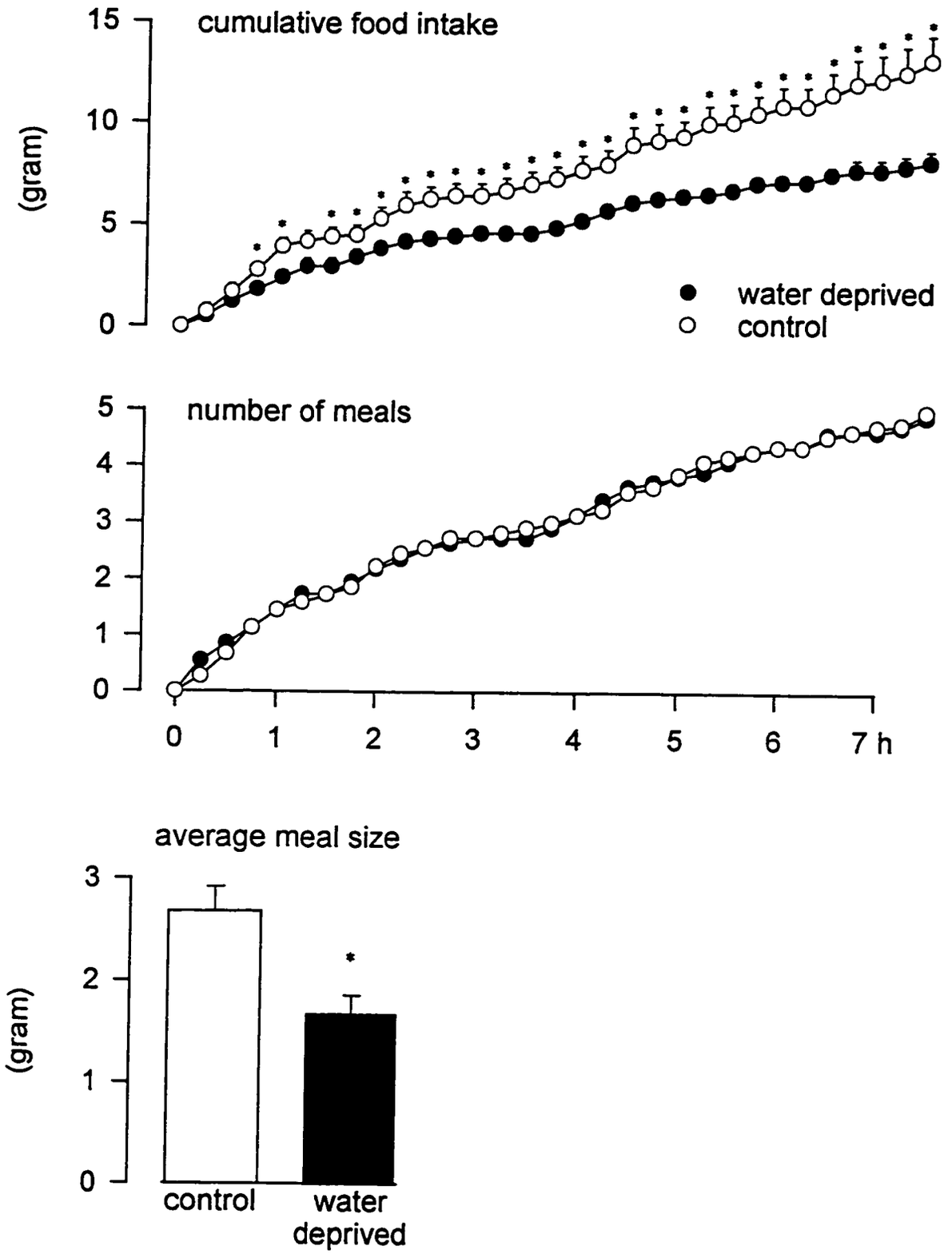

Figure 8.2 Cumulative food intake, number of meals and average meal size during 7.5 hours of water deprivation. * indicates diffence between water deprived rats and water replete controls ($p<0.05$, t test, $n=11$).

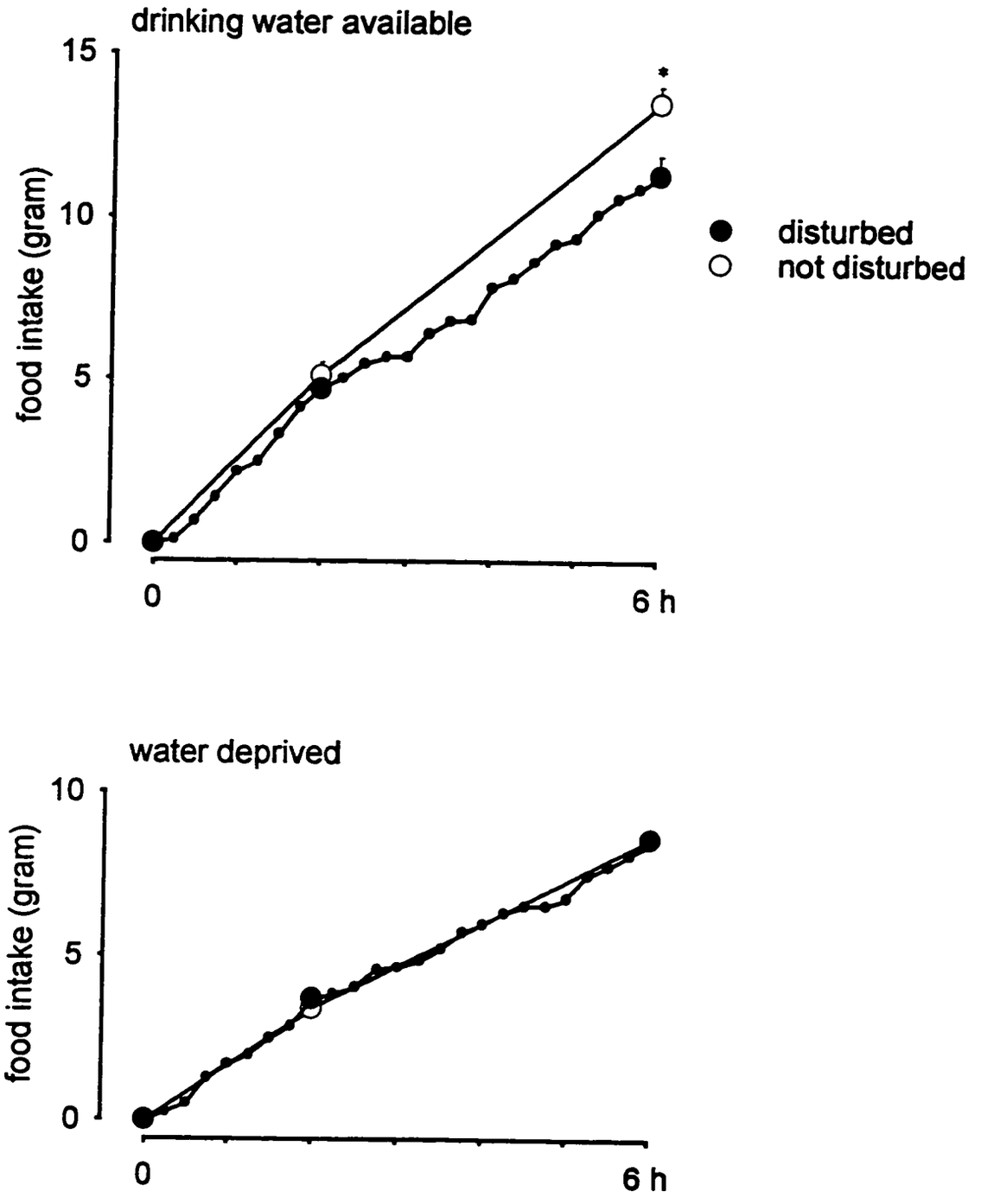

Figure 8.3. Food intake in rats that were disturbed for measurement of food intake every 15 min or only at 0, 2, and 6 hours. * indicates difference between disturbed and undisturbed ($p<0.05$, t test, $n=8$).

they have access to water only one hour each day. In goats, water deprivation reduces meal size as well (Langhans et al. 1991).

