ATRIAL NATRIURETIC FACTOR IN TWO CANINE MODELS OF ASCITES: CARDIAC RELEASE AND HETEROGENEITY OF RENAL NATRIURETIC RESPONSE

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ANF IN ASCITES: CARDIAC RELEASE AND HETEROGENEOUS RENAL RESPONSE

ABSTRACT

In response to i.v. ANF at 175 ng/kg/min, normal dogs increase sodium excretion (ΔUNaV=150 uEq/min) independent of changes in GFR and RPF. In contrast, when ANF was infused into chronic caval dogs (TIVC) or cirrhotic dogs (Cir) retaining sodium in the presence of ascites, they divided 50:50 into those who had a marked natriuretic response (responders, R) and chose who had no natriuretic response (non-responders. NR). Of 46 TIVC dogs, 22 R had ΔUNaV of 185 ± 35 uEq/min and 24 NR had ΔUNaV = 2 ± 1 uEq/min. In 19 Cir dogs, 9 R had ΔUNaV=60 ± 10 uEq/min and 10 NR had ΔUNaV=1.3 ± .6 uEq/min. R and NR could not be diffentiated in terms of atrial content of ANF, plasma iANF, ANF T1/2, plasma levels of renin and aldosterone, systemic hemodynamics, plasma volume, or papillary plasma flow. All dogs generated plasma and urinary cGMP equally. Renal denervation or vasodilatation did not increase sodium excretion in response to ANF in NR. When NR dogs returned to sodium balance in the presence of ascites, the natriuretic response was restored (ΔUNaV=90-340 uEq/min) and was not different from R dogs in this phase. Cir dogs studied sequentially in the pre-ascitic phase responded normally to ANF infusion when they were in sodium balance but split 50:50 into R and NR at week 4 during a period of sodium retention, plasma volume expansion, elevated plasma iANF and normal renin and aldosterone. We conclude that the blunting of UNaV in response to ANF is a characteristic of the sodium-retaining kidney, is reversible when sodium balance is restored and occurs at a tubular level, most likely in the medulla.

RESUMÉ

En réponse a une dose de ANF de 175 ng/kg/min, des chiens normaux ont augmentes leur sécretion de sodium (ΔUNaV=150 uEq/min) independemment des changements en GFR et RPF. Par contraste, une infusion de ANF dans des chiens traites chroniquement par obstruction de veine cavale (TIVC) ou avec cirrhose (Cir), et qui retiennent sodium en presence d'ascites, sont devises 50:50 entre ceux qui avaient une réponse natriuretique marquee (Repondant, R) et ceux qui n'avaient pas de reponse natriuretique (Non-repondant, NR). De 46 chiens avecTIVC, 22 R avaient une ΔUNaV de 185+35 uEq/min et 24 NR avaient une ΔUNaV de 2±1uEq/min. De 19 chiens avec Cir, 9 R avaient une ΔUNaV de 60±10uEq/min et 10 NR avaient une ΔUNaV de 1.3±0.6uEq/min. R et NR ne pouvaient pas être differenciés en termes de quantitee de ANF dans l'atrium, de iANF dans le plasma, de hemodinamique systemique, de volume de produits des quantitées égale de cGMP dans le plasma et dans l'urine. Denervation due rein ou vasodilatation n'ont pas augmentes la secretion de sodium en presence de ANF dans les chiens NR. Après le retour des chiens NR en balance de sodium par formation d'ascites, la reponse natriuretique est revenue (ΔUNaV=90-340 uEq/min) et n'etait pas differente des chiens R dans cette phase. L'etude sequentiella des chiens Cir dans la phase pre-ascitic a revelee une réponse normale a l'infusion de ANF quand ils etaient en balance de sodium mais a revelee une response partagee, 50:50, entre R et NR a la 4 eme semaine de retention de sodium, d'expansion de volume de plasma, d'elevation de plasma iANF et de niveaux normal de renin et d'aldosterone. Nous concluons que l'attenuation de UNaV en reponse d'infusion de ANF est characteristique du rein en phase de retention de sodium, est reversible quand la balance de sodium est retournee et est localisee au niveau tubulaire probablement dans le medulla.

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PREFACE: CLAIMS TO ORIGINALITY

The following results are original observations which are presented in several papers for publication which are presently under review.

1) Normal dogs have higher left atrial than right atrial iANF content. Caval and cirrhotic dogs with sodium retention and ascites have reduced left atrial iANF content and thus homogeneous concentrations in the right and left atria.

2) Caval and cirrhotic dogs with sodium retention and ascites divide 50:50 into natriuretic responders and non-responders in response to ANF infusion. This has recently been confirmed in cirrhotic patients with sodium retention and ascites.

3) Caval dogs, non-responding in the phase of sodium retention and ascites regain normal natriuretic response when sodium balance (in the presence of ascites) is restored demonstrating that the phenomenon of heterogeneity is functional and reversible. Similarly,
the heterogeneous natriuretic response was demonstrated in the pre-ascitic cirrhotic dogs during a period of sodium retention, plasma volume expansion, and elevated plasma

iANF. The dogs regain normal natriuretic reponse when sodium balance is restored in this pre-ascitic phase but divide again in the presence of sodium retention and ascites.

4) Sequential measurements of plasma iANF demonstrated a biphasic pattern, being depressed during the first three weeks after surgery, rising to 2x normal during a period from 4-6 weeks, and fall again to low levels when ascites is present. Caval dogs have depressed plasma iANF by 24 hours post-caval constriction. These levels remain low throughout the phase of sodium retention and ascites.

5) Several parameters were examined in order to differentiate responding and non-responding dogs:

a) cGMP - both caval and cirrhotic responding and non-responding dogs generate plasma and urinary cGMP to the same degree as normal dogs.

b) Papillary plasma flow - ANF infusion did not change papillary plasma flow in normal dogs. Caval responders and non-responders had depressed papillary flow rates during ANF infusion to the same degree in each group. Cirrhotic dogs had higher than normal papillary plasma flow than control dogs in response to ANF infusion and, like the caval dogs, papillary flow was the same in the responding and non-responding dogs. Papillary plasma flow could not

be correlated to the natriuretic response to ANF.

c) Renal denervation augmented the natriuretic response to ANF in caval and cirrhotic responding dogs whereas the complete attenuation of natriuresis was maintained in the non-responding dogs. Renal vasodilatation did not reverse the responsiveness in caval or cirrhotic non-responders.

6) Acute selective intrahepatic hypertension did not alter the natriuretic responsiveness to infused ANF.

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CHAPTER 1: INTRODUCTION

The maintenance of normal extracellular fluid volume, particularly that component within the vascular compartment, is essential in order to maintain cardiac output within narrowly defined physiological limits and so to ensure adequate perfusion of the vital organs. Extracellular fluid volume is regulated by three main mechanisms: 1) The partitioning of extracellular fluid volume between the vascular and extravascular space. 2) Adjustments in peripheral vascular resistance. 3) Control of renal excretion of salt and water (Seely, Levy, 1981). These mechanisms are most clearly illustrated by the following examples. Upon assuming an upright posture (orthostasis), there is a shift of a large volume of blood into the dependent parts of the circulation due to a hydrostatic gradient which effectively reduces ventricular filling and stroke volume. Much of this volume is translocated from thoracic vessels. If uncompensated, cardiac output would fall markedly. Rapid adjustments are triggered by the autonomic nervous system which, with a very short latency (2-5 seconds) produces venoconstriction and an increase in peripheral vascular resistance to maintain a normal cardiac output (Kuchel et al., 1970). The second example, hemorrhage, similarly elicits compensatory reflex mechanisms. A short latency mechanism again involves activation of the sympathetic nervous system with actions similar to those described above (Gill,

Casper, 1972). In addition, a humoral mechanism involving the release of vasopressin from the neurohypophysis and activation of the renin-angiotensin system, enhances renal sodium and water retention in order to restore plasma volume (Goetz et al., 1970).

The interdependency of extracellular fluid volume and the control of sodium excretion is apparent from data on sodium intake. When daily salt intake increases, water is retained isotonically to maintain plasma osmolality and a state of positive sodium balance is achieved. Sodium excretion gradually increases over a 2-3 day period until a new steady state equilibrium is reached. Sodium balance is restored at the increased intake level (Seely and Levy, 1981). The phenomenon of deoxycorticosterone acetate (DOCA) "escape" represents similar changes in renal sodium handling. Following mineralocorticoid administration to normal subjects, sodium and water are retained for a period of 3-5 days. Then, despite continued mineralocorticoid administration, sodium excretion gradually returns to normal and sodium balance is restored at a higher level of extracellular fluid volume (Reineck, Stein, 1978).

The first part of this introduction will be a review of the various factors which monitor extracellular fluid volume status and the effector mechanisms which control sodium excretion.

1.1 AFFERENT LIMB OF VOLUME CONTROL

The afferent limb of extracellular volume control is comprised of a series of pressure/volume receptors which send information via afferent nerves to the central nervous system. The efferent limb of this neural loop consists of sympathetic preganglionic neurons located in the lateral horn of the spinal cord, which pass their axons into the periphery and synapse onto the sympathetic postganglionic neurons in the paravertebral chain which, in turn, send their adrenergic axons to cardiovascular and visceral (including kidney) targets. The efferent limb of volume control, therefore, is the integration of this information into a mechanism which can alter the renal handling of sodium to effect changes in extracellular fluid volume.

1.1.1 Volume Receptors

The concept of volume receptors located on the high-pressure side of the circulation has been discussed for many years and is the basis for the hypothesis that the kidney retains or excretes sodium in response to the degree of filling of some critical portion of the arterial tree (Epstein, 1956). The volume of blood capable of stimulating these receptors is termed the "effective arterial blood volume". Although no volume receptors have been identified as yet, there is considerable evidence that the hypothesis is well-founded. Acute opening and closing

of arterio-venous fistulas in Korean war veterans provided a technique to study the cardiovascular and renal response to abrupt changes in the circulation without a change in blood volume (Epstein, Post, McDowell, 1952). During acute closure of the fistula, the arterial tree empties more slowly and less completely than when the fistula is open. This results in a fall in right atrial and pulmonary vascular pressures, an increase in mean arterial pressure and a fall in cardiac output as heart rate and stroke volume decrease. These cardiovascular changes are accompanied by a brisk natriuresis. The opposite responses are recorded when the fistula is opened, allowing blood to empty quickly from the arteries into the veins. Mean arterial pressure falls as right atrial and great vein pressure increases, producing a rise in cardiac filling pressure as evidenced by a rise in cardiac output. Concomitant with these changes is a decrease in sodium excretion. It is important to note that during both manipulations renal blood flow and glomerular filtration rate remained constant despite large fluctuations in blood pressure. A different experimental approach yielded similar results. Arterial blood pressure was held constant in anaesthetized dogs at the level of an external reservoir of blood to which the dog's arterial system was connected. Ganglionic blockade with hexamethonium abolished neurogenic vasoconstrictor tone, thereby increasing compliance of the

vasculature, in particular the capacitance vessels (veins). Blood was infused from the reservoir without changing arterial pressure and produced an increase in sodium excretion which was not altered by previous renal denervation (Goodyer, Jaeger, 1955). These animals clearly responded to an increase in volume, since arterial pressure was held constant. It was concluded from these studies that the kidney was responding to the degree of filling of the arterial tree, not to changes in mean arterial pressure, right atrial pressure, cardiac output, renal perfusion or glomerular filtration rate.

The question of where the volume receptors (functional baroreceptors in the circulation) are located has been examined in great detail over the past three decades. The venous side of the circulation contains approximately 85% of the circulating blood volume and is a highly distensible system. The arterial side of the circulation contains only 15% of the blood volume, is characterized by very low distensibility and thus small changes in blood volume are reflected as changes in blood pressure (Gauer, Henry, 1976). Since regulation of extracellular fluid volume actually represents regulation of the plasma volume relative to the holding capacity of the circulation (Gauer, Henry, 1976), it follows that the venous bed is well suited to detecting changes in blood volume.

1.1.2 Cardiopulmonary Receptors

Two types of atrial receptors, originally described in the cat (Paintal, 1953), have been identified in dog atria: "A" receptors respond to changes in atrial pressure, discharging when the pressure wave develops in atrial systole. The "B" receptors discharge in response to atrial distension and are characterized by a late burst of discharge corresponding to the v wave of atrial filling (Coleridge et al., 1957). The receptors are found in both right and left atria, concentrated at the atrio-venous junctions and may serve as a sensory mechanism in the reflex regulation of blood volume by control of urine output. Experimental evidence to support this hypothesis is presented in this section.

Henry, Gauer and Reeves (1956) observed that elevation of left atrial pressure and pulmonary vascular pressures by inflation of a balloon in the left atrium elicited a diuresis in anaesthetized dogs. This observation, in addition to an increase in heart rate, was confirmed by others using the same technique (Ledsome, Linden, 1968; Karim et al., 1972). Schultz et al. (1982) demonstrated that this diuretic response resulted from the increase in left atrial pressure since selective elevation of pulmonary vein or artery pressure had no diuretic effect. Since right atrial pressure does not change during inflation of a balloon in the left atrium, it was concluded

that the diuresis associated with increased left atrial pressure was a result of stimulation of stretch receptors in the left atrium. Indeed, cardiac denervation abolished the diuretic response in anaesthetized dogs (Fater et al., 1982). More specific determination of the pathway mediating this reflex was obtained from studies demonstrating that the increase in urine flow can be prevented by cooling the cervical vagi (Kappagoda et al., 1974; Ledsome, Linden, 1968).

Given the presence of receptors in the right atrium responsive to changes in atrial and transmural pressure (Coleridge et al., 1957) and that a 15% increase or decrease in blood volume produces similar changes in right and left atrial pressures (Henry, Gauer, Sieker, 1956), several investigators examined the effect of right atrial stimulation on urine flow with conflicting results. Mills and Osbaldiston (1968) and Malvin, Jochim, Roberts (1971) found no effect of right atrial stretch on urine flow or sodium excretion in anaesthetized dogs. However, Kappagoda, Linden and Snow (1973), also using anaesthetized dogs, reported that distension of a balloon in the right atrial appendage and at a junction between the superior vena cava and right atrium elicited qualitatively similar responses to those produced by left atrial distension which could be abolished by vagal cooling. A comparison of the renal effects of distension of the left and the right atria

in conscious dogs demonstrated that only increased left atrial pressure produced a diuretic and natriuretic response (Schultz et al., 1982). Although the functional link between atrial pressure and sodium and water excretion is unsettled, the more important relationship is between blood volume and sodium excretion. The next section will describe other effects of atrial receptors on factors known to be involved in the control of sodium excretion.

Activation of cardiac afferents by the increase in left atrial pressure is purported to have effects on vasopressin release from the posterior pituitary, efferent renal sympathetic nerve activity, and renin release, all of which are implicated in the renal control of extracellular fluid volume. Several studies of the diuretic response to left atrial distension reported a fall in plasma vasopressin concomitant with a rise in left atrial pressure (Gauer, Henry, 1963; Johnson, Moore, Segar, 1969; Brennan et al., 1971) suggesting that the efferent limb of this reflex at least partially involves reflex inhibition of vasopressin release from the neurohypophysis in response to left atrial receptor activation. This is however, controversial. Dogs infused with high doses of vasopressin to keep the plasma levels elevated still had a diuretic response to left atrial distension (Ledsome, Linden, O'Connor, 1961). Kappagoda et al (1974) reported that the diuretic response associated with left atrial distension was not consistently

accompanied by a fall in plasma vasopressin. Similarly, reduction of mean atrial transmural pressure by atrial tamponade in conscious dogs reduced renal sodium excretion and urine flow but had no effect on plasma vasopressin levels (Goetz et al., 1970). Even more convincing is that dogs in which the pituitary gland has been destroyed have the same increase in urine flow as dogs with intact pituitary glands (Kappagoda et al., 1975).

There are several reports that stimulation of the atrial receptors lead to tonic inhibition of renal sympathetic nerve activity (Weaver, 1977; Linden, Mary, Weatherill, 1980) which can be abolished by vagotomy (Karim et al., 1972). Comparison of the effects of left atrial distension on innervated and denervated kidney demonstrated that the natriuresis occurred only in the innervated kidney while the increase in urine flow was maintained in both kidneys (Kappagoda et al., 1979). In addition, there is data to suggest that during volume expansion, activation of the cardiac afferents from atrial receptors result in decreased spontaneous renal nerve discharge (Weaver, 1977). Indeed, it has been demonstrated that the response of the sympathetic nervous system to activation of cardiopulmonary vagal afferents is nonuniform and can be directionally opposite, increasing sympathetic activity to the heart while decreasing activity to the kidney (Shepherd, 1973).

Atrial receptors also appear to contribute to the reflex control of renin secretion. Balloon inflation in the left atrium of dogs reduced plasma renin activity (Brennan et al., 1971; Zehr et al., 1976), a response which was abolished by vagotomy (Zehr et al., 1976). There is evidence that the cardiopulmonary receptors exert a tonic inhibition on renin release since vagal cold block produced a 5 fold increase in renin secretion in dogs with aortic nerve section and constant perfusion of the carotid sinus (Mancia, Romero, Shepherd, 1973).

Of interest is that cardiac denervation attenuated the diuretic and natriuretic response to acute volume loading with saline to 3% of body weight (Gilmore, Daggett, 1966) suggesting that afferent activity from the cardiopulmonary receptors does contribute to the renal tubular response to volume expansion. However, there is data which argues against the importance of this reflex. Infusion of 6% dextran, representing a 16% expansion of blood volume, produced the same diuretic and natriuretic response in cardiac-denervated and cardiac innervated conscious dogs (Fater et al., 1982). Moreover, renal denervation, in addition to large doses of vasopressin, was unable to abolish the diuretic response to left atrial distension (Ledsome, Linden, O'Connor, 1961). Thus, surgical removal of the afferent signal from the atrial receptors or the efferent signal to the kidney does not inhibit the renal

response to an increase in left atrial pressure. Furthermore, cross-perfusion experiments demonstrated that the recipient kidney, isolated from the neural influence of the donor heart had a diuretic response to balloon inflation in the left atrium (Carswell, Hainsworth, Ledsome, 1970). This experiment provides clear evidence that the renal response is at least in part humorally mediated.

A technique employed to demonstrate the influence of intrathoracic blood volume on renal function is thermoneutral water immersion to the neck. This manouever produces a brisk natriuresis and diuresis (Graveline, Jackson, 1962; Epstein, Duncan, Fishman, 1975) which is associated with increased central blood volume and central venous pressure and suppression the renin-angiotensin system. The rise in cardiac output is similar to saline expansion to 3% of body weight (Epstein, 1978). This technique is advantageous in the study of volume control since it provides a volume stimulus without a rise in total blood volume. The mechanism of the diuresis and natriuresis has been examined in relation to several variables. Administration of vasopressin during the initial four hours of water immersion in human subjects abolished the diuresis but did not alter the natriuresis (Epstein, Ulano, 1976). The data suggests that inhibition of vasopressin release from the neurohypophysis may be the

main mechanism mediating the diuresis of immersion and provides evidence that the natriuresis is accomplished through a different pathway.

1.1.3 Hepatic Baroreceptors

A second type of baroreceptor which may participate in the control of sodium excretion and therefore extracellular fluid volume are low-pressure mechanoreceptors located on the portal venous side of the hepatic circulation (Niijima, 1976). The existence of these receptors were proposed by Ohm and Haberich (1969 - as quoted by Sawchenko, Friedman, 1979) to explain a rapid decrease in urine flow rate following brief or prolonged increases in portal venous pressure in the rat. A similar observation was made in dogs where elevating portal venous pressure of up to 15 cm H2C above normal produced a 2 to 3 fold increase in urine flow within 2-5 seconds (Liang, 1971). Interestingly, this diuretic response was inhibited by increases in portal pressure greater than 15 cm H_2O. These responses were suggested to be neural in nature since it had already been shown in dogs that increased hepatic and portal pressure, due to thoracic inferior vena caval constriction, produced action potentials in the hepatic nerves (Andrews, Palmer, 1967). Direct stimulation of hepatic afferents elevated renal and cardiopulmonary efferent nerve activity without changing heart rate (Kostreva, Castaner, Kampine, 1980) and

could not be altered by carotid sinus denervation, bilateral vagotomy or phrenectomy (Kostreva, Castaner, Kampine, 1980). Thus the activity of this loop is sympathetic, both afferent and efferent nerves travelling in spinal nerves. This differs from atrial receptors where the afferents travel in the vagus nerve (Kappagoda et al., 1974). A recent study in anaesthetized dogs demonstrated that inflation of a balloon in the portal vein to raise portal pressure produced an increase in renal nerve activity, a response which could be abolished by hepatic denervation (Koyamo et al., 1988). Hepatic, rather than portal congestion, produces a more consistent renal effect - reduction in urine flow, sodium excretion, CPAH and cortical blood flow (Lautt, 1983). The circuitry exists whereby increases in hepatic and portal venous pressures could send afferent sympathetic activity to the central nervous system which would subsequently send efferent sympathetic nerves to the heart and the kidney. However, it is presently unclear whether this system is functional and if it plays a role in normal sodium physiology.

1.1.4 Arterial Baroreceptors

A third type of pressure receptor which may send afferent sensory information to the kidney is the high pressure arterial baroreceptor. These slowly adapting mechanoreceptors are located in the aortic arch and carotid

sinus and function mainly in the reflex regulation of blood pressure (Kirchheim, 1976). Denervation of the carotid sinus results in an immediate and marked increase in systemic blood pressure which is attributed to removal of tonic inhibition from this baroreceptor (Shepherd, 1973). Unilateral stimulation of carotid receptors by carotid traction in unanesthetized rats produces a large increase in sodium excretion which can be abolished by denervation of the carotid sinus (Keeler, 1974). Although carotid sinus stimulation produces a reduction in renal nerve activity (Wilson et al., 1971), a manouever known to produce a natriuresis (DiBona, 1982), the natriuresis associated with carotid sinus stimulation is not a neural reflex response since the natriuresis is maintained in a denervated kidney (Keeler, 1974). The release of renin, however, by a decrease in carotid sinus pressure when renal arterial pressure is constant is neurally-mediated, since renal denervation abolishes this response (Jarecki, Thoren, Donald, 1978). These data suggest that the carotid sinus can influence renal function both by a neural pathway as well as a humoral pathway. Of importance in assessing the role of these baroreceptors in the renal regulation of extracellular fluid volume is their response to changes in blood volume. Kumada and Sagawa (1970) demonstrated that changes in blood volume of 10-20% produce a 21-31% increase in impulse activity in rabbit aortic nerves despite only a

6% increase in arterial pressure. These investigators suggested that the arterial baroreceptors can act as volume receptors as well as pressure receptors.

An important site of afferent control is the kidney itself. There are several sensing mechanisms within the kidney including the juxtaglomerular apparatus, tubuloglomerular feedback initiated at the macula densa and response to changes in interstitial pressure. These will be discussed in the next section.

1.2 EFFERENT LIMB OF VOLUME CONTROL

1.2.1 Glomerular Filtration Rate (GFR) and Renal Perfusion

Glomerular filtration is governed by Starling forces acting at the level of the glomerulus. Net filtration pressure, the result of hydrostatic pressure in the glomerular capillary opposed by the sum of the hydrostatic pressure in Bowman's space and plasma oncotic pressure, is maximal at the afferent arteriolar side of the glomerular capillary. The plasma oncotic pressure rises along the length of the capillary as a result of the formation of a protein-free ultrafiltrate and thus approaches 0 by the end of the capillary (Brenner, Troy, Daugharty, 1971).

In normal man, the filtered load of sodium is approximately 14 mEq per minute while the urinary sodium excretion is 0.14 mEq per minute. That is, 99% of the

filtered load is reabsorbed with only 1% being excreted into the final urine. Thus a small increase in GFR would produce large changes in sodium excretion if there was no coupling between filtered and reabsorbed sodium (Reineck, Stein, 1978). In fact, the extent to which changes in GFR can influence sodium excretion and thereby extracellular fluid volume is limited by two mechanisms operating to keep GFR constant. The first is glomerulotubular balance where changes in GFR are accompanied by proportional changes in proximal tubular sodium reabsorption resulting in the maintenance of a constant fractional reabsorption of sodium in this segment of the nephron (Lindheimer, Lalone, Levinsky, 1967). The modulation of the net rate of sodium reabsorption described by glomerulotubular balance appears to be a result of changes in oncotic pressure in the peritubular capillaries (Brenner, Troy, Daugharty, 1973). Thus there is no linear relationship between GFR and increasing sodium excretion. The second mechanism is tubuloglomerular feedback whereby events in the distal tubule are linked to glomerular function. The distal delivery of sodium chloride is sensed by the macula densa cells of the juxtaglomerular apparatus which sends information back to the glomerulus to modify GFR (Wright, Briggs, 1979). Although the mechanism of this feedback has not been clearly elucidated, it has been proposed that some aspect of chloride transport at the macula densa

initiates the feedback (Wright, Briggs, 1979) and that local changes in angiotensin II may at least partially mediate changes in afferent and efferent arteriolar resistances which will alter GFR (Burg, 1981). As mentioned previously, manipulation of an arteriovenous fistula produces wide fluctuations in mean arterial pressure but does not alter GFR or renal blood flow (Epstein, Post, McDowell, 1952). This phenomenon of autoregulation does not apply to renal perfusion pressure which rises linearly within the peritubular capillaries in direct relation to mean arterial pressure and, furthermore, is directly correlated to increased urinary sodium excretion. Fluid exchange in the peritubular capillaries is governed by the balance between peritubular hydrostatic pressure and colloid osmotic (oncotic) pressure. Thus raising hydrostatic pressure favors sodium excretion while increased protein concentration favors reabsorption. These changes occur to a large extent in the proximal tubule (Seely, Levy, 1981).

1.2.2 Sympathetic Nervous Activity

Many studies have demonstrated that anatomic or pharmacologic unilateral renal denervation leads to an increase in sodium excretion (Bricker et al., 1958; Blake, Jurf, 1968; Bonjour, Churchill, Malvin, 1969) which has been attributed to small increases in GFR rather than to

any direct neural effect (Gottschalk, 1979). However, more detailed investigation into the role of the renal efferent sympathetic nerves in the tubular handling of sodium, has indicated that the phenomenon of denervation natriuresis may be a result of removal of the neural influence on sodium reabsorption.

As previously described, stimulation of the low pressure baroreceptors in the atria lead to a tonic inhibition of renal nerve activity and renin release (Karim et al., 1972; Weaver, 1977). The increase in sodium and water excretion following acute renal denervation is related to a fall in fractional and absolute sodium reabsorption in the proximal tubule (Bello-Reuss et al., 1975). Alternatively, renal nerve stimulation is associated with a 25% fall in urine flow rate and sodium excretion (Blendis et al., 1972; Gill, Casper, 1972; Bello-Reuss, Trevino, Gottschalk, 1976; DiBona, 1982). Under these conditions, GFR and RPF are unchanged. Micropuncture analysis demonstrated that during stimulation, sodium and water reabsorption in the proximal tubule increases without a change in single-nephron GFR or the filtered load of sodium (Bello-Reuss, Trevino, Gottschalk, 1976). Although it has been shown that a normal response to volume expansion requires an intact renal nervous system, it is not clear whether this is a result of its direct effects on tubular sodium handling or through its interaction with the renin-angiotensin system

(Gottschalk, 1979).

The sympathetic nervous system in the kidney richly supplies the cortical vasculature exclusively with noradrenergic innervation (Barajas, 1978). The hypothesis just presented, that renal nerves may in some way modulate sodium reabsorption, is strongly supported by direct anatomical evidence of innervation to the various parts of the renal tubule. A recent study employing light microscopy autoradiography demonstrated that the highest degree of innervation occurs in the thick ascending limb of the loop of Henle followed by the distal tubule and then the proximal tubule (Barajas, Powers, 1988). In addition, direct neural contact was shown with the juxtaglomerular cells of the macula densa strongly suggesting a direct neural influence on the release of renin, independent of any effect of atrial receptors. Electrical stimulation of renal sympathetic nerves at intensities that do not alter renal blood flow or perfusion pressure results in an increase in renin secretion (LaGrange, Sloop, Schmid, 1973). The functional significance of the innervation of the specific segments has not yet been completely identified although there is sufficient evidence to support an important role of the renal nerves in the direct control of sodium handling in the kidney.

1.2.3 The Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system provides an important pathway for the control of both blood pressure and extracellular fluid volume through the control of sodium excretion. The enzyme renin is synthesized in and stored by the juxtaglomerular cells of the afferent arteriole in intimate contact with the distal tubule and macula densa, thus forming the juxtaglomerular apparatus (Edelman, Hartroft, 1961; Davis, Freeman, 1976). Release from the macula densa can be stimulated by an increase in sodium chloride delivery to the distal tubule, activation of the afferent arteriolar baroreceptor (Levens, Peach, Carey, 1981), or an increase in renal nerve activity (DiBona, 1982). Renin catalyzes the conversion of angiotensinogen, an alpha-globulin synthesized by the liver, to the largely inactive decapeptide, angiotensin I. The two C-terminal amino acids, histidyl and leucine, are removed by a specific converting enzyme, dipeptidyl carboxypeptidase, to form angiotensin II. Although this conversion takes place mainly in the lungs, evidence suggests that there is also conversion within the kidney (DiSalvo et al., 1971; Bailie, Rector, Seldin, 1971) in particular at the juxtaglomerular apparatus where there is a high concentration of converting enzyme (Granger, Dahlheim, Thurau, 1972). Angiotensin II is a potent vasoconstrictor in the peripheral circulation and, as such,

maintains blood pressure under conditions of decreased blood volume. It also stimulates the synthesis of renal prostaglandins which stimulate further release of renin from the macula densa. In this positive feedback system, the enhanced release of renin will produce enhanced levels of angictensin II. Interestingly, in the systemic circulation, the effect of angiotensin II is part of a negative feedback system since prostaglandins antagonize its vasoconstrictive actions on the vasculature (Lee, 1980).

One of the most important effects of angiotensin II is the stimulation of aldosterone release from the zona glomerulosa of the adrenal cortex. Aldosterone is a potent activator of the Na/K ATPase in the distal tubule increasing sodium reabsorption and potassium secretion and, as such, was heralded as "the" controller of sodium handling in the control of extracellular fluid volume. Aldosterone is clearly important in the control of sodium balance as volume contraction stimulates, and volume expansion depresses, its secretion (Reineck, Stein, 1978). Similarly, a low sodium diet is associated with high circulating aldosterone levels while high sodium intake is associated with low levels (Sealey, Laragh, 1974). However, this apparent cause and effect relationship breaks down following prolonged elevation of aldosterone. As previously described, animals given high levels of DOCA

retained sodium for a period of a few days after which time sodium excretion returned to normal despite the continued elevation of aldosterone (Knox, 1980). Further evidence for a less important role of aldosterone in the control of sodium is that sodium balance can be maintained by patients with adrenal insufficiency on a fixed dose of mineralocorticoid despite their inability to regulate the endogenous levels of aldosterone (Rosenbaum, Papper, Aschley, 1955).

1.2.4 Intrarenal Prostaglandins

The potential role of the renal prostaglandin system in the control of sodium and water excretion and renin release has received a great deal of attention in the past several years. Intrarenal prostaglandins are synthesized primarily from the polyunsaturated fatty acid, arachidonic acid (Isakson et al., 1977), by synthetases found mainly in the medullary collecting tubule and, to a lesser extent, in the cortical collecting tubule and the thin limb of the loop of Henle (Bonvalet, Pradelles, Farman, 1987). A natriuretic role for the renal prostaglandins is suggested by the increase in sodium excretion following infusions of prostaglandin A, I and E as well as sodium retention following prostaglandin inhibition with indomethacin (Donker et al., 1976; Bolger et al., 1976). It has been established that prostaglandins stimulate renin release by

a direct action on the juxtaglomerular cells of the macula densa in a mechanism which modulates the rate of renin secretion as determined by other factors (Freeman, Davis, Villarreal, 1984). The prostaglandins may also contribute to water homeostasis by modulating the action of vasopressin (Berl et al., 1977; Fejes-Toth, Magyar, Walter, 1977). Although the complex interactions between prostaglandins, the renin-angiotensin system, and vasopressin in the regulation of sodium excretion are not completely elucidated, the prostaglandins appear to play a determining role in the natriuretic response to extracellular fluid volume expansion (Epstein et al., 1979). They will be discussed later in terms of their role in edema states.

A landmark study by De Wardener and colleagues (1961) demonstrated that saline infusion in dogs with decreased GFR and filtered sodium load and extremely high aldosterone levels was still capable of producing a natriuretic response. This study clearly de-emphasized the role of both GFR and aldosterone in the control of extracellular fluid volume and raised the possibility that a natriuretic factor was intimately involved in normal volume control. The newly described hormone, atrial natriuretic factor is presently the focus of a great deal of investigation in terms of the control of extracellular fluid volume and will be discussed in great detail in later sections.

In summary, the control of extracellular fluid volume is accomplished by adjustments in sodium handling by the kidney through a complex series of pathways including intrarenal adjustments of pressure and flow rates, hormones acting at various sites of the renal tubule and nervous input to the renal vasculature and specific tubule sites. Although the mechanisms which operate to apprise the kidney of the extracellular fluid volume are not yet understood, it is clear that there is a neural component, acting either through the cardiopulmonary receptors or high pressure baroreceptors in the aortic arch and carotid sinus, a humoral component, possibly vasopressin, the renin-angiotensin system, or a natriuretic hormone, as well as a volume component, that part of the arterial circulation which is capable of stimulating "volume" receptors.

1.3 GENERALIZED EDEMA

Edema formation, characterized by continuous accumulation of salt and water within the interstitial space, reflects two major problems. Firstly, there is a persistent low-volume stimulus to the kidney signalling sodium and water retention. Secondly, there is no feedback by which the retained fluid can terminate the low-volume stimulus. Thus, the kidney behaves as if it is underperfused even when plasma volume is expanded (Gauer, Henry, 1979). This

may be attributed to disturbances in the Starling forces governing the distribution of fluid in the extracellular compartment (Seldin, 1975).

Transcapillary movements of fluid depend primarily on the relationship between the Starling forces in the capillary and interstitial space. Hydrostatic and oncotic pressure in the capillary are opposed by interstitial hydrostatic pressure and interstitial oncotic pressure. Under normal conditions, fluid filtered from the capillary into the interstitium and not reabsorbed enters the lymphatic vessels and is returned to the circulation via the thoracic duct thus preventing the accumulation of fluid in the interstitial space. Three "safety factors" normally operate to prevent the accumulation of large amounts of interstitial fluid. 1) Lymphatic vessels can increase their flow rates considerably such that the bulk of fluid is returned to the circulation. 2) As lymph flow increases, the normal interstitial fluid protein concentration falls. Thus the oncotic gradient is such that fluid is reabsorbed back into the capillary. 3) The hydrostatic pressure gradient between capillary and interstitial space is minimized by the low compliance of the interstitial space. Increased interstitial fluid increases the tissue hydrostatic pressure which decreases the net driving pressure on fluid moving out of the capillary (Coggins, 1978). However, an imbalance in the

Starling forces, such as produced by increased capillary hydrostatic pressure when plasma volume is expanded, a decrease in plasma protein as in nephrotic syndrome or inadequate lymph removal will produce enough interstitial fluid to raise the interstitial pressure several millimeters of mercury higher than normal. At a critical pressure (the inflection point), the compliance of the interstitial space increases allowing large amounts of interstitial fluid to accumulate without a further rise in hydrostatic pressure (Guyton, 1964). The extent to which fluid can accumulate in the interstitial space should be limited by the size of the plasma volume which would quickly become depleted. However, as the accumulated fluid expands the plasma volume, the low volume stimulus to the kidney is not terminated, resulting in progressive sodium retention and extracellular volume expansion, and accumulation of fluid in the interstitial space. Fluid collecting in the abdomen is referred to as ascites.

The traditional explanation for edema formation centers on a primary disturbance in the Starling forces favoring excess capillary filtration leading to a decrease in plasma volume. The kidney will respond by retaining extra sodium and water to replenish the vascular volume. There is, however, an alternate theory to explain edema formation. It has been suggested that the kidney receives a signal independent of plasma volume to retain sodium and water and

thereby expands the plasma volume. An increase in plasma volume raises capillary hydrostatic pressure and lowers the capillary protein concentration both of which favor the outward movement of fluid from the capillary into the interstitial space. According to this theory, edema is the end result of an appropriate response to an overfilled circulation in the face of primary and progressive sodium retention.

Two major edematous disorders which have been studied extensively in clinical settings as well as in animal models are congestive heart failure and cirrhosis of the liver. Although the etiologies differ, these two disease states exhibit classic characteristics of edema - avid sodium retention, plasma volume expansion, and activation of the renin-angiotensin-aldosterone system.

1.3.1 Congestive Heart Failure

Two theories have been advanced to explain the sodium retention and edema formation of congestive heart failure. The backward failure theory, proposed in the 1800's (Hope, 1832 and Starling, 1896 as quoted by Warren, Stead, 1944) postulated that progressive heart failure, accompanied by increased right atrial and peripheral venous pressure, alters Starling forces to favor net transudation of fluid out of the capillary. As a result, the kidney responds by reabsorbing sodium and water to replenish the now

underfilled circulation. Alternatively, the forward failure theory, proposed in the early 1940's (Warren, Stead, 1944), does not depend on a primary perturbation in the Starling forces. The failing heart is associated with a fall in cardiac output resulting in decreased organ perfusion. The kidney responds to a fall in renal blood flow by retaining sodium and water in an attempt to elevate cardiac output and, in doing so, expands the plasma volume. According to this model, progressive sodium retention elevates venous pressure and produces edema. A matter complicating the distinction between these two interpretations of edema formation in heart failure is the subdivision of congestive heart failure into those conditions exhibiting elevated cardiac output such as anaemia, hyperthyroidism and beriberi and those exhibiting decreased cardiac output including coronary artery disease, hypertension and primary myocardopathies (Gill, 1970). However, both high- and low-output heart failure patients exhibit avid sodium retention and ascites suggesting a common response to the same or perhaps differing afferent signals.

A canine model used extensively to study low-output heart failure is the dog with thoracic inferior vena caval constriction (the caval dog). This is not a model of congestive heart failure as such but rather simulates the hemodynamic and renal consequences of impaired cardiac

performance. The dogs are characterized by increased venous pressure distal to the constriction, increased circulating renin and aldosterone, progressive sodium and water retention followed by accumulation of ascites in the peritoneal cavity 7-10 days post ligation (Davis, Howell, Southworth, 1953). Cardiac output and mean arterial pressure, although decreased initially, rise back to normal in the caval dog (Gill, 1970). Ganglionic blockade with hexamethonium deprives the circulation of the stabilizing effects of the adrenergic nervous system as evidenced by a fall in mean arterial blood pressure. Thus, the normal response to inadequate arterial filling, an increase in baroreceptor firing, produces a decrease in myocardial contractility and a decrease in peripheral arterial resistance to restore normal cardiac output and arterial blood pressure in these dogs.

Intravenous infusion of angiotensin II at a dose which produces a significant increase in blood pressure in normal dogs, has an attenuated effect in caval dogs (Davis et al., 1962; Johnson and Davis, 1973). To elucidate the mechanism of this decreased pressor response, both normal and caval dogs were infused with an angiotensin II-antagonist, saralasin. Blood pressure was unaffected in normal dogs but fell markedly in caval dogs suggesting that the maintenance of mean arterial pressure in the caval dogs is angiotensin- as well as adrenergic-dependent (Davis and

Johnson, 1973). Additional evidence was obtained when angiotensin converting enzyme inhibitors (CEI) became commercially available. Low dose infusion of CEI in caval dogs to prevent a rise in plasma angiotensin II prevented the restoration of blood pressure (Watkins et al., 1976). Although the mechanism of this apparent dependency of blood pressure on angiotensin II has not yet been completely elucidated, there are several possibilities. Caval dogs, known to have elevated plasma renin levels, have high circulating angiotensin II which may saturate the receptor sites on arteriolar smooth muscle thereby reducing the effectiveness of further increasing plasma angiotensin II levels. Blockade of endogenous angiotensin II would produce massive unloading of angiotensin II vascular smooth muscle receptors resulting in peripheral vasodilatation and a fall in mean arterial pressure. Alternatively, the angiotensin II receptor density or affinity in the vascular smooth muscle may be lowered thereby reducing the response to infused angiotensin II.

In search for the factor mediating the sodium retention and ascites of the caval dog, the effect of GFR was studied extensively. As described previously, an elevation in GFR can markedly enhance sodium excretion through an increase in distal delivery of sodium. It was thus proposed that the antinatriuresis of caval constriction may be a result of depressed GFR. However, it has been shown that GFR

remains relatively constant and if any changes occur, they cannot be correlated with the decrease in sodium excretion (Porush et al., 1967; Kaloyanides et al., 1969; Levinsky, Lalone, 1965). It is thus generally agreed that the sodium retention of caval dogs cannot be explained by changes in glomerular filtration rate.

Another focus has been on the effect of changes in total renal blood flow as well as its redistribution within the kidney in the development of ascites. A manoeuvre often used to raise renal blood flow is renal vasodilatation by intrarenal infusion of acetylcholine. In normal dogs, it produces a significant elevation in renal blood flow and sodium excretion without a concomitant increase in glomerular filtration (Friedler et al., 1967). The mechanism of this vasodilatation includes reductions in both afferent and efferent arteriolar resistances and ultrafiltration coefficient, with little or no change in glomerular hydrostatic pressure or the glomerular transcapillary hydrostatic pressure difference (Baylis and Brenner, 1978). While producing a normal increase in renal blood flow, intrarenal acetylcholine infusion in the caval dog raises sodium excretion only slightly (Friedler et al., 1967) indicating that enhanced renal vascular resistance does not contribute to the sodium retention seen in these dogs. Interestingly, however, intravenous infusion of angiotensin II at a dose sufficient to raise

blood pressure and therefore perfusion pressure will produce a brisk, potent natriuresis in the vasodilated kidney while having no effect on the normotensive kidney (Friedler et al., 1967). This is controversial since Porush et al. (1967) demonstrated a natriuretic response to either intravenous or intrarenal angiotensin II administration in caval dogs. The natriuresis is unlikely to be due to a specific action of angiotensin II in the kidney but rather to the increase in perfusion pressure since a similar natriuresis was recorded in caval dogs with acetylcholine-induced renal vasodilatation and noradrenaline induced elevated perfusion pressure (Levy, 1972). These results suggest that the combined effect of reducing renal vascular resistance and elevating perfusion pressure will overcome the sodium retaining lesion, although does not imply what the lesion actually is or that alterations in renal blood flow are associated with it. Rather, in fact, the results further underline the likelihood that the direct cause of the sodium retention in caval dogs is an alteration in the tubular handling of sodium.

Dogs with acute caval constriction cannot respond to saline expansion of the extracellular fluid (Dirks et al., 1967). Normal dogs respond to the same manoeuvre with an immediate and brisk natriuresis (Levinsky and Lalone, 1965) through an inhibition of proximal tubular fractional

reabsorption of sodium (Dirks et al., 1965). It was postulated, therefore, that the sodium retaining lesion in caval dogs was either maintenance or enhancement of fractional reabsorption of sodium in the proximal tubule. Micropuncture of proximal and distal segments of normal and chronic caval dog nephrons revealed that fractional reabsorption of sodium within the proximal tubule as well as distal tubule fluid delivery were similar in both groups (Levy, 1972) providing evidence against an important role of the proximal tubule in the sodium retention of caval dogs and, in fact, strongly suggested that the loop of Henle and the early distal tubule were the major sites involved. Further evidence was derived from measurement of distal Na/K exchange ratio ($U_K/U_{Na}+U_K$) which was elevated in caval as compared to control dogs (Kaloyanides et al., 1969) indicating enhanced sodium reabsorption in this segment of the nephron.

If indeed the loop of Henle and distal tubule play an important role in the sodium retention of edema, then the role of aldosterone as a primary mediator of this retention must be questioned. As expected, plasma aldosterone levels are markedly elevated in caval dogs (Davis et al., 1953; Watkins et al., 1976) reflecting both increased synthesis and release from the adrenal cortex and decreased hepatic degradation. The importance of these high levels was investigated in adrenalectomized dogs maintained on DOCA.

These caval dogs required large doses of corticosterone to maintain the sodium retention and ascites indicating a major role of this hormone in the pathogenesis of edema in this model. Of interest, however, is that normal adrenalectomized dogs given similar doses of DOCA never develop ascites and, in fact, "escape" from the sodium retaining effects of this hormone within a few days (Davis et al., 1953). Therefore, while aldosterone clearly is important in enhancing sodium reabsorption in caval dogs, it is not the only mechanism acting on the tubule to precipitate such profound and continuous sodium retention.

Another mechanism which has been considered as a possible afferent stimulus for sodium retention in the caval dogs is hepatic congestion. Acute caval constriction below the hepatic veins but above the renal veins does not produce an antinatriuresis (Schrier, Humphreys, 1971). Whether selective hepatic congestion can alter tubular sodium handling directly was investigated in dogs with hepatic vein hypertension induced by acute partial balloon occlusion of the TIVC. The investigators showed that hepatic congestion per se does not alter renal function significantly (Priebe, 1980). Elevating intrahepatic pressure selectively by intraportal histamine infusion suggested an alternate conclusion. In these dogs, a rise in intrahepatic pressure was associated with a significant antinatriuresis (Levy, 1974). It seems likely that such a

mechanism could be acting since, as previously described, a reflex loop between the liver, cardiopulmonary region and the kidneys is activated by increments in hepatic pressure (Niijima, 1977). The efferent modulation of this reflex loop is presently unknown but may involve activation of a humoral factor having important effects on the kidney.

It has become clear from the studies of Flombaum et al. (1978) and Faubert et al. (1982) that the caval dog is characterized by two distinct pathophysiological states. The first, as previously described in detail, reflects avid sodium retention with ascites formation, activation of the renin-angiotensin-aldosterone system, saralasin sensitivity and inability to excrete a saline load. The second state, however, normally occurs after two to three weeks of sodium retention and is characterized by a return to sodium balance with a normalized renin-angiotensin-aldosterone system, loss of saralasin sensitivity, and a renewed ability to excrete a sodium load despite the persistence of ascites and an expanded plasma volume. This phenomenon of return to sodium balance in caval dogs provides an avenue to investigate those variables that may differ between these two phases of sodium handling so as to elucidate the mechanism of sodium retention.

To examine the possibility that changes in inner medullary blood flow may contribute to the sodium retention of caval dogs, papillary plasma flow was measured during

sodium retention and sodium balance. Caval dogs in the sodium retaining phase had significantly reduced papillary plasma flow as compared to control dogs. The return to sodium balance in these dogs was associated with a recovery of papillary plasma flow (Faubert et al., 1982). Supporting the hypothesis that low papillary flow could maintain or enhance medullary hypertonicity was the finding that papillary osmolality and sodium content were significantly greater in the sodium retaining as compared with either the control dogs or those who had returned to sodium balance.

1.3.2 Cirrhosis Of The Liver

Progressive cirrhosis of the liver is characterized by necrosis and inflammation of the liver leading to nodular regeneration and fibrotic bridging. Postsinusoidal resistance rises resulting in progressive hepatic venous outflow obstruction, portal-systemic shunting of blood and eventually portal venous hypertension. The later stages are characterized by progressive sodium and water retention with marked edema accumulating in the peritoneal space as ascites (Levy, 1984). The stimulus for the sodium retention is not known but is likely to be extrarenal and not a function of primary pathological disturbances in the kidney itself since a kidney from a cirrhotic patient transplanted to a noncirrhotic patient will function

normally.

The traditional explanation for the relationship between urinary sodium retention, plasma volume and ascites formation is the vascular underfill theory. It was suggested that ascites formation begins when the increase in intrahepatic sinusoidal pressure produces a critical imbalance of the Starling forces in the hepatic sinusoids and splanchnic capillaries. When the amount of lymph formation exceeds the capacity of the thoracic duct to return it to the circulation, ascites will accumulate in the peritoneal space and the vascular compartment subsequently becomes underfilled. This reduction in plasma volume is thought to be the afferent signal to the kidney to reabsorb extra sodium and water to replenish this compartment.

Clinical studies in the late 1960's of patients with alcoholic cirrhosis of the liver revealed four lines of evidence which questioned the validity of the underfill theory. First, cirrhotic patients had an expanded plasma volume as opposed to the predicted contracted plasma volume. Second, portal pressure does not fall during spontaneous loss of ascites and total plasma volume remains constant, indicating that the effective plasma volume (that capable of stimulating volume receptors) does not contract during ascites formation. Thirdly, the rate of ascites formation does not increase following paracentesis (removal

of ascites) nor does plasma volume contract and finally, it was shown directly that ascites can form in patients previously free of ascites as a consequence of volume expansion by administration of the sodium retaining hormone, DOCA. These observations were the basis for the overflow theory of ascites formation proposed by Lieberman, Denison and Reynolds (1970) which focused on increased renal retention of salt and water as the primary event leading to the development of ascites. The sodium retention leads to expansion of the plasma volume including the splanchnic bed. As a result, portal and intrahepatic pressure rise sufficiently to alter the Starling forces in the hepatic sinusoid to favor sequestration of fluid into the peritoneal space. As ascites accumulates, the circulation will become underfilled obligating a second phase of sodium retention. Of importance is that it is impossible to tell these two theories apart once ascites has occurred since both predict underfilling and thus a phase of sodium and water retention.

In attempting to discern whether diminished effective arterial blood volume was actually present in cirrhotic patients and if it could be an afferent signal for renal sodium retention, cirrhotic patients underwent head-out water immersion. As previously described this manoeuvre results in a prompt redistribution of blood volume with a sustained increase in central blood volume. Immersion

produced a variable response in the cirrhotic patients; one group had a normal natriuretic response, one group had a greater than normal natriuretic response and a third group had no natriuretic response. It was suggested that these data indicate that a diminished effective arterial blood volume could be involved in signalling the kidney to enhance sodium reabsorption (Epstein, 1988). However, it is precisely this spectrum of responsiveness which can be predicted from the overflow theory of ascites formation which predicts both vascular overfill and underfill as related to the stage of the disease. Those patients responding normally to water immersion would have normal plasma volume, those with an enhanced natriuretic response would be volume expanded and those not responding would be volume depleted. This type of analysis emphasizes the importance of identifying the hemodynamic and hormonal characteristics of each patient studied in order to glean important information from the clinical data.

An experimental model used to study liver cirrhosis under more controlled conditions is the cirrhotic dog, either hepatotoxic (dimethylnitrosamine-induced (DMA)) or obstructional (bile duct ligated). Similar models have been developed in rats (carbon tetrachloride inhalation or bile duct ligation). The time course of sodium retention and ascites formation was evaluated in the DMA cirrhotic dogs (Levy, 1977a). The dogs were fed a control diet

containing 45 mEq of sodium per day and housed in metabolic balance cages for the collection of timed urine samples. Ten to fifteen days following the onset of cirrhosis, the dogs began to retain sodium although ascites did not appear until approximately ten days later. The plasma volume was expanded during the phase of sodium retention. Similar results have been reported in cirrhotic rats in which plasma volume expansion and sodium retention are demonstrated at 5 weeks post carbon tetrachloride inhalation, approximately 2 weeks prior to the appearance of ascites (Lopez-Novoa, Rengel, Hernando, 1980). These data clearly support the overflow theory since renal sodium retention and plasma volume expansion both precede the appearance of ascites.

One would naturally assume that the sodium retention and volume expansion in the pre-ascitic phase represents the renal response to the perception of an underfilled circulation. It has been suggested that most of the retained sodium is directed toward replenishing the expanding splanchnic bed and portasystemic collaterals (Levy, 1977a). To examine this, dogs with surgically created end-to-side portacaval fistulas prior to induction of cirrhosis were studied sequentially as cirrhosis developed. Despite the normalization of portal venous pressure and lack of portacaval collaterals, the dogs exhibited sodium retention and plasma volume expansion

prior to the appearance of ascites (Levy, 1977a). This experiment clearly dismisses portal hypertension or the need to fill up venous collaterals as possible afferent signals in the pre-ascitic phase. In addition, several investigators have shown that the pre-ascitic volume expansion is associated with a suppression of plasma renin and aldosterone (Wernze, Speck, Muller, 1978; Wilkinson, Williams, 1980; Bernardi et al., 1983) indicating that the circulation is adequately "filled". This pre-ascitic sodium retention therefore, must be a response to a signal not related to the plasma volume. Indeed, plasma volume expansion appears to be a secondary effect of the sodium-retaining stimulus during this phase. This data provides important and convincing evidence for the overflow theory of ascites formation.

In attempting to elucidate the factor or factors responsible for the first phase sodium retention, micropuncture and whole kidney function studies in cirrhotic dogs and rats showed that there is no change in GFR, renal plasma flow, filtration fraction or renal vascular resistance during sodium retention prior to or during ascites formation (Better, Massry, 1972; Bank, Aynedjian, 1975; Levy, 1977a). Since proximal tubular function is normal, the site of enhanced tubular sodium reabsorption must be distal to this portion of the nephron (Levy, 1977b). These characteristics of cirrhotic kidney

function are identical to those recorded in the caval kidney which is retaining sodium in the presence of ascites. This is not surprising when one considers that both are models of intrahepatic and portal hypertension and edema but may indeed be surprising if compromised systemic hemodynamics is considered to be an important afferent signal apprising the kidney of the fullness of the circulation in edema as will be described in the next section.

Cardiac output increases by 20% in cirrhotic (bile-duct ligated) dogs, accompanied by a fall in peripheral vascular resistance while arterial blood pressure remains normal (Shasha et al., 1976; Levy, 1983). Plasma renin and aldosterone remain normal and there is no change in blood pressure in response to saralasin infusion prior to the appearance of ascites (Schroeder et al., 1976). These dogs differ from the caval dogs in which cardiac output and mean arterial pressure are initially low but are restored to normal as the elevated renin generates high levels of angiotensin II to maintain blood pressure and, at the same time, leading to angiotensin II-dependence as reflected by saralasin sensitivity. It is important to note that cirrhotic patients and animals have high circulating renin and aldosterone levels (Levy, 1977b; Better, Massry, 1972) and are saralasin sensitive when ascites is present (Schroeder et al., 1976). These data suggest that

although both edema models exhibit marked sodium retention and ascites formation, the initiating events that produce the afferent signal to the renal tubule may be different. The mechanisms mediating this enhanced sodium retention may also be different in these two edema states. While intrarenal vasodilatation with acetylcholine effectively raises renal blood flow in both caval and cirrhotic dogs, superimposition of increased perfusion pressure by noradrenaline or angiotensin II infusion does not produce a natriuresis in the cirrhotic animal while restoring sodium excretion to normal in the caval dog (Levy, 1977b). This data suggests that the site of maximal sodium retention in cirrhosis is beyond the nephron segment usually responsive to altered renal hemodynamics in the caval kidney.

It has been suggested that prostaglandins may play an important role in the control of renal function in cirrhosis by preserving renal perfusion (Boyer, Zia, Reynolds, 1979). There is considerable evidence that renal prostaglandins are increased in cirrhotics (Epstein et al., 1979; Zambraski, Dunn, 1984) and that administration of indomethacin will depress GFR and renal blood flow without an effect on blood pressure (Zambraski, Dunn, 1984; Levy, Wexler, Fechner, 1983) in the presence of ascites. The renal prostaglandin system in cirrhosis is stimulated by an increased activity of the renin-angiotensin system and may

serve to offset the vasoconstrictive effects of angiotensin II thus maintaining normal renal perfusion (Levy, Wexler, Fechner, 1983).

The importance of changes in renal sympathetic activity have also been assessed in cirrhosis. Plasma norepinephrine concentrations, used as an index of the activity of the sympathetic nervous system, are elevated in cirrhotic patients with ascites but not in the pre-ascitic stage (Arroyo et al., 1983; Henriksen, Christensen, Ring-Larsen, 1981; Burghardt, Wernze, Diehl, 1986). The activation of the sympathetic nervous system in cirrhosis has been suggested as an important homeostatic mechanism in advanced liver disease (Bichet, Van Putten, Schrier, 1982; Ring-Larsen, Henriksen, Christensen, 1983). However, there is evidence which indicates that the sympathetic nervous system may not play such a major role in the sodium retention of cirrhosis. Cirrhotic patients with sodium retention and ascites, subjected to thermoneutral water immersion all had a diuresis, most had a natriuresis, but in only 1/2 was plasma norepinephrine depressed. Indeed, plasma norepinephrine was dissociated from the natriuretic response (Epstein, 1986). In addition, renal denervation or infusion of alpha- or beta-blocking agents to saline-loaded cirrhotic dogs with ascites did not induce a natriuresis (Levy, 1977b).

Although portal pressure was normalized in the cirrhotic

dogs with an end-to-side portacaval anastomosis, intrahepatic pressure remained significantly elevated thus raising the possibility that intrahepatic pressure itself could be an afferent stimulus. A series of experiments were performed in cirrhotic dogs where intrahepatic and portal pressure were normalized by creation of a side-to-side portacaval anastomosis which serves as an outflow tract from the liver. These dogs did not exhibit sodium retention or plasma volume expansion and remained free of ascites for as long as twelve weeks after surgery while ingesting 35 mEq of sodium per day. Therefore, the normalization of intrahepatic pressure in the cirrhotic dog abolished the sodium retention and, as predicted by the overflow theory, prevented the formation of ascites (Unikowsky, Wexler, Levy, 1983).

The majority of data from both cirrhotic patients and experimental animals seems to support a major role for the liver in initiating sodium retention. Intrahepatic hypertension per se, shown to be an antinatriuretic signal to the renal tubule (Levy, 1974), could activate a neural pathway through pressure-dependent baroreceptors or stretch receptors within the liver. Alternatively, the liver may generate a humoral, metabolic or hemodynamic signal as a result of deterioration in hepatic function or elevation of sinusoidal pressure. The major focus of this thesis is the relationship between the cardiac hormone, atrial

natriuretic factor, and the control of sodium excretion in caval and cirrhotic dogs as compared to normal dogs. Therefore, having described the some of the important factors in extracellular fluid volume control under normal conditions and in edema, the second part of this introduction will focus on the state of the current knowledge about this new hormonal system.

1.4 ATRIAL NATRIURETIC FACTOR

1.4.1 History

Early electron microscopy of guinea pig heart revealed the presence of a population of dense granules within the atrial, but not ventricular, myocytes (Kisch, 1956). A detailed morphological examination of rat atria (Jamieson, Palade, 1964) demonstrated that the granules are spherical, average between 0.25 um and 0.5 um in diameter and are surrounded by a membrane of approximately 18 A in diameter. They are located adjacent to one or both poles of the nucleus of the central sarcoplasmic core, closely associated with the Golgi complex. While being located in both right and left atria, the highest density is found in the anterior wall of the right atrium while the lowest is found near the sulcus terminalis. Comparison of granules from rat, cat, dog and human atria demonstrated that granule size and frequency tend to decrease with increasing size of the animal (Jamieson, Palade, 1964).

