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Responses of water and salt regulating hormones during acute cold exposure in the rat

Dice, Margaret Sibyl, Ph.D.

University of Hawai'i, 1992
RESPONSES OF WATER AND SALT REGULATING HORMONES
DURING ACUTE COLD EXPOSURE
IN THE RAT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
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TO MY GRANDFATHER:  DR. LEE R. DICE

Professor Emeritus of the University of Michigan, in whose honor the Lee R. Dice Chair in Genetics was established there.

ALSO, AND ESPECIALLY

TO MY PARENTS
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This research effort was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals. The authors adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1985.
ABSTRACT

Soon after exposure to cold, humans and some animals experience a dilute diuresis, which is often accompanied by a natriuresis. Plasma vasopressin has been reported to decrease, possibly due to a central shunting of blood from peripheral vasoconstriction, and subsequent activation of the "Gauer-Henry reflex". The sodium regulating hormones atrial natriuretic factor (ANF), and the renin-aldosterone system have not been measured acutely in the cold.

The present study used the conscious, chronically instrumented rat to assess plasma vasopressin, aldosterone, renin, ANF, and urinary vasopressin during acute cold exposures of 1 hour at 13° C., or time control at 26° C.. Urine flow, osmolality, electrolyte excretion rates, and creatinine, osmotic, and free water clearances were measured at 10 minute intervals. Hematocrit, plasma electrolytes and osmolality, and all hormones were obtained using a donor replacement protocol at baseline, at 20, and 60 minutes of cold exposure, and at 1 hour recovery. Rectal temperature, heart rate, and mean arterial blood pressure were monitored, and to evaluate the degree of cold-induced thoracic engorgement, central venous pressure was measured in one series of experiments.

In all series of experiments, urine flow increased to double or more baseline values by 20 minutes of cold exposure (p < .05). By 60 minutes, in the cold, however, flow had returned toward baseline, and was different neither from baseline nor from time control. The free water component of the diuresis followed the same pattern, while urinary osmolality decreased biphasically as well. Osmotic clearance significantly increased in all series, and creatinine clearance was unchanged.
Plasma vasopressin was significantly reduced compared to baseline at 20 minutes of cold exposure in all three series in which it was measured. Values had returned to baseline levels by 60 minutes in the cold. Mean arterial blood pressure and heart rate increased significantly in the cold (p < .01), while all but one experimental series showed no change in plasma osmolality, and in no series was there a change in rectal temperature. Mean central venous pressure was unaffected by cold exposure, although systolic and pulsatile central venous pressure increased.

Cold was without effect on sodium excretion, with both control and cold-exposed groups significantly increasing their rate of excretion. Similarly, there was no change in plasma ANF, although both renin and aldosterone increased progressively during cold exposure.

These results suggest that there is no consistent natriuresis in the rat during acute cold exposure, and that an increase in arterial blood pressure may be the driving force behind the observed reductions in plasma vasopressin.
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CHAPTER 1
INTRODUCTION

COLD DIURESIS: DESCRIPTION AND HISTORICAL BACKGROUND

Upon being exposed to a cold environment, humans and many animals experience a phenomenon generally termed "cold diuresis": an increase in urine flow and electrolyte excretion beginning within the first hour of exposure and sustained for a lengthy period. The period of increased urine flow has been reported to last at least 5 days in humans (Granberg et al., 1971a), and at least 30 days in rats (Fregly, 1982). By 6 weeks, however, cold-exposed rats exhibit no greater urine flows than those maintained in a thermo-neutral environment (Delost, 1988).

Apparently the first person to recognize and study the effects of cold on urine flow was Gibson in 1909, who termed it: "the diuresis of chill" (Gibson, 1909). Preceding that were several clinical reports of increased urine flow during cold baths (Sutherland, 1764; Leichtenstern, 1885) in which the effects of temperature on urine flow are confounded by hydrostatic effects.

SPECIES SPECIFICITY OF COLD DIURESIS

Although cold diuresis is well documented in humans, it is absent in many other species, including all ruminants examined to date, and the pig (Fregly, 1982; Forsling, 1976; Thomson, 1980). In other mammals, such as the dog, the few available studies have failed to demonstrate a significant diuresis (Sadowski, 1972). (These few studies, however, suffer from methodological difficulties, discussed later.) Still other mammals, even closer relatives of humans, (rhesus monkey), appear to exhibit no increased urine
flow with cold exposure, although this is an inference drawn from measurements of water turnover rate; urine flow was not recorded in the one available study (Oddershede, 1980).

The rat develops a cold diuresis with a character similar to that of humans: a decrease in urinary osmolality (Morgan, 1983), and, at least during chronic exposures, an increased sodium excretion (Canguilhem, 1967; Fregly, 1982; Selaititskaia et al., 1985; Smith and de Jong, 1975). The similarity to humans in the urinary response to cold makes the rat an appropriate animal for the present study. In addition, the use of rats in the present study allows comparisons with previous literature - also largely utilizing the rat as the experimental animal. The bulk of the ensuing discussion, therefore, will deal with responses seen in rats and humans.

**ACUTE VERSUS CHRONIC COLD EXPOSURES.**

There is evidence that the characteristics of acute cold exposure are quite different from those of long duration. This distinction has received surprisingly little attention. Although Fregly, in his 1982 review divided the literature into short, intermediate, and long term cold exposures, the range of durations in the "short term" category (1 hr to 30 days) was too broad and tended to obscure important response differences between acute studies (lasting 4 hours or less), and those of longer duration. Nowhere is there a comparison of the acute with the chronic exposure, or an investigation of the time course of urine flow, hematologic changes, and hormonal responses from the onset of the cold stimulus through longer term exposures. Data of this type would probably reconcile many discrepancies in the literature, as it is likely that different factors predominate at different durations of cold exposure. The ensuing discussion, therefore, will attempt to distinguish between acute and chronic responses where that
distinction is known. Acute exposures for the purposes of this review are defined as those lasting 4-6 hours or less, and chronic exposures are defined as lasting longer than 6 hours and less than 30 days.

CHARACTER OF COLD-INDUCED DIURESIS

Urine Flow. The character of cold-induced diuresis is little disputed. Urine flow typically doubles within the first 1-2 hours (Bader, et al., 1952; Segar and Moore, 1968). This increase appears to be more rapid the lower the temperature (Gibson, 1909), but the magnitude of the increase as reported by Adolph and Molnar (1946) is maximized at the relatively mild temperature of 15°C. Urine flow rates during cold exposure are reportedly less in upright subjects compared to supine (Adolph and Molnar, 1946). Cold-induced diuresis has also been shown to be abolished by exercise (Bader et al., 1952), and reduced or abolished when subjects are in a dehydrated condition prior to the onset of cold exposure (Bass and Henschel, 1954; Hillier, 1969; Wallenberg and Granberg, 1974).

In a pattern similar to that of humans, urine flow in rats also doubles within the first hour of cold exposure (Morgan, 1983), and is diminished by dehydration (Fregly and Tyler, 1972)

Longer term human studies show urine flow still significantly elevated after 5 days in light clothing at 15°C. (Granberg et al., 1971a). Likewise in rats, significant urine flow increases have been reported to persist for the duration of a 30 day cold exposure (Fregly, 1982).

Urinary Osmolality. Urinary osmolality responds similarly to cold in rats and humans, and seems to depend on the duration of the cold exposure. Acute cold exposures in humans are accompanied by decreases in urinary osmolality (Lennquist,
1972; Segar and Moore, 1968) or by decreases in urinary specific gravity (Gibson, 1909; Adolph and Molnar, 1946; Bader, 1952). Rat's responses are similar to that of humans (Fregly, 1967; Morgan, 1983).

During longer term exposures, however, average daily urinary osmolalities for both rats and humans are unchanged (Lennquist, 1972; Fregly, 1972).

**Electrolyte Excretion.**

**SODIUM AND CHLORIDE.** Early reports of acute cold-induced chloriuresis in humans (Adolph and Molnar, 1946), were subsequently supported by Bass and Henschel, who also found sodium excretion increased (1954). Since then a natriuresis has almost invariably been shown to accompany acute cold exposures (Segar and Moore, 1968; Lennquist, 1972b).

Similarly, during longer term cold exposures sodium excretion is increased, even when dietary intake is held constant (Granberg et al., 1971a; Granberg et al., 1971c).

Though an acute cold-induced natriuresis has been a nearly universal finding in humans, the pattern in rats is less clear. In an acute setting, some investigators (Morgan et al., 1983) observed no increase in sodium excretion in rats exposed to moderate cold (13-15° C) for 1 hr or less. Others, using lower temperatures (4° C) and longer durations (3 hours), did see a natriuresis (Smith and de Jong, 1975). In rats, then, it is possible that natriuresis is dependant on the duration and severity of exposure. The sampling interval is also extremely important, especially in accurately observing a potentially transient natriuresis; too long an interval and the transient will be obscured by the subjects' having returned to sodium balance (Delost et al., 1988).
Study of this question, then, requires sequential measurements of sodium excretion over a period of days or less. Chronic exposures of several days have been conducted by Canguilhem, (1967), Seliatitskaia et al., (1985), and Fregly (1968), who all show significant increases in sodium excretion. Fregly also observed an increased sodium intake, and corrected for it by expressing his sodium excretion per unit of sodium ingestion. He found that even when this was done, cold-exposed rats excreted more sodium than did controls.

Cold-induced natriuresis is a universal finding in humans, exhibited in both long and short term exposures. In rats, increased sodium excretion is found during chronic exposures of several days or more, but is seen only inconsistently in an acute setting of 4-6 hours or less. There is some evidence that natriuresis during acute exposures may depend on the temperature and duration of the exposure.

MAGNESIUM. Bass and Henschel (1954) reported an increase in magnesium excretion during 2 hours of exposure to 15° C. These results were not confirmed by Lennquist during a similar set of experiments (Lennquist, 1972b). In longer term exposures (3 days), magnesium excretion was moderately increased during the first 24 hours, but returned to baseline by day 2 (Granberg et al., 1971a).

POTASSIUM. During 2 hours of cold exposure, Lennquist (1971) reported potassium excretion increased in human subjects exposed to 15° C. This conflicts with Bass and Henschel's (1954) finding of no consistent change under similar conditions. A longer (6 hrs) and colder (8° C.) exposure by Purshottam et al. (1978) also reported no change in potassium excretion, indicating that the inconsistency in results is not stabilized by increasing the intensity and duration of exposure.
During chronic cold exposure Granberg et al., (1971a & 1971d) saw no change in average daily potassium excretion over 5 and 3 days, but did observe a transient increase during the first 4 hours. Although the findings of Rogers et al. (1964) of a chronic kaliuresis seem to contradict these results, Rogers attributed his subject’s increased potassium excretion to their state of semi-starvation. He hypothesized an accelerated release of intracellular potassium as a result of starvation-induced cell catabolism.

Although human studies are few and conflicting, they suggest overall that potassium excretion is probably unchanged by chronic cold exposures, and during acute exposures of 6 hrs or less, shows consistent pattern. The sampling interval may be important for the latter, however, as some studies show a transient rise very acutely at 2-4 hours (Lennquist, 1971; Granberg et al., 1971b & 1971d).

Unlike the response seen in humans, longer term exposures in rats have shown an increased potassium excretion, which peaks during the second day of cold and diminishes almost to baseline by the end of a 5 day exposure period (Canguilhem, 1967). The kaliuresis in this experiment, however, may not be caused solely by cold. Although it was not measured, potassium intake almost certainly increased since hyperphagia is known to accompany cold exposure. This suggests that potassium intake may account for the differences between Granberg’s human study - where potassium excretion was unchanged and food intake was standardized - and Canguilhem’s rat study in which potassium excretion increased, but food intake was ad libitum and undoubtedly increased.

A study by Fregly (1968) argues against this, however. Fregly corrected for cold-induced hyperphagia by measuring food intake and expressing his excretion rates per
unit of potassium ingested. He found that over a 10 day exposure to 6° C, potassium excretion per unit of intake was greater in cold-exposed rats than in controls, indicating that even when intake is considered, rats may exhibit a kaliuresis during chronic cold exposures.

Although the longer term exposures seem to elevate potassium excretion in the rat, acute exposures of 1 to 3 hours, even at the rather severe temperature of 4° C., do not (Smith and de Jong, 1975).

The evidence seems to suggest, therefore, divergent potassium excretory responses between rats and humans. Chronic exposures appear to increase potassium excretion in rats, whereas in humans, only acute exposure has proved (though inconsistently) kaliuretic.

Other electrolytes reported to change in the cold are calcium and phosphate. Granberg et al. (1971d) also measured calcium and phosphate excretions during both acute and chronic cold exposures, and found them to be significantly elevated (1971d).

**Glomerular Filtration Rate and Renal Plasma Flow.** Increases in renal plasma flow or glomerular filtration rate (GFR) can produce a diuresis, and thus are potential contributors to the diuresis of cold exposure. This seems unlikely since both are observed to be either unchanged or reduced in the cold.

As an estimate of GFR, creatinine clearance was first measured by Bader et al. (1952) during acute (2.5 hr) exposures of humans to 15.5° C. He observed no change either in creatinine clearance or the clearance of para-aminohippurate (PAH), an estimate of renal plasma flow. Similar acute experiments by Lennquist (1972) actually reported a decrease in creatinine clearance which was corroborated by simultaneous determination of inulin clearance. These were also unaccompanied by
changes in the clearance of PAH (Lennquist, 1972). Finally, Segar and Moore (1968) report no change in creatinine excretion.

Even when exposures were very severe (2 to -5°C.), acute cold did not produce an increase in creatinine clearance (Wilson et al., 1970). This typical response of no change or a slight reduction does not appear to vary with hydration state, as Wallenberg and Atterhog and colleagues (1974 & 1975) reported no change in the clearance of inulin, and a slight decrease in PAH clearance during 1 hr exposures of both water-loaded and hydropenic men.

Rats have not been so extensively studied acutely, but available indications are that, as is seen in humans, acute cold exposures of 1 hr or less effect no change in GFR or renal blood flow as estimated by inulin and PAH clearances respectively (Morgan, 1983).

Chronic studies lasting longer than 6 hours show the same patterns as those seen in acute studies. Cunningham et al., (1985) report no change in GFR as suggested by unchanged creatinine excretion in rats exposed to 5 days of cold. In humans, creatinine clearance over the same time period (Lennquist, 1972) was also found to be unchanged. Lennquist did note an increased creatinine clearance during the first 4 hours of cold (15°C) exposure, however.

**Osmotic Clearance.** In view of the substantial solute excretion, it is not surprising that an increased osmotic clearance has been observed in both acute and chronic cold exposures in humans (Lennquist, 1972; Segar and Moore, 1968). In rats, however, the one published report of osmotic clearance shows no change during 45 minutes of cold exposure (Morgan et al., 1983). There are indications that the rats in this study may have been slightly hydrated, as their urinary osmolalities were 679 mOsm/kg during the
control period. Normal values for Sprague Dawley rats with free access to water are closer to the 1500-2000 mOsm/kg range (Fregly and Tyler, 1972).

**Free Water Clearance.** Free water clearance in chronically exposed humans has been reported to decrease (Lennquist, 1972). An acute study (2 hrs) by the same investigator, however, showed the opposite response, suggesting that free water clearance may increase acutely, and then return to baseline or below in the longer term.

Segar and Moore’s studies using a 31-hour exposure would seem not to support this, as free water clearances calculated from their data decrease rather than increase. Their subjects were hydropenic, however, a condition that has been shown to affect the character of the diuresis (Wallenberg and Granberg, 1974).

Free water clearances have not been reported in rats exposed chronically to cold. In the acute case, the exposure of rats to 45 minutes of 15° C. produced significant increases, even to the point of generating free water (Morgan et al., 1983).

**HEMATOCRIT AND PLASMA VOLUME**

In humans, acute cold exposures of 1-4 hours almost invariably produce hematocrit increases of 2-3 percent within the first 30 minutes of exposure (Bass and Henschel, 1956; Fregly, 1982; Atterhog et al., 1975). Plasma proteins are elevated as well (Bass and Henschel, 1956).

Calculations of plasma volume changes using various radio-labelling techniques in acutely cold-exposed humans have demonstrated a 7-8% reduction when urinary fluid losses are continuously replaced (Bass and Henschel, 1956; Atterhog, 1975). Although net urinary fluid losses undoubtedly augment plasma volume contraction, reductions in plasma volume have also been seen in the absence of urine flow.
increases, either because of maximal vasopressin dosage, (Eliot et al., 1949) or as a result of water loading the subjects before the start of the experiment (Atterhog et al., 1975).

Further evidence that hemoconcentration as measured by an increased hematocrit is not dependant upon urinary fluid losses comes from a human study by Wilkerson et al. (1974) in which hematocrit became elevated within 30 seconds of exposure to 5°C.

Studies of chronic (3 day) human cold exposures where the first samples are obtained after 12 hours, report that hematocrits are not significantly increased until the second or third day, indicating that any acute change had subsided by the first sampling period (Granberg et al., 1971a). Further, it suggests a different mechanism for the increased hematocrit in acute versus chronic cold exposures.

In rats, four out of five studies of cold exposures lasting 24 hours or less show an increase in hematocrit; the fifth showing no change (Fregly, 1982). Neither hematocrit nor blood volume have been reported to change in rats exposed to cold longer than 24 hours, and in exposures lasting 3 weeks or longer, while hematocrit responses remain unchanged, all 3 studies reporting plasma volume determinations show an increase (Fregly, 1982).

POTENTIAL MECHANISMS FOR COLD DIURESIS

While the character of the diuresis has become reasonably well established, its cause is still the subject of debate. Early explanations (Cushny, 1917) invoked a reduction in evaporative fluid loss due to reduced sweating, obligating the body to void the water through another means. Although not intending to test this hypothesis, Adolph and Molnar (1946) measured evaporative water loss by weighing and
subtracting urinary losses. They did not directly compare cold-induced reductions in evaporation to urinary increases, but rough extrapolations from their figures suggest that urinary losses exceeded the modest gains made through reductions in evaporation. Granberg et al. (1971a) who measured evaporation from the skin using an "evaporometer", reported that reductions in evaporative loss were only 29 percent of the increase in urine flow.

Reduced evaporation can therefore be eliminated as a major contributor to cold-induced diuresis, even though it is not by itself a mechanism. Another proposal was advanced by Segar and Moore in their seminal paper on vasopressin in the cold (1968). They reasoned that the peripheral vasoconstriction which is a part of the normal thermoregulatory response to cold should result in a shunting of blood to the body's core, and thus produce a central volume expansion. The proposal that cold increases central blood volume, is of interest because central blood volume fluctuations are most widely believed to be the stimulus for correcting small changes in body fluids. According to this prominent theory (termed the Gauer-Henry hypothesis after it's originators) fluid volume changes too small to affect arterial blood pressure are sensed by stretch receptors in the left atrium and possibly the pulmonary vein/atrial junctions. Because pressures in these areas are much lower than arterial, and can show small changes in the absence of changes in arterial pressure, this system is also called the "low pressure system" of fluid volume regulation (as opposed to the "high pressure system" of the arterial baroreceptors).

In support of the proposal that central volume expansion is the stimulus for cold diuresis is its similarity in urinary character to that of such known means of expanding central volume as immersion and left atrial balloon inflation. In all of these
manipulations, a decrease in urinary osmolality and an increased sodium excretion accompany the increased urine flow (Goetz et al., 1975; Segar and Moore, 1968; Krasney et al., 1989). Additionally, the character of the diuresis undergoes similar changes with the state of hydration. Hydropenic subjects increase osmotic clearance in the cold (Segar and Moore, 1968), and during immersion (Epstein et al., 1978; Menninger, 1985) In the euhydrated, free water clearance increases as well, during balloon inflation (Krasney, 1989), immersion (Norsk and Epstein, 1988), and cold exposure (Morgan, 1983, Sadowski, 1972).

In its original form, the efferent limb of Gauer and Henry’s reflex consisted of vasopressin, a suppression of which was proposed as the mechanism for the dilute diuresis following volume expansion (Henry et al., 1956). As data has accumulated, the list of potential effectors has enlarged to include the hormones aldosterone, atrial natriuretic factor, and changes in renal nerve activity as well (DiBona, 1981). The proposed afferent limb continues to be nerve impulses carried centrally via the vagus.

**Vasopressin As a Mechanism for Cold-Induced Diuresis.** Cold diuresis has in common with such known central-volume-expanding maneuvers as water immersion and left atrial balloon inflation a decreased urinary osmolality accompanying the increase in urine flow. It has long been thought that reductions in plasma vasopressin may participate in the dilute diuresis attending these latter manipulations (Ledsome, 1986; Norsk and Epstein, 1988). If cold likewise produces an atrial volume expansion, a similar decrease in vasopressin might be expected.

This role, however, is not universally accepted (Fregly, 1989), as there appear to be species differences in the vasopressin response, and because the strategy of administering exogenous vasopressin has not always abolished the diuresis.
Bader et al. (1952) may have been the first to suggest a role for decreased vasopressin in the cold, based on their observations of a dilute diuresis coupled with a lack of significant change in other factors influencing urine flow such as GFR and renal plasma flow. Until quite recently, few measurements of vasopressin during cold exposure existed (Itoh, 1954; Moore & Segar, 1968), and attempts to suppress cold-induced diuresis using endogenous vasopressin have yielded equivocal results (Bader, 1952; Itoh et al., 1959; Granberg, 1971b.)