Even the first meal during water deprivation was smaller. This suggests that the signal that inhibits food intake develops during the meal, and causes the early termination of the meal. This is surprising, because rats do not drink much during a meal: only about 15% of the total water intake is within a meal, according to Kissileff (1969). Alternatively, the rats may already have been dehydrated at the start of the first meal. Rats often drink in the 10 min before a meal starts (Kissileff 1969). Water deprived rats lacked the opportunity to do so. Also, rats usually started eating in the last hour of the light period. Rats that ate just before the start of the period of water deprivation did not have their normal postprandial drink.

8.2 Effect of a meal on plasma osmolality and blood volume

8.2.1 Introduction Even very short periods of water deprivation reduce food intake. Because the first meal during water deprivation is smaller it is possible that the stimulus that reduces feeding develops within the first meal. It is not clear whether such a rapid response rules out sensors located outside the gut. Therefore I measured the changes in blood volume and tonicity after rats ate a normal sized meal when no drinking water was available. The results show that eating has rapid effects on systemic hydration.

8.2.2 Methods I took blood samples (0.35 mL each) from 6 rats (410$\pm$23 g) that had venous cannulas. (These rats had bladder cannulas as well). Rats were food deprived

overnight (from about 16:00 to 11:00). They were transferred to restraining cages, and offered 3 gram food, but no water. In control experiments rats did not eat or drink. All rats received both treatments in mixed order. Period between experiments was at least 5 days. In 4 of these rats blood was also collected after they were allowed to drink during the feeding period. In rats allowed to drink, drinking water was removed as soon as all food was eaten (usually within 20 min).

8.2.3 Results Plasma solute concentrations before the start of the experiment are shown in table 8.1. Rats allowed food ate rapidly (Figure 8.4). When drinking water was not available, plasma osmolality and $[Na^+]$ increased rapidly. Blood volume fell (calculated from changes in plasma protein concentration, see Appendix equation 3). Changes in plasma $[K^+]$ and [urea] after eating were small and not significant.

Rats allowed water and food drank 7.4 ± 0.8 mL (2.0 ± 0.6 mL during the first 5 min). Changes in plasma tonicity and blood volume in these rats were not significant. Changes in rats allowed no food or water were very small and not significant.

8.2.4 Discussion When no drinking water was present, eating caused changes in plasma tonicity within 10 min. Blood volume was reduced within 10 min as well. The assumption made in estimating the changes in blood volume (no change in the total amount of plasma protein except for the amount removed by sampling) is likely valid over such short periods. Rapid changes in plasma osmolality after eating, when no water is available have been described before (Deaux et al. 1970). Changes in osmolality in the GI tract, liver and portal vein may be even larger and faster than those measured here.

Table 8.1 Plasma solute concentrations before eating a meal. At the start of the experiment, rats had been food deprived for 17 hours.

	$[Na^+]$ mM	osmolality mosmol/kg	hematocrit %	[protein] mg/dL
no food, no water	141.1±0.9	288.8±0.9	43.6±0.8	6.4±0.1
food, no water	141.4±0.5	288.9±1.4	44.6±0.9	6.3±0.1
food and water	141.4±0.3	291.1±1.8	42.9±1.2	6.7±0.2

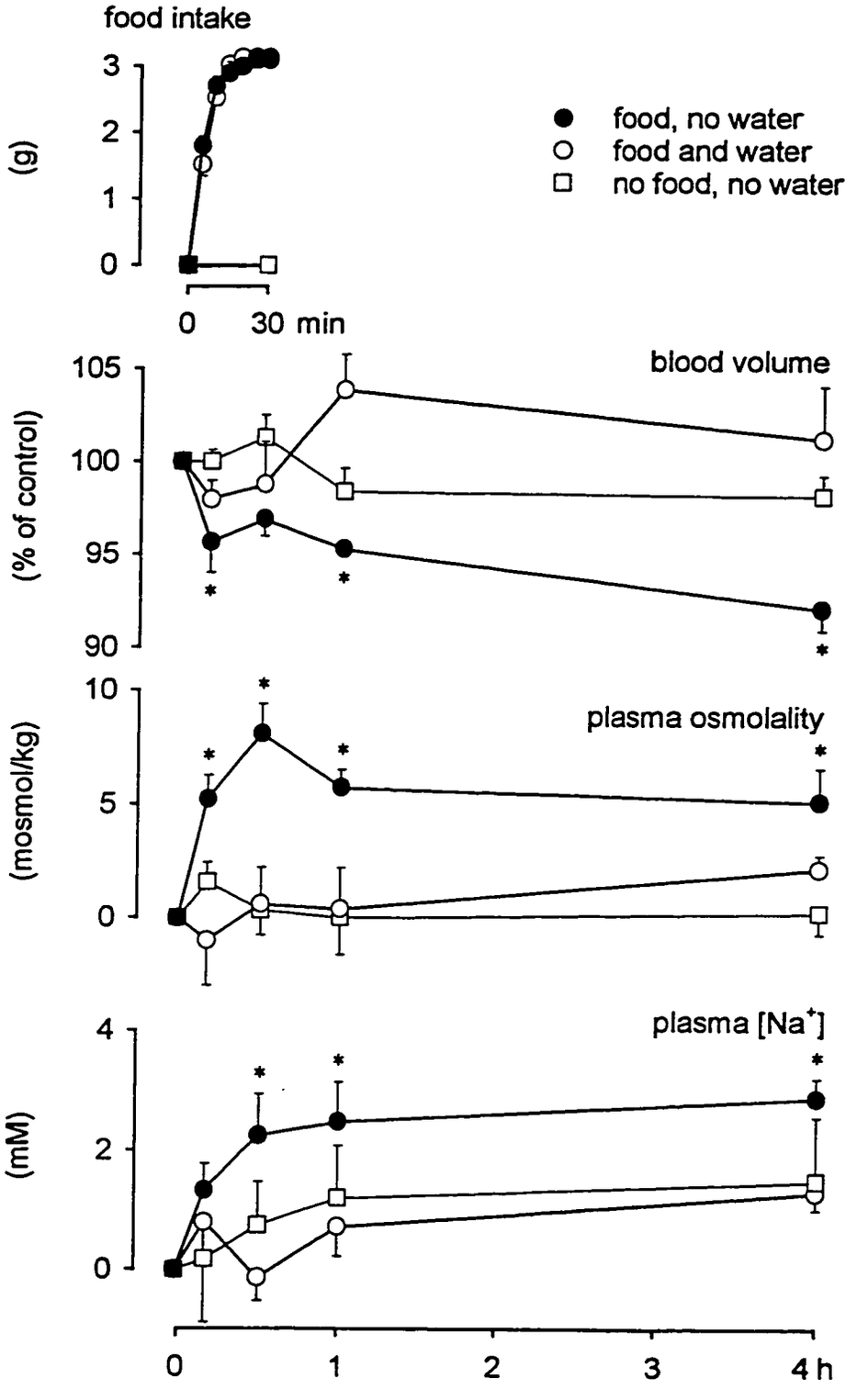

Figure 8.4 Food intake, changes in blood volume and plasma osmolality and plasma [Na^+] after a meal. Hungry rats were allowed only food (n=6), or water and food (n=4). Controls not allowed to eat or drink are also shown (n=6). * indicates difference from t=0 ($p<0.05$, Dunnet's t test).