Although no functional significance was originally attributed to the granules, their morphological similarity to secretory granules supported the idea that they were a storage site for cardiac catecholamines. In 1979, De Bold reported that water deprivation and sodium deficiency increased granularity while the combined administration of DOCA and 2% sodium chloride in the drinking water decreased granularity. This apparent relationship between granularity and regulation of water-electrolyte balance prompted De Bold to assess the acute renal effects of infusion of a crude extract of rat atrial myocardium (De Bold et al., 1981). The infusion produced a rapid and potent natriuresis and diuresis in anaesthetized rats; infusion of ventricular extract had no effect. It was concluded that the extract contained a powerful inhibitor of renal tubular sodium chloride reabsorption.

Since this initial observation there has been an explosion of investigation into the biochemistry, pharmacology, physiology and pathophysiology of this factor. In a short period of time, several investigators isolated a series of closely related peptides with very similar bioactivity. A variety of terms have been used to refer to the peptides including: atrial natriuretic factor (De Bold et al., 1984; Cantin, Genest, 1985), atriopeptin (Currie et al., 1984), cardionatrin (Flynn, De Bold, De Bold, 1983), auriculin (Atlas et al., 1984), atrial

natriuretic polypeptide (Kangawa, Matsuo, 1984), and atrin (Ballerman, Brenner, 1986). Recently, a committee on nomenclature has named the hormone atrial natriuretic factor. In addition, different sequences are used when referring to specific peptides and numbering amino acid residues (Cernacek, Crawhall, Levy, 1988). For purposes of simplicity in this thesis, the peptide will be referred to as atrial natriuretic factor (ANF) and will be numbered 1 - 28 beginning with the N-terminal amino acid, Ser, as indicated in Figure 1.1.

Cloning and sequence analysis of complementary DNA clones raised to atrial mRNA, identified the structure of the rat preprohormone, consisting of 152 amino acids (151 in human) (Yamanaka et al., 1984; Oikawa et al., 1984; Oikawa et al., 1985). The sequence of prepro-ANF appears in phylogenesis (Cantin, Genest, 1985) and shows considerable homology between species. The signal peptide is cleaved during processing to produce the prohormone which consists of 126 amino acids. Direct sequence analysis has recently demonstrated that only pro-ANF is stored in the atrial granules (Thibault et al., 1987) and further processing liberates a peptide of 28 amino acids, termed alpha-ANF (Kangawa, Matsuo, 1984), now considered to be the major circulating form of the peptide (Thibault et al., 1985; Schwartz et al., 1985). Sequence analysis of the alpha form revealed a high degree of homology between species;

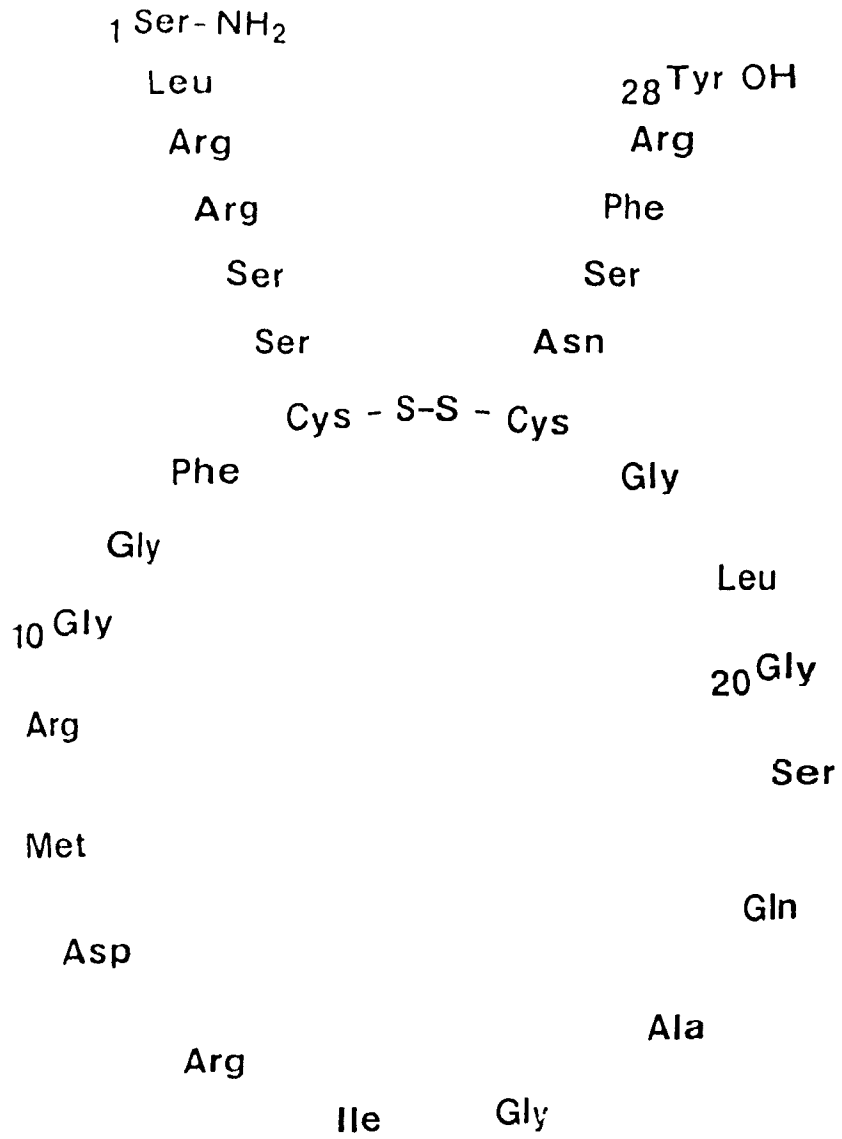

FIGURE 1.1 Primary structure of human ANF(1-28)

dog, human and bovine are identical and differ from that of rat and rabbit by only one amino acid; isoleucine at position 12 is replaced by methionine (Flynn, Davies, 1985). The core structure of ANF is a 17-membered ring formed by a disulfide bond between Cys-7 and Cys-23, as illustrated in Figure 1.1, and is essential for biological activity (Misono et al., 1984). While the C-terminal sequence Phe-Arg-Tyr is also necessary for full bioactivity, N-truncated variants seem capable of normal action (Thibault et al., 1984; Garcia et al., 1985).

Prepro-ANF mRNA has been identified in cardiac atria, the aortic arch and the distal thoracic aorta (Gardner, Deschepper, Baxter, 1987). The existence of mRNA transcripts as well as high concentrations of ANF in the adventitia of the aortic arch demonstrate that the protein is being synthesized in these key places rather than simply binding to the surface receptors and may indeed indicate an important relationship between arterial baroreceptors and the secretion of ANF.

1.4.2 Degradation

The mechanism of ANF clearance from the circulation is presently unknown. Pharmacokinetic analysis of exogenously administered ANF has demonstrated that the half-life of the peptide is very short. T1/2 in humans ranges from 1.7 (Nakao et al., 1986) to 3.1 minutes (Yandle et al., 1986),

similar to dogs in which T1/2 ranges from 1.9 (Verberg et al., 1986) to 4.5 minutes (Ledsome, 1986). Rats have considerably shorter T1/2, ranging from 16-31 seconds (Murthy et al., 1986; Luft et al., 1986; Katsube, Schwartz, Neeleman, 1986) presumably due to the short circulation time. Possible clearing sites include kidney, liver, lung, heart and plasma, although it is generally agreed that very little processing takes place in the plasma. An early study of ANF incubation with various rat tissue homogenates indicated that the kidney had the highest ANF-degrading activity (Tang et al., 1984). An in vivo examination of ANF survival through various organs in the dog demonstrated that 80% of the peptide survived circulation through the lungs while only 20% remained following circulation through the kidney (Weselcouch, Humphrey, Aiken, 1985). Rats with bilateral nephrectomy had increased ANF T1/2 as compared to normal rats (Luft et al., 1986; Katsube, Schwartz, Needleman, 1986) although not to the degree that would be predicted if the kidney were the major route of degradation. The role of the liver in ANF degradation is controversial. A substantial arterio-venous difference has been measured across the liver thus suggesting that it is part of the degradative pathway, although hepatectomy did not alter the T1/2 of infused ANF (Murthy et al., 1986).

Since many small peptides are metabolized in the proximal

tubule (eg. LHRH, angiotensin), ANF degradation has been assessed using several in vitro preparations of proximal tubular cells. Incubation of ANF with rat kidney cortex membrane and rabbit brush border leads to formation of one major degradation product corresponding to a single cleavage between Cys-7 and Phe-8 leaving the disulfide bridge intact (Olins et al., 1987; Koehn et al., 1987). A similar product was obtained during the incubation of pig brush border (Stephenson, Kenny, 1987). The enzyme responsible for the cleavage has recently been identified as endopeptidase 24.11, a peptidase in the microvilli with its active site protruding into the tubular lumen (Stephenson, Kenny, 1987). An important consideration in the site and mechanism of ANF degradation is that the peptide must survive passage through the tubule until it reaches the collecting duct or it must exert its actions on the contraluminal side of the tubule.

1.4.3 Regulation of ANF Secretion From Atrial Myocytes

A major focus of the physiology of ANF has been on the mechanism of release from the atrial myocyte and the associated natriuresis. Release is believed to involve exocytosis and extrusion of pro-ANF or the biologically active ANF, although this was not demonstrated in electron micrographs of normal atrium in the early observations. Page et al. (1986) provided the first ultrastructural

evidence for exocytotic extrusion in both thin-sectioned and freeze-fractured atrial myocytes.

The direct effect of selective elevation of right or left atrial pressure on plasma iANF was investigated in dogs with mitral obstruction, pulmonary artery constriction or inflation of a balloon in the right atrium and showed that plasma iANF increased in direct relation to atrial pressure (Ledsome et al., 1985; Metzler et al., 1986; Akabane et al., 1987). Since atrial stretch occurs as a result of volume expansion or water immersion, it was suggested that changes in transmural pressure, due to an increase in central venous filling pressure, signal ANF release from the atrial myocyte and thereby contribute to the natriuresis of volume expansion and water immersion. The effects of volume expansion were similar in humans (Weil et al., 1985; Yandle et al., 1986), dogs and rats (Lang et al., 1985), producing an acute, rapid increase in plasma iANF which was directly correlated to right atrial pressure in dogs (Goetz et al., 1986). Interestingly, both the increase in ANF levels and sodium excretion were attenuated by atrial appendectomy in rats (Veress, Sonnenberg, 1984; Schwab et al., 1986). To evaluate this apparent relationship between the increase in ANF and the natriuresis of volume expansion, rats were infused with a dose of ANF sufficient to produce the same plasma levels as incurred by volume expansion (Khraibi et al., 1987).

The ensuing natriuresis was 40% of that caused by expansion, providing evidence that ANF, although contributing significantly to the natriuresis of volume expansion, is most likely only part of the response mechanism.

Similar results were found with water immersion in a thermoneutral bath which, as previously described, elevates venous pressure and central venous volume and is associated with a brisk natriuresis (Epstein, Duncan, Fishman, 1975). Water immersed subjects had a rapid increase in plasma iANF which was correlated with urinary sodium excretion (Epstein et al., 1986; Anderson et al., 1986; Ogihara et al., 1986). In rats, horizontal water immersion had little effect on right atrial pressure and no effect on plasma iANF while vertical immersion elevated both right atrial pressure and plasma iANF (Katsube et al., 1985). Water immersed dogs also tended to have elevated plasma iANF although it was dissociated from changes in sodium excretion (Miki et al., 1986).

Postural change, associated with altered sodium handling, also effects changes in plasma iANF. Recumbency, associated with an increase in central venous filling pressure and sodium excretion, elevated plasma iANF while return to the upright position, associated with a decrease in central venous filling pressure, depressed plasma iANF levels (Hollister et al., 1986; Ogihara et al., 1986;

Solomon et al., 1986).

Several studies have investigated the effects of dietary sodium intake on plasma iANF levels. Normotensive subjects maintained on a low sodium diet (10 mmoles per day) have lower plasma iANF than subjects maintained on a normal scdium intake while subjects ingesting a high sodium diet (350 mmoles per day) had higher than normal basal iANF levels (Sagnella et al., 1985; Hollister et al., 1986). Interestingly, plasma iANF was remarkably stable at the new levels for as long as the subject was maintained on a given diet (Sagnella et al., 1986) suggesting a tight coupling between plasma sodium and ANF.

It is clear from these studies that ANF is released in response to a variety of stimuli which alter atrial transmural pressure. However, since cardiac mechano-receptors, located in the walls of the right atrium respond to changes in atrial transmural pressure, it is possible that release of ANF can be mediated by a neural reflex mechanism rather than simply a mechanical response.

Pharmacological doses of adrenergic agonists (isoproterenol, phenylephrine, clonidine) can stimulate ANF secretion in vivo (Rankin, Wilson, Ledsome, 1987; Baranowska et al., 1987). The isolated perfused rat heart releases ANF in response to epinephrine or norepinephrine infusion while the b-adrenergic agonist, isoproteronol, and the a-2 agonist, BHT-920 have no effect. The a-1 agonist,

phenylephrine, released ANF in a dose-dependent manner, an effect which was abolished by infusion of the a-antagonist, phentolamine (Currie, Newman, 1986). In vitro evidence supports a role for the sympathetic nervous system in release of ANF. Extracts of atria preincubated with epinephrine produced a significant natriuresis and diuresis when injected into rats while preincubation with epinephrine and the a-antagonist, phentolamine, largely abolished the renal response (Sonnenberg, 1984). The question of whether direct sympathetic stimulation to the heart can release ANF directly has not yet been addressed. There is clear evidence that ANF can be released in the absence of vagus and sympathetic innervation. Bilateral vagotomy and cardiac b-blockade in dogs did not prevent the increase in plasma iANF when atrial pressure was elevated by mitral obstruction (Ledsome, 1986). Similarly, cardiac denervated dogs increased plasma iANF in response to atrial stretch although there was no associated natriuresis (Goetz et al., 1986).

These studies clearly show that ANF release from the atrial myocyte can be effected simply by increasing transmural pressure as well as suggest that stimulation of cardiac nerves may represent a second mechanism of release. However, it is presently not clear what the relative roles of these two pathways are under physiological or pathological conditions.

It was recently suggested that the anterior pituitary may play a modulating role in ANF release since acute hypophysectomy in rats produced an attenuated increase in plasma iANF in response to volume expansion. Interestingly, the normal response was restored when the pituitary was ectopically transplanted to the kidney (Zamir et al., 1987). The importance of this modulation has not yet been elucidated but further indicates that ANF secretion is controlled by a variety of mechanisms, the interactions of which have not yet been described.

1.4.4 ANF Receptors

Autoradiographic studies localized ANF binding sites to the renal glomerulus, zona glomerulosa cells, adrenergic and noradrenergic cells of the adrenal medulla, hepatocytes, and various cells in the small and large intestine (Bianchi et al., 1985). A high density of receptors have been found in the hypothalamus and lower densities have been reported in the central nervous system (Nakao, 1986). Within the kidney, specific receptors have been localized in the glomeruli, vasa recta, and medullary collecting duct (DeLean, Vinay, Cantin, 1985; Ballerman et al., 1985; Murphy et al., 1985). Comparing receptor density in the various parts of the kidney demonstrated that greater than 90% of the total binding sites are in the cortex while only 2% are found in the papilla. Moreover,

the papillary receptors have a 10 fold lower affinity for ANF than the cortical receptors (Koseki et al., 1986; Suzuki et al., 1987). Binding characterization of ANF to rat renal glomeruli localized the receptor to the cultured mesangial but not epithelial cell (Ballerman et al., 1985). However, a recent study has characterized an epithelial cell ANF receptor in suspensions of inner medullary collecting duct cells (Gunning et al., 1988). Both glomerular receptor density and affinity can be altered by sodium intake as rats on a low sodium diet upregulate their receptors while those on a high sodium diet downregulate the receptors (Ballerman et al., 1985; Gauquelin et al., 1988). Similar results have been obtained with water deprivation in rats (Gauquelin et al., 1988).

The mechanism through which ANF exerts its natriuretic effect following receptor binding involves the activation of a second messenger system. Hamet and his colleagues made an important observation in 1984 when they demonstrated that injection of crude atrial, but not ventricular, extract elevated urinary cGMP excretion and sodium excretion in the rat. This has since been confirmed in the dog (Seymour et al., 1985), rat (Hamet et al., 1986), and man (Gerzer et al., 1985). The renal effects will be described in detail in the next section.

Waldman, Rappaport, and Murad (1984) and Hamet et al. (1984) demonstrated that ANF activates particulate

guanylate cyclase in a concentration- and time-dependent manner in crude kidney membrane preparations while having no effect on soluble guanylate cyclase. The distribution of particulate guanylate cyclase in the various parts of the nephron correlated with ANF-induced increases in cGMP in the segments (Tremblay et al., 1985). This is a fascinating observation since ANF is the first endogenous agonist of the particulate fraction to be identified. Similar results have been obtained in vascular smooth muscle cells of the rabbit aorta (Winquist et al., 1984), human renal glomeruli (Ardaillon, Nivez, Ardaillon, 1985), and kidney cortical (Hamet et al., 1984) and collecting duct cells (Nonoguschi, Knepper, Maganiello, 1987). Further evidence reveals that the vasorelaxant effects in vascular smooth muscle cells, mediated by cGMP do not depend on an intact endothelium (Winquist et al., 1984; Ohlstein, Berkowitz, 1985).

Direct evidence that cGMP mediates ANF's action in vivo is not as easy to obtain. Huang et al. (1986) demonstrated that the source of cGMP in Bowman's space is not from blood via filtration but rather from glomerular mesangial or epithelial cells. It has been demonstrated that cGMP does not increase in the proximal tubule (Stokes, McConkey, Martin, 1986) whereas cGMP increases two fold in the thick ascending limb, three fold in the collecting ducts and 50 fold in the glomerulus (Tremblay et al., 1985). Despite

significantly lower affinity of ANF for the glomerular mesangial receptor, rats fed a low salt diet accumulated cGMP in the glomeruli to the same degree as rats on a high salt diet (Ballerman et al., 1985). Similarly, saline loaded dogs had elevations in cGMP higher than when infused with ANF, neither of which could be correlated to sodium excretion (Seymour et al., 1985). These data indicate that although the sites of cGMP elevation correlate with ANF's known effects on sodium excretion, the direct relationship between increased cGMP and the natriuretic response to ANF has not been clearly shown in vivo.

Affinity cross-linking techniques in combination with cGMP measurements, used to examine the ANF receptor, provided strong evidence for the existence of two distinct ANF-binding sites of Mr approximately 66,000 and 130,000 (Leitman et al., 1986). The smaller receptor site accounts for 94% of binding sites in cultured endothelial cells but does not elicit an increase in cGMP in response to ANF binding and thus is most likely not coupled to guanylate cyclase (Scarborough et al., 1986). These receptors have been termed "silent receptors" (C-ANF receptors) and, although they do not mediate any of the known physiological effects of ANF, they may be specific storage-clearance binding sites for ANF (Maack et al., 1987). The larger receptor, accounting for only 6% of the total receptors liberates cGMP in a dose-dependent manner and appears to

link ANF to its biological actions (Leitman et al., 1986; Maack et al., 1987). The significance of two separate binding sites with two distinct second messenger systems has not yet been determined but will undoubtedly provide important clues as to both the mechanisms of ANF action and its degradation.

1.4.5 Renal Response to Infused ANF

The commercial availability of 1-28 human and rat ANF prompted many studies assessing the natriuretic and diuretic effects of ANF under a variety of conditions. ANF given as a bolus or continuous infusion produces a dose-dependent increase in urinary sodium excretion in control animals. In dogs, doses range from 30 to 600 ng/kg/min and evoke potent natriuretic responses which are usually accompanied by a significant diuresis. Several laboratories report a maximum sodium excretion of 150-200 ueq/min (Blaine et al., 1986; Pollack, Arendshorst, 1986; Seymour et al., 1985) although larger natriuretic response have been obtained in other laboratories (Burnett, Opgenorth, Granger, 1984). ANF infusion in human subjects at a dose in the upper physiological range also enhanced sodium excretion (Anderson et al, 1987) providing important evidence that endogenous ANF is not simply an emergency hormone but one which most likely plays a role in the day to day regulation of sodium and volume homeostasis. The

following sections will present an overview of the physiological (and pharmacological) effects of ANF and the present knowledge regarding the mechanisms of these actions.

The effect of ANF on GFR has been an area of controversy over the past several years. Many investigators report a significant increase in GFR (Cogan, 1986; Kramer et al., 1986) which is postulated to be due either to afferent arteriolar vasodilatation and efferent arteriolar vasoconstriction (Maack et al., 1984; Ichikawa et al., 1985; Marin-Grez, Fleming, Steinhausen, 1986; Fried et al., 1986) leading to an increase in glomerular capillary hydrostatic pressure or a combined effect of efferent vasoconstriction and increased glomerular ultrafiltration coefficient (Fried et al., 1986). ANF infusion in rats produced an increase in glomerular filtration pressure (Schnermann, Marin-Grez, Briggs, 1986) while both an increase in glomerular capillary hydrostatic pressure and ultrafiltration coefficient were reported for the isolated perfused dog glomerulus (Fried et al., 1986). The importance of increased GFR in determining the natriuresis was further underlined by several studies assessing response to ANF when GFR could not rise. Under conditions of decreased renal perfusion pressure (aortic or renal arterial clamp), GFR did not rise and the natriuresis was abolished (Burnett, Opgenorth, Granger, 1986; Sosa et al.,

1986). However, there are several studies which report a normal natriuretic response with no associated increment in GFR (Seymour et al., 1985; Pollack, Arendshorst, 1986; Seymour, Smith, Mazack, 1987) and, in fact, it has been reported in conscious dogs GFR increases in response to ANF infusion only at large doses (Johannessen et al., 1986). This disparity between results cannot be ascribed to anaesthetic effects, species differences, peptide differences (rat vs. human) or method of infusion. It seems likely that an increase in GFR and therefore increased filtered load of sodium can contribute to the natriuretic effect of ANF although is not a major determinant of the ensuing natriuresis.

ANF produces a transient rise in renal blood flow following bolus injection in rats although has no effect during a continuous infusion in both dogs and rats (Maack et al., 1986; Seymour et al., 1985; Dunn et al., 1986). The combined effects of ANF on GFR and renal blood flow are reflected in some studies by an increase in filtration fraction (Burnett, Granger, Opgenorth, 1984). The effect of ANF on renal hemodynamics, while possibly augmenting sodium chloride delivery to the distal tubule, does not appear to be the primary determinant of its natriuretic action thus raising the possibility that the natriuretic action may be through a direct tubular effect.

As previously mentioned, there are no receptors for ANF

in the proximal tubule (Stokes, McConkey, Martin, 1986). Micropuncture and microperfusion of proximal tubular segments has demonstrated that there is no increase in sodium delivery out of this segment (Briggs et al., 1982). ANF applied to luminal brush border membrane vesicles prepared from normal rat kidneys decreased sodium reabsorption linked to both bicarbonate and phosphate reabsorption (Hammond et al., 1985). Stop-flow perfusion of ANF in dogs indicated that, despite any small changes in sodium reabsorption in the proximal tubule, there is no net change in sodium handling in this segment (Steigerwalt, Carretero, Beierwaltes, 1986). Similarly, micropuncture and microperfusion have shown that ANF has no effect on sodium or water handling in the thin loops of Henle (Sonnenberg, 1982; Roy, 1986) or sodium chloride transport out of the thick ascending limb (Peterson et al., 1987). It also does not interfere with Na/K/ATPase in rabbit outer medullary collecting duct cells or thick ascending limb of Henle (Zeidel et al., 1986) and, in fact, the data suggest a primary effect of ANF on sodium channels in the medullary collecting duct. A recent micropuncture study of rat papillary collecting duct demonstrated that while late distal tubule chloride delivery was not influenced by ANF infusion, fractional delivery to the base of the papillary collecting duct was significantly greater and the percent reabsorption (of fraction delivered) along the papilla was

less during ANF infusion (Fried, Osgood, Stein, 1988).

There is accumulating evidence that ANF-induced alterations in peritubular Starling forces governing tubule fluid reabsorption may play a major role in preventing sodium reabsorption in the late collecting duct. Elevation of renal perfusion pressure by angiotensin II infusion in rats enhanced the natriuresis evoked by ANF infusion while elevation of oncotic pressure in the postglomerular capillaries by hyperoncotic albumin exchange abolished the natriuresis (Mendez et al., 1986). This was confirmed in dogs where constriction of the renal artery to produce a 32% decrement in perfusion pressure abolished the natriuresis (Sosa et al., 1986), an effect which is not seen in the contralateral control kidney (Davis, Briggs, 1987). Changes in papillary vasa recta hydraulic pressures as found by microperfusion of rat kidney (Dunn et al., 1986) may also contribute to the natriuretic effect of ANF. Fluorescence videomicroscopy of ANF action in the exposed rat renal papilla demonstrated that ANF does increase vasa recta flow although later than the initial diuresis and natriuresis (Kiberd et al., 1987).

These studies demonstrate that the modulation of sodium chloride handling by ANF does not take place in the proximal tubule, loop of Henle, or distal tubule but rather the majority of action takes place in the medullary and papillary collecting duct. Indeed, the importance of

inhibition of sodium reabsorption in the medullary collecting duct, regardless of the mechanism, is relatively undisputed. Micropuncture of the outer medullary collecting duct demonstrated that the decrease in fractional reabsorption in this segment accounts for approximately 80% of the natriuresis (Sonnenberg, 1982) and appears to represent both an inhibition of sodium reabsorption, possibly through modulation of a sodium channel, as well as secretion into this segment of the nephron.

1.4.6 Cardiovascular Response to Infused ANF

Pharmacological doses of ANF given as either a bolus or continuous infusion produce an acute decrease in mean arterial pressure in humans and most experimental animals which appears to be dose-dependent (Hirata et al., 1985; Biollaz et al., 1986; Richards et al., 1985). This hypotensive response is accompanied by a fall in cardiac output and either no change or an increase in total peripheral resistance (Kleinert et al., 1986; Zimmerman et al., 1987). Since blood pressure is a product of cardiac output and peripheral vascular resistance, the decrease in blood pressure must be due to a decrease in cardiac output which was not due to a decrease in heart rate in animals studied (Zimmerman et al., 1987), and, in fact, an increase in heart rate was reported in human subjects (Richards et

al., 1985; Fujio et al., 1986; Bie et al., 1988). Therefore, ANF's effect on cardiac output must be a result of a decrease in stroke volume and/or a change in cardiac contractility. This hypothesis is consistent with the observation that ANF produces a decrease in central venous filling pressure in dogs and rats (Goetz, 1986). By the Starling mechanism, this would result in a decrease in stroke volume and hence cardiac output. Cardiac contractility (dP/dt) has been shown to decrease in isovolumically paced rat heart and thus may also contribute to the decrease in cardiac output.

The observed decrease in mean arterial pressure is consistent with the demonstration that ANF relaxes vascular smooth muscle preparations which have been precontracted with a variety of vasoconstrictors including angiotensin II, norepinephrine, and vasopressin as well as the nonhormonal agonist ouabain (Aalkjaer, Mulvany, Nyborg, 1985; Faison et al., 1985; Ishihara et al., 1985). The vasorelaxant effect of ANF is most likely a direct effect on the vascular smooth muscle since it has been shown that the intact endothelial lining is not required to achieve relaxation (Winquist et al., 1984).

These in vitro studies, in addition to the demonstration that ANF reduces human forearm vascular resistance (Bolli et al., 1987), appear to be in contrast with the increase in total peripheral resistance measured in response to ANF

in intact animals. This apparent vasoconstrictor action may, in fact, be a secondary reflex effect of a decrease in mean arterial pressure which produces an unloading of the aortic and carotid baroreceptors resulting in an increase in sympathetic activity and thus an increase in peripheral arteriolar resistance (Holtz et al., 1986). The decrease in baroreceptor activity may also account for the increase in heart rate reported for human subjects. The physiological significance of the cardiovascular effects of ANF can only be assessed at plasma ANF levels approximating those seen under normal conditions. ANF infused into conscious dogs to produce plasma ANF levels approximately three times normal did not change arterial pressure, cardiac output or peripheral vascular resistance while significantly decreasing cardiac filling pressure (Bie et al., 1988).

1.4.7 ANF and the Renin-Angiotensin-Aldosterone System

There has been a great deal of investigation into the relationship between ANF and the renin-angiotensin-aldosterone system. Despite an acute fall in blood pressure, ANF infusion has also been shown to depress plasma renin activity (Freeman, Davis, Vari, 1985; Maack et al., 1984) at pharmacological levels as well as at physiological levels (Zimmerman et al., 1987; Brands, Freeman, 1988) although many investigators find no change

in renin with either acute or chronic ANF infusion (Anderson et al., 1987; Cody et al., 1986). The inconsistency in these results may be explained in part by the baseline plasma renin activity in a given animal. Infusion of ANF into spontaneously hypertensive rats, in which basal plasma renin is high, produces a marked decrease in renin. Interestingly, the hypotensive effects of ANF are also more pronounced in hypertensive, renin-dependent animals (Volpe et al., 1985). Infusion into dogs with low or normal plasma renin activity does not necessarily further depress renin. The mechanism most often invoked to explain the drop in renin with ANF infusion is an increased sodium load to the macula densa (Maack et al., 1984; Burnett, Granger, Opgenorth, 1984). However, a recent study of ANF's effect in a denervated, non-filtering kidney (Villarreal et al., 1986) demonstrated a decrease in plasma renin comparable to that measured in the control kidney thus suggesting that an increase in filtered load to the macula densa is not necessary for the renin response to ANF. It has been suggested that ANF may interfere directly with the renal vascular receptor for renin or directly inhibit the juxtaglomerular cells (Obana et al., 1985; Kurtz et al., 1986) but direct evidence is still lacking.

ANF's effects on aldosterone secretion is more firmly documented. In isolated adrenal glomerulosa cells, ANF

causes a dose-dependent inhibition of basal aldosterone secretion, as well as that stimulated by angiotensin II, ACTH and potassium (Atarashi, Mulrow, Franco-Saenz, 1985; Aguilera, 1987). Detailed investigation of the underlying mechanism has demonstrated that ANF inhibits the early path of steroidogenesis, decreasing the conversion of cholesterol to pregnanolone which is stimulated normally by adrenocorticotropin and angiotensin II. In agreement with the in vitro data, infusion of ANF produces decreases in plasma aldosterone (Brands, Freeman, 1988) and attenuates the secretory response to angiotensin II (Anderson et al., 1986).

1.4.8 ANF and Vasopressin Interactions

Intravenous infusion of ANF to normal human subjects or anaesthetized dogs does not alter plasma levels of vasopressin (Biollaz et al., 1986; Gnadinger et al., 1986; Kimura et al., 1986) although markedly reduces hemorrhage-induced elevated vasopressin levels (Samson, 1985). Incubation of posterior pituitary with ANF gives contradictory results, stimulating vasopressin release in one preparation (Januszewicz et al., 1985) and inhibiting its release in another (Obana et al., 1985). As these data suggest, the interaction between plasma ANF and vasopressin is, at present, quite confusing.

1.5 ANF IN EDEMA

As previously described, the pathogenesis of edema is only partially understood. With the discovery of ANF, it was suggested that an impaired secretion of, or an impaired renal response to this important natriuretic peptide may be involved in the progressive sodium and water retention of edema (Goetz, 1978). Recently, a great deal of investigation has focused on defining the relationship between ANF and the sodium retention of edematous patients, in particular those with cirrhosis of the liver, congestive heart failure and nephrotic syndrome, as well as various experimental models.

There are several avenues for potential disturbances in the pathway of ANF's function in extracellular fluid volume control including 1) an absolute or relative deficiency of releasable ANF, 2) secretion of abnormal or less bioactive forms of ANF, 3) a decrease in target organ responsiveness. These possibilities will be discussed in brief in terms of what is presently known about the role of ANF in edema.

An inability to transmit the signal of increased atrial transmural pressure, impaired granular release or a deficiency of atrial ANF would result in a deficiency of circulating ANF and thus permit unopposed action of the renin-angiotensin system. Congestive heart failure patients, however, with a variety of underlying etiologies

generally have very high circulating plasma ANF levels (Cody et al., 1986; Riegger et al., 1986). Moreover, elevation of cardiac filling pressure (Burnett et al., 1986) as well as exercise (Dietz et al., 1986) are associated with further increases in plasma iANF in these patients indicating that the relationship between secretory stimulus and granular release is normal. Therefore, the sodium retention and edema of congestive heart failure is not an ANF deficiency state and, in fact, ANF release is enhanced to compensate for chronic volume overload. This raises the question of the relative effectiveness of the renin-angiotensin system and ANF in volume control in edema states since, despite elevated levels of ANF, sodium and water retention persist.

Studies of patients with cirrhosis of the liver and nephrotic syndrome have revealed some interesting results. These patients have low, normal, or elevated plasma iANF levels (Brabant et al., 1986; Burghardt, Wernze, Diehl, 1986) which can be further elevated in response to thermoneutral water immersion (Epstein et al., 1986). However, despite an apparently normal response of the atria, both cirrhotic and nephrotic patients have a blunted natriuretic response to water immersion. In fact, there is a great deal of variation in individual responsiveness between the patients, despite uniform elevation of plasma ANF (Leung et al., 1987). This attenuated natriuresis was

also demonstrated by intravenous infusion of ANF to cirrhotic and congestive heart failure patients (Hricik et al., 1986; Riegger et al., 1987; Wambach et al., 1988). The clinical data strongly suggests that plasma ANF in these disease states, reflecting normal stimulus-release coupling, is ineffective in producing a normal natriuretic response at the levels of the renal tubule. This may explain, at least in part, the apparent dominance of the renin-angiotensin system over ANF in edema. Elevated aldosterone enhances sodium retention in the distal tubule while high concentrations of ANF cannot increase sodium excretion and bring the system back into equilibrium.

The investigation of various experimental edema models supports such a conclusion. The dissociation between plasma iANF and the renal response to the peptide is emphasized by comparing two canine models of congestive heart failure. Caval dogs have low circulating plasma ANF levels (Paganelli et al., 1988) while dogs with aortocaval fistulas have high ANF levels (Villarreal et al., 1987). Both retain sodium, have an activated renin-angiotensin system and an impaired natriuretic response to infused ANF (Freeman et al., 1985; Chou, Reiser, Porusch, 1987).

There is presently very little data to explain the attenuated natriuretic response to ANF in edema although a recent study suggests that the problem may be at the levels of the medullary ANF receptor. Rats with congestive heart

failure due to myocardial infarction have elevated plasma iANF and reduced ANF binding in the inner renal medulla. This decrease in binding is due to a decrease in receptor density while the affinity of the receptor for ANF is unchanged (Tsunoda et al., 1988). In addition, Koepke, Jones, DiBona (1987) and colleagues demonstrated that renal denervation of cirrhotic and nephrotic rats, previously weakly responsive to ANF infusion, now had the full natriuretic response restored suggesting that the renal nerves are antagonizing the effects of ANF.

1.6 PURPOSE OF THESIS EXPERIMENTS

The experiments described in this thesis were designed to assess the role of ANF in two canine models of edema: the dog with thoracic inferior vena caval constriction (caval dog) and the dog with biliary cirrhosis of the liver (cirrhotic dog).

The caval dog is a well characterized model of low-output congestive heart failure. Its major advantage in the study of sodium retention and ascites is that it shows a biphasic pattern of sodium handling. Following a period of sodium retention in the presence of ascites, the dogs will return to sodium balance. The cirrhotic dog model closely mimics the human disease and has also been well characterized. Unlike chronic caval dogs, cirrhotic dogs do not return to sodium balance in the presence of ascites.

However, the pre-ascitic phase, lasting approximately 8-12 weeks, provides the opportunity to study the role of ANF during the development of cirrhosis of the liver.

In these studies, the profile of atrial ANF in terms of cardiac storage and release was assessed by comparing atrial content, plasma iANF levels, molecular forms of the peptide in the atria and plasma, disappearance time and metabolic clearance rate of normal dogs with both the caval and cirrhotic dogs with sodium retention and ascites. In addition to establishing these characteristics in normal dogs, these data permitted the examination of one area where ANF physiology could be abnormal in the genesis and maintenance of edema.

The renal response to exogenous ANF infusion in normal dogs was also compared to that in caval and cirrhotic dogs. When it became clear that the caval and cirrhotic dogs in sodium retention and ascites divide 50:50 into natriuretic responders and non-responders, many parameters were examined in detail to attempt to delineate the factor(s) which produce this heterogeneous renal response.

CHAPTER 2: METHODS

2.1 SURGICAL TECHNIQUES

2.1.1 General Preparation and Recovery

All dogs prepared for sterile surgery were handled in the same manner. Healthy, chronically conditioned male and female dogs who had been in the McIntyre Animal Center for at least 3-4 weeks were fasted overnight before the day of surgery and allowed water ad lib. Surgery was carried out under sterile conditions using either inhalation (methoxyfluorane) or pentothal (2.5% sodium thiopental, Abbott Laboratories, Montreal, 25 mg/kg i.v.) anaesthesia. Dogs anesthetized with pentothal were intubated with a cuffed endotracheal tube and placed on a Harvard respirator. The area of the incision was cleansed with soap and water, and sterilized with alcohol and betadine. After surgery, all dogs were returned to the Recovery Room in the McIntyre Animal Center, and treated with Penlong-S (rogar/STB Inc., Montreal, PQ) 2ml/day (approximately 400,000 I.U. Penicillin G/day) for 5 days.

2.1.2 Cirrhosis - Bile Duct Ligation

Through a midline abdominal incision, the common bile duct was identified and doubly ligated above the entrance of the pancreatic duct and as high as possible to prevent later fistulous reattachment of the proximal stump to the duodenum. Care was taken to identify and ligate all accessory branches to the common bile duct including the

cystic duct. While cholychystectomy was performed in most dogs, technical difficulties precluded this procedure in some dogs. No differences in the recovery from surgery or development of cirrhosis and ascites were seen and therefore all dogs were considered as a uniform group. The bile duct was divided between the ligatures. The abdomen was carefully closed in two or three layers.

2.1.3 Thoracic Inferior Vena Cava Constriction

Through a right thoracotomy between the 5th and 6th ribs, the vagosympathetic trunk was gently freed from its advential attachments to the inferior vena cava, and then the cava was constricted approximately 50% with stout umbilical tape. The chest was closed with 1-0 chromic (Ethicon) sutures around both ribs. Care was taken to expel all air from within the thoracic cavity before complete closure. The chest was closed in 2-3 muscle layers.

2.2 General Protocols (Detailed methods for each protocol will be described in later sections.)

2.2.1 Normal Dogs

1. ANF-Dose Response

Changes in renal function and blood pressure at four different doses of ANF were assessed in five normal

conscious dogs standing quietly in the Pavlov sling. Following control measurements, ANF was infused at 50 ng/kg/min, 75 ng/kg/min, 125 ng/kg/min and 175 ng/kg/min for 45 minutes each while repeating the measurements taken in the control phase.

2. Determination of Papillary Plasma Flow

8 dogs were prepared for the determination of papillary plasma flow. 4 dogs were studied in the control state and 4 were studied during the intravencus infusion of ANF at 125 ng/kg/min.

2.2.2 Caval Dogs

1. Sequential Response to ANF Infusion

14 chronic caval dogs were studied in the control phase and again following TIVC constriction in 2 phases when ascites was present - sodium retention and when the dogs had returned to sodium balance. The dogs were re-studied following mobilization of ascites with the LeVeen peritoneovenous valve.

2. Hemodynamic Studies

A group of caval dogs was prepared with chronic indwelling catheters for the determination of changes in systemic hemodynamics (cardiac output, mean arterial pressure, central venous pressure, and plasma volume). The

dogs were studied in the pre-surgical control phase and in the presence of ascites, both in the phases of sodium retention and sodium balance. The hemodynamic parameters were measured during a control period and again during the infusion of 175 ng/kg/min ANF.

3. Determination of Papillary Plasma Flow

10 caval dogs were prepared for the determination of papillary plasma flow during the infusion of ANF at 125 ng/kg/min.

4. Effects of Renal Denervation and Renal Vasodilatation

Response to ANF (125 ng/kg/min) was determined in a series of anaesthetized dogs with left renal denervation (the right kidney served as the control). In dogs not responding to the infusion under control conditions or following denervation, acetylcholine was infused in the left renal artery at a dose sufficient to raise renal blood flow and the response to ANF was determined.

5. Sequential Measurement of Plasma Hormones

8 dogs were used for the sequential measurement of plasma iANF, renin, aldosterone and ADH. Samples were obtained prior to caval constriction and, following surgery, daily until the appearance of ascites. All samples were taken in the conscious state, with the dogs standing quietly in the

Pavlov sling.

2.2.3 Cirrhotic Dogs

1. Response to ANF Infusion

9 chronic dogs were studied in the control phase and following the development of cirrhosis, as determined by liver function tests, and ascites. LeVeen peritoneovenous shunt was inserted into 3 cirrhotic dogs and the response to ANF reevaluated.

2. Determination of Papillary Plasma Flow

7 cirrhotic dogs with sodium retention and ascites were prepared for the determination of papillary plasma flow during the infusion of ANF at 125 ng/kg/min.

3. Effects of Renal Denervation and Renal Vasodilatation

The response to ANF was measured under control conditions, and following left renal denervation (the right kidney served as control). When baseline sodium excretion had been restored, acetylcholine was infused into the left renal artery and the response to ANF reevaluated.

4. Sequential Evaluation of Cirrhotic Dogs

A series of 9 cirrhotic dogs were followed weekly after bile-duct ligation for the determination of plasma volume, sodium balance, plasma iANF, renin and aldosterone. In

addition, the response to ANF was studied in the conscious dogs biweekly from week 2 to week 8 and once again when ascites had developed.

5. Acute Intrahepatic Hypertension

6 acute, anaesthetized dogs were prepared for the determination of ANF responsiveness under control conditions and again during the intraportal infusion of histamine.

2.3 Surgical Techniques

2.3.1 LeVeen Peritoneovenous Valve

A two inch longitudinal incision was made in the abdomen medial to the anterior axillary line, lateral to the rectus sheath and below the liver edge taking care to avoid distended varices on the abdominal surface. The abdominal muscles were separated between fibres until the peritoneum was exposed. Two purse string sutures (3-0 nylon, Ethicon, Montreal) were sewn loosely in concentric 1 and 11/4 inch circles, leaving at least 3 inch ends free on each suture. A longitudinal incision was then made overlying the left jugular vein. A tunnelling instrument was routed subcutaneously from the incision at the neck and exteriorized through the abdominal incision. The solid venous tubing was attached to the instrument and pulled through the subcutaneous tunnel into the incision at the

neck. A small incision was made in the center of the purse string sutures and the perforated collecting tube was passed through this incision into the peritoneal cavity. The sutures were pulled tightly and tied securely to ensure that no ascites could leak around the shunt. When flow was established through the valve, the end of the solid tubing was inserted into the internal jugular vein through a longitudinal venotomy. The vein was tied with 2-0 silk sutures above and below after insertion of the tubing. The abdominal muscles were pulled over the valve and sutured with 2-0 chromic suture to protect the valve. The abdominal and neck wounds were closed with 3-0 nylon sutures in the skin.

2.3.2 Chronic Catheters

Chronic indwelling catheters were placed in the right jugular vein and right carotid artery for measurement of cardiac output, arterial blood pressure and central venous pressure. A longitudinal incision was made overlying the right jugular vein. Both the jugular vein and carotid artery were isolated, ligated and catheterized with polyethylene transmission tubing catheters. Catheters were routed subcutaneously and exteriorized through a small incision at the back of the neck. The catheters were fitted with 3-way stopcocks, flushed with heparinized saline and tied securely into a neck band. The catheters

were flushed daily with heparinized saline to maintain patency.

2.4 Sling Studies

A series of experiments were carried out on alert, unanesthetized control, caval, and cirrhotic dogs standing quietly in a Pavlov sling. All dogs were mildly sedated with 1.0 mg i.m. of atropine sulfate and 20-30 mg xylazine at the commencement of the study. A Foley catheter was inserted into the bladder for collection of urine by a standard washout technique using 10 ml of sterile distilled water. Catheters were inserted into the antecubital veins by direct venipuncture for the infusion of either inulin (to measure GFR) and para-aminohippurate (PAH, to measure renal plasma flow) on one side or for the infusion of rat 1-28 ANF (obtained from either Peninsula Laboratories, Belmont, CA or Armand Frappier, Montreal, Quebec) at 175 ng/kg/min. Inulin and PAH were infused at a priming dose of 6 ml/min for 10 minutes (150 mg/min inulin; 30 mg/min PAH) followed by a continuous infusion at 0.6 ml/min (15 mg/min inulin; 3 mg/min PAH). Venous blood was sampled from a polyethylene catheter inserted into the abdominal vena cava by direct puncture of the saphenous vein. Prior to the control clearance collections (4 collections of at least 10 minutes duration) plasma was taken for the measurement of plasma ANF, plasma protein, hematocrit,

sodium. Following control clearance collections, ANF was infused at 175 ng/kg/min (0.58 ml/min in normal saline with a constant infusion syringe pump). The same pump was used for each animal. After a 10 minute equilibration period, another 4 clearance periods were collected. Blood was sampled once more at the midpoint of these 4 clearance periods for the measurement of ANF, renin, aldosterone, and cGMP, and at the mid point of each clearance period for the measurement of plasma inulin and PAH. 5 ml aliquots of urine were taken from two clearance periods and frozen for measurement of cGMP. Generally, no more than 7-10 ml of blood was sampled for determinations at any one time (blood for plasma hormone levels being staggered over 10 minutes) and was always replaced with an equal volume of isotonic saline, as were all urinary losses.

2.5 Balance Studies

In order to document the status of sodium balance, dogs were placed in large metabolic cages for the collection of 24 hour urine. Food was standard dog chow containing 0.1 mEq/gm sodium. The dogs were fed approximately 45 mEq sodium per day with free access to water. Balance studies were carried out for at least 48 hours to ensure accurate measurements.

2.6 Hemodynamic Parameters

2.6.1 Cardiac Output:

Cardiac output was measured by dye dilution technique using indocyanide green (Cardiogreen, Hyson, Westcott and Dunning). The carotid arterial catheter was connected to a densitometer (Densitometer, D402A, Waters Instrument Inc.) and a dye injection system (3-way stopcock) was connected to the jugular vein catheter. Prior to the determination of cardiac output a standard curve was constructed using blank blood containing 2-16 mg/ml cardiogreen. Blood was withdrawn at a constant rate from the arterial catheter with a constant infusion/withdrawal pump (Sage Instruments Orion Research) and 0.5 ml cardiogreen injected quickly through the jugular catheter and the density curve inscribed several seconds later. To compute the area under the curve, the downslope of the curve is extrapolated to zero concentration on 4 cycle semi-log paper, beyond the beginning of recirculation. Because of exponential decay, the extrapolation is best effected on semilogarithmic paper which converts exponential curves to straight lines. The formula used to compute the cardiac output in liters per minute is:

C.O. = (I x 60 sec)/(E x K) where I=amount of dye injected, E=total area of the curve, and K=slope constant of the calibration curve.

2.6.2 Mean Arterial Blood Pressure:

Mean arterial blood pressure was determined by mercury manometry through the catheter in the carotid artery.

2.6.3 Central Venous Pressure:

Central venous pressure was determined by saline manometry through the catheter in the jugular vein. The reference point was taken at the anterior axillary line with the dog standing on all four legs.

2.6.4 Plasma Volume:

Plasma volume was determined by dye-dilution technique with T1824 Evan's Blue (Fisher Scientific, Montreal) prepared as a 5% solution. Blank plasma was obtained prior to infusion for the preparation of standards. 1.0-1.5 ml of dye (determined by gravimetric techniques) was injected. Blood samples were collected every 7.5 minutes until 30 minutes had elapsed. Spectrophometric determination of the concentration of the dye was carried out at 620 nm.

2.7 Preparation of Anaesthetized Dogs

Caval or cirrhotic dogs with ascites were anaesthetized with nembutal, (Abbott Laboratories, Montreal) 25 mg/kg intravenous, intubated with a cuffed endotracheal tube and ventilated with a Harvard respirator when necessary. Mean arterial pressure was recorded by a mercury manometer attached to a polyethylene catheter (PE 205) in a femoral

artery. Arterial blood samples were collected from a catheter placed in the other femoral artery. A PE 190 catheter was placed in a femoral vein for the infusion of ANF. A PE 50 catheter was placed in the right jugular vein for infusion of inulin and PAH. Through flank incisions, each ureter was cannulated with PE 90 tubing to the level of the renal pelvis with great care being taken to avoid tearing the peritoneum and loss of ascites. Dogs were positioned on their right side to allow easy access to the left kidney for further manipulation. All infusions were administered with constant infusion pumps.

2.7.1 Left Renal Denervation

The left kidney was denervated by stripping the renal artery free of all attachments. The artery was then painted with a 5% phenol solution. In addition, the fibrofatty attachments surrounding the kidney and upper ureter and pelvis were stripped away as much as possible without causing bleeding or perforation of the peritoneum.

2.7.2 Intrarenal Acetylcholine

A 23 gauge needle was inserted and fixed in the left renal artery, attached to a PE 50 catheter, and connected to a constant infusion Harvard syringe pump. Normal saline was infused into the renal artery at 0.5 ml/min to keep the needle patent and was continued during the control

clearance period. Thereafter, acetylcholine bromide (Sigma Chemical Co., New York) was infused at 60-80 ug/minute without changing the pump speed.

2.7.3 Acute Intrahepatic Hypertension

The splenic pedicle was clamped and a PE 190 or 205 polyethylene catheter was inserted through the splenic vein into the portal vein and advanced as closely as possible to the porta hepatis. Through a 3-way stopcock, this catheter was used either for the infusion of histamine or for the measurement of portal venous pressure by saline manometry. Histamine, as the diphosphate (containing 30% free base) was infused into the portal vein in order to take advantage of its ability to selectively cause postsinusoidal hepatic venoconstriction in the dog. The infusion of small doses (approximately 3-6 ug/min free base) was sufficient to significantly elevate portal, and thus intrahepatic pressures. Similar doses infused into a femoral vein were without effect on central hemodynamics.

In control studies, the portal venous catheter was left filled with heparinized saline at 0.5 ml/min and a histamine infusion at the same rate (3-6 ug/min free base) was administered in the contralateral catheter. After a set of 3 clearance experiemtns, the saline infusion was replaced with an infusion of ANF at 175 ng/kg/min and a second set of clearances obtained. Following this latter

set of clearance, the animals were allowed a 30 minute resting period. A third set of clearances were taken with isotonic saline running in one femoal venous catheter and histamine in the portal catheter sufficient to cause a significant increment in portal venous pressure. After this set of clearances, with portal hypercension maintained, the femoral catheter was used to infuse ANF at the same dose as used during the control periods. During the initial clearance periods in the control phase of the study, and where ANF was being infused, the urine collections were 10-15 minutes in duration and comparable between all dogs. During the control collections where IHH was present, urine was collected for 20 minutes for all dogs.

2.8 Papillary Plasma Flow

A catheter (transmission tubing) was placed in the right jugular vein, fitted with a three-way stop-cock and closed. A second cannula, fitted with a 3-way stop-cock was placed in the left femoral artery and threaded up into the aorta to a level just below the renal arteries. A PE-90 catheter was placed in the left femoral vein for the infusion of ANF at 125 ng/kg/min. The left kidney was exposed through a flank incision without disturbing the peritoneal cavity. The renal pedicle was gently dissected and a piece of umbilical tape passed around it, and a

loosely tied knot made. Prior to determination of papillary plasma flow, transit time was measured for each dog. Saline containing a small amount of cardiogreen (give supplier name) was infused through the jugular vein and blood was withdrawn from the left femoral artery both attached to a reciprocal action pump (Harvard Apparatus Co., Mills, MA). The withdrawal catheter was attached to a densitometer (Gilson Medical Electronics, Middleton, WI) which was connected to the Harvard pump. Transit time was taken as the time for cardiogreen to appear at the level of the renal artery as determined by the densitometer. ANF was infused at 125 ng/kg/min for 40 minutes and 2-3 clearance periods collected. With the continued infusion of ANF, Albumin I125 (Dupont Canada, Mississauga, Ontario), 100 uCi/ml, was infused through the jugular catheter by the Harvard pump at a rate of 4 uCi/sec. After the transit time had elapsed, blood was withdrawn from the aortic catheter attached to the Harvard pump to exactly match the albumin infusion. After a simultaneous perfusion and collection period of 20-30 seconds, the umbilical tape knot around the left renal pedicle was pulled tight and the blood stopped at the same time. The kidney was removed immediately, placed in ice and frozen at -10 C for 20-30 minutes. The blood was placed in a heparinized tube, spun at 2500 x g for 10 minutes and plasma separated. The papilla was cut into 50-100 mg pieces, weighed and counted

together with 1 ml and 0.5 ml aliquots of the plasma for 1 minute in a gamma counter. PPF was calculated according to the formula:

PPF (ml/min/100 g papilla) = CPM/100 g papilla/CPM/ml plasma x 60 sec/perfusion time (sec)

Mean plasma CPM/ml = 140,000 with a range of 47,000 - 330,000 CMP/ml Two dogs with PPF of 1 ml/min/100 g were discarded because of the presence of kidney stones.

2.9 Radioimmunoassays

2.9.1 Atrial Natriuretic Factor - Plasma and Atria

Atrial Extraction: Pieces of atrial tissue (approximately 50 mg) were cut out of left and right atria and from the atrial septum in control dogs. Samples were immediately rinsed with ice-cold saline and homogenized with Polytron (Brinkman) for 30 seconds in 1 ml of ice-cold 0.1 M acetic acid with 0.02 N HCl, rinsing the probe 3 times with the same solution. Boiling of the samples was omitted since the preliminary experiments did not show any effect of this step either on total iANF level, or on its chromatographic profile. The homogenate was centrifuged for 20 minutes at 30,000 g (4°C) and the supernatant stored at -20°C for a maximum of 1 week until further analysis. 3 ml of blood were collected in a chilled polystyrene tube containing the following protease inhibitors, EDTA (1 mg/ml), pepstatin (5 uM), and phenylmethyl sulfonyl

fluoride (PMSF) (10uM). The tubes were immediately centrifuged for 20 minutes at 4°C at 1800 x g. The plasma was decanted and stored at -85°C and extracted within 3 days.

Plasma Extraction: 1-3 ml of plasma were acidified with 1M HCl to pH 4 and loaded on SEP-PAK C18 cartridge (Waters Associates, Millford, MA) activated by rinsing with methanol, urea (8M) and water. After washing the cartridge with water and 4% acetic acid, iANF was eluted with 3 ml of 90% ethanol in 0.4% acetic acid. The eluate was evaporated to dryness under a stream of air and stored at -20°C. The extraction of ANF from dog plasma by this procedure is 94%, as estimated by the recovery of 125I-human ANF99-126 added to plasma. Immediately prior to the radioimmunoassay of iANF, the dried eluate was reconstituted in 0.4 ml of assay buffer. RIA of iANP was performed in 0.1M sodium phosphate buffer (pH 7.4) containing 0.05 M NaCl, 0.1% bovine serum albumin (RIA grade, Sigma, St. Louis), 0.1% Triton X-100, and 0.01% sodium azide. 0.1 ml of reconstituted plasma, atrial extract (diluted 1/1000 to 1/4000 in RIA buffer) or 0.1 ml of standard (1.8 to 120 pg of human ANP99-126, obtained from Peninsula Laboratories, Belmont, CA) was incubated at 4°for 24 hours with 0.1 ml of antiserum recognizing the C-terminal portion of ANP (rabbit anti-human ANP99-126antiserum, Peninsula). After addition of the radioligand (125I-ANF99-126, Amersham, Oakville,

Ontario) and further incubation for 24 hours, the bound radioligand was precipitated with goat anti- rabbit IgG antiserum in the presence of normal rabbit serum. Radioactivity of centrifuged and washed pellets was measured in an automatic gamma-counter (Automatic Gamma Counter, Micromedic Systems 4-600) Unknowns and standards were evaluated by logit-log transformation of data after correction of nonspecific binding. Maximum binding of the radioligand averaged 35%, nonspecific binding was less than 2%.

2.9.2 Plasma Renin Activity

Plasma renin activity was determined by the quantization of generated Angiotensin I by a commercial RIA kit, RIANEN Angiotensin-I (125I) (New England Nuclear Products, Dupont Canada, Mississauga, Ontario). 7.0 ml of blood were collected into a chilled glass test tube containing EDTA (1mg/ml), placed on ice and centrifuged at 4 ^{o}C for 15 minutes at 1200 x g. Plasma was separated and stored at -20 ^{o}C until assay at which time the samples were thawed in the refrigerator. Angiotensin I was generated as follows. To 0.5 ml plasma sample, the converting enzyme inhibitor and angiotensinase inhibitors, dimercaprol and 8-hydroxyquinoline, were added. pH was adjusted to pH 6.0 with maleic acid, 1 ml aliquots of each sample were incubated for 1 hour at 37 ^{o}C while the remainder was

incubated for 1 hour at 4°C. Standards (0.1 - 10.0 ng/ml) and both samples generated at 37°C and 4°C were then incubated overnight at 4°C with radiolabeled Angiotensin I (5-L-isoleucine)[tyrosyl-125I] and rabbit antiserum (in Tris-acetate buffer containing 0.1% sodium azide. A second incubation for 30 minutes at room temperature with a second antibody (anti rabbit gamma globulin in Tris-acetate buffer, 0.1% sodium azide and polyethylene glycol) was performed to precipitate the primary antigen-antibody complex, thus separating bound from free antigen. Following centrifugation at 100 x g for 10 minutes at room temperature, the supernatant was discarded and the pellet counted in a gamma counter for 1 minute. Plasma renin activity is expressed as ng/ml/hr.