PLASMA VASOPRESSIN MEASUREMENTS. Measurements of plasma vasopressin in humans and rats show a decrease with cold exposure (Segar and Moore, 1968; Morgan, 1983; Itoh, 1954), while in dogs, vasopressin is reported to increase (Sadowski et al., 1972), and in goats, show no change (Thomson et al., 1980). Neither of the latter two species, however, has demonstrated a significant cold-induced diuresis. Another study in dogs by the Sadowski et al. (1975) showed no change in either urine flow or plasma vasopressin. Studies in dogs are few, and interpretation of these is hampered by confounding factors such as lengthy transitions between control and cold periods (one study took 50 minutes to effect the temperature change (Sadowski et al., 1972), and the physical activity (walking) of the dog during the cold exposure (Sadowski, 1975). In humans, walking abolishes the diuretic response to cold entirely (Bader, 1952).

Since the development of sensitive radioimmunoassays for vasopressin, many more measurements of plasma vasopressin have been conducted during cold exposure (Morgan, 1983; Gibbs, 1984; Seliatitskaia et al., 1985; Thornton et al., 1989) including one abstract (Reddix-Cheri and Martin, 1990), and one published report (Berecek et al., 1988) which have appeared since the inception of the present study.
Segar and Moore's study using human subjects, and 4 out of the 7 studies in rats report suppressed plasma vasopressin in response to cold exposure. The three exceptions include Gibbs (1984) who found no change in very young (200-250 gm) rats subjected to the severe cold of 4° C. for 30 minutes, and experiments in spontaneously hypertensive rats (SHR). Berecek et al. (1988) reported an approximate tripling of vasopressin in SHR after 4 hours at 4° C., and Thornton et al. (1989) in high sodium-fed SHR only, found vasopressin elevated after 90 minutes at 6° C. In SHR fed a normal sodium diet, plasma vasopressin was unchanged after either 45 or 90 minutes of cold.

ADMINISTRATION OF EXOGENOUS VASOPRESSIN. In addition to measuring plasma levels, the contribution of vasopressin to cold diuresis has been assessed by administering exogenous vasopressin. Presumably, if reductions in vasopressin play a role in cold diuresis, preventing this reduction by raising circulating levels should suppress the diuresis.

Some authors report an abolition of the diuresis in humans when Pitressin is infused during acute cold exposures (Bader et al., 1952). Granberg et al. (1971b), in a longer study over 24 hours, injected Pitressin at 4 hour intervals and found only the immediate peak (within two hours of exposure) to be abolished.

In rats, a similar pattern with respect to the duration of cold exposure emerges. Following intraperitoneal injections of Pitressin, Itoh (1959) observed an abolition of cold diuresis for 60-75 minutes. When Fregly (1972) injected cold exposed rats subcutaneously with Pitressin, he found the volume of urine excreted to be approximately equal to baseline, but significantly higher than control rats given the same dose.
By inference from these studies, then, it appears that vasopressin may be primary in the acute phase of cold diuresis, whereas other factors assume greater importance as the diuresis continues.

POSSIBLE REGULATORS OF VASOPRESSIN IN THE COLD.

Central volume expansion. The explanation given by Gauer and Henry (1963) and echoed by Segar and Moore (1968) for cold-induced vasopressin reduction invoked a central shunting of blood due to constriction of peripheral vessels. The atrial stretch thus produced would then reflexively inhibit vasopressin via the so-called Gauer-Henry reflex.

In an attempt to evaluate this hypothesis, Lennquist (1971) measured right atrial pressures in 3 human subjects exposed to 15° C. on a hypothermic operating table. Immediate responses were variable, as right atrial pressure increased, decreased, and didn't change in the three subjects. By the end of the 60 minute cold period, however, all pressures were slightly below baseline.

Another approach that has been used in dogs as well as humans is the measurement of central blood volume by dye dilution in which flow was estimated from dye transit times between an injection site outside the right atrium, and a sampling site in the aorta (Atterhog et al., 1975; Sobocinska and Kozlowski, 1987). Volume was calculated from flow.

Results of Sobocinska and Kozlowski's (1987) measurements in 4 dogs during exposures of variable lengths and temperatures (+1° to -8° C. for 1-3 hours) were equivocal due to the small numbers and variability of temperatures and durations of exposure. Atterhog's (1975) experiments using water-loaded human subjects were better controlled, consisting of 1 hour on a hypothermic operating table at 15° C.
By 30 minutes of cold exposure central blood volume had increased by an average of 160 ml.

*Mean arterial blood pressure; heart rate.* While the role of central volume expansion in modulating plasma vasopressin is still somewhat controversial (Goetz et al., 1975), there is little debate about the responsiveness of plasma vasopressin to changes in arterial blood pressure; increases in arterial pressure act powerfully to decrease vasopressin (Robertson, 1985). This raises the possibility that arterial pressure changes could be either wholly or in part, mediating plasma vasopressin levels in the cold.

In humans, cold-induced increases in arterial blood pressure have been seen by nearly all investigators (Satow, 1937; Adolph and Molnar, 1946; Budd and Warhaft, 1966; Suzuki et al., 1967; Granberg et al., 1971a; Atterhog et al., 1975; Wallenberg, 1975; Wallenberg and Granberg, 1974; Wallenberg and Granberg, 1975; Hiramatsu et al., 1984; Kawahara et al., 1989), both in chronic and in acute exposures.

The response of arterial blood pressure in rats apparently depends on the strain of rat and the duration of the study. Thus, while many acute studies of 24 hours or less report an immediate and sustained hypertension in Sprague-Dawley (Morgan et al., 1983; Fisher et al., 1985) and spontaneously hypertensive rats (SHR) (Minami et al., 1982; Thornton et al., 1988) others (Thornton et al., 1988) observe cold-induced hypertension in SHRs but not their normotensive controls, Wistar-Kyoto.

In contrast, durations of longer than 24 hours (in Sprague Dawley rats) give 10 days to 4 weeks as the time to the onset of hypertension (Fregly, 1954; Schectman et al., 1989). Any immediate change in blood pressure would have been missed in these studies, however, as the minimum sampling period was twice weekly. In addition,
blood pressure was sampled by tail cuff sphygmo-manometry where in the acute studies listed above it was measured directly.

The above suggests that the acute blood pressure increase seen in shorter term studies may be transient. It further suggests a different mechanism for the delayed increase in blood pressure observed after 10 days.

Studies of heart rate in cold-exposed rats invariably report an increase despite variations in strain and duration of exposure (Fisher et al., 1985, Thornton et al., 1988).

In humans during acute cold exposures, heart rate has been reported elevated (Adolph and Molnar, 1946; Atterhog et al., 1975), unchanged (Raven et al., 1970; Hiramatsu et al., 1984) or decreased (Budd and Warhaft, 1966). The discrepancy could partially be explained by differences in body temperature, as heart rate has been reported to decrease linearly with rectal temperature (Burton, 1955).

Burton (1955) describes a general pattern for changes in blood pressure and heart rate as:

"an initial marked peripheral vasoconstriction with a rise in blood pressure and heart rate. During this stage the rectal temperature does not fall. If cooling is continued or intensified, rectal temperature starts to fall. As it does so the heart rate decreases... Blood pressure falls during this period but only gradually."

In those studies where heart rate increased, rectal temperature increased initially, then returned to baseline (Adolph and Molnar, 1946; Atterhog et al., 1975). Rectal temperature was not measured in the studies reporting no change in heart rate (Raven et al., 1970; Hiramatsu et al., 1984); however the exposure temperature was lower than the others, (4 and 5° C. compared to 15° C.), so it is possible a slight decrease in rectal temperature occurred, and if so, may have counteracted the expected acute increase in heart rate. A slight lowering of body temperature can occur within 30
minutes at similar temperatures (6.5° C.), and are associated with significant decrements in heart rate (Arnett and Watts, 1960). Rectal temperatures during Budd and Warhaft's 2 hour exposure to 10° C. (1966) decreased significantly, as did heart rate.

Subjects in Granberg et al.'s much longer exposure (5 days at 15° C.) experienced a significant drop in heart rate along with rectal temperature during the first 24 hours. Heart rate in the normothermic control group, however, also decreased, presumably without a drop in rectal temperature although this data was not reported (1971a).

Plasma osmolality. Another factor influencing plasma vasopressin levels besides hemodynamics is the osmolality of the plasma; vasopressin levels increase with increasing plasma osmolality. It is therefore essential that this be considered in any discussion of changes in vasopressin.

Plasma osmolality has been reported not to change significantly in humans exposed acutely to 15° C. temperatures for short periods (Segar and Moore, 1968). Even during chronic exposures of several days, plasma osmolality undergoes no significant change at 15° C. (Granberg, 1971a).

While the acute (1-4 hr) response in rats is a similarly unchanged osmolality (Morgan et al., 1983), during longer studies, Fregly and colleagues found increased serum osmolalities as early as 1 day which persisted for as long as 48 days of cold exposure (Fregly et al., 1976; Fregly, 1982b).

Glucocorticoids. In humans, plasma cortisol and urinary cortisol metabolites are known to be elevated during acute (Hiramatsu et al., 1984; Suzuki et al., 1967), as well as chronic (Granberg, 1971e) cold exposures. Likewise in rats, plasma corticosterone and corticosterone excretion increase following both acute (Paris, 1987;
Berecek et al., 1988) and chronic ( Bertin, 1971; Gerlinskaia and Moshkin, 1984; Cunningham et al., 1985; Delost et al., 1989) sojourns in cold environments.

Since cortisol has been reported to tonically inhibit vasopressin release, and to attenuate its release in response to known stimuli ( Raff, 1987), plasma glucocorticoids should be included in a list of potential influences on vasopressin in the cold. Indeed, at least one author has invoked this relationship in explaining his failure to observe vasopressin elevations in cold-exposed dogs ( Sadowski et al., 1975).

Glucocorticoid responses to cold may vary with the temperature and duration, however. In contrast to longer studies and those at more severe temperatures, plasma cortisol measurements taken every 10 min during the exposure of two men to 15° C. show no deviation from the expected circadian decline during the first hour ( Wilkerson et al., 1974). Values began to rise at 60 minutes and were indistinguishable from baseline at 2 hours. These results are essentially identical to those of Wilson et al. ( 1970), although temperatures in the latter study were lower ( -5° to +2° C.).

In addition, although cortisol reduces the degree of vasopressin stimulation, no depression of normal vasopressin levels by glucocorticoids has been demonstrated ( Raff, 1987). In experiments by Cornette-Finn ( 1987) cortisol infusions in dehydrated dogs have no effect on plasma vasopressin.

Cold Diuresis: Potential Mechanisms other than Vasopressin.

GLUCOCORTICOIDS. As detailed above, both acute and chronic cold exposures elevate glucocorticoid levels in both humans and rats. While it seems unlikely that glucocorticoids are inhibiting vasopressin in the cold, glucocorticoids may have other renal effects besides a modulation of vasopressin.
Cortisol infusions in dogs have been shown to augment water load excretion by a mechanism seemingly independent of changes in plasma vasopressin levels; when administered prior to water ingestion, urine flow was enhanced in the absence of changes in vasopressin (Cornette-Finn, 1987). Cold-induced increases in cortisol/corticosterone, therefore, could contribute to cold diuresis, even without affecting plasma vasopressin.

The rat's small size severely limits the number of hormones that can be assayed in a single protocol. Because simple cortisol infusion in dehydrated and in normally hydrated dogs does not affect urine flow (Cornette-Finn, 1987), and because pharmacologically blocking cortisol increments did not alter the urinary responses of cold-exposed dogs (Sadowski, 1975), glucocorticoids were not considered a sufficiently strong contributor to cold diuresis to warrant an additional experimental series devoted to their study. In addition, the human studies suggest that glucocorticoid levels are unchanged during mild, short term exposures such as those used in the present experiment (13°C for 1 hour) (Wilson et al., 1970, Wilkerson et al., 1974).

CATECHOLAMINES. By far the most well-documented hormonal effect of cold exposure is an increase in catecholamine excretion rates, and, more recently, plasma levels. Increases in excretion and/or plasma levels have been found in humans exposed acutely to cold (Arnett and Watts, 1960; Wilson et al., 1970; Lennquist, 1972a; Lamke et al., 1972; Wilkerson et al., 1974; Wagner et al., 1987; Kawahara et al., 1989). More chronic exposures of humans (greater than 24 hours) report similar results (Granberg et al., 1971e; Radomski and Boutelier, 1982). Earlier literature is reviewed by Granberg (1971e), and is in agreement with the studies cited above.
Catecholamine responses in rats are similar to those seen in humans, showing elevated excretions of both norepinephrine and epinephrine during acute exposures (Leduc, 1960; Shum et al., 1969; LeBlanc et al., 1971; Bibbiani and Viola-Magni, 1971; Appelgren et al., 1982; Minami et al., 1982; Yamori et al., 1985; Nakashima and Esashi, 1987; Berecek et al., 1988; Ueda et al., 1990) as well as those longer than 24 hours (Leduc, 1961; Shum et al., 1969; Bertin, 1971; Sellers et al., 1971; Sellers et al., 1974; Young and Landsberg, 1981; Storm et al., 1981; Fregly, 1989).

The general finding on long term urinary excretion, as stated by Leduc (whose studies are still the most complete on the subject) is that norepinephrine excretion increases rapidly in the cold (within the first 12 hours) and remains high, whereas the excretion of epinephrine rises more slowly, peaking at about 6 days, and thereafter declines, although not completely to baseline (Leduc, 1961).

In humans this is supported by the finding that plasma norepinephrine concentrations (Wilkerson et al., 1974; Wagner et al., 1987) and urinary excretion of norepinephrine (Lennquist, 1971a; Lamke, 1973) are generally greater than that of epinephrine during the first several hours of cold exposure, although this is not always the case (Arnett and Watts, 1960; Wilson et al., 1970). Differences may be related to nutritional status of the subject, as fasting has been shown to increase adrenal medullary secretions in response to a depression of sympathetic nervous system activity (Young et al., 1984).

The magnitude of the catecholamine response, and especially the relative magnitude of the epinephrine as compared to the norepinephrine response, seems to depend on both the intensity and duration of the exposures.
Urinary excretion of both hormones increases with the severity of the temperature. The response in plasma levels, on the other hand, is quite variable (Wilkerson et al., 1974). Based on the extremely limited evidence available, epinephrine at the moderate temperature of 15° C. appears to rise slightly within the first 20 minutes and fall thereafter so as to be indistinguishable from baseline at 60 minutes.

Norepinephrine on the other hand, increases to a greater degree but does so more slowly and unlike epinephrine, continues to rise throughout the 2 hour cold exposure (Wilkerson et al., 1974). Paradoxically, the pattern is exactly reversed at the colder temperature of 10° C. with norepinephrine increasing acutely and then falling, while epinephrine rises continuously and to a much greater degree.

No comparable time course data is available in rats, as no acute plasma measurements exist.

The importance of these hormone increases is that both catecholamines are known to affect renal function. Epinephrine is thought to stimulate alpha 2 receptors in rats, functionally antagonizing vasopressin and causing a water diuresis (Gellai, 1991). The effect is not seen in dogs, however, and its importance in humans is not established (Brooks et al., 1991).

Infused Norepinephrine decreases urinary osmolality in rats (McDonald et al., 1977). In dogs, infusion at high physiologic rates (125 ng/kg/min) produces significant increases in urine flow and decreases in sodium and potassium excretion (Johnson and Barger, 1981). In contrast, higher doses (300 ng/kg/min) produce opposite effects - an increased vascular resistance "in all renal vascular segments", and antidiuresis (Liang and Yang, 1972).
In neither study did norepinephrine have a diuretic effect when administered intra-renal, indicating no direct tubular action. Since norepinephrine infusion causes an increased arterial blood pressure and no change in GFR or renal plasma flow (Johnson and Barger, 1981) when infused at the above rates, the dilute diuresis which accompanies it is thought to be due to reduced plasma vasopressin, hemodynamically-mediated (Robertson, 1985; Schrier and Kim, 1987).

In the study by Johnson and Barger (1981), a 25 ng/kg/min infusion rate was without effect on urinary parameters. The larger dose of 125 ng/kg/min increased urine flow and sodium excretion, but elevated plasma norepinephrine concentration to 1,602 ± 311 pg/ml, approximately 16 times the baseline value of 101 ± 18 pg/ml. The authors comment that although these levels are in the physiological range, they are "ordinarily associated with high sympathetic nervous system activity,...and under such circumstances the role of circulating norepinephrine would be expected to be minor." (Johnson and Barger, 1981). That is, circulating norepinephrine during high stress situations is found to increase due to spillover from accelerated sympathetic nerve terminal discharge. If sympathetic activity is increased to such an extent that this spillover increases plasma norepinephrine significantly, the effect of the neurotransmitter on the nerve terminal itself would be so great as to dwarf the effects of circulating norepinephrine.

**POTENTIAL MECHANISMS OF COLD NATRIURESIS**

If a central shift of blood in the cold is plausible, it could bring about not only the dilute diuresis, but also the natriuresis of cold exposure through its effect on the renin-angiotensin system. And, while probably not under Gauer-Henry reflex control (Brenner et al., 1990), atrial natriuretic factor (ANF) is nevertheless a volume sensitive
hormone, and so is also a candidate for producing and/or contributing to the natriuresis of cold exposure.

**Volume Sensitive Hormones.** The hormones vasopressin, ANF, and aldosterone, then, can be seen as possible contributors to the renal responses to cold; they are diuretic and/or natriuretic, and at the same time could be influenced by central blood volume expansion. The increase in sodium excretion is a characteristic that cold exposure shares with water immersion, fluid volume expansion, and left atrial balloon inflation: all known means of increasing intrathoracic fluid volume and thus atrial pressures. The natriuresis in all three of these latter is usually accompanied by decreases in aldosterone and/or plasma renin activity (Zehr et al., 1976; Reinhardt et al., 1980, Schultz et al., 1982; Bishop et al., 1983; Zimmerman et al., 1987). Only rarely has this failed to be the case (Zucker et al., 1979).

However, sodium excretion during these procedures increases within the first 20-40 minutes (Epstein, 1978; Reinhardt, 1987); too soon for changes in aldosterone to be the sole cause of the natriuresis. More rapidly acting factors, therefore, must account for at least the early natriuretic response. One candidate is atrial natriuretic factor, a recently discovered hormone which is known to respond to atrial stretch and is reported to increase following numerous means of increasing atrial volume, including water immersion, volume expansion, and atrial balloon inflation (Goetz, 1988). Its action is quite rapid (de Bold et al., 1981), and therefore could produce the changes seen.

**RENIN-ALDOSTERONE RESPONSE TO COLD.** Given the natriuresis of cold exposure (Canguilhem, 1967; Fregly, 1982; Seliatitskaia et al., 1985; Smith and de Jong, 1975), aldosterone would be expected to decrease if it is involved in the
response. This would seem logical considering the responses reported above for various means of volume expansion. In the few reports of aldosterone in the cold, however, this has not been the case. During a 3 day exposure of humans to 15° C., (Lennquist, 1971) found along with the increased sodium excretion, an increase, not a decrease in urinary aldosterone excretion.

In an even longer term cold exposure of Long-Evans rats (six weeks at 15° C.), Delost et al. (1988) reported plasma aldosterone levels which were 156% higher than control rats maintained at 28° C.. Caution should be exercised in interpreting results from rats, however, as the response may vary with the strain. A study by Gerlinskala and Moshkin (1984) show urinary aldosterone excretion enhanced after 4 weeks at 10° C. in only one strain of rats (August), while in Sprague-Dawley and WAG rats, it was diminished.

The above studies are all long term, and their increased aldosterone may be explained on the basis of a common, although not universal (Delost et al., 1988) finding in chronic cold exposure that plasma volume is reduced (Bass and Henschel, 1956). Reduced volume resulting from a negative sodium balance would tend to increase aldosterone levels. A difficulty with this formulation is that renin, the volume-sensitive portion of the renin-aldosterone system, appears normal after several weeks of cold exposure (Fregly, 1982; Delost et al., 1988), and therefore does not seem to account for the greatly augmented plasma aldosterone concentration (Delost et al., 1988).

It seems surprising that renin would be normal in the cold if vasoconstriction translocates blood to the thorax, and this is maintained during chronic cold exposure.
The central volume expansion thus produced might be expected to depress plasma renin levels.

While the longest-term exposures are absent the expected decrease in renin, and aldosterone is if anything, increased, sodium has come back into balance (Delost et al., 1988), and there is thus no relative natriuresis. One must therefore look to more acute studies for clarification of the interaction between cold-induced natriuresis and the renin-aldosterone system.

In this regard, it has been found that shorter term (1 hour) exposures of humans to 4°C do reduce plasma renin activity (Hiramatsu et al., 1984), although longer (4 hour) studies in rats at the same temperature (Berecek et al., 1988) show no significant change.

The literature, therefore, does not strongly support a role for the renin-angiotensin-aldosterone system in cold natriuresis, although it has not been adequately studied during very acute exposures (1 hour or less).

ATRIAL NATRIURETIC FACTOR. Even if renin and aldosterone were consistently altered in the appropriate directions during cold exposure, the same timing mismatch which has been noted for immersion applies as well to cold: sodium excretion increases too rapidly for aldosterone to be the sole mediator.

No known hormone then, had been able to account for the natriuresis of central volume expansion. As a result, when atrial natriuretic factor (ANF) was discovered in 1981 (deBold et al., 1981), it stimulated a great many investigations of its contribution, not only to the natriuresis of immersion (Katsube et al., 1985, Anderson et al., 1986; Gerbes et al., 1986; Muller et al., 1986; Miki et al., 1986 & 1988; Pendergast et al.,
1987; Rabelink et al., 1989), but to other central volume expansion strategies as well (Veress and Sonnenberg, 1984; Hirata et al., 1989).

Although atrial natriuretic factor increases dramatically during these, and in fact, any procedure which expands the atria (Brenner et al., 1990), in general it is not well correlated with sodium excretion during immersion (Miki et al., 1986 & 1988; Pendergast et al., 1987), or left atrial balloon inflation (Hirata, 1989). In the former case the time courses of the two events are asynchronous. In the latter, results are inconclusive because a rather small increment in pressure was used, rendering the sodium excretory response non-significant (Hirata, 1989).