Food intake after 10 min was 2.7 gram. This is more than total food intake at the onset of dehydration anorexia (1.85 g). Therefore the changes at the onset of dehydration anorexia may be smaller than those measured here.

In conclusion, changes in systemic osmolality after a meal occur so fast that a sensor outside the gut could account for an early termination of the first meal taken during water deprivation. The next section investigates if the small changes in plasma tonicity that occur in rats that eat a meal, but are not allowed to drink, are sufficient to account for the feeding inhibition.

8.3 Sensitivity of feeding to changes in plasma tonicity

8.3.1 Introduction As shown in section 8.2, plasma tonicity increases when rats are allowed to eat a meal while drinking water is witheld. Whether these changes in plasma tonicity are sufficient to account for the feeding inhibition is not known. Therefore I measured the sensitivity of the mechanism to changes in tonicity. In this experiment I altered plasma tonicity by infusing water or hypertonic saline at a fairly high rate through a venous cannula, and measured the effect of the infusions on food intake.

8.3.2 Methods Eight rats (body weight 511 $\pm$ 13 g) with venous cannulas were used. They were housed in metabolism cages.

To measure sensitivity to hypotonicity, rats were water deprived for 18 hours, from 17:00 to 11:00 the next morning. Then food was removed, cannulas were connected, and sterile water was infused through the venous cannula at a rate of 1.1 mL/min.

Infusions were 0, 5, 10 or 15 mL. Food was returned 6 min after the end of the infusion, and food intake was measured for 1 hour. Drinking water was not available during the feeding period. All rats received all treatments in mixed order. Period between experiments was at least 2 days.

To measure sensitivity to hypertonicity, rats were deprived of food for 10 hours, from 1:00 in the morning until 11:00. Then drinking water was removed, and 300 mM sterile NaCl solution was infused through the venous cannula. Infusion speed and other experimental details were the same as in the previous experiment.

I also measured the effect of the intravenous infusions on plasma osmolality and $[Na^+]$. Rats were water and fluid replete at the start of this experiment, but were not allowed to eat or drink during the experiment. I took a blood sample (0.35 mL) from the venous cannula, infused 10 mL water or 300 mM NaCl at a rate of 1.1 mL/min, waited 6 min, and took another blood sample.

8.3.3 Results Infusion of 10 mL water reduced plasma osmolality from 295 $\pm$ 1 to 282 $\pm$ 2 mosmol/kg and plasma $[Na^+]$ from 140 $\pm$ 1 to 134 $\pm$ 1 mM. The infusion did not cause obvious hemolysis. Infusion of 10 mL 300 mM NaCl increased plasma osmolality from 295 $\pm$ 1 to 308 $\pm$ 2 mosmol/kg and plasma $[Na^+]$ from 139 $\pm$ 0.3 to 147 $\pm$ 1 mM.

Intravenous infusion of water in water deprived rats stimulated feeding (Figure 8.5). The effect appears to be dose dependent. Infusion of 300 mM NaCl in hungry rats appeared to reduce feeding dose dependently, although only the largest infusion had a significant effect.

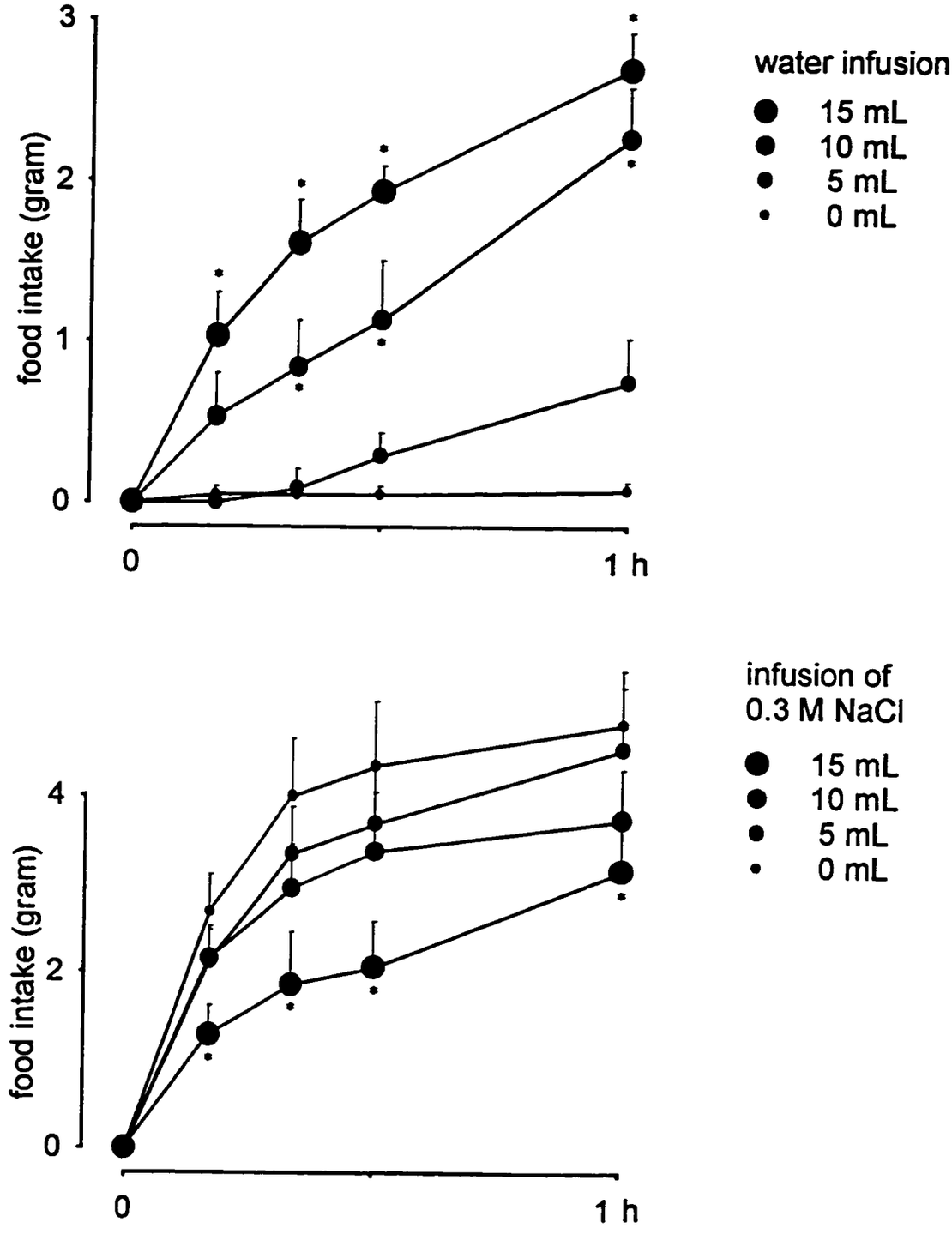

Figure 8.5 A) Intravenous infusion of water in water deprived rats stimulated feeding. B) Intravenous infusion of 300 mM NaCl in rats that had been food deprived for 10 hours reduced food intake. * indicates difference from no infusion (Dunnett's multiple comparisons test, $p<0.05$). All $n = 8$.