2.9.3 Serum Aldosterone Concentration

Serum aldosterone concentration was measured using a commercial RIA kit, RSL (125I)Aldosterone (Radioassay Systems Laboratories, Inc., Carson, CA). Care was taken to avoid excessive light exposure. 10.0 ml of blood were collected into glass test tubes and allowed to clot for 30 minutes at room temperature. Plasma was separated and stored at -20°C until assay. Plasma samples were thawed at room temperature. 0.6 ml of plasma was extracted with ethyl acetate:hexane (3:2), evaporated under air and

reconstituted with the steroid diluent, 0.005% rabbit gamma globulins in 0.1M phosphosaline gelatin buffer at pH 7.0. Samples and aldosterone standards (2-200 pg/ml) were incubated at room temperature for 60 minutes in the dark with radioiodinated aldosterone and specific antiserum. After a second 60 minute incubation at 4 °C bound aldosterone was precipitated with a second antibody (goat anti-rabbit gamma globulin in phosphosaline buffer and polyethylene glycol). Following centrifugation at 1000 x gfor 20 minutes, the supernatant was decanted and the pellet was counted in a gamma counter for 1 minute and evaluated as described for ANF. Maximum binding of the radioligand averaged 34%, nonspecific binding was less than 1.5%. Plasma aldosterone concentration is expressed as ng/dl.

2.9.4 Plasma and Urinary Cyclic GMP

Plasma and urinary cGMP were determined with a commercial RIA kit, cGMP-I125 (New England Nuclear Products, Dupont Canada, Mississauga, Ontario). 10.0 ml of blood were collected in cold, heparinized polyethylene tubes, and centrifuged at 2500 x g for 10 minutes at 4°C. Plasma was separated and stored at -20°C until assay. 5 ml aliquots of undiluted urine were stored at -20°C until assay. All samples were thawed in an ice bath before RIA. 1.0 ml plasma was extracted with 1.0 ml cold 10% TCA, centrifuged

and 100 ul of the supernatant used in the assay. Urine samples collected during ANF infusion were diluted 1:10 with 0.05 M sodium acetate buffer, pH 6.2. All other urine samples were assayed undiluted. cGMP standards (1-100 pmol/ml) and samples were incubated for 18 hours at 4°C with the radioligand (succinyl tyrosine I125methyl ester derivative of cGMP in 1.0% normal rabbit serum and 0.05 M sodium acetate buffer) and a pre-reacted antibody complex. At the end of incubation, 1.0 ml cold sodium acetate buffer, pH 6.2, was added. Following centrifugation at 2000 x g for 15 minutes at 4°C, the supernatant was decanted and the pellet counted in a gamma counter for 1 minute and evaluated as described for ANF. Maximum binding of the radioligand averaged 55%, nonspecific binding was less than 3%. Plasma cGMP is expressed as pmol/ml and urinary cGMP is expressed as pmol/min.

2.9.5 Plasma Vasopressin

RIA of vasopressin was performed with an aliquot of the same dried ethanolic extract of plasma as that used for the assay of iANF. Standards (Arg8-vasopressin, 0.4 to 40 pg/tube, Peninsula Laboratories) and samples reconstituted in RIA buffer (composition according to the manufacturer) were incubated for 24 hours at 4°C with antiserum (rabbit anti-Arg8-serum, RAS8103, Peninsula Laboratories). After addition of the radioligand and the second 24 hour

incubation, bound and free antigen were separated by precipitation with goat anti- rabbit IgG serum and the bound radioactivity was counted in a gamma counter for 20 minutes. The standard curve was stable with the maximum binding of 22.5% and non-specific binding of 2%.

2.10 Reverse Phase-High Pressure Liquid Chromatography

(RP-HPLC) was performed on a uBondapak C18 column (3.9x300mm) eluted with a linear gradient of acetonitrile from 20 to 50% in 0.1% trifluoroacetic acid, with a flow rate of 1 ml/min and slope of 0.5%/min. Fractions were collected, lyophilized, reconstituted in RIA buffer and assayed for immunoreactive ANF.

2.11 Gel Permeation Chromatography

Gel permeation chromatography was used to estimate the molecular weight of the main iANF-fractions, obtained during RP-HPLC. Lyophilized fractions were dissolved in 1 ml of the elution solvent (0.1 M acetic acid containing 1 g of bovine serum albumin per liter) and chromatographed ir separate runs on Sephadex G50 column (98x1.5 cm), eluted with a flow rate of 0.5ml/min. 1 ml fractions were collected, lyophilized, and assayed for iANF as described above. The void (Vo) and total (Vt) volumes of the column were determined with blue dextran and sodium iodide, respectively. The column was calibrated with a series of

small proteins of known molecular weight determining their elution volume (Ve) by monitoring of absorbance at 280 nm. Molecular weight versus distribution coefficient (Kav), using the following formula to calculate the latter:

$$Kav = (Ve - Vo)/(Vt - Vo)$$

2.12 Analysis

2.12.1 Inulin/PAH

Inulin in urine and plasma was measured by an anthrone method (Fuhr, Kaczmarczyk, Kruttgen, 1955). PAH was measured by the autoanalyzer method of Bratton and Marshall (1939) as modified by Smith et al. (1945).

2.12.2 Plasma and Urine Na

Plasma and Urinary sodium and potassium were evaluated by flame photometry (Flame Photometer 443, Instrumentation Laboratories) against an internal lithium standard. Results are reported as uEq/ml.

2.12.3 Liver Function

10 ml of blood were collected in a glass test tube and allowed to clot for 30 minutes. Serum was separated and stored at $-10^{o}C$ until assay.

Serum Bilirubin:

Total serum bilirubin was measured in 0.2 ml serum with

a commercial kit (Hycel Bilirubin Test, Fisher Scientific) based on the Evelyn and Malloy method. Bilirubin is reacted with diazotized sulfanilic acid at a moderately acid pH to form azo-bilirubin, producing a strong purple color which is read at 550 nm (Beckman Spectrophotmeter). Methyl alcohol is used as the accelerator to cause unconjugated bilirubin to react thus giving total serum bilirubin.

Serum Alkaline Phosphatase:

Serum alkaline phosphatase was measured in 0.1ml serum sample with a commercial kit (Hycel Alkaline Phosphatase, Fisher Scientific). Magnesium thymophthalein monophosphate is hydrolyzed by serum alkaline phosphatase at pH 9.85 to thymolphthalein and phosphate ions. The enzymatic reaction is stopped and free thymolphthalein is converted from the colorless to the blue form by the addition of alkali. Peak absorbance is 590 nm in the blue region where there is minimal interference from bilirubin (both are usually elevated in cirrhosis).

Serum Glutamate Oxalacetate Transaminase (SGOT):

SGOT was determined in 0.2 ml sample with a commercial kit (SGOT, Dade) which is a modified version of the method by Reitman and Frankel (1957). GOT catalyzes the exchange of an amino group of aspartate for an alpha-keto group of

alpha-ketoglutarate forming glutamate and oxalacetate. The addition of sodium hydroxide and 2,4Dinitrophenyl-hydrazine forms a colorless complex which is read against a standard curve at 505 nm.

2.13 Determination of Kinetic Parameters

Metabolic clearance rate (MCR) was calculated by dividing the infusion rate by the steady-state plasma concentration of iANF. The latter was obtained by averaging the results of 3 plasma samples taken at the plateau during the infusion. Pharmacokinetic parameters of iANF elimination from plasma after the end of infusion were obtained using a computer-assisted curve-stripping procedure. The best fit of the post-infusion iANF levels was obtained with a two-compartment model described by the equation $Ct = A*e^{-\alpha t} + B*e^{-\beta t}$ where Ct is plasma iANF concentration at time t after completion of the infusion, A and B are constant coefficients giving the y-axis intercepts of the fast and of the slow component of the decay curve, and a and b are rate constants derived from the slope of the two components of the decay curve. The half-time (t1/2) corresponding to each component was calculated using the formula:

$$t1/2 = \ln 2/\alpha \text{ and } t1/2 = \ln 2/\beta.$$

The apparent volume of distribution during the steady state was calculated as MCR/ke, where ke represents the overall elimination rate constant given by the formula:

$$ke = (A + B)/(A/\alpha + B/\beta).$$

2.14 Statistical Analysis

Data was analyzed by the paired t-test since each dog served as its own control. Where multiple analysis was required, the data was analyzed by analysis of variance or the Sign-Rank test. Statistics were carried out on an IBM-PC using the statistics package, Epistat, or on a Hewlett packard Model 41CX calculator equipped with a statistics program.

Data is reported as mean $\pm$ standard error. Significance is taken at the 5% probability level.

CHAPTER 3: ANF IN NORMAL, CONSCIOUS DOGS

3.1 INTRODUCTION

The discovery of granules in mammalian hearts responsive to changes in extracellular fluid volume (deBold, Salerno, 1983) and the subsequent identification of ANF purified from rat atria suggested that the heart is an endocrine gland which has a major role in fluid homeostasis. In determining the physiological importance of this natriuretic factor, histochemical and immunocytochemical analysis and bioassays were performed in cross-species comparative studies. Extracts of canine atria evoked a natriuretic and diuretic response upon intravenous infusion to the same extent as human atria although to a much lesser extent than rat or mouse atrial extract (de Bold, Salerno, 1983). In addition, histochemical analysis of canine atria showed a rather weak immunostaining reaction as compared to that observed in rats (Chapeau et al., 1985). These studies confirmed the presence of iANF in all mammalian vertebrates hearts studied, and suggested that canine and human atria contain substantially less immunoreactive and bioactive ANF than rodent atria. Although the dog is often used in the study of ANF physiology, neither the total immunoreactive ANF nor the nature of the immunoreactive forms present in the canine atria have been described. Plasma iANF has been quantified under basal conditions in the dog (Ledsome et al., 1985; Granger et al., 1986; Verburg et al., 1986) although the nature of the

circulating forms of the peptide has not been established.

The first series of experiments presented in this chapter were designed to quantify ANF in the plasma and in several sites of both atria using a C-terminus radioimmunoassay. Atrial extracts and plasma samples, extracted on SepPak cartridge were then subjected to reverse phase-high pressure liquid chromatography to investigate the molecular heterogeneity of iANF. The atrial extracts were then analyzed by gel permeation chromatography to estimate the molecular weights of the separated fractions. The second series of experiments are an investigation of the pharmacodynamics and pharmacokinetics of infused rat-ANF in the dog. The renal and blood pressure responses to 4 doses of ANF were measured in normal dogs and a dose-response curve constructed to enable accurate interpretation of the pharmacodynamics of ANF under varying experimental conditions and also to titrate the optimal dose for further studies in caval and cirrhotic dogs.

3.2 DISTRIBUTION OF iANF IN CANINE ATRIA

The distribution of iANF in the atrium is presented in Table 3.1. iANF was found in each site analyzed, in concentrations approximately 5 - 10 times lower than that reported for rat, hamster (Chapeau et al., 1985) and

TABLE 3.1

Immunoreactive ANF (iANF) in Different Sites of Dog Atria

Site	μg iANF/g wet weight	
	Right	Left
Appendage	18.2 ±3.1	32.3** ±1.5
Anterior Wall	14.7 ±2.2	23.1*** ±1.3
Posterior Wall	15.8 ±1.7	19.6* ±1.1
Atrial Septum	14.0 ±2.2	

Values are mean ± standard error (n=7). Level of significance of the left vs right side as evaluated by paired Student's t-test: *P < 0.05, **P < 0.02, ***P < 0.001.

rabbit (Synhorst, Gutkowska, 1988). iANF was consistently higher on the left side at each site analyzed with the greatest concentration found in the left appendage. Note that with the exception of the appendage, iANF is homogeneously distributed throughout the atrium (including the septum).

3.3 CHROMATOGRAPHIC ANALYSIS OF CANINE ATRIA

The RP-HPLC profile of atrial appendage extract, shown in Figure 3.1, reveals three distinct fractions of immunoreactive material. Fraction A, representing 4% of the total iANF is eluted 3 minutes earlier than synthetic h-ANF99-126, and may represent one of the N-terminal truncated variants. 30-35% of the total iANF is eluted in fraction B while the remaining 65% of iANF, fraction C, elutes 8 minutes later.

The molecular weights of fractions B and C were estimated using gel permeation chromatography on a Sephadex G50 column (Figure 3.2). The column was calibrated with 5 molecular weight markers: 1-aprotinin (6500), 2-cytochrome C (12,400), 3-beta-lactoglobulin (18400), 4-soybean trypsin inhibitor (20,100) and 5-trypsinogen (24,000). From the log-linear plot of molecular weight versus the distribution coefficient, the molecular weights of fractions B and C were estimated as 11,700 and 16,300 respectively.

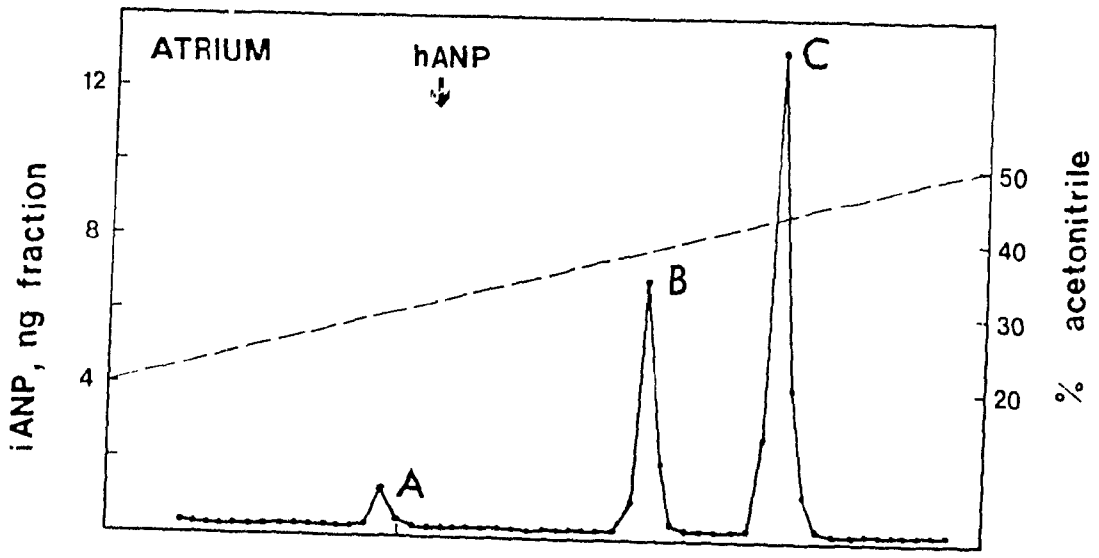

FIGURE 3.1 Reverse phase HPLC of atrial extract obtained from normal dog atrium. The arrow indicates the elution time of human ANF(99-126).

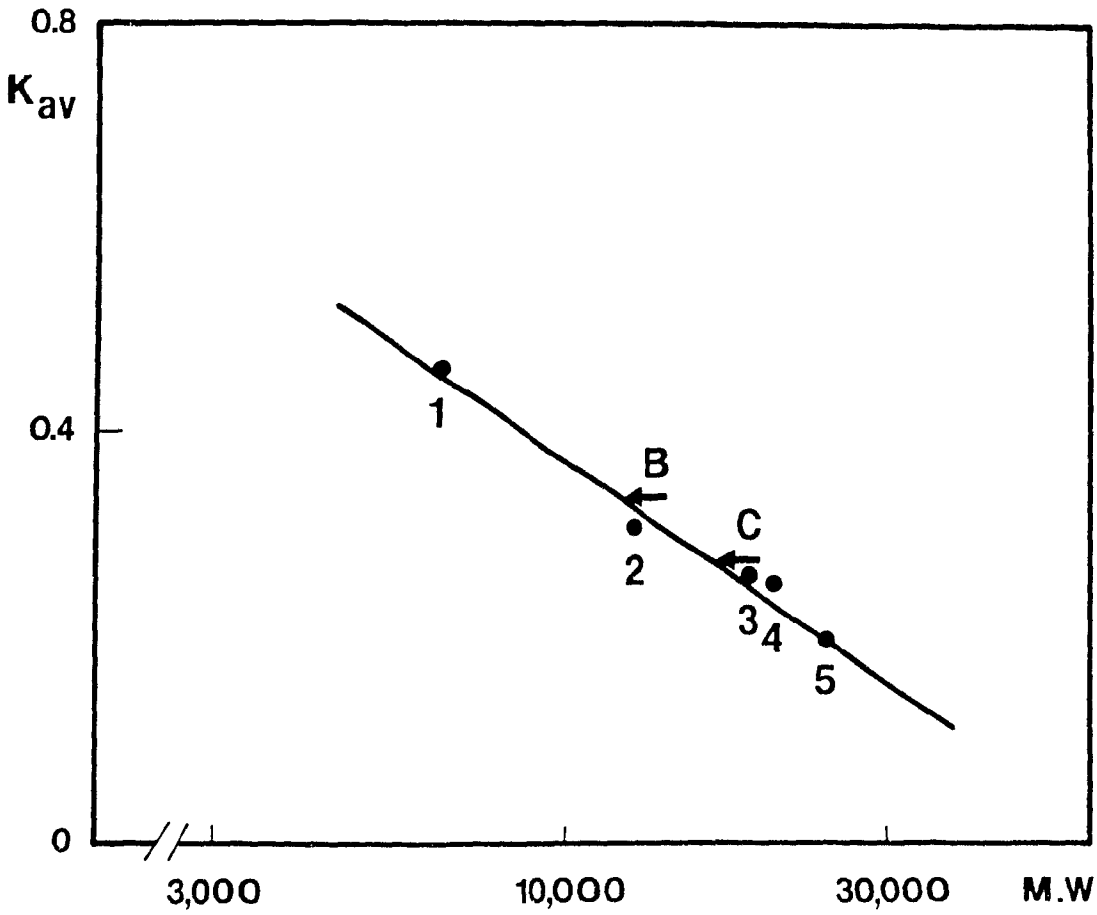

FIGURE 3.2 Molecular weight estimation of fractions B and C on Sephadex G50 column. The arrows mark the Kav values of the indicated fractions.

3.4 CHROMATOGRAPHIC ANALYSIS OF CANINE PLASMA

RP-HPLC of normal canine plasma is presented in Figure 3.3, panel A. 92% of immunoreactive material is eluted from the column as a single sharp peak with the same retention time as both synthetic human and rat ANF(99-126). 8% of iANF eluted at the same time as fraction C found in the atrial extract. The chromatographic profile of plasma sampled during the infusion of rat ANF at 175 ng/kg/min (panel B) displayed a sharp peak of iANF eluting at the same time as the control sample. No degradation products of the infused peptide were detected.

3.5 RIA OF PLASMA iANF

3.5.1 Directed Versus Extracted Plasma

The original RIA of ANF involved the direct assay of plasma samples (Tanaka, Misono, Inagami, 1984). However, the analysis of human plasma revealed that some component of the plasma interfered in the assay leading to substantially higher levels than obtained following extraction (Gutkowska et al., 1984). Thus, direct assay was believed to be unreliable in the determination of human plasma ANF. In order to establish a reliable method for the measurment of ANF in dog plasma, both the direct and extracted methods were performed on 98 samples

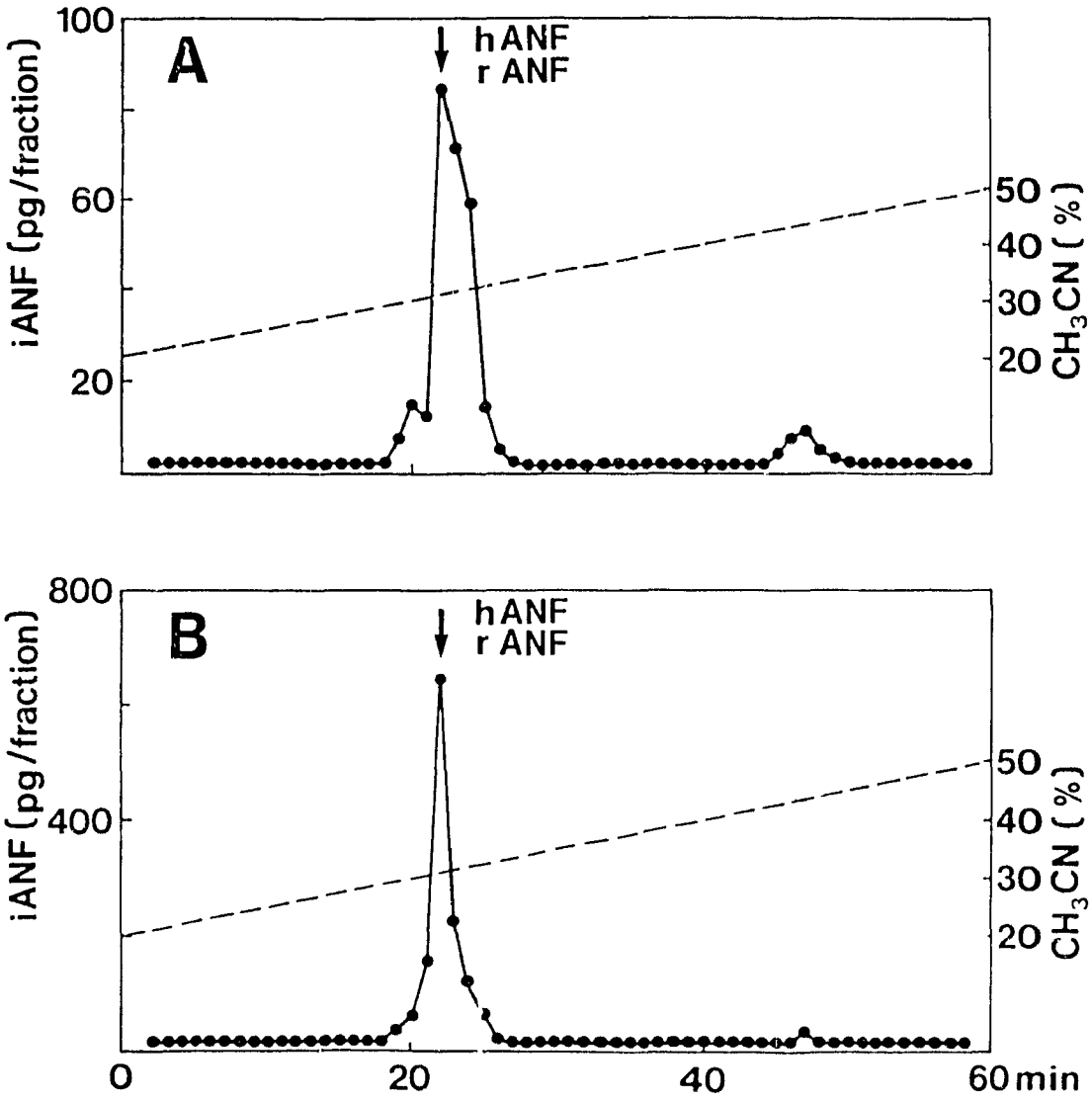

FIGURE 3.3 HPLC-profile of dog plasma extract obtained before the infusion (panel A) and during rat ANF infusion (panel B). Arrows indicate the retention time of Both rat ANF (99-126) and human ANF (99-126).

obtained from dogs prior to or during the infusion of rat ANF.

Figure 3.4 depicts the relationship between plasma iANF levels as measured with the direct assay and the same plasma samples obtained following extraction on a SepPak C18 cartridge over a range from 9.5 - 4000pg/ml. A direct correlation with coefficient of variation, r = 0.95 was derived for the two methods. Despite this strong correlation, the slope of 1.1 indicates a systematic proportional error of at least 10% in addition to a systematic constant error of 115.5 pg/ml (y intercept) in levels measured with the direct assay thus rendering it analytically unacceptable.

3.5.2 Validation of the RIA

The results of the RIA are expressed as weight equivalents of ANF99-126, as read from the standard curve (%B/BO versus log pg ANF99-126 per tube). Maximum binding of the radioligand averaged 35%, non-specific binding was less than 2%. The mid-point of the standard curve (ID50) was 12.6 $\pm$ 0.7 pg/tube (mean $\pm$ SE from 9 consecutive assays), with the detection limit of 1 pg/tube at 95% level of confidence. The assay was appropriately reproducible (intra-assay coefficient of variation (CV) of 6.7%, inter-assay CV of 12%) and

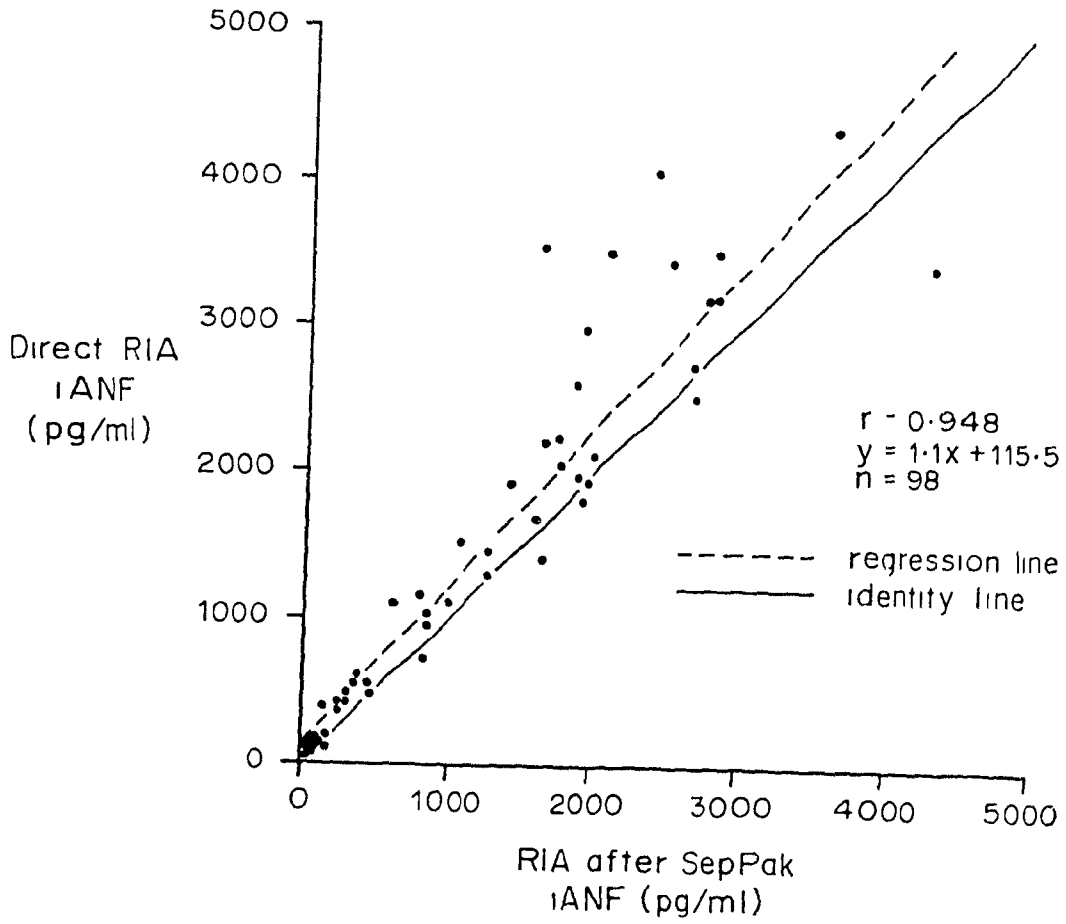

FIGURE 3.4 Comparison of direct RIA of plasma iANF (y-axis) with RIA after SepPak-extraction of plasma (x-axis).

and accurate (recovery of 85% of synthetic ANF added to the plasma. Serial dilutions of atrial extracts inhibited the binding of the radioligand in parallel with the standard curve. No inhibition was found with ventricular extracts in dilutions as low as 1/50.

3.6 DOSE-RESPONSE TO GRADED ANF INFUSION

The renal and blood pressure responses to ANF infused intravenously at doses gradually increasing from 50 to 75, 125, and 175 ng/kg/min were measured in 5 conscious dogs, each dose being infused for 45 minutes. As presented in Figure 3.5, ANF infusion produced a dose-dependent linear increase in sodium excretion (r=.991) and fractional excretion of sodium (r=.986) while urine flow remained constant. Sodium excretion rose from a control of 10 $\pm$ 4 ueq/min to 50 ueq/min at 50 ng/kg/min and 150 ueq/min at 175 ng/kg/min, apparently not achieving a maximum at this highest dose. Despite the marked natriuresis at each dose, ANF had no effect on GFR, RPF or FF (Figure 3.6). Maximum hypotension was reached at a dose of 75 ng/kg/min, higher doses producing no further drop in blood pressure. As depicted in Figure 3.7, fractional excretion of sodium rose linearly despite the fall in blood pressure to 83 mmHg.

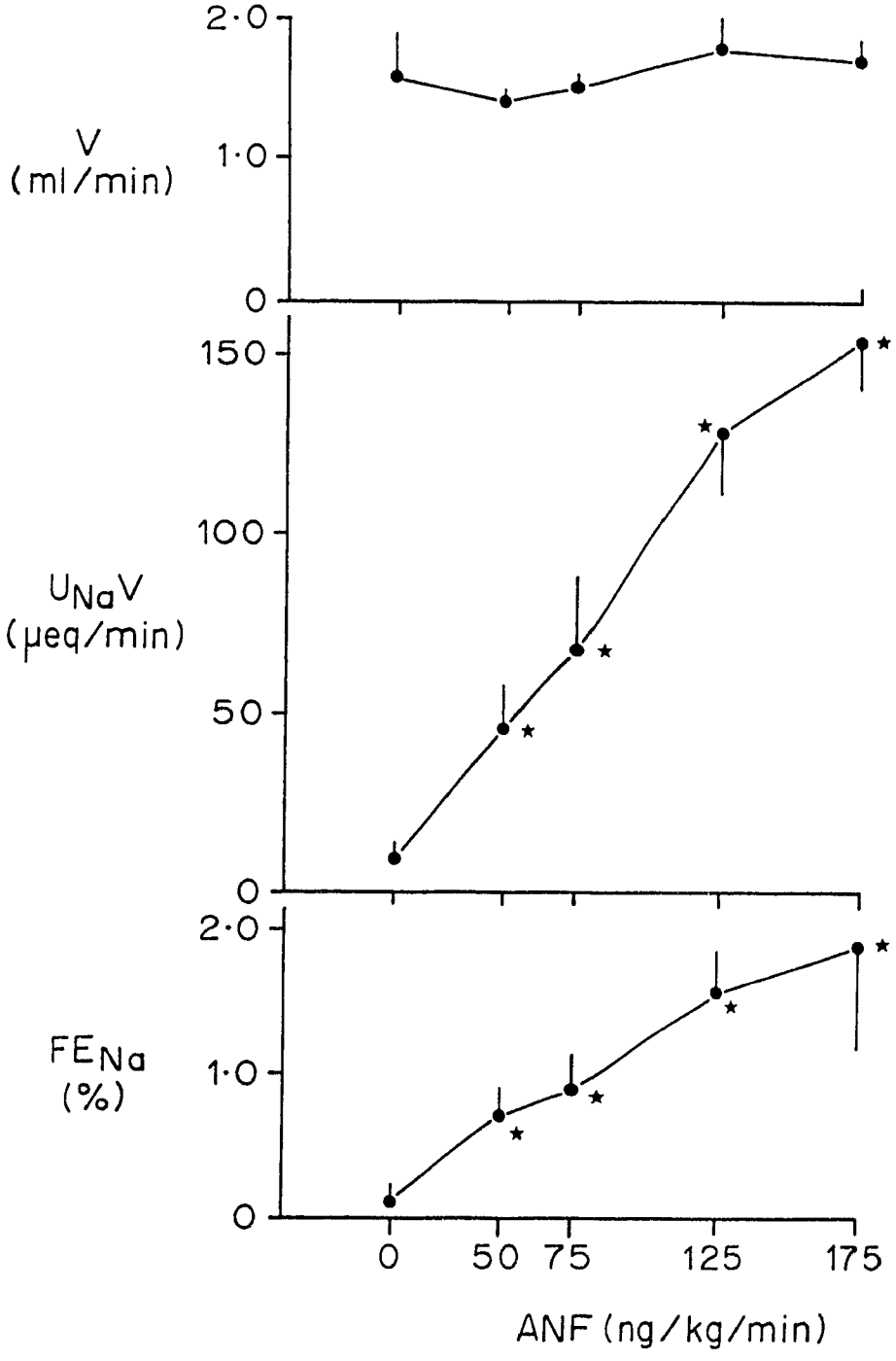

FIGURE 3.5 Effect of graded infusion of ANF (each dose during 45 minutes) on urine flow, sodium excretion, and fractional excretion of sodium. * $p<0.05$ versus control

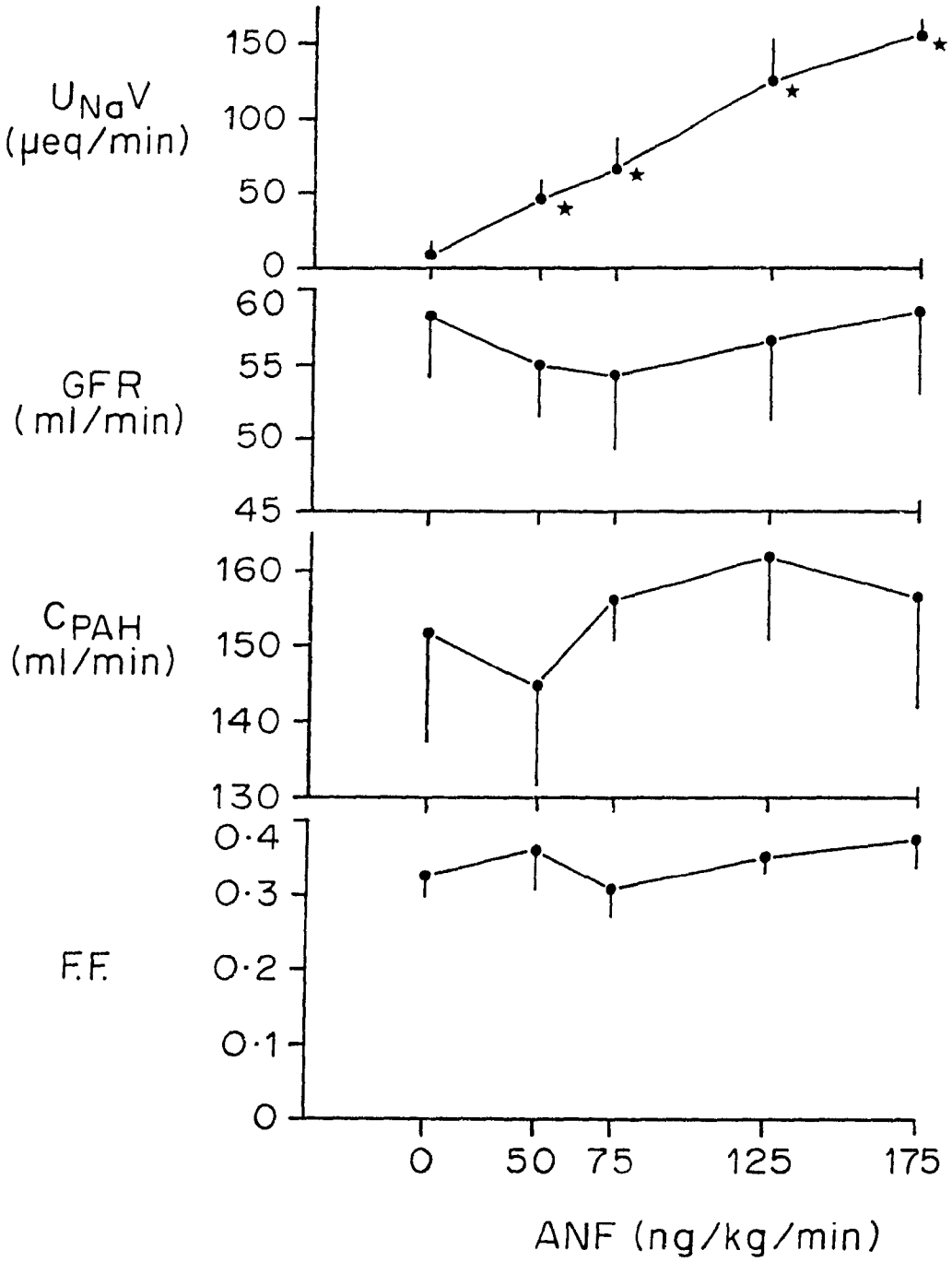

FIGURE 3.6 Effect of graded infusion of ANF (each dose 45 minutes) on sodium excretion, GFR, CPAH, and FF* $p<0.05$ versus control

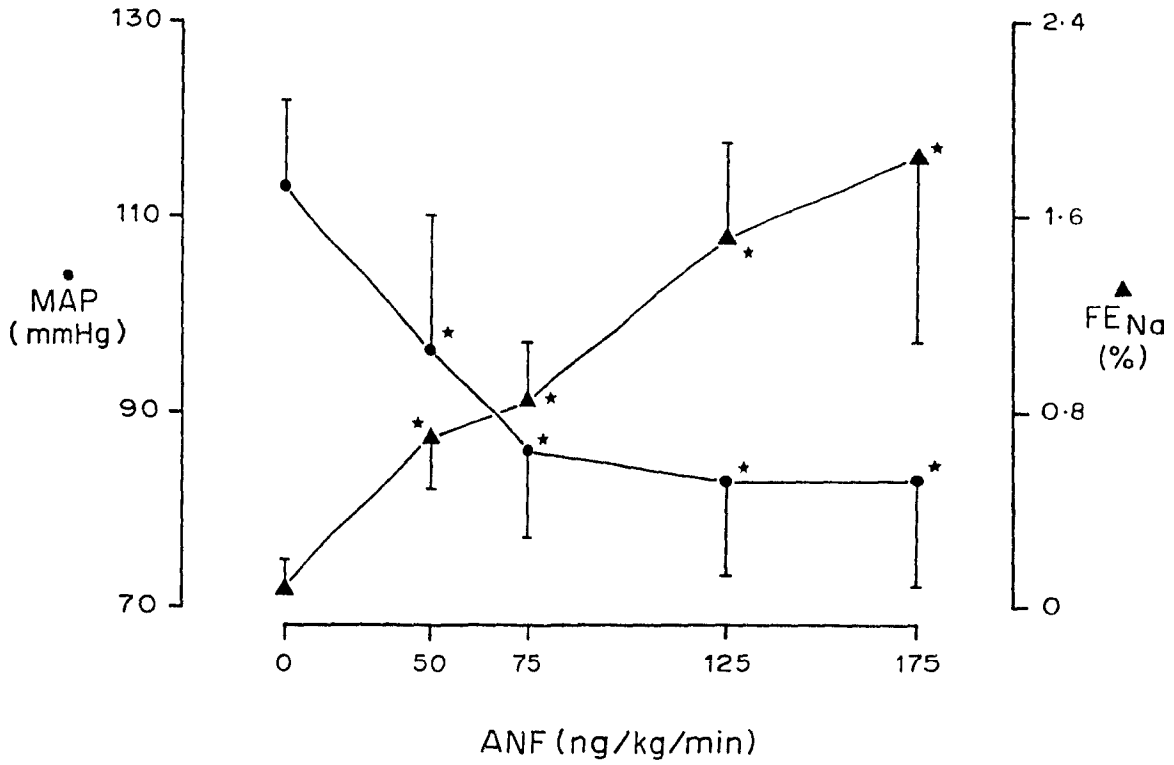

FIGURE 3.7 Effect of graded infusion of ANF on Mean Arterial Blood Pressure (circles) and Fractional Excretion of sodium (triangles) * $p<0.05$ versus control

Pharmacokinetic parameters of ANF were estimated during infusion of 175 ng/kg/min in 13 conscious dogs. Renal response to the infusion, shown in Table 3.2, does not vary significantly from the five dogs used to examine the dose-response relationship.

Plasma iANF (as measured in extracted samples) increased rapidly from the pre-infusion level of 99±14.8 pg/ml to more than 2300 pg/ml during the infusion (Figure 3.8). Repeated measurements during the infusion indicated that steady state plasma levels were achieved in the first 10 minutes and remained stable throughout the infusion. Baseline plasma iANF measured in 27 conscious dogs in our laboratory averaged 71.6 ± 9.5 pg/ml. Metabolic clearance rate calculated as (infusion rate)/(steady state plasma iANF - baseline plasma iANF) was 1.09±.19 l/min. At the end of the infusion, plasma iANF declined to below the pre-infusion level, remaining low for at least 30 minutes suggesting a suppression of the endogenous secretion of ANF by the infused peptide. The rate of disappearance was linear (r=0.98) over the first 6 minutes, as shown in Figure 3.8, panel B, corresponding to first order kinetics during this period with T1/2 of 1.8 minutes. However, the disappearance of ANF from the circulation is best described

TABLE 3.2

	Urine Flow ml/min	Sodium Excretion. μmol/min	Potassium Excretion. μmol/min
Control Mean ± SE	1.87±0.29	55.0±14.4	35.7±5.2
ANF Mean ± SE	2.64±0.33*	243.0±53.7*	60.9±5.5

	GFR ml/min	RPF ml/min	FF %
Control Mean ± SE	46.5±4.1	124.4±12.1	37.9±1.3
ANF Mean ± SE	48.3±3.5*	116.5± 9.5*	41.8±1.6*

Data were obtained in 13 conscious dogs infused with rat atrial natriuretic factor (ANF)(175ng/kg/min). FGR glomerular filtration rate; RPF, renal perfusion flow; FF filtration fraction. Level of significance of differences between control (preinfusion) and ANF infusion values (paired t-test) are *P<0.02; P<0.001.

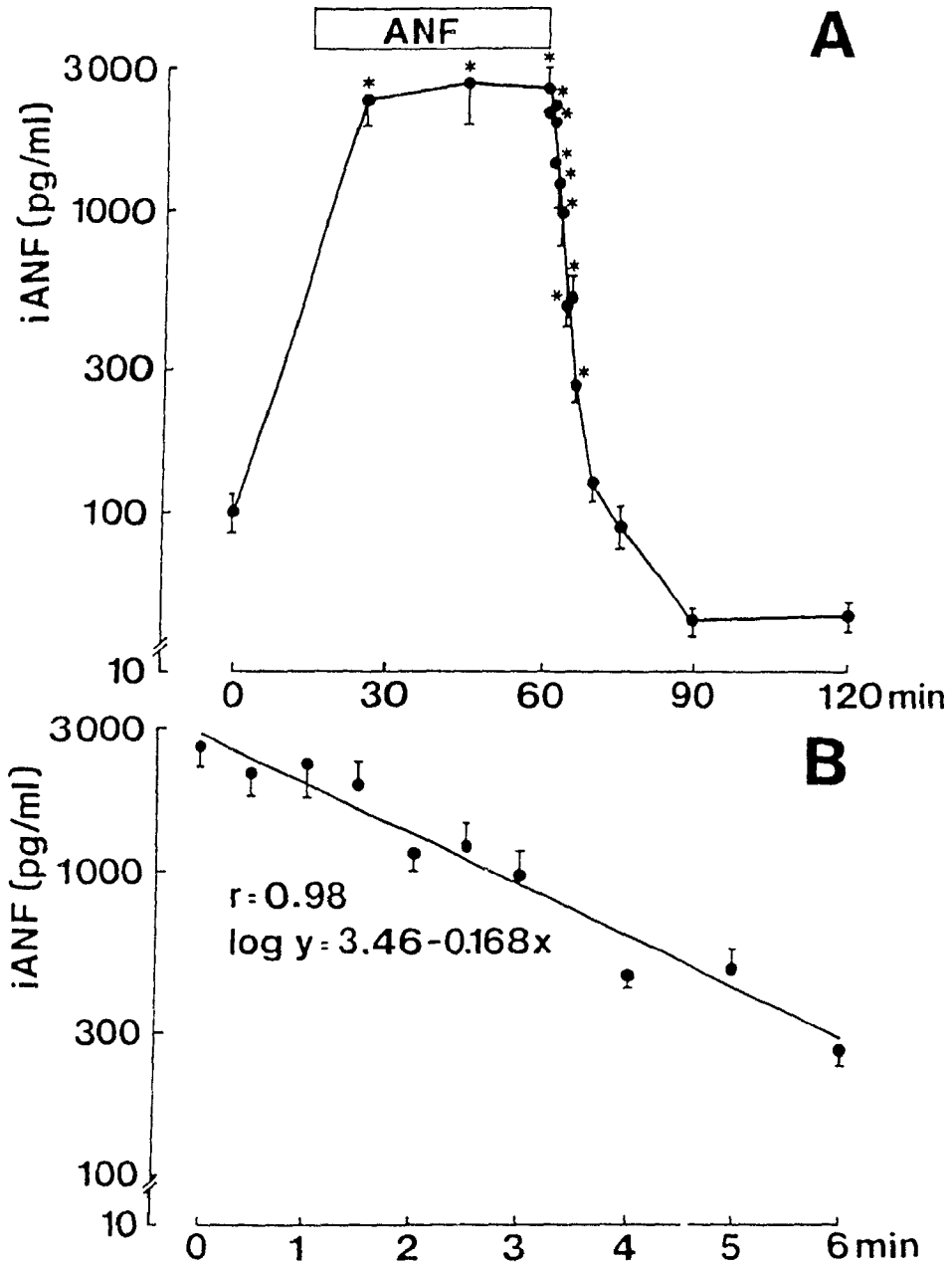

FIGURE 3.8 A: Plasma iANF level before, during and after infusion of ANF (175 ng/kg/min) in 13 dogs. Each point is mean of at least six measurements. B: Developed view of plasma iANF during first 6 minutes post-infusion. *$p<0.05$ vs. control.

by a biexponential equation, Ct=269e-0.48t+299e-.067 t (r=0.91). Disappearance half-time, calculated from the corresponding rate constants a (rapid phase) and B (slow phase), is 1.44 minutes during the rapid phase, and 10.3 minutes during the slow phase of elimination. The apparent distribution volume during the steady state is 3.66 L.

3.8 DISCUSSION

The presence of iANF was demonstrated by immunohisto- and immuno-cytochemical techniques both in dog atrial myocytes (Chapeau et al., 1985) and in canine plasma (Ledsome et al., 1985). Release of ANF in the dog is stimulated by atrial distension (Ledsome et al., 1985), acute volume expansion (Salazar et al., 1986) and high salt intake (Verburg et al., 1986) in a manner similar to that described in other species including man (Hollister et al., 1986). Although the complete amino acid structure of rat and human ANF have been described (Thibeau et al., 1985; Kangawa, Matsuo, 1984; Schwartz et al., 1985), neither atrial nor plasma canine forms have been purified or submitted to structural analysis. However, the structure of dog prepro-ANF was deduced from the nucleotide sequences of cloned cDNAs of mRNAs encoding these peptides (Oikawa et al., 1985). Comparison with other species indicates a high degree of homology of proANF structure in man, dog, beef, rat, mouse and rabbit (Oikawa et al., 1985). It also

strongly suggests that the C-terminal sequence of 28 amino acids which constitutes the circulating hormone is identical in man, beef and dog, differing from that in small rodents by a single substitution, the replacement of isoleucine in position 12 by methionine.

The RP-HPLC profile of canine atrial extract revealed 3 distinct immunoreactive fractions. The dominant form (fraction C), having an estimated molecular weight of 16,300 most probably corresponds to the high molecular weight hormone precursor, pro-ANF. One-third of the iANF (fraction B) has a molecular weight of 11,700. No corresponding peptide has been identified in the sequence analysis of the fractions from human (Miyata et al., 1985) or rat atria (Thibeau et al., 1985) although it has recently been shown that rat atria do, in fact, contain a small quantity of iANF fragment of approximately the same molecular weight as we found in the dog (Dolan, Doborsz, 1987). The physiological significance of this immunoreactive fragment is presently unclear. It may represent an intermediary product of intraatrial processing of the prohormone or may simply be an artifact due to the proteolytic degradation during the extraction procedure. Human atria contain a dimer of ANF, B-ANF, which has not been identified in any other species and appears to be cleaved prior to or during release from the atria (Miyata et al., 1985).

The HPLC profile of plasma is more homogeneous than that of the atria and is very similar to the profile obtained for human (Yamaji, Ishibashi, Takaku, 1985) and rat plasma. The single peak coeluting with hANF and rat ANF (99-126) is further evidence that the 28-residue peptide is the circulating form in the dog. It is apparent that the replacement of isoleucine, an amino acid with a rather hydrophobic side chain, by methionine, one with a rather polar side chain, does not take place in a position of the ANF molecule which is sufficiently exposed to alter the resulting hydrophobicity appreciably. This is reflected by the inability of even high-resolution chromatographic systems, such as the 0.5%/min- acetonitrile gradient used in our study, to separate rat from human ANF-(99-126) and thus only purification and complete amino acid sequencing will confirm the structure of canine ANF. However, the plasma iANF profile obtained by HPLC in addition to the data obtained by cDNA sequencing (Oikawa et al., 1985) certainly indicates that canine and human circulating ANF are identical. The presence of only a small amount of pro-ANF in the HPLC profile is consistent with data from other species suggesting that small amounts of the prohormone are either released from or leak out of the atria (Yamaji, Ishibashi, Takaku, 1985).

Of interest is the lack of circulating immunoreactive fragments which one would expect to find in the plasma as

a result of degradation. This may be explained by the rapidity of ANF disappearance from the circulation. In our normal dogs, the metabolic clearance rate of rat-ANF was 1.09 liter/minute, agreeing well with the clearance rate of 1.08 liter/minute measured during the infusion of human-ANF in conscious dogs (Verburg et al., 1986) and very similar to data by Bie et al (1988). The disappearance half-time found in our experiments (1.44 minutes in the rapid phase and 10.3 minutes during the slow phase) is considerably shorter than the time reported by Verburg et al. (1986). Their rapid-phase half-time of 3.8 minutes and slow-phase half-time of 21 minutes are most likely overestimates since the disappearance curves were constructed from a few samples taken over long intervals (10 minutes). Our curves, however, were constructed from samples obtained every 30 seconds within the first 3 minutes of the post-infusion period thus ensuring a more accurate estimate of the half-life in the circulation. A recent examination of the hydrolysis of ANF in rat circulation using sequence analysis demonstrated that a 16-amino acid fragment was generated within the first 45 seconds (the earliest sampling period) of infusion and was followed by the generation of at least 3 other fragments by 2 minutes (Condra et al., 1988). It is presently unknown whether these fragments are large enough to exhibit appreciable cross-reactivity with the C-terminal polyclonal antibody

used in our assay and thereby ensure detection. Alternatively, there is evidence that ANF may be internalized and degraded intracellularly (Hirata et al., 1985) in which case the fragments would not appear in the plasma. The quantitative measurement of plasma ANF by radioimmunoassay has been one of the most important tools which has enabled rapid progress in this field. However, it was found in the early studies that the direct RIAs of plasma iANF gave considerably higher values than those obtained after various extraction procedures. In human plasma, the positive bias of the direct RIA is as large as 80 to 200 pg/ml (Gutkowska et al., 1986; Yandle et al., 1986; Cernacek et al., 1988) thus giving higher values by a factor of greater than 3 when compared with the average normal levels in man in the range from 10 to 30 pg/ml (Gutkowska et al., 1986; Cernacek et al., 1988). The difference between the direct RIA and the values after the SepPak-extraction which we found in dog plasma is strikingly similar to that in other species, pointing to a common cause of this phenomenon, now generally assumed to be due to interference of plasma proteins in the RIA (Cernacek et al., 1988). In the present study, the results obtained by the direct RIA were compared with those after extraction over a very broad concentration range, from normal to more than 4500 pg/ml. The combination of a 10% systematic proportional error of approximately 100 pg/ml

with a constant error leads to overestimation of plasma iANF levels when using the direct RIA. Plasma samples should therefore be extracted before assay.

The quantification of atrial iANF in the present study demonstrated that canine iANF content is approximately 5-10 times lower than that reported for rat (Chapeau et al., 1985) and rabbit (Synhorst, Gutkowska, 1988) atria whether expressed per mg tissue weight or per mg protein. iANF was consistently higher in the canine left atrium as compared to the right, the same pattern which has recently been described in rabbits (Synhorst, Gutkowska, 1988). The opposite relationship is found in rat atria (Tanaka et al., 1984; Ding et al., 1987) while human atria contain equivalent amounts of the stored peptide. The importance of this species-specific difference is unclear and may not be physiologically significant as both rats and dogs have similar natriuretic responses to infused ANF (Lang et al., 1985) and can release similar quantities upon atrial stimulation. Indeed, this asymetry in ANF content does not reflect a difference at the level of gene expression since it has been shown in rats that the baseline pro-ANF mRNA content is the same in both atria and increases to the same level with DOCA-salt treatment (Lattion et al., 1986). Baseline plasma iANF reported in 27 conscious dogs (71.6 $\pm$ 9.5 pg/ml), determined using extracted plasma, are remarkably consistent with levels previously reported for

normal anesthetized dogs (74 pg/ml (Ledsome et al., 1985) and 68.8 pg/ml (Zimmerman et al., 1987)) and conscious dogs (75 pg/ml (Verburg et al., 1986) and 69 pg/ml (Granger et al., 1986)). Rat (1-28) ANF was chosen for the infusion studies before it became clear that dog and human ANF are identical. However, a comparison of rat and human (1-28) ANF infusion in conscious dogs demonstrated that the two peptides elicit the same renal and systemic hemodynamic effects (Seymour et al., 1986). The dose-response of our 5 conscious dogs to infused rat ANF was studied at pharmacological levels, the lowest dose leading to a 12 fold increase in plasma ANF. Within the relatively narrow dose range, plasma iANF and sodium excretion rose as a direct linear function of the infusion rate indicating a closely dose-dependent natriuretic response. The natriuretic response did not appear to achieve a maximum at the highest dose of ANF (175 ng/kg/min). This is confirmed by a study in anesthetized dogs where intrarenal infusion of ANF at 300 ng/kg/min produced an almost 2 fold higher natriuretic response than what we measured with 175 ng/kg/min (Burnett, Granger, Opgenorth, 1984).

Natriuretic response to various doses of ANF producing plasma levels in the physiological range has recently been reported in conscious dogs (Bie et al., 1988) demonstrating that even at these low dose infusion rates, sodium

excretion is enhanced and rises linearly in a dose-dependent manner.

It has been suggested that changes in renal hemodynamics play a major role in determining the natriuretic response to ANF (Goetz, 1988). As discussed in Chapter 1, the role of GFR has been particularly controversial. In the present study, GFR did not change at any dose of ANF, thus apparently having no role in the ensuing natriuresis. This, of course, does not suggest that an increase in GFR will not contribute to the natriuresis but rather emphasizes a more important role for the tubule in the natriuretic response to ANF.

It has been demonstrated that reduction of renal perfusion pressure (Burnett, Opgenorth, Granger, 1986; Sosa et al., 1986) abolishes the natriuretic response to ANF. The natriuretic response to ANF was linear even in the presence of marked hypotension. However, it is possible that higher perfusion pressure would have permitted an even greater increase in sodium excretion. That is, preventing a fall in blood pressure by administration of a vasoconstrictor may enhance the natriuresis at each dose, although, clearly, a more substantial reduction in blood pressure would be required to abolish the natriuretic response in the conscious dogs.

CHAPTER 4: HETEROGENEOUS RENAL RESPONSES TO ATRIAL NATRIURETIC FACTOR: CHRONIC CAVAL DOGS

4.1 INTRODUCTION

It is clear from the investigation of normal dogs that ANF is secreted from canine atria, circulates with an average T1/2 of 2 minutes, enhances sodium excretion and, as such, is most likely an important modulator or regulator of ECF volume in health. However, as discussed in section 1.5, its role in the pathophysiology of edema is less certain. Atria of edematous patients or animals release ANF normally in response to physiological stimuli such as volume expansion (Burnett et al., 1986) and water immersion (Epstein et al., 1986; Leung et al., 1987). However, the kidney excretes only a fraction of the sodium normally excreted in response to these stimuli.

The experiments described in this chapter were undertaken to closely examine the profile of the renal and systemic hemodynamic response to infused ANF and its relationship to plasma and atrial iANF in the chronic caval dog. This model of low-output heart failure was chosen because it is well defined and, following a variable period of avid sodium retention, the dogs will return to urinary sodium balance despite the persistence of ascites. Sequential observations thus makes it possible to study the renal response to ANF when sodium handling is either normal or abnormal, both phases co-existant with volume expansion and edema formation, and where the renin-angiotensin system (which exercises a reciprocal effect to ANF on the renal

tubule) is either normal or activated. Each dog was studied prior to caval constriction to establish its control response to ANF thus providing a basis for comparison with the other phases.

4.2.1 CHARACTERISTICS OF TIVC DOGS

Table 1 summarizes data obtained in 14 TIVC dogs studied in a control phase and again during a phase of active sodium retention and ascites formation. Ten of these animals were re-studied when they had returned to a state of urinary sodium balance despite the persistence of ascites. The dogs were maintained on 45 mEq Na/day during all three phases of the study. Note that body weight increases as ascites collects within the peritoneal compartment, and that plasma volume increased from control levels. ABP and CO remained unchanged in the salt-retaining phase of the study despite a decline in CVP. The UNa concentration in aliquots of urine collected at the start of an experiment reflects the degree of sodium retention at the time and is confirmed by the 24 hour urinary sodium excretion data and the UK/UNa + UK ratio, an index of distal tubular cationic exchange. GFR and renal perfusion were unchanged regardless of the state of sodium balance. Plasma renin activity and aldosterone are elevated during the phase of avid sodium retention. The decline in aldosterone to control levels when sodium balance had been

restored indicated a normalization of the renin-angiotensin-aldosterone system in this phase.

4.2.2 HPLC OF ATRIAL EXTRACTS

Figure 4.1 compares the RP-HPLC profile of immunoreactive iANF from an extract of TIVC atria to that from an extract of normal dog atria. This qualitative analysis reveals no atypical peak of immunoreactivity in the TIVC atria. The presence of the same fractions in both extracts indicates that the intracellular processing of the prohormone is normal in the TIVC dog with sodium retention and ascites.

4.2.3 ATRIAL iANF

The atrial iANF content in 7 normal dogs and 8 TIVC dogs with avid sodium retention and ascites is presented in Figure 4.2. As reported in Chapter 3, atrial content of normal dogs is higher on the left side. This difference disappears in the TIVC dog as left atrial iANF as measured in the atrial appendage falls to levels similar to that obtained from the right side. Note that the right atrial concentration is unchanged from control.