Two additional findings speak against an exclusive role for ANF in immersion natriuresis. Kurosawa et al. (1988) showed that dehydration eliminates the ANF response to immersion but not the natriuretic response. Rabelink et al., (1989) used infusion of exogenous ANF to demonstrate that it requires plasma levels fivefold greater than those seen during immersion to produce an equivalent diuresis.

COLD AS A MODEL FOR THE GAUER-HENRY HYPOTHESIS

Among the diuretic maneuvers which prompted Gauer and Henry to formulate their hypothesis were negative pressure breathing, and atrial balloon inflation (Gauer and Henry, 1963). Since then, they and others have utilized various techniques of mitral valve obstruction, lower body negative and positive pressure, head down and head up tilt, water immersion to various depths, hemorrhage and volume expansion, atrial tamponade, and chemical stimulation (Goetz et al., 1975) in an attempt to validate or
disprove this widely accepted but still controversial (Goetz et al., 1975; Kappagoda et al., 1974) theory.

The controversy has continued primarily because no single means of expanding atrial volume has succeeded in eliminating all confounding hemodynamic variables (Goetz et al., 1975). Thus, while cold exposure is not without its confounding factors, it is without some of the key hemodynamic changes which have hampered interpretation of other methods for central volume expansion.

Unlike immersion and saline infusion, the central volume expansion associated with cold exposure is localized to the thorax, and there is no hemodilution, which in itself could effect a diuresis and natriuresis (Krasney, 1989). In fact, cold exposure invariably results in hemoconcentration, which if anything, would tend to work against the urinary responses. No changes in GFR have been reported, and the only reported alteration in renal plasma flow has been a reduction. This, observed in water-loaded humans (Atterhog et al., 1975), is again a change working the "wrong" direction.

In addition, cold exposure is a "physiological" situation, which can be induced in conscious animals whose state of hydration and vasopressin levels are normal: a feat which has proved difficult to achieve by other means (Ledsome, 1985). Unlike balloon inflation, it does not require the insertion of catheters into the atria via the atrial appendages - a technique which may differentially mechanically stimulate the appendage receptors during the tachycardia following inflation of the balloon.

Disadvantages of cold exposure as a means of testing the Gauer-Henry hypothesis are the increase of corticosteriods and catecholamines, (Leduc, 1961; Lennquist, 1972; Delost et al., 1988) which may interfere with renal concentrating ability. Lennquist (1972), however, in comparing cold to psychological stress, found that while increases
in epinephrine and cortisol were similar, cold produced a greater diuresis than did psychological stress.

Finally, a great disadvantage to cold exposure is that arterial blood pressure is also increased, and therefore both high pressure and low pressure systems are involved.

STATEMENT OF QUESTION

The hypothesis to be tested was suggested by Segar and Moore in 1968. They postulated a central shunting of blood due to cold-induced constriction of peripheral vessels. The atrial stretch thus produced would reflexively inhibit vasopressin and bring about the observed diuresis. The present study was designed to test this hypothesis in the conscious, cold-exposed rat by measuring plasma and urinary vasopressin at the same time, measuring an index of central volume expansion - central venous pressure.

To assess hormonal determinants of sodium excretion: renin, aldosterone, and atrial natriuretic factor were evaluated as well. By this means, the present study hoped to clarify the role of these hormones in the genesis of "cold diuresis", and further define the pattern of their response to central volume expansion.

Experiments focussed on acute rather than chronic cold exposure, and on the time course of the examined variables. The acute diuresis and natriuresis of cold exposure resembles that of the central volume expanding maneuvers used to study fluid and electrolyte homeostasis; procedures such as balloon inflation, immersion, volume expansion, and tilting which change vascular or central thoracic volumes within
minutes. Acute, as opposed to long term cold exposure therefore, is of interest because it allows comparison with these studies.

Acute cold exposure also permits following the time course of the response as it proceeds from the stimulus, while at the same time monitoring supposed mediators. Although a correspondence in time course cannot establish causality, a comparison of time courses can help eliminate a potential mediator should changes in its activity not correspond with observed responses or observed stimuli.

In addition, the strategy of narrowly spaced, simultaneous measurements of stimulus, response, and potential mediators has value in distinguishing between two potential mediators (for example, whether the "high pressure" or the "low pressure" receptors are activated coincident with decreases in vasopressin). Lastly this approach may also help reconcile divergent reports in the literature which may conflict only because they measure a biphasic phenomena at differing times.
CHAPTER 2
GENERAL MATERIALS AND METHODS

ANIMALS

Animals used for these experiments were male Sprague-Dawley rats 400-500 grams, obtained from Charles River Laboratories (Wilmington, MA). They were obtained at least one week prior to surgery, and maintained at 21°C in a 12 hour light/dark cycle. Diet was tap water and Purina Laboratory Chow ad libitum.

SURGERY

A minimum of 3 days prior to the experiment, animals were anesthetized with halothane (Halocarbon Laboratories, N. Augusta, SC) using a 2% setting on either a Ohio Vetaflex or Ohmeda Modulus anesthesia machine, and placed on a pad heated to 37°C. Indwelling bladder, femoral arterial, and venous cannulas were implanted using sterile technique. In those rats in which central venous pressure was measured, an additional catheter was placed in the right superior vena cava. All implanted material was gas sterilized prior to surgery.

Immediately following surgery animals were injected with antibiotic (0.3 ml 30,000 U, Flocillin, Fort Dodge Drug Co.) subcutaneously, and placed on a warmed operating table to recover for several hours before being returned to the animal facility. Rats were maintained in separate cages for post-surgical care until the experiment. This period of time ranged from 3-5 days before being first used in an experiment. Rats
with still-patent catheters were re-used for one more experiment which occurred within 5-6 additional days.

**BLADDER CANNULATION**

**Bladder Cannula Construction.** Three-flanged bladder cannulas were constructed entirely of Silastic (Dow Corning, Midland, MI) according to techniques outlined by Gellai and Valtin (1979), and modified by Gellai (personal communication). Three pieces of Silastic sheeting were cut for each cannula: one 0.020-inch thick for the top flange, and two 0.010 inch thick for the bottom two flanges. Each piece was approximately 15 mm square. A 0.095-inch diameter hole was cut in the center of each before threading onto Silastic tubing (i.D. 0.062 inch, o.D. 0.095 inch) cut to a length of approximately 2 cm, cut at an angle on one end and straight across at the other. Care was taken to place the top (0.020-inch thick) flange square with the straight-cut end, and to place the bottom flange 8 mm from the top flange. The middle flange between them was not anchored to the tubing, and so placement was not important.

Top and bottom flanges were secured to the tubing with Silastic medical adhesive (no. 891) which was thinned with xylene and placed in a 1 ml plastic syringe (23 gauge blunted needle) for application to both top and bottom surfaces of these two flanges at their junctions with the tubing. The center flange was left unglued. Cut edges of the top flange and tubing were beaded over with the adhesive mixture to prevent tissue irritation (personal modification of Gellai's procedures, which seemed to extend the period of cannula patency)

Cannula's thus prepared were placed in a tissue drying oven (Blue M Electric. Co. Blue Island, IL) at 75-80° C. for several hours or preferably overnight to cure. Curing
did not occur if cannulas were left in room air. When completely solid, cannulas were soaked in xylene briefly to swell the Silastic material and allow easy insertion of a stainless steel tube (14 gauge, 1.6 cm long) into the Silastic tube, just fractionally below the level of the top flange (again, to reduce tissue irritation). When these were dried a second time, the Silastic material closed tightly on the steel tube. Edges of all three flanges were then shaped and rounded, and the cannula was ready for use.

Mr. Gellai uses a small stainless steel screw covered in Silastic as a plug for his cannulas. These plugs were prone to removal by the rat. To solve this problem, a plug was developed by the investigator that would be almost completely inaccessible to the rat. The plug consisted of a hardened Silastic cylinder with an attached wire loop. The entire plug fit inside the stainless tube of the bladder cannula and could be retrieved via the wire loop using very fine forceps. When in place, however, it could be recessed far enough inside the tubing as to be inaccessible to the rat's teeth or claws.

Construction starts with a fine stainless steel dressmakers pin, the head of which is as broad as possible and still allow insertion into the stainless tube of the bladder cannula. Thin-walled Silastic tubing of an internal diameter that would fit over the pin head with a slight but not excessive stretch is cut into lengths of approximately 1 cm. These are filled with undiluted Silastic adhesive, and slid onto the pin from the pointed end so that most of its length is above the pin head and only 1-2 mm of tubing remains below. This action scrapes adhesive from inside the tubing with the pin head and pushes an excess underneath. This blob of adhesive filling the tubing below the pin head and protruding below it is used to glue on a small circle cut of 0.020-inch Silastic sheeting threaded onto the pin by piercing its center with the pointed end. The
diameter of the circle should be just slightly smaller than that of the stainless tube of the bladder cannula.

The next step is to fill the now-empty tubing above the pin head with undiluted Silastic adhesive, and to dry this much in the drying oven overnight or until cured.

When completely hardened, the adhesive-filled tubing above the pin head is trimmed close, leaving only 1-2 mm above the top of the pin. A small circle cut from 0.020-inch Silastic sheeting is glued to the trimmed top of the tube, and 2-3 more to the bottom by threading onto the pin from below, making sure that there is adequate diluted adhesive between the layers to secure them. These should all be slightly smaller in diameter than the stainless tube, with insertion and removal of the bladder cannula plug being easier if the very top circle is very slightly smaller still.

After this last step has dried in the oven, the completed plug is dipped into thinned adhesive a few times to cover rough edges and make sure the plug fits snugly in the stainless tube. If this last step is done too many times or the adhesive is not sufficiently thinned, the plug becomes too large. It is easier to dip it one more time in adhesive than to trim it evenly enough to make a good seal with the stainless tube.

Once the adhesive coating is dry, the plug is inserted into a completed bladder cannula until the top of the plug protrudes slightly beyond the top flange. The sharp pin point is grasped with pliers flush with the bottom of the stainless tube and bent at right angles. The plug is finished by continuing to bend and squeeze the sharp end until it crosses over and makes a tight enough loop to fit into the bladder cannula, after which the sharp excess is cut off with wire cutters.
Bladder Cannula Implantation. Bladder cannula and plug are gas sterilized prior to surgery. All instruments to be used are either gas sterilized or cold sterilized with Cidex (Surgikos, Arlington, TX).

After sterile preparation of the abdomen with Betadine Scrub (7.5% Povidone Iodine, Purdue Frederick, Norwalk, CT) followed by isopropyl alcohol, a transverse abdominal incision (1 cm) is made with scissors just below an imaginary line drawn across the top of the haunches. Underlying abdominal fascia is nicked and the bladder is exposed by spreading longitudinally along the midline of the rectus abdominus. The bladder is brought outside and secured to abdominal skin with micro "bulldog" clips so that the apex is toward the head of the rat.

Under a dissecting microscope at low magnification (Zeiss), a purse-string circle with 6-0 polypropylene suture (Ethicon tapercut cc-1, #8707h, Somerville, NJ) about 5 mm in diameter is made slightly below the midline, avoiding blood vessels where possible. This is grasped at the top edge with forceps and pierced at the center with an 18 gauge sterile needle. The hole is stretched and enlarged slightly with forceps to allow insertion of the top flange of the bladder cannula.

Sutures are tightened and secured, and the moveable center flange slid over them to make a tight seal. The bladder is returned to the abdominal cavity with the flanges arranged so that the middle flange is below the abdominal musculature, and the bottom (now the top as the rat is viewed) is between the muscle and the skin.

The cannula is tightly secured within the abdomen by suturing the abdominal muscle and fascia around it in two steps: the first closes the abdominal muscle longitudinally by placing sutures (4-0 silk, Ethicon, Somerville, NJ) on the head and tail
side of the cannula. The second closes the fascia around the cannula with sutures on the right and left sides.

Once this is done, the skin is cleared from underlying structures for a short space, and a hole made in the skin with an 18 gauge needle along the midline and about 5-8 mm caudal to the edge of the incision. The hole is enlarged slightly by spreading with a pair of hemostats, and the sharpened free end of Silastic tubing on the bladder cannula brought through from below. The hole should not be over-enlarged as the skin must fit very snugly around the cannula.

Excess Silastic tubing is trimmed away from the stainless steel tube of the bladder cannula, and the incision is closed with 4-0 stainless steel braided suture. (Davis and Geck, CE-4 cutting, American Cyanamid, Danbury, CT). The bladder is flushed with an antibiotic solution in the concentration of 1 ml Neosporin G.U. (Burroughs Wellcome) to 250 ml sterile saline, and plugged with the plug described. Betadine solution (Povidone-Iodine, 10%, Purdue Frederick, Norwalk, CT) is applied to the wound.

**FEMORAL ARTERIAL AND VENOUS CANNULATION**

**Femoral Arterial and Venous Construction.** Vascular catheters are constructed as described by Gellai and Valtin (1979). Catheter material is Medical grade tygon tubing (S-54-HL, I.D. 0.015, O.D. 0.030 inch, Norton Co, Akron, OH). A 24 inch length of tubing is stretched by grasping at the center and pulling in opposite directions. In the center where the tubing has been slightly narrowed by stretching, it is cut into two catheters approximately 12 inches long. Two "dumbells" previously made of lengths of PE-60 tubing (Intramedic) heat-expanded on either side (near a soldering iron) are slipped over the narrowed ends and advanced along the length of the catheter until
they fit snugly about 5 mm apart. The narrowed end is then cut to the length of 4.5 cm and catheters are gas sterilized in preparation for surgery.

**Femoral Arterial and Venous Catheter Implantation.** Techniques are as described by Gellai and Valtin (1979). Just prior to surgery, using sterile technique, catheters are attached to 3 ml syringes containing sterile saline via 26 gauge needles and filled with the saline. After sterile preparation of the inner hindlimb, craniodorsal back, and a flank area just caudal to the lower ribs, a 1 cm incision is made with scissors along the inner fold-line of the left hind limb. The femoral artery and vein are exposed by bluntly dissecting away muscle and fascia. The vein is catheterized first. Under a dissecting microscope at high magnification, one 4-0 silk suture is passed beneath the vessel at points both proximal and distal to the site of catheter insertion. The distal suture is tied off, and the proximal suture is lifted so as to temporarily occlude blood flow from the opposite (proximal) direction. A nick is made distal to the lifted suture with small scissors and spread slightly to allow insertion of the narrowed end of the catheter. The catheter is advanced all the way to the first dumbbell, releasing the lifted suture enough to allow the catheter to slip through, but not so much there is a backflow of blood from the vessel. That suture is then tied while still slightly lifted around the vessel and catheter together so as to secure the catheter and prevent leakage of blood. Ends from the distal suture that had been previously tied off are now looped around the first dumbbell and tied securely. This procedure is repeated for the artery. Catheter tips are now lying in the abdominal vessels past the point of femoral bifurcation.

Following insertion and securing to the vessel, both catheters are tunneled under the skin in a 14 gauge trochar (biopsy needle) to the mid-flank location where they are
brought out through a stab wound or a small incision. An additional dumbbell is attached to each to anchor it, and they are now tunneled individually in a 19-20 gauge trochar to exit through a stab wound between the scapulae, about 8 mm apart. Locations had been previously marked with surgical marker.

The rat is returned to his back and the muscle is sewn over and around the catheters with 4-0 silk, covering both dumbbells and making the closure around the catheters as tight as possible. Skin is then closed with braided stainless steel suture.

In the final step, 0.2-0.3 ml of a mixture of equal parts sodium heparin (1000 U/ml, Upjohn, Kalamazoo, MI) and 50% dextrose (500 mg/ml, Abbott Hospital Products) is injected into each catheter. The ends are trimmed to protrude about 3 cm from the skin and plugged with a stainless steel pin (dressmakers pin from which the head was later cut) Important details for the procedure include insertion of the pin as far as possible into the catheter to protect it from destruction by the rat, and release of the clip holding the arterial line closed prior to pushing the pin all the way into the lumen of the catheter. This pushes a small amount of the heparin mixture out into the vessel and helps prevent clotting. Betadine is applied to all wounds.

CENTRAL VENOUS CATHETERIZATION

Central Venous Catheter Construction. Catheters are constructed of 15 cm lengths of medical grade tygon tubing (0.025 I.D., 0.040 O.D., unstretched). "Dumbbells" of PE-100 tubing are positioned 3.5 cm from one end. The length was predetermined by dissections to fall just opposite the right heart.

Central Venous Catheter Implantation. All cannulas are implanted during one surgery with central venous catheterization following both bladder and vascular catheterization. The catheter is first filled with sterile saline by attaching it via a 20
gauge needle and teflon tubing to a pressure transducer and a chart recorder (Gould Instruments, 2600S, Cleveland, OH). The rat is positioned head down with the tail facing away from the investigator. After shaving and sterile preparation of the skin with Betadine followed by isopropyl alcohol, a 1 cm transverse incision is made with scissors 2-5 mm rostral to the clavicle and approximately 1 cm to the right of the midline. Catheterization procedures are identical to those above for arterial and venous catheterization, except that catheter position is adjusted while watching the pressure recording to optimize the pressure pulse before finally securing. Pressure tracing will be considered optimal at the point where pulse pressure excursions are greatest and the pressure waves from different portions of the cardiac cycle are most clearly differentiated.

Following insertion and securing to the vessel, the catheter is tunneled under the skin in an 18 gauge trochar to a point 5-8 mm caudal to the exit points of the arterial and venous catheters, and centered between them. It is brought out through a stab wound with the trochar, filled with 0.2-0.3 ml of the heparin-dextrose solution, trimmed to be about 3 cm from the exit point, and plugged with a stainless steel dressmaker’s pin. The pin must be inserted as far as possible into the catheter to protect it from destruction by the rat, and the clip holding the line closed must be released PRIOR to pushing the pin all the way into the lumen of the catheter. The pin head is then cut off with wire cutters.

The rat is returned to his back and the muscle is sewn over and around the catheters using 4-0 silk, covering the dumbbell and making the closure around the catheters as tight as possible. Skin is then closed with braided stainless steel suture, and Betadine is applied to all wounds.
TRAINING OF ANIMALS AND MAINTENANCE OF PREPARATION

Animals were held for all experiments in plastic restrainers either obtained commercially (Braintree Scientific, Braintree, MA) or constructed by the investigator. Figure 1 illustrates the position of the rat in the restrainer, including connections for measuring urine flow, rectal temperature, and blood pressures. In-house construction of restrainers became necessary when animals which were used twice (once as an experimental animal and once as a time control) outgrew the commercial restrainers in the interval between experiments. Since larger restrainers were not available commercially, restrainers were constructed from a particular brand of plastic soft-drink bottle which was shaped with the appropriate taper at the neck - one which would prevent the rat from turning its head during the experiment.

TRAINING IN PLASTIC RESTRAINERS

Rats were made accustomed to experimental conditions by placing them in the restrainers for 1 hour per day for 2-3 days prior to surgery, and 1-2 hours per day for 4-5 days prior to an experiment. Rats so conditioned were shown to be calm as indicated by heart rates of 300-420 beats per minute and mean arterial blood pressures in the range of 95-110 mmHg.

MAINTENANCE OF CATHETERS

Bladder cannulas were flushed on alternate days with the Neosporin solution mentioned previously. To insure vascular catheter patency, 0.1-0.2 ml of a fibrinolytic solution (5 mg Streptase 750,000/150 mg, Hoechst-Roussel, dissolved in 1 ml heparin/dextrose solution) was injected into each arterial catheter, and 0.05 ml into each venous catheter 1-2 days preceding the experiment.
Figure 1. Position of rat in restrainer for all experiments. Catheters for drawing blood and recording pressures are shown exiting from behind the head. Rectal probe shown in place. Urine tubes could be easily changed without disturbing the rat.
Due to the rat's small blood volume, sufficient blood to assay all hormones could only be obtained by conducting several series of experiments. Two hormones were assayed for each series in addition to plasma and urinary osmolality, electrolytes, and creatinine. Details of individual series are given in the separate sections which follow; only those elements common to all experiments will be described here. Table 1 summarizes all series, which differ in hormones measured, in the timing of blood and urine collection, and in state of hydration.

PROCEDURES COMMON TO ALL EXPERIMENTS

General Form for Experimental Protocols. The general form for Series 1 experiments is given in the next chapter on Preliminary Experiments. All experiments for series 2-4 can be divided into the following sections:

1 HOUR EQUILIBRATION. During this period blood pressure transducers, infusion lines, sampling lines, and rectal probes were connected.

1 HOUR CONTROL PERIOD. Baseline values were established during this period. If, at the end of 1 hour, the animal was judged not to be in a steady state (blood pressure or heart rate was not within normal limits, or urine appeared too dilute), the control period was extended until these values became normal.

The presence of dilute urine deserves comment here. Rats almost invariably undergo a dilute diuresis upon being first placed in the restrainer. It can be assumed that the mechanism is stress-related, although the precise cause is not known. This underscores the need for rats to remain calm throughout the experiment. Although
Table 1

SUMMARY OF EXPERIMENTAL SERIES

<table>
<thead>
<tr>
<th>SERIES NUMBER</th>
<th>TIMING (minutes)</th>
<th>HORMONES MEASURED</th>
<th>HYDRATION</th>
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<tr>
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<td></td>
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</tr>
<tr>
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<td>60 control</td>
<td>ANF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 experimental</td>
<td>vasopressin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>60 control</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 recovery</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Dehydrated</td>
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<td>60 control</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 &amp; 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>experimental</td>
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<td></td>
</tr>
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<td>ANF</td>
<td></td>
</tr>
<tr>
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<td>renin</td>
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<tr>
<td>4.</td>
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</tr>
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<td>60 control</td>
<td>vasopressin</td>
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<td>60 control</td>
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<tr>
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<td>experimental</td>
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</table>
urine samples were collected during this time, values have not yet reached steady state and so are not reported.