8.3.4 Discussion Infusion of 10 mL of these solutions caused changes in plasma osmolality and [Na^+] between 4.3 and 5.5%, 6 min after the end of the infusion. Infusion volume, 10 mL, was about 3.0% of the amount of water in the body of these rats (assuming body water was 65% of the body weight). Therefore, at the start of the feeding period the fluid infused had not yet completely mixed with the body fluids. Thus osmotic changes in poorly perfused tissues may have been smaller. Infusion of 10 mL water or 300 mM NaCl caused changes in plasma tonicity that are slightly larger than changes after a meal. Plasma osmolality increased by 2.8%, and [Na^+] by 2.2%, when rats were allowed to eat a meal, but no drinking water was present (section 8.2). Changes in plasma solutes at the onset of dehydration anorexia may be even smaller.

Fairly small changes in plasma tonicity do cause changes in food intake. This confirms data from Novin et al. (1966), who found that intravenous infusion of water stimulated food intake in rats that had been water deprived for 1 day, but infusion of 0.15 mM NaCl did not. The changes caused by the hypertonic infusions however are probably a bit larger than those seen at the onset of dehydration anorexia. This does not rule out a role for plasma tonicity in feeding during water deprivation. The physiological states that rats are in at the onset of dehydration anorexia and after the infusions may be quite different. For example, metabolic deficits would be larger in the infusion experiment. It is possible that sensitivity to osmotic signals is different in the two conditions. It is not known if smaller changes in plasma tonicity can affect food intake.

8.4 Infusion of water in the stomach restores feeding

8.4.1 Introduction Eating and drinking often occur together. Normally rats drink most of their water immediately before, during or shortly after a meal (Kissileff 1969, Fitzsimons and Le Magnen 1969, Oatley 1971, Kraly 1984).

There is evidence that suggests that pre-absorptive signals are important in this coupling of food and water intake. When rats are put on a novel diet that requires a high water intake, initially much of the water is taken outside meal time, but after a few days most of the drinking is associated with meals (Fitzsimons and LeMagnen 1969). This suggests that systemic dehydration is the cause for drinking on the novel diet, and that pre-absorptive signals become the stimulus for drinking when rats are getting used to the diet. Also, as shown in section 7.2, pre-absorptive signals caused by drinking also play a role in the feeding seen when water is returned after a period of water deprivation.

Therefore I measured if the act of drinking is needed for normal food intake. I infused water into the stomach of rats that were not allowed to drink, bypassing receptors in the mouth and pharynx. Because infusion of water in the stomach completely restored food intake, dehydration anorexia is not caused by pre-absorptive signals associated with drinking.

8.4.2 Methods I measured food intake in rats (504±22 g, n=8) that had gastric cannulas. They were housed in metabolism cages.

The rats had no drinking water between 17:30 and 9:00 the next morning. During this period water was slowly infused into their stomach. The amount infused (47 mL in 15.5 h, about 50 μL/min) was slightly larger than the amount normally drunk (40.0 ±

4.4 mL). In control experiments cannulas were connected, but rats received no infusion (sham infusion). In other control experiments rats had drinking water available, and also received infusions or sham infusions. All rats received all four treatments in mixed order. Time between experiments was 2-3 days.

8.4.3 Results When allowed to drink during the test period, rats drank 40 $\pm$ 4 mL. During water deprivation rats reduced food intake 43%, compared with when they were allowed to drink (Figure 8.6). Intragastric infusion of water during deprivation completely restored food intake. The infusion did not alter food intake in rats that were allowed to drink.

8.4.4 Discussion Rats that were not allowed to drink but received an infusion of water ate as much as rats allowed to drink. This demonstrates that the act of drinking is not necessary for normal food intake. The results suggest that instead hydration of the body is critical. The pattern of water intake does not appear to be important. Rats ate the same amount whether water was infused continuously or they were allowed to drink. The data however do not rule out the possibility that meal pattern in water deprived rats infused with water is different.

Note that gastric infusions will affect the whole body: the infused water is absorbed and hydrates the whole body. Therefore the results do not provide information on the location of the sensor. It could be located anywhere in the body. For example, the infusions will hydrate the oropharyngeal area. Thus the results do not rule out a dry mouth as a cause for the feeding inhibition.

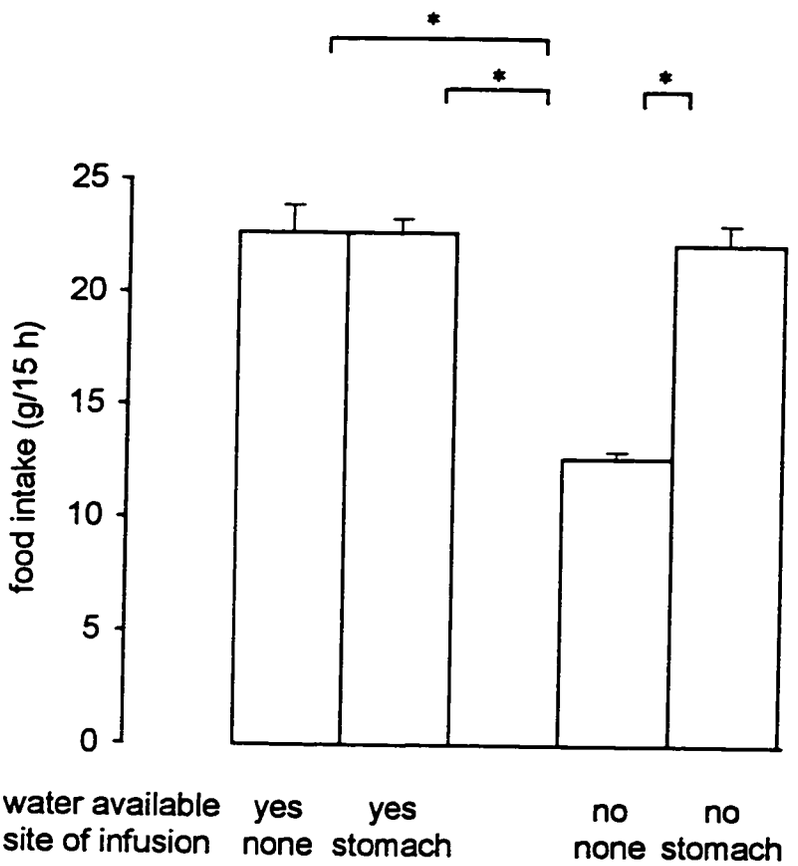

Figure 8.6 Infusion of water in the stomach restored food intake in rats not allowed to drink. Rate of infusion was 47 mL/15.5 h. Normal water intake during this period was 40 ± 4 mL. * indicates difference ($p < 0.05$, Student Newman Keuls test, $n=8$).

8.5 Location of the sensor

8.5.1 Introduction There are several possible locations for the sensors that reduce food intake during water deprivation. The mouth and throat, gastrointestinal tract, portal vein, liver and brain seem the most likely places (see section 1.4.1). To distinguish between these locations water was infused during water deprivation in the stomach, vena cava, jejunum or cecum, and food intake was measured. Such infusions have different effects on sensors in different places. For example water infused in the stomach will have more effect on osmoreceptors located in gut and liver than intravenous infusions would. A stronger effect of the intragastric infusion would support a sensor in the gut or liver, whereas similar effects of the infusions would support a location outside the gut, for example in the brain. To minimize the effect on sensors located far away from the infusion site infusion speed in these experiments was lower than the normal water intake. This also helps prevent unphysiologically large changes in tonicity.