4.2.4 SEQUENTIAL MEASUREMENT OF PLASMA HORMONES

Figure 4.3 depicts the sequential changes in plasma ANF, renin plasma renin activity, aldosterone and

TABLE 1

SOME EXPERIMENTAL OBSERVATIONS IN TIVC DOGS WITH ASCITES

	Control (N = 14)	Sodium Retention & Ascites (N = 14)	Sodium Balance & Ascites (N = 10)
Body weight - kg	14 ± 1.2	15.4 ± 1.3[e]	16.2 ± 1.3[f]
Plasma volume - ml	532 ± 57	635 ± 43[e]	-
Hematocrit - %	49 ± 2	35 ± 2[e]	37 ± 2
ABP - mmHg	117 ± 3	118 ± 3	-
CVP - cm H_2O	4.2 ± 0.3	1.0 ± 0.2[e]	-
CO - litres/min	2.06± 0.10	2.01± 0.21	-
PVR - mmHg/L/min	62 ± 6	68 ± 8	-
P_{Na^+} - mEq/L[a]	149 ± 2	147 ± 2	149 ± 3
P_{K^+} - mEq/L[b]	4.3 ± 0.1	4.6 ± 0.3	3.9 ± 0.3[f]
GFR - ml/min	42.3 ± 3.3	42.2 ± 2.4	41.7 ± 4.3
C_{PAH} - ml/min	104 ± 8.4	108 ± 8.5	113 ± 26
FF - %[c]	41 ± 2	42 ± 4	43 ± 5
U_{Na} - mEq/L	106 ± 11.7	3.1 ± 0.9[e]	93.8 ± 15.1[f]
$Uk/U_{Na} + U_k$ - %[d]	44 ± 4	96 ± 1[e]	50 ± 5[f]
24 hr sodium excretion - mEq/day	44.6 ± 1.8	3.3 ± 0.8[e]	44.2 ± 1.4[f]
Plasma aldosterone ng/100 ml	7.6 ± 1.6	146 ± 31.9[e]	8.7 ± 3.3[f]
Plasma Renin activity ng/ml/hr	2.36± 0.54	12.65 ± 2.46[e]	-
Plasma AVP pg/ml	5.76± 1.08	10.37 ± 2.7[e]	-

[a] plasma sodium

[b] plasma potassium

[c] filtration fraction (GFR/C_{PAH})

[d] U_{Na} = urinary sodium concentration; U_k = urinary potassium concentration. The ratio $U_k/U_{Na} + U_k$ has been used as an index for distal tubular cationic exchange.

[e] $P < 0.05$ compared to control phase

[f] $P < 0.05$ compared to previous phase

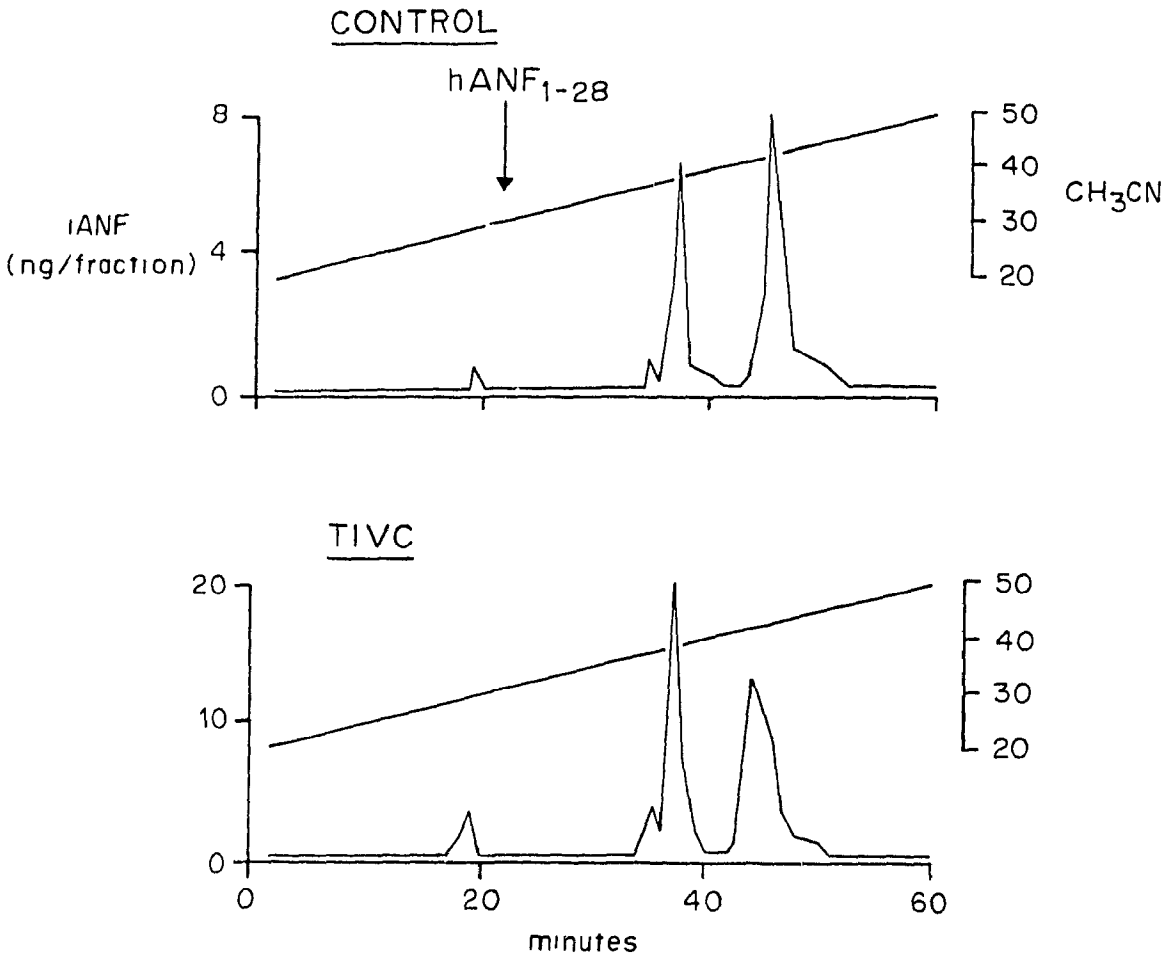

FIGURE 4.1 RP-HPLC profile of atrial extracts from normal dog (upper panel) and TIVC dog (lower panel). Arrow indicates retention time of human ANF (99-126).

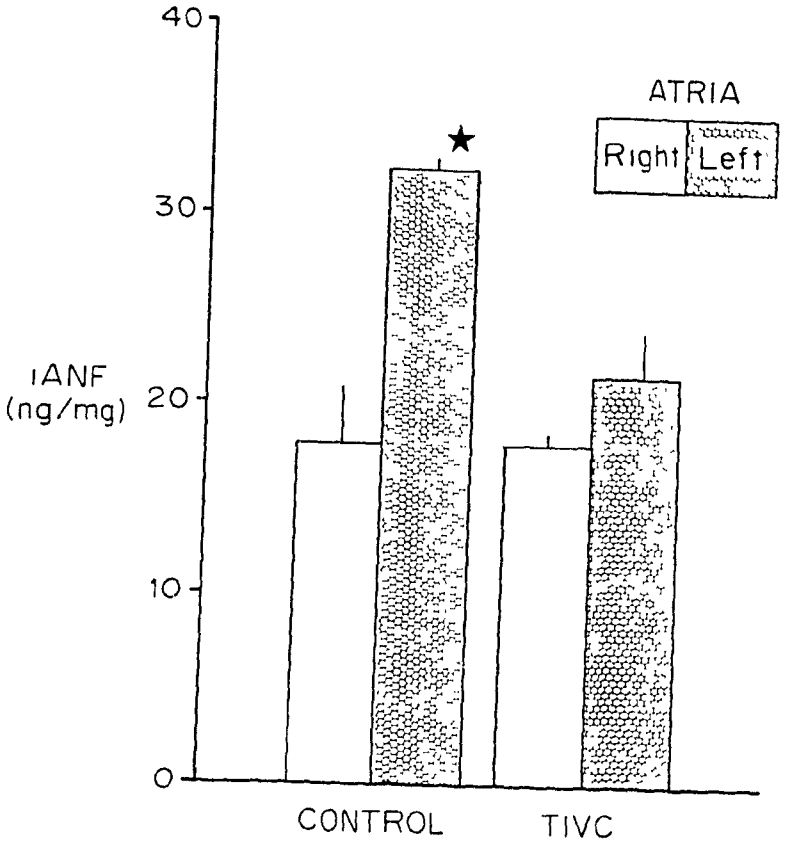

FIGURE 4.2 Comparison of atrial iANF content (ng/mg wet atrial weight) in control and TIVC dogs. right atrium, left atrium. *p<0.05 vs. right side.

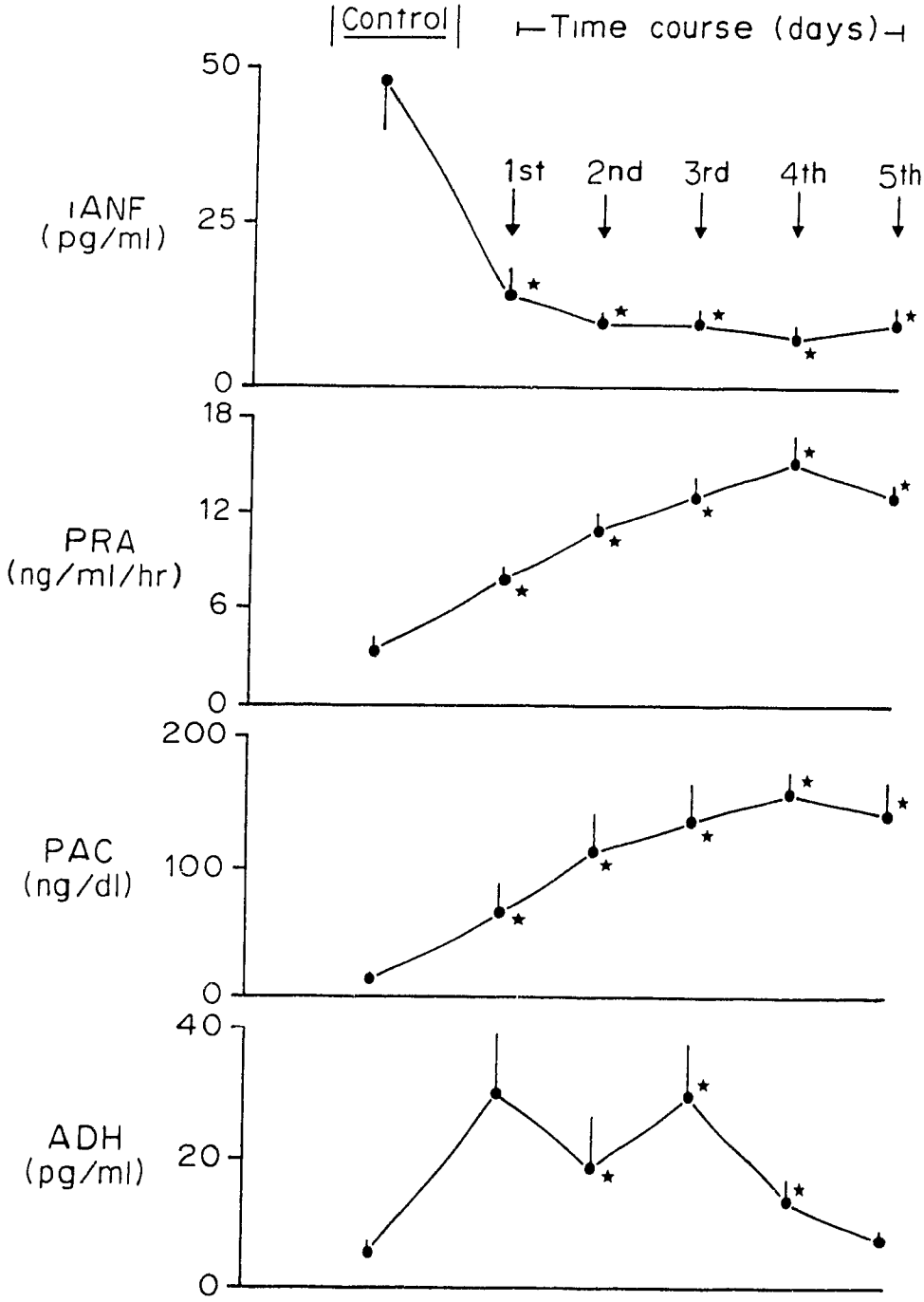

FIGURE 4.3 Time course of plasma hormones, iANF, renin (PRA), aldosterone (PAC), vasopressin (ADH) from control to Day 5 in TIVC dogs. *p<0.05 vs. control

vasopressin from a pre-surgical control state to the appearance of ascites in 8 TIVC dogs. Ascites was present in all dogs by 5 days post-constriction. Plasma iANF fell from 48.1±9.5 to 13.9±4.6 pg/ml on day 1 ($p<.05$) and remained at this low level throughout the period of sodium retention. Similarly, plasma renin and aldosterone are elevated by 24 hours after surgery, rising even further to a maximum by day 4. Plasma vasopressin, while elevated until day 3, returned to normal by day 5.

4.2.5 Pharmacokinetics of infused ANF

T1/2 averaged 1.99 ± 0.09 minutes in the normal dogs and 1.88 ± 0.19 minutes in the TIVC dogs ($p>0.05$). Metabolic clearance rate during the phase of active sodium retention and ascites (1.11 ± .19 l/min) was unchanged from the control rate of 1.09 ± .19 l/min.

4.3 RENAL RESPONSE TO ANF INFUSION

The study of the dose-response to ANF in normal unanesthetized dogs (Figure 3.5) showed a consistent natriuretic response with as little as 50 ng/kg/min. However, the highest dose, 175 ng/kg/min, was chosen for this study in order to enable comparison of the data with a previously published study in the caval dog also using 175 ng/kg/min (Freeman et al., 1985). Figure 4.4 summarizes the diuretic and natriuretic response to i.v.

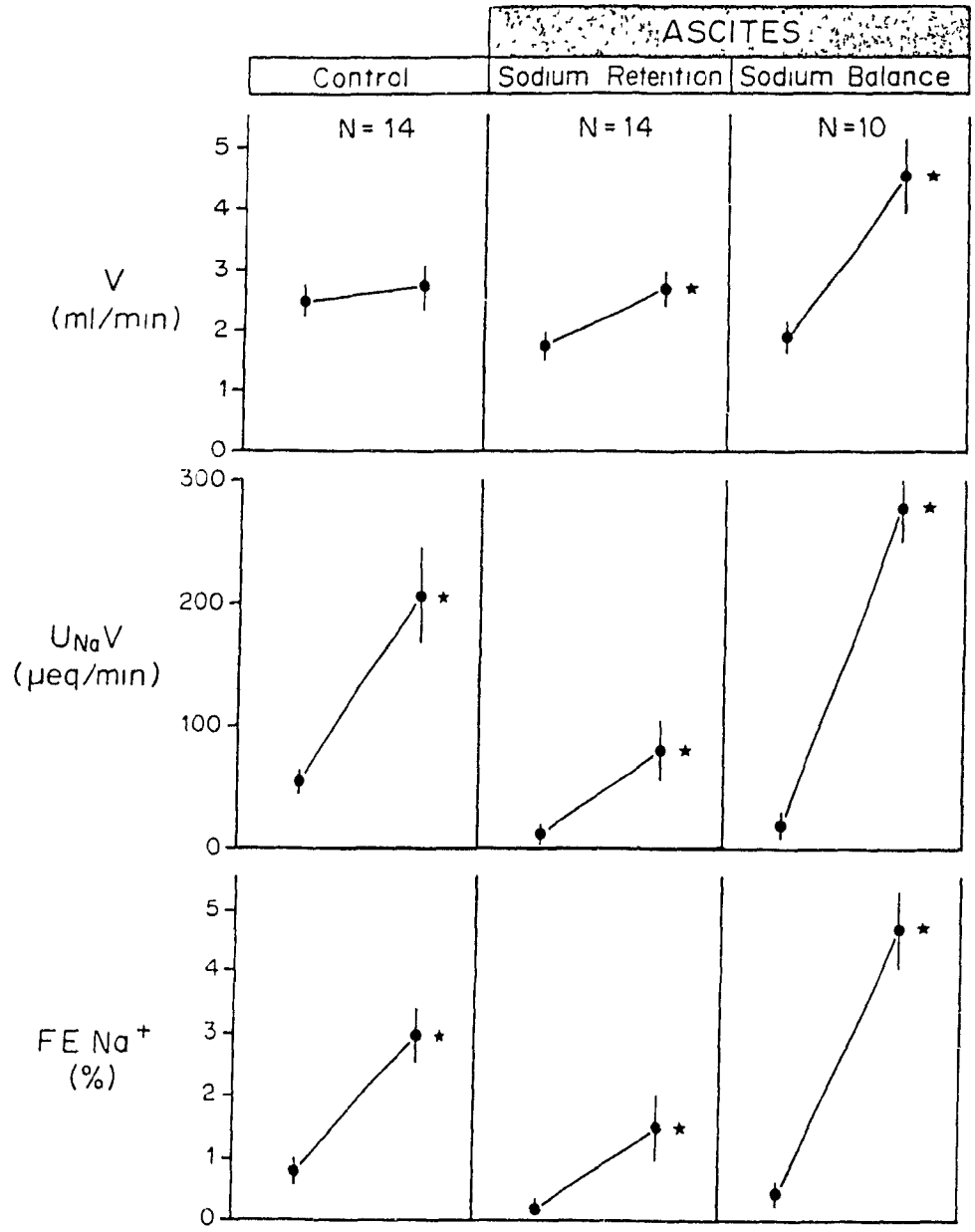

FIGURE 4.4 Diuretic and natriuretic response to rat (1-28) ANF i.v. at 175 ng/kg/min in 14 dogs prior to surgery and in chronic caval phase with ascites, both in sodium retention and sodium balance. Values are mean + SEM of 3-4 clearance periods in each phase. *$p<0.05$ vs. pre-infusion.

ANF in 14 TIVC dogs studied sequentially from the control phase through the phase of active sodium retention in the presence of ascites to the phase of restored sodium balance.

During the control phase of the study, there was no diuretic response to ANF, but a brisk and highly significant natriuretic response, with UNaV rising from 56.3 $\pm$ 9.8 uEq/min to 206 $\pm$ 14.1 uEq/min. Fractional excretion of water rose by only 0.4% (NS). During the accumulation of ascites and active sodium retention, a diuretic response appeared and was magnified ('V = 0.76 vs, 2.64 ml/min) during the balance phase. F.E. H2O rose by 0.7% during the sodium retention phase ($p<0.05$) and by 4.8% ($p< 0.05$) during the final phase, this latter figure being significantly different from that obtained during the previous phase. Of interest was that the natriuretic response was severely blunted during the phase of sodium retention only to become magnified during the final experimental phase. Figure 4.5 compares the diuretic and natriuretic responses between the control and sodium balance phases of the study. ΔUNaV was 132 uEq/min during the control period; 67 uEq/min during the ascitic-sodium retaining phase and 265 uEq/min during the final ascitic-Na balance phase. Fractional excretion of sodium was 2.8% post-ANF in the control phase but averaged 4.7% in the final TIVC phase. Similarly,Δ V

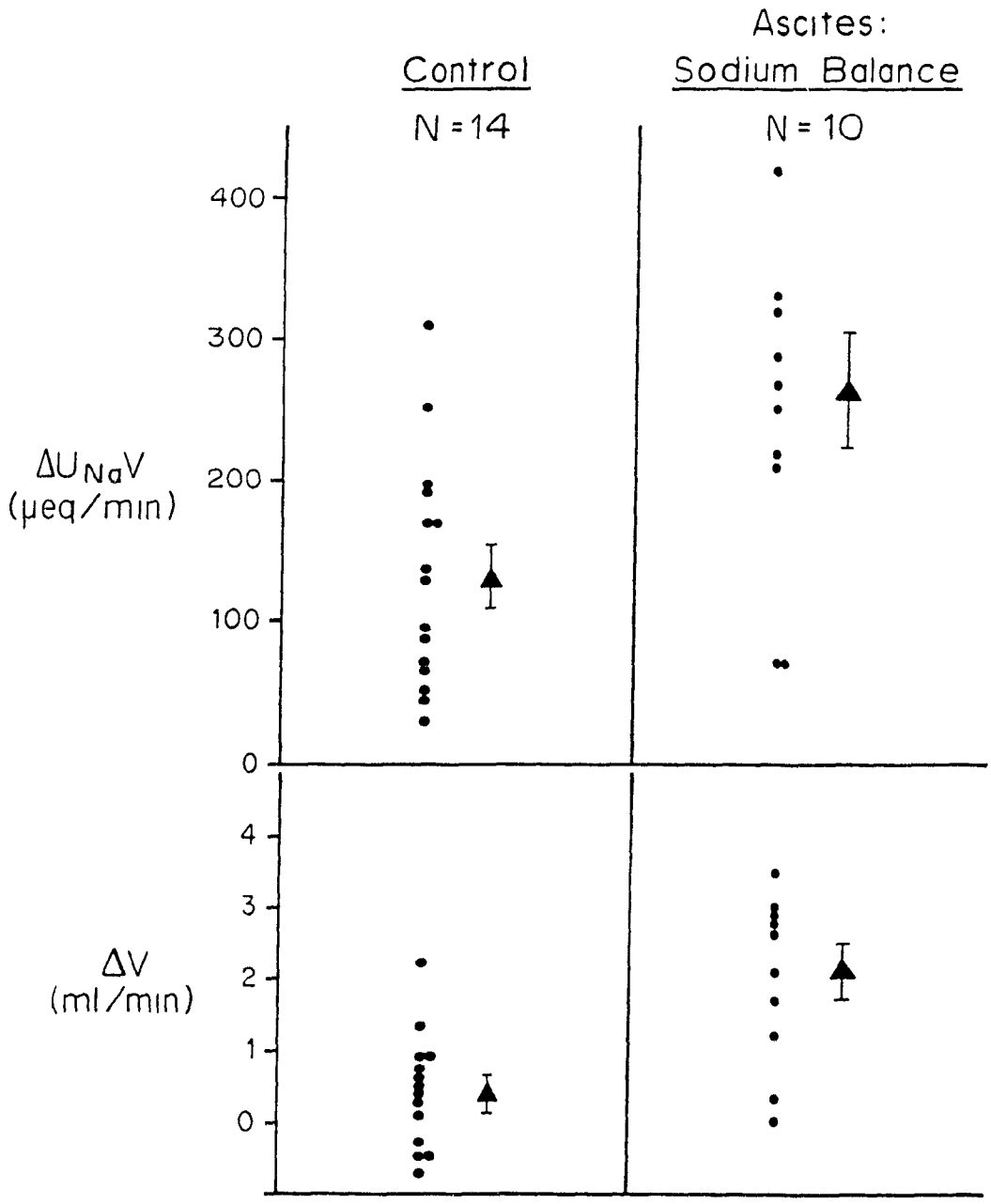

FIGURE 4.5 A comparison of the changes in both sodium and water excretion in response to ANF in dogs in the pre-surgical phase and in the caval phase with ascites in sodium balance.

increased from 1.5 ml/min in the control phase to 2.2 ml/min ($p<0.05$) in the balance phase.

Figure 4.6 summarizes the renal hemodynamic changes in response to ANF for each experimental phase. The GFR response was quite variable, being absent for control dogs and present to a variable extent for the TIVC dogs. CPAH did not change in any phase of the experiment.

4.4 IDENTIFICATION OF HETEROGENEOUS NATRIURETIC RESPONSE

When the natriuretic response of the caval dogs was examined in detail during the sodium-retaining phase, it became clear that the animals were dividing into natriuretic "responders", i.e. those dogs that excreted a dUNaV of at least 30 uEq/min in response to ANF, and those who were completely "non-responders". Figure 4.7 summarizes these data in relation to changes in renal plasma flow and GFR. Of 14 dogs, there was a 50:50 division into 7 responders (mean ΔUNaV = 133 ± 32 uEq/min; range: 30-265 uEq/min) and 7 non-responders (mean ΔUNaV = 1 ± 1 uEq/min) following ANF infusion. The natriuretic response or lack of response was correlated neither to changes in renal perfusion, GFR nor filtration fraction. This feature was also independent of the degree of daily sodium retention. Responders excreted on average 3.9 ± 2 mEq/day (91.3% dietary sodium retention) and the

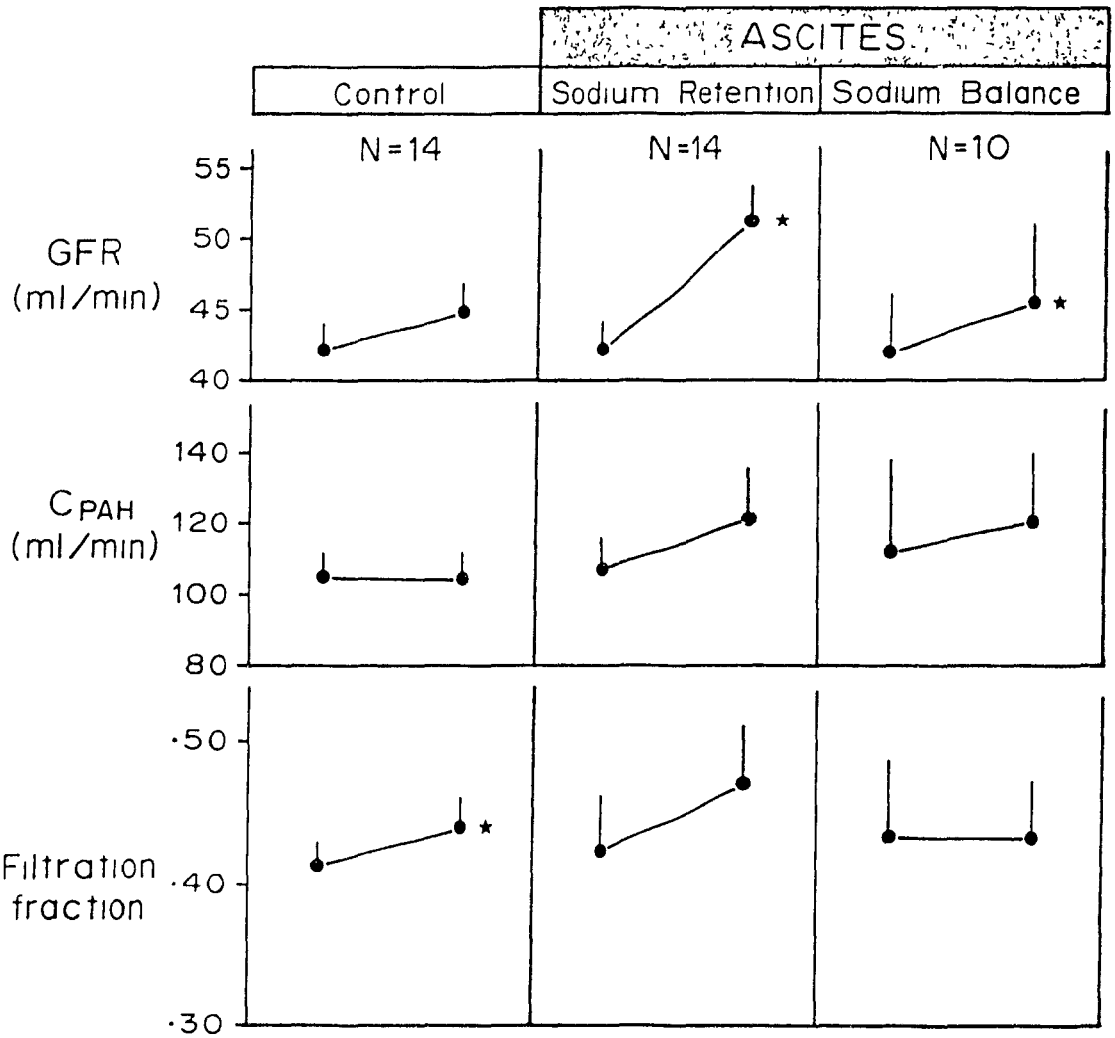

FIGURE 4.6 Renal hemodynamic response to ANF in 14 dogs in the pre-surgical phase and in the chronic caval phase with ascites during both urinary sodium retention and balance. *p<0.05 vs. pre-infusion

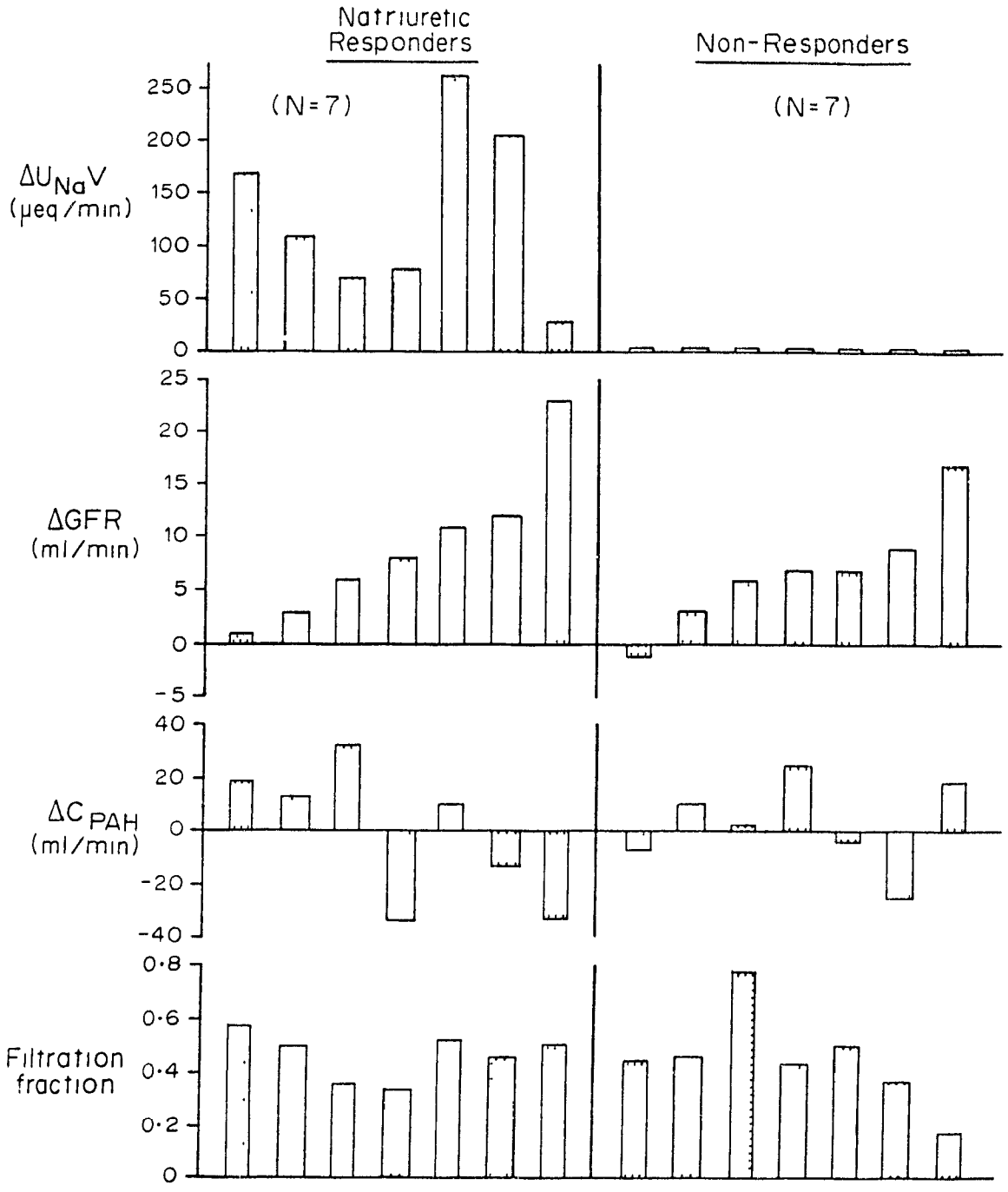

FIGURE 4.7 The relationship of magnitude of natriuretic response in chronic caval dogs with ascites either responding or non-responding to ANF to GFR and renal perfusion.

non-responders excreted 2.9 ± 0.7 mEq/day (93.5% retention). These differences were not significant. The dissociation of GFR from the natriuretic response was also observed in dogs in the control and TIVC-sodium balance phases. All dogs in each of these phases responded to ANF. This data is summarized in Figure 4.8. Note that in these experimental phases the range of natriuretic response was 30-250 uEq/min for 14 control dogs, and 70-415 uEq/min for 10 dogs studied while caval and in sodium balance with ascites. Since completing the sequential studies reported here, another 32 TIVC dogs have been prepared for other aspects of this investigation. When ANF was administered in the ascitic-salt retaining phase only, the 32 animals divided into 15 responders with a mean ΔUNaV = 185 ±35 uEq/min and 17 non-responders having a mean ΔUNaV = 2 ± 1 uEq/min. These two groups did not appear to differ from the original 14 dogs and within the group could not be differentiated (natriuretic responders vs. non-responders) in terms of GFR: 39 ± 7 versus 41 ± 3 ml/min (NS), CPAH: 123 ± 10 versus 129 ± 9 ml/min (NS), UNa of an aliquot of urine taken from a 24 hour collection: 18.5 ± 1.8 versus 15.3 ± 2.4 mEq/L (NS). Though ascites volume was not actually measured, the animals appeared to be randomly distributed with regard to this variable in each of the groups. Animals with minimal amounts of ascites were not

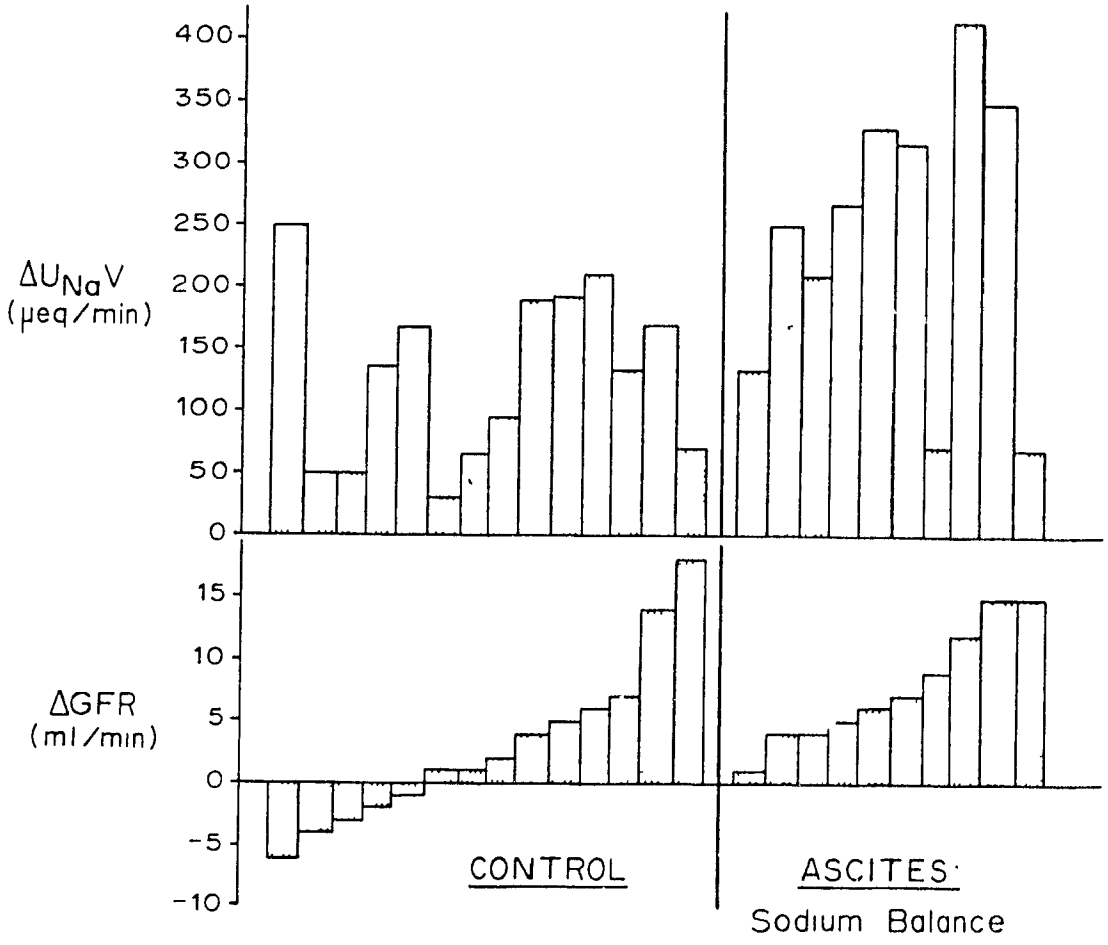

FIGURE 4.8 Relationship of change in sodium excretion to change in GFR in control dogs and again in the chronic caval phase with ascites and sodium balance.

relegated to the responders and dogs with large volumes of ascites did not appear only among the non-responders. The natriuretic response to ANF is summarized in Figure 4.9 for these 32 dogs. As far as can be determined, these two groups of dogs did not differ from each other in terms of ABP, mean GFR, mean CPAH and the volume of ascites present.

4.5 RESPONDERS AND NON-RESPONDERS IN SODIUM BALANCE WITH ASCITES

Of the 14 dogs studied in the ascites-sodium retention phase, we were able to bring 10 (5 natriuretic responders and 5 non- responders) into the final sodium balance phase. Figure 4.10 compares for each of these dogs, their response to ANF in each of the last two experimental phases when ascites is present. Note that the 5 responding dogs still respond to ANF, and that the 5 non-responders now all demonstrate a significant natriuretic response, where none was demonstrated before. The dogs previously non-responding now showed a dUNaV in response to ANF that ranged between 90 and 340 uEq/min. It is worth noting that although time-control studies for the repetitive administration of ANF to normal dogs in sequential fashion has not been carried out, we have never found a single normal dog (of more than 80 now studied in our laboratory) that did not have a significant and pronounced natriuresis in response

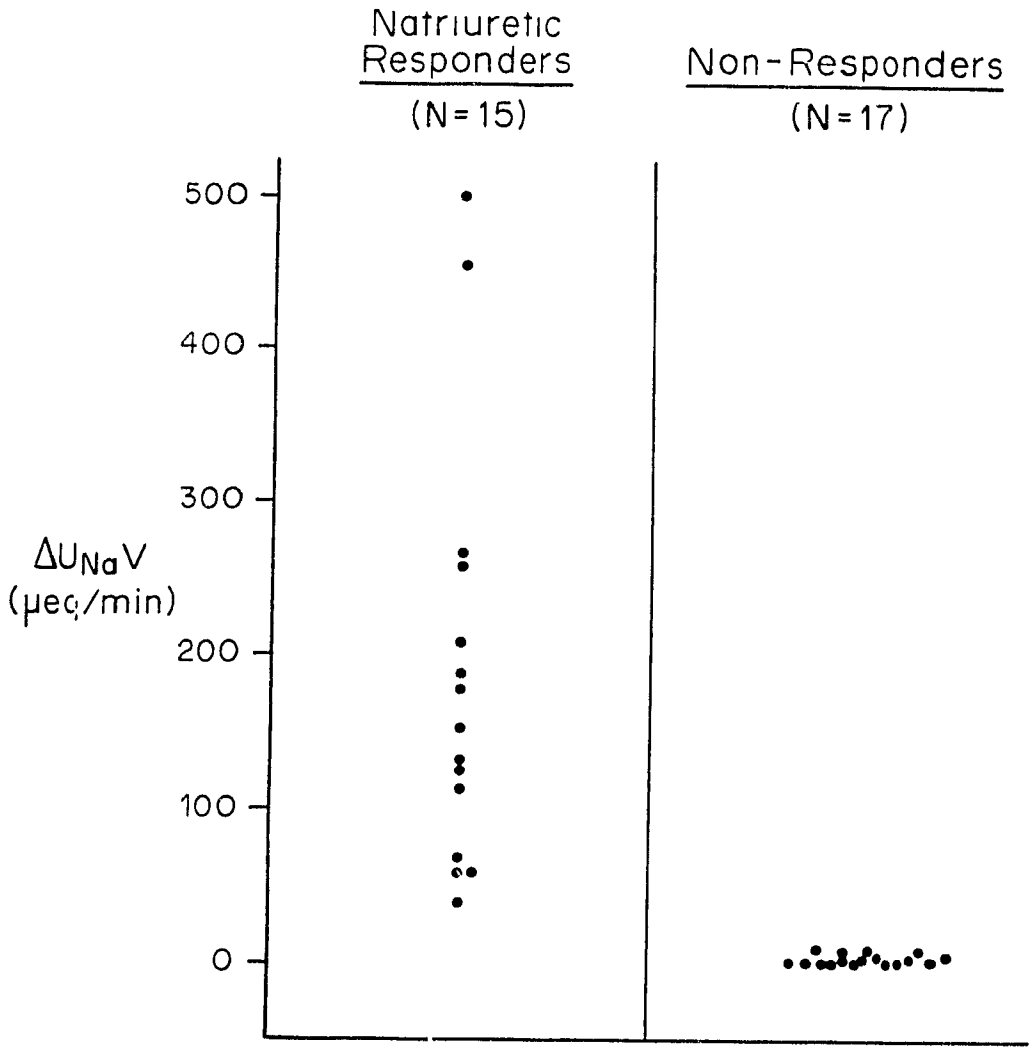

FIGURE 4.9 Natriuretic response to ANF in 32 TIVC dogs with ascites and sodium retention.

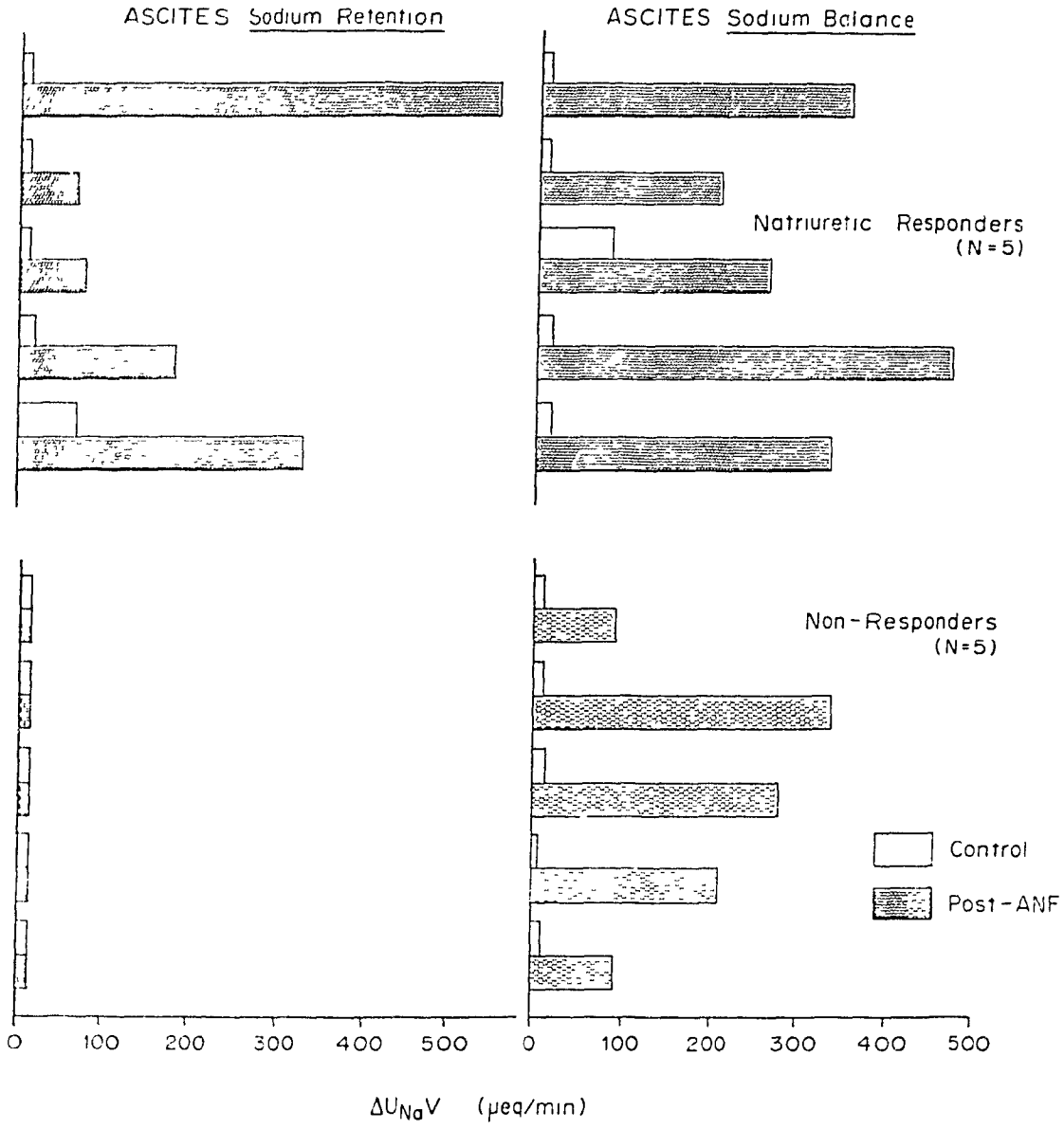

FIGURE 4.10 Natriuretic responses of TIVC dogs with ascites studied sequentially in the sodium retaining and sodium balance phases.

to ANF administered at either 175 ng/kg/min or 125 ng/kg/min intravenously.

4.6 RESPONDERS VS. NON-RESPONDERS: PLASMA AND ATRIAL iANF

A variety of data was examined in an attempt to determine important physiological differences between the TIVC natriuretic responders and non-responders. Figure 4.11 summarizes the plasma iANF levels for each phase of the experiment. As shown previously in Figure 4.3, plasma iANF falls to very low levels as the dogs develop ascites, but a return to sodium balance is associated with a return to normal levels. When the plasma values were analyzed during the sodium retaining phase for these dogs, it was seen that plasma iANF for responders was slightly but significantly higher than in non-responding dogs (15.8 $\pm$ 2.1 vs. 10.1 $\pm$1.8 pg/ml ($p<0.05$). These data are shown in FIgure 4.12. Even when the lowest out-lyer for the non-responders was removed from the calculations, these results differed significantly. This difference in plasma iANF did not exist for these two groups of dogs when separated in the control and balance (with ascites) phases of the experiment. In the control phase, plasma iANF was 39.4 $\pm$5.9 pg/ml (N=11) for a group of responders and 52.8 $\pm$ 7.6 pg/ml (N=8) for non-responders ($p>0.05$). In a balance phase, 4 responders had 67.6 $\pm$ 35.2 and 64.6 $\pm$ 20.6 pg/ml

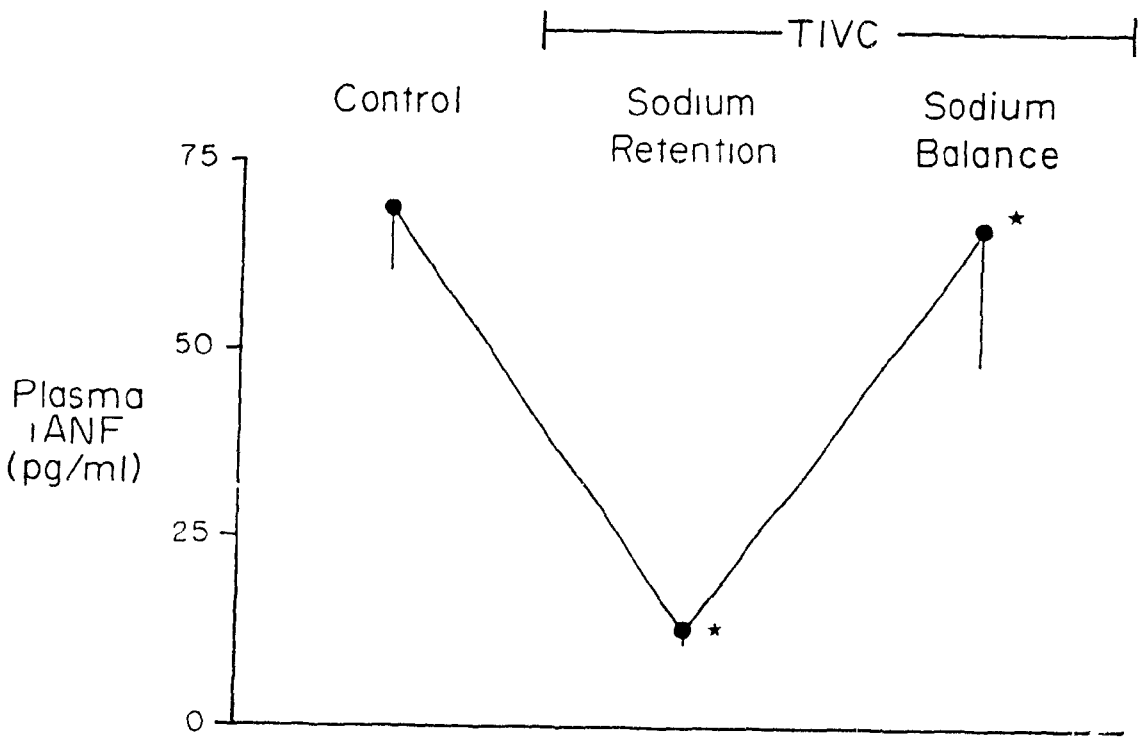

FIGURE 4.11 Plasma levels of iANF in control (mean of 30 dogs), TIVC with sodium retention (mean of 25 dogs), TIVC in sodium balance (mean of 9 dogs). *$p<0.05$ vs. previous phase.

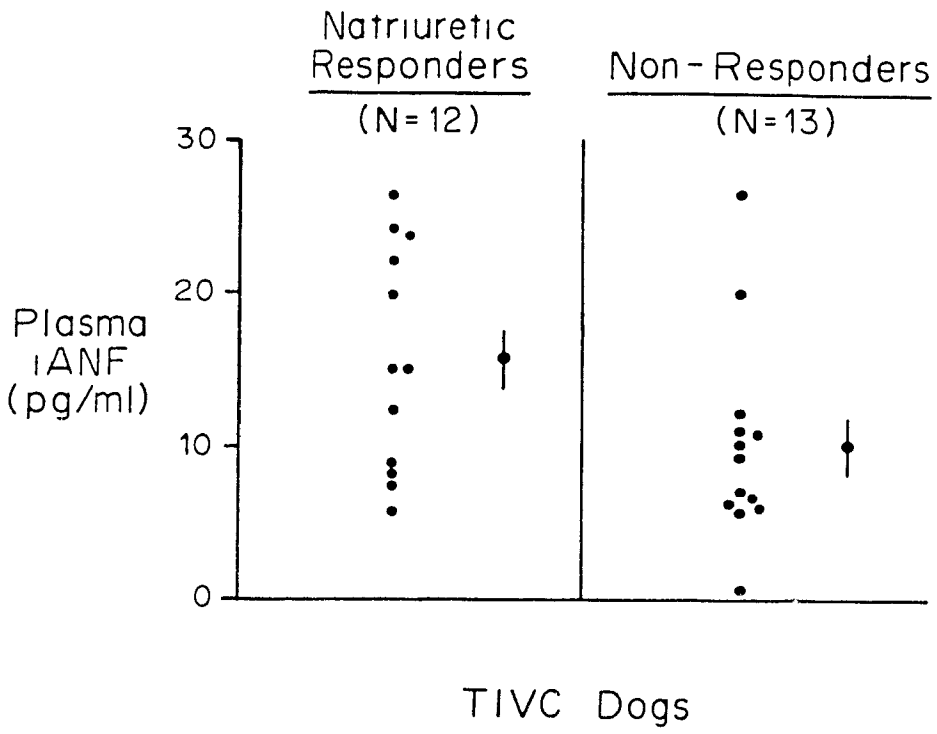

FIGURE 4.12 Plasma iANF in natriuretic responders and non-responders during TIVC phase with sodium retention and ascites. Mean + SEM for each group given at right of each panel.

(N=5) was measured for the non-responders. The different natriuretic response between the responding and non-responding dogs was not due to differences in plasma levels of iANF produced by exogenous infusion. Plasma iANF was 2532 ± 315 pg/ml for all dogs in the control phase. In the sodium retention phase, the responders averaged 2141 ± 301 pg/ml while the non-responders averaged 2260 ± 421 pg/ml. None of these values are significantly different from each other. During the balance phase, despite a significant natriuretic response, the level at the end of the infusion was less than previously achieved (1403 ± 137 pg/ml), though this difference was not statistically significant.

When the responding and non-responding dogs were categorized according to atrial content of iANF, the following data were obtained (Figure 4.13). For 5 responding dogs, the left atrium contained 19.3 ± 3.5 ng iANF/mg tissue and the right 18.1 ± 2.4 ng/mg tissue. Three non-responding dogs had 24.9 ± 4.9 ng/mg right atrium and 21.3 ± 3.1 ng/mg left atrium. No value was significantly different from each other. Thus, albeit in a small sample of dogs, atrial content of iANF did not determine the magnitude of the natriuretic response in the salt- retaining ascitic phase of chronic caval constriction.

There was no indication of altered ANF elimination from

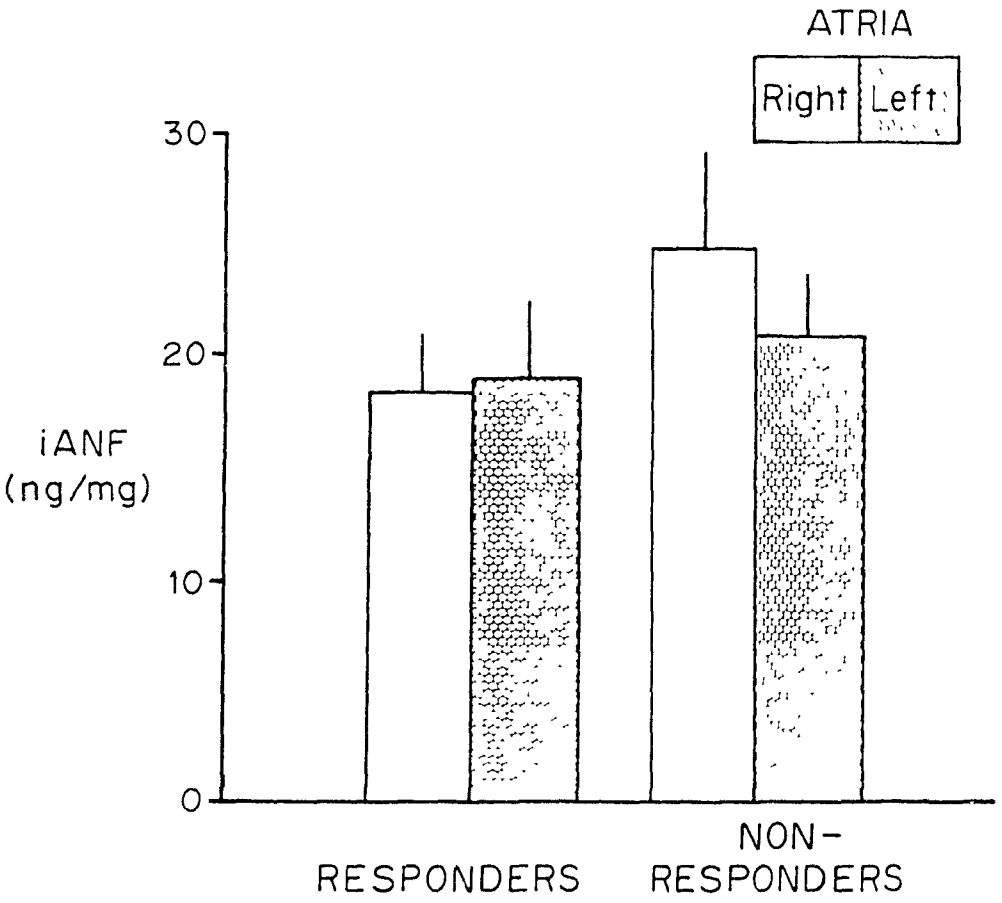

FIGURE 4.13 Atrial iANF (ng/mg wet tissue weight) in natriuretic responders and non-responders.

the circulation of either group. Plasma T1/2 averaged 2.10 ± 32 minutes in 4 responders and 1.71 ±.26 in 5 non-responders ($p>0.05$). Similarly, metabolic clearance rate did not differ between the two groups. Responding dogs (N=6) cleared 1.12 ± .21 l/min as compared to 1.10 ± .16 l/min in the non-responders (N=7).

4.7 RESPONDERS VS. NON-RESPONDERS: SYSTEMIC AND RENAL HEMODYNAMICS

In an attempt to define responding and non-responding TIVC dogs, the baseline hemodynamics and renal function were examined prior to and following standard infusion of ANF in a group of dogs separate from those reported in Table 4.1. These data are presented in Table 4.2. The responding and non-responding dogs could not be differentiated prior to the infusion of ANF in terms of blood pressure or cardiac output. There was insufficient CVP data in these dogs, due to technical problems with the venous catheters, to be useful in separating responders and non-responders. However, because baseline cardiac output and blood pressure during ANF infusion were not different between these two groups, it is unlikely that CVP differed. Similarly, plasma volumes, baseline GFR, CPAH, urine flow rate (V) and UNaV did not differ between the two groups.

Following the ANF infusion, only V and UNaV were significantly less for the non-responding dogs. GFR,

	ANP RESPONDERS (N = 7) Pre-ANP	Post-ANP	ANP NON-RESPONDERS (N = 7) Pre-ANP	Post-ANP
ABP - mmHg	123 ± 5	110 ± 4*	114 ± 3	89 ± 10*
CO - litres/min	1.95 ± 0.39	1.45 ± 0.31*	2.09 ± 0.25	1.53 ± 0.17*
GFR - ml/min	42 ± 4	51 ± 4*	37 ± 5	49 ± 4*
C_{PAH} - ml/min	114 ± 11	115 ± 6	87 ± 18	94 ± 8
FF	0.36 ± 0.0	0.37 ± 0.02	0.42 ± 0.02	0.44 ± 0.02
V - ml/min	2.1 ± 0.2	3.2 ± 0.6*	1.7 ± 0.3	1.9 ± 0.3
$U_{Na}V$ - μEq/min	13.6 ± 8.9	146 ± 39*	8.0 ± 6.0	10 ± 6

For abbreviations see text and previous table.

* $P < 0.05$ compared to pre-ANP phase.

For each variable, pre-ANP values were not different between the two groups of dogs. Except for V and $U_{Na}V$, all post-ANP values were also not significant between the two groups.

TABLE 4.2 Responders vs. Non-responders

CPAH, cardiac output and blood pressure were not different post-ANF for each group. The degree of avidity of sodium retention of the TIVC dogs also did not correlate with the magnitude of post-ANF natriuretic response to ANF infusion. For 7 responding dogs, the range of UNa was between 1-12 mEq/L (normal dogs 80 ± 9 mEq/L on similar diet) while in the responding dogs (n=7) ranged between 1-5 mEq/L. The mean for the former group was 3.8 mEq/L and 4.9 mEq/L for the responders (NS). Table 4.3 describes parameters relating to sodium handling in the distal tubule. The filtered load of sodium was the same in both groups suggesting that distal delivery of sodium was also equivalent. There was no difference in baseline sodium excretion, 24 hour sodium excretion or distal cationic exchange.