1 HOUR EXPERIMENTAL PERIOD. The period of cold exposure for experimental animals.

Cooling Procedures and Time Control Conditions. To maintain conditions that are as close to identical as possible for experimental and time control rats, the two were run side by side; one rat was cooled while the other remained at room temperature. Blood sampling was as close to simultaneous as possible for the two rats.

Cooling for half of series 2 rats was accomplished by wheeling into a pre-cooled (13° C.) walk-in refrigerator after the baseline sample was taken. Time control rats remained outside at 26° C. but were jostled to an extent thought equivalent to wheeling into the refrigerator.

Mid-way through series 2, the refrigerator broke down and could not be repaired. To replace it, the following cooling apparatus was designed and constructed by the investigator (Figure 2). Procedures for operating the chamber are as follows.

Just prior to the experiment, the rear chamber was filled with crushed ice and rock salt in a ratio of 6 parts of ice to 1 part of rock salt. To insulate the rat from cool air during the control period, a thick foam baffle was fit into the opening between the chambers, and the top was placed on the ice-filled chamber. In this way the ice-filled chamber was completely enclosed and insulated from the rat. The restrainer containing the experimental rat was placed in the front chamber, which, for the baseline portion of the experiment was open to room temperature of 26-27° C. After the time control rat was situated in its stand several feet away, urine collection was begun and all lines were connected.
Figure 2. Cooling chamber for experimental series 2-4. Air cooled by circulating through an ice and salt filled rear chamber is pumped gently and continuously over and around rat, exiting at lower front. Thermister above continuously records chamber temperature; mercury thermometer below monitors chamber temperature homogeneity.
Following the baseline blood sample, plexiglas covers were positioned over the front chamber and secured with tape. The chamber was rapidly cooled by blowing air (Gast pump model 0322-V4B-G18DX) through the coiled tubing in the rear chamber and into a network of perforated plastic tubes which surrounded the rat and distributed cold air to all parts of the chamber. An additional, smaller pump (Gast model DOL-101-AA) exhausted through a lengthwise perforated tube at the back of the rear chamber, moving air slowly across the ice and over the rat, finally flowing out the bottom rear opening. Through this constant flow of cold air over the rat, temperature could be maintained even though the bottom rear of the chamber was left open to allow for frequent urine collections.

The rat was protected by the restrainer from any localized stream of cold air. To test whether the airstream alone caused a diuresis, urine was collected from two rats using the same experimental procedures but without ice in the rear chamber. No diuresis was observed.

Both pumps were initially located equidistant between experimental and time control rats with the idea that the considerable pump noise would affect both rats equally. Time control rats occasionally experienced a diuresis, however, and it was thought that stress caused by the pump noise may have been a contributing factor. Pumps were therefore moved to a cooled enclosure which virtually eliminated the noise. Since urine flows did not differ statistically between the two setups, results for the two are pooled.

Once the pumps were activated, temperatures in the front chamber rapidly (within 5 minutes) reached 13-15° C. This was verified by a thermistor (YSI, Yellow Springs, OH) suspended approximately 1 inch above the rat, and connected to a chart recorder.
for continuous recording of temperature. The temperature recording was monitored during the experiment, and further drops to below 13° C. were prevented by reducing the airflow through the Gast pump. To assure adequate homogeneity of temperature, mercury thermometers were placed at other locations (Fig. 2) and monitored at intervals throughout the experiment. Those beneath the rat routinely read about 0.5° C. higher than the thermistor. Time control temperature was also continuously recorded.

**Blood Sampling.** Blood samples for all experiments were collected during the last 5-10 minutes of the control period, at 20 minutes into the experimental period, and 5-10 minutes before the end of the experimental period.

So that sample removal would not cause volume changes and thus affect results, all samples were replaced simultaneously via the venous line as blood was withdrawn from the abdominal aorta. Replacement was with an equal amount of blood from donor rats taken the morning of the experiment.

Donor blood was obtained by decapitation under Ketamine anesthesia (44 mg/kg, Vetlar, Parke Davis). Rats 800-1000 gm were decapitated and then inverted over a beaker-funnel combination for blood collection. Clotting was minimized by the addition of 0.8 ml sodium heparin (1000 U/ml) to the beaker and 0.2 ml dotted evenly around the funnel sides. Clots were removed by straining through sterile gauze or the filter of a blood infusion set, and blood was gently aliquotted into sterile syringes for injection during sampling. Capped syringes were kept at room temperature until just prior to sampling, at which time the blood was warmed to 37° C. in a sterile saline bath.

To minimize variation due to donor blood, both time control and experimental rats received the same blood for all blood samples. Blood from all donor rats was pooled.
before straining and aliquotting into individual syringes, thus maintaining uniformity between groups and over the experimental time period.

Sampled blood was drawn into heparinized syringes, immediately decanted into pre-chilled tubes containing anticoagulants appropriate for the assay (Vacutainer), and kept on ice until termination of the experiment. Hematocrit was taken in duplicate from the heparinized Vacutainer.

Dead space in the connections to the vascular catheters was slightly less than 0.5 ml. Preceding each blood draw a 1 ml syringe was filled with 0.5 ml heparinized saline (1 part heparin (1,000 U/ml) to 10 parts sterile saline; this may be increased if necessary to 1 to 6). At 5-10 minutes before the end of each experimental period, the saline-containing syringe was fastened to one stopcock, and the syringe containing pre-warmed donor blood was attached to the other. To start the blood draw, 0.5 ml was drawn into the 1 ml syringe, which was then removed and replaced with a heparinized syringe for sample collection. After drawing the desired amount (replaced simultaneously with donor blood), the sample syringe was removed, and the 1 ml syringe replaced. 0.5 ml was then injected at a moderate speed to flush the line, and blood samples were aliquotted. After aliquotting the blood, the 1 ml syringe was attached to the venous line and this was cleared with the remaining 0.5 ml of solution. Time for the entire process was 3-5 minutes.

At the end of the experiment, vascular catheters were disconnected, re-filled with 0.5 ml heparin/dextrose solution, and re-plugged with the stainless steel pins.

**Urine Sampling.** At the start of the experiment, rats were placed in restrainers, their bladder cannula plugs removed, and bladders flushed with 1 ml neosporin solution. A 1 mm extension of Silastic tubing (0.078 inch I.D, 0.125 inch O.D.) was
fitted over the exposed stainless steel portion of the bladder cannula. The distal end of the extension was cut at an angle so as to facilitate release of urine droplets. The rat was then positioned in the experimental stand so that the cannula with extension was directly above a small funnel which channelled urine into the collecting test tube. At the end of the experiment, bladders were again flushed with 1 ml Neosporin solution, and the plug re-inserted.

Volume of urine was estimated by weighing, using the assumption that the mass of urine does not differ significantly from that of water. Collection tubes were pre­weighed, and this amount was subtracted from total weight to obtain final weight or volume.

**Arterial Blood Pressure and Heart Rate Measurement.** Mean arterial pressure was continuously recorded using Statham P23 Db or P23 ID pressure transducer linked to a Beckman 4511A chart recorder set at 0.1 mm/sec. For heart rate determinations, the recorder was switched from the "mean" setting to direct pulse pressure measurement and the speed increased to 10 mm/sec for several seconds 1-2 minutes before each blood draw. Heart rate was calculated by counting the number of pulses in a 10 sec. strip and multiplying by 6.

**Infusions and Fluid Added During Experiment.** As stated above, a total of 1.5 ml heparinized saline per rat was injected in the process of flushing vascular lines after blood sampling. Of this, the baseline and 20 minute samples (a total of 1.0 ml) were injected within 30 minutes of starting the experimental period, and so should be included in any consideration of water balance for the experiment.

At the start of the equilibration period, vascular lines were clamped and the plugs removed. Ends were cut at an angle with small scissors, and inserted into connecting
tubes, (Tygon microbore tubing, I.D. 0.30 inch, wall thickness 0.030 inch). After achieving a tight pressure fit, clamps were released and any clots were cleared with injections of 0.025-0.5 ml heparinized saline. In rare cases up to 1.0 ml was used for this purpose. Injected amounts were equalized for both experimental and time control rat; i.e., if 0.5 ml was required to open the line in the experimental rat, 0.5 ml total was injected into the time control rat's line.

Following the establishment of open lines, an infusion of heparinized normal saline (proportions above) was initiated into the arterial line via a syringe pump (Harvard Apparatus) at the rate of 0.6 ml/hr. This was maintained throughout to assure catheter patency.

In central venous cannulated rats, volume used to clear the line was kept below 0.2 ml. Once lines were cleared, they were filled with undiluted heparin solution (1,000 U/ml) and a very slow infusion (0.03 ml/hr) of this solution was begun using the same Harvard infusion pump as the arterial line. If this level of infusion increased the recorded central venous pressure, the rat was not used in the experiment.

Rectal Temperature. For measurement of rectal temperature a YSI temperature probe, (#402) was inserted 5 cm past the anal sphincter and taped to the tail for the duration of the experiment. For approximately half of the experiments, temperature was read using a YSI model 43TA, which was observed at the end of each sampling period and temperature recorded. For the remainder of the experiments, continuous on-line temperatures were obtained using a Hewlett Packard patient monitor, model 66.

SERIES 2

Hormones Measured. In addition to plasma and urinary osmolality, electrolytes, and creatinine, plasma AVP and plasma ALDO were measured in this series.
**Sampling Procedures and Timing.** Urine was collected in 13 x 100 mm screw-capped tubes (Falcon), and changed at the end of equilibration and baseline periods, and at 20 and 60 minutes of cold exposure.

Blood collections were at the end of the control period, and at 20 and 60 minutes of cold exposure. Total volume of each blood sample was 4 ml, all of which went into a 5 ml heparin-containing Vacutainer.

**SERIES 4**

**Hormones Measured.** Urinary as well as plasma vasopressin were the only hormones measured in this series. Osmolality, electrolytes, and creatinine were measured as usual in plasma, but in order to conserve sample for urinary vasopressin measurements, urine was analyzed for osmolality and creatinine concentration only.

**Sampling Procedures and Timing.** As in series 2 experiments, blood was drawn after the control period, and at 20 and 60 minutes following cold exposure. Each 3 ml sample was transferred into a 5 ml Vacutainer containing sodium heparin.

Urine, however, was sampled more frequently during cold exposure. Samples were collected after the equilibration and control periods, and at 10 minute intervals after the onset of cold exposure. 10 minute urine samples were also collected from the time control group simultaneously with those of the cold exposed group so as not to portray a false steadiness in the controls. For these smaller sample collections, Eppendorf 1.5 ml microtubes were used to increase accuracy and minimize evaporation.

**Central Venous Pressure Measurement.** Only rats which exhibited substantial pulse pressure excursions were used for central venous pressure measurements as previous attempts with partially clotted lines showed numerous artifacts. Pressure was
recorded on a Gould model 2600 chart recorder, with mean pressure recorded continuously at a chart speed of 0.1 mm/sec.

For initial experiments, only mean pressure was recorded, as this was thought to be an adequate index of central filling. After two experiments, however, it became clear that mean pressure was being influenced by a cold-induced increase in depth and frequency of breathing. This greater ventilatory excursion decreased intrapleural, and thus mean central venous pressure in the cold. To overcome this problem, an estimate of central venous pressures was obtained from expanded tracing of the pressure wave at critical intervals during the experiment. These were taken 1-2 minutes before the end of the control period, immediately after cold induction, and at 20 and 60 minutes of cold exposure. The recorder was switched to direct pressure recording, and the chart speed increased to 25 mm/sec. Pressure tracings were enlarged by 100% and analyzed according to the following rationale:

A key assumption was that at end-expiration intrapleural pressure can be assumed to be zero and would therefore not affect central venous pressure. Systolic and diastolic pressures taken at end-expiration, then, should be free of respiratory artifacts. In each tracing, end-expiration was assumed to coincide with the most positive excursion of the systolic portion, and the most positive (least negative) nadir of the diastolic. Ten of each were averaged for an estimate of systolic and diastolic central venous pressures, and an estimate of mean pressure was obtained using the standard formula of 1/3 pulse pressure added to diastolic. This procedure makes no provision for the possibility of a forced expiration in which intrapleural pressure might be slightly positive at end expiration.
At the end of the experiment, the ability of the preparation to measure small volume changes was verified by the injection of 2 ml whole donor blood into the venous line. This was always followed by a slight rise in pressure of approximately 0.5 mmHg.

**SERIES 3**

**Hormones Measured.** In addition to plasma and urinary osmolality, electrolytes, and creatinine, plasma ANF and Plasma Renin Activity (PRA) were measured in this series.

**Sampling Procedures and Timing.** Urine was collected as in series 4, with collections after equilibration and control periods, and then at 10 minute intervals throughout the experimental period for both time control and cold-exposed rats. Blood samples were taken as usual after the control period and at 20 and 60 minutes of cold exposure. Of each 4 ml sample, 3 ml was aliquotted into a 5 ml EDTA-containing Vacutainer for measurement of ANF and PRA. The remainder was transferred to a 3 ml heparin-containing tube.

**SAMPLE PROCESSING AND ANALYSIS**

**BLOOD**

During the experiment, 2 hematocrit tubes per blood sample were filled from a heparin-containing Vacutainer. These are reserved until other blood had been processed, and then were spun on a Hermle centrifuge (National Labnet, Woodbridge, NJ) for 5 minutes and read immediately.
All blood samples were spun at 2,000 rpm in a refrigerated centrifuge for 30 minutes. Plasma was removed into storage tubes and frozen until analyzed. Freund (1990) has shown that plasma electrolytes and osmolality are not affected by freezing.

**Hormone Analysis.**

**VASOPRESSIN.** To minimize the effects of inter-assay variability, all experimental and time control samples for a single experiment were assayed together in the same assay. 1 ml aliquots of plasma were acidified with .1 ml 1N HCl and frozen until assayed using a radioimmunoassay developed in this laboratory.

**Antibody production.** Antibody to arginine vasopressin was produced in this laboratory using the method of Skowsky and Fisher (1972). Briefly, rabbits were injected at 2 week intervals with a conjugate of 8-lysine vasopressin and thyroglobulin (Sigma) and 1-ethyl-3-(3-dimethylaminopropyl) and carbodiimide Hcl (The Ott Company, Muskegon, MI) with a conjugation molar ratio of 118:1:200, respectively. They were bled after a minimum of three booster series, and the titer was checked following heating of the serum to 56° C. for 30 minutes to destroy the complement. This was repeated for several months as the titer usually improved with additional booster series. Antisera so developed had a cross-reactivity of less than .05% with oxytocin, and is designated as antisera #96 in previous publications (Uyehara and Claybaugh, 1989).

Final dilution of antisera for the radioimmunoassay was 1:600,000. This is higher than the dilution normally used in this laboratory, but it provides the greater sensitivity necessary for the small sample.

**Extraction.** Plasma for vasopressin assay was first extracted using a modification of the octadecyl silane extraction method of LaRochelle et al. (1980).
Cartridges containing octadecylsilica (Sep-Pak C-18; Water Associates; Milford, MA) were primed with 5 ml methanol, 5 ml 8M urea, and 10 ml distilled water before the application of sample. Plasma was passed through the cartridge slowly. The cartridge was then washed with 10 ml distilled water followed by 10 ml 4% acetic acid. 3 ml of eluate, consisting of 40% ETOH - 4% acetic acid was collected in glass test tubes coated with assay buffer, and dried under vacuum (Savant Speed Vac Concentrator, SVC 200). The mean recovery of added hormone to plasma samples was 65%.

**Assay buffer.** The assay buffer consisted of 0.33% NaCl and 0.1% bovine serum albumin (BSA) in 0.1M sodium phosphate buffer at Ph 7.2. This was made fresh the day of the assay.

**Assay procedure.** The radioimmunoassay procedure used was a 72 hour/48 hour disequilibrium assay similar to those described by Skowski et al. (1974), and Miller and Moses (1972). Standard curves were generated in duplicate using arginine-vasopressin standards (V-0377, Synthetic; Sigma; St. Louis, Missouri) at concentrations of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0, μU/tube assay buffer. The standard curves generated with this standard and the U.S.P. Pharmacopeia reference standard are identical on all doses tested.

Dried samples were reconstituted in 0.4 ml assay buffer, and because of their small original volume, placed whole in the assay as a single unknown. This is a departure from the usual practice in this laboratory of assaying the sample at several dilutions, but based on earlier trials, it was considered necessary in order to maximize sensitivity and precision.

Antiserum at the dilution listed above was added in a 50 μl volume, and the assay was then kept at 4° C. for 72 hours to allow vasopressin to bind to the antibody.
After 72 hours, $^{125}\text{I}$-AVP (New England Nuclear, Boston, MA) was diluted to obtain approximately 3000 counts per minute (CPM) in 50 $\mu$L. 50 $\mu$L of $^{125}\text{I}$-AVP was added to each assay tube and allowed to incubate at 4° C. for another 48 hours. The final volume of the assay tubes was 0.5 ml.

Antiserum-bound $^{125}\text{I}$-AVP was separated from unbound $^{125}\text{I}$-AVP by adding 300 $\mu$L of BSA-coated charcoal (0.8% Norit A charcoal 0.16% BSA in assay buffer to each tube and centrifuging at 2000 rpm for 20 minutes in a refrigerated centrifuge. Calculations of AVP concentration used both bound and unbound counts.

Control samples were run at the start and end of each assay.

Assay statistics. Inter-assay coefficient of variability (C.V.) was 8.86%. The intra-assay C.V. was 4.64%. The lower limit of sensitivity for the assay was 0.25 $\mu$L/ml.

ALDOSTERONE. On the day of the experiment, 0.5 ml aliquots were reserved and frozen until the day of assay. Samples were assayed in duplicate using a commercial kit (Diagnostic Products, Los Angeles, CA).

PLASMA RENIN ACTIVITY. 0.5 ml plasma was frozen the day of the experiment for determination of plasma renin activity (PRA). These were thawed on wet ice the day of the assay and PRA was determined in duplicate using a kit manufactured by Dupont, Wilmington, Delaware (Rianen).

ATRIAL NATRIURETIC FACTOR. Following separation from cells on the day of the experiment, 25 $\mu$L of a 10,000 KIU/ml solution of Aprotinin (Sigma Chemicals, cat. no. 4529) was added to each 1 ml aliquot of plasma.

Plasma concentrations of ANF were determined using a radioimmunoassay developed by Wilson et al. (1986).
Antibody. Rabbit Anti-Alpha atrial natriuretic factor serum was purchased from Peninsula Laboratories, Belmont, CA (RAS-8798). This antiserum cross-reacts 100% with rat atriopeptin III.

Extraction. Plasma was first extracted on non-polar silica gel columns (Absorbex RP-18, cat #19840, Merck, Rahway, NJ). Columns are first primed with 8 ml methanol and 8 ml distilled water. The 1 ml sample is applied, and the column is again washed with 8 ml distilled water before eluting into plastic tubes with 3.4 ml 80% methanol 0.1% trifluoroacetic acid. Samples were dried overnight under vacuum (Savant Speed Vac Concentrator).

Assay buffer. A buffer of 0.1 M sodium phosphate at Ph 7.4, 0.05 M NaCl, 0.1% Triton X-100, 0.01% NaN₃, and 0.1% BSA was used. A solution of double all components except the BSA was prepared in advance. The day of the assay, the buffer was diluted with an equal amount of distilled water, and the BSA was added.

Peptide iodination. Iodination was performed using the method developed by Hunter and Greenwood (1964). Ten µl 0.05 M HOAc and 20 µl phosphate buffer (0.5 M, Ph 7.4) were added to 10 µg of freeze dried peptide (the same as was used for standards). This was followed by addition of 10 µl chloramine T (Eastman Kodak no. 1022, 1 mg/ml in water), and 1-1.5 mCi of Na¹²⁵I (Amersham, IMS-30). The reaction was allowed to proceed for 45-60 seconds.

100 µl of BSA solution (50 mg/200 µl saline, Pentex, Miles Labs Inc, 81-001-2) was then added followed by 50 mg AGI-X10 (Biorad, no. 9995) in 200 µl water. The mixture was centrifuged for 3 min, and the supernate transferred to an SP Sephadex C-25 column at 4° C. as described for purification of rat ANF by Currie et al. (1984).
Purified iodinated material was stored at 4°C. The specific radioactivity of iodinated ANF (peak tube) was calculated using self-displacement (Morris, 1976).

**Assay procedure.** This RIA was a 48 hour/24 hour disequilibrium assay in which antibody is allowed to act on standards and unknowns before the radiolabel is added. Atriopeptin III for standards was purchased from Peninsula Laboratories (rat, no. 8799). The concentrations were corrected for % peptide content. A stock solution of peptide (0.5 μg/μl) was made in 0.1 M HOAc, aliquoted and stored at -20°C. For each radio immunoassay (RIA) a fresh set of standards was prepared, containing 1, 2.5, 5, 10, 25, 50, and 100 pg/100 μl in the RIA buffer.

The RIA incubation mixture consisted of 100 μl of unknown (dried sample reconstituted in 0.6 ml assay buffer), and 100 μl of antiserum at 4°C. After preincubation for 36 hours, 100 μl 125I-ANF (approximately 3,000 CPM) was added, and the incubation continued for 24 hours. At the end of this period, separation of antibody-bound from free 125I-ANF was achieved using polyethelene glycol (Carbowax PEG 8000, Fisher Sci., 100 g plus 400 ml H2O, 600 ul/tube) and gamma globulin (Sigma BG II, 8 mg/ml, 100 ul/tube).