8.5.2 Methods Eight rats (368 $\pm$ 12 g) were used that had cannulas in the stomach and in the jejunum, about 7 cm from the pylorus. Eight rats (404 $\pm$ 3 g) had cannulas in the stomach and cecum. Eight rats (552 $\pm$ 13 g) had cannulas in the stomach and in the vena cava. All rats were housed in metabolism cages.

Rats were water deprived for 6 hours, starting at the beginning of the dark period. During this period sterile distilled water was slowly infused through a cannula. The rate of infusion was 10 mL/6 hours, which was less than the amount these rats normally drank during this period. (Rats with duodenal cannulas normally drank 14.3 $\pm$ 1.5 mL, rats with cannulas in the cecum drank 14.7 $\pm$ 1.0 mL, and rats with venous cannulas

drank 18.6 ± 1.3 mL during the test period). Food intake was measured every 15 min in the rats with venous cannulas, and only at the end of the test period in the other experiments.

In control experiments food intake was measured in rats that were water deprived, or had drinking water available, but received no infusion. In these experiments rats were not connected to infusion tubing. All rats received all treatments in mixed order. The period between experiments was at least 2 days.

In other control experiments I tested whether intravenous infusions have a non-specific effect on food intake. Food intake was measured when water (10 mL/6 h) was infused in rats allowed to drink, and compared with food intake when rats received no infusion. Rats receiving no infusion were not connected to the infusion tubing.

8.5.3 Results Water deprivation reduced food intake. Infusion of water in the stomach stimulated food intake (Figure 8.7). Infusions in jejunum and cecum were as effective as gastric infusions, but intravenous infusions did not stimulate food intake. The infusions did not change urinary water loss (Table 8.2).

Intravenous infusion of water did not influence food intake in rats allowed to drink. Rats ate 12.9 ± 0.6 g when they received no infusion, and 12.9 ± 0.6 g when they did.

Meal pattern was monitored in rats given gastric or venous infusions. Compared to controls allowed to drink, water deprived rats reduced food intake within 2 h (3.3 ± 0.4 vs. 4.6 ± 0.6 g, $p<0.05$, t test). As in section 8.1, water deprivation reduced meal size (from 2.9 ± 0.2 to 2.2 ± 0.3 gram, $p<0.05$, t test), but had no effect on the number of meals (4.9 ± 0.6 during water deprivation, 4.9 ± 0.3 when water was available). The first meal during water deprivation tended to be smaller (2.4 ± 0.4 vs 3.0 ± 0.3

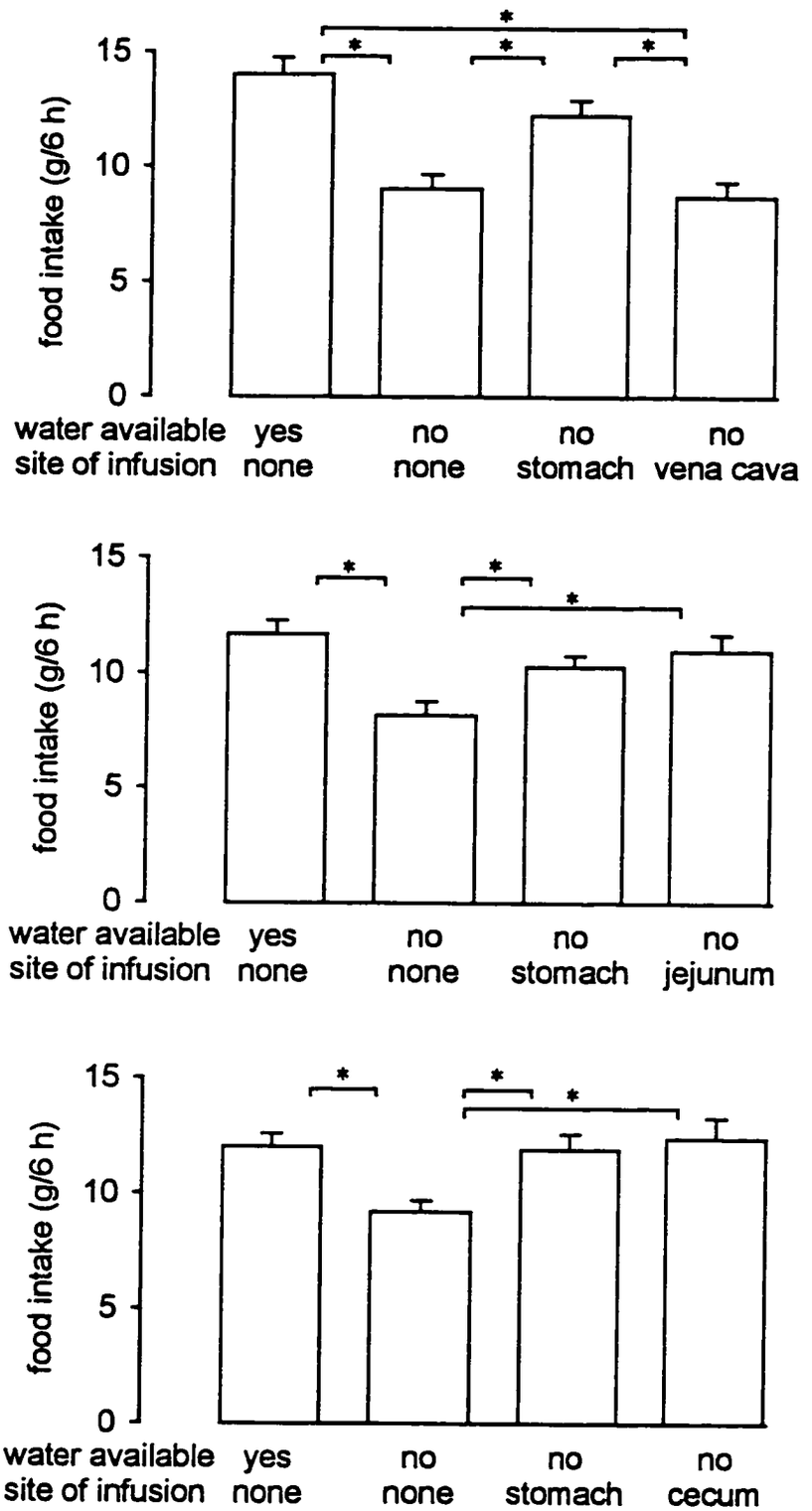

Figure 8.7 Effect of infusion of water in stomach, jejunum, cecum, or vena cava on food intake during 6 hours of water deprivation. Rate of infusion was 10 mL/6 hours. Normal water intake was 17 mL. * indicates difference ($p<0.05$, Student Newman Keuls test, $n=8$).

Table 8.2 Infusion of water (10 mL/6 h) in rats not allowed to drink did not alter urine volume.

	vena cava	jejunum	cecum
drinking water available, no infusion	6.0 ± 0.9	7.0 ± 1.0	7.6 ± 0.6
water deprived, no infusion	6.5 ± 0.3	5.9 ± 0.6	6.6 ± 0.5
water deprived, gastric infusion	6.3 ± 0.9	6.5 ± 0.6	6.9 ± 0.5
water deprived, test infusion	6.5 ± 0.5	6.5 ± 0.4	7.6 ± 0.6

Urine volume (± SEM) in mL/6h. Differences were not statistically significant (one way ANOVA, n=8).

g) but the difference was not significant.