4.8 RESPONDERS VS. NON-RESPONDERS: PLASMA HORMONES

The responders and non-responders could not be differentiated in terms of plasma levels of renin, aldosterone, or vasopressin. Plasma renin in the control phase (n=18) was 2.36 ± 0.54 ng/ml/hr. This rose to 12.7 ± 2.5 following the induction of ascites and sodium retention in TIVC dogs ($p<0.05$). For 4 responding and non-responding dogs each, the pre-iANF levels were 11.5 ± 3.7 and 14.1 ± 1.6 ng/ml/hr respectively (NS).

Control plasma levels of aldosterone (n=12) were 7.6 ±

TABLE 4.3

Effects of ANF on distal tubular function.

	Responders	Non-Responders
$U_{Na}V$ (μEq/min)	13.6 ±8.9	8.0 ±6.0
24-hour $U_{Na}V$ (mEq/day)	3.9 ±2.0	2.9 ± .81
$\frac{U_K}{U_{Na}+U_K}$	0.95± .02	0.96± .01
FL_{Na} (μEq/min)	5.9 ± .4	6.2 ± .7

1.6 ng/dl and this rose to 146 ± 31.9 ng/dl for TIVC dogs retaining sodium with ascites ($p<0.05$). For 4 responding and non-responding dogs, these pre-ANF levels were 130 ± 50 and 177 ± 42.3 ng/dl respectively (NS).

Control vasopressin levels were 5.76 ± 1.08 pg/ml (n=8) and this rose to 10.37 ± 2.70 in TIVC dogs with ascites and sodium retention but did not reach statistical significance. For 4 responding and non-responding dogs each, the pre-ANF levels were 12.16 ± 5.45 and 8.59 ± 1.47 pg/ml respectively (NS). Thus, for each of these 3 hormones, there were no significant differences between the two groups of dogs. Figure 4.14 summarizes the response of renin and aldosterone to ANF infusion in several of the TIVC dogs. Although the data are limited, no consistent pattern appears which would enable the delineation of the two groups.

4.10 TIVC Natriuretic Response at 75 ng/kg/min

It is important to emphasize that this phenomenon is not a unique feature of the infused dose of ANF (175 ng/kg/min). Figure 4.15 summarizes the natriuretic response of 22 anesthetized TIVC dogs in the phase of active sodium retention and ascites to ANF infused at 75 ng/kg/min. Note that the dogs divide into 13 responders (mean ΔUNaV = 139 ± 28 uEq/min) and 9 non-responders (mean Δ UNaV = 2 ± 1 uEq/min).

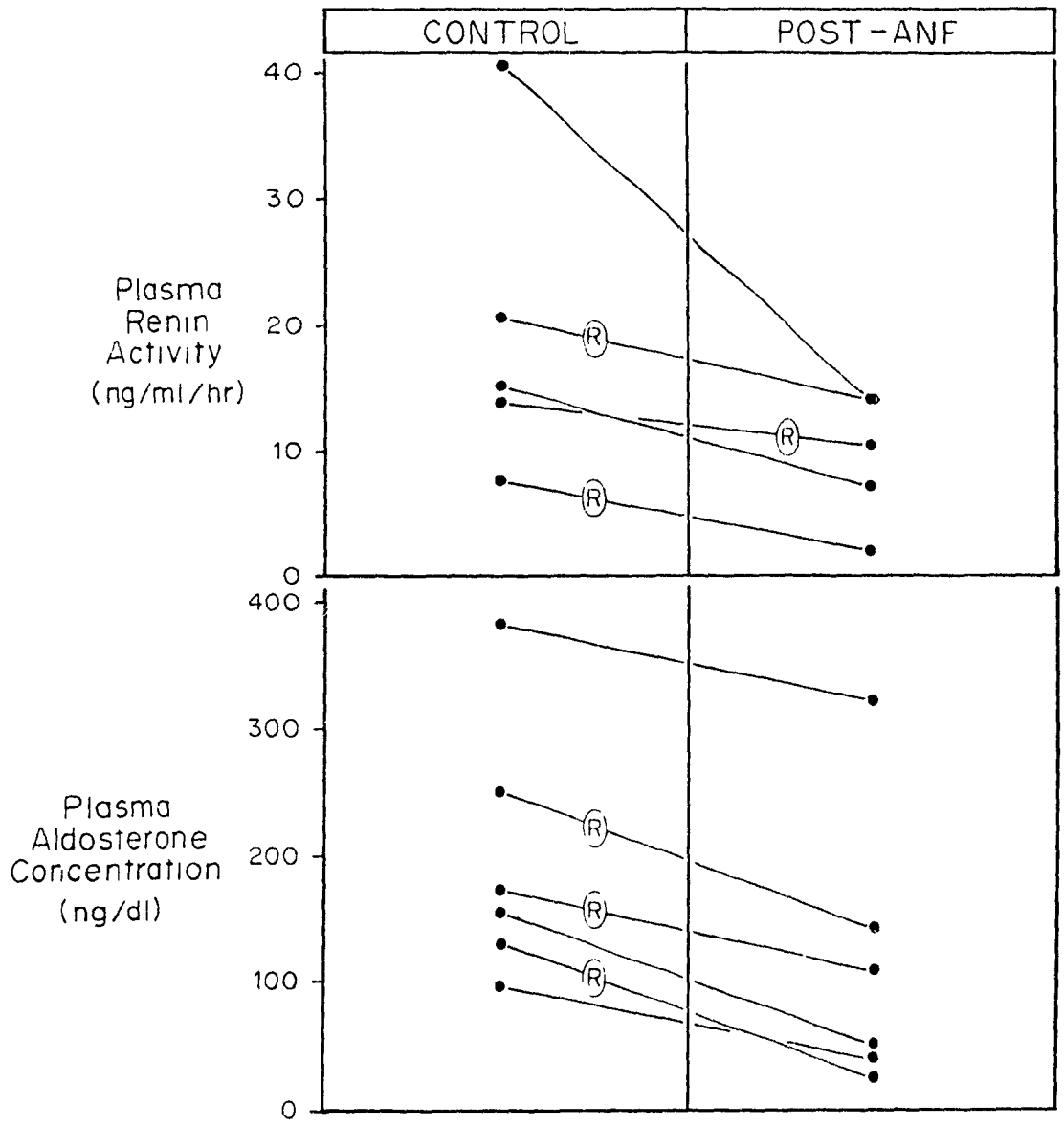

FIGURE 4.14 Response of plasma renin and aldosterone to and i.v. ANF infusion in TIVC dogs with ascites and sodium retention.

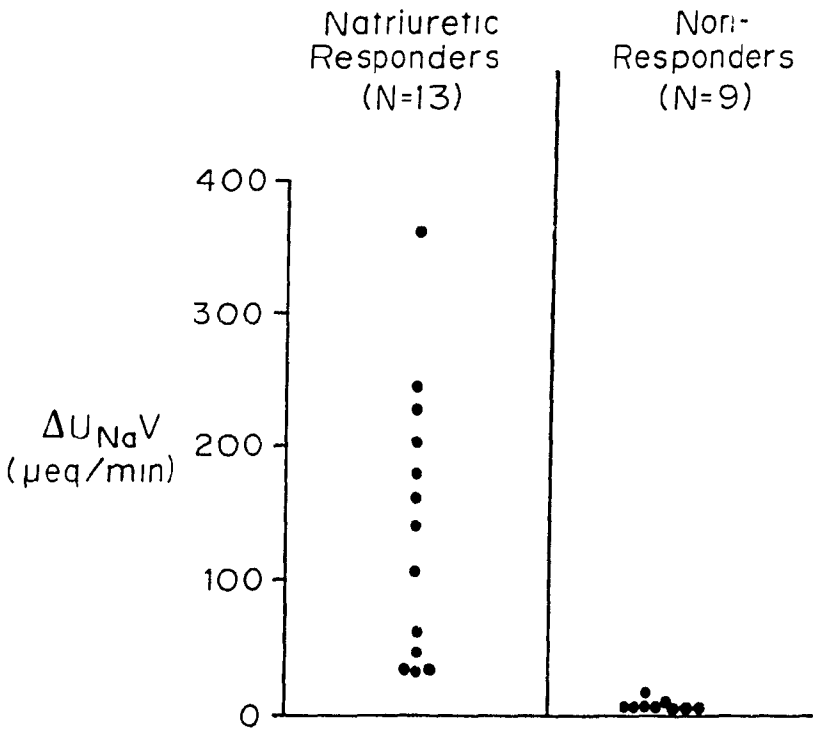

FIGURE 4.15 Natriuretic response to ANF infusion at 75 ng/kg/min in 22 TIVC dogs with ascites and sodium retention.

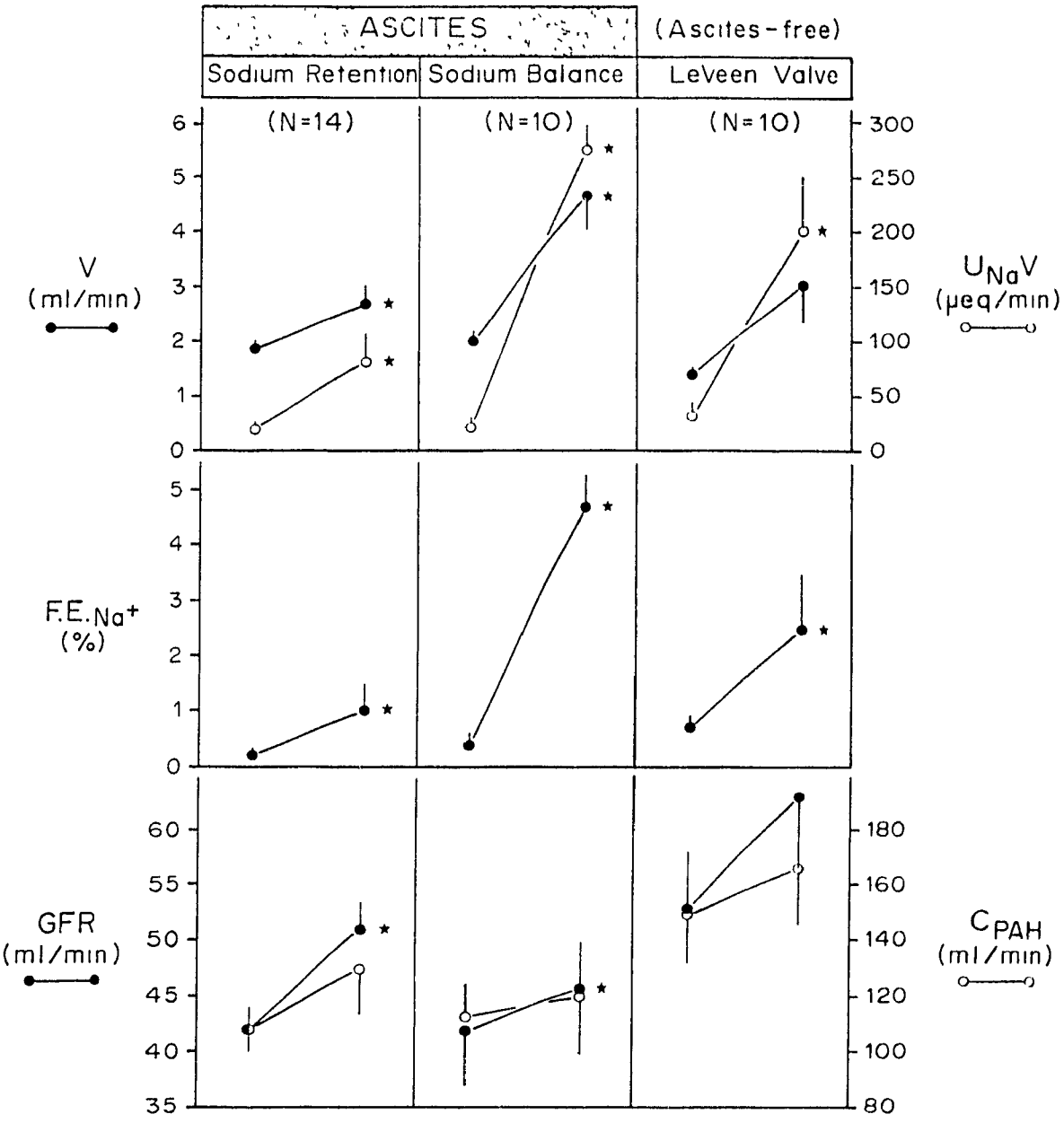

FIGURE 4.16 Comparison of natriuretic and renal hemodynamic response to ANF in dogs where ascites has been mobilized by LeVeen peritoneovenous shunting to earlier phases.

4.11 MOBILIZATION OF ASCITES WITH LEVEEN PERITONEOVENOUS VALVE

Figure 4.16 compares the ANF response of all dogs once the ascites had been mobilized with a LeVeen peritoneovenous valve to the responses obtained when the dogs had ascites but had returned to sodium balance. Previous data collected in our laboratory reveals that this shunting procedure is associated with increments of plasma volume and CVP and normalization of plasma levels of renin and aldosterone (Levy, Wexler, McCaffrey, 1979). In addition, plasma iANF was 70.0 ±18.8 pg/ml, not different from control levels but different than during sodium retention. Note that these levels were measured 1-2 days after the shunt was inserted. Both urine flow and UNaV increase comparable to the control state, but the response is not different from the "sodium balance" phase despite the removal of sequestered ascites volume and a replete expanded plasma volume.

4.12 GENERATION OF CYCLIC GMP (cGMP)

The relationship between ANF natriuretic effects and its ability to generate cGMP is depicted in Figure 4.17. The response in normal dogs is shown for comparison. Despite the lack of natriuretic response in the non-responders, ANF infusion produced a marked increment in both plasma and

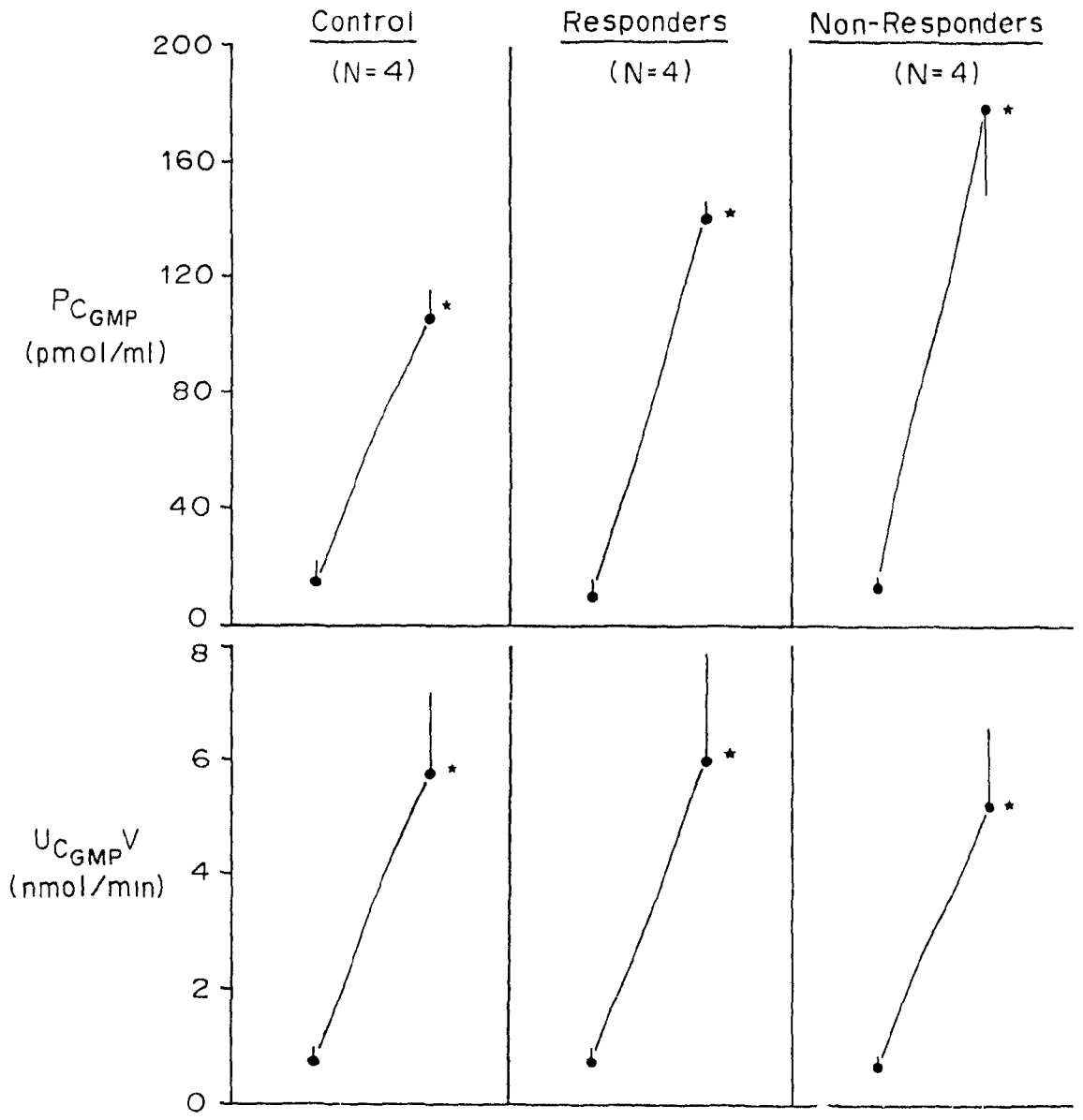

FIGURE 4.17 Changes in plasma and urinary cGMP in response to ANF infusion in control, TIVC responders and non-responders. * $p<0.05$ compared to pre-infusion.

urinary cGMP excretion which was comparable to both TIVC-responders and normal dogs.

4.13 ROLE OF RENAL SYMPATHETIC NERVES

The role of the renal sympathetic nerves in the natriuretic response to ANF was investigated by acute renal denervation in 5 responding and 5 non-responding TIVC dogs in the phase of active sodium retention and ascites. These data are summarized in Figure 4.18. The ANF-induced natriuretic response in responding dogs was 132 $\pm$ 38 uEq/min which was markedly magnified following renal denervation to 298 $\pm$ 63 uEq/min ($p<0.05$). ANF had no effect on sodium excretion in the non-responding dogs either prior to or after renal denervation (UNaV = 9 $\pm$ 8 uEq/min). Denervation produced a slightly enhanced urine flow rate in both groups while GFR and renal perfusion did not change in either group prior to or following denervation.

4.14 PAPILLARY PLASMA FLOW

To investigate the role of the renal papilla in determining the natriuretic response to ANF, the Lillienfield albumin uptake method was employed to measure papillary plasma flow (Lillienfield, Maganzini, Bauer, 1961). The critical assumption of this technique is that no tracer leaves the tissue and that the reference sample

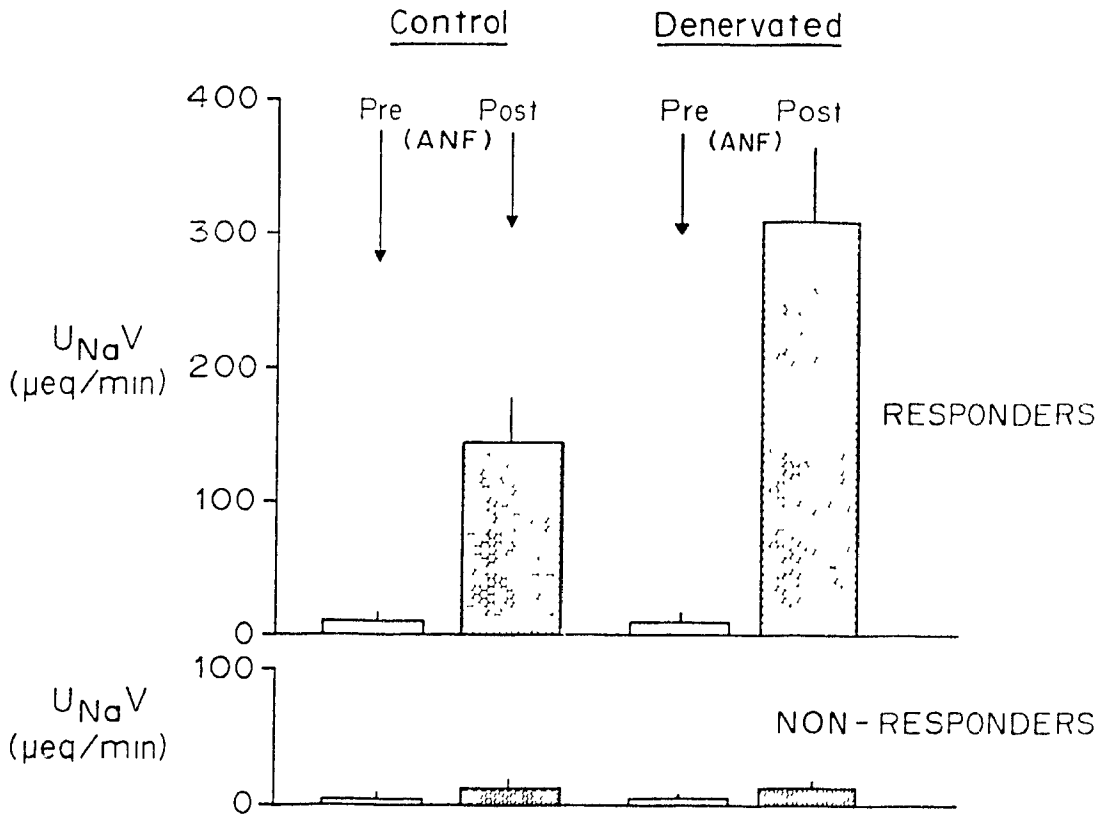

FIGURE 4.18 Effects of ANF infusion in TIVC dogs with sodium retention and ascites with renal nerves intact and following acute unilateral renal denervation. Results are experimental kidney only.

accurately reflects the concentration of tracer in the blood reaching the tissue. It has been shown that I125 uptake is linear up to 60 seconds in the dog and therefore mean transit time is long enough to allow for 30 second tracer infusion.

The blood sampling catheter was placed in the abdominal aorta just below the renal artery to ensure that the concentration of tracer in the plasma sample reflected the concentration entering the kidney. The transit time was measured for each dog to know precisely the arrival time of the tracer. The data is presented in Figure 4.19. The pre- and post-infusion PPF could not be measured in each dog since the technique requires sacrifice of the kidney. PPF measured in 4 normal dogs during ANF infusion (125 ng/kg/min) was 35.5 $\pm$ 4.0 ml/min/100g papilla, unchanged from baseline flow = 39.6 $\pm$ 3.2 ml/min/100 g papilla. 10 TIVC dogs had an average PPF = 10.7 $\pm$ .7 ml/min/100 g during ANF infusion, significantly lower than during ANF infusion in the normal dogs. When the TIVC dogs were divided into natriuretic responders and non-responders, no difference in PPF during ANF infusion was demonstrated. Baseline blood pressure and renal blood flow as well as ANF-induced changes in these parameters were the same in both groups of dogs.

Although the Lillienfield technique gives consistent, reproducible rates of papillary plasma flow, it is based

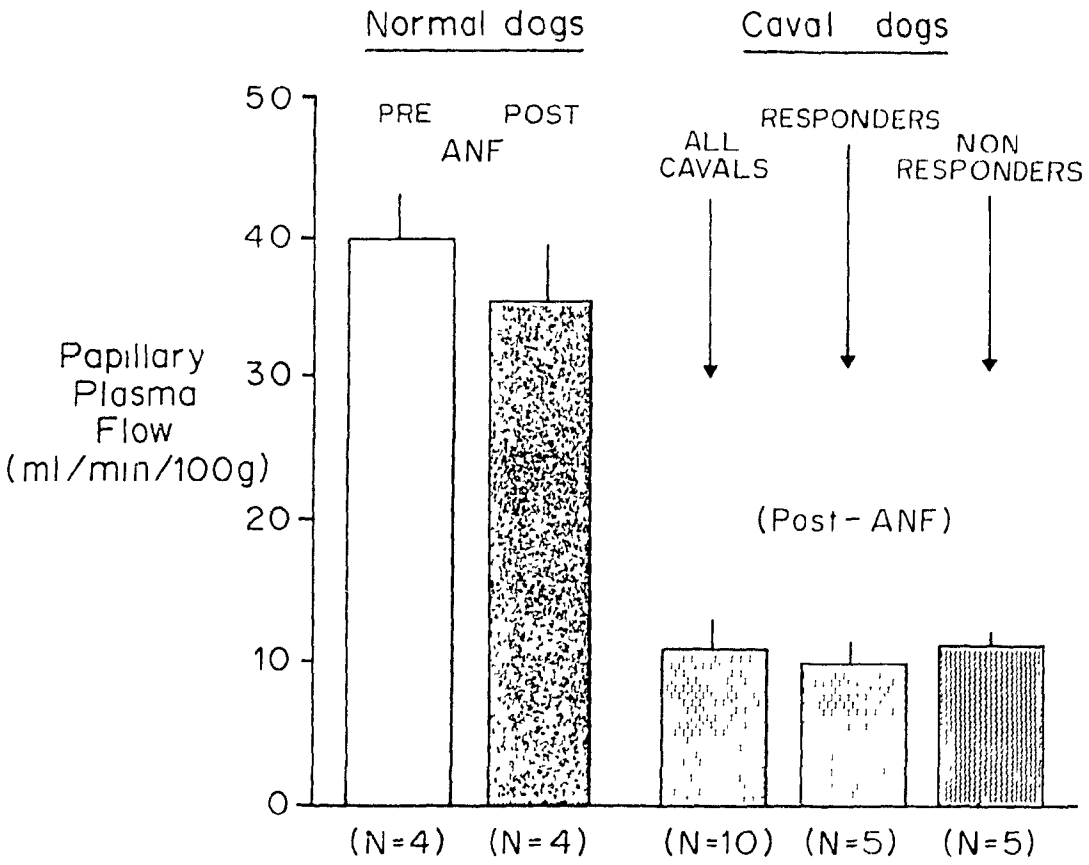

FIGURE 4.19 Effect of ANF on papillary plasma flow in normal and TIVC dogs. Normal dogs are shown pre- .nd post-infusion, TIVC dogs are post-infusion. TIVC dogs are shown as a group and split into repsonders and non-responders.

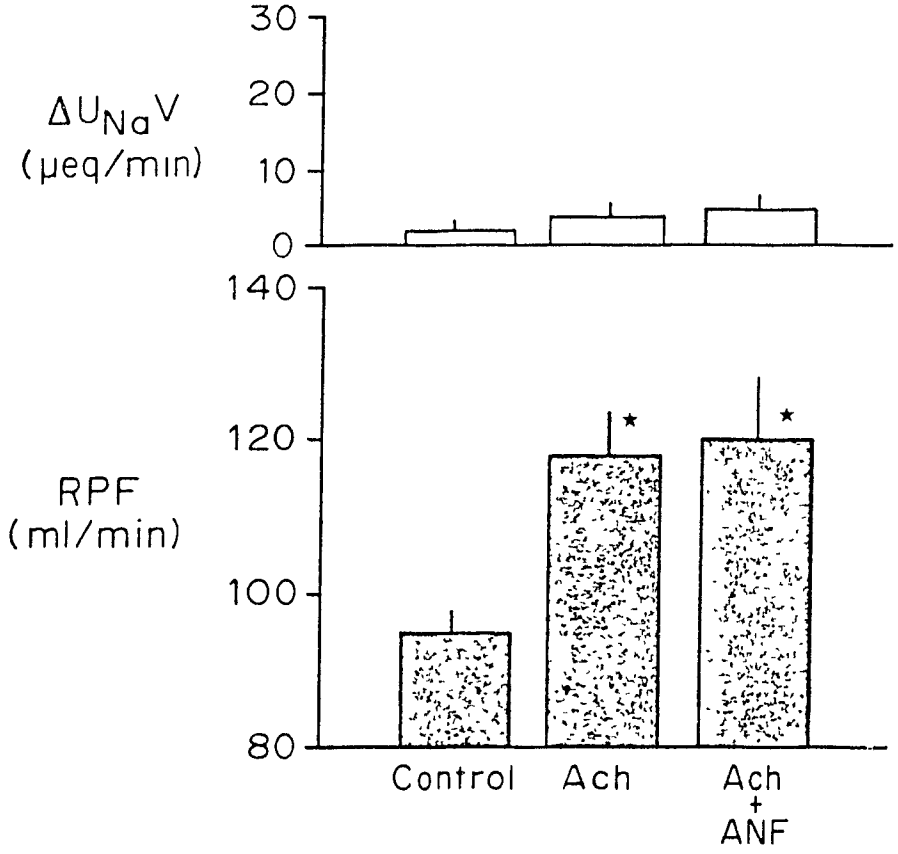

FIGURE 4.20 Renal vasodilatation in non-responding TIVC dogs.

on several assumptions (outlined previously) which are difficult to verify. Recognizing the limitations of the method, a group of non-responding dogs were subjected to renal vasodilatation with intrarenal acetylcholine infusion. The data are presented in Figure 4.20. While vasodilation raised renal plasma flow, infusion did not produce a natriuretic response. These data support the conclusion that changes in renal plasma flow, and papillary plasma flow in particular, do not alter the tubular responsiveness to ANF.

4.15 DISCUSSION

Following constriction of the thoracic inferior vena cava, dogs retain sodium, the renin-angiotensin system is activated and significant ascites develops. As demonstrated in Figure 4.3, the advent of ascites and avid sodium retention is accompanied by an early fall in plasma iANF concomitant with a rise in plasma renin and aldosterone, a relationship which persists throughout the phase of sodium retention and ascites. As ANF release is largely a result of atrial stretch, this rapid fall in plasma iANF appears to be an appropriate response to the drop in CVP produced by caval constriction. This is supported by the data demonstrating that plasma T1/2 and the metabolic clearance rate are unchanged from the control state. Accompanying the fall in plasma iANF is a reduction

in atrial iANF content. Since atrial iANF was measured several days after the appearance of ascites and therefore an even greater time following the initial decline in plasma ANF levels, the reduction in atrial content most likely represents a new equilibrium between the quantity of ANF released from the atria and that of pro-ANF synthesized and subsequently stored. These data suggest that a feedback mechanism exists in which the quantity of ANF released from the atria regulates the rate of synthesis. Though the possibility of such a mechanism has not been investigated in this study one could measure atrial iANF content or pro-ANF mRNA level along the same time course as that reported for plasma levels to evaluate the sequential changes in both parameters.

To address the possibility that low plasma ANF levels result from an impaired coupling between atrial stimulation and ANF release, 2 caval dogs with active sodium retention and ascites were given a 7% body weight acute saline load. Figure 4.21 shows that both dogs with low baseline plasma iANF and CVP were able to release ANF in direct relation to the rise in CVP as produced by the saline infusion. Moreover, once the infusion was complete, plasma ANF fell as CVP returned to baseline. These data demonstrate that stimulus-release coupling is normal in the caval dogs and, furthermore, emphasizes that plasma iANF can be rapidly

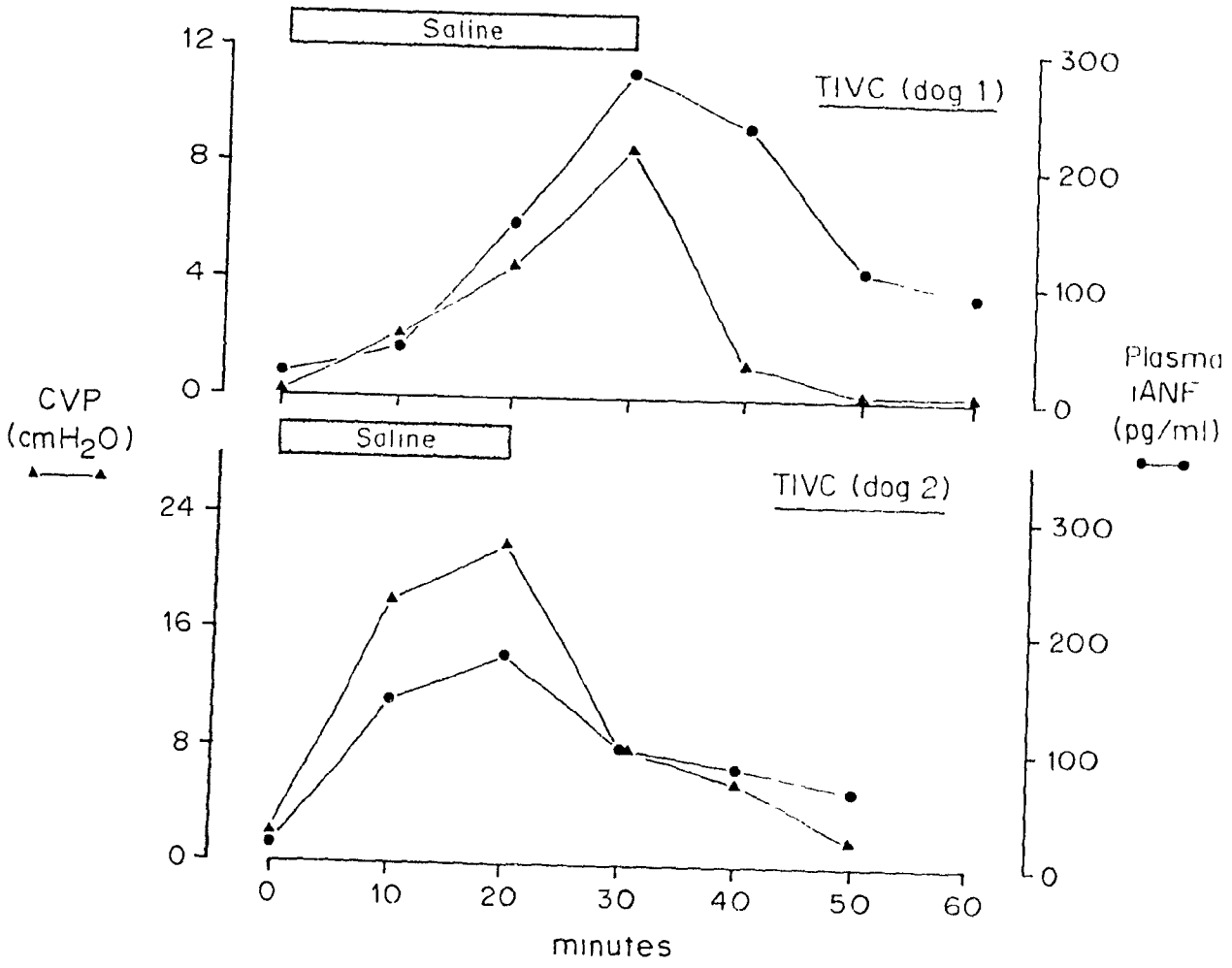

FIGURE 4.21 Effects of saline infusion on CVP (x-x) and plasma iANF (.-.) to 2 TIVC dogs with sodium retention and ascites. Each panel represents 1 dog's response.

altered as a result of changes in CVP.

As the caval dogs returned to sodium balance, the plasma levels of iANF and aldosterone returned to baseline levels. Although technical difficulties prevented the measurement of CVP as the dogs returned to sodium balance, a recent study showed a correlation between the rise in CVP and plama iANF as one caval dog returned to sodium balance (Freeman et al., 1987). It is not clear from the data whether the changing plasma level of ANF, or rather the reciprocal relationship between ANF and mineralocorticoids is the prime mover in causing both the sodium retention and return to sodium balance. Indeed, several investigators have questioned whether or not in the physiological range observed in our study, ANF may have a role to play in the regulation of sodium excretion (Anderson et al., 1987; Cuneo et al., 1987). To address this question, Paganelli et al (1988) released the venous constriction in chronic caval dogs and observed a 6-fold increment in daily sodium excretion, but a return of depressed plasma ANF only to control levels, despite a marked increment in right atrial pressure. It was thus concluded that non-ANF factors were playing a major role in producing the natriuresis. Conversely, in the phenomenon of mineralcorticoid escape, it seems clear that elevated levels of plasma iANF, in the presence of excess mineralcorticoid, may be mediating much of the natriuresis (Ballermann et al., 1986).

Despite our observations that support a role for ANF in both the sodium retention and balance phases of the TIVC dogs with ascites we have apparently uncovered a unique response of the renal tubule of these edematous animals to exogenously administered ANF. In the phase of sodium retention with the ascites, approximately 50% of conscious TIVC dogs will respond with a normal natriuresis to a large dose of ANF (175 ng/kg/min) while 50% do not. This phenomonon is also evident in anesthetized dogs at 75 and 125 ng/kg/min. The lack of natriuretic response should not be viewed as some unique attribute of these animals, but rather as a distinct physiological response, functional and reversible, since dogs who did not have a natriuretic response to ANF in the phase of sodium retention had previously responded normally in the control state and responded again when sodium balance had been restored. It is also worth emphasizing that available data suggest that while "low" doses of ANF (50 ng/kg/min) may not cause a brisk natriuresis in either human subjects or experimental animals, "moderate" doses (50-500 ng/kg/min) and "high" doses (500 ng/kg/min) appear to cause only a brisk natriuresis without causing aberrant physiological responses (Goetz, 1988).

Our finding of a heterogeneous natriuretic response in TIVC dogs is different from that obtained by other investigators using this model (Freeman et al., 1985)).

These investigators however, administered ANF to only 5 TIVC dogs and it is possible that they missed this natriuretic response pattern. Of the 46 caval dogs tested, runs of 5-6 dogs showing a single pattern of natriuretic response have often been noted. We have also observed this heterogeneity of natriuretic response in dogs with experimental hepatic cirrhosis (Chapter 5). Although investigators using other models, e.g. cirrhotic rat (Olivera et al., 1988), nephrotic rat (Koepke, and DiBona, 1987) and dogs with heart failure other than caval constriction (Chou et al., 1987) have reported absent or blunted natriuretic effects, small numbers were tested and no comment is made upon the degree of heterogeneity of the natriuretic response. Several investigators however, have reported heterogeneous natriuretic responses to ANF in other volume-overload conditions. Winaver et al. (1988) administered ANF to rats with high output heart failure due to an aortocaval fistula. Balance studies showed two distinct patterns of sodium handling in this model. Rats with progressive sodium retention developed very high levels of plasma renin activity and did not respond to exogenous ANF with either a diuresis or natriuresis. Sixty % of rats returned to sodium balance within 7-10 days after surgery, did not show elevated plasma levels of renin activity, and showed a significant natriuresis in response to exogenous ANF while 40% did not return to balance.

Hildebrandt and Banks (1988) evaluated the renal effects of ANF in rats with nephrotic syndrome at varying time intervals after induction of the glomerulopathy. Despite increasing proteinuria and falling GFR up to 14 days post-aminonucleoside treatment, ANF produced a blunted natriuresis at 2 days compared to controls, no natriuresis whatsoever at 4 and 6 days and a return of the natriuretic response (albeit attenuated) at 14 days. The authors postulated that this changing renal responsiveness to ANF might be due to increased ABP levels at 14 days. This however, would not explain the lack of response at 4 and 6 days since ABP was unchanged from control levels at this time.

The novel observation of this study is both the heterogeneity of natriuretic response in well defined ascitic dogs with sodium retention, and the reappearance of a natriuretic response in the non-responders when they regain sodium balance. What is the physiological basis for this phenomenon? Whatever the explanation, it is possible that the tubular unresponsiveness to ANF in the sodium retaining state is peculiar to this peptide. Preliminary data in our laboratory (Maher, E., Levy, E. unpublished observations) suggest that natriuretic non-responders will react normally to various diuretics. It is of interest to note that caval dogs with a blunted response to ANF will respond normally to furosemide and hydrochlorothiazide

infusion (Blaine et al., 1986). Similarly, Hildebrandt and Banks (1988) have demonstrated that nephrotic rats not responding to ANF will respond to a bolus of furosemide.

Our results show that the TIVC responders and non-responders cannot be differentiated in terms of breed, gender, supply source, length of stay in the Animal Center and previous dietary history. They were all studied in the forenoon at an equivalent time period physiologically post-caval constriction. In terms of systemic hemodynamics, renal perfusion, plasma volume, plasma levels of renin and aldosterone (as well as the response of these variables to ANF), and baseline sodium excretion, both the responders and non-responders were equivalent. The only differentiating factor that we have adduced to date is the lower plasma iANF level in the non-responders prior to receiving the exogenous infusion. It is difficult to see, however, how this small difference could be physiologically important, especially since several dogs with the same plasma iANF level had such remarkably different natriuretic responses. Furthermore, if this difference is important, one might expect ANF receptors to be up-regulated in the non-responders and produce both a greater natriuresis and hypotension. Moreover, the plasma levels of ANF reached extremely high levels during the infusion and were equivalent in each group. Since it was the response to exogenous ANF that determined whether or not a TIVC dog was

a natriuretic responder, it seems unlikely that the endogenous plasma levels of ANF could be playing a determinant role between these two groups.

The biphasic response of the renal tubule to an exogenous infusion of ANF in 50% of tested TIVC dogs is perhaps best explained in terms of the events that occur as TIVC dogs with ascites pass from a state of avid tubular sodium retention to that of tubular sodium rejection and the re-attainment of sodium balance. Several laboratories have produced evidence that this physiological progression is associated with expansion of the plasma volume, return of the plasma levels of renin and aldosterone to normal, a probable decrease in renal sympathetic nervous activity, a rise in CVP, and loss of angiotensin dependence of the arterial blood pressure. In a similar progression, as caval dogs retain sodium and develop ascites papillary plasma flow falls to approximately 30% of normal and return to sodium balance is associated with a recovery of papillary flow to baseline (Faubert et al., 1982). The relationship between low papillary plasma flow and enhanced sodium reabsorption in the caval dog may be of some importance since it has been suggested that ANF-induced increments in papillary flow mediates in large part the natriuretic effect of this peptide. Indeed, rats with selective destruction of the renal papilla by BEA were unable to respond to ANF infusion (Fried et al., 1987).

Moreover, dogs with aortocaval fistula infused with ANF have a blunted natriuretic response and papillary plasma flow only 30% of normal. While these data suggest an important role for increments in papillary plasma flow in mediating the natriuretic response to ANF, the two events may not be causally related. Fluorescent videomicroscopy of the normal rat papilla demonstrated that vasa recta flow increases with ANF infusion although not until after the diuretic and natriuretic response has been initiated (Kiberd et al., 1987).

Papillary plasma flow was measured in this study by the Lillienfield radiolabelled albumin uptake method. ANF infusion at 125 ng/kg/min produced a dramatic increase in sodium excretion (264 $\pm$ 53 uEq/min) in four normal dogs while papillary plasma flow did not change indicating a dissociation between papillary flow and ANF's natriuretic effect. Furthermore, the lack of natriuretic response in 50% of TIVC dogs is not a result of the reduction in papillary plasma flow during the phase of sodium retention and edema, since 50% of TIVC dogs with the same reduced papillary plasma flow had a normal natriuretic response to ANF infusion. Recently, DiBona and colleagues (1987) have presented evidence that renal denervation will reverse the blunted natriuresis to ANF in both nephrotic and cirrhotic rats. It is therefore possible that altered renal sympathetic nervous activity or the intrarenal

catecholamine profile may be playing a role in differentiating non- responders from responders. In this study, acute renal denervation enhanced the ANF-induced natriuresis in responding dogs although did not produce a natriuresis in the non-responding dogs. It seems likely therefore that the renal sympathetic nerves are antagonistic to ANF in the caval dog such that the sympathetic tone serves to limit the degree to which sodium excretion can be enhanced. Thus removal of this sympathetic tone allows complete expression of ANF's natriuretic effect. Reversal of the blunted natriuresis by renal denervation in cirrhotic and nephrotic rats may indeed reflect removal of the inhibitory effect of the renal nerves on ANF action since it been shown that baseline efferent renal nerve activity is enhanced in cirrhotic and nephrotic rats (DiBona, 1988). This, however, does not imply that the complete lack of response in the non-responding caval dogs is a result of an even greater sympathetic tone, since renal denervation had no effect in these animals. Rather, it provides clear evidence that the renal nerves are not involved in the inhibition of the natriuretic response in 50% of caval dogs and, in fact, only after the ability to respond to ANF is restored will the modulatory role of the renal nerves also be restored.

This heterogeneous natriuretic response to ANF in TIVC

dogs with sodium retention and ascites cannot be explained on the basis of renal hemodynamics, systemic hemodynamics, the renin-angiotensin-aldosterone system, renal nerve activity or changes in medullary or papillary blood flow. All the analysis suggests that there is a problem at the level of ANF receptors in the inner medulla. An indirect measure of the binding of ANF to its receptor and the transduction of the signal is the generation of ANF's second messenger, believed to be cyclic GMP (Hamet et al., 1984).

In the present study, there is a complete dissociation of cGMP generation and natriuretic response in the TIVC dogs. A comparable increase in plasma cGMP is not surprising since both groups show a similar degree of hypotension. In fact, the enormous addition of cGMP to the glomerular filtrate may mask small but important changes in cGMP production along the nephron. Moreover, it has been demonstrated that in response to ANF cGMP increases only 2-fold in the thick ascending limb and 3-fold in the medullary collecting duct where ANF is believed to exert its major natriuretic effect. Conversely, the glomerulus produces a 50-fold increase in cGMP and yet the involvement of GFR in ANF-induced natriuresis is minimal. The existence of a second receptor of MW 60,000 accounts for 94% of all ANF endothelial receptors and, as discussed earlier (1.5), is not coupled to cGMP generation although

its second messenger system has not yet been identified. The relative density of receptors or proportion of cGMP generated by ANF does not necessarily assign a relative importance to receptors in a given area. However, the observation that both plasma and urinary cGMP rises to normal levels in non-responding dogs is certainly suggestive of a dissociation between this response and the ability to produce a natriuresis in caval dogs with avid sodium retention and ascites. This dissociation has also been reported in normal dogs where ANF was infused intrarenally to produce a natriuresis in only the infused kidney. Of interest was that cGMP rose comparably in both kidneys (Seymour et al., 1985). Similarly, water immersed cirrhotic patients not undergoing a natriuresis will demonstrate elevated cGMP (Skorecki, 1988). Thus, it is clear that ANF can bind to its receptor, couple to guanylate cyclase and generate cGMP normally. However, none of the data address the possibility that the lack of natriuretic response is a result of an event occurring later in the transduction of the signal. Specific in vitro receptor studies need to be conducted in order to examine this possibility.

CHAPTER 5: RESPONSE TO ATRIAL NATRIURETIC FACTOR IN DOGS WITH ACUTE SELECTIVE INTRAHEPATIC HYPERTENSION

5.1 INTRODUCTION

Acute intrahepatic sinusoidal hypertension is generally associated with a reduction in the urinary excretion of sodium in a variety of species, including man and a variety of experimental animals (Levy, 1974; Campbell et al., 1982; Blanchart et al., 1987) and usually occurs without simultaneously changing systemic hemodynamics. If prolonged, this experimental manipulation is associated with the production of large volumes of ascites (Levy, 1978) and, in dogs, with a reduction in endogenous serum levels of ANF and unresponsiveness to exogenous infusions of this peptide (Chapters 4,6).

The heterogeneous natriuretic response to exogenous ANF in caval dogs with chronic intrahepatic and portal hypertension is not determined by GFR, renal plasma flow, papillary plasma flow, renal nerve activity or systemic hemodynamics (Chapter 4). To assess the role of acute intrahepatic hypertension (IHH) per se, without confounding physiological disturbances (e.g. activated renin-angiotensin system) in producing natriuretic unresponsiveness to ANF infusions, a series of dogs were prepared with selective acute IHH by histamine infusion. Responses to infusions of ANF were compared in both the absence and presence of IHH.

5.2 Effect of Systemic Histamine Infusion

The average body weight of the dogs used in this study was 12.7 kg and the average dose of histamine base was 4.07 ug/min (range 3-5). Compared to the pre-infusion state, the administration of small doses of histamine into the femoral vein did not alter arterial blood pressure (98.0 ± 4.4 vs 94.2 ± 49 mm Hg; p>0.05); central venous pressure (4.8 ± 0.42 vs 4.6 ± 0.44 cm H2O p>0.05) or portal venous pressure (13 ± 0.2 vs 12.9 ± 0.3 cm H2O P>0.05). Though not measured in this study, previous investigations made in our laboratory with identical infusion rates of histamine base, have confirmed that GFR, renal plasma flow, urine flow, urinary sodium excretion and cardiac output are not altered when this substance is infused into the femoral vein at these dosage levels (Levy, 1974).

5.3 Effect of ANF in the Presence of Normal Intrahepatic Pressure

Figure 5.1 summarizes the effect of ANF infused into the contralateral femoral vein at 175 ng/kg/min on renal function while histamine was continued. Both sodium excretion and urine flow rose dramatically without changes in GFR or CPAH , though filtration fraction rose significantly. The fractional excretion of sodium rose from 0.82 ± 0.14 to 4.3 ± 0.4% (P<0.05). Fractional excretion of water rose from 1.7 ± 0.12 to 5.8 ± 0.14%

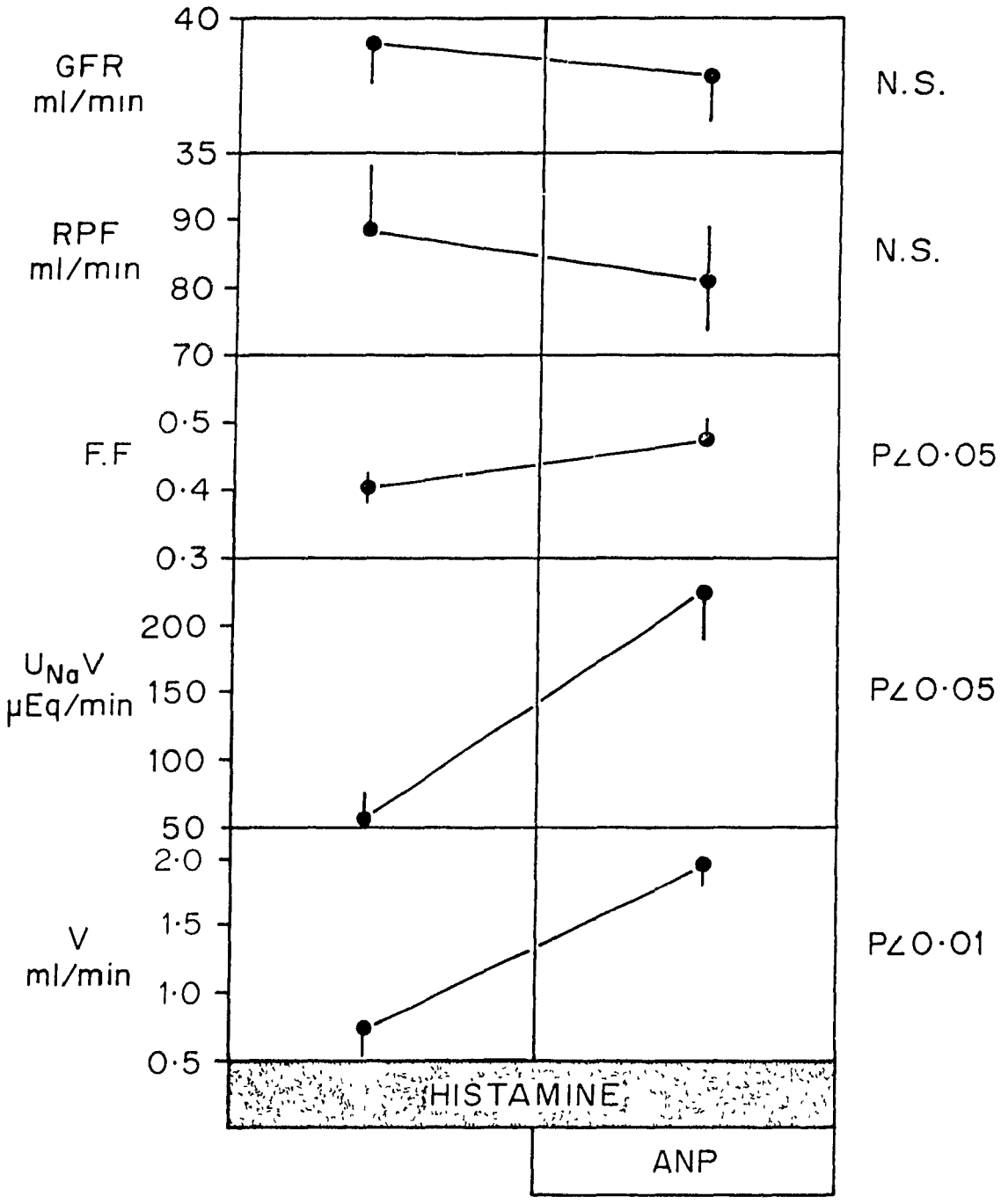

FIGURE 5.1 Effect of ANF on renal function in 6 dogs receiving histamine into a femoral vein. Portal pressure is normal.

($P<0.05$). Blood pressure declined significantly from 94.2 $\pm$ 4.9 to 83.3 $\pm$ 5.1 mm Hg $P>0.05$, while portal venous pressure remained constant (13.0 $\pm$ 0.2 vs 13.3 $\pm$ 0.8 cm H2O $P>0.05$).

5.4 Effect of Intraportal Infusion of Histamine

When histamine was infused into the portal vein, portal pressure rose from 13 $\pm$ 0.3 cm H2O to 19 $\pm$ 2.2 cm H2O ($P<0.05$), a 46.2% increment in pressure. The remaining changes are summarized in Figure 5.2. Arterial blood pressure did not change significanly, GFR and CPAH now fell significantly and urinary sodium excretion declined, but did not reach statistical significance.

5.5 Effect of ANF in the Presence of Intrahepatic Hypertension

Figure 5.3 summarizes the response to ANF while histamine in an equivalent dose to that previously infused into the femoral vein, was being infused into the portal vein to maintain intrahepatic pressures. GFR rose significantly; CPAH also increased, but this change did not achieve statistical significance. As a result of these increments in renal hemodynamics, FF remained constant. As previously noted for the control phase of the experiment, both sodium excretion and urine flow increased dramatically. In terms of fractional excretion, the F.E. of water increased from

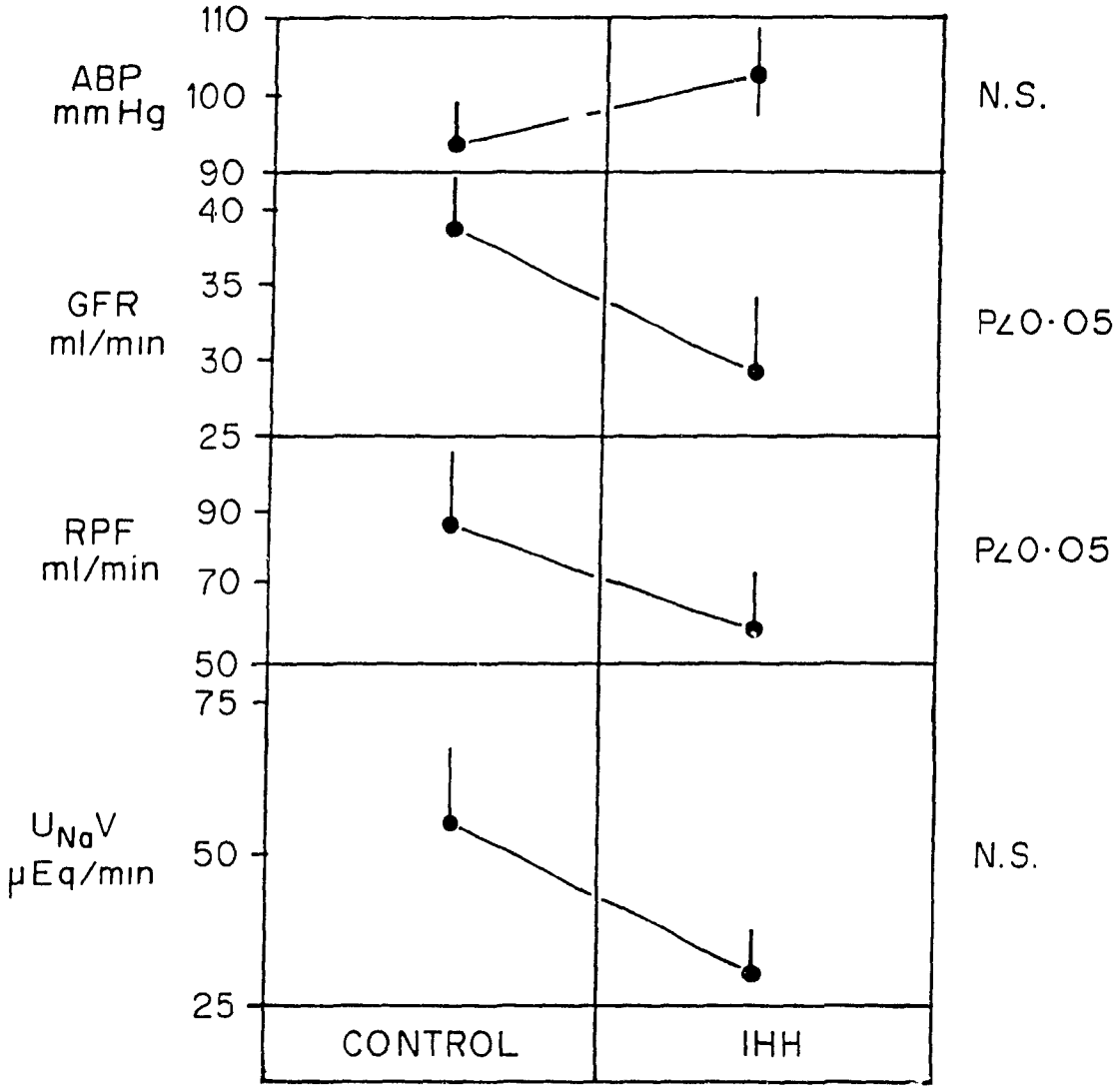

FIGURE 5.2 Changes in arterial pressure and renal function when histamine is infused in the portal vein to produce intrahepatic hypertension.