Both bound and free fractions were counted (LKB Clinigamma 1272 Quatro), and the results corrected for non-specific binding.

**Assay statistics.** Intra-assay C.V. for this assay is 7.9%, inter-assay C.V. is 7.6%.

**Osmolality.** Plasma and urinary osmolality was measured by means of freezing point depression (Advanced Instruments Micro Osmometer, model #3 MO, Needham Hts, MA).
Electrolytes and Creatinine. Sodium, Potassium, and Creatinine were measured in plasma and urine by ion permeable electrode (Astra automated systems model 4, Beckman Instruments)

URINE

All urine samples were put on ice immediately after collection. Following the experiment they were weighed and frozen for later determination of osmolality, sodium, potassium, and in some cases vasopressin. If urinary vasopressin was to be done, 50 µl was immediately removed and frozen in separate plastic tubes.

Hormone Analysis.

VASOPRESSIN. All methods for determination of urinary vasopressin were identical to that of plasma except for the following.

Extraction. After preparing columns as previously detailed, sample was applied by diluting each 50 µl aliquot with 1 ml saline at Ph 2, and pouring onto the column. Thereafter the only difference between the procedures for urine and those of plasma is the reduction in the eluate volume to 2 ml rather than 3.

Assay procedure. The assay for urinary vasopressin is a 24-hour equilibrium assay; the antibody and $^{125}$I vasopressin are both added at the start, and the assay is separated and counted 24 hours later.

Standards are 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 Uu/ml, which is a shift toward higher concentrations when compared to the standard curve for plasma. This reflects the higher concentration of urinary vasopressin.
CHAPTER 3
PRELIMINARY EXPERIMENTS

METHODS

EXPERIMENTAL PROTOCOL

SERIES 1

The aim of initial experiments was to establish the rat as a model for the renal effects of acute cold exposure, in its handling of both sodium and of free water.

Though similar in most aspects of the cold diuretic response, the rat and human appeared to differ in sodium excretory responses to exposures of 1-2 hours. Humans have invariably displayed a natriuresis during this time period (Segar and Moore, 1968 and others), while in the single report of acute exposures in rats, Morgan et al. (1983) failed to observe a change in sodium excretion during 45 minutes in the cold.

One explanation for Morgan's negative finding could have been the relatively short duration of her study. At only 45 minutes, her study was shorter than all human studies. Since it has been suggested that in comparison to humans, rats exhibit a longer latency to the onset of the cold diuretic response (Fregly, 1982), it seemed possible that lengthening the period of exposure might reveal a natriuresis in the rat.

The length of preliminary studies was accordingly set at one hour of cold exposure to correspond to the majority of acute cold exposures in humans. With Fregly's comments in mind, it was anticipated that the duration might have to be lengthened.
Hormones Measured. Since an investigation of sodium excretion was a major focus of preliminary studies, and because atrial natriuretic factor (ANF) had not been measured in the cold, the hormones ANF and aldosterone were assayed in the first group of preliminary experiments.

In a second set of preliminary experiments, vasopressin was measured to verify a correspondence in the rat between plasma levels of the hormone, and urinary parameters.

Sampling Procedures and Timing. The general procedures for these experiments followed the form:

1 HOUR EQUILIBRATION. Identical to equilibration periods in other experimental series, this period was the time for connecting and clearing infusion and recording lines, and inserting rectal probes.

1 HOUR CONTROL PERIOD. This period, during which a baseline in all parameters was established, was also identical to control periods in other experiments.

1 HOUR EXPERIMENTAL PERIOD. Unlike experiments in other series, blood and urine were not collected at any intermediate times during this period. Blood and urine were collected at the end of the control period, and one hour later at the end of the experimental period.

1 HOUR RECOVERY. In preliminary experiments only, a one hour recovery period followed cold exposure. Blood and urine were again collected at the end of this period. In subsequent series, the recovery period was eliminated for two reasons. First, the demonstration of a return to baseline after cold exposure was no longer necessary. A second reason was that a recovery sample in experimental series 2-4

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would bring the total number of blood samples to 4 in 2 hours, and the total amount of
donor blood infused to 16 ml - about half the blood volume of a rat this size.

As described below, donor blood for sample replacement was obtained by
withdrawal from the abdominal aorta rather than by decapitation.

**Cooling Procedures and Time Control Conditions.** Cooling was accomplished by
wheeling the rats into a 13° C. walk-in refrigerator for the experimental period, and
then out again for recovery. Control rats remained outside at 26° C., and their cart
was shaken gently to simulate the jostling of being wheeled in and out of the
refrigerator.

**Blood Sampling.** Blood sampling procedures were as described in the methods
section with the exception of collection of donor blood. All donor blood for this first
series was drawn rapidly from the abdominal aorta of halothane anesthetized rats
(within 30-45 seconds). Preliminary experiments determined that removal of even
large volumes during this time period did not cause a hemorrhage response.

Rats were anesthetized with halothane (Ohio Vetaflex anesthesia machine). The
abdomen was shaved and opened via midline and transverse incisions, and the
viscera moved to one side. Blood was withdrawn from the abdominal aorta via
puncture with an 18 gauge needle and 20 ml syringe. Processing of donor blood was
as previously described.
RESULTS

URINARY PARAMETERS

The two groups of rats described in this preliminary series will be referred to by the major hormone measured in each group. The two groups will therefore be called the ANF group and the vasopressin group.

Urine Flow. Urine flow in cold-exposed rats increased by 60% in the ANF group as can be seen in Figure 3. This increase was not significant, however, due to the large standard error seen during the recovery period. Eliminating recovery renders the increase significantly different from baseline.

Figure 4 shows urine flow in the vasopressin group approximately doubled. This difference was significantly different both from baseline and from time control values.

Urinary Osmolality. A slight dilution of urine occurred in the ANF group as shown by the changes in urinary osmolality (Figure 5). Urinary osmolality decreased to a point significantly different from time control but not from baseline values.

Sodium Excretion. Sodium excretion and fractional sodium excretion (ratio of amount excreted to amount filtered) are shown in Figure 4. As can be seen, neither are significant when all three time periods are included. When recovery is omitted, however, the increase in sodium excretion becomes significantly different from baseline.

Potassium Excretion. Cold exposure does not affect potassium excretion as indicated in Figure 4.
Figure 3. Series 1, ANF group urine flow and urinary osmolality in euhydrated rats (water removed 3 hrs. before the experiment) subjected to 13° C. temperatures (Cold), or 28° C. temperatures (Time Contd). Bars represent ± S.E.. No significant change was seen in either parameter.
Figure 4. Series 1 ANF group sodium excretion, fractional sodium excretion, potassium excretion, and plasma atrial natriuretic factor (ANF) for euhydrated rats (water removed 3 hrs before experiment) subjected to 13°C temperatures (Cold), or 26°C temperatures (Time Cont'l). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Control group.
Figure 5. Series 1 vasopressin group urine flow and plasma vasopressin for euhydrated rats (water removed 3 hrs. before experiment) subjected to 13° C. temperatures (Cold), or 26° C. temperatures (Time Contl). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Control.
HORMONE MEASUREMENTS

**Plasma ANF.** Contrary to expectations, plasma ANF decreased significantly in cold exposed rats and remained depressed during recovery. An additional group of rats was tested at one time point only (1 hour of cold exposure and time control) in an attempt to validate these results. Due to high mortality in this group, the number of subjects were too few for reliable statistics. In four cold exposed animals, however, plasma ANF after 1 hour was 76.5±16.0 while four values for four time controls averaged 105.6±35.3.

**Plasma Vasopressin.** As shown in Figure 5, plasma vasopressin measured in four cold-exposed rats and four time controls at these sampling periods showed no change during cold exposure.

DISCUSSION

The major findings of this preliminary series were a decrease in plasma ANF after one hour of cold exposure no change in plasma vasopressin in the same time period. If recovery values for sodium excretion are not used for statistical analysis, sodium excretion is significant at one hour of cold exposure. When this is expressed as fractional sodium excretion, however, the result becomes non-significant.

**FREE WATER EXCRETION AND PLASMA VASOPRESSIN**

These studies show a tendency for a dilution of urine during cold exposure. Urinary osmolality is 634±52.9 at baseline and 513±61.8 mOsm/Kg, at 60 minutes of cold exposure. The baseline value for the ratio of free water to osmotic clearance ($C_{\text{osm}}/C_{\text{H2O}} \times 10$) is -5.6±0.59 and the value at 60 minutes of cold exposure is -
3.9±1.02. Both cold-exposed urinary osmolality and clearance ratios are significantly different from time control at 60 minutes of cold exposure. Despite these indices of free water excretion, plasma vasopressin measured at these time points remains unchanged.

Since Morgan et al. (1983), who measured urine at intervals shorter than 1 hour, reported a nadir for urinary osmolality at 20 minutes, it seemed possible that vasopressin suppression was a transient which was being missed by the 1 hour sampling interval. Accordingly, we measured urine flow and plasma vasopressin at varying times throughout a cold exposure of 1 hour or more. Our results suggested that vasopressin may be decreased at 20 minutes of cold exposure, but returned to baseline values by 60 minutes in the cold.

**SODIUM EXCRETION AND PLASMA ANF**

Sodium excretion was significantly increased in these experiments if recovery is omitted from statistical calculations. This result was not definitive and needed further study.

ANF has been shown to facilitate movement of fluid from the vasculature by increasing vascular permeability (Brenner et al., 1990). If central volume expansion occurred in the cold, this should release ANF, which, together with the increase in mean arterial pressure, would act to move fluid out of the vascular space, increasing hematocrit and decreasing plasma volume. The decreased plasma volume would then act to reduce plasma ANF and at the same time, increase plasma vasopressin.
STATEMENT OF HYPOTHESIS FOR REMAINING EXPERIMENTS

The remaining series of experiments were conducted to test the accuracy of the following predictions: Intrathoracic volume and thus pressure was expected to increase acutely upon exposure to cold. This would elevate plasma ANF, and would suppress both renin activity, and plasma vasopressin. The increased ANF levels would, in combination with blood pressure elevations, decrease total plasma volume. That this does occur is suggested by a significantly increased hematocrit at 20 minutes of cold exposure (34.8±1.8 to 36.3±2.1). A decrease in total plasma volume would then return intrathoracic volume and pressures to normal. This would bring the volume sensitive hormones ANF, renin, and vasopressin to normal as well. If this pattern did hold, that is if these hormones were significantly altered at 20 minutes and, following central venous pressure, returned to normal at 60 minutes, then urine flow, urinary osmolality, and sodium and potassium excretion would be expected to follow the same pattern.

The primary means by which the remaining experiments would test these predictions was by measuring these parameters at narrowly spaced intervals to examine the correspondence in the time course of changes in these parameters.
CHAPTER 4
EFFECTS OF COLD ON RENAL HANDLING OF FREE WATER

RESULTS

URINARY PARAMETERS

Urine Flow. Figures 6 and 7 show urine flow for Series 2 and 4 respectively. In all but the dehydrated group, urine flow increased following cold exposure and reached a peak at 20 minutes. Thereafter, it declined steadily to a point where, at 60 minutes of cold exposure, it was different neither from baseline nor from corresponding control group values. This pattern was observed in all experiments, regardless of the timing of urine collections; urine collections divided into 10 minute periods followed the same pattern as that seen when urine was collected only at 20 and 60 minutes during cold exposure. The more frequent collections demonstrate that urine flow was significantly increased as early as 10 minutes into the period of cold exposure.

Urine flow in the animals deprived of water for 24 hours (Fig. 6) was not significantly affected by cold. In the dehydrated control group, however, urine flow by the 60 minute time period was slightly but significantly different both from baseline values and from the corresponding value for cold exposed rats.

Urinary osmolality and Clearances.

URINARY OSMOLALITY. The pattern for changes in urinary osmolality, also shown in Figures 6 and 7, was by its biphasic nature similar to that of urine flow. Urinary osmolality decreased during cold exposure and reached a nadir at 20-30 minutes. From this point it returned toward baseline, and in series 2, was not
Figure 6. Series 2 urine flow and urinary osmolality for euhydrated rats (water withdrawn 3 hrs. before experiment), and dehydrated rats (water withdrawn 24 hrs. prior to experiment) subjected to either 13° C. temperatures (Cold) or 26° C. (Time Contl). Bars represent ± S.E.. * P < .05 compared to time zero (baseline). + P < .05 compared to baseline.
Figure 7. Series 4 urine flow and urinary osmolality for euhydrated rats (water withdrawn 3 hrs. before experiment) subjected to either 13° C. temperatures (Cold), or 26° C. temperatures (Time Contl). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Control.
significantly different from baseline at the 60 minute sample period (Fig. 6). Although
the 60 minute sample in Series 4 was still different from baseline, it had increased
significantly (P < .01) from the 30 minute nadir (Fig. 7).

In marked contrast to responses of rats in a normal state of hydration, urinary
osmolality of the dehydrated animals showed a tendency to increase (N.S.) throughout
the experiment in both control and cold exposed groups. In this dehydrated group,
urinary osmolalities in cold-exposed rats were inexplicably slightly higher than controls
at all time periods.

CREATININE CLEARANCE. Although creatinine clearance (Table 2) tended to
increase in all groups (cold exposed as well as control and both levels of hydration),
changes were not significant in any group except in dehydrated rats exposed to cold.
For this parameter, however, the number of animals in each group (cold exposed and
time control) was reduced to three due to an instrument malfunction which destroyed
several blood samples. For this reason, and the lack of significant changes in all other
experimental series, although it is interesting that in dehydrated rats only, creatinine
clearance increased significantly in the cold, no conclusions can be drawn without
further study. Taken together, these results suggest the glomerular filtration rate
(GFR) as estimated by creatinine clearance, is not affected by cold exposure, except
possibly in dehydrated rats, although the small number of data points in that study
precludes drawing any conclusions.

OSMOTIC CLEARANCE. Most noteworthy regarding osmotic clearance (C_{osm})
(Table 2) is the transient increase in cold-exposed rats at 10 minutes in series 4. C_{osm}
increased from 71.25 ± 9.70 to 103.36 ± 10.79 in the first 10 minutes, but by 20
minutes had decreased to a point not statistically different from baseline and had
# TABLE 2

<table>
<thead>
<tr>
<th>SERIES</th>
<th>HYDRATION</th>
<th>BASELINE</th>
<th>10</th>
<th>20</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Creat (ml/ min/100gm)</td>
<td>(Euhyd.) Cold</td>
<td>1.10 ± 0.14</td>
<td>1.44 ± 0.14</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>(Euhyd.) Contl.</td>
<td>0.87 ± 0.08</td>
<td>0.79 ± 0.20</td>
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<td>0.86 ± 0.20</td>
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<td>Creat (ml/ min/100gm)</td>
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<td>0.96 ± 0.20</td>
<td>0.86 ± 0.20</td>
</tr>
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<td>Creat (ml/ min/100gm)</td>
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<td>Cosm (ml/min)</td>
<td>(Euhyd.) Cold</td>
<td>71.25 ± 9.70</td>
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<td>81.20 ± 7.72</td>
</tr>
<tr>
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<td>66.79 ± 8.73</td>
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<td>Cosm (ml/min)</td>
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<td>74.30 ± 10.29</td>
<td>74.30 ± 10.29</td>
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<td>CH2O (ml/min)</td>
<td>(Euhyd.) Cold</td>
<td>−50.95 ± 6.98</td>
<td>−56.86 ± 10.63</td>
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<td>−47.12 ± 7.65</td>
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<td>CH2O (ml/min)</td>
<td>(Dehyd.) Cold</td>
<td>−34.81 ± 5.78</td>
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<td>−44.31 ± 7.97</td>
</tr>
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<td>−39.41 ± 3.33</td>
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<td>−44.31 ± 7.97</td>
<td>−44.31 ± 7.97</td>
</tr>
<tr>
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<td>CH2O (ml/min)</td>
<td>(Euhyd.) Cold</td>
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</tr>
<tr>
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<td>−40.34 ± 6.67</td>
<td>−41.07 ± 7.09</td>
<td>−41.07 ± 7.09</td>
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</tbody>
</table>

Mean ± S.E. * P < .05 compared to baseline (time 0). + P < .05 compared to baseline (time 0).
CREATININE CLEARANCE (Creat), OSMOTIC CLEARANCE (Cosm), FREE WATER CLEARANCE (CH2O), FOR TO EXPERIMENT), AND EUHYDRATED (WATER REMOVED 3 HR BEFORE THE EXPERIMENT) RATS SUBJECTED TO 13

<table>
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<th>30</th>
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<th>50</th>
<th>60</th>
</tr>
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<td>± 0.14 +</td>
<td>1.09 ± 0.09</td>
<td>0.85 ± 0.03</td>
<td>0.70 ± 0.09</td>
<td>0.78 ± 0.06</td>
<td>0.86 ± 0.05</td>
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</tr>
<tr>
<td>± 0.20</td>
<td>0.96 ± 0.20</td>
<td>0.86 ± 0.14</td>
<td>1.03 ± 0.13</td>
<td>0.88 ± 0.07</td>
<td>0.82 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>1.14 ± 0.25</td>
<td>0.72 ± 0.14</td>
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</tr>
<tr>
<td>0.76 ± 0.20</td>
<td>1.04 ± 0.11</td>
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<tr>
<td>1.10 ± 0.13</td>
<td>0.83 ± 0.15</td>
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<tr>
<td>0.85 ± 0.09</td>
<td>0.87 ± 0.10</td>
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<tr>
<td>10.79 **</td>
<td>81.20 ± 7.72</td>
<td>63.04 ± 4.41</td>
<td>60.03 ± 6.77</td>
<td>59.08 ± 5.38</td>
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<tr>
<td>9.91</td>
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<td>68.74 ± 8.38</td>
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<td>73.84 ± 5.51</td>
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<td>61.60 ± 11.02</td>
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<tr>
<td>52.29 ± 9.35</td>
<td>70.94 ± 7.76 **</td>
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<td>79.73 ± 9.96</td>
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<td>74.30 ± 10.29</td>
<td>65.62 ± 7.71</td>
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<tr>
<td>7.28</td>
<td>-55.36 ± 7.77</td>
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<td>-54.04 ± 9.54</td>
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<tr>
<td>-44.31 ± 7.97</td>
<td>-59.03 ± 6.72</td>
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<tr>
<td>-24.15 ± 8.22</td>
<td>-22.13 ± 9.43</td>
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<td></td>
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<tr>
<td>-41.07 ± 7.09</td>
<td>-41.89 ± 4.15</td>
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</tr>
</tbody>
</table>

compared to baseline (time 0). + P<.05 compared to Time Control
completely returned to initial values by 30 minutes into the cold. Since the large immediate transient was seen in other series having 10 minute urine collections, it probably occurred in Series 2 as well, and would have been seen if urine had been collected at 10 minutes of cold exposure.

FREE WATER CLEARANCE. Table 2 shows that although euhydrated rats in Series 2 and 4 increased their urine flow dramatically in the first 20 minutes of cold exposure, \( C_{\text{osm}} \) increased to a greater extent. This had the effect of blunting increments in free-water clearance (\( C_{\text{H}_2\text{O}} \)) (since \( C_{\text{H}_2\text{O}} = V - C_{\text{osm}} \)). In Series 4 rats, due to the large transient increase in \( C_{\text{osm}} \), \( C_{\text{H}_2\text{O}} \) decreased (became more negative) immediately upon cold exposure (10 min). Thereafter it increased so rapidly that at 20 minutes rats were generating free water. By the 60 minute time period, however, in a pattern that is by now typically biphasic, \( C_{\text{H}_2\text{O}} \) had returned toward baseline and was different neither from baseline nor from time control.

\( C_{\text{H}_2\text{O}} \) in Series 2 Euhydrated rats (Table 2) showed a non-significant tendency to rise as well. The pattern would probably have more closely resembled that of series 4 if urine collections had been more frequent in this series.

The free water component of the diuresis can be estimated from the ratio of free water to osmotic clearances (\( C_{\text{H}_2\text{O}}/C_{\text{osm}} \)). When this is done (Figure 8), a significant increase of the Free Water component at 20 minutes becomes evident in Series 2 euhydrated rats, making the response similar to Series 4 rats in which urine collections were more frequent.

\( C_{\text{H}_2\text{O}} \) in dehydrated rats responds quite differently from euhydrated rats, showing a downward trend but no significant change. This trend is eliminated and the curve flattened completely when the free water component is expressed as \( C_{\text{H}_2\text{O}}/C_{\text{osm}} \).
Figure 8. Series 2 and 4 ratios of free water to osmotic clearances ($C_{\text{H}_{2}O}/C_{\text{osm}}$) for euhydrated rats (water withdrawn 3 hrs. before experiment), and dehydrated rats (water withdrawn 24 hrs. prior to experiment) subjected to either 13° C. (Cold), or 26° C. (Time Contl). Bars represent ± S.E.. * P < .05 compared to time zero (baseline). + P < .05 compared to Time Control.
VASOPRESSIN VALUES

**Plasma Vasopressin.** In Figures 9 and 10 the plasma vasopressin responses are shown along with corresponding plasma osmolalities and blood pressures (discussed in the next section). For reference, the urine flow values already shown (Figs. 6 and 7) are indicated by hatched lines.

As Figure 9 shows, basal vasopressin values for 24-hr dehydrated rats in series 2 were an expected 5 to 6-fold higher than in euhydrated rats. Superimposition of cold exposure on the rats in the two states of hydration caused a reduction in vasopressin in both series at the 20 minute time period. This is confirmed by the results of series 4 in euhydrated rats (Fig. 10). In all three experiments, the two euhydrated and one dehydrated, plasma vasopressin returned to baseline values, as did urine flow, at 60 minutes of cold exposure.