Infusion of water in the stomach did not alter meal frequency and size. During gastric infusion rats ate 5.8 ± 0.7 meals and each was on average 2.4±0.3 g. While water deprived, these rats ate 4.9 ± 0.6 meals of 2.2 ± 0.3 g. During venous infusions rats tended to eat fewer, but larger meals (3.8 ± 0.5 meals, each 2.5 ± 0.3 g large) than during water deprivation, but the difference was not significant.

8.5.4 Discussion Gastric infusions will cause larger changes in tonicity in gut and liver than intravenous infusions do. The effect of intragastric infusion on tonicity in the rest of the body would be either smaller or similar to intravenous infusion. If the water infused in the stomach is immediately absorbed, the effects of the infusions on the rest of the body would be similar, because the infusions did not alter urinary water loss. It seems more likely not all water infused in the stomach immediately enters the circulation, and intragastric infusions may have had less effect outside the gut. Water deprived rats ate more when water was infused in the stomach than in the blood. This supports a gut sensor for dehydration anorexia, or possibly a sensor in the liver or portal circulation. Because the infusions were very slow (10 mL/6 h), slower than normal drinking, it is not likely that gastric and intravenous infusions caused unphysiologically large changes in tonicity anywhere in the body.

Water deprivation reduced meal size, but had no effect on meal frequency (see also section 8.1, Bolles 1961, Langhans et al. 1991). If drinking and intragastric infusions stimulate feeding by the same mechanism one would expect that intragastric infusions increase meal size, but do not alter meal frequency. It remains to be seen whether that is true. Gastric infusions did not significantly increase meal size, but meal size did not

differ from water replete controls either. Failure of gastric infusions to increase meal size is however not incompatible with a sensor in gut or liver. For example, the infusions do not mimic the normal drinking pattern. Much of the normal drinking occurs immediately before, during or immediately after a meal (Kissileff 1969). This would reduce changes in gut tonicity during a meal. The slow, continuous infusions may have little effect on the presumably rapid rise in gut tonicity during a meal, and thus not prevent early termination of a meal. Instead, they may accelerate the slow fall to normal tonicity after the meal ends. This could facilitate initiation of the next meal. Therefore an increase in meal frequency during slow infusions is compatible with a gut or liver sensor.

Infusion of water into the vena cava had no significant effect on meal size and meal frequency. The trend toward fewer, larger meals is puzzling. The cause is not clear.

If intravenous infusions had made the rats sick (by causing hemolysis for example, or by introducing bacterial contamination) the results could be compatible with a sensor outside the gut. In this case intravenous infusions would have to affect food intake in two ways that exactly cancel each other. Clearly this is not the case: the infusions did not alter food intake in rats allowed to drink. Intragastric infusions also do not affect food intake in rats allowed to drink (section 8.3.1).

The results are not compatible with a brain sensor. Intravenous infusions would have at least as much effect on brain tonicity as intragastric infusions, yet did not stimulate feeding. There is additional evidence that no brain sensor is involved. Rolls (1975) found that brain lesions that abolish drinking to injections with hypertonic saline do not prevent dehydration anorexia.

The results also are not compatible with a dry mouth as a cause for the inhibition of feeding. Both intravenous and intragastric infusions would have rehydrated sensors in the mouth and throat.

The importance of gastro-intestinal osmoreceptors is well established (see Hunt, 1983, for review). Duodenal osmoreceptors are important in controlling gastric emptying (Hunt 1983), and receptors in the jejunum may play a role as well (Cooke 1977). Gut osmoreceptors are also known to play a role in control of food intake. Houpt et al. (1979) found that infusion of small amounts of hypertonic saline in the duodenum reduced food intake in pigs not allowed to drink. Similar infusions in the portal vein had no effect, and they concluded that an osmoreceptor in the gut influences feeding. The feeding inhibition was abolished after vagotomy, suggesting the signals from the sensors travel through the vagus nerve to the brain.

Recent experiments from our lab support Houpt's conclusion (Houpt et al. 1979) that the sensors are not in the liver. Infusion of water in the portal vein, just proximal of the liver, did not restore food intake during water deprivation (Durant et al. 1996).

The exact location of the sensor is not clear, but the most likely explanation of the results is that osmosensitive receptors are all over the GI tract or portal circulation. Infusions in the jejunum or cecum were as effective as intragastric infusions. The blood that drains from the cecum mixes with blood draining from the stomach, duodenum and jejunum only a few cm before the liver (Greene 1935). The finding that infusions in the stomach and cecum restore feeding suggests that the sensors are either in the portal circulation just before the liver, or else they are dispersed throughout the intestine, portal circulation, or both. Of course, the cecum and blood draining from it are not likely to mediate the feeding inhibition during water deprivation. It seems unlikely that large

changes in tonicity of the cecum occur during the first hour of water deprivation. However, the infusions in the cecum, although they were slow, may have caused changes in local tonicity much larger than those seen during water deprivation or rehydration.

In conclusion, the sensors that mediate the feeding inhibition during water deprivation are likely in the stomach, the first part of the intestine, or in the circulation that drains these areas, but before the liver. There are probably also osmoreceptors in more distal parts of the intestine that influence food intake, but these are probably not the cause of the changes in food intake during water deprivation.

As a last note, it appears that tonicity of the stomach itself has little effect on food intake. In section 7.2, rats with gastric fistulas had the stomach rinsed with water before the start of the experiment. Whatever stomach content was left must have been isotonic or hypotonic. Flushing the stomach with water had little effect on food intake: controls not allowed to drink ate little. This suggests that hypertonicity of stomach contents is not the cause of the anorexia seen during water deprivation.

8.6 General discussion

During water deprivation rats reduced food intake within an hour: even the first meal during water deprivation was smaller. Such rapid changes in food intake do not rule out a sensor outside the gut: during eating, changes in systemic hydration develop so fast that they could account for the changes in food intake seen during water deprivation. Indeed, infusions that caused reductions in plasma tonicity of a few percent did stimulate drinking

in water deprived rats, and infusions that increased plasma tonicity by a few percent reduced feeding in hungry rats.

However, experiments in sections 8.4 and 8.5 show the sensor is in the gut. First, the act of drinking is not necessary: feeding was restored when water was infused in the stomach of rats not allowed to drink (section 8.3). The oropharyngeal signals caused by drinking are not necessary for normal food intake during water deprivation. This is surprising, because oropharyngeal signals contributed to drinking on rehydration (section 7.2). The reason for this apparent discrepancy is not clear. Rats receiving an infusion during water deprivation did not become dehydrated, and it may be that oropharyngeal signals are more important when the animal is already dehydrated. It may also be that that lack of oropharyngeal signals associated with drinking influenced meal pattern in rats receiving an infusion during water deprivation. Oropharyngeal signals are often involved in feed-forward mechanisms that contribute to more rapid and accurate restoration of a deficit. Their effects may be short lasting, because rats allowed to drink while their gastric fistula was open completed feeding within 30 min (Figure 7.2). Signals from the gut and postabsorptive signals may last longer: rats that received an infusion with water in the stomach appeared to continue eating longer (Figure 7.2). It is possible that rats receiving a gastric infusion of water would have eaten as much as rats allowed to drink, had they been allowed more time.

Second, intravenous infusion of water did not restore food intake in rats not allowed to drink, while intragastric control infusions did (section 8.4). This strongly suggests the sensor is in the gut, portal circulation, or liver. As explained in section 6.5.4, it seems likely that the sensor is in the first part of the small intestine or in the portal circulation that drains this segment. Signals from these sensors may travel through the vagus nerve

to the brain (Houpt et al. 1979). Whether the same sensor is involved in the feeding inhibition during long periods of water deprivation is not known. Other mechanisms may play a role during longer periods of water deprivation. For example, lack of saliva may make swallowing impossible (Wolf 1957).