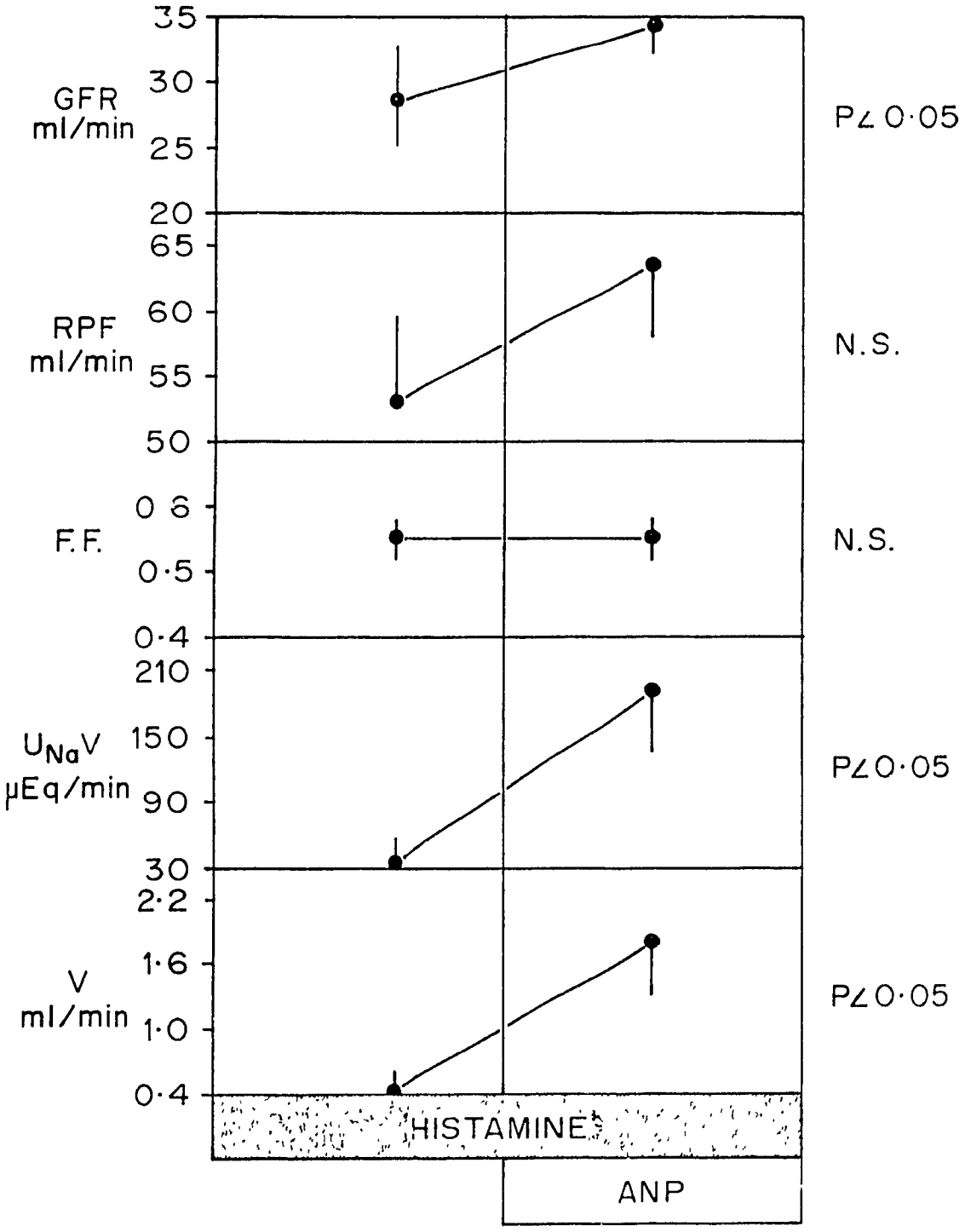

FIGURE 5.3 The effect of ANF on renal function in 6 dogs receiving histamine into the portal vein. Portal pressure elevated.

1.37 ± 0.14% to 5.17 ±0.15%, P<0.05. F.E. of sodium changed from 0.67 ± 0.17 to 3.6 ± 1.5% (P<0.05).

The changes in fractional excretion of sodium and water were not significantly different from each other when ANF was administered either during the control or experimental phase (portal pressure elevated).

Figure 5.4 summarizes the changes in the major variables in both control and experimental phases of the experiment. Though there were minor differences in the response of renal hemodynamics to the ANF infusion between the control and experimental phases of the study, there was no blunting of either water or sodium excretion during IHH.

5.6 DISCUSSION

As noted previously in our laboratory, the infusion of small doses of histamine base into the systemic circulation does not influence caval or portal pressure, arterial blood pressure or renal function (Levy, 1974). When this same dose is infused into the portal vein, there is a significant increment in portal (and presumably intrahepatic) pressure without any change in ABP. Our laboratory (Levy, 1974), as well as others (Campbell et al., 1982; Blanchart et al., 1987) using mechanical means to raise intrahepatic pressure have shown that intrahepatic hypertension (but not elevated portal venous pressures)

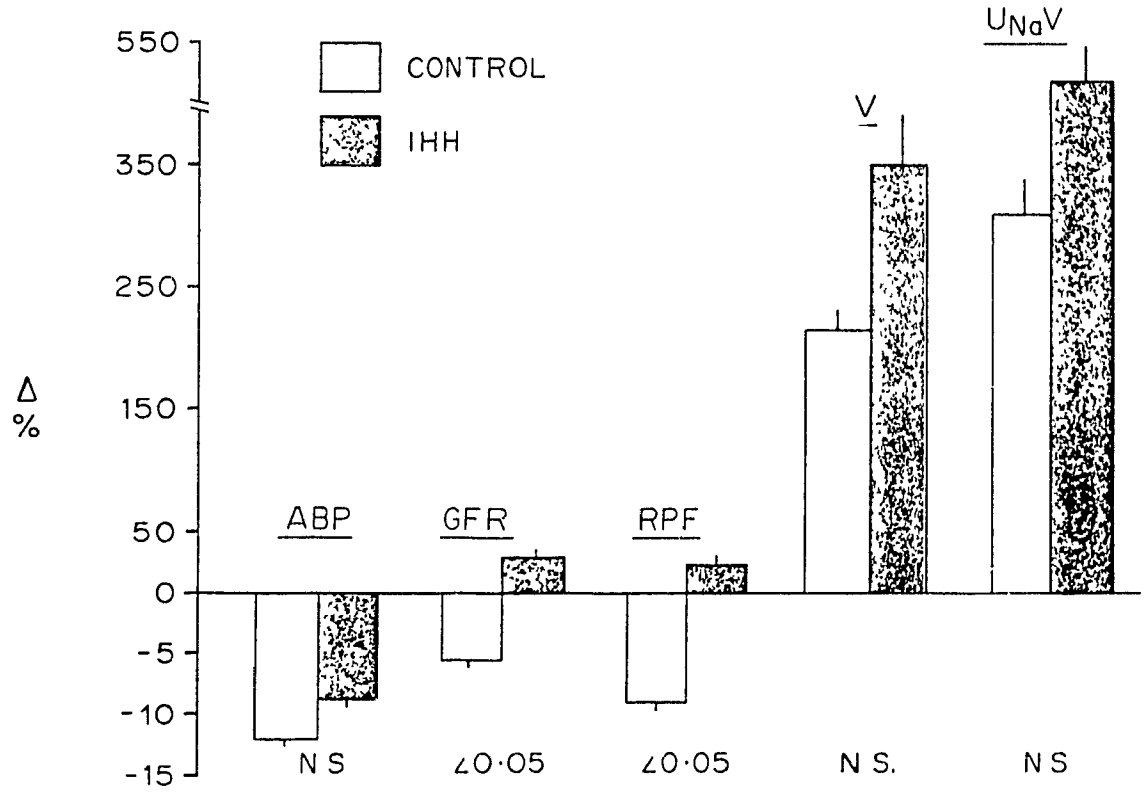

FIGURE 5.4 Percentage change in arterial blood pressure and some parameters of renal function during a control phase and during IHH. Probability levels compare changes in response to ANF infusion between phases.

whether produced in this way or with histamine, is generally associated with the tubular retention of sodium. It should be noted that in the present study, IHH was associated with a decline in urinary sodium excretion, though the change did not reach statistical significance. This was probably due to experimental design; in the present study dogs were hydropaenic; in the previous study demonstrating IHH-induced anti-natriuresis the dogs were saline loaded (Levy, 1974). This study was performed in hydropenic rather than volume-loaded dogs in order to "sensitize" the tubule to the effects of ANF in a physiological setting of IHH uncomplicated by other possible interfering physiological variables, particularly altered renal responsiveness to exogenous ANF in the presence of acute saline expansion.

When ANF was infused into the systemic circulation, there was the anticipated drop in ABP, concomitant with a significant increase in the renal excretion of water and sodium. This natriuresis occurred without any significant increment in GFR or CPAH, though filtration fraction did increase. When ANF was infused in the presence of IHH, GFR rose, though not renal plasma flow or filtration fraction and there was still a marked natriuresis and diuresis. It is not clear why GFR rose in this latter instance when it did not during the initial control periods. It could not be the influence of histamine since this base was infused

in identical amounts during each phase of the experiment. It is possible, however, that since IHH depressed GFR (and RPF) from control levels, ANF either suppressed an angiotensin effect or in other ways the renal micro- vasculature was now more sensitive to a potent vasodilator.

As discussed in section 1.2, stimulation of low-pressure mechanoreceptors located on the portal venous side of the hepatic circulation, increases renal efferent sympathetic nerve activity, and reduces urinary sodium excretion and renal blood flow. Of interest is whether this is simply a neural reflex loop whereby the increase in renal nerve activity directly enhances sodium reabsorption or acts in some way to modify a humoral pathway. It has been shown that hepatic denervation abolishes the responses to elevated hepatic and portal pressure (Lautt, 1983) but this may be the result of removing the neural afferent input to a humoral mechanism. In particular, it is presently unknown if stimulation of hepatic mechanoreceptors can alter the release of ANF or, conversely, alter the renal responsiveness to the peptide.

Chronic IHH could alter renal sodium excretion through a signal to inhibit the release of ANF from the atrial myocyte. Indeed, both caval and cirrhotic dogs with sodium retention and ascites have chronically low plasma iANF levels. However, more important is that 50% of caval and

cirrhotic dogs do not respond to exogenous ANF infusion where plasma levels reach up to 2500 pg/ml. Therefore, if IHH is modulating ANF action, it must be at the level of the kidney. It is unlikely that the increase in renal sympathetic nerve activity as produced by the rise in intrahepatic pressure is antagonizing the renal response to ANF since renal denervation did not restore the natriuretic response in caval non-responding dogs.

If one compares the response of ANF in both the control and hepatic hypertensive phase of the experiment, there were no differences in the blood pressure response or sodium and water excretion. During IHH, ANF increased GFR and CPAH significantly compared to the response during the control phase and yet sodium excretion did not increase out of proportion emphasizing that the natriuretic effect of ANF is independent of the GFR effect. These data are perhaps best compared with the chronic caval dog which, despite the presence of ascites and IHH, has returned to sodium balance. In these dogs, as in the dogs with histamine-induced IHH, the natriuretic response to ANF is normal.

These data permit the conclusion that acute IHH, by itself, without any confounding physiological disturbances, does not prevent a normal renal tubular response to ANF. Thus, it is unlikely that chronic IHH, per se, is the primary cause of the heterogeneous natriuretic response to

ANF in chronic caval and cirrhotic dogs with sodium retention and ascites.

CHAPTER 6: HETEROGENEOUS RENAL RESPONSES TO ATRIAL NATRIURETIC FACTOR: CIRRHOTIC DOGS

6.1 INTRODUCTION

The role of ANF in cirrhosis of the liver is quite confusing. Data from cirrhotic patients indicates that plasma iANF is low (Bonkovsky et al., 1986), normal (Henriksen et al., 1986; Burghardt Wernze, Diehl, 1986; Shenker et al., 1985), or elevated (Leung et al., 1987) while the renal response to manoeuvers raising endogenous ANF (volume expansion and water immersion) or exogenous ANF infusion is largely blunted. A recent investigation of water immersed cirrhotic patients with ascites demonstrated the phenomenon of heterogeneous natriuretic response similar to that described in the caval dogs (chapter 3) where 6 patients had a normal natriuretic response and 6 patients had no natriuresis.

The experiments presented in this chapter were designed to characterize ANF in a canine model of experimental cirrhosis in terms of its relationship to renin and aldosterone and plasma volume as the disease progresses. The renal response to exogenous ANF was measured several times prior to the development of ascites and again in great detail when ascites was present in order to define the possible heterogeneity of response.

6.2 CHARACTERISTICS OF CIRRHOTIC DOGS

6.2.1 Baseline Features

Table 6.1 summarizes the data obtained from 9 cirrhotic dogs followed sequentially from the pre-surgical phase to the phase of cirrhosis and ascites accumulation. Unlike chronic caval dogs, presented in Chapter 4, cirrhotic dogs do not ordinarily return to a state of sodium balance coexistent with the presence of ascites. The loss of body weight despite the accumulation of ascites is repeatedly observed in this canine model and is due to progressive muscle atrophy. Note that renal function is maintained despite plasma volume expansion and the severe deterioration of liver function. Plasma levels of renin and aldosterone are elevated. Though not measured in this particular group of dogs, arterial blood pressure is invariably slightly reduced, cardiac output increases and central venous pressure remains unchanged (Levy, Allotey, 1978). As observed for the chronic caval dogs, plasma levels of iANF were reduced in cirrhotic dogs with ascites, falling from a control level of 71.6 $\pm$ 9.5 pg/ml to 34.7 $\pm$ 5.4 pg/ml ($p<0.05$).

6.2.2 HPLC of Atrial Extracts

Figure 6.1 compares the RP-HPLC profile of atrial iANF from a cirrhotic dog with avid sodium retention and ascites to the iANF profile obtained from normal canine

	CONTROL (N = 9)	CIRRHOSIS (N = 9)
Body weight kg	15.9 ± 1.4	13.2 ± 1.1*
Plasma volume ml	632 ± 34	789 ± 24*
P_{Na^+} mEq/L	149 ± 1.0	148 ± 2
P_{k^+} mEq/L	4.3 ± 1.2	4.2 ± 0.2
U_{Na^+} mEq/L	106 ± 11.7	3.5 ± 1.0*
24 hr urinary Na^+ mEq/day	45.1 ± 1.9	1.9 ± 1.2*
$U_{Na}V$ - μEq/min †	56.3 ± 9.8	6.1 ± 2.2*
$U_k/U_{Na}+U_k$ %	44 ± 4	91 ± 2*
GFR - ml/min	42.3 ± 3.3	37.9 ± 4.6
C_{PAH} ml/min	104 ± 8.4	110 ± 15
FF % ≠	41 ± 2	40 ± 5
SGOT IU	29 ± 3	86 ± 7*
Serum Bilirubin mg/dl	0.22 ± 0.10	8.3 ± 0.4*
Serum Alkaline Phosphatase IU	24 ± 5	380 ± 41*
Plasma protein gm/dl	5.3 ± 0.16	4.0 ± 0.14*
Hematocrit %	48 ± 1.1	40 ± 1.2*
Plasma renin activity ng/ml/hr	1.41± 0.47	17.1 ± 6.9*
Plasma aldosterone ng/100 ml	4.33± 0.31	27 ± 9.7*

≠ FF = filtration fraction; * $p < 0.05$; † $U_{Na}V$ = urinary sodium excretion in acute clearance study.

TABLE 6.1: Characteristics of cirrhotic dogs

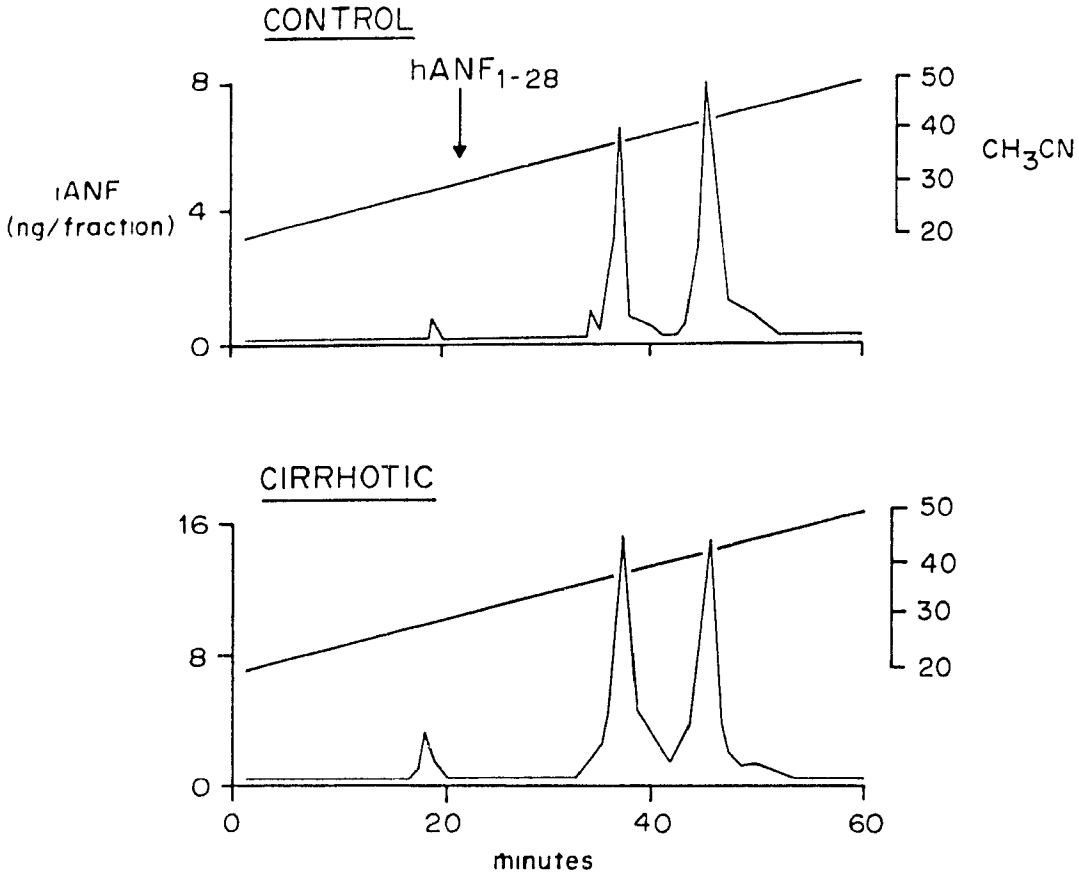

FIGURE 6.1 RP-HPLC profile of atrial extracts from normal dog (upper panel) and cirrhotic dog (lower panel). Arrow indicates retention time of human ANF (99-126).

atria. Three peaks of immunoreactivity eluted from the column with the same retention time in each extract indicating that the intraatrial processing of pro-ANF is normal in the cirrhotic dog.

6.2.3 Atrial iANF

The atrial content of iANF in cirrhotic dogs is compared to iANF content of normal atria in Figure 6.2. Left atrial content fell from 32.3 $\pm$ 1.5 to 24.2 $\pm$ 2.8 ng/mg atrium ($p<0.05$) in the cirrhotic dog while right atrial content did not change. Of interest, this is the same pattern as observed in the TIVC dogs, and, in fact, left atrial content was the same in the caval (21.4 $\pm$ 2.7 ng/mg atrium) and cirrhotic dogs.

6.2.4 Plasma T1/2

Plasma T1/2 did not change once the dogs became cirrhotic and developed ascites. Control T1/2 = 1.99 $\pm$ 0.09 min versus cirrhotic T1/2 = 2.15 $\pm$ .11 min.

6.3 RENAL RESPONSE TO ANF INFUSION

Nine animals were tested in a control phase and again when they were clearly cirrhotic with significant amounts of detectable ascites and avid urinary sodium retention. In 3 dogs the ascites was mobilized with a LeVeen peritoneovenous shunt and the response to ANF was

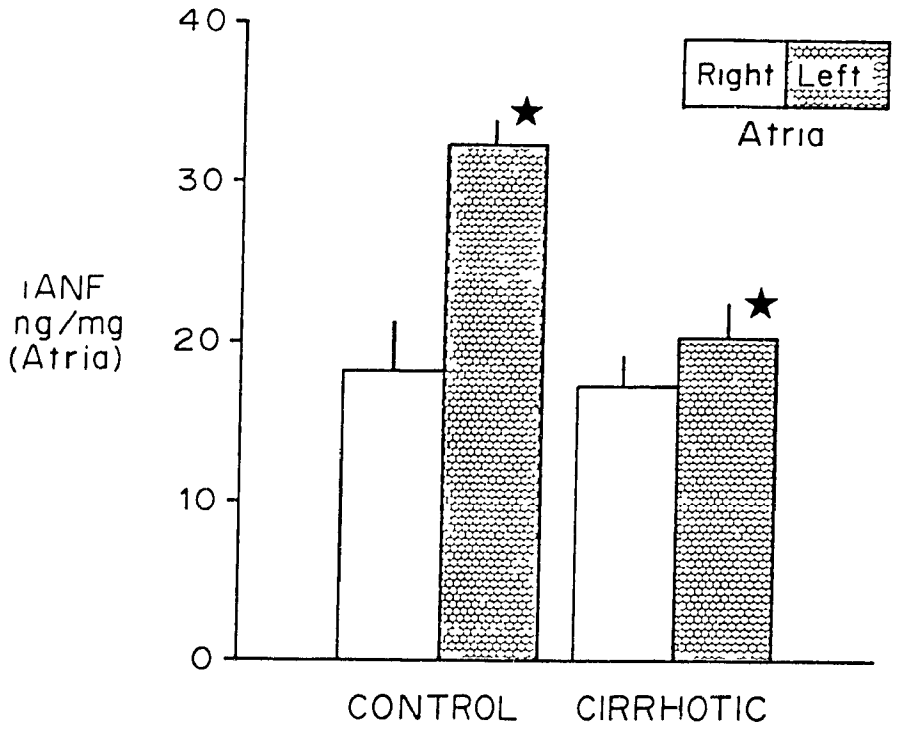

FIGURE 6.2 Comparison of atrial iANF content (ng/mg wet weight) in control and cirrhotic dogs. *p<0.05 vs. right side.

reevaluated. All dogs were handled identically, appeared to be uniform in their pathophysiological evolution and generally were studied 10-12 weeks post bile duct ligation. Figure 6.3 summarizes the effect of intravenous rat 1-28 ANF infusion at 175 ng/kg/min on urine flow and sodium excretion in all 3 phases of the experiment. There was no diuresis in any phase of the experiment, a significant natriuresis in the control phase (ΔUNaV = 122 $\pm$ 22 uEq/min) and a markedly attenuated natriuresis in the remaining two phases (ΔUNaV = 29 $\pm$ 12.5 uEq/min and 34 $\pm$ 18.9 uEq/min respectively). Fractional excretion of sodium was significantly increased as well in each phase, though markedly blunted in the last two. Figure 6.4 summarizes the effect of exogenous ANF on renal hemodynamics in each phase of the experiment. None of the variables depicted changed in response to ANF for each experimental phase.

6.4 IDENTIFICATION OF THE HETEROGENEOUS NATRIURETIC RESPONSE

When the natriuretic response to ANF infusion was examined in greater detail during the cirrhotic phase, the dogs were seen to divide into two groups, natriuretic responders and non-responders. Data for the original 9 dogs plus an additional 10 dogs studied in the presence

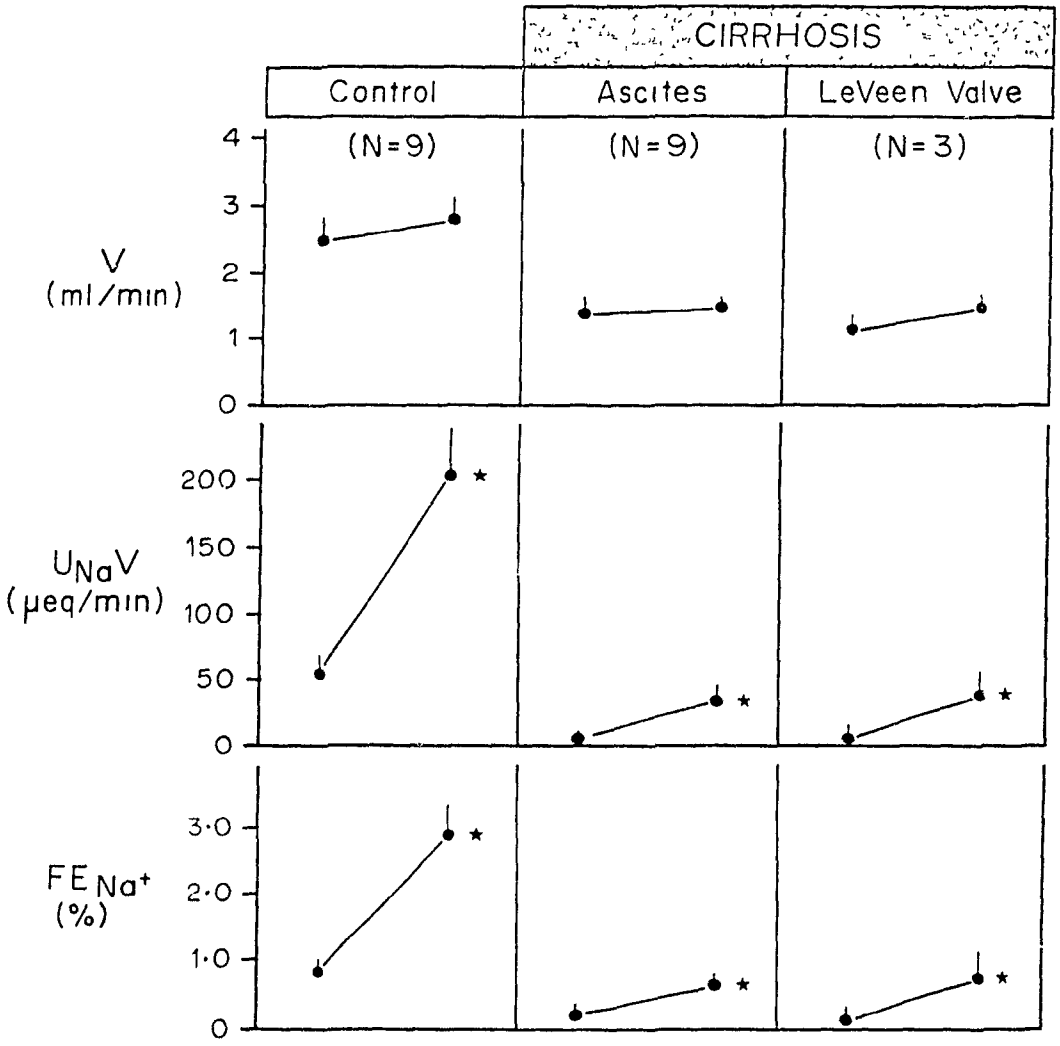

FIGURE 6.3 The effect of ANF (175 ng/kg/min) on sodium and water excretion in 3 experimental phases
*p<0.05 vs control

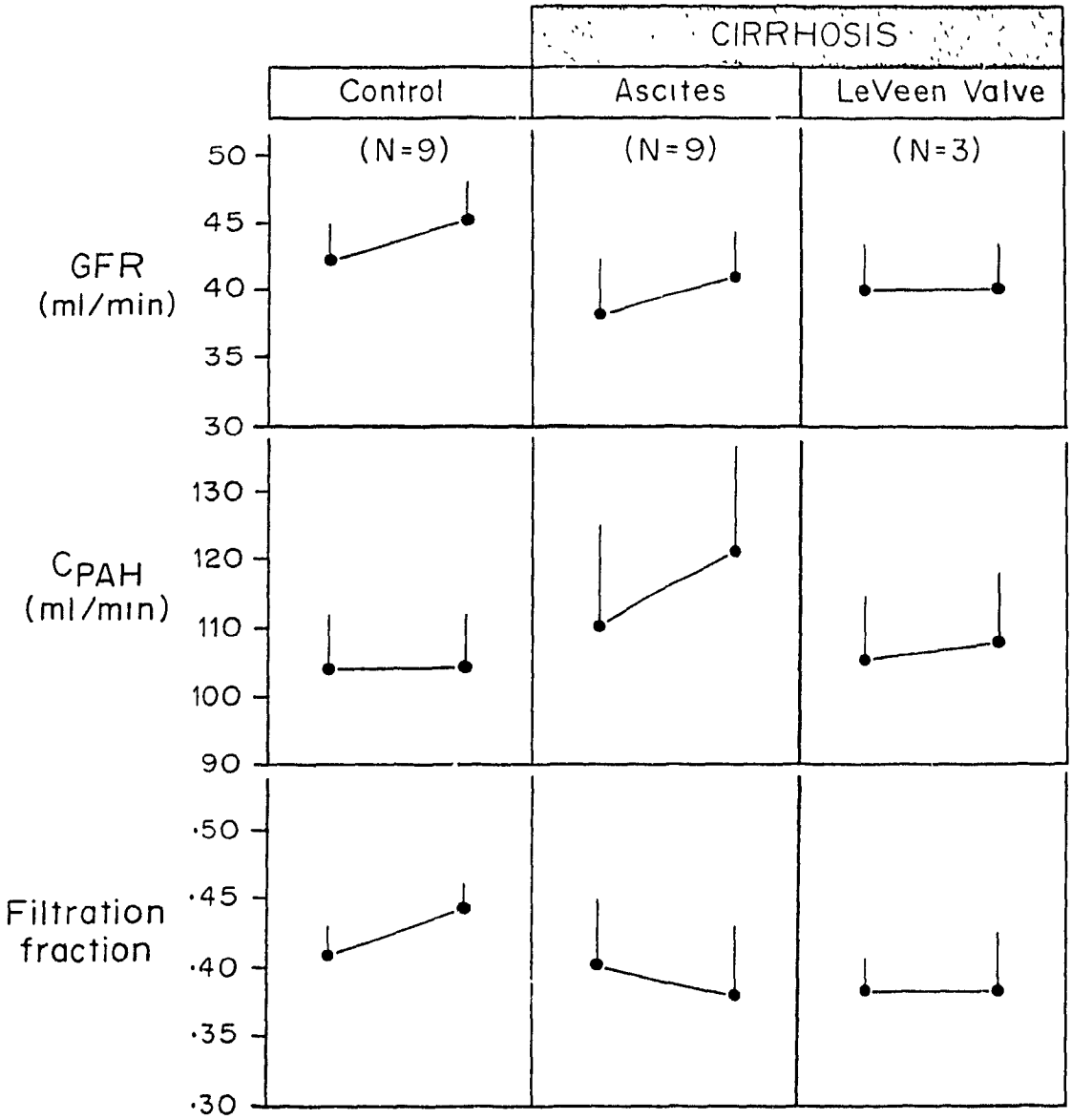

FIGURE 6.4 The effect of ANF on renal perfusion

of sodium retention and ascites is presented in Figure 6.5. The responding dogs excreted dUNaV of at least 20 uEq/min and ranged as high as 114 uEq/min with a mean ΔUNaV of 59.6 ± 10.6 uEq/min. The non-responders excreted virtually no extra sodium except for one dog whose dUNaV was 6 uEq/min. On average, the non-responders excreted an increment of 1.3 ± 0.6 uEq/min urinary sodium. Figure 6.6 correlates the sodium excretory data to changes in GFR, CPAH and filtration fraction. Neither the magnitude of the natriuretic response nor the lack of such response could be correlated to equivalent changes in GFR or renal perfusion.

6.5 RESPONDERS VERSUS NON-RESPONDERS

6.5.1 Plasma and Atrial iANF

Differences in plasma levels of iANF did not differentiate responding from non-responding dogs. These data are presented in Figure 6.7. The mean plasma iANF level of 40.2 ±11.4 pg/ml in the responders was not significantly different from the value of 34.2 ± 11.1 pg/ml obtained in the non-responders. Atrial content of iANF was comparably depressed in responding and non-responding dogs (Figure 6.8). Left atrial content averaged 24.8 ± 4.5 ng/mg atrium for the responders and 20.1 ± 2.1 ng/mg atrium for the non-responders ($p>0.05$).

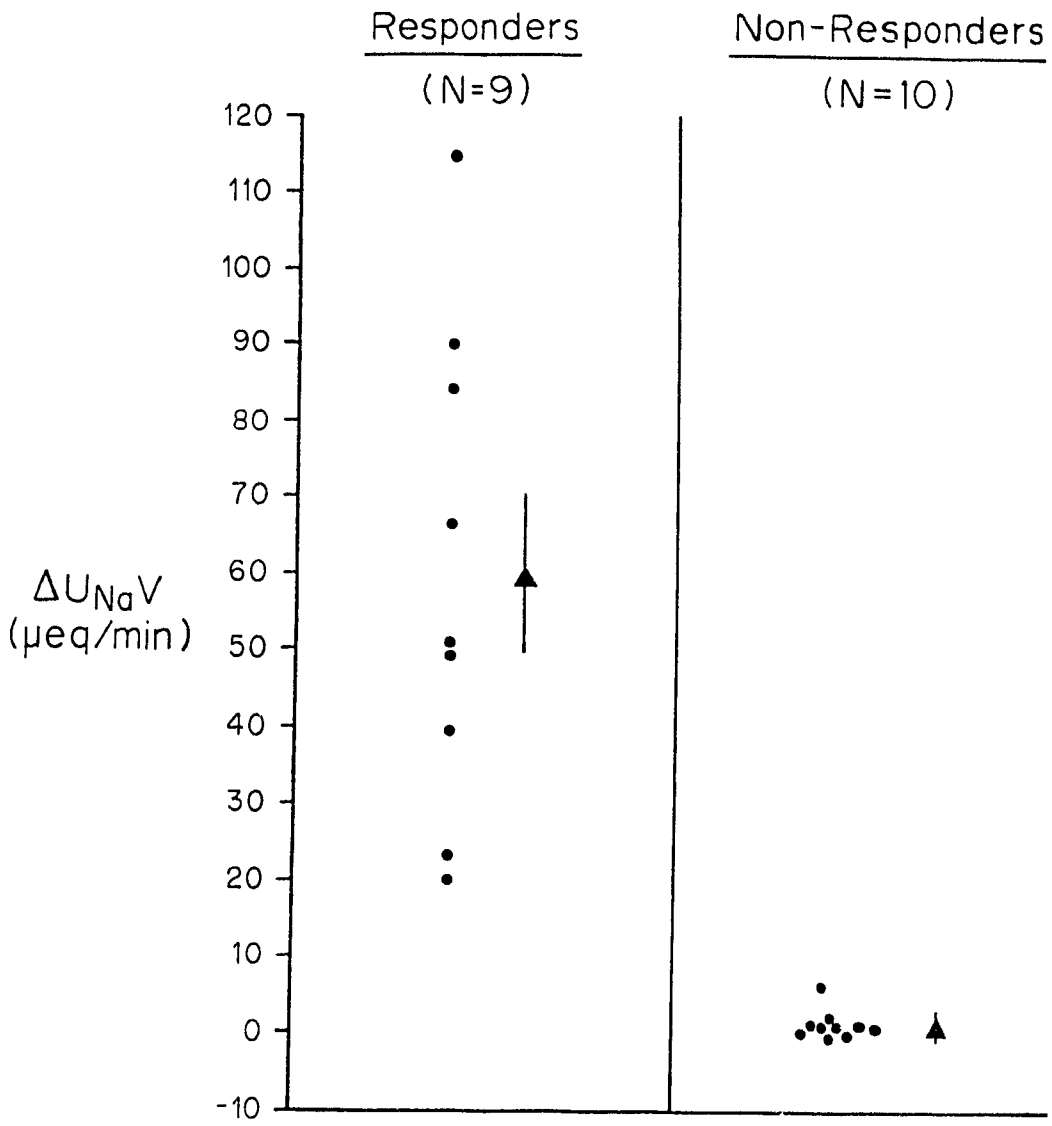

FIGURE 6.5 Sodium excretion in 19 cirrhotic dogs with ascites and sodium retention in response to i.v. ANF (175 ng/kg/min). Triangle represents the mean ± SE for each group.

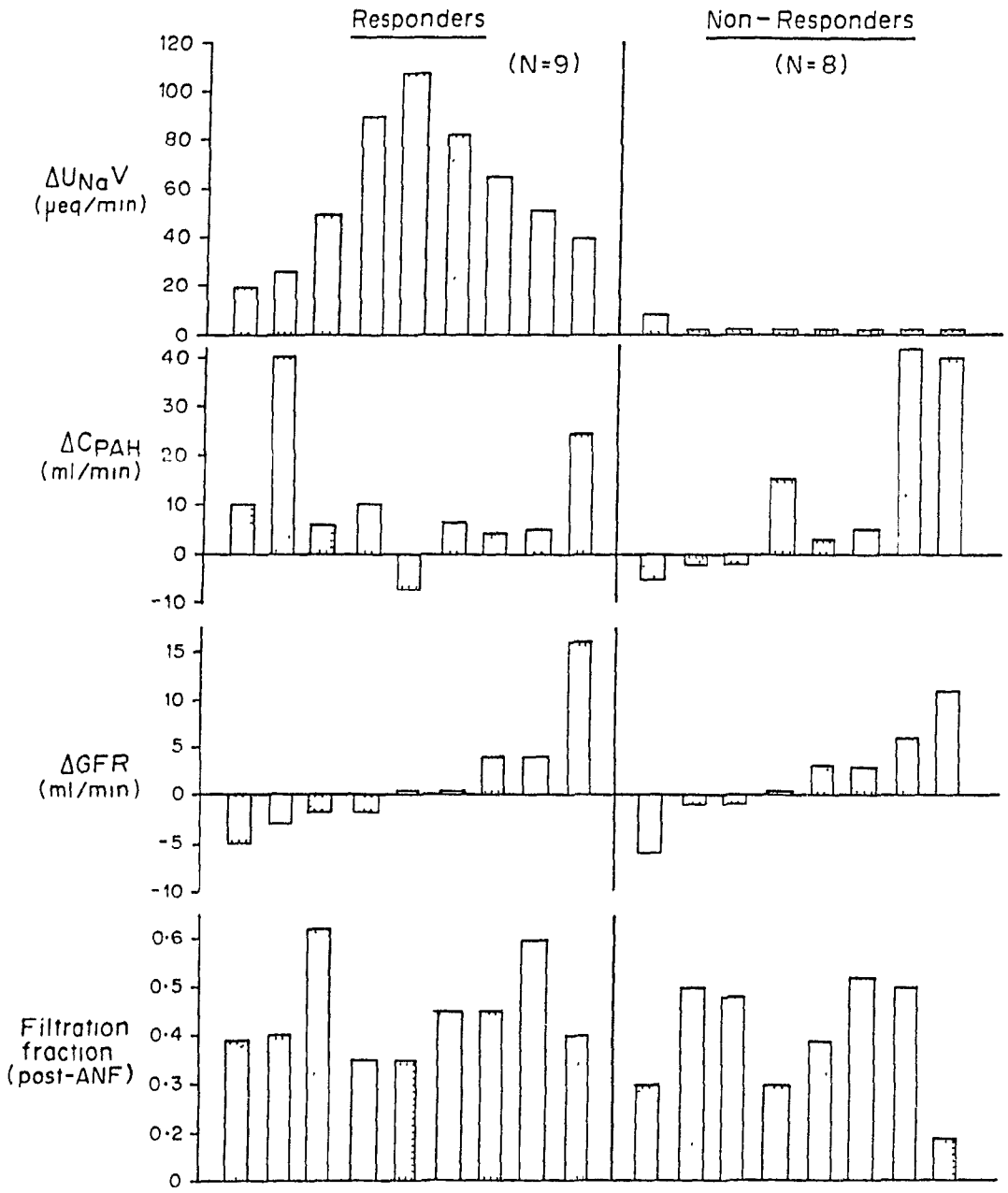

FIGURE 6.6 The correlation of ΔUNaV post-ANF infusion during cirrhosis and ascites to changes in renal hemodynamics.

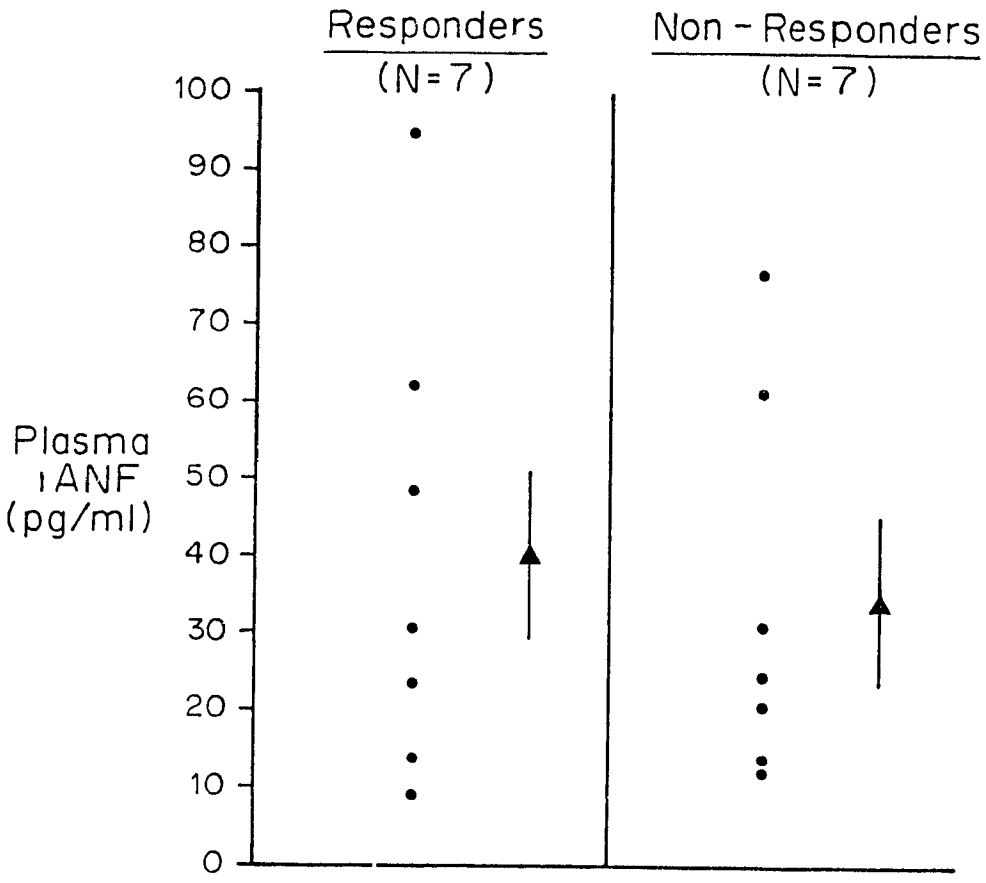

FIGURE 6.7 Plasma iANF in 7 responders and 7 non-responders. Triangles represent the mean of each group.

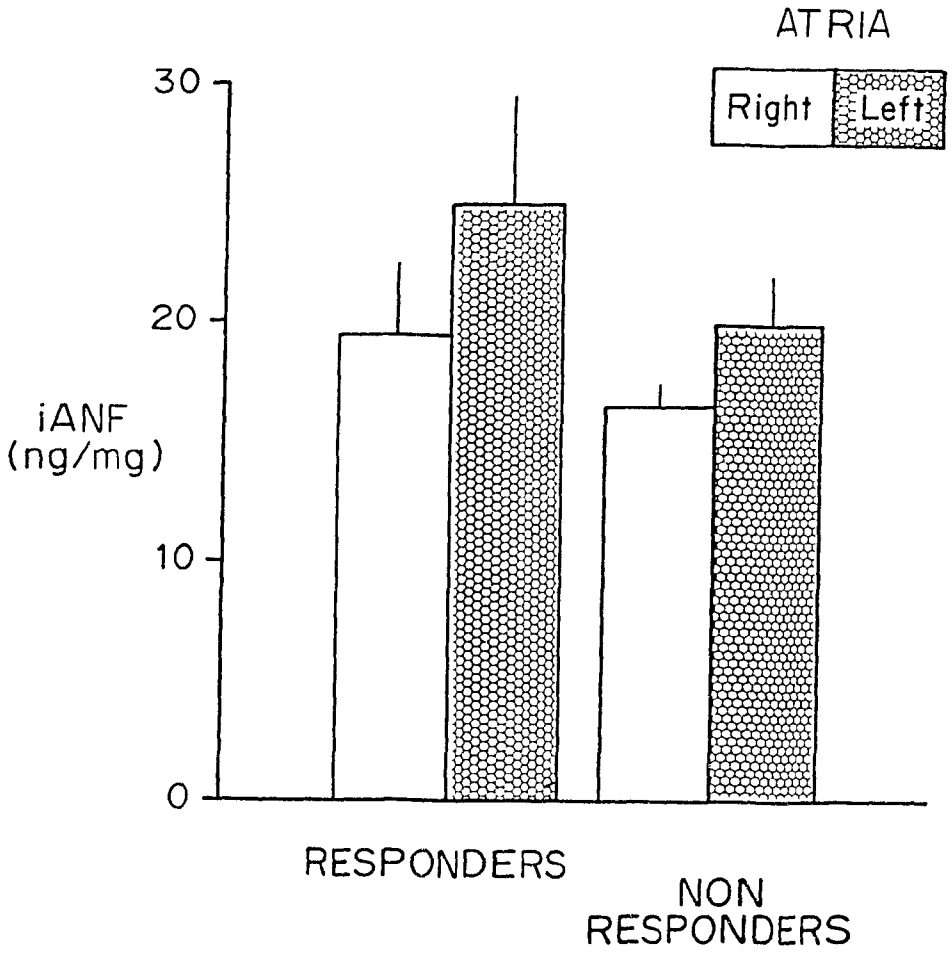

FIGURE 6.8 Atrial iANF content responding and non-responding cirrhotic dogs. No differences were measured.

Right atrial content averaged 18.8 ± 2.9 ng/mg atrium for the responders and 16.3 ± 2.6 ng/mg atrium for the non-responders.

6.5.2 Renal Hemodynamics

Figure 6.9 summarizes some renal functional data of the original 9 cirrhotic dogs before and after exogenous administration of ANF. There were no differences in urine flow, GFR or CPAH at baseline or post-ANF between the two groups.

6.6 cGMP Generation

The generation of cGMP in response to ANF infusion in normal and cirrhotic dogs is presented in Figure 6.10. Despite a blunted natriuretic response in 5 responding cirrhotic dogs and a complete lack of natriuresis in 4 non-responding cirrhotics, both groups demonstrated similar elevations of plasma and urinary cGMP as observed in normal dogs.

6.7 Effects of Renal Denervation and Vasodilatation

The natriuretic response to renal denervation and renal vasodilatation are presented in Figure 6.11. Baseline sodium excretion was not affected by denervation in either responding or non- responding dogs. The natriuretic response to ANF was magnified in the

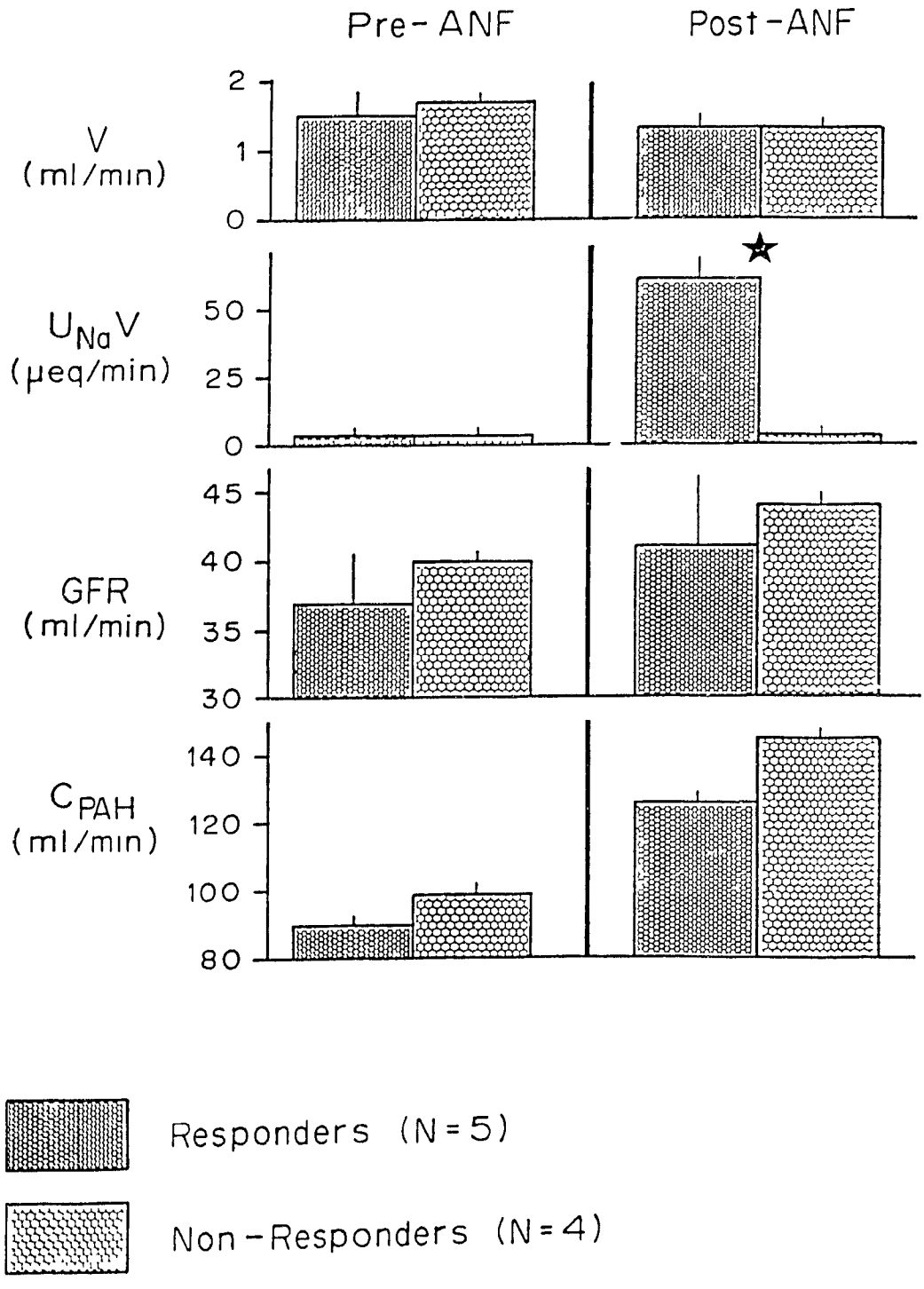

FIGURE 6.9 Effects of ANF on renal perfusion

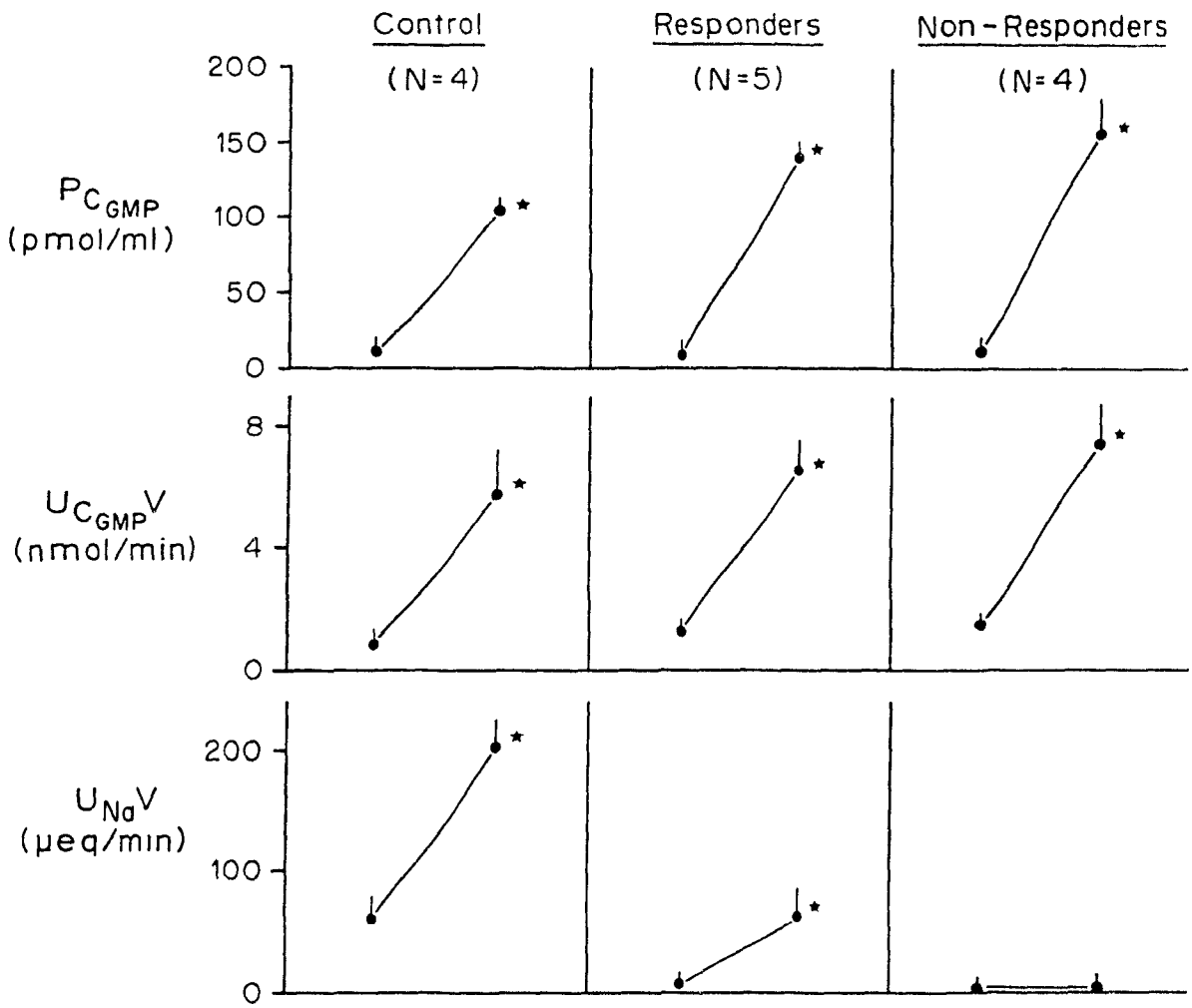

FIGURE 6.10 Changes in plasma and urinary excretion of cGMP in response to ANF infusion in normal and Cirrhotic responders and non-responders as compared to the natriuretic response in each group. *P<0.05

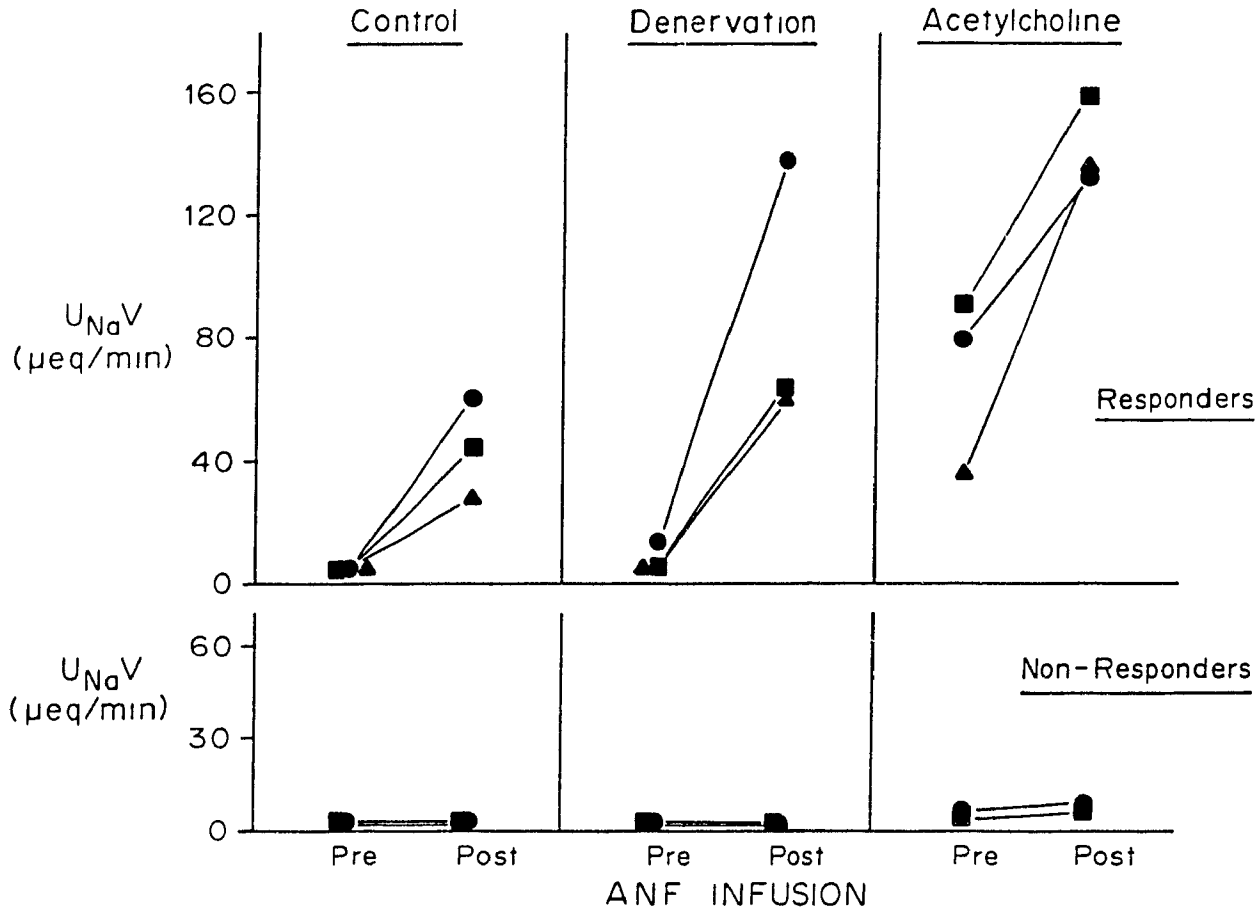

FIGURE 6.11 Natriuretic response to ANF following renal denervation and vasodilatation as compared to the control response. Data from 1 kidney.

responding dogs in the denervated kidney (84.3 ± 22 uEq/min vs 44 ± 10 uEq/min, $p<.05$). Non-responding dogs remained non-responders. Similar results were obtained following intrarenal infusion of Ach-Br to raise renal blood flow. Baseline sodium excretion and the natriuretic response was again magnified in the responding dogs. Although Ach-Br increased renal blood flow in the non-responding dogs, there was still complete attenuation of the natriuretic response in these dogs during ANF infusion.

6.8 Papillary Plasma Flow

Figure 6.12 summarizes the effect of ANF infusion at 125 ng/kg/min on papillary plasma flow, as measured by the Lillienfield technique, in 4 normal and 7 cirrhotic dogs with avid sodium retention and ascites. Papillary plasma flow was elevated in the cirrhotic dogs (48.3 ± 1.1 ml/min/100g) as compared to the normal dogs (35.5 ± 4.0 ml/min/100g) despite a markedly blunted natriuretic response to ANF. When the cirrhotic dogs were divided into responders and non-responders no difference in papillary plasma flow was demonstrated between the two groups.