In dehydrated animals only, vasopressin values in the time control group were significantly elevated at the 60 minute collection. A possible explanation is an increase in rectal temperature which accompanied the rise in vasopressin, since heating has been reported to stimulate vasopressin release (Segar and Moore 1968). The temperature increase, seen in these rats but in no other time control group, was in turn most likely due to dehydration-induced impairment of thermoregulatory mechanisms.

**Urinary Vasopressin.** Central to the present investigation is the attempt to distinguish potential mediators of cold diuresis by measuring these factors in shortly spaced intervals. A correspondence in time course of changes between these and urinary variables is taken to suggest a causal relationship, and a non-correspondence, as evidence against a role for that factor. Since more frequent assessment of vasopressin in plasma was prohibited by the rat's small blood volume, urinary
Figure 9. Series 2 plasma osmolality, vasopressin, and mean arterial blood pressure (MAP), for euhydrated rats (water removed 3 hrs. before experiment), and dehydrated rats (water removed 24 hrs. prior to experiment) subjected to 13° C. temperatures (Cold), and 26° C. (Time Contd). Bars represent ± S.E. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Control.
Figure 10. Series 4 plasma osmolality, vasopressin and MAP for euhydrated rats (water removed 3 hrs. before experiment) subjected to 13°C temperatures (Cold) or 26°C temperatures (Time Contl). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Control.
vasopressin was assayed in the 10 minute urine collections from Series 4 rats and is shown in Table 3. Data is expressed both as vasopressin excretion/min, and as µU of vasopressin excreted per mg of excreted creatinine. The latter corrects for transient changes in GFR and any incomplete urine collections.

There were no detectable decreases in urinary vasopressin excretion. This could be due to altered renal tubular metabolism of vasopressin (Keeler et al., 1991). Thus, when urine flow increases, the intraluminal exposure time of vasopressin in various areas of the nephron decreases, and the degree of metabolism (usually about half of that which is filtered) is reduced. This effect tends to blunt urinary vasopressin responses and could account for our inability to confirm the decreases in plasma vasopressin.

HEMATOLOGIC AND HEMODYNAMIC DATA

Plasma Osmolality. The dehydrated rats of Series 2, and euhydrated Series 4 rats experienced no change in plasma osmolality. Series 2 euhydrated rats, on the other hand, had slightly but significantly increased plasma osmolalities over baseline at the 20 and 60 minute collection periods.

Mean Arterial Blood Pressure, Heart Rate. In all rats in series 2 and 4, heart rate (Table 4) and mean arterial blood pressure (Figs. 9 and 10) significantly increased within 10 minutes of cold exposure and remained elevated throughout. The maintenance of this response is in contrast to the return toward baseline exhibited by both urine flow and plasma vasopressin.

Hematocrit. Like the urinary parameters, the response of blood hematocrit in all Series followed a biphasic time course, becoming significantly elevated at 20 minutes at both hydration states, and returning to baseline by the 60 min. sample (Fig @3/6).
TABLE 3  
URINARY VASOPRESSIN FOR SERIES 4. VASOPRESSIN EXCRETION (UADH V), AND VASOPRESSIN/CREATININE EXCRETION RATIOS (UADH/UCRE) FOR EUHYDRATED (WATER REMOVED 3 HR PRIOR TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) or 26 deg C (Contl) FOR ONE HOUR. NO CHANGE IN UADH V OR UADH/CRE WAS STATISTICALLY SIGNIFICANT.

<table>
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<tr>
<th>TIME (minutes)</th>
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<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
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<td>UADH V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cold</td>
<td>1.71 ± 0.44</td>
<td>2.03 ± 0.36</td>
<td>1.93 ± 0.22</td>
<td>1.68 ± 0.18</td>
<td>1.63 ± 0.22</td>
<td>1.36 ± 0.14</td>
<td>1.42 ± 0.17</td>
</tr>
<tr>
<td>Contl</td>
<td>1.22 ± 0.08</td>
<td>1.40 ± 0.23</td>
<td>1.68 ± 0.48</td>
<td>1.60 ± 0.23</td>
<td>2.13 ± 0.34</td>
<td>1.93 ± 0.20</td>
<td>1.86 ± 0.17</td>
</tr>
<tr>
<td>UADH/UCRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>11.22 ± 2.58</td>
<td>10.17 ± 2.16</td>
<td>11.00 ± 1.39</td>
<td>12.20 ± 0.99</td>
<td>15.79 ± 3.31</td>
<td>10.86 ± 0.85</td>
<td>10.12 ± 1.08</td>
</tr>
<tr>
<td>Contl</td>
<td>9.68 ± 0.93</td>
<td>12.89 ± 1.37</td>
<td>12.20 ± 1.73</td>
<td>14.08 ± 2.10</td>
<td>15.25 ± 2.08</td>
<td>16.72 ± 2.63</td>
<td>16.79 ± 3.09</td>
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</table>
TABLE 4 MEAN ARTERIAL BLOOD PRESSURE (MABP), HEART RATE (HR), AND RECTAL TEMPERATURE (Trect) IN DEHYDRATED (WATER REMOVED 24 HR BEFORE EXPERIMENT) AND EUHYDRATED (WATER REMOVED 3 HR PRIOR TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) OR 26 deg C (Contl) FOR ONE HOUR.

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<th>20</th>
<th>60</th>
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<td>2</td>
<td>MABP</td>
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<td>104.9 ± 3.7</td>
<td>127.6 ± 4.2</td>
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<td>(mmHg)</td>
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<td>103.6 ± 3.3</td>
<td>104.7 ± 4.1</td>
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<td>(mmHg)</td>
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<td>113.4 ± 6.7</td>
<td>115.2 ± 5.7</td>
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<td>106.3 ± 3.7</td>
<td>126.9 ± 2.1</td>
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<td>(mmHg)</td>
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<td>293.3 ± 8.8</td>
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<td>(bpm)</td>
<td>Contl.</td>
<td>338.6 ± 16.9</td>
<td>334.9 ± 14.4</td>
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<tr>
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<td>2</td>
<td>HR</td>
<td>Cold</td>
<td>280.5 ± 13.7</td>
<td>443.5 ± 28.7</td>
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<tr>
<td></td>
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<td>(bpm)</td>
<td>Contl.</td>
<td>304.8 ± 15.6</td>
<td>281.4 ± 10.1</td>
</tr>
<tr>
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<td>4</td>
<td>HR</td>
<td>Cold</td>
<td>349.7 ± 14.6</td>
<td>499.2 ± 10.6</td>
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<td>(bpm)</td>
<td>Contl.</td>
<td>349.7 ± 24.1</td>
<td>354.0 ± 23.7</td>
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<td>T rect</td>
<td>Cold</td>
<td>37.8 ± 0.25</td>
<td>38.3 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(deg C)</td>
<td>Contl.</td>
<td>37.6 ± 0.13</td>
<td>37.9 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>T rect</td>
<td>Cold</td>
<td>37.6 ± 0.13</td>
<td>37.8 ± 0.11</td>
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<td>(deg C)</td>
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<td>37.6 ± 0.39</td>
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<td>4</td>
<td>T rect</td>
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<td>38.1 ± 0.27</td>
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<tr>
<td></td>
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<td>(deg C)</td>
<td>Contl.</td>
<td>37.6 ± 0.52</td>
<td>37.6 ± 0.56</td>
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Mean ± S.E. * P<.05 compared to baseline (time 0).
+ P<.05 compared to Time Control
The hematocrit increase at 20 minutes suggests a reduction in circulating blood volume which returns to normal by the end of the experimental period.

**Central Venous Pressure.** Central venous pressure (CVP) was measured in Series 4 only; responses are shown in Figure 12. Contrary to what might be expected from the hematocrit data which, if symptomatic of a reduced blood volume should produce lower central venous pressures at 20 minutes, the slight downward trend in
Figure 12. Series 4 central venous pressures (CVP) in euhydrated rats (water removed 3 hrs. before experiment) subjected to 13° C. temperatures (Cold) or 26° C temperatures (Time Contl.). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Contl..
CVP at that time was not significant. Significant increases were seen in systolic-diastolic (or pulsatile) central venous pressure by 20 minutes, and in systolic alone at the 60 minute sampling period.

**Rectal Temperature.** Although there was a tendency for rectal temperature to decrease by the end of the experiment (Table 4) this decrease was not significant. In the initial 20 minutes of cold exposure, the response was in the opposite direction, showing a nonsignificant tendency to increase. This early increasing tendency coincides with the reduced plasma vasopressin, and so appears to rule out as a cause of the vasopressin response, a cold-induced reduction in deep body temperature which may have affected the hypothalamus directly.

**DISCUSSION**

These studies have demonstrated a substantial diuresis during cold exposure which is comprised of both an osmotic component, and a large free water component. The osmotic component is quite transient, and is caused by the relatively slower reduction in urine osmolality in relation to the urine flow increase. As discussed below, the lag in measured changes in urinary osmolality relative to urine flow is probably due to the presence in the present experiments of dead space distal to the nephron. Residual osmotic material in the ureters, bladder, and bladder cannula render the initial diuresis more concentrated than instantaneous samples of tubular fluid would have indicated.

In contrast to the transient (evident at 10 min. only) changes in osmotic clearance, indicators of a free water component such as urinary osmolality and ${C_{\text{HCO}_3}}/{C_{\text{osm}}}$, followed
urine flow in describing a biphasic time course: peaking at 20 minutes of cold exposure, and decreasing toward baseline by the end of the 60 minute exposure period. The pattern of changes in plasma vasopressin was biphasic during the same time periods in directions consistent with the changes in urine flow and free water indicators.

In searching for a mechanism which would explain changes in vasopressin alterations, it was found that hematocrit showed the same biphasic increase, while elevations of arterial blood pressure and heart rate did not.

Contrary to our hypothesis, mean central venous pressure did not increase significantly in the cold, although central venous pulse pressure and central venous systolic pressure showed steady increases. The rise in pulse pressure became significant at the 20 minute sampling period, while systolic pressure reached significance at 60 minutes into the cold.

ROLE OF VASOPRESSIN IN COLD DIURESIS

ACUTE RESPONSE

Published measurements of plasma vasopressin in humans (Segar and Moore, 1968), and rats (Itoh, 1954; Morgan et al., 1983; Seliatitskaia et al., 1985) have shown acute decreases on exposure to moderate cold. One abstract published since the inception of the present study (Reddix-Cheri and Martin, 1990), as well as three separate experimental series in the present investigation, have found plasma vasopressin decreased acutely in cold-exposed rats.
Against this evidence is one report of no change in young Sprague-Dawley rats (Gibbs, 1984), and two reports of elevated or unchanged plasma vasopressin in spontaneously hypertensive cold-exposed rats (Berecek et al., 1988; Thornton et al., 1988).

It is possible that SHR and its normotensive control, Wistar Kyoto (WKY) have an altered responsiveness to cold and/or the stimuli that normally affect vasopressin. This is suggested by the lack of a cold-induced blood pressure increase in either WKY or normal SHR, and in contrast, an exaggerated arterial pressure response in SHR fed a high sodium diet. In spite of the large blood pressure elevation (an approximately 25 mmhg increase) in the latter group, plasma vasopressin was significantly increased (Thornton et al., 1988). Both the lack of blood pressure elevation in the cold, and the lack of vasopressin responsiveness to such large elevations in arterial pressure appear to be unique to SHR and WKY.

In addition to strain of rat used, a potentially confounding factor in all three of these studies is that blood for vasopressin analysis was collected by decapitation, a procedure which requires handling the animal, and if not carefully done, has been reported to stimulate vasopressin (Husain et al., 1979). Gibb's study (1984) contains the greatest potential for bias in this regard, as only in cold-exposed rats was a rectal thermometer inserted just prior to decapitation.

Most indications, then, are that plasma vasopressin is reduced during cold exposure both in rats and in humans. What is its contribution to the diuresis? Some clarification can be obtained by following the time course of the changes in each factor.
Time Course of Responses

URINE FLOW AND VASOPRESSIN. When urine is collected at 10 minute intervals, it reveals a pattern of urine flow rapidly increasing within the first 10 minutes of cold exposure and peaking at 20 minutes. Plasma vasopressin levels follow this pattern in reverse, reaching a nadir at 20 minutes, and returning to baseline at 60. Blood samples were taken at only the 20 and 60 minute time periods, however, as the volume of blood necessary for all assays precluded more frequent sampling. As a result, the exact timing of the low point in plasma vasopressin can only be estimated from these data.

URINARY OSMOLALITY, FREE WATER CLEARANCE. Urinary osmolality decreases biphasically during cold exposure (Figs. 6 and 7). The free water component of the diuresis as calculated from the ratio of free water to osmotic clearances increases biphasically in a mirror image of the urine osmolality in all series with 10 minute urine collections (series 3 and 4). The response is considered biphasic because the free water component is significantly increased by cold exposure, and returns to a value not different from baseline at the last sampling period. In contrast to this are the euhydrated rats in series 2 which do not seem to follow a biphasic pattern - having a still-elevated free water component at the 60 minute collection. That this is an artifact of sampling interval is made clear when the experiments with 10 minute urine collections (which are biphasic) are re-arranged to simulate the 20 and 60 minute urine collections of Series 2. When this is done, they too appear not to be biphasic, as the free water component of the last urine sample (comprising the last 40 minutes of cold exposure) is still significantly above baseline.
There is an apparent non-correspondence of time course in series 3 and 4 between urine flow, urine osmolality and the free water component of the diuresis. The nadir of urinary osmolality and the peak of the free water component coincide at 30 minutes of cold exposure. The highest rate of urine flow, however, occurs at 20 minutes. This, as well as a transient increase in osmotic clearance at 10 minutes in both series, is a consequence of a urine flow changing more rapidly than urinary osmolality. This could be due to residual osmoles left in the tubule and bladder catheter which are flushed out by and tend to concentrate the dilute fluid which follows.

Alternatively, it could indicate an osmotic component to the diuresis, (perhaps urea, since electrolyte excretion is unchanged) which is transient and augments the effects of vasopressin.

In general, the time course of changes in plasma vasopressin follow changes in relevant urinary variables such as free water and urine osmolality. While such a correspondence cannot prove a causal role for vasopressin in the diuresis of cold exposure, it is consistent with such a role.

**Vasopressin Elevations.** Another means by which a role for vasopressin could be disproved but not proved is by elevating circulating levels. Elimination of the diuresis by antidiuretic levels of vasopressin would not be conclusive since large doses of vasopressin might eliminate a diuresis regardless of original cause. A failure for it to do so, however, could be considered proof that changes in vasopressin do not solely determine the response.

**DEHYDRATION.** One means of elevating plasma vasopressin is by dehydration. In the present study, rats deprived of water for 24 hours developed plasma vasopressin levels approximately five times that of their normally hydrated
counterparts (2.79 ± 0.46 compared to 0.69 ± 0.14 and 0.47 ± 0.09 Uu in series 2 and 4 respectively). In agreement with studies by Hillier (1969), Wallenberg (1975), and Wallenberg et al. (1974), this degree of dehydration eliminates the diuresis following exposure to 13° C. temperatures for 1 hour. Lesser degrees of dehydration only reduce the diuresis (Lennquist, 1972), not abolish it.

EXOGENOUS VASOPRESSIN ADMINISTRATION. The most common means of elevating plasma vasopressin is by administering exogenous hormone, either by injection (Itoh, 1959; Granberg et al., 1972; Fregly and Tyler, 1972), or infusion (Bader et al., 1952). In all cases the result was an acute reduction in urine flow which was reversed wholly or in part by 2-8 hours after administration. Where vasopressin was injected as a single bolus, the resumption of the diuresis could be due to the hormone's being gradually cleared from the circulation. Since plasma vasopressin was not measured, this cannot be established.

Arguing against this explanation is the one study where vasopressin was continuously infused, presumably maintaining plasma levels at a constant value (Bader et al., 1952). The vasopressin-induced inhibition of the diuresis persisted in this study only in the highest dose used (0.5 mU/kg/hr). For all other doses, a transient suppression of the diuresis was followed by a steady rise in urine flow to an extent that was dose-dependent: the lower the dose, the greater the "escape" from vasopressin inhibition.

Elevated plasma levels of vasopressin, then, seem to affect the urinary response to cold exposure in a consistent fashion: an acute suppression of the diuresis followed by an apparent reduction in the antidiuretic action of the hormone.
Depressed Vasopressin. Dehydrated rats in the present experiments exhibited significant cold-induced decreases in plasma vasopressin, although values remained in the antidiuretic range. Efforts to completely eliminate vasopressin changes as a potential cause of cold diuresis have focussed on the use of congenitally vasopressin deficient animal models (such as the Brattleboro rat, or Diabetes Insipidus rat) or reducing plasma levels essentially to zero by maximal water loads. The failure using either technique to observe a significant cold diuresis is further suggestive evidence for an etiologic role for vasopressin in renal responses to cold.

DIABETES INSIPIDUS RATS. Diabetes Insipidus (DI) rats are congenitally deficient in vasopressin. Morgan et al. (1983) used a 0.02 mU/100 gm body weight/hr vasopressin infusion to induce in DI rats a urinary osmolality comparable to that of normal Sprague-Dawleys (400-1200 mOsm/kg). With vasopressin thus held constant, subjection to 45 minutes of 13° C. temperatures produced no significant increase in urine flow. Mean arterial blood pressure increased less in these animals than in normal rats, however, leaving open the possibility that the reduced urinary response was due to a reduced "pressure diuresis".

WATER LOADED HUMANS. Water diuresis has been shown in several human studies to eliminate the diuresis of cold exposure (Atterhog et al., 1975; Wallenberg, 1974; Wallenberg and Granberg, 1974). Plasma vasopressin can be assumed to be negligible in these experiments in view of the very large water load given (tap water equal to 2% body weight, maintained every 30 minutes by 0.5% of body weight) (Atterhog et al., 1975). Although the absence of a diuresis with vasopressin kept constant in this way suggests a role for vasopressin, these findings cannot be considered conclusive, as water loading eliminates other potential causes of cold-
induced diuresis at the same time it eliminates changes in vasopressin. One of these is the so-called "pressure diuresis", which figures prominently on a list of potential causes. At least one proposed mediator of "pressure diuresis" - a "washout" of the renal medullary interstitial gradient (Selkurt et al., 1965) - would be compromised by water loading; medullary hypertonicity can be assumed to be reduced if not completely "washed out" after 90-120 minutes of water diuresis prior to cold exposure (Atterhog, 1975).

**CHRONIC RESPONSE**

Several pieces of evidence suggest that the acute and chronic responses to cold exposure may have different etiologies. In the present experiments, changes in plasma vasopressin show an acute suppression followed by a return to baseline values within 60 minutes after the onset of cold exposure. While in these experiments urine flow has returned nearly to baseline as well, Fregly (1982) and others report elevated urine flows in rats exposed to cold for as long as 30 days.

One explanation for the discrepancy is that the return of urine flow to baseline in the present experiments is a transient, due to dehydration from unreplenished urinary losses. Since subjects in chronic experiments are able to replace fluid through drinking, dehydration is avoided and urine flow is maintained. An attractive aspect of this formulation is that it eliminates the necessity for multiple explanations of cold diuresis. Conceivably, if fluid were continuously replaced, plasma vasopressin may remain at low levels, and could thus account for the chronic as well as the acute response to cold. This possibility has not been tested since no chronic measurements of vasopressin have been conducted. If the return of vasopressin to basal levels is caused by dehydration, the question arises as to how this is mediated. Plasma
osmolality is not statistically changed, and such volume indicators as hematocrit and central venous pressure are either returned to normal or significantly elevated. Both changes are in the wrong direction to affect plasma vasopressin.

**Elevated Vasopressin.** Acute and chronic responses to the administration of exogenous vasopressin appear to differ, as indicated by the transience of the antidiuresis following vasopressin administration; after 1-4 hours, urine flow for all but the very highest dose of vasopressin has equalled or exceeded that of cold-exposed, non vasopressin-supplemented controls (Bader et al., 1952; Itoh, 1959; Granberg et al., 1971b). This seems to indicate a reduction of vasopressin effectiveness with time.

This is the conclusion arrived at by Fregly and Tyler (1972) in interpreting the data from their studies of chronically cold exposed rats. They found that the urinary response of their cold exposed rats to Pitressin administration or 24 hours of water deprivation was less than that of room temperature controls. In the acute setting, Morgan et al. (1983) tested the possibility of reduced sensitivity to vasopressin by administering high doses (500 μU) to water-diuresing rats in 15° C. temperatures and found their urinary concentrating ability no different from that of rats kept at 30° C.

Since the duration of the exposure was 15 minutes only, this does not address the question of whether vasopressin responsiveness is reduced chronically, but it does suggest, together with the effectiveness of hydropenia in eliminating the diuretic response to cold exposures of 1 hour or less, an ability for elevated vasopressin levels to abolish the immediate diuretic response to cold.

Besides a reduction in sensitivity, the inability for high levels of vasopressin to maintain an antidiuresis in the face of cold exposure could be explained by the antagonism of another substance that increases slowly in the plasma. One candidate
for this substance is cortisol, which has been extensively investigated for possible interaction with vasopressin in regulating free water (Raff, 1987). The time course for cortisol increases in human plasma appears roughly appropriate for this role, as levels begin to rise after 1 hour at 15°C. (Wilkerson et al., 1974).

Other hormones whose plasma levels increase with the proper timing are epinephrine and norepinephrine, although their respective incremental patterns seem strongly dependent on exposure temperature (Wilkerson et al., 1974). Both hormones increase urine flow and reduce urinary osmolality when infused into whole rats (McDonald et al., 1977), or isolated rat kidneys (Besarab et al., 1977). A full discussion of the importance of these hormones in cold diuresis, however, will be reserved for separate sections below.