9 PERSPECTIVES AND CONCLUSIONS

9.1 Perspectives

One can gauge our knowledge of body fluid regulation from our understanding about how an organism deals with very common disturbances, such as a short period of water deprivation. My studies on water deprivation and rehydration highlight the role that changes in salt intake and salt excretion play in body fluid regulation. It has been known for a long time that such changes play an important role in the regulation of ECF and blood volume, but my experiments show that they play a very important role in osmoregulation as well.

In rats, even short periods of water deprivation are a threat to body fluid maintenance because normally, water intake is high. During 10 hours of water deprivation rats lost close to 8% of their body water, and blood volume fell by about 6.5%. Osmoregulation appeared to be a more important concern than maintenance of blood volume: plasma tonicity increased by only 1 or 2%.

This thesis shows that during water deprivation rats osmoregulated mainly by reducing the amount of salt in the body. Rats reduced food intake and excreted more sodium, potassium and chloride. This is very different from textbook explanations of

osmoregulation, which focus almost exclusively on the role of ADH and urine concentrating mechanisms.

During 10 hours of water deprivation, urinary water loss changed little, and urine concentration increased only slightly. Longer periods of water deprivation cause larger increases in urine osmolality (Woods and Johnston 1983), but because the amount of solute that is excreted falls to low levels (Taffel and Elkinton 1942, Ben Goumi et al 1993), the associated water savings are not large. Water deprivation is not the only condition where the role of ADH appears limited. For example, increases in dietary salt intake cause an increase in water intake and urine volume in dogs, but plasma ADH levels do not rise, and urine tonicity does not increase (Cowley et al. 1983). Ramsay and Thrasher (1991) have argued that at the normal set point for plasma osmolality, urine concentration approaches its maximum value, at least in rats and dogs. Changes in ADH may be more important in preventing overhydration than in preventing hypertonicity.

The most important osmoregulatory response during water deprivation was a reduction in food intake. This had several beneficial effects: eating less allowed absorption of water already in the gut, it reduced the amount of sodium and potassium absorbed from the gut, and it may also reduce evaporative water loss. Reduced food intake is important during longer deprivation periods as well, as food intake falls progressively during longer periods of water deprivation (Ben Goumi et al. 1993).

The mechanism of the feeding inhibition has hardly been investigated. Much of the research on dehydration anorexia is old and poorly controlled. Instead, our best knowledge about the role of tonicity in control of feeding has come from a different approach: the study of the effect of hypertonic infusions into the gut on food intake. Small infusions of hypertonic solutions into the duodenum reduce food intake in animals

not allowed to drink (Houpt et al. 1979). A gut sensor is involved, because infusion into the portal vein does not inhibit food intake (Houpt et al. 1979). These infusions probably did not cause sickness, because animals did not develop a conditioned taste aversion to the infusions (Houpt et al. 1979). Houpt suggested that duodenal tonicity may be an important factor in termination of normal meals.

My results indicate that the mechanism that causes dehydration anorexia may be similar to the mechanism that reduces food intake after hypertonic infusions in the gut. My results show that the act of drinking (taste of water, swallowing) is not necessary for normal food intake. The sensor that mediates the feeding inhibition during water deprivation is not located in the brain, and the feeding inhibition also is not caused by a dry mouth. Instead, the sensor is located in the gut or in the portal circulation. The exact location of the sensor is not clear. My results suggest that sensors monitoring hydration may be located throughout the gastro-intestinal tract. Measurement of changes in tonicity during water deprivation throughout the gastrointestinal tract could provide more information on the location of the sensors. My finding that even the first meal during water deprivation was smaller suggests that the mechanism may be important in termination of normal meals.

The type of sensor is not known. Houpt et al. (1979) found that intestinal infusions with hypertonic NaCl or glucose had more effect on feeding than equally hypertonic infusions with sorbitol or mannitol. In contrast to NaCl and glucose, sorbitol and mannitol are poorly absorbed. Houpt also found that the feeding inhibition was reduced when the vagus nerve was cut, suggesting that the signal travels through the vagus nerve to the brain.

Increased excretion of osmotically active solutes also contributed to osmoregulation during water deprivation. This response may be less important during longer periods of water deprivation because then excretion of sodium falls to very low values (Taffel and Elkinton 1942, Ben Goumi et al. 1991). Potassium excretion continues, but part of the potassium excretion may be related to the breakdown of intracellular protein that occurs during longer periods of deprivation (Taffel and Elkinton 1942).

It is generally thought that dehydration natriuresis is caused by a brain sensor. However, the brain infusions that have been used to investigate the mechanism have invariably caused changes in brain tonicity that are far larger than those seen during water deprivation. So far, it has not been shown that brain infusions that cause small changes in CSF [Na^+], such as occur during water deprivation, affect solute excretion.

My research shows that urinary changes after brain infusions and after water deprivation and rehydration are similar in several ways, suggesting that indeed the same mechanism is involved. The same solutes are involved, the time course of the changes is similar, and the effect of brain infusions depends on hydrational state. My research also suggests that the sensor that causes the urinary changes during brain infusions is sensitive to changes in the ECF of the brain rather than CSF. The effect of brain infusions on [Na^+] at the sensor site is not known.

Brain infusions probably acted on a sensor within the blood-brain barrier. Whether the sensor for dehydration natriuresis is inside the blood-brain barrier remains to be seen. Tissue within the blood brain barrier can be dehydrated selectively by infusions with hypertonic urea, glucose or glycerol into the carotid artery. Such infusions may also cause an osmotic diuresis, which could affect electrolyte excretion, but infusion of the same solutions into the vena cava could control for this effect.

9.2 Conclusions

1 Rats are highly capable of keeping tonicity of the body fluids constant during water deprivation. Although rats lost about 8% of their body water during 10 hours of water deprivation, plasma [Na^+] rose by only 1-2%. Blood volume fell by 6.5%.

2 During 10 hours of water deprivation rats reduced food intake, increased excretion of sodium, potassium and chloride, and produced a more concentrated urine. Reduced food intake, increased solute excretion and increased urinary concentration reduced the amount of water that would be needed to keep plasma tonicity constant by about 48, 17 and 10%, respectively.

3 Reduced food intake allowed rats to absorb water already in the gut. It also reduced absorption of sodium and potassium from the gut, reducing the amount of solute to be excreted. The results also suggest that by reducing food intake rats reduced evaporative water loss.

4 Rats allowed to drink after 10 hours of water deprivation drank sufficient to restore or almost restore their fluid deficit. They also rapidly reduced excretion of sodium, potassium and chloride. These changes in solute excretion would contribute to osmoregulation.

5 Rats allowed to eat and drink after water deprivation drank more, and excreted more solute. As a result of the changes in intake and excretion, changes in plasma tonicity and blood volume were similar whether rats were allowed to eat or not.

6 Infusions in the lateral brain ventricle that caused changes in CSF [Na^+] caused urinary changes that are similar to those seen during water deprivation and rehydration: the same solutes were involved, and the time course of the changes was

similar. This suggests that the mechanisms are similar. The fact that the effect of brain infusions depended on hydrational status of the rat supports this hypothesis.

7 Water deprived rats ate smaller meals, but ate as often. Even the first meal was smaller. Eating a normal sized meal, when drinking water was not available, caused a rapid increase in plasma osmolality, and reduced blood volume.