6.9 Sequential Plasma Hormones

A group of cirrhotic dogs were studied weekly (or

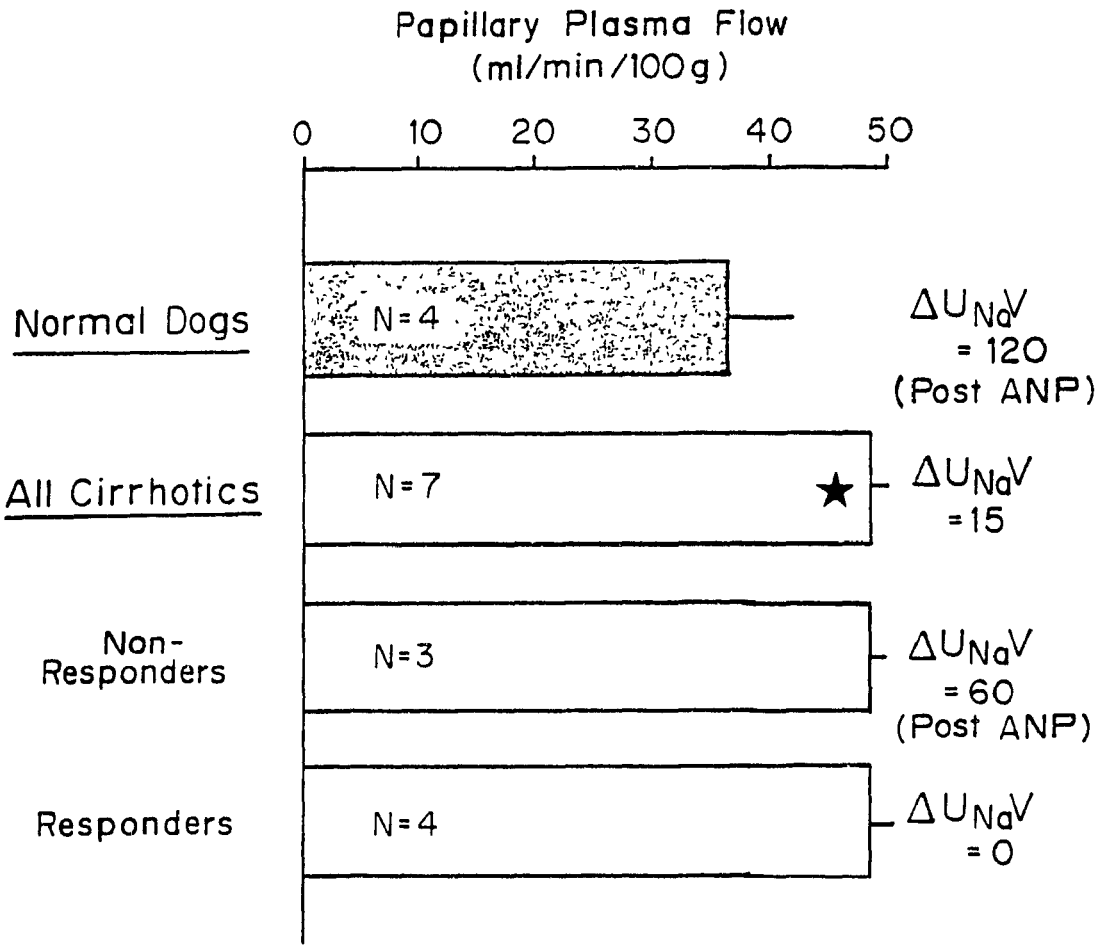

FIGURE 6.12 Papillary plasma flow in normal and cirrhotic dogs. 7 cirrhotic dogs are split into responders and non-responders.

bi-weekly) to determine the relationship between changes in plasma volume, plasma iANF, renin and aldosterone, and state of sodium balance as the disease progresses and the animals developed ascites. Figure 6.13 summarizes the data for plasma volume as a percentage of body weight, plasma iANF, and 24 hour sodium excretion. In the control state, plasma volume was 3.7 $\pm$ .2 %BWt., increased to a peak of 5.2 + .4 % at week 4 during the pre-ascitic phase, and rose even higher to 5.7 $\pm$ .3 % when the dogs had developed ascites. Plasma iANF fell to significantly lower levels than control for the first three weeks, was elevated at week four reaching a peak at week 5 at a level 2x greater than control and 5x greater than the first three weeks (150 $\pm$ 31 pg/ml; $p<0.05$ vs. control). These levels declined to control by week 7 but were markedly depressed when ascites was present at levels similar to the first three weeks. The dogs were in sodium balance at each of the time periods measured during the first 8 weeks except for a period of marked sodium retention at week 4 (daily sodium excretion = 11 $\pm$ 3 mEq/day). Figure 6.14 depicts the changes in plasma renin activity and plasma aldosterone concentration as compared to plasma iANF. Although no samples were taken during the first 3 weeks after surgery, both renin and aldosterone were normal at four weeks and remained at these levels until 7 weeks. It is interesting to note

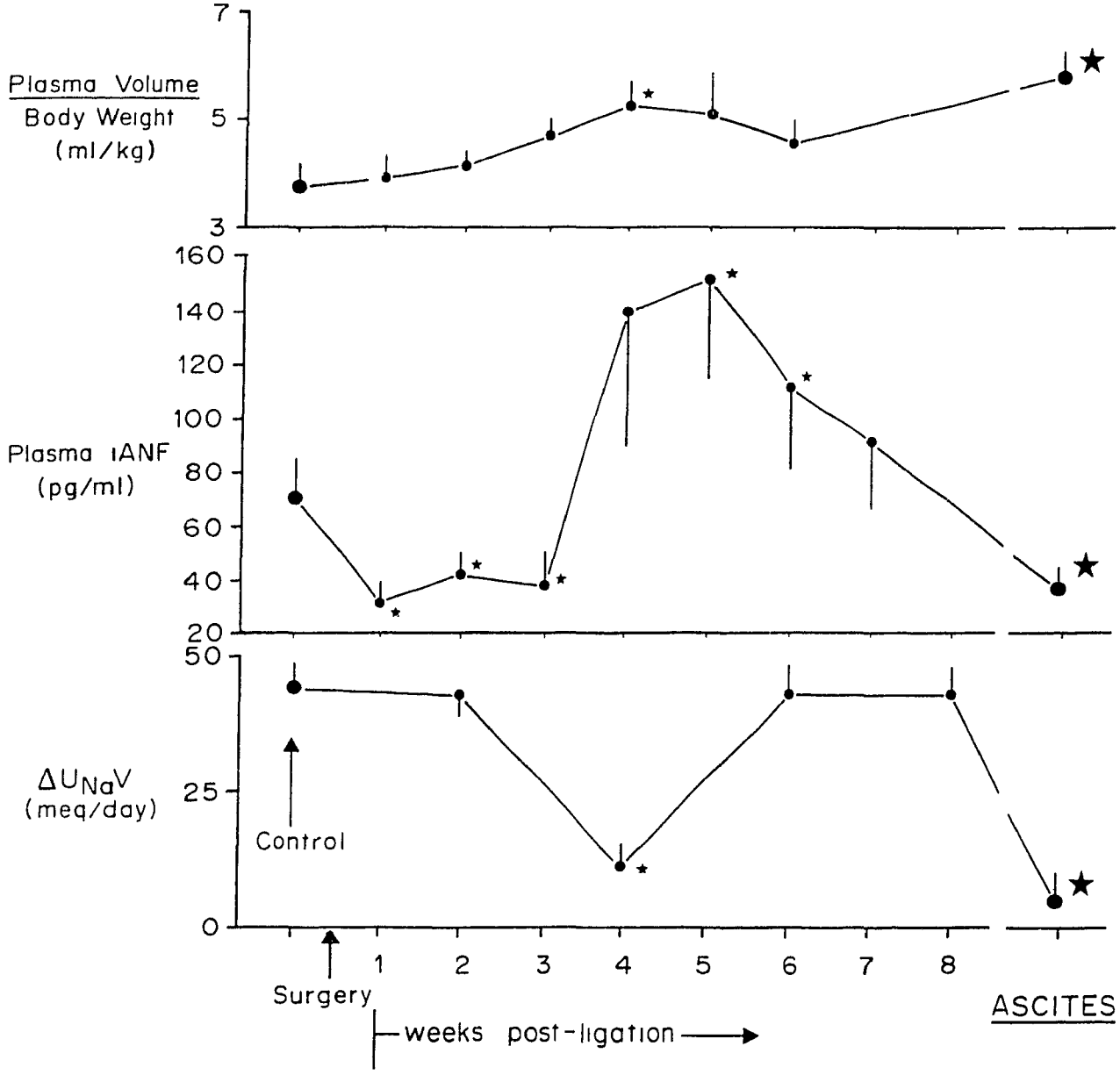

FIGURE 6.13 Sequential changes in plasma volume/body weight, plasma iANF, and 24 urinary sodium excretion in 9 cirrhotic dogs in control, pre-ascitic and ascitic phase.

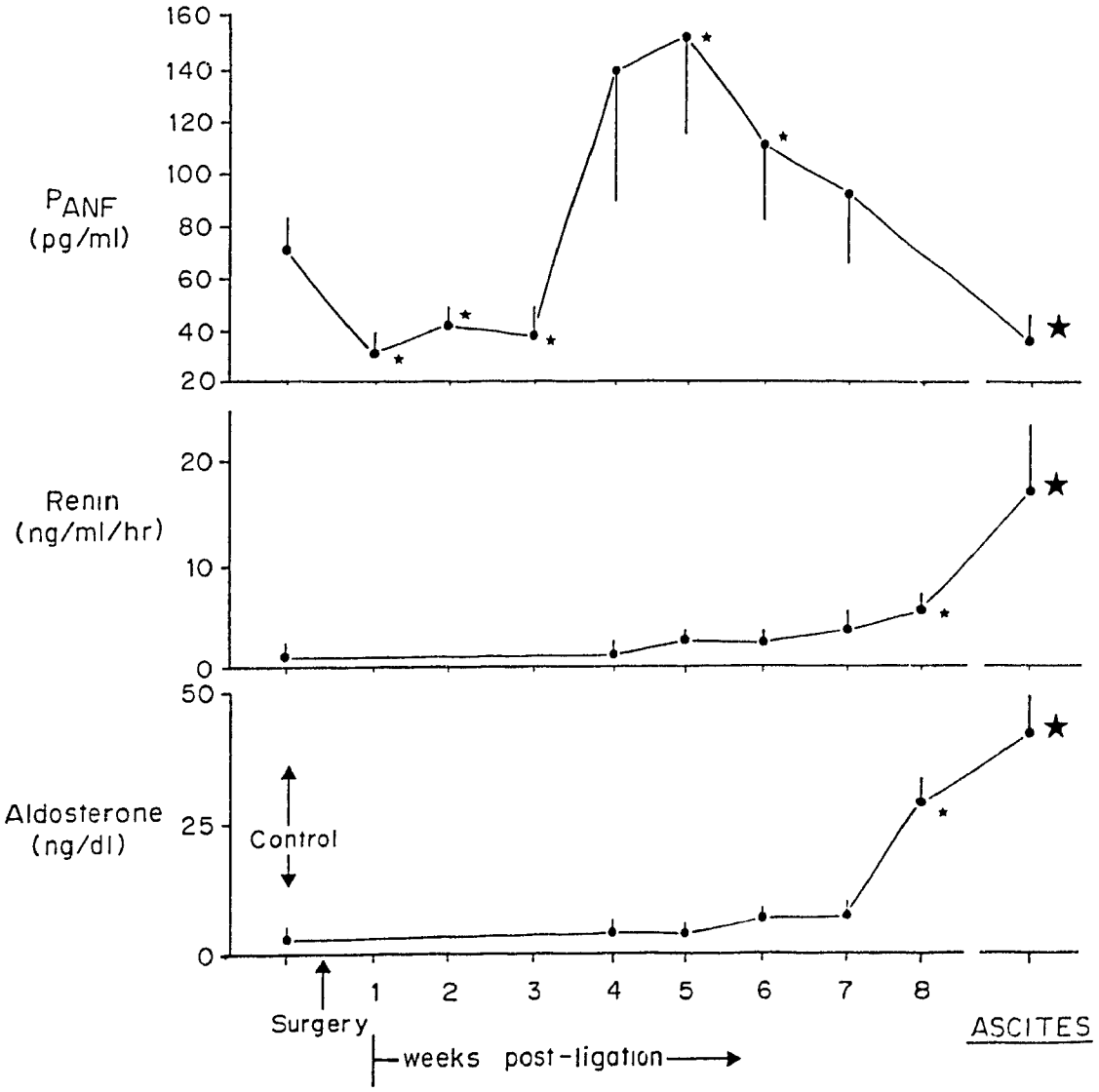

FIGURE 6.14 Sequential changes in plasma renin activity, aldosterone, and iANF in the control, pre-ascitic, and ascitic phase. *p<0.05 vs. control.

that both renin and aldosterone were normal during the period when plasma iANF was elevated and did not rise until iANF had returned to at least baseline levels.

6.10 Sequential Response to ANF Infusion

The renal response to ANF was measured at biweekly intervals from week 2 to week 8 after bile-duct ligation and again when ascites had developed (Figure 6.15). The heterogeneous natriuretic response reported previously for the cirrhotic dogs with ascites was also present at week 4, the dogs splitting into 5 responders and 4 non-responders. The mean natriuretic response was the similar to the control response in all other weeks studied. As shown in Figure 6.16, all dogs consumed 45 mEq/day during week 4 and excreted only minimal amounts of sodium. The natriuretic response of the individual dogs is shown in Figure 6.17 for week 4 and the ascitic phase. Note that two responders in week 4 became non-responders while 2 responders remained responders when ascites had developed. Similarly, 3 non-responders became responders while 1 non-responder again did not respond in the ascitic phase.

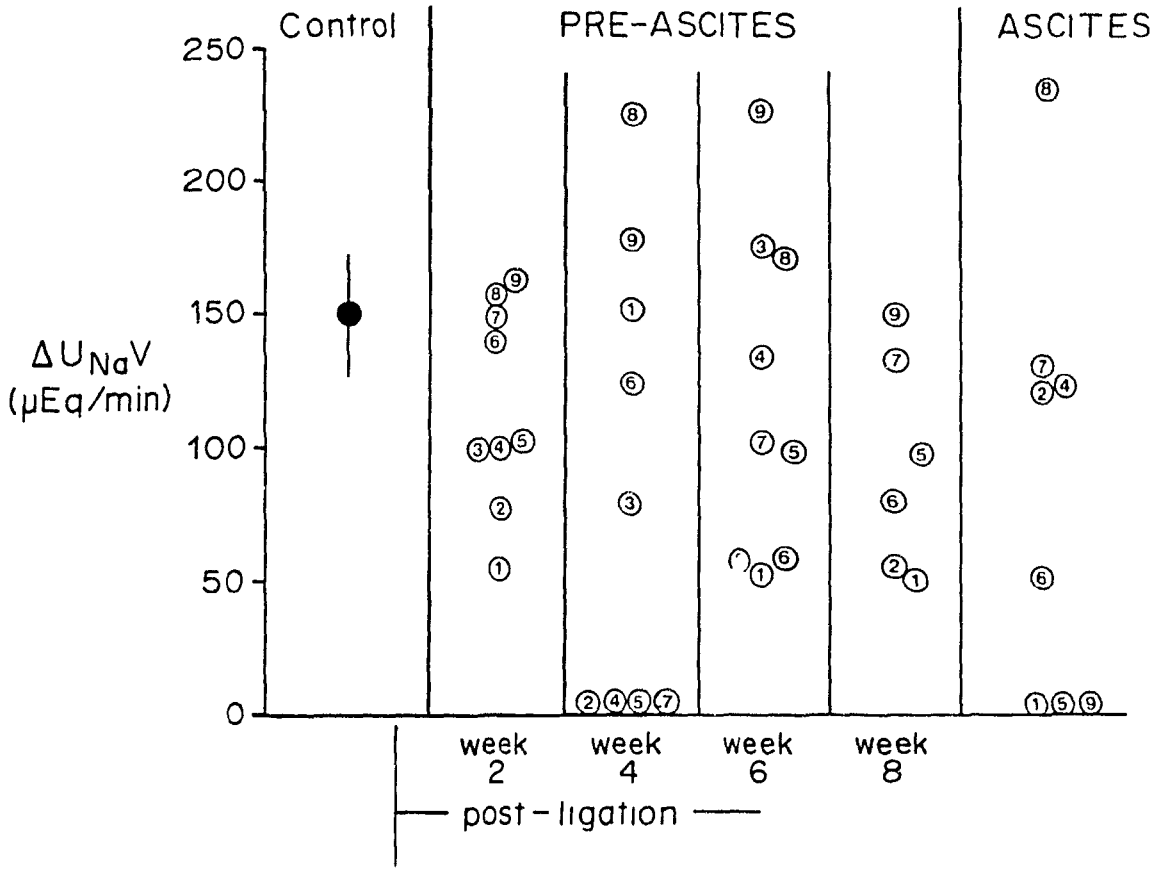

FIGURE 6.15 Natriuretic response to ANF measured biweekly after bile-duct ligation and in ascites. Sequential responses are shown for 9 individual dogs.

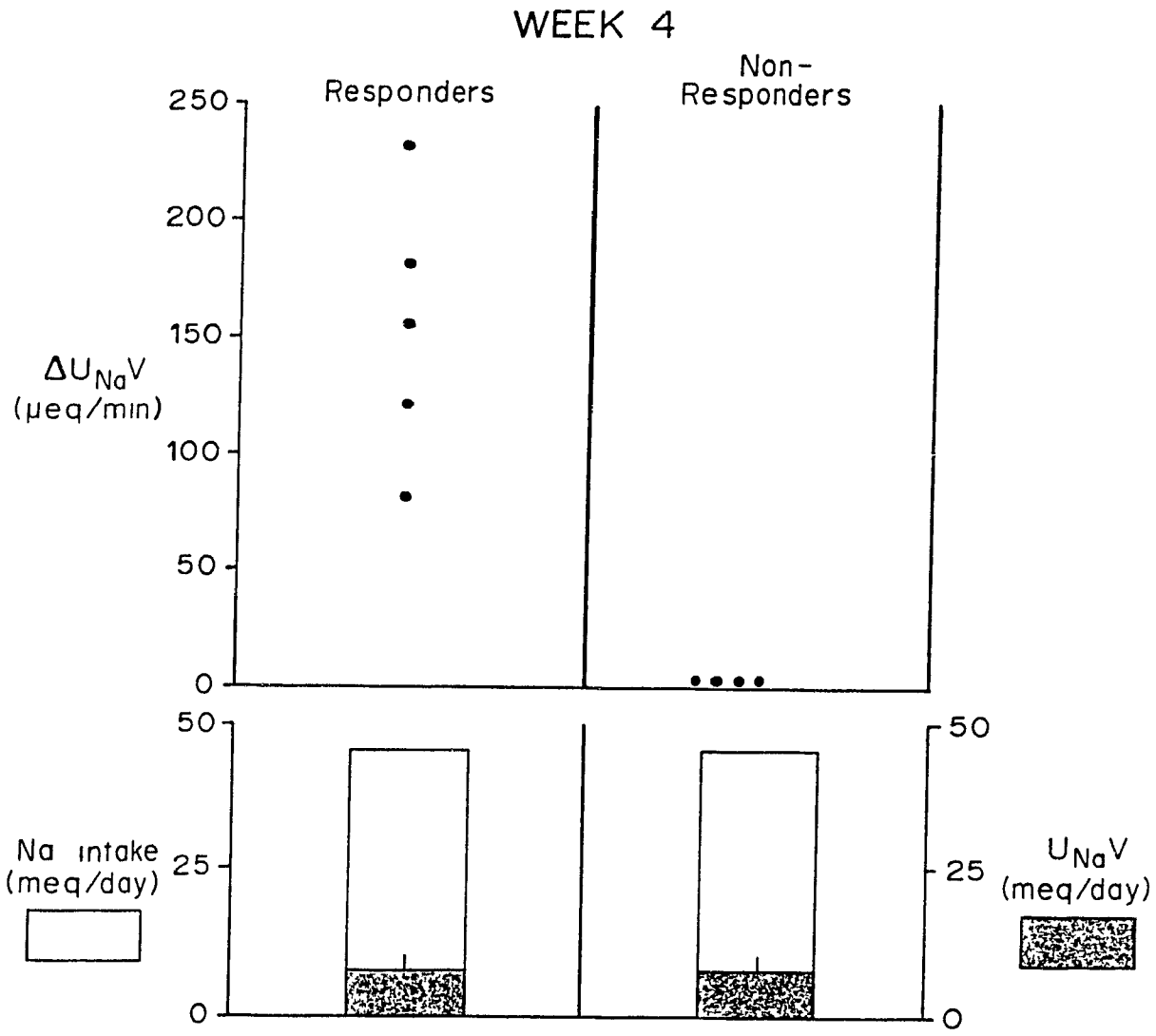

FIGURE 6.16: Responders and non-responders at week 4: natriuretic response as compared to daily sodium intake and 24 hour sodium excretion.

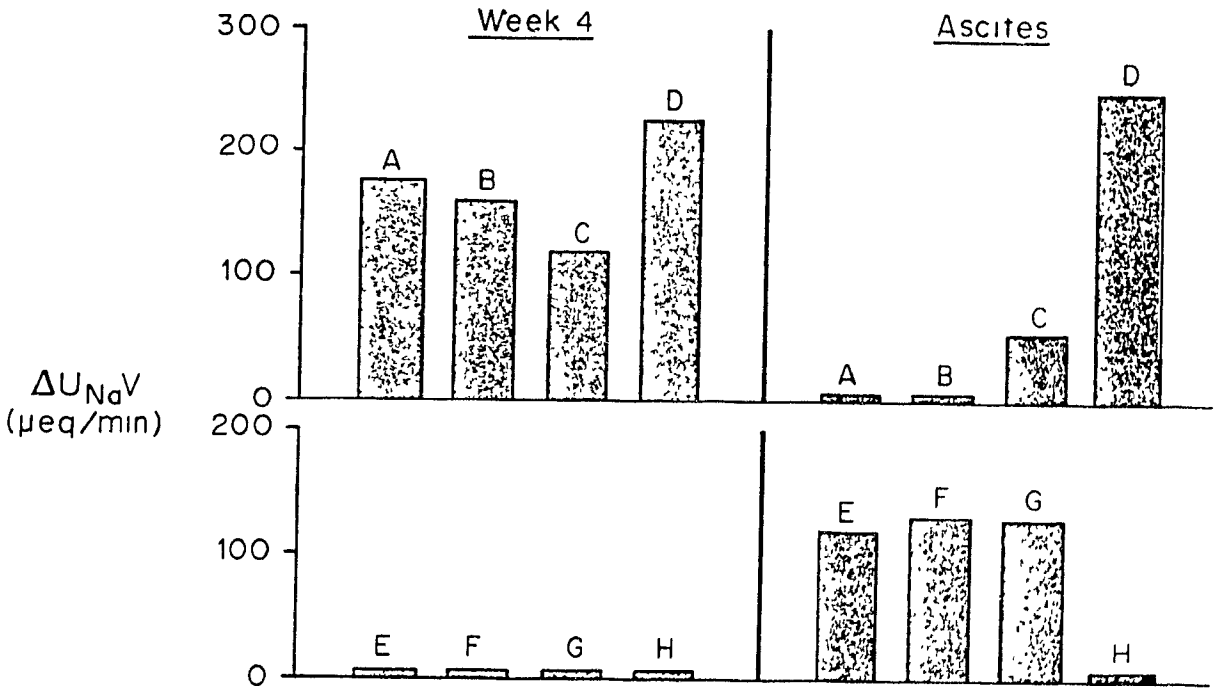

FIGURE 6.17 Natriuretic response of 8 cirrhotic dogs studied at week 4 and in the presence of ascites.

6.9 DISCUSSION

The present study was designed to assess baseline plasma and atrial ANF levels as well as the renal response to exogenous ANF infusion at defined physiological time points as cirrhosis of the liver developed in dogs with bile-duct ligation. This model is characterized by progressive degeneration of liver function accompanied by sodium retention and ascites formation. Weekly plasma iANF measurements in the dogs demonstrated a biphasic pattern. Plasma iANF levels fell dramatically by 1 week post-biliary ligation, remained low for approximately 4 weeks, then rose to a peak 2x greater than normal and 5x greater than levels during the first three weeks. Plasma levels fell steadily after week 6, reaching the levels approximating those measured during the early post-ligation period when the dogs were retaining sodium in the presence of ascites. Plasma levels of renin and aldosterone were normal in these dogs from four to eight weeks post- ligation at which time they rose to 200% of normal and increased even further when the dogs developed ascites. A study of the early time course of hormones in cirrhotic dogs (Better et al., 1988) demonstrated a marked rise in renin, aldosterone, and angiotensin II in the first week after surgery followed by a return towards normal and levelling off at a level slightly greater than normal during the first 5 weeks. We did not measure renin and aldosterone samples in the first

three weeks and therefore it is possible that we missed an early change in these hormone levels.

The pre-ascitic rise in plasma iANF presumably reflects a true increase in effective plasma volume which supports the pathogenesis of ascites as described by the overflow theory. In addition, the fall in plasma iANF concomitant with the rise in renin and aldosterone just prior to the development of ascites is further support for this theory. Although in this study the early rise in plasma volume was not statistically significant, the ratio of plasma volume/body weight was significantly elevated concomitant with the rise in plasma iANF and a period of sodium retention at around the four week post-ligation period. Interestingly, Olivera et al (1988) have demonstrated a 62% increment in plasma iANF in cirrhotic rats in the pre-ascitic sodium retaining phase, a period which they previously reported is associated with volume expansion in these rats (Lopez-Novoa et al., 1980). Unfortunately they did not measure plasma ANF in the presence of ascites although other investigators have reported a marked elevation in cirrhotic rats with ascites (Vakil et al., 1988; Koepke, Jones, DiBona, 1986). Plasma iANF varies considerably in patients with cirrhosis and ascites, some studies reporting elevated levels (Leung et al., 1987), while some report normal or even low levels (Burghardt, Wernze, Diehl, 1986). These data are difficult to

interpret since sodium intake, diuretic therapy and stage of the disease are factors which can influence the plasma levels.

In both the cirrhotic and caval dogs with sodium retention and ascites, the reduction in plasma iANF was accompanied by a decrease in left atrial content. The disappearance time (T1/2) and metabolic clearance rate of infused ANF are normal in these dogs indicating that the low plasma levels are a consequence of a decrease in peptide release. Under these conditions, without a change in the rate of ANF synthesis, one would expect a rise in atrial ANF content. There are two possible mechanisms which would result in both low plasma and atrial ANF levels. First, the stimulus for release may be normal while the stimulus for synthesis is reduced. If, under steady state conditions, release is a constant percentage of atrial content, then a reduction in stored ANF would produce a decrease in the absolute quantity of ANF released. Secondly, the stimulus for release and synthesis may both be reduced resulting in a fall in both plasma and atrial levels. This is certainly a possibility in the chronic caval dog where, despite elevation of renin and aldosterone and plasma volume expansion, central venous pressure and thus right atrial pressure remain significantly lower than normal. This fall in transmural pressure would produce a decrease in ANF release and may

also influence the rate of ANF synthesis. Indeed, plasma ANF levels remain low until the dogs return to sodium balance, a phase associated with normalization of CVP. Atrial content has not yet been measured in this phase.

The reduction in atrial content has also been demonstrated in cirrhotic rats (Jimenez et al., 1986) and cardiomyopathic hamsters (Edwards et al., 1986). However, both have elevated plasma ANF levels indicating that the reduction in atrial content may be a result of atrial oversecretion. This may also be the case for cirrhotic dogs despite low plasma ANF levels in the ascitic phase since there is marked elevation of plasma ANF for at least a two week period during the pre-ascitic phase. If there is no stimulus for an increase in synthesis, then atrial content will remain low. It is not clear why plasma levels are reduced in the cirrhotic dog in the presence of ascites since central venous pressure is normal in these animals. Perhaps high bilirubin or some other consequence of acute biliary obstuction can alter atrial ANF release. This, however, does not explain why cirrhotic dogs have low plasma levels while cirrhotic patients and rats with presumably the same degree of hepatic dysfunction have normal or elevated ANF levels in the presence of ascites.

Although the dogs in the control phase of the study responded to infused ANF with a dramatic natriuresis, when retested in the presence of advanced hepatic cirrhosis and

ascites there was a significant blunting of the natriuretic response by an average of 78%. This degree of blunting is slightly higher than the 55% suppression observed in the caval dogs with sodium retention and ascites (chapter 3) and probably reflects the lower baseline blood pressure in the cirrhotic dogs. In 9 dogs studied sequentially through 3 phases of the experiment, and an additional 10 cirrhotic dogs studied only in the presence of ascites, there was approximately a 50:50 division into natriuretic responders (N=9) and non-responders (N=10) when sodium retention and ascites are present. This 50:50 heterogeneity of natriuretic response to infused ANF was also demonstrated in the caval dogs (chapter 3). The only difference in the profile of responses in these two models is that cirrhotic responders had an attenuated natriuretic response relative to the control phase while the caval responders showed a normal natriuretic response.

Although it is generally agreed that the natriuretic renal response to infused ANF is attenuated in cirrhotic patients (Fyhrquist et al., 1985; Hricik et al., 1986; Brabant et al., 1986) and rats with experimental cirrhosis (Koepke, Jones, DiBona, 1986), the heterogeneity of natriuretic response has also been demonstrated. Cirrhotic patients undergoing thermoindifferent water immersion have elevations in plasma iANF levels comparable to normal subjects although only half exhibit an increase in sodium

excretion (Leung et al., 1987). Salerno et al (1988) recently reported that ANF (1 ug/kg) administered to cirrhotic patients without or with only modest retention produced a natriuresis comparable to that observed in normal subjects. However, when ANF was given to cirrhotics with avid sodium retention, the natriuretic response was completely or markedly attenuated. Of the 14 patients studied, 7 responded while 7 did not respond to the exogenous infusion. Since the responders were generally without ascites and had normal or only modest sodium retention while the non-responders had ascites and were avidly retaining sodium, it was concluded that the natriuretic responsiveness to ANF infusion was dependent on basal sodium retention. This result differs from our cirrhotic dogs where basal sodium retention was equivalent in the responding and non-responding dogs. It is important to point out that the response to ANF was measured in cirrhotic dogs with ascites at approximately the same time frame in their physiological expression (i.e. sodium retention, volume of ascites). Looking more closely at the study by Salerno et al. (1988), the 7 patients with 2-3+ ascites divided 4:3 into non-responders and responders. Thus, even in this group, the heterogeneity of natriuretic response is present. Responding and non-responding cirrhotic dogs could not be differentiated by degree of hepatic dysfunction, the liver function tests being

equivalent in both groups. Although the volume of ascites was not measured, it was clear by gross examination that responding dogs were not solely those with little ascites and neither were the non-responders solely those with large volumes of ascites. Plasma levels of iANF as well as infusion-induced increments in the plasma iANF were also equivalent in the two groups. In addition, responders could not be separated from non-responders either by baseline or ANF-induced changes in renal hemodynamics. In fact, neither in the pre- cirrhotic control phase nor the ascitic phase did the ANF infusion influence GFR, CPAH or filtration fraction in either group.

Since ANF is believed to exert its major natriuretic effects in the distal nephron (Goetz, 1986) we examined the possibility that differences in the fractional distal delivery of sodium could explain variable responses to infused ANF. 24 hour sodium balance, urinary concentration of sodium and the distal cationic exchange ratio (Uk/UNa+UK) did not differ between the two groups. Furthermore, baseline sodium excretion, as well as baseline and post-ANF filtered load of sodium during the acute clearance experiments were the same in responders and non-responders. These data strongly suggest that the distal sodium delivery is equivalent in the two groups under steady-state conditions thus making it unlikely that the lack of natriuretic response in 50% of the cirrhotic

dogs is related to the fractional sodium delivery to the distal tubule during ANF infusion.

Other possible causes of the blunting or complete attenuation of the natriuretic response in the cirrhotic dogs include changes in systemic hemodynamics or the renin-angiotensin system. Blood pressure and cardiac output were not measured in the cirrhotic dogs. However, it was shown in the caval dogs (chapter 3) that a 40% fall in cardiac output accompanied by a 15% drop in blood pressure was associated with a potent natriuresis in one dog and no natriuretic response whatsoever in another dog. Indeed, neither baseline nor ANF-induced changes in cardiac output or blood pressure differed between the two groups. Thus it is unlikely that exaggerated hypotension or fall in cardiac output can account for the heterogeneous response in the cirrhotic dogs.

Although not large numbers of renin and aldosterone were measured in the cirrhotic dogs, there did not appear to be a difference between responding and non-responding dogs. This is supported by the data from caval dogs which showed no differences between the groups for renin, aldosterone or vasopressin. However, it is possible that high intrarenal angiotensin II is stimulating cGMP hydrolysis such that there is insufficient cGMP effectively generated to produce the natriuretic actions of ANF. This is supported by a recent study by Smith and Lincoln (1987) showing that

Angiotensin II decreases cGMP accumulation in cultured aortic smooth muscle cells by stimulating cGMP hydrolysis. To determine if elevated angiotensin II (resulting from elevated renin) was offsetting the natriuretic potential in cirrhotic rats, Koepke, Jones and DiBona (1987) administered the converting-enzyme inhibitor, captopril, at a dose which did not influence blood pressure. They found that inhibition of the renin-angiotensin system did not restore the natriuretic response to ANF in cirrhotic rats nor augment the response in control rats. Further evidence indicating that abnormally high levels of these hormones is unlikely to produce the attenuated responsiveness in the cirrhotic dog is that mobilization of ascites with a LeVeen peritoneovenous valve, a procedure known to result in normalization of plasma renin and aldosterone, further plasma volume expansion (Levy, Wexler, McCaffrey, 1979) and marked elevation of plasma ANF (Campbell et al., 1987; Burghardt, Wernze, Diehl, 1986), no augmentation of the natriuretic response occurred in the three cirrhotic dogs tested. Two of the dogs had been natriuretic responders and one was a non-responder prior to ascites mobilization. The responding dogs now showed a similar natriuretic response as in the ascitic phase, remaining blunted as compared to control, while the non-responder again had no natriuretic response to the exogenous infusion. Unfortunately, an insufficient number of cirrhotic dogs

could be studied in this phase as a result of deteriorating condition of the dog or failure of the LeVeen valve we do not know if the normal natriuretic response to ANF would be restored in this final phase of the experiment. However, it is unlikely since these cirrhotic dogs are known to remain as salt retainers despite complete mobilization of ascites (Levy, Wexler, McCaffrey, 1979). Furthermore, chronic caval dogs not responding to ANF in the presence of ascites and sodium retention regain responsiveness only when sodium balance has been restored despite the presence of ascites. Moreover, mobilization of ascites did not enhance the natriuretic response to infused ANF further indicating that the state of sodium balance is the determining factor in ANF action. Thus, the heterogeneous natriuretic response persists in the face of abnormal tubular handling of sodium.

Other possible factors mediating the blunted or completely attenuated response to ANF in the cirrhotic dog with ascites were divided into 3 categories: 1) enhanced sympathetic nerve activity, 2) intrarenal hemodynamics, 3) signal transduction at the receptor level. Each of these factors has been discussed in detail in chapter 3 as they pertain to the caval dogs and thus will be addressed here only as they pertain to cirrhosis. It is generally agreed that efferent renal sympathetic nerve activity (ERSNA) plays an important role in the control of sodium excretion

and renin release (DiBona, 1982; others). It has been suggested that specific stimuli involved in the pathology of the cirrhosis serve to enhance ERSNA which, in turn, promotes renal sodium retention. Indeed, there is evidence that ERSNA is enhanced in cirrhosis (DiBona, 1984; DiBona et al., 1988) although neither acute unilateral renal denervation nor renal arterial infusion of the a-adrenergic blocker, phenoxybenzamine, were able to increase sodium excretion in bile-duct ligated cirrhotic dogs (Chaimovitz et al., 1977). Although the interaction between renal nerve activity and ANF action has not yet been characterized, it is possible that enhanced ERSNA in cirrhosis could prevent the full expression of ANF natriuretic action. This was investigated in cirrhotic rats with ascites by chronic renal denervation. Rats with previously blunted natriuretic responses to infused ANF now responded normally despite the presence of ascites and sodium retention (Koepke, Jones, DiBona, 1987). The investigators concluded therefore that enhanced renal nerve activity mediated the blunted natriuretic response in cirrhosis with ascites. The data from the cirrhotic dogs does not support such a conclusion. Although only 3 non-responding cirrhotic dogs were studied following acute renal denervation, it is clear that removing renal sympathetic nerve activity was ineffective in producing a natriuretic response to ANF. This is consistent with the

data from caval non-responding dogs (Chapter 3) where renal denervation also had no effect. Of interest, was that in the cirrhotic responding dogs, as in the caval responders, renal denervation exaggerated the natriuretic response to ANF. This data supports the conclusion that renal sympathetic tone limits the degree to which sodium excretion can be enhanced by ANF although is not the primary factor producing complete inhibition of natriuretic responsiveness in 50% of caval and cirrhotic dogs with ascites and sodium retention. The role of inner medullary hemodynamics in ANF action is currently under investigation. As described in chapter 3, augmentation of renal blood flow does not appear to play a role in the natriuretic response to ANF. Indeed, renal blood flow did not change when ANF was infused in cirrhotic dogs with ascites. However, redistribution of blood flow, in particular an increase in papillary flow has been invoked as a major mechanism of ANF action (Goetz, 1986).

Papillary plasma flow in control dogs was unaffected by ANF infusion despite an increase in sodium excretion of 240 uEq/min. In addition, papillary plasma flow is approximately 30% of normal in caval dogs with sodium retention during ANF infusion and does not differ between responding and non-responding dogs. The data in cirrhotic dogs provides even stronger evidence that flow rate in the papilla is unrelated to ANF's evoked natriuresis.

Papillary plasma flow in these dogs was significantly greater than normal dogs during ANF infusion yet 50% had a markedly blunted natriuretic response and 50% had no natriuretic response whatsoever. The flow rates in the cirrhotic dogs reached levels comparable to that measured in dogs vasodilated with bradykinin or acetylcholine (Fadem et al., 1982). Of interest is that these increases in papillary plasma flow have been invoked to explain the natriuresis associated with intrarenal infusion of these vasodilators. Since ANF had no effect on papillary plasma flow in normal dogs or caval dogs with ascites and sodium retention (i.e. post-ANF levels equivalent to baseline levels previously measured in caval dogs (Faubert et al., 1982)), the elevated papillary plasma flow in cirrhotics would also be expected under baseline conditions, especially since renal plasma flow is unchanged from baseline. Why PPF would be so markedly elevated under conditions of almost complete sodium retention is unclear. Previous measurements of the distribution of renal blood flow in cirrhotic patients using the Xenon22 washout technique have indicated that there is a fall in cortical blood flow while inner medullary flow is maintained. Clearly, whatever signals the renal tubule to retain sodium and water in cirrhosis is not influenced by changes in inner medullary hemodynamics. Even raising renal blood flow by intrarenal acetylcholine, a procedure associated

with a potent natriuresis in normal dogs (Fadem et al., 1987) was ineffective in overcoming the tubular resistance to ANF in 50% of the cirrhotic dogs.

The data strongly suggests that there is a problem at the level of the renal tubular receptors which produces the blunted or completely attenuated responsiveness in cirrhotic dogs. Generation of second messenger, cGMP, was measured in these dogs, yielding the same results as described in the caval dogs (chapter 3). Plasma and urinary cGMP were equivalent in the responding and non-responding dogs despite the heterogeneity of natriuretic response. Consistent with this finding is that cirrhotic patients undergoing thermoindifferent water immersion have equivalent increases in plasma iANF and urinary cGMP excretion despite only half of the patients having a natriuretic response (Leung et al., 1987).

Perhaps the most interesting observation in these cirrhotic dogs was that the heterogeneity of natriuretic response to exogenous ANF infusion was demonstrated in the pre-ascitic phase during a period of sodium retention, volume expansion and elevated plasma iANF levels. Of 9 dogs tested biweekly throughout this phase, 5 were natriuretic responders and 4 were non-responders. This period of partial renal refractoriness to ANF infusion has also been reported in cirrhotic rats in the pre-ascitic phase when they were also retaining sodium, volume expanded

and had elevated plasma iANF levels (Olivera et al., 1988). The most interesting point in the sequential measurement in the cirrhotic dogs is that natriuretic responsiveness had been present in all dogs prior to week 4 and was restored in the non-responders by week 6 and maintained during the week 8 measurement. Furthermore, the heterogeneity was reestablished when the natriuretic response to ANF was examined in the presence of ascites. It is important to note that there was no correlation between a given response at 4 weeks and that measured when ascites had developed. These data are very similar to the caval dog where natriuretic responsiveness was restored in the non-responding dogs once they had returned to sodium balance. These results emphasize that the heterogeneity of natriuretic response to ANF infusion is a physiological phenomenon related to the salt-retaining kidney rather than some unique characteristic of an individual dog.

CHAPTER 7: GENERAL DISCUSSION

7.1 DOES ANF HAVE A ROLE IN THE REGULATION OF EXTRACELLULAR FLUID VOLUME?

Distension of a balloon in the left atrium of an anaesthetized dog elicits a diuresis and natriuresis (Henry. Gauer, Reeves, 1956; Ledsome, Linden, 1968; Kappagoda, Linden, Snow, 1973; Shultz et al., 1982). As discussed in chapter 1 (section 1.1), many studies were conducted in the 1960's and 70's examining the mechanism of this reflex renal response in order to gain further understanding about the regulation of extracellular fluid volume. It was demonstrated that cardiopulmonary atrial receptors initiate a neural reflex which involves the vagus and possibly the renal efferent sympathetic nerves (Karim et al., 1972; Weaver, 1977; Linden, Mary, Weatherill, 1980). This neural component however, only partially mediates the renal response as concluded from an elegant study by Carswell, Hainsworth and Ledsome (1970). In cross-perfusion experiments, the donor dog atrium was stretched and a diuretic response was elicited in the recipient dog thus providing clear evidence that a humoral mechanism must, in part, be responsible for the observed renal response to atrial distension. Since left atrial distension was associated with a fall in plasma vasopressin concomitant with a rise in left atrial pressure (Gauer, Henry, 1963; Johnson, Moore, Segar, 1969; Brennan et al., 1971) it was postulated that reduction of vasopressin was

the humoral mechanism mediating this reflex. However, the importance of the inhibition of vasopressin release in response to distension is minimal since several studies report a normal renal response in the presence of high vasopressin levels (Ledsome, Linden, O'Connor, 1961) or an ablated pituitary gland (Kappagoda et al., 1975).

The observation that secretory granules within the atrial cardiocytes (Kisch, 1956) are responsive to changes in sodium and water balance (De Bold, 1979) led to the discovery of ANF (DeBold et al., 1981), a new hormonal system believed to exert an important influence on extracellular fluid volume regulation. Two questions have been a major focus in the investigation of this new hormonal system. 1) Does ANF mediate the renal effects induced by a variety of physiological stimuli which elevate atrial pressure and 2) Does it have a primary role in the normal regulation of sodium balance and extracellular fluid volume. If ANF is to be considered as a possible mediator of the renal effects of atrial stretch, it must be shown that stretch elicits release of ANF and, furthermore, that the concentrations are sufficient to produce a diuretic and natriuretic response of the same magnitude as occurs with atrial stretch.

Release of ANF has been convincingly demonstrated under a variety of experimental conditions. Mechanical distension of the atria in an isolated rat heart-lung

preparation results in increased plasma natriuretic activity in relation to changes in central venous pressure (Dietz, 1984). Acute volume expansion, eliciting a significant increase in central venous pressure, decrease in arterial pressure and increase in urinary sodium excretion also stimulates iANF release in direct proportion to the volume load in man (Sagnella, 1985; Takaku, 1985), dogs (Salazar et al., 1986) and rats (Petterson et al., 1985; Schwab et al., 1986; Veress, Sonnenberg, 1984). That an important link exists between the rise in ANF and the natriuretic response to volume expansion is supported by studies demonstrating attenuation of both the rise in plasma ANF and sodium excretion after saline expansion in atrial appen-dectomized rats (Veress, Sonnenberg, 1984; Schwab et al., 1986). It is important to note that in addition to release being stimulated by acute volume expansion, it has been demonstrated that acute volume contraction, such as hemorrhage, produces a fall in plasma ANF (Verberg et al., 1986). This capability to modulate levels in a bidirectional manner is an important criterion for a volume regulatory hormone.

Similar conclusions have been drawn from studies of water immersion, a procedure which produces marked and sustained central hypervolemia without the necessity of exogenous volume expansion (Epstein, 1978). Three hour water immersion to the neck in sodium-replete humans is

associated with an increase in plasma iANF which promptly returns to normal during the recovery period (Epstein et al., 1986; Anderson et al., 1986). Moreover, the water immersion-induced increase in plasma iANF in rats was correlated with increases in right atrial pressure (Katsube et al., 1985). The functional relationship between these parameters has not been clearly established however, since, in dogs, there was no correlation between hormone levels and the natriuresis observed during 1 1/2 hours of water immersion (Miki et al., 1986). It is interesting that the nocturnal inhibition of the renal responses to water immersion are not mediated by a similar circadian rhythm in plasma ANF since water immersion in humans induced comparable elevations in plasma levels at night as measured during the day (Miki et al., 1988).

Another experimental paradigm used to examine the same question is postural changes since atrial pressure is higher in the supine as compared with the upright position (Guyton, 1973). As would be predicted, iANF was also elevated in the supine position and was higher in subjects on a high salt intake indicating that both posture and sodium intake can influence the plasma levels of ANF (Hollister et al., 1986).

These studies clearly demonstrate that ANF can be released in response to stimuli which alter atrial pressures most likely as a function of increasing

transmural pressure (See Introduction, section 1.1). That pharmacological doses of ANF infused under steady-state conditions produce a natriuresis in a dose-dependent manner has been shown in our conscious dogs (Chapter 3), confirming the findings of other investigations in dogs (Seymour et al., 1985), and rats (Pollack, Arendshorst). Dose response has also been demonstrated in the physiological range in man (Anderson et al., 1987) and dogs (Bie et al., 1988). It is generally agreed, therefore, that ANF can be released in response to acute changes in plasma volume, sodium balance, and posture and is natriuretic in all mammals studied. The inhibition of vasopressin release by atrial stretch may be a direct effect of ANF. Intravenous ANF administration is a potent inhibitor of hemorrhage- or dehydration-induced vasopressin release in normal rats (Samson, 1985). However, it is not clear what contribution activation of ANF makes as compared to that made by the neural component in the reflex response to atrial distension. Chronic cardiac denervation in dogs abolished the renal response to left atrial distension (Fater et al., 1982; Kamarczyk et al., 1981) even though plasma iANF rises normally in these dogs (Goetz et al., 1986). Although these data suggest that ANF is not mediating the natriuresis of atrial stretch, the data is rather confusing. Plasma levels of ANF measured during atrial stretch in the cardiac denervated dogs were

comparable to levels achieved by ANF infusion at 25 ng/kg/min, a dose shown previously to be natriuretic in dogs (Bie et al., 1988), yet did not increase sodium excretion in either the cardiac denervated or normal dogs. Moreover, infusion at 100 ng/kg/min, a dose which produced a marked natriuretic response in our normal dogs, also was not natriuretic in the experiments described above. Thus the data from cardiac denervation must be interpreted with caution and the question of the relative contributions of ANF and neural reflex in mediating the natriuretic response to atrial stretch must remain unresolved.

To more directly assess the role of ANF in the natriuresis associated with volume expansion (and therefore atrial stretch) Khraibi et al. (1987) measured plasma iANF and sodium excretion in rats expanded with 5% body weight saline infusion. Upon return to baseline levels, exogenous ANF was infused to produce the same plasma levels as measured during saline expansion. The associated natriuresis was approximately 40% of that observed with volume expansion establishing that ANF can contribute a major proportion of the natriuresis but is only one pathway in the mechanism. It is thus generally agreed that ANF is an important hormonal component of the daily and long-term regulation of sodium balance in the control of extracellular fluid volume.

7.2 IS THERE AN ABNORMALITY IN ANF PHYSIOLOGY IN EDEMA STATES?

Recently, the role of ANF in the pathophysiology of edema has become the focus of a great deal of investigation. It has been postulated that a disturbance in normal ANF physiology may contribute to the sodium retention and ascites formation in edema (Goetz et al., 1986). In order to determine the validity of this hypothesis, two questions need to be addressed. 1) Is ANF being released normally? 2) Is the kidney responding normally to the hormone? The purpose of this thesis was to examine, in great detail, the relationship between ANF and the handling of sodium in two canine models of edema. The dog with thoracic inferior vena caval constriction (caval dog) was chosen because it represents a condition of altered systemic hemodynamics (fall in central venous filling pressure) and a period of sodium retention with the development of ascites followed by return to a state of sodium balance. Thus, this model presented the unique opportunity to study ANF in relation to changes in systemic hemodynamics, sodium balance and the renin-angiotensin system. The dog with biliary cirrhosis of the liver was chosen in order to study, as in the caval dog, the interaction between ANF and changes in the factors which determine the fullness of the circulation (i.e. plasma volume, renin, aldosterone) over a period of time when the dogs progress from health through the various

stages of cirrhosis of the liver leading eventually to unrelenting sodium retention, ascites, and plasma volume expansion. This experimental model has been well described and closely mimics the human disease state.

Plasma levels of ANF have been measured in a variety of edema states as an index of atrial release. Congestive heart failure patients have very high circulating plasma levels regardless of the etiology of the disease, demonstrating that this disease state does not result from a deficiency of ANF. This has been confirmed in cardiomyopathic hamsters (Edwards et al., 1986) as well as rats and dogs with A-V fistulas (Winaver et al., 1988; Chou et al., 1987). The chronic caval dog, however, has very low circulating plasma ANF levels as confirmed by Freeman et al., 1987. As shown in the daily measurements of plasma levels in these dogs, ANF falls dramatically by 24 hours after surgery (the earliest measurement) and remains remarkably constant at this low level throughout the period of sodium retention. It may be that release is normal, as suggested by the congestive heart failure data, but the atria is responding to a sustained inhibitory stimulus, most likely the fall in central venous pressure. Indeed, acute saline infusion in 2 caval dogs produced a rapid rise in plasma iANF levels in direct proportion to the rise in central venous pressure (Figure 4.2). Following the infusion, recovery of central venous pressure was

accompanied by a return of plasma iANF levels to baseline. These data, in addition to the studies demonstrating that plasma ANF can be further increased in congestive heart failure patients by elevation of cardiac filling pressure (Burnett et al., 1986) and exercise (Dietz et al., 1986) confirm that release of ANF is normal in congestive heart failure and caval constriction. In fact, the difference in plasma levels, despite many common disturbances in sodium and water balance (i.e. sodium retention, plasma volume expansion, activation of the renin-angiotensin system), suggests that ANF's ability to act as a regulator of extracellular fluid volume is being overridden by a strong antinatriuretic signal to some part of the nephron (the possible sites will be discussed later).

Similar conclusions have been drawn from cirrhotic patients and animal models. Plasma iANF can be low (Bonkovsky et al., 1986), normal (Shenker et al., 1985; Burghardt, Wernze, Diehl, 1986) or elevated in cirrhotic patients (Gines et al., 1988), is high in cirrhotic rats (Vakil et al., 1988; Koepke, Jones, DiBona, 1986) and depressed in our cirrhotic dogs. The variation in plasma levels in cirrhotic patients may be due to timing of the samples and the conditions under which they were obtained. As demonstrated in the sequential measurement of plasma levels in the cirrhotic dogs, there are two periods when plasma iANF is markedly depressed (i.e. first 3 weeks post

bile-duct ligation and in the presence of ascites) and a period when the levels are substantially elevated. In addition, as previously discussed, variables such as daily sodium intake and posture can significantly alter plasma iANF levels. It is therefore critical to study the hormonal system under strictly defined physiological conditions including presence of ascites, plasma volume, sodium intake, sodium balance and body position during sampling.

The data on atrial release of ANF in cirrhotic patients is similar to that obtained in congestive heart failure patients and caval dogs. Cirrhotic patients with sodium retention, ascites and high plasma iANF showed a comparable increase in the plasma levels to normal subjects in response to water immersion (Epstein et al., 1986; Skorecki et al., 1988). It is important to note that the RP-HPLC from cirrhotic and caval dog atria as well as plasma did not show any aberrant forms of the peptide (Chapter 4, 6), indicating that the intraatrial processing and therefore the circulating form of ANF are normal in these two models.

The metabolic clearance rate and disappearance half-time (T 1/2) of ANF were assessed in the normal dogs to enable comparison with the caval and cirrhotic dogs since the effectiveness of released ANF could be reduced by enhanced degradation of the peptide. Although major strides have been made in the molecular biology of ANF and its

physiological actions, very little is known about the mechanism of clearance from the circulation. In humans, the T 1/2 varies from 1.7 (Nakao et al., 1986) to 3.1 minutes (Yandle et al., 1986) which is similar to the time we measure in the normal dog (1.9 minutes). There is conflicting evidence for the role of the liver in ANF degradation. A substantial arterio-venous difference was measured across the liver although hepatectomy did not alter the T 1/2 of infused ANF. Both T 1/2 and the metabolic clearance rate were unchanged from control in the caval and cirrhotic dogs and similar results have been reported in cirrhotic patients (Olivera et al., 1988). These data minimize a role for the liver in the metabolic pathway of ANF since cirrhotic dogs with severe hepatic dysfunction have the same T 1/2 as caval and normal dogs in which liver function is normal. These data permit the conclusion that in the caval and cirrhotic dogs, intraatrial processing and release of ANF is normal and that the 1-28 form of the peptide circulates in the plasma and is degraded and cleared from the circulation at the same rates as measured in normal dogs. Thus any breakdown in ANF action must be at the target-organ level.

The second question regarding ANF in edema therefore, concerns whether the kidney is responding normally to the hormone. This has been examined mainly by exogenous infusion of ANF to edematous patients and animal models and

consistently demonstrates that there is some degree of renal refractoriness in virtually all edema models tested including congestive heart failure (Cody et al., 1986; Winaver et al., 1988), cirrhosis of the liver (Skorecki et al., 1988; Olivera et al., 1988; Epstein et al., 1986), and nephrotic syndrome (Hildebrant, Banks, 1988; Koepke, DiBona, 1987). The major observation of this thesis is that chronic caval and cirrhotic dogs divide 50:50 into responders and non-responders when the natriuretic response to ANF is considered. Many parameters have subsequently been examined in these canine models of ascites in order to ascertain the level at which the kidney loses the ability to excrete sodium in the presence of ANF. Although each model has been discussed in detail in the previous chapters, the results will be summarized here and discussed in relation to each other and in terms of normal ANF physiology.

A source of great controversy over the past few years has been the role of GFR in mediating the natriuretic response to ANF. There are many studies which report that ANF infusion increases GFR (Cogan, 1986; Kramer et al., 1986; Huang et al., 1985; Mendez et al., 1988) due to an increase in glomerular capillary hydrostatic pressure as produced primarily by efferent arteriolar vasoconstriction (Maack et al., 1984; Ichikawa et al., 1985; Fried et al., 1986) although afferent arteriolar vasodilatation and an

increase in Kf contribute to this increase (Fried et al., 1986). However, there are many studies which report that GFR is constant (Seymour et al., 1985; Pollack, Arendshorst, 1986; Seymour, Smith, Mazack, 1987; Zimmerman et al., 1987) and some in which GFR is reduced (Roy, 1986). While GFR was constant at all doses in our normal and cirrhotic dogs, it rose significantly in our caval dogs. Perhaps in caval dogs, with activated renin-angiotensin system and thus higher levels of circulating angiotensin II than normal dogs, ANF's vasoconstrictive effect at the efferent arteriole is synergistic with the angiotensin II vasoconstrictor effect already present, resulting in a rise in GFR. Of importance is that the mean increase in GFR in the caval dogs in the phase of sodium retention was the same regardless of whether the dog was a responder or non-responder. Indeed, evaluating the change in GFR as compared to the change in sodium excretion revealed no relationship between the two parameters emphasizing that an increase in GFR, while possibly contributing to the rise in sodium excretion, does not mediate the natriuretic action of ANF.

It is generally agreed that ANF does not influence renal plasma flow more than perhaps by producing an acute reflex increase at the beginning of an infusion. This was confirmed in the present experiments in normal as well as caval and cirrhotic dogs in both the conscious or

anaesthetized state.

There is a great deal of evidence to support the hypothesis that changes in inner medullary sodium handling, through medullary hemodynamic events or direct inhibition of sodium transport in this segment, mediates the natriuretic action of ANF (Briggs et al., 1982; Sonnenberg et al., 1982). Several investigators report that ANF can increase medullary blood flow which would washout the medullary concentration gradient, and thus reduce sodium reabsorption from the ascending thin limb of Henle (Maack et al., 1985; Sonnenberg et al., 1986). Borenstein et al. (1983) demonstrated that infusion of atrial extracts in anaesthetized rats resulted in a rise in blood flow to the inner medulla and papilla. In addition, papillary necrosis abolished the increase in sodium excretion produced by ANF infusion in rats (Chen, Caldwell, Hsu, 1984) providing evidence that the papilla plays an important role in mediating the natriuretic response to ANF. ANF infusion in euvolemic rats raised vasa recta hydraulic pressure in excess of the rise in efferent arteriolar hydraulic pressure demonstrating that ANF preferentially vasodilates the medullary vasculature (Mendez et al., 1988). However, it has also been shown that a rise in papillary flow occurred after the initial natriuretic response to ANF infusion in normal rats (Kiberd et al., 1987) and rats with renal denervation and adrenalectomy (Takezawa et al., 1987)

indicating that the change in vasa recta blood flow may be a consequence of the natriuretic action of ANF rather than its primary mediator.

Papillary plasma flow was measured in the present experiments to assess whether changes in inner medullary hemodynamics could account for the heterogeneity of natriuretic response to ANF in the caval and cirrhotic dogs with sodium retention in the presence of ascites. Normal dogs were also examined under control conditions and during the infusion of ANF to establish ANF's effect on papillary plasma flow under control conditions. Since the technique requires sacrificing the kidney, it was not possible to get pre- and post-infusion measurements in each dog. ANF at 125 ng/kg/min had no effect on papillary plasma flow in normal anaesthetized dogs despite an increase in sodium excretion of greater than 200 uEq/min. Demonstrating even more clearly this lack of relationship between the natriuretic response to ANF and papillary plasma flow was that in both caval and cirrhotic dogs with sodium retention and ascites, the 50% which had a natriuretic response to ANF had the same papillary flow rate as those who did not respond to the infusion. This data is interesting, not only in terms of the mechanism of action of ANF, but also in relation to the mechanism invoked to explain the natriuretic response to vasodilators bradykinin and acetylcholine (Fadem et al., 1982). It has been suggested

that without a rise in papillary plasma flow, as is seen with infusion of the vasodilator, secretin, no natriuresis will occur. Clearly this is oversimplified since ANF, a potent vasodilator, can evoke a marked natriuresis without any change in papillary plasma flow.