An equally plausible explanation for the failure of chronically cold-exposed rats to maximally concentrate their urine is a "washout" of the renal medullary gradient due to increased flow through the distal nephron (Berliner and Bennett, 1967). This would explain the lack of complete efficacy of a single dose of Pitressin or single day of dehydration (Fregly and Tyler, 1972), since, once the osmotic gradient is "washed out" by a sustained water diuresis, the medullary hypertonicity may take several days to reestablish (Harrington and Valtin, 1968). Experiments with Diabetes Insipidus (DI) rats (those congenitally lacking vasopressin) showed that urinary and medullary tonicity was still well below normal even after 3 days of supramaximal doses of vasopressin (Harrington and Valtin, 1968). When these authors repeated their measurements after 28 days of treatment, normal hypertonicity had been achieved.

Reduced sensitivity to large doses of vasopressin given chronically is not confined to cold exposure. Sawyer (1974) commented upon the apparent rapid "escape" from
the antidiuresis of vasopressin administration in ethanol anesthetized rats used for bioassay. Mechanisms may involve interstitial hypoosmolality secondary to water diuresis, but are by no means clear.

POTENTIAL EFFECTORS OF PLASMA VASOPRESSIN IN THE COLD

Segar and Moore’s original explanation for the cold-induced reduction in plasma vasopressin was an increase in central venous filling brought about by cold vasoconstriction (1968). Although they did not measure arterial blood pressure, they found the other major influence on vasopressin: plasma osmolality, to be constant at 293 mOsm/kg.

CENTRAL VENOUS PRESSURE

Preliminary experiments in the present investigation indicated a depressed level of plasma vasopressin at 20 minutes of cold exposure, which had returned to baseline levels after 60 minutes in the cold. If central volume was the major factor determining vasopressin level, then a similarly biphasic change in central venous pressure might be expected. That total plasma volume as indicated by hematocrit was decreased at 20 minutes led to formulation of the following hypothesis: a cold-induced vasoconstriction shunts blood into the thoracic cavity, increasing central volume and reflexively suppressing vasopressin. A subsequently decreased plasma volume reduces central filling and thus the stimulus for vasopressin suppression, causing vasopressin to rise toward the end of cold exposure. To test this hypothesis, vasopressin and central venous pressure - an index of central filling - were measured simultaneously.
Results indicate no significant change in estimated mean central venous pressure. These measurements, then, do not support the hypothesis that a biphasic change in central blood volume is the determining factor for plasma vasopressin in the cold.

Certain limitations of the measurement, however, must be taken into consideration. The first is that the measurement is not continuous, and values are taken from estimates made only at certain times throughout the experiment. Initially, since mean pressure was thought to be an adequate indicator of the overall response, systolic, diastolic, and pulse pressures were not recorded. The presence of a large respiratory artifact soon became evident, however, as the frequency and the depth of respiration increased dramatically in the cold. This was not anticipated, as it is absent in humans (Adolph and Molnar, 1946).

The effect of the greater ventilatory depth was an increased chest negativity which was in turn reflected as a decrease in mean central venous pressure. The continuously recorded mean pressure, therefore, did not represent actual transmural pressures and volumes affecting the cardiac receptors. When this was discovered after the first two experiments, direct pressure tracings were made at selected times throughout the experiment, and central venous pressure was estimated from these. An accurate time course for the central venous pressure response is consequently not available. Existing estimates from pressure pulse recordings are limited in number as a result of their being taken only at intervals during the experiments, and not at all prior to the discovery of the ventilatory artifact.

Another limitation of these measurements may be the compliance of the right atrium and vena cava into which the catheter was placed. Since in the rat heart the junction between the superior vena cava and the right atrium is an open conduit, it was
assumed that the pressures in the vena cava at the caval-atrial junction accurately reflected right atrial pressures. It is possible, however, that the total compliance of these structures is too great to allow small volume increases into increases to be reflected in increments in pressure.

The central venous pressure measurement was chosen as one which has long been used as an index of central filling (Gauer and Henry, 1963). That catheters in a dog's right atrium could detect small volume changes is indicated by Reinhardt's experiments (1963) which showed increases in pressure following a high sodium and fluid-containing meal. Further evidence that volume increases of this magnitude would be recorded as changes in central venous pressure is given in an abstract published after the start of the present experiments. In it, central venous pressure was reported to increase two-fold during cold exposure (Reddix-Cheri and Martin, 1990).

Changes in central filling have not always translated into pressure increases, however. Atterhog et al. (1975) measured both and found that while central blood flow (measured by dye dilution) increases in cold-exposed humans, right atrial pressure does not. In view of this and the inability to evaluate exact methodology in the Reddix-Cheri and Martin abstract (1990), the question of compliance must remain open.

The only central hemodynamic change in the present experiments is an increase in pulse pressure due almost completely to an increased systolic pressure. Pulse pressure is significantly elevated over baseline at 20 minutes of cold exposure, while the increase in systolic central venous pressure does not become significantly different from baseline until the 60 minute time period. Although mean central venous pressure is significantly elevated over the time control group at these time periods, this should
not be given too much emphasis since it was also significantly about time control values during the baseline collection period.

Assuming surrounding structures are stiff enough to reflect volume changes, this increase in systolic and pulse pressures alone suggests an augmented contractility without changes in the degree of cardiac filling, since diastolic pressure remained the same. A cause for the increase in contractility could be the increased sympathetic activity during cold exposure.

**MEAN ARTERIAL BLOOD PRESSURE**

The suppression of plasma vasopressin by increases in arterial blood pressure has been well established (Robertson, 1985). Given its powerful effect on vasopressin, the cold-induced increments in arterial blood pressure must be considered a primary candidate for regulation of that hormone in the cold. A disparity of the time course of changes in the two factors, however, is difficult to reconcile; mean arterial blood pressure seems unlikely to be the only regulator of vasopressin in the cold since it remains elevated while vasopressin returns to baseline after 60 minutes of exposure.

On the other hand, baroreceptor reflexes are known to accommodate, and reductions of efferent nerve traffic from the receptors have been observed to decrease over time. This could be one explanation for the return of vasopressin to normal in the face of still-elevated arterial blood pressure.

**PLASMA OSMOLALITY**

Plasma osmolality is a powerful regulator of vasopressin and at times can take precedence over baroreflex control mechanisms (Robertson, 1985). Consequently, an initial suppression of vasopressin by the increment in arterial pressure, might be speculated to be overcome despite continued baroreceptor activity, by a slight,
statistically insignificant but possibly physiologically significant increase in plasma osmolality at the 60 minute time period. This could explain why vasopressin has returned to baseline by the end of cold exposure. What makes this formulation plausible is the finding by Dunn et al. (1973), that a change of as little as 2.9 mOsm/kg in rats can produce vasopressin increases of approximately 2.4 pg/ml.

The average increase in the 60 minute plasma osmolality over baseline in the present study was 2-2.5 mOsm/kg in series 2 and 4, but the average increase in vasopressin compared to baseline was less than 0.17 pg/ml, indicating a relative unresponsiveness to plasma osmolality. A further indication of a relative unresponsiveness of vasopressin to osmotic stimuli is the finding that plasma osmolality is also 1-2 mOsm/kg above baseline by 20 minutes in the cold, yet vasopressin is significantly reduced.

One explanation for this unresponsiveness is given by Robertson (1985) who suggests an increased osmotic threshold, and possibly a reduced sensitivity of vasopressin to osmotic stimuli in hypervolemia and hypertension.

ALTERNATIVES TO VASOPRESSIN AS A MECHANISM FOR COLD DIURESIS

PRESSURE DIURESIS

The classic experiments of Selkurt et al. (1965) demonstrated that elevations in renal arterial pressure can increase sodium and free water excretion in anesthetized dogs in the absence of a change in glomerular filtration rate. There has been a great deal of speculation as to whether this "pressure diuresis" is responsible for the renal effects of cold exposure (Wallenberg and Granberg, 1976).
The "pressure diuresis" described is very similar in character to that of cold diuresis. The same discrepancies in time course observed with vasopressin, however, apply here: arterial pressure increases and remains high even though urine flow returns to baseline by 60 minutes of cold exposure. Another difficulty in ascribing cold diuresis purely to elevations in renal perfusion pressure (if there is, in fact, an elevation; since renal plasma flow does not increase, this may indicate little increase in renal perfusion pressure) is the magnitude of the diuresis. In the cold urine flow typically increases to double or more that of baseline. In the experiments of Selkurt et al. (1965), elevations in renal perfusion pressure of as much as 46 mmhg increased urine flow by only 81%. The 10-15 mmhg increase in arterial pressure seen in the cold would presumably result in lesser increases still.

**GLUCOCORTICOIDs**

Glucocorticoids are known to increase in both cold-exposed humans and rats, and thus may affect cold diuresis either by suppressing vasopressin or blocking its action at the distal nephron. Available evidence does not support this conclusion, however.

Although cortisol may tonically inhibit vasopressin release and attenuate the vasopressin response to known stimuli (Raff, 1987), no depression of normal vasopressin levels by glucocorticoids has been demonstrated. Cortisol infusions in dehydrated dogs do not change plasma vasopressin (Cornette-Finn, 1987).

In addition, suggestions that cortisol inhibits the action of vasopressin at the tubule appear to be controverted by the studies of Lindeman et al. (1961) which show no effect of exogenous cortisol on the renal function of men receiving submaximal doses of vasopressin. Further support is given by the finding of unchanged urine flow in normally hydrated dogs given cortisol infusions (Cornette-Finn, 1987).
A further reason for not placing too much emphasis on a potential glucocorticoid role in the present studies is that glucocorticoids are probably not elevated in such a short exposure to such moderate temperatures (Wilkerson et al., 1974).

CATECHOLAMINES

Both epinephrine and norepinephrine increase in the cold and have been shown in several experiments to produce a dilute diuresis when given to intact animals (McDonald et al., 1977; Johnson and Barger, 1981). When physiologic levels are infused into the renal arteries of conscious dogs, however, no increase in arterial blood pressure, and no diuresis is detected (Johnson and Barger, 1981). This, together with the exclusively free water character of the diuresis when catecholamines are infused systemically, led the authors to conclude that changes seen with systemic administration were due to baroreceptor-mediated reductions in vasopressin. Attempts to keep vasopressin constant by infusing it in water-loaded subjects either used pharmacological doses of both vasopressin and norepinephrine (Fisher, 1968), or were also accompanied by increases in arterial pressure, which itself could not be eliminated as a cause of the relative diuresis (Klein et al., 1971).

Many of the above studies were done in dogs (Johnson and Barger, 1981; Klein et al., 1971). Recent experiments have suggested that an important diuretic action of circulating catecholamines, particularly of epinephrine, is accomplished through stimulation of alpha 2 adrenoreceptors (Gellai, 1991), and that this is absent in dogs (Brooks et al., 1991). Consequently the importance of catecholamines to the renal responses seen in the present study is difficult to evaluate in terms of findings obtained using dogs as subjects.
The data for physiological increases in norepinephrine in dogs indicate that it produces a free water diuresis and a decrease in sodium excretion, only half of the response to cold seen in humans, since a natriuresis in addition to the dilute diuresis is a hallmark of the human response. The present study did not find sodium excretion increased in rats exposed acutely to cold, but it was not reduced either, indicating that some other factor besides or in addition to increased plasma epinephrine is probably operative.

Based on the studies of Gellai and colleagues (1991), increases in circulating epinephrine may be important in the present rat studies, but it may be species-specific, and its importance to human cold diuresis is not yet determined. Further, its action in rats is to functionally antagonize vasopressin, an action that would presumably be less important in a situation such as the present, in which vasopressin levels are reduced.

Even though plasma epinephrine was not measured in the present studies it is doubtful that there is large increase based on findings in both rats and humans that norepinephrine, not epinephrine, is the catecholamine most affected acutely (Lennquist, 1972a; Leduc, 1961). In 3 studies of acutely cold-exposed rats in which epinephrine excretion was measured, two reported increased norepinephrine but not epinephrine excretion (Nakashima et al., 1987; Shum et al., 1969). One which did report increased epinephrine excretion was of longer duration (24 hours as opposed to 4 hours or less), and was in SHR (Minami et al., 1982).
CHAPTER 5
EFFECTS OF COLD ON URINARY SODIUM EXCRETION

RESULTS

URINARY PARAMETERS

Urine Flow. Table 5 shows urinary parameters for Series 3. Urine flow responses to cold were similar to those observed during Series 2 and 4; flow increased following cold exposure, reached a peak at 20 minutes, and then declined steadily to a point where, at 60 minutes of cold exposure, it was different neither from baseline nor from corresponding control group values.

Urinary Osmolality and Clearances.

URINARY OSMOLALITY. As was seen in Series 2 and 4, the pattern for changes in urinary osmolality for Series 3, also shown in Table 5, was by its biphasic nature similar to that of urine flow. Urinary osmolality was decreased by cold exposure and reached a nadir at 30 minutes. From this point it returned toward baseline although unlike urine flow, at 60 minutes it was still significantly reduced.

CREATININE CLEARANCE. As seen in Table 5, no significant change in creatinine clearance was observed in Series 3 rats.

OSMOTIC CLEARANCE. $C_{\text{osm}}$ increased at 10 minutes but was not significantly different from baseline at 20 minutes, and returned almost to initial values by 30 minutes into the cold (Table 5).
TABLE 5  
URINARY PARAMETERS FOR SERIES 3. URINE FLOW (V), URINARY OSMOLALITY (mOsm/l), CREATININE CLEARANCE (Cosm), FREE WATER CLEARANCE (CH20), AND FREE WATER/OSMOTIC CLEARANCE (WATER REMOVED 24 HR PRIOR TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) or 26 deg C (Control) for 60 minutes.

<table>
<thead>
<tr>
<th>TIME (minutes)</th>
<th>BASELINE</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ul/min)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cold</td>
<td>15.3 ± 2.9</td>
<td>31.6 ± 6.1</td>
<td>51.0 ± 11.5*+</td>
<td>46.1 ± 7.1*</td>
</tr>
<tr>
<td>Contl.</td>
<td>17.1 ± 3.7</td>
<td>22.3 ± 4.9</td>
<td>22.0 ± 4.3</td>
<td>32.0 ± 7.9</td>
</tr>
<tr>
<td>Uosm (mOsm/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>1237.3 ± 107.8</td>
<td>1029.9 ± 119.4</td>
<td>662.4 ± 122.7*+</td>
<td>539.7 ± 87.8*+</td>
</tr>
<tr>
<td>Contl.</td>
<td>1151.0 ± 118.9</td>
<td>1133.8 ± 140.1</td>
<td>1090.9 ± 132.5</td>
<td>931.1 ± 109.6</td>
</tr>
<tr>
<td>Ccreat (ml/min/100gm)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>0.70 ± 0.04</td>
<td>0.90 ± 0.06</td>
<td>0.85 ± 0.09</td>
<td>0.75 ± 0.04</td>
</tr>
<tr>
<td>Contl.</td>
<td>0.69 ± 0.09</td>
<td>0.83 ± 0.15</td>
<td>0.77 ± 0.17</td>
<td>0.84 ± 0.11</td>
</tr>
<tr>
<td>Cosm (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>56.89 ± 4.38</td>
<td>92.48 ± 11.86*</td>
<td>78.94 ± 8.17*</td>
<td>68.46 ± 7.21</td>
</tr>
<tr>
<td>Contl.</td>
<td>59.95 ± 9.65</td>
<td>76.37 ± 11.96</td>
<td>73.10 ± 13.13</td>
<td>87.71 ± 14.15*</td>
</tr>
<tr>
<td>CH2O (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>-41.55 ± 3.16</td>
<td>-60.86 ± 9.46</td>
<td>-27.99 ± 12.76*+</td>
<td>-22.39 ± 6.45*+</td>
</tr>
<tr>
<td>Contl.</td>
<td>-42.84 ± 6.58</td>
<td>-54.08 ± 10.11</td>
<td>-51.07 ± 11.19</td>
<td>-55.67 ± 7.57</td>
</tr>
<tr>
<td>CH2O/Cosm X 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>-7.39 ± 0.32</td>
<td>-6.59 ± 0.59</td>
<td>-3.29 ± 1.88*</td>
<td>-3.25 ± 1.00*+</td>
</tr>
<tr>
<td>Contl.</td>
<td>-7.21 ± 0.28</td>
<td>-6.99 ± 0.47</td>
<td>-6.83 ± 0.50</td>
<td>-6.49 ± 0.37</td>
</tr>
</tbody>
</table>

Mean ±S.E.  * P<.05 compared to baseline (time 0).  + P<.05 compared to Time Control
TABLE 3. URINE FLOW (V), URINARY OSMOLALITY (mOsm/l), CREATININE CLEARANCE (Ccreat), OSMOTIC CLEARANCE (CH2O), AND FREE WATER/OSMOTIC CLEARANCE RATIO (Cosm/CH2O) FOR EUHYDRATED TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) or 26 deg C (Contl) for one hour.

<table>
<thead>
<tr>
<th>TIME (minutes)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.6 ± 6.1</td>
<td>51.0 ± 11.5 *+</td>
<td>46.1 ± 7.1 *</td>
<td>45.3 ± 8.6 *</td>
<td>37.8 ± 6.0 *</td>
<td>30.4 ± 7.1</td>
</tr>
<tr>
<td>20</td>
<td>2.3 ± 4.9</td>
<td>22.0 ± 4.3</td>
<td>32.0 ± 7.9</td>
<td>27.1 ± 5.3</td>
<td>27.1 ± 7.2</td>
<td>28.9 ± 7.4</td>
</tr>
<tr>
<td>30</td>
<td>9.9 ±119.4</td>
<td>662.4 ±122.7 *+</td>
<td>539.7 ± 87.8 *+</td>
<td>555.8 ± 78.9 *+</td>
<td>618.6 ± 90.1 *+</td>
<td>722.8 ±109.1</td>
</tr>
<tr>
<td>40</td>
<td>3.8 ±140.1</td>
<td>1090.9 ±132.5</td>
<td>931.1 ±109.6</td>
<td>940.0 ± 88.4</td>
<td>926.2 ±131.4</td>
<td>1025.6 ±145.5</td>
</tr>
<tr>
<td>50</td>
<td>60.0 ± 0.06</td>
<td>0.85 ± 0.09</td>
<td>0.75 ± 0.04</td>
<td>0.79 ± 0.09</td>
<td>0.76 ± 0.07</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>83 ± 0.15</td>
<td>0.77 ± 0.17</td>
<td>0.84 ± 0.11</td>
<td>0.70 ± 0.07</td>
<td>0.60 ± 0.08</td>
<td>0.82 ± 0.10</td>
</tr>
<tr>
<td>70</td>
<td>48 ±11.86 *</td>
<td>78.94 ± 8.17</td>
<td>68.45 ± 7.21</td>
<td>69.13 ± 6.92</td>
<td>66.49 ± 6.86</td>
<td>61.09 ±10.69</td>
</tr>
<tr>
<td>80</td>
<td>37 ±11.96</td>
<td>73.10 ±13.13</td>
<td>87.71 ±14.15 *</td>
<td>77.01 ± 9.35</td>
<td>66.76 ± 9.47</td>
<td>80.13 ±12.70</td>
</tr>
<tr>
<td>100</td>
<td>08 ±10.11</td>
<td>-51.07 ±11.19</td>
<td>-55.67 ± 7.57</td>
<td>-49.88 ± 5.40</td>
<td>-39.66 ± 5.27</td>
<td>-51.21 ±10.38</td>
</tr>
<tr>
<td>110</td>
<td>59 ± 0.59</td>
<td>-3.29 ± 1.88 *</td>
<td>-3.25 ± 1.00 *+</td>
<td>-3.28 ± 1.29 *+</td>
<td>-4.16 ± 0.96 *</td>
<td>-4.82 ± 1.07</td>
</tr>
<tr>
<td>120</td>
<td>99 ± 0.47</td>
<td>-6.83 ± 0.50</td>
<td>-6.49 ± 0.37</td>
<td>-6.63 ± 0.32</td>
<td>-6.23 ± 0.57</td>
<td>-6.37 ± 0.81</td>
</tr>
</tbody>
</table>

compared to baseline (time 0). + P<.05 compared to Time Control
FREE WATER CLEARANCE. Table 5 shows that due to the large transient increase in $C_{\text{osm}}$, $C_{\text{H}2\text{O}}$ decreased immediately upon cold exposure (10 min). Thereafter it increased rapidly and at 20 minutes was significantly different from time controls, although not significantly different from baseline. By the 60 minute time period, however, in a biphasic pattern similar to that of other series, $C_{\text{H}2\text{O}}$ had returned toward baseline and was no longer different from time control.

The free water component of the diuresis can be estimated from the ratio of free water to osmotic clearance. These results are shown in Table 5. Expressed in this manner, a significant increase at 20 minutes becomes evident. Again, by 60 minutes, values for this ratio are no different from baseline or time control.

SODIUM EXCRETION. Sodium excretion for all Series except Series 4 is shown in Figures 13 and 14. (In Series 4, urinary sodium and potassium determinations were omitted so that samples could be used for urinary vasopressin assays.) Sodium excretion and fractional sodium excretion in Series 2 showed a tendency to increase over time, which reached significance at 60 minutes in all groups except dehydrated cold exposed rats. This response was not dependent upon cold or state of hydration. In Series 4 the same pattern was seen in the control group only.