8 The sensor that causes dehydration anorexia is located in the gut or portal circulation.

9 On rehydration, oropharyngeal as well as post-oropharyngeal signals caused by drinking stimulate food intake.

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APPENDIX ESTIMATION OF BLOOD VOLUME

Changes in hematocrit, hemoglobin concentration and plasma protein concentration can be used to estimate changes in blood volume.

The relation between hematocrit changes and blood volume changes can be derived as follows: (hct is true hematocrit, that is, average hematocrit of all blood in the body, bv is blood volume, rcv is volume of all red cells, and the subscripts indicate different time points).

$hct = rcv/bv$. rearrange:

$bv = rcv/hct$.

$bv_2/bv_1 = (rcv_2/hct_2) / (rcv_1/hct_1)$. If $rcv_2 = rcv_1$ then

$= (1/hct_2) / (1/hct_1)$.

$= hct_1/hct_2$.

The observed hematocrit is higher than the true hematocrit. First, some plasma is trapped between the packed cells. Also, hematocrit in large vessels is higher than hematocrit in the rest of the body (see Erslev 1990). According to Ware et al. (1984), observed hematocrit * 0.9 equals true hematocrit. Substituting observed hematocrit * 0.9 for hct has no effect on the result because the 0.9 divides out. Therefore we can use observed hematocrit instead of true hematocrit. As shown, the formula assumes that total red cell volume does not change. Fortunately rats do not have a contractile spleen. Note however that red cell volume does change if plasma tonicity changes. If red cells are removed during repeated sampling, the formula can be corrected: (bv_i = initial blood volume)

$$\mathbf{bv_2/bv_1 = hct_1/hct_2 \times (1\text{-sample volume}/bv_i)} \quad (1)$$

If sample volume is small relative to bv_i, bv_i can be estimated without effect on the result. In the calculations I used 6.4 mL blood/kg body weight (Altmann and Dittmer 1974).

To estimate blood volume changes from changes in hemoglobin concentration in blood, a similar formula can be derived: ([hg] is true hemoglobin concentration, that is, average hemoglobin concentration of all blood, thg is total amount of hemoglobin in the blood)

$thg = [hg]*bv$. rearrange:

$bv = thg/[hg]$.

$bv_2/bv_1 = (thg_2/[hg_2] / (thg_1/[hg_1])$. If $thg_2 = thg_1$ then

$= (1/[hg_2) / (1/[hg_1])$.

$= [hg_1]/[hg_2]$.

As was the case for hematocrit, it is correct to substitute observed hemoglobin concentration for true hemoglobin concentration. Note that the only assumption is that the total amount of circulating hemoglobin does not change. Thus this method, unlike hematocrit, is not sensitive to changes in plasma tonicity. If hemoglobin is lost with repeated sampling, the formula can be adapted:

$$\mathbf{bv_2/bv_1 = hg_1/hg_2 \times (1\text{-sample volume}/bv_i)} \quad (2)$$

Deriving blood volume changes from changes in plasma protein and hematocrit is slightly more complicated: (tpp is total amount of circulating protein, and [pp] is 'true' plasma protein concentration)

bv = pv/(1-hct). Also,

tpp = pv*[pp]. rearrange:

pv = tpp/[pp]. Substituting pv in the first line gives

bv = (tpp/[pp]) / (1-hct).

= tpp / ([pp]*(1-hct)).

bv_2/bv_1 = $(tpp_2/([pp_2])*(1-hct_2)) / (tpp_1/([pp_1]*(1-hct_1))$. If $tpp_2 = tpp_1$ then

= $(1/([pp_2])*(1-hct_2)) / (1/[pp_1])*(1-hct_1))$.

= $[pp_1]*(1-hct_1) / ([pp_2*(1-hct_2))$.

= $[pp_1]/[pp_2] * (1-hct_1)/(1-hct_2)$. (see Houpt and Yang 1995)

Given the even distribution of protein throughout the blood, the observed plasma protein concentration can be used instead of the true plasma protein concentration. However, we should use 0.9 * observed hematocrit to get true hematocrit. After correcting for loss of protein due to repeated sampling the formula is:

$$bv_2/bv_1 = (pp_1/pp_2)x(1-hct_1x0.90)/(1-hct_2x0.90)x(1-\text{sample volume}/bv_1) \quad (3)$$

This formula assumes the total amount of plasma protein does not change, but does not make assumptions about total red cell volume. However there are several factors that may alter the amount of plasma protein. First, changes in capillary permeability can alter the amount of plasma protein. Unfortunately, several hormones involved in body fluid regulation may affect vascular permeability (Svensjö and Grega 1986). Second, turnover of plasma proteins is faster than that of red blood cells, therefore changes in protein production rate are more likely to affect the calculations.

I tested the validity of the assumptions used in deriving the formulas with data from Kutscher (1971). He measured distribution volume of Evans Blue dye (this is plasma volume), plasma protein concentration, and hematocrit in rats after 48 or 96 hours of deprivation of water, food or both. From these data we can calculate total amount of circulating protein and total volume of red cells (see column 5). The results show that total volume of red blood cells did not change much, but the treatments reduced the amount of plasma protein. Thus equations 1 and 2 would be more accurate than 3.

Next I compared the changes in blood volume estimated with equations 1 and 3 with those calculated from distribution volume of Evans Blue dye and hematocrit (column 6). As expected, the changes calculated from hematocrit changes are close to those calculated with the reference method (dye) but estimates based on plasma protein concentration are useless, at least after these relatively long deprivation periods. If the amount of plasma protein remains constant, for example when the experiment is short, plasma protein is a very good indicator.

Table A Effect of 48 or 96 hours of water deprivation, food deprivation or water + food deprivaton on indicators for blood volume, on amount of circulating protein and total volume of red cells, and on estimates of blood volume.

	plasma vol.	hct (%)	[pp] (g/dL)	total pp	rcv	estimated bv (%)		
						dye	hct	pp
control	3.7	43.1	6.0	222	2.31	100.0		
w48	2.7	53.0	7.0	189	2.47	85.9	81.3	100.3
w96	2.6	52.4	6.8	177	2.32	81.9	82.3	102.2
w+f48	2.8	51.5	6.2	174	2.41	86.7	83.7	110.4
w+f96	2.3	55.2	6.2	143	2.29	76.3	78.1	117.7
f48	3.0	49.4	6.1	183	2.39	89.6	87.2	108.4
f96	2.6	52.0	5.6	146	2.29	81.3	82.9	123.3

Calculations are based on data from Kutscher (1971). Kutscher measured distribution volume of Evans Blue dye (plasma volume), hematocrit (hct), and plasma protein concentration ([pp]) in rats after 48 or 96 hours of deprivation of water, food or both. His data are shown in the left 3 columns (plasma volume in 100 g predeprivation body weight).

From his data I calculated total amount of circulating protein (total pp = plasma volume x [pp], in mg/100 g pre-deprivation body weight, column 4) and total volume of red cells (rcv = plasma volume x hct x 0.9 / (1-hct x 0.9), in mL/100 g pre-deprivation body weight, column 5). Deprivation sharply reduced the amount of circulating protein, but had little effect on total red cell volume.

From Kutcher's data we can also calculate blood volume after deprivation (blood volume = pv+rcv). It can be expressed as % of blood volume of controls (column 6). Then I used formulas 1 and 3 to estimate blood volume changes from changes in hematocrit (column 7) and plasma protein (column 8). If we compare columns 7 and 8 with 6, it appears that changes in hematocrit are a good indicator for blood volume changes, but estimates based on plasma protein are way off.