Another point of interest is the relationship between papillary flow rate and the sodium retaining state in these two edema models. The reduction in papillary flow in the caval dogs from 35 to 15 ml/min/100 g papilla has been suggested to play a determining role in the progressive sodium retention in this model and, in fact, only when papillary plasma flow rises to normal do the dogs regain sodium balance in the presence of ascites (Faubert et al., 1978). However, this may not represent a cause and effect phenomenon but may rather be two consequences of some other change occurring in these dogs since cirrhotic dogs, also with progressive sodium retention and ascites have papillary plasma flow rates 37% higher than normal dogs and 337% higher than caval dogs. Unfortunately these flow rates were measured only in the presence of ANF as technical constraints precluded the measurement under baseline conditions. However, since papillary plasma flow did not change in the normal dogs where the natriuresis was large, it is unlikely that ANF produced such an enormous increase in papillary plasma flow without any increase in sodium excretion in half of the cirrhotic dogs and an

attenuated response in the remaining 50%. The major question arising from these results is why the tubule is retaining sodium when vasa recta flow is so greatly enhanced. The inner medullary solute gradient should be washed out, as it is when papillary plasma flow increases to comparable levels with acetylcholine or bradykinin infusion in normal dogs, thus inhibiting the sodium retaining mechanisms. These results clearly suggest that the signal for sodium retention in these cirrhotic dogs and, by extrapolation, in the caval dogs must be so strong that it can overcome the hemodynamic forces normally producing a massive natriuresis and thus may explain why these animals are unable to excrete a saline load.

A second mechanism of ANF action in the medulla is thought to be through direct inhibition of sodium transport at the tubular level. A comparison of ANF infusion with KCl infusion in anaesthetized rats demonstrated that, although both increase sodium delivery to the medullary collecting duct (whether by washout or an increase in GFR), the natriuretic effect of ANF was considerably greater (Sonnenberg et al., 1986). These investigators concluded that ANF inhibits sodium reabsorption from the medullary collecting duct. This was shown directly by sodium transport studies of inner medullary, outer medullary, and thick ascending limb cell suspensions. ANF inhibited oxygen consumption in inner medullary collecting duct cells

by a mechanism similar to that of amiloride indicating that ANF reduces Na/K/ATPase activity by inhibiting sodium channel-mediated sodium entry into inner medullary collecting duct cells (Zeidel et al., 1986). No effect of ANF on oxygen consumption was found in thick ascending limb or outer medullary collecting duct cell suspensions, confirming the data obtained from micropuncture studies of juxtamedullary nephrons (Roy, 1986; Peterson et al., 1987).

It is generally agreed that ANF induces a marked hypotension under most experimental conditions (Hirata et al., 1985; Biollaz et al., 1986; Richards et al., 1985) which can be attributed, at least in part, to a fall in cardiac output (Kleinert et al., 1986; Zimmerman et al., 1987). In our conscious normal dogs, maximum hypotension was reached at a dose of ANF of 75 ng/kg/min. A further fall in blood pressure with higher doses of ANF (when sodium excretion increased linearly) may be prevented by reflex increases in peripheral resistance by unloading of the arterial baroreceptors. It has been demonstrated that ANF induces a greater fall in blood pressure in dogs with sinoaortic denervation and vagotomy than neuraxis intact dogs (Koyama et al., 1986) supporting a role of the baroreceptors in minimizing the hypotension induced by ANF.

In examining the heterogeneous natriuretic response to

ANF it was clear that changes in blood pressure and thus changes in renal perfusion pressure could alter the natriuretic response since an increase in renal perfusion pressure is normally associated with inhibition of tubular reabsorption in the juxtamedullary nephrons (Haas, Granger, Knox, 1985). Increasing renal perfusion pressure by partially clamping the abdominal aorta and partially constricting the mesenteric and celiac arteries enhanced the natriuretic response to ANF in renal denervated and adrenalectomized rats (Takezawa et al., 1987). Similarly in normal rats, intravenous angiotensin II infusion superimposed on ANF infusion enhanced the natriuresis in direct relation to the rise in blood pressure and thus renal perfusion pressure (Mendez et al., 1986). Confirming this relationship between perfusion pressure and the natriuretic effect of ANF is the data demonstrating that pre-renal arterial clamping blunts or abolishes the ANF-induced natriuresis (Sosa et al., 1986; Davis, Briggs, 1987).

In the 50% of caval dogs with sodium retention and ascites which did not have a natriuretic response to ANF infusion, blood pressure and cardiac output fell to the same degree as those dogs which responded normally. Although perfusion pressure was not measured directly, if one accepts that it is a direct reflection of blood pressure, then the lack of natriuretic response is not due

to a more substantial decrease in perfusion pressure than the responding dogs.

One of the most important "clues" as to the source of heterogeneity of natriuretic response in the caval and cirrhotic dogs with sodium retention and ascites is that when caval dogs return to sodium balance in the presence of ascites and when the pre-ascitic cirrhotic dogs are in sodium balance, the natriuretic response to ANF is normal. Therefore, there is a mechanism acting in the salt-retaining kidney which can override the natriuretic effects of ANF in 50% of the dogs. Since the renin-angiotensin-aldosterone system is activated in these two models in the presence of sodium retention and ascites, perhaps it can override the effects of ANF when these hormone levels reach some critical concentration. Preliminary renin and aldosterone levels in the caval salt retaining dogs indicated that neither baseline nor ANF-induced decreases in either hormone differed between the responding and non-responding dogs.

It has recently been shown that intrarenal angiotensin infusion at a non-pressor dose of 1.5 ng/kg/min in anaesthetized dogs markedly attenuated the rise in both absolute and fractional excretion of sodium (Showalter et al., 1988). It is possible therefore that a difference in the intrarenal concentration of angiotensin II could produce the heterogeneous natriuretic response. In fact,

angiotensin II levels increasing by only 10-15 pg/ml in the renal arterial blood reportedly reduce sodium excretion markedly (Fagard et al., 1976; Waugh, 1972). ANF's suppressive effect on renin and aldosterone must be taken into consideration when evaluating this hypothesis since it is the lack of response to infused ANF which differentiates responding from non-responding dogs. Considering that renin and aldosterone, and therefore intrarenal angiotensin II, fall during the infusion of ANF, the baseline angiotensin II activity must be sufficiently elevated such that a decrease in these levels will still blunt the natriuresis. This seems unlikely as angiotensin II is a function of plasma renin activity which was not different between the two groups. Furthermore, levels of intrarenal angiotensin II as described above would likely manifest other observable differences between the two groups. Enhanced levels would decrease baseline renal blood flow and probably raise GFR as well (Hall, 1986). However, no differences in these parameters were demonstrated between responders and non-responders in either caval or cirrhotic dogs. Moreover, infusion of captopril to reduce intrarenal angiotensin II levels did not reverse the attenuated natriuretic response in cirrhotic rats with sodium retention and ascites (Koepke, Jones, DiBona, 1987).

Since the systemic and renal hemodynamics do not appear to play a role in producing the heterogeneity of

natriuretic response, we postulated that the problem could be at the ANF receptor level. As we were unable to directly measure receptor binding, we examined the ANF effect on cyclic GMP (cGMP) generation. It is well established that cGMP is the second messenger of one class of ANF receptor (Hamet et al., 1984; Gerzer et al., 1985; Seymour et al., 1985) through the activation of particulate guanylate cyclase (Waldman, Rappaport, Murad, 1984; Hamet et al., 1984). However, it has not clearly been shown that the activation of this system accounts for the biological activity of ANF. Comparing cGMP activity in various parts of the nephron, Tremblay et al (1985) demonstrated that ANF infusion produces the greatest elevation in cGMP in the glomerulus (50 fold), although ANF's effect on GFR are minimal. Data from both plasma and urinary excretion of cGMP in normal dogs appeared to correlate well with the ANF-induced increase in sodium excretion. However, both caval and cirrhotic dogs demonstrated a similar rise in cGMP despite a markedly blunted natriuretic response. These data indicate that the increase in cGMP is functionally dissociated from the natriuretic action of ANF. It has been suggested that cGMP in Bowman's space (and therefore the final urine) is from glomerular mesangial or epithelial cells, not from the blood via filtration (Huang et al., 1986). However, in the normal, caval, and cirrhotic dogs, the increase in plasma cGMP was

substantial and thus contributed to the final urinary excretion of cGMP. In fact, it is possible that small changes occurring in the collecting duct, where ANF exerts a major influence and where cGMP rises only 2 fold, cannot be detected because of the enormous outpouring of cGMP from the glomerulus or filtered from the blood. The calculated values for nephrogenous cGMP (excreted load - filtered load) were far too variable to be able to dissect out any small differences between responding and non-responding dogs. A second possible explanation for the apparent dissociation of ANF from the natriuretic response is that there is a defect in the pathway at a step after cGMP has been generated which functionally dissociates the receptor from the target response. Thus, in the non-responding dogs, ANF may bind normally to its receptor, couple to guanylate cyclase, liberate cGMP but may not elevate intracellular calcium levels.

The second class of receptors, the so-called silent receptors (C-ANF receptors) were first identified in vascular smooth muscle cell and endothelium and are not coupled to cGMP (Leitman, et al., 1986; Scarborough et al., 1986). It has recently been shown that the silent receptors comprise the majority of renal receptors and do not mediate any of the known renal effects of the hormone (Maack et al., 1987). It is not known whether these receptors produce a functional response which has not yet

been identified. The evidence suggests that they serve as specific storage-clearance binding sites for ANF (Maack et al., 1987) and that the high ratio of C-receptors to the B-receptors (biologically active) indicates a very controlled regulation of ANF action. Future investigation of the second messenger of this receptor site in addition to the relative affinities of these two receptors for ANF under conditions of sodium loading and depletion and in the presence of edema may add to the understanding of the mechanisms of ANF action.

Perhaps the most intriguing finding of the experiments presented in this thesis is that the heterogeneous natriuretic response to ANF was found in pre-ascitic cirrhotic dogs. The profile of cirrhotic dogs, in terms of liver function, plasma hormones, renal sodium handling, and plasma volume status changes during the pre-ascitic phase. Thus the profile of the renal response to ANF over the same time course provides insight into the mechanisms of ANF action and the development of cirrhosis and ascites.

At week 2 post-biliary ligation, all dogs, with markedly elevated bilirubin levels and depressed plasma iANF, had a normal natriuretic response to ANF infusion. It is unclear what stimulates the fall in plasma iANF and what maintains these low levels for the first 3 weeks following surgery. Since the dogs are in sodium balance and plasma volume is normal, one would expect normal plasma iANF levels. It is

important to note that these levels are not a consequence of interference from bilirubin in the radioimmunoassay, as determined in our laboratory. However, the marked elevation in circulating bilirubin or some consequence of biliary obstruction may somehow inhibit the release of ANF. At present however, there are no data regarding this possible interaction.

The dogs divided 50:50 into responders and non-responders when the natriuretic response to ANF was assessed at 4 weeks post biliary ligation. During this time period, the dogs were retaining sodium and plasma volume was increased. Plasma renin and aldosterone were normal while plasma iANF was increased (the rise was not statistically significant at 4 weeks due to the large variability between dogs in the degree to which plasma ANF rose during the initial increase). Three interesting questions arise from these data. 1) What causes the sodium retention during this period? 2) What stimulates the rise in plasma iANF? 3) Why do the dogs divide into responders and non-responders during this period?

The pre-ascitic sodium retention is predicted by the overflow theory of ascites formation (as described in Chapter 1, Section 1.5). This sodium retention appears to be a consequence of two factors: 1) the defense of the extracellular fluid volume including the need to fill venous collaterals in the splachnic circulation and 2)

extra sodium retention, mediated by intrahepatic hypertension (Unikowsky, Wexler, Levy, 1983). The present data extend these observations. Plasma volume as a function of body weight is increased by 4 weeks while plasma renin and aldosterone are normal. If one uses these hormones as markers for the "fullness of the circulation", then it would appear that the circulation is adequately filled. Thus the stimulus for this pre-ascitic sodium retention must be some factor other than the need to replenish the extracellular fluid volume. Confounding this analysis is the dramatic rise in plasma iANF. Plasma levels remain elevated for a period of approximately three weeks and, in fact, are still elevated when sodium balance has been restored. One could argue that it is precisely this rise in ANF, in response to expansion of the plasma volume or possibly a feedback mechanism initiated by sodium retention, which brings the dogs back into sodium balance. Indeed, plasma volume is also reduced as compared to week 4. However, it can also be argued that the factor stimulating sodium retention at week 4 also stimulates ANF release. Since we did not measure sodium balance or response to ANF during either week 3 or week 5, we do not know how long the period of sodium retention persisted and therefore cannot distinguish between these two possibilities.

The heterogeneous natriuretic response to ANF was seen

twice in these dogs; once, as described above, at 4 weeks post-ligation, and again when ascites was present. It is important to note that for a given dog, a response in week 4 was not necessarily the same response measured during the ascitic phase (i.e. a responder could become a non-responder or vice versa) which emphasizes that this heterogeneity is not a unique feature of individual dogs. The baseline characteristics of the dogs in these two time periods are quite different. Renin and aldosterone are normal in week 4 but markedly elevated in the presence of ascites. Conversely, ANF is elevated during week 4 but markedly depressed in the ascitic phase. Plasma volume, while increased at week 4, is further increased when ascites develops. Clearly, the depression of plasma ANF prior to and during the ascitic phase is a response to a non-volume stimulus since one would expect a marked rise in this hormone when plasma volume is expanded. Despite the different profiles of plasma hormones and plasma volume in these two time periods, the dogs divide 50:50 into natriuretic responders and non-responders. Thus it is clear that none of the parameters describe above influence the natriuretic responsiveness to ANF. However, the common feature in these two periods is avid sodium retention. Moreover, return to sodium balance in the pre-ascitic phase restored the natriuretic responsiveness regardless of the status of the plasma hormones or plasma volume. This is

similar to the chronic caval dogs, where non-responding dogs in the phase of active sodium retention and ascites regained natriuretic responsiveness when they returned to sodium balance despite the persistence of ascites and plasma volume expansion.

These data permit the conclusion that the heterogeneity of natriuretic response to exogenous ANF infusion is a property of the sodium-retaining kidney. This phenomenon is not influenced by renal or systemic hemodynamics, papillary plasma flow, renal sympathetic efferent nerve activity or plasma levels of renin, aldosterone or ANF. This phenomenon may not be strictly a consequence of the edema state but rather may be a consequence of sodium retention. In the canine models described in this thesis, chronic sodium retention manifested itself as ascites. It is important to emphasize that our results indicate that the presence of ascites itself does not influence the heterogeneous nature of the natriuretic response. Furthermore, I would predict that any state which is characterized by sodium retention will demonstrate this heterogeneous response to exogenous ANF infusion.

Preliminary studies in our laboratory have shown that the non-responding dogs will have a normal natriuretic response to furosemide, hydorchlorathiazide and acetazolamide, indicating that this heterogeneous response to ANF is unique for this hormone. Although we were not

able to precisely determine the site at which ANF interacts with sodium handling to produce the heterogeneity of natriuretic response, it may be predicted in general terms from the knowledge of ANF action. The role of GFR, as discussed in detail in this thesis, appears to be minimal in influencing the natriuretic response to ANF. Although ANF may inhibit bicarbonate-coupled sodium transport in the proximal tubule and thereby increase sodium delivery out of this segment of the nephron, these changes are most likely to be minimal since the proximal tubule lacks ANF receptors. Similarly, the loop of Henle has been shown to be relatively unresponsive to ANF, producing only modest increases in the delivery of sodium to the distal nephron.

There is substantial evidence, as described in detail above, supporting the hypothesis that ANF acts primarily at the level of the medullary and papillary collecting duct, most probably influencing a sodium channel (Zeidel et al., 1987). The mechanism of ANF interaction with the sodium channel is presently unknown. However, as a point of speculation, the heterogeneity of natriuretic action when avid sodium retention is present must ultimately involve an alteration in the ANF-receptor coupling with the sodium channel. Implicit in this hypothesis is the suggestion that the interaction between ANF and the sodium channel is modulated by some mechanism sensing the degree of sodium retention by the kidney.

Aalkjaer, C., Mulvany, M., Nyborg, N. Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries. Br. J. Pharmacol 86:447-453, 1985.

Aguilera, G. Differential effects of atrial natriuretic factor on angiotensin II - and adrenocorticotropin - stimulated aldosterone secretion. Endocrinology 120(1):299-304, 1987.

Akabane, S., Kojima, S., Igarashi, Y., Kawamura, M., Matsushima, Y., Ito, K. Release of atrial natriuretic peptide by graded right atrial distension in anesthetized dogs. Life Sci 40(2):119-125, 1987.

Anderson, J., Struthers, A., Payne, N., Slater, J., Bloom, S. Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. Clin Sci 70:507-512, 1986.

Anderson, J., Donckier, J., Payne, N., Beacham, J., Slater, H., Bloom, S. Atrial natriuretic peptide. Evidence of action as a natriuretic hormone at physiological plasma concentrations in man. Clin Sci 72:305-312, 1987.

Anderson, J., Millar, N., O'Hare, J., Mackenzie, J., Corrall, R., Bloom, S. Atrial natriuretic peptide: Physiological release associated with natriuresis during water immersion in man. Clin Sci 71:319-322, 1986.

Andrews, W., Palmer, J. Afferent nerve discharge from the canine liver. QJ Exp Physiol 52:269-276, 1967.

Ardaillou, N., Nivez, M., Ardaillou, R. Stimulation of

guanylate cyclase by atrial natriuretic factor in isolated human glomeruli. FEBS Letters j189(1):8-12, 1985.

Arroyo, V., Palnas, R., Gaya, J., Januszwicz, A., Sealey, J., Laragh, J., Schilling, J., Lewicki, J., Johnson, L., Maack, T. Sympathetic nervous activity, renin-angiotensin system and renal excretion of prostaglandin E2 in cirrhosis. Relationship to functional renal failure and sodium and water excretion. Eur J Clin Invest 13:271-278, 1983.

Atarashi, K., Mulrow, P., Franco-Saenz, R. Effect of atrial peptides on aldosterone production. J Clin Invest 76:1807-1811, 1985.

Atlas, S.A., Kleinert, H.D., Camargo, M.J. Deulofeu, R., Rimola, A., Perez-Ayuso, R., Rivera, F., Rodes, J. Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide. Nature 309:717-719, 1984.

August, J., Nelson, D., Thorn, G. Response of normal subjects to large amounts of aldosterone. J Clin Invest 37:1549-1555, 1958.

Bailie, M., Rector, F., Seldin, D. Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. J Clin Invest 50:119-126, 1971.

Ballerman, B., Brenner, B. Role of atrial peptides in body fluid homeostasis. Circ Res 58:619-630, 1986.

Ballermann, B.J., Bloch, K., Seidman, J. Brenner, B.

Atrial natriuretic peptide transcription, secretion, and glomerular receptor activity during mineralcorticoid escape in the rat. J Clin Invest 78:840-843, 1986.

Ballermann, B.J., Hoover, R.L., Karnovsky, M.J., Brenner, B.M. Physiologic regulation of atrial natriuretic peptide receptors in rat renal glomeruli. J Clin Invest 76:2049-2056, 1985.

Bank, N., Aynedjian, H. A micropuncture study of renal salt and water retention in chronic bile duct obstruction. J Clin Invest 55:994-1002, 1975.

Barajas, L. Innervation of the renal cortex. Fed Proc 1192-1201, 1978.

Barajas, L., Powers, K. Innervation of the thick ascending limb of Henle. Am J Physiol 255:F340-F348, 1988.

Baranowska, B., Gutkowska, J., Cantin, M., Genest, J. Plasma immunoreactive atrial natriuretic factor (I-ANF) increases markedly after alpha$_2$-adrenergic stimulation with clonidine in normally hydrated rats. Biochem Biophys Res Commun 143:159-163, 1987.

Bello-Reuss, E., Colindres, R., Pastoriza-Munoz, E., Mueller, R., Gottschalk, C. Effect of acute unilateral renal denervation in the rat. J Clin Invest 56:208-217, 1985.

Bello-Reuss, E., Trevino, D., Gottschalk, C. Effect of renal sympathetic nerve stimulation on proximal water and sodium reabsorption. J Clin Invest 57:1104-1107, 1976.

Berl, T., Raz, A., Wald, H., Horowitz, J., Czaizkes, W. Prostaglandin synthesis inhibition in the action of vasopressin: studies in man and rat. Am J Physiol 232:F529-F537, 1977.

Bernardi, M., Trevisani, F., Santini, R., DePalma,R., Gasbarrini, G. Aldosterone related blood volume expansion before and during the early phase of ascites formation. Gut 24:761-766, 1983.

Better, O., Massry, S. Effect of chronic bile duct obstruction on renal handling of salt and water. J Clin Invest 51:402-411, 1972.

Better, O., Schrier, R. Disturbed volume homeostasis in patients with cirrhosis of the liver. Kid Inter 23:303-311, 1983.

Bianchi, C., Gutkowska, J., Thibault, G., Garcia, R., Genest, J., Cantin, M. Radioautographic localization of I^{125}ANF in rat tissues. Histochemistry 82:441-452, 1985.

Bianchi, C., Gutkowska, J., Thibault, G., Garcia, R., Genest, J., Cantin, M. Distinct localization of atrial natriuretic factor and angiotensin binding sites in the glomerulus. Am J Physiol 251:F594-602, 1986.

Bichet, D., Van Putten, V., Schrier, R. Potential role of increased sympathetic activity in impaired sodium and water excretion in cirrhosis. New Engl J Med 307:1552-1557, 1982.

Bie, P., Wang, B., Leadley, R., Goetz, K. Hemodynamic and

renal effects of low-dose infusions of atrial peptide in awake dogs. Am J Physiol 254:R161-169, 1988.

Biollaz, J., Nussberger, J., Porchet, M., Brunner-Ferber, F., Otterbein, E.S., Gomez, H., Waeber, B., Brunner, H.R. Four-hour infusions of synthetic atrial natriuretic peptide in normal volunteers. Hypertension 8, Supl. II:II96-II105, 1986.

Blaine, E., Seymour, A., Marsh, E., Napier, M. Effects of atrial natriuretic factor on renal function and cyclic GMP production. Fed Proc 45:2122-2127, 1986.

Blanchart, A., Rodriguez-Puyal, D., Hernando, L., Lopez-Novoa, J.M. Effect of chronic and propensive hepatic outflow blockade on renal function in rats. J Lab Clin Med 109:718-723, 1987.

Blake, W., Jurf, A. Renal sodium reabsorption after acute renal denervation in the rabbit. J Physiol 196:65-73, 1968.

Blendis, L., Auld, R., Alexander, E., Levinsky, N. Effect of renal beta and alpha adrenergic stimulation on proximal sodium reabsorption in dogs. Clin Science 43:569-576, 1972.

Bolger, P., Eisner, G., Ramwell, P., Slotkoff, L. Effect of prostaglandin synthesis on renal function and renin in the dog. Nature 259:244-245, 1976.

Bolli, P., Muller, F.B., Linder, L., Raine, A.E.G., Resink, T.J., Erne, P., Kiowski, W., Ritz, R., Buhler, F.R. The

vasodilator potency of atrial natriuretic peptide in man. Circulation 75:221-228, 1987.

Bonjour, J., Churchill, P., Malvin, R. Change in tubular reabsorption of sodium and water after renal denervation in the dog. J Physiol 204:571-582, 1969.

Bonkovsky, H., Hartle, D., Simon, D., Meller, M., Kutner, K., Salambos, J. Decreased plasma arterial natriuretic peptides in cirrhotic ascitic patients. Abstract Hepatology 6:1213, 1986.

Bonvalet, J., Pradelles, P., Farman, N. Segmental synthesis and actions of prostaglandins along the nephron. Am J Physiol 253:F377-F387, 1987.

Borenstein, H., Cupples, W., Sonnenberg, H., Veress, A. The effect of a natriuretic atrial extract on renal hemodynamics and urinary excretion in anesthetized rats. J Physiol 344:133-140, 1983.

Boyer, T., Zia, P., Reynolds, T. Effects of indomethacin and prostaglandin A1, on renal function and plasma renin activity in alcoholic liver disease. Gastroenterology 77:215-222, 1979.

Brabant, G., Juppner, H., Kirschner, M., Boker, K., Schmidt, F., Hesch. Human atrial natariuretic peptide (ANP) for the treatment of patients with liver cirrhosis and ascites. Klin Wochenschr 64(Supp II):108-111, 1986.

Brands, M., Freeman, M. Aldosterone and renin inhibition by physiological levels of atrial natriuretic factor.

Am J Physiol 254:R1011-R1016, 1988.

Brennan, L., Henninger, A., Jochim, K., Malvin, R. Relationship between carotid sinus pressure and plasma renin level. Am J Physiol 227(2):295-299, 1974.

Brennan, L., Malvin, R., Jochim, R., Roberts, D. Influence of right and left atrial receptors on plasma concentration of ADH and renin. Am J Physiol 221:2273-2278, 1971.

Brenner, B., Troy, J., Daugharty, T. Dynamics of glomerular ultrafiltration in the rat. J Clin Invest 50:1776-1779, 1971.

Brenner, B., Troy, J., Daugharty, T. Quantitative importance of changes in postglomerular colloid osmotic pressure in mediating glomerular tubular balance in the rat. J Clin Invest 52:190-197, 1973.

Bricker, N., Staffon, R., Mahoney, E., Merrill, J. The functional capacity of the kidney denervated by autotransplantation in the dog. J Clin Invest 37:185-193, 1958.

Briggs, Steipe, B., Schubert, G., Schnermann, J. Micropuncture studies of the renal effects of atrial natriuretic substance. Pflugers Arch 271-276, 1982.

Burg, M. Renal handling of sodium chloride, water, amino acids, and glucose. In: Brenner, B., Rector, F., (ed.), The Kidney, W.B. Saunders Co., Philadelphia Vol. 1, 328-370, 1981.

Burghardt, W., Wernze, H., Diehl, T. ANP in hepatic cirrhosis: relation to stage of disease, sympathoadrenal system and renin-aldosterone axis. Klin Wochenschr (Supp VI):103-107, 1986.

Burnett, J., Granger, J., Opgenorth, T. Effects of synthetic atrial natriuretic factor on renal function and renin release. Am J Physiol 247:F863-F866, 1984.

Burnett, J., Kao, P., Hu, D., Heser, D., Heublein, D., Granger, J., Opgenorth, T., Reeder, G. Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 231:1145-1147, 1986.

Burnett, J., Opgenorth, T., Granger, J. The renal action of atrial natriuretic peptide during control of glomerular filtration. Kid Inter 30:16-19, 1986.

Campbell, V., Greig, P.D., Granford, N. A comparison of acute reversible pre- and post-sinusoidal portal hypertension in salt and water retension in the dog. Hepatology 2:54-58, 1982.

Campbell, P., Leung, W., Logan, A., Blendis, L., Skorecki, K. Comparison of the effects of α-H atrial natriuretic peptide infusion with peritoneovenous shunting in cirrhosis. (Abstract) 20th Annual Meeting Am Soc Neph p. 110A, 1987.

Cantin, M., Genest, J. The heart and the atrial natriuretic factor. Endocrine Rev 6:107-127, 1985.

Carswell, F., Hainsworth, R., Ledsome, J. The effects of

left atrial distension upon urine flow from the isolated perfused kidney, QJ Exp Physiol 55:173-182, 1970.

Cernacek, P., Crawhall, J., Levy, M. Atrial natriuretic peptide: blood levels in human disease and their measurement. Clin Biochem 21:5-17, 1988.

Chaimovitz, C., Massry, S., Friedler, R., Coburn, J. Effect of renal denervation and alpha-adrenergic blockade on sodium excretion in dogs with chronic ligation of the common bile duct. J Lab Clin Med 89:1053-1065, 1977.

Chapeau, C., Gutkowska, J., Schiller, P., Milne, R., Thibault, G., Garcia, R., Genest, J., Cantin, M. Localization of immunoreactive atrial natriuretic factor (ANF) in the heart of various animal species. J Histochem Cytochem 33:541-550, 1985.

Chen, P., Caldwell, R., Hsu, C. Role of the renal papillae in the regulation of sodium excretion during acute elevation of renal perfusion pressure in the rat. Hypertension 6:893-898, 1984.

Chou, S., Liebman, P., Ferder, L., Levin, D., Cacciaguida, R., Porush, J. Effects of α-adrenergic blockade on sodium excretion in normal and chronic salt retaining dogs. Can J Phys Pharm 54:209-218, 1976.

Chou, S., Reiser, I., Porush, J. Failure of atrial natriuretic factor t induce natriuresis in dogs with aortocaval fistula. (Abstract) 19th Meeting, ASN, p. 107A, 1987.

Cody, R., Atlas, S., Laragh, J., Kubo, S., Covit, A., Ryman, K., Shaknovitch, A., Pondolfino, K., Clark, M., Camargo, M., Scarborough, R., Lewicki, J. Atrial natriuretic factor in normal subjects and heart failure patients. Plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. J Clin Invest 78:1362-1374, 1986.

Cogan, M.G. Atrial natriuretic factor can increase renal solute excretion primarily by raising glomerular filtration. Am J Physiol 250(Renal Fluid Electrolyte Physiol 19):F710-F714, 1986.

Coggins, C. Nephrotic and nephritic edema. In: Sodium and water homeostasis. Brenner, B., Stein, J. (eds.). Churchill Livingstone, New York, Vol. 1:117-130, 1978.

Coleridge, J., Hemingway, H., Holmes, R., Linden, R. The location of atrial receptors in the dog: a physiological and histological study. J Physiol 136:174-197, 1957.

Condra, C., Leidy, E., Bunting, P., Colton, C., Nutt, R., Rosenblatt, M., Jacobs, J. Clearance and early hydrolysis of atrial natriuretic factor in vivo. J Clin Invest 81:1348-1354, 1988.

Cuneo, R., Espiner, E., Nicholls, M., Yandle, T., Livesey, J. Effect of physiological levels of atrial natriuretic peptide on hormone secretion: inhibition of angiotensin-induced aldosterone and renin release in normal man. J Clin Endo Metab 65:765-772, 1987.

Cuneo, R.C., Espiner, E.A., Nicholls, M.G., Yandle, T.G., Joyce, S.L., Gilchrist, N.L. Renal, hemodynamic, and hormonal responses to atrial natriuretic peptide infusions in normal man, and effect of sodium intake. J Clin Endocrin Metab 63:946-953, 1986.

Currie, M.G., Geller, D.M., Cole, B.R., Siegel, N.R., Fok, K.F., Adams, S.P., Eubanks, S.R., Galluppi, G.R., Needleman, P. Purification and sequence analysis of bioactive atrial peptides (atriopeptins). Science Wash, DC 223:67-69, 1984.

Currie, M.G., Newman, W.H. Evidence for α_1-adrenergic receptor regulation of atriopeptin release from the isolated rat heart. Biochem Biophys Res Commun 137:94-100, 1986.

Davis, C., Briggs, J. Effect of reduction in renal artery pressure on atrial natriuretic peptide - induced natriuresis. Am J Physiol 252:F146-F153, 1987.

Davis, J., Freeman, R. Mechanisms regulating renin release. Physiol Rev 56:1-56, 1976.

Davis, J., Hartroft, P., Titus, E., Carpenter, C., Ayers, C. Spiegel, H. The role f the renin-angiotensin system in the control of aldosterone secrtion. J Clin Invest 41:378, 1962.

Davis, J., Howell, D., Southworth, J. Mechanisms of fluid and electrolyte retention in experimental preparations in dogs. III. Effect of adrenalectomy and subsequent

desoxycorticosterone acetate administration on ascities formation. Circ Res 1:260-270, 1953.

Davis, J., Olichney, M., Brown, T., Binnion, P. Metabolism of aldosterone in several experimental situations with alteredaldosterone secretion. J Clin Invest 44:1433-41, 1965.

De Bold, A. Heart atrial granularity. Effects of changes in water - electrolyte balance. Proc Soc Exp Biol Med 161:508-511, 1979.

De Bold, A., Borenstein, H., Veress, A., Sonnenberg. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 28:89-94, 1981.

De Bold, A., Salerno, T. Natriuretic activity of extracts obtained from hearts of different species and from various rat tissues. Can J Physiol Pharm 61:127-130, 1983.

DeLean, A., Vinay, P., Cantin, M. Distribution of atrial natriuretic factor receptors in dog kidney fractions. FEBS Lett 193:239-241, 1985.

De Torrente, A., Robertson, G., McDonald, K., Schrier, R. Mechanism of diuretic response to increased left atrial pressure in the anesthetized dog. Kid Inter 8:355-361, 1975.

De Wardener, H., Mills, I., Clapham, W., Hayter, C. Studies on the efferent mechanism of the sodium diuresis

which follows the administration of intravenous saline in the dog. Clin Science 21:249-258, 1961.

DiBona, G. Neural control of renal tubular sodium reabsorption in the dog. Fed Proc 37:1214-1217, 1978.

DiBona, G. The functions of the renal nerves. Rev Physiol Biochem Pharmacol 94:75-181, 1982.

DiBona, G. Renal nerve activity in hepatorenal syndrome. Kid Int 25:841-853, 1984.

Dietz, J. Release of natriuretic factor from rat heart-lung preparation by atrial distension. Am J Physiol 247:R1093-R1096, 1984.

Dietz, R., Purgaj, J., Lang, R., Schomig, A. Pressure dependent release of atrial natriuretic peptide in patients with chronic cardiac diseases: does it reset? Klin Wochenschr 64(Supp VI):42-46, 1986.

Ding, J., Thibault, G., Gutkowska, J., Garcia, R., Karabatsos, T., Jasmin, G., Genest, J., Cantin, M. Cardiac and plasma atrial natriuretic factor in experimental congestive heart failure. Endocrin 121:248-257, 1987.

Di Salvo, J., Peterson, A., Montefusco, C., Menta, M. Intrarenal conversion of angiotensin I to angiotensin II in the dog. Circ Res 29:398-406, 1971.

Dirks, J., Cirksena, W., Berliner, R. The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J Clin Invest 44:1160, 1965.

Dolan, L., Dobroszi, D. Atrial Natriuretic polypeptide in the fetal rat: Ontogeny and characterization. Ped Res 22:115-117, 1987.

Donker, A., Arisz, L., Brentjens, J., Venderhem, G., Hollemans, H. The effect of indomethacin on kidney function and plasma renin activity in man. Nephron 17:288-296, 1976.

Dunn, B.R., Ichikawa, I., Pfeffer, J.M., Troy, J.L., Brenner, B.M. Renal and systemic hemodynamic effects of synthetic atrial natriuretic peptide in the anesthezied rat. Circ Res 59:237-246, 1986.

Edelman, R., Hartroft, P. Localization of renin in the juxtaglomerular cells of the rabbit and dog through the use of the fluorescent-antibody technique. Circ Res 9:1069-1077, 1961.

Edwards, B., Ackermann, D., Schwab, T., Heublein, D., Edwards, W., Wold, L., Burnett, J. The relationship between atrial granularity and circulating atrial natriuretic peptide in hamsters with congestive heart failure. Mayo Clin Proc 61:517-521, 1986.

Epstein, F., Renal excretion of sodium and the concept of a volume receptor. Yale J Biol and Med 29:282-298, 1956.

Epstein, F., Post, R., McDowell, M. The effect of an arteriovenous fistula on renal hemodynamics and electrolyte excretion. J Clin Inves 32:233-?, 1953.

Epstein, M. Renal effects of head-out water immersion in

man. Implications for an understanding of volume homeostasis. Phys Rev 58(3):529-581, 1978.

Epstein, M. Increased sympathetic activity in cirrhosis. New Engl J Med 30:1030, 1983.

Epstein, M. Renal prostaglandins and the control of renal function in liver disease. Am J Med. 80(Suppl A):46-55, 1986.

Epstein, M., Lifschitz, M., Hoffman, D., Stein, J. Relationship between renal prostaglandin E and renal sodium handling during water immersion in normal man. Circ Res 45:71-80, 1979.

Epstein, M., Loutzenhiser, R., Friedland, E., Aceto, R., Camargo, M., Atlas, S. Increases in circulating atrial natriuretic factor during immersion-induced central hypervolemia in normal humans. J Hypertension 4 Supp2:S93-S99, 1986.

Epstein, M., Saruta, T. Effect of water immersion on renin-aldosterone and renal sodium handling in normal man. J. App. Phys. 31(3):368-374, 1971.

Epstein, M., Ulano, H. Role of vasopressin in mediating the diuresis of water immersion to the neck. Proc. 9th Ann. Meeting, Am Soc Nephrol p. 96, 1976.

Epstein, M., Duncan, D., Fishman, L. Characterization of the natriuresis caused in normal man by immersion in water. Clin Sci 43:275-287, 1972.

Epstein, M. Sodium homeostasis in liver disease. In:

Epstein M. (ed.). The Kidney in liver disease, 3rd ed. Williams, Wilkinson, 1988.

Fadem, S., Hernandez-Llamas, G., Patak, R., Rosenblatt, S., Lifschitz, M., Stein, J. Studies on the mechanism of sodium excretion during drug-induced vasodilatation in the dog. J Clin Invest 69:604-610, 1982.

Fagard, R., Cowley, A., Navar, L., Langford, H., Guyton, A. Renal responses to slight elevation of renal arterial plasma angiotensin II concentration in dogs. Clin Exp Pharm Physiol 3:539-544, 1976.

Faison, E., Siegel, P., Morgan, G., Winquist, R. Regional vasorelaxant selectivity of atrial natriuretic factor in isolated rabbit vessels. Life Sci 37:1073-1079, 1985.

Fater, D., Schultz, H., Sundet, W., Mapes, J., Geotz, K. Effects of left atrial stretch in cardiac-denervated and intact conscious dogs. Am J Physiol 242:H1056-H1064, 1982.

Faubert, P., Chou, S., Porush, J., Belizon, I., Spitalewritz, S. Papillary plasma flow and tissue osmolality in chronic caval dogs. Am J Physiol 242:F370-F378, 1982.

Fejes-Toth, G., Magyar, A., Walter, J. Renal response to vasopressin after inhibition of prostaglandin synthesis. Am J Physiol 232:F416-423, 1977.

Flombaum, C., Chou, S., Porush, J., Slater,P., Ferder, L., Levin, D., Gadhok, R. Serial evaluation of the renin-

angiotensin aldosterone system in caval dogs. Circ Res 42:778-786, 1978.

Flynn, T.G., De Bold, M.L., De Bold, A.J. The amino acid sequence of atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun 117:859-865, 1983.

Flynn, T.G., Davies, P.L. The biochemistry and molecular biology of atrial natriuretic factor. Biochem J 232:313-321, 1985.

Franch, H., Heinel, L., Callahan, L., Blaine, E. Elevated circulating levels of atrial natriuretic factor in cardiomyopathic hamsters. In: Biologically Active Atrial Peptides, Vol., 1., Am Soc Hypertension Symposium Series, ed. B. Brenner, J. Laragh, Raven Press, New York, p. 89-95, 1987.

Freeman, R., Davis, J., Vari, R. Renal response to atrial natriuretic factor in conscious dogs with caval constriction. Am J Physiol 248:R495-R500, 1985.

Freeman, R., Davis, J., Villarreal, D. Role of renal prostaglandins in the control of renin release. Circ Res 54(1):1-9, 1984.

Freeman, R., Villarreal, D., Vari, R., Verburg, K. Endogenous atrial natriuretic factor in dogs with caval constriction. Circ Res 61(Supp I):I96-I99, 1987.

Fried, T., McCoy, R., Osgood, R., Reineck, J., Stein, J. Effect of atriopeptin II on determinants of glomerular

filtration rate in the in vitro perfused dog glomerulus. Am J Physiol 250:F1119-F1122, 1986.

Fried, T., Osgood, R., Stein, J. Tubular site(s) of action of atrial natriuretic peptide in the rat. Am J Physiol 255:F313-F316, 1988.

Friedler, R., Belleau, L., Martino, J., Earley, L. Hemodynamically induced natriuresis in the presence of sodium retention resulting from constriction of the thoracic inferior vena cava. J Lab Clin Med 69(4):565-583, 1966.

Fujio, N., Ohashi, M., Nawata, H., Kato, K., Ibayashi, H., Kangawa, K., Matsuo, H. α-Human atrial natriuretic polypeptide reduces the plasma arginine vasopressin concentration in human subjects. Clin Endocrinol 25:181-187, 1986.

Fyhrquist, F., Totterman, K., Tikkanen, I., Infusion of atrial natriuretic peptide in liver cirrhosis with ascites. Lancet, Dec. 21/28, p. 1439, 1985.

Garcia, R., Thibault, G., Seidah, N.G., Lazure, C., Cantin, M., Genest, J. Chretien, M. Structure-activity relationships of atrial natriuretic factor (ANF). II. Effect of chain length modifications on vascular reactivity. Biochem Biophys Res Commun 126:178-184, 1985.

Gardner, D.G., Deschepper, C.F., Baxter, J.D. The gene for the atrial natriuretic factor is expressed in the aortic

arch. Hypertension 9:103-106, 1987.

Gauer, O., Henry, J. Circulatory basis of fluid volume control. Physiol Rev 43:423-481, 1963.

Gauer, O., Henry, J. Neurohormonal control of plasma volume. International Rev Physiol, ? Physiol II, Vol 9:146-189, 1976.

Gauquelin, G., Garcia, R., Carrier, F., Cantin, M., Gutkkowska, J., Thibault, G., Schiffrein, E. Glomerular ANF receptor regulation during changes in sodium and water metabolism. Am J Physiol 254:F51-F55, 1988.

Gerzer, R., Vitzgall, M., Tremblay, J., Cutkowska, J., Flamert, P. Rapid increase in plasma and urinary cGMP after bolus injection of ANF in man. J Clin Endo Metab 61(6):1217-1219, 1985.

Gill, J., Carr, A., Fleischmann, L., Casper, A., Bartter, F. Effects of pentolinium on sodium excretion in dogs with constriction of the vena cava. Am J Physiol 212:191-196, 1967.

Gill, J., Casper, G. Role of the sympathetic nervous system in the renal response to hemorrhage. J Clin Invest 48:915-922, 1969.

Gilmore, J., Daggett, W. Response of the chronic cardiac denervated dog to acute volume expansion. Am J Physiol 210(3):509-512, 1966.

Gines, P., Jimenez, W., Arroyo, V., Navasa, M., Lopez, C., Tito, L., Serra, A., Bosch, J., Sanz, G., Rivera, F.,

Rodes, J. Atrial natriuretic factor in cirrhosis with ascites: Plasma levels, cardiac release and splanchnic extraction. Hepatology 8(3):636-642, 1988.

Gnadinger, M.P., Weidmann, P., Rascher, W., Lang, R.E., Hellmuller, B., Uehlinger, D.E. Plasma arginine-vasopressin levels during infusion of synthetic atrial natriuretic peptide on different sodium intakes in man. J Hypertens 4:623-629, 1986.

Goetz, K.L. Atrial receptors, natriuretic peptides, and the kidney - current understanding. Mayo Clin Proc 61:600-603, 1986.

Goetz, K., Bond, G., Hermreck, A., Trank, J. Plasma ADH levels following a decrease in mean atrial transmural pressure in dogs. Am J Physiol 219(5):1424-1428, 1970.

Goetz, K., Wang, B., Bie, P., Leadley, R., Greer, P. Natriuresis during atrial distension and a concurrent decline in plasma atriopeptin. Am J Physiol 255:R259-R267, 1988.

Goetz, K., Wang, B., Geer, P., Leadley, R., Reinhardt, H. Atrial stretch increases sodium excretion independently of release of atrial peptides. Am J Physiol 250:R946-R950, 1986.

Goodyer, A., Jaeger, C. Renal response to non-shocking hemorrhage. Role of the autonomic nervous system and of the renal circulation. Am J Physiol 180:69-74, 1955.

Gottschalk, C. Renal nerves and sodium excretion. Ann Rev

Physiol 41:229-240, 1979.

Granger, J., Opgenorth, T., Salazar, J., Romero, J., Burnett, J. Long-term hypotensive and renal effects of atrial natriuretic peptide. Hypertension 8(Supp II):II-112-II-116, 1986.

Granger, P., Dahlheim, H., Thurau, K. Enzyme activities of the single juxtaglomerular apparatus in the rat kidney. Kidney Inter 1:78-88, 1972.

Graveline, D., Jackson, M. Diuresis associated with prolonged water immersion. J App Phys 17(3):519-524, 1962.

Gunning, M., Ballermann, B., Silva, P., Brenner, B., Zeidel, M. Characterization of ANP receptors in rabbit inner medullary collecting duct cells. Am J Physiol 255:F324-F330, 1988.

Gutkowska, J., Bonan, R., Roy, D., Bourassa, M., Garcia, R., Thibault, G., Genest, J., Cantin, M. Atrial natriuretic factor in human plasma. Biochem Biophys Res Comm 139:287-295, 1986.

Guyton, A. Interstitial fluid pressure. Pressure-volume curves of interstitial space. Circ Res 16:452-460, 1964.

Guyton, A., Coleman, T., Granger, H. Circulation: Overall Regulation. Ann Review of Physiology 34:13-45, 1972.

Haas, J., Granger, J., Knox, F. Effect of renal perfusion pressure on sodium reabsorption from proximal tubules of superficial and deep nephrons. Am J Physiol 249:F315-

F318, 1985.

Hall, J. Control of sodium excretion by angiotensin II: Intrarenal mechanisms and blood pressure regulation. Am J Physiol 250:R960-R972, 1986.

Hamet, P., Tremblay, J., Pang, S.C. Garcia, R., Thibault, G., Gutkowska, J., Cantin, M., Genest, J. Effects of native and synthetic atrial natriuretic factor on cyclic GMP. Biochem Biophys Res Commun 123:515-527, 1984.

Hamet, P., Tremblay, J., Pang, S., Skuherska, R., Schiffrin, E., Garcia, R., Cantin, M., Genest, J., Palmour, R., Ervin, F., Martin, S., Goldwater, R. Cyclic GMP as mediator and biological marker of atrial natriuretic factor. J Hypertension 4, Suppl. 2:S49-S56, 1986.

Hammond, T., Yusufi, A., Knox, F., Dousa, T. Administration of atrial natriuretic factor inhibits sodium-coupled transport in proximal tubules. J Clin Invest 75(6):1983-1989, 1985.

Henriksen, J., Christensen, N., Ring-harsen, H. Noradrenaline and adrenaline concentration decompensated cirrhosis. Relation to hemodynamics. Clin Physiol 1:293-304, 1981.

Henriksen, J., Schutten, H., Bendtsen, F. Circulating atrial natriuretic peptide (ANP) and central blood volume (CBV) in cirrhosis. Liver 6:361-368, 1986.

Henry, J., Gauer, O., Reeves, J. Evidence of the atrial

location of receptors influencing urine flow. Circ Res 4:85-90, 1956.

Hildebrandt, D., Banks, R. Effect of atrial natriuretic factor on renal function in rats with papillary necrosis. Am J Physiol 252:F977-F980, 1987.

Hirata, Y., Tomita, M., Yoshimi, H., Masao, I. The effects of human atrial 28-amino acid peptide on systemic and renal hemodynamics in anesthetized rats. Circ Res 57(4):634-639, 1985.

Hoffmann, A., Burnett, J., Haramati, A., Winaver, J. Effects of atrial natriuretic factor in rats with experimental high-output heart failure. Kid Int 33:656-661, 1988.

Hollister, A., Tanaka, I., Imada, T., Onrot, J., Biaggione, I., Robertson, D., Inagami, T. Sodium loading and posture modulate human atrial natriuretic factor plasma levels. Hypertension 8(Supp. II):II 106-II 111, 1986.

Holtz, J., Sommer, O., Bassenge, E. Inhibition of sympathoadrenal activity by atrial natriuretic factor in dogs. Hypertension 9:350-354, 1987.

Holtz, J., Stewart, D.J., Elsner, D., Bassenge, E. In vivo atrial peptide-venodilation: minimal potency relative to nitroglycerin in dogs. Life Sci 39:2177-2184, 1986.

Hricik, D., Appel, R., Simonson, M., Green, J., Smith, R., Dunn, M. Effects of human atrial natriuretic factor in patients with nephrotic syndrome and cirrhosis with

ascites. (Abstract)A.S.N., 19th Annual Meeting, p. 116, 1986.

Huang, C., Lewicki, J., Johnson, L., Cogan, M. Renal mechanism of action of rat atrial natriuretic factor. J Clin Invest 75:769-773, 1985.

Ichikawa, I., Dunn, B., Troy, J., Maack, T., Brenner, B. Influence of atrial natriuretic peptide on glomerular microcirculation in vivo. Clin Res 33:487A, 1985.

Isakson, P., Raz, A., Denny, S., Wyche, A., Needleman,P. Hormonal stimulation of archidonate release from isolated perfused organs: Relationship to prostaglandin biosynthesis. Prostaglandins 14:853-871, 1977.

Ishihara, T., Aisaka, K., Hattori, K., Hamasaki, S., Morita, M., Noguchi, T., Kangawa, K., Matsuo, H. Vasodilatory and diuretic actions of α-human atrial natriuretic polypeptide (α-hANP). Life Sci 36:1205-1215, 1985.

Jamieson, J., Palade, G. Specific granules in atrial muscle cells. J Cell Biol 23:151-172, 1964.

Januszewicz, P., Gutkowska, J., DeLean, A., Thibault, G., Garcia, R., Genest, J., Cantin, M. Synthetic atrial natriuretic factor induces release (possibly receptor mediated) of vasopressin from rat posterior pituitary. Proc Soc Exp Biol Med 178:321-325, 1985.

Jarecki, M., Thoren, P., Donald, D. Release of renin by the carotid baroreflex in anesthetized dogs. Role of

cardiopulmonary vagal afferents and renal arterial pressure. Circ Res 42(5):614-619, 1978.

Jimenez, W., Martinez-Pardo, A., Arroyo, V., Gaya, J., Rivera, F., Rodes, J. Atrial Natriuretic Factor: reduced cardiac content in cirrhotic rats with ascites. Am J Physiol 250:F749-F752, 1986.

Johannessen, A., Schutten, H., Torp-Pedersen, C., Warberg, J., Bie, P. Effects of human atrial naatriuretic peptide (hANP) in conscious dogs. Acta Physiol Scand 127:449-454, 1986.

Johnson, J., Davis, J. Angiotensin II. Important role in the maintenance of arterial blood pressure. Science, ?906-907, 1973.

Johnson, J., Moore, W., Segar, W. Small changes in left atrial pressure and plasma antidiuretic titers in dogs. Am J Physiol 217:210-214, 1969.

Johnston, C., Davis, J. Evidence from cross circulation studies for a humoral mechanism in the natriuresis of saline loading. Proc Soc Exp Biol Med 121:1058-1063, 1966.

Karzmarczyk, G., Drake, A., Eisele, R., Mohnhaupt, R., Noble, M., Simgen, B., Stubbs, J., Reinhardt, H. The role of the cardiac nerves in the regulation of sodium excretion in conscious dogs. Pflugers Arch 390:125-130, 1981.

Kaloyanides, G., Cacciaguida, R., Pablo, N., Porush, J.

Increased sodium reabsorption in the proximal and distal tubule of caval dogs. J Clin Invest 48:1543-1551, 1969.

Kangawa, K., Matsuo, H. Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (α-ANP). Biochem Biophys Res Comm 118:131-139, 1984.

Kappagoda, C., Linden, R., Snow, H. Effect of stimulating right atrial receptors on urine flow in the dog. J Physiol 235:493-502, 1973.

Kappagoda, C., Linden, R., Snow, H., Whitaker, E. Left atrial receptors and the antidiuretic hormone. J Physiol 237:663-683, 1974.

Kappagoda, C., Linden, R., Snow, H., Whitaker, E. Effect of destruction of the posterior pituitary on the diuresis from left atrial receptors. J Physiol 244:757-770, 1975.

Kappagoda, C., Linden, R., Sivananthan, N. The nature of atrial receptors responsible for a reflex increase in heart rate in the dog. J Physiol 291:393-412, 1979.

Karim, F., Kidd, C., Malpus, C., Penna, P. The effects of stimulation of left atrial receptors on sympathetic efferent nerve activity. J Physiol 227:243-260, 1972.

Katsube, N., Schwartz, D., Needleman, P. Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. Biochem Biophys Res Comm 133:937-944, 1985.

Katsube, N., Schwartz, D., Needleman. Atriopeptin

turnover: Quantitative relationship between in vivo changes in plasma levels and atrial content. J Pharm Exp Therap 239:474-479, 1986.

Keeler, R. Natriuresis after unilateral stimulation of carotid receptors in unanesthetized rats. Am J Physiol 226(3):507-511, 1974.

Khraibi, A., Granger, J., Burnett, J., Walker, K., Knox, F. Role of atrial natriuretic factor in the natriuresis of acute volume expansion. Am J Physiol 252:R921-R924, 1987.

Kiberd, B., Larson, T., Robertson, C., Jamieson, R. Effect of atrial natriuretic peptide on vasa recta blood flow in the rat. Am J Physiol 252:F1112-F1117, 1987.

Kimura, T., Abe, K., Shoji, M., Tsunoda, K., Matsui, K., Ota, K., Inoue, M., Yasujima, M., Yoshinaga, K. Effects of human atrial natriuretic peptide on renal function and vasopressin release. Am J Physiol 250:R789-R794, 1986.

Kirchheim, H. Systemic arterial baroreceptor reflexes. Phys Rev 56(1):100-176, 1976.

Kisch, B. Electron microscopy of the atrium of the heart: guinea pig. Exp Med Surg 14:99-112, 1956.

Kleinert, H., Volpe, M., Odell, G., Mario, D., Atlas, S., Camargo, M., Laragh, J., Maack, T. Cardiovascular effects of atrial natriuretic factor in anesthetized and conscious dogs. Hypertension 8:312-316, 1986.

Knox, F. Escape fromthe sodium retanine effects of

mineralcorticoids. Kid Inter 17:263-276, 1980.

Koehn, J., Norman, J., Jones, B., LeSuer, L., Sakane, Y., Ghai, R., Degradation of atrial natriuretic factor by kidney cortex membranes. J Biol Chem 262(24):11623-11627, 1987.

Koepke, J., DiBona, G. Blunted natriuresis to atrial natriuretic peptide in chronic sodium retaining disorders. Am J Physiol 252:F865-F871, 1987.

Koepke, J., Jones, S., DiBona, G. Attenuated natriuresis to atrial natriuretic peptide in conscious cirrhotic rats. (Abstract), ASN 19th Annual Meeting, p. 119A, 1986.

Koepke, J., Jones, S., DiBona, G. Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats. Am J Physiol 252:R1019-R1023, 1987.

Koseki, C., Hayashi, Y., Torikai, S., Furuya, M., Ohnuma, N., Imai, M. Localization of binding sites for α-rat atrial natriuretic polypeptide in rat kidney. Am J Physiol 250:F210-F216, 1986.

Kostreva, D., Castaner, A., Kampine, J. Reflex effects of hepatic baroreceptors on renal and cardiac sympathetic nerve activity. Am J Physiol 238:R390-R394, 1980.

Kostreva, D., Zuperku, E., Purtock, R., Coon, R., Kampine, J. Sympathetic afferent nerve activity of right heart origin. Am J Physiol 229(4):911-915, 1975.

Koyama, S., Kanai, K., Aibiki, M., Fujita, T. Reflex

increase in renal nerve activity during acutely altered portal venous pressure. J Aut Nerv Syst 23:55-62, 1988.

Koyama, S., Nishida, Y., Hosomi, H., Abe, Y. Participation of baroreceptor reflexes in blood pressure and sympathetic nerve responses to a synthetic human atrial natruiretic peptide in anesthetized dogs. Eur J Pharm 127:43-48, 1986.

Kramer, H., Kipnowski, J., Stekle, B., Baker, A., Predel, H., Dusing, R. Studies on the tubular effects of atrial extract and of atriopeptin III in conscious rats. Klin Wochenschr (Supp) 6:31-36, 1986.

Kuchel, O., Horky, K., Gregorova, I., Marek, J., Kopecha, J., Kobilkova, J. Inappropriate response to upright posture: a precipitating factor in the pathogenesis of idiopathic edema. Ann Inter Med 73:245, 1970.

Kumada, M., Sagawa, K. Aortic nerve activity during blood volume changes. Am J Physiol 218:961-965, 1970.

Kurtze, A., Bruna, R., Pfeilschifter, J., Tauigner, R., Bauer, C. Atrial natriuretic peptide inhibits renin release from juxtaglomerular cells by a cGMP-mediated process. Proc Natl Acad Sci 83:4769-4773, 1986.

LaGrange, R., Sloop, C., Schmid, T. Selective stimulation of renal nerves in the anesthetized dog. Effect on renin release during controlled changes in the renal hemodynamics. Circ Res 33:704-712, 1973.

Lang, R., Tholken, H., Ganten, D., Luft, F., Ruskoaho, H.,

Unger, T. Atrial natriuretic factor - a circulating hormone stimulated by volume loading. Nature Lond 314:264-266, 1985.

Lattion, A., Michel, J., Arnauld, E., Corvol, P., Soubrier, F. Myocardial recruitment during ANF mRNA increase with volume overload in the rat. Am J Physiol 251:H890-H896.

Lautt, W. Afferent and efferent neural roles in liver function. Prog in Neurobio 21:323-348, 1983.

Ledsome, J. The courser of release of ANP(Z) in the anesthetized dog. Can J Physiol Pharm 64(7):1017-1022, 1986.

Ledsome, J., Linden, R. The role of left atrial receptors in the diuretic response to left atrial distension. J Physiol 198:487-503, 1968.

Ledsome, J., Linden, R., O'Connor, W. The mechanisms by which distension of the left atrium produces a diuresis in anesthetized dogs. J Physiol 159:87-100, 1961.

Ledsome, J.R., Wilson, N., Courneya, C.A., Rankin, A.J. Release of atrial natriuretic peptide by atrial distension. Can J Physiol Pharmacol 63:739-742, 1985.

Ledsome, J., Wilson, N., Rankin, A., Courneya, C. Time course of release of atrial natriuretic peptide in the anesthetized dog. Can J Phys Pharm 64:1017-1022, 1985.

Lee, J. Prostaglandins and the renin-angiotensin axis. Clin Nephrology 14(4):159-163, 1980.

Leitman, D., Andresen, J., Kuno, J., Kamisaki, Y., Chang,

J., Murad, F. Identification of multiple binding sites for atrial natriuretic factor by affinity cross-linking in cultured endothelial cells. J Biol Chem 261(25):11650-11655, 1986.

Leung, W., Skorecki, K., Campbell, P., Debowski, T., Blendis, L., Logan, A. Role of atrial natriuretic response to head-out water immersion