POTASSIUM EXCRETION. Series 4 potassium excretion was unchanged, as can be seen in Figure 14. This was not the case in Series 2, where euhydrated rats in both control and cold-exposed groups displayed an increased potassium excretion at 20 minutes. Dehydrated rats did not exhibit the same response (Fig. 13). The only significant change in potassium excretion in these rats was in the control group, where an increase in urine flow at the 60 minute time period produced not only a kaliuresis, but the natriuresis described above.
Figure 13. Series 2 electrolyte excretion and plasma aldosterone of euhydrated rats (water removed 3 hrs. before experiment), and dehydrated rats (water removed 24 hrs prior to experiment) subjected to 13° C. temperatures (Cold), or 26° C. temperatures (Time Contl.). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Contl..
Figure 14. Series 3 electrolyte excretion, plasma renin activity, and plasma ANF for euhydrated (water removed 3 hrs. before experiment) subjected to 13° C. temperatures (Cold) or 26° C temperatures (Time Contt.). Bars represent ± S.E.. * P < .05 compared to zero time (baseline). + P < .05 compared to Time Contt..
PLASMA HORMONES

**Plasma Aldosterone.** While a central volume expansion hypothesis would argue for a reduced aldosterone, cold exposure as shown in Figure 13 for Series 2, was accompanied by an elevation in plasma aldosterone that was significantly different from both Baseline and time controls. The response was particularly striking in the dehydrated animals.

By 60 minutes in euhydrated rats, the increase was still elevated over baseline although it was no longer significant with respect to time controls. Dehydrated animals, on the other hand, increased plasma aldosterone still further by the 60 minute sample period.

**Plasma Renin Activity.** Figure 14 shows data for plasma renin activity in Series 3 rats, since small sample size precluded assaying renin in Series 2 when aldosterone measurements were made (vasopressin was to be measured as well). Given the similarity in other responses across experimental series, however, it seems reasonable that the significantly increased renin activity in Series 3 suggests a similar elevation with aldosterone in series 2. It is assumed, therefore, that both renin and aldosterone are stimulated by cold exposure.

**Plasma Atrial Natriuretic Factor.** Plasma atrial natriuretic factor (ANF) was not affected by cold exposure (Fig. 14). The significant decrease seen in the time control group may reflect dilution by donor blood which, at approximately 18 pg/ml was considerably lower than the 43.9 to 62.1 pg/ml basal levels seen in the experimental animals. The absence of a similar decrease in the cold-exposed animals may indicate an effect of cold exposure, although this is speculative.
HEMATOLOGIC AND HEMODYNAMIC DATA

**Plasma Osmolality.** Plasma osmolality for all Series is shown on Table 6. As discussed previously, the only significant change over baseline in any Series was for euhydrated rats in Series 2. Series 3 rats showed a tendency to increase at 20 minutes but this was significantly different only from the time control value.

**Plasma Electrolytes.** Plasma sodium was unchanged in all groups and time periods with the exception of dehydrated time controls, which at 20 minutes was increased by 5 mEq/L over baseline (Table 6). By 60 minutes, values had returned toward baseline and were not different either from baseline levels or from corresponding cold group values.

Baseline plasma potassium values were high due to the injection of donor blood which was slightly hemolyzed from having been collected by decapitation. Where this might be expected to gradually raise plasma potassium over the course of three blood samples and subsequent replacement with donor blood, this was not the case. In fact the opposite response was observed: a striking, progressive decrease of approximately 1 Meq/L occurred in all time control groups. Cold exposed groups, however, demonstrated no statistically significant change in plasma potassium concentration (Table 6). It appears that the donor blood or sampling procedure itself lowers plasma potassium, and that this effect is delayed or blunted by exposure to cold.

**Mean Arterial Blood Pressure, Heart Rate.** In all rats heart rate (Table 7) and mean arterial blood pressure significantly increased within 10 minutes of cold exposure and remained elevated throughout.

**Rectal Temperature.** Cold exposure produced no significant change in rectal temperature throughout the 1 hour exposure period (Table 7).
TABLE 6  PLASMA CONSTITUENTS FOR SERIES 2-4. PLASMA OSMOLALITY (Posm), PLASMA SODIUM (PNa+), AND PLASMA POTASSIUM (PK+) FOR DEHYDRATED (WATER REMOVED 24 HR BEFORE EXPERIMENT) AND EUHYDRATED (WATER REMOVED 3 HR PRIOR TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) OR 26 deg C (Conti) FOR ONE HOUR.

<table>
<thead>
<tr>
<th>SERIES</th>
<th>HYDRATION</th>
<th>TIME (minutes)</th>
<th>BASELINE 20</th>
<th>BASELINE 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Posm</td>
<td>Cold</td>
<td>290.9 ± 1.3</td>
<td>293.7 ± 1.5 * 293.5 ± 0.9 *</td>
</tr>
<tr>
<td>2</td>
<td>(mOsm/l)</td>
<td>Contl</td>
<td>293.2 ± 1.5</td>
<td>293.6 ± 1.1 292.0 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>Posm</td>
<td>Cold</td>
<td>298.2 ± 1.7</td>
<td>297.5 ± 2.4 298.1 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>(mOsm/l)</td>
<td>Contl</td>
<td>299.1 ± 2.0</td>
<td>298.8 ± 2.0 298.2 ± 2.0</td>
</tr>
<tr>
<td>3</td>
<td>Posm</td>
<td>Cold</td>
<td>297.2 ± 1.2</td>
<td>298.3 ± 1.4 + 296.9 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>(mOsm/l)</td>
<td>Contl</td>
<td>295.6 ± 1.2</td>
<td>295.5 ± 0.9 295.5 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>Posm</td>
<td>Cold</td>
<td>291.9 ± 2.3</td>
<td>292.7 ± 1.4 294.0 ± 1.6 +</td>
</tr>
<tr>
<td>4</td>
<td>(mOsm/l)</td>
<td>Contl</td>
<td>288.3 ± 2.1</td>
<td>288.2 ± 1.4 289.8 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>PNa+</td>
<td>Cold</td>
<td>147.7 ± 1.9</td>
<td>147.7 ± 1.2 150.7 ± 2.3</td>
</tr>
<tr>
<td>2</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>145.5 ± 0.8</td>
<td>147.3 ± 1.1 147.9 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>PNa+</td>
<td>Cold</td>
<td>149.7 ± 0.8</td>
<td>149.7 ± 1.2 154.7 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>149.2 ± 1.2</td>
<td>154.3 ± 0.9 * 152.3 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>PNa+</td>
<td>Cold</td>
<td>149.6 ± 0.9</td>
<td>151.0 ± 1.3 + 151.1 ± 1.0 + 151.1 ± 1.0 +</td>
</tr>
<tr>
<td>3</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>149.3 ± 0.8</td>
<td>149.7 ± 1.0 149.5 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>PNa+</td>
<td>Cold</td>
<td>144.3 ± 0.4</td>
<td>145.3 ± 0.5 146.6 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>143.8 ± 1.2</td>
<td>145.1 ± 0.3 145.8 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>PK+</td>
<td>Cold</td>
<td>5.7 ± 0.3</td>
<td>5.8 ± 0.2  5.3 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>5.7 ± 0.3</td>
<td>5.3 ± 0.2 *+ 4.8 ± 0.2 *+</td>
</tr>
<tr>
<td>2</td>
<td>PK+</td>
<td>Cold</td>
<td>5.6 ± 0.1</td>
<td>5.6 ± 0.1  5.3 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>5.4 ± 0.2</td>
<td>5.0 ± 0.2 *+ 4.7 ± 0.2 *+</td>
</tr>
<tr>
<td>3</td>
<td>PK+</td>
<td>Cold</td>
<td>5.9 ± 0.2</td>
<td>6.1 ± 0.3  5.4 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>5.9 ± 0.3</td>
<td>5.2 ± 0.2 *+ 5.0 ± 0.2 *</td>
</tr>
<tr>
<td>4</td>
<td>PK+</td>
<td>Cold</td>
<td>5.1 ± 0.2</td>
<td>5.4 ± 0.1  4.7 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>(mEq/l)</td>
<td>Contl</td>
<td>5.1 ± 0.1</td>
<td>4.6 ± 0.2 *+ 4.3 ± 0.1 *+</td>
</tr>
</tbody>
</table>

Mean ± S.E. * P<.05 compared to baseline (time 0).
+ P<.05 compared to Time Control
TABLE 7 MEAN ARTERIAL BLOOD PRESSURE (MABP), HEART RATE (HR), AND RECTAL TEMPERATURE (T rect) IN EUHYDRATED (WATER REMOVED 3 HR PRIOR TO EXPERIMENT) RATS SUBJECTED TO 13 deg C (Cold) OR 26 deg C (Contl) FOR ONE HOUR.

<table>
<thead>
<tr>
<th>SERIES</th>
<th>HYDRATION</th>
<th>TIME (minutes)</th>
<th>BASELINE</th>
<th>20</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>MABP (mmHg) (Euhyd.)</td>
<td>Cold</td>
<td>103.2 ± 1.8</td>
<td>114.8 ± 1.4 *+</td>
<td>117.8 ± 2.7 *+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contl.</td>
<td>102.8 ± 1.5</td>
<td>106.1 ± 2.3</td>
<td>106.1 ± 2.3</td>
</tr>
<tr>
<td>3</td>
<td>HR (bpm) (Euhyd.)</td>
<td>Cold</td>
<td>356.0 ± 14.2</td>
<td>438.6 ± 17.7 *+</td>
<td>478.9 ± 13.5 *+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contl.</td>
<td>388.9 ± 13.6</td>
<td>369.0 ± 18.9</td>
<td>366.0 ± 19.9</td>
</tr>
<tr>
<td>3</td>
<td>T rect (deg C) (Euhyd.)</td>
<td>Cold</td>
<td>37.5 ± 0.16</td>
<td>37.9 ± 0.19</td>
<td>36.9 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contl.</td>
<td>37.1 ± 0.11</td>
<td>37.8 ± 0.28</td>
<td>37.7 ± 0.19</td>
</tr>
</tbody>
</table>

Mean ± S.E. * P<.05 compared to baseline (time 0).  
+ P<.05 compared to Time Control.
DISCUSSION

ELECTROLYTE HANDLING IN COLD DIURESIS

SODIUM EXCRETION

In none of the 4 series (involving 5 groups of rats) in the present investigation did cold exert an independent natriuretic effect. This is in agreement with Morgan et al. (1983), and with an abstract published since the start of this study (Reddix-Cheri and Martin, 1990), who also failed to observe an increase in sodium excretion in rats exposed to moderate cold (13-15° C.) for 1 hour or less.

Since humans do increase sodium excretion under identical conditions, this raises the possibility of a species difference in this response. The intensity of the cold stimulus, however, may be less for a furred animal than for the human, and this may account for the lack of effect on sodium excretion. In support of this possibility are Fregly's studies at colder (6° C.) temperatures which did show an increased sodium excretion in rats per unit of sodium intake (1968). These were chronic studies, however, and the effect of a lower temperature cannot be separated from the effect of longer duration in contributing to the natriuresis. A much shorter duration study was conducted by Smith and de Jong (1975), who reported a natriuresis in rats exposed for only 3 hours to still colder temperatures (4° C.).

In conclusion, while the rat appears to differ from the human in its acute sodium excretory response to moderate cold, this cannot be said with certainty until acute experiments are conducted at temperatures which, to the rat, are equal in severity to 15° C. in humans.
Since the rat does not experience an increased sodium excretion during the present experiments, yet does undergo an arterial blood pressure increase of 15-20 mmhg, the absence of a "pressure natriuresis" should be explained.

It is possible that the observed pressure increase is not sufficient to cause a pressure natriuresis in the whole animal. Reports of pressure natriuresis often involve the isolated kidney in which pressure is varied directly at the renal artery (Selkurt et al., 1965), or extreme pressure elevations of 50-100 mmhg (Selkurt et al., 1965; Guyton, 1991, p.206).

Another explanation might involve the increase in aldosterone, which could block cold-induced natriuresis. That aldosterone may be acting to decrease sodium excretion is suggest by comparing fractional sodium excretions at the two levels of hydration in Series 2. In this series, fractional sodium excretion in time controls becomes significantly elevated under both euhydrated and 24-hour water deprived conditions. Cold-exposed rats, on the other hand, in the presence of significantly increased aldosterone under euhydrated conditions, have a delayed natriuresis. Finally, when rats are deprived of water for 24 hours, the cold-induced aldosterone increase is several times what it is during normal hydration, and fractional sodium excretion is completely suppressed. In Series 4, the same pattern was observed; although aldosterone was not measured, fractional sodium excretion was significantly increased only in time controls. Aldosterone may not be the only anti-natriuretic influence involved in the cold. Several indicators indirectly suggest an elevated sympathetic activity: the increase in heart rate, excretion of catecholamines, and renin activity. Since an elevation in sympathetic renal nerve activity reduces sodium
excretion (DiBona, 1982), this may help explain why, in two out of three experiments, fractional sodium excretion increases in time controls, and not in rats exposed to cold.

**RENIN-ALDOSTERONE STIMULATION**

Hematocrit increases very rapidly following cold exposure in both humans and rats. Since this is suggestive of a decreased blood volume and is accompanied by increases in renin and aldosterone, it might be concluded that renin and aldosterone are responding appropriately to volume reduction.

Alternatively, cold-induced elevation in sympathetic tone may be responsible for the increased plasma renin activity seen in Series 3. Possibly sympathetic stimuli override any input which might come from central volume expansion via cardiac reflexes, which would work to decrease renin. In humans, exposures of equal duration and colder temperatures (4°C: Hiramatsu et al., 1984) do result in renin activity reductions which might suggest a species-dependent variation in cardiac reflex response, in sympathetic response, or simply in a non-species-related factor such as intensity of the cold stimulus. Colder temperatures produce more rapid vasoconstriction (Adolph and Molnar, 1946) which might be translated to more rapid and greater central volume expansion, and thus a renin reduction. On the other hand, colder temperatures produce greater catecholamine excretions, presumably as a result of greater sympathetic activity (Wilkerson et al., 1974). Plasma renin activity levels, then, may be the resultant of these two and perhaps other, as yet undetermined factors.

When the volume expansion stimulus is very large, as it is during immersion, aldosterone is depressed even with the superimposition of cold (K. Shiraki, personal communication). This may mean either that cardiac receptor input takes precedence, or that the volume stimulus was simply the greater of the two in this case.
ATRIAL NATRIURETIC FACTOR

If the atria expand during cold exposure, then ANF should increase as it is a very sensitive marker for atrial stretch (Brenner et al., 1990). That our measurements show no increase could be interpreted as a failure of central volume expansion to occur. Studies published after the start of the present experiments, however, report not only increases in central venous pressure (Reddix-Cheri and Martin, 1990), but also increases in plasma ANF (Reddix-Cheri and Martin, 1990; Askew et al., 1991)

It is possible that plasma ANF would have shown an increase in our experiments if sampled blood had not been successively diluted with the lower levels in donor blood. It might be argued that, if cold had no effect on plasma ANF, the amount of hormone in the plasma of cold exposed rats would have been reduced to the same extent as controls.

Although this could explain the discrepancy between our results and those of others, it is not consistent with our finding of no change in diastolic central venous pressure. An unchanged diastolic pressure would argue against central volume expansion, unless there is a question, given the distensibility of the structures surrounding the central catheter, about the ability of our apparatus to measure small volume changes as changes in pressure.
CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

DISCUSSION

Major findings of this study include the demonstration of a biphasic time course to the diuresis following exposure to cold. Also biphasic were decreases in urinary osmolality and plasma vasopressin, and increases in free water clearance. Hematocrit increased biphasically, while elevations of arterial blood pressure and heart rate did not. Contrary to expectations, mean central venous pressure did not change significantly in the cold, although central venous pulse pressure and central venous systolic pressure showed steady increases. The rise in pulse pressure became significant at the 20 minute sampling period, while systolic pressure reached significance at 60 minutes into the cold. As previously discussed, our inability to detect an increase in mean central venous pressure may be a consequence of high caval compliance, which could render small volume changes difficult to detect as changes in pressure.

Osmotic clearance increased, but the urinary excretion of neither sodium or potassium was significantly altered by cold exposure. Plasma levels of both renin and aldosterone rose throughout the 60 minute exposure, and no changes were seen in plasma ANF.

URINARY BIPHASIC RESPONSE

The biphasic nature of the acute diuresis and plasma vasopressin response is a new finding and does much to reconcile a number of discrepant reports in the
literature. As an example, Granberg and colleague's (1971b) report of no effect of exogenous vasopressin on cold diuresis does is shown not to disagree with Bader's (1952) study showing a suppression, if the two studies are compared during the acute time period when urine flow is maximal. Then both show that administration of exogenous vasopressin causes a reduction in urine flow.

Our finding that plasma vasopressin returns to normal within 1-2 hours suggests that any continuance or renewal of the diuresis may be caused by other factors, and could explain why Granberg and colleagues found the effectiveness of exogenous vasopressin administration diminished after the first 4-8 hours (1971b). One possible "other factor" which may explain the reduced effectiveness of exogenous vasopressin is a washout of medullary hypertonicity with the initial diuresis. Since vasopressin acts by allowing fluid to move down an osmotic gradient into the medullary interstitium, the absence of such a gradient would greatly reduce its effectiveness.

Possible Effectors of the Biphasic Response.

DEHYDRATION. One obvious criticism of the present study is that responses may have been made artificially biphasic by not replacing fluid excreted during cold exposure. Thus, the resulting dehydration could be what returns plasma vasopressin and so urine flow to normal levels by the end of the exposure period. If this were true, it would be consistent with the hypothesis that vasopressin is responsible for acute cold-induced diuresis: urine flow follows the biphasic vasopressin levels, not the monophasic changes in other potential effectors such as arterial blood pressure, and urinary catecholamines.

"Dehydration", however, is not a stimulus for vasopressin release. Vasopressin is increased during dehydration by the combined stimuli of increased plasma osmolality
and decreased blood volume (Dunn et al., 1973). In the present studies, plasma osmolality is not significantly increased. While blood volume may be decreased at 20 minutes as indicated by increased hematocrit, hematocrit has returned to normal at 60 minutes when any dehydration should be maximal, and therefore does not appear to be affected by urinary fluid losses. In any case, the means by which volume depletion is proposed to affect plasma vasopressin is via central volume receptors which, in the present experiments, undergo no change in activation (assuming they respond to mean central venous pressure which is unchanged throughout cold exposure). In these experiments, then, it is difficult to conceive of a mechanism by which dehydration might be returning plasma vasopressin to normal levels.

Another argument for this response being intrinsically biphasic is that urinary patterns during balloon inflation frequently follow the same pattern - diminishing over time, even though the left atrial pressure does not decrease, and urinary losses are continuously replaced (Kappagoda, 1974).

ACCOMMODATION. One explanation for the biphasic response is that the receptors accommodate, diminishing their firing frequency after the initial acute burst. There is evidence that both the high and low pressure baroreceptors are subject to accommodation.

BIPHASIC RESPONSE IN HEMATOCRIT

While hematocrit is invariably reported to increase in the cold, one of the most interesting new finding in this series of experiments is its return to normal by 60 minutes of cold exposure. Besides plasma vasopressin and indices of free water excretion, this is the only measured parameter which is biphasic. There is thus a
temptation to try and associate free water, vasopressin and hematocrit responses with some common causative agent.

One causative agent for increased hematocrit might be sympathetic activity as indicated by increased heart rate, blood pressure and plasma renin activity. An increase in sympathetic activity would cause splenic contraction, and thus increase hematocrit. It would be difficult to tie the vasopressin and urinary responses to sympathetic activity, however, since neither catecholamine excretion, nor any of the above indices of sympathetic activity is biphasic.

HORMONAL MODULATORS OF SODIUM EXCRETION

The present group of studies includes the first report of plasma ANF in the cold (Dice et al., 1989), and the first measurement of plasma renin activity in normal rats exposed acutely to cold (Berecek et al., 1988) measured renin in SHR only. We found no consistent effect of cold on either plasma ANF or sodium excretion. This is in contrast to humans where not only is there a natriuresis acutely, but ANF has been reported to increase (Agnew et al., 1991). The increased renin in our experiments is also consistent with the lack of observed natriuresis. Again this differs from human studies which report a decrease in plasma renin activity in response to acute cold exposure (Hiramatsu et al., 1984).

These distinctions between the rat and human responses may help clarify the roles of the high and low pressure systems in maintenance of sodium and water homeostasis. It could be speculated that the differences in response between rat and human reflects a different predominant pressure receptor system. Our unchanged central venous pressures suggest that in the rat, vasopressin is suppressed by elevated arterial pressure, and that increased sympathetic outflow overcomes the
inhibitory effect of increased arterial pressure on renin secretion. The failure of central pressures to increase could be explained by the relatively small skin surface area exposed to the cold and available for vasoconstriction. (It could also indicate insufficiently cold temperatures).

Increases in central blood flow have been reported in humans, along with decreases in renin and increases in plasma ANF. These are consistent with the natriuresis which accompanies acute cold exposure. Further, these results suggest that central volume expansion may be a more powerful suppressor of renin than elevated arterial pressure, since it appears to overcome the increase in sympathetic nervous activity which follows cold exposure.

CONCLUSIONS

The present results are equivocal with respect to a role for the Gauer/Henry reflex in the diuresis of the acutely cold-exposed rat. They do, however, strongly support a role for vasopressin. Although a Gauer-Henry mediated contribution to the suppression of vasopressin cannot be ruled out, the major mechanism for vasopressin suppression is most likely increased arterial pressure, and the biphasic nature of the response is due probably to accommodation of arterial baroreceptors and reduction of their nervous outflow over time.

No conclusions can be drawn from the responses of plasma ANF and sodium excretion as no consistent effect of cold could be demonstrated. Increases in renin suggest that the stimulatory effects of increased sympathetic activity over-ride the
inhibitory influence of increased arterial pressure on plasma renin activity under these conditions.


Sutherland, A. (1764). Of the dropsy. In: An attempt to ascertain and extend the virtues of bath and bristol waters (2nd ed.). Frederich & Leake. Ch. 8, p. 213-218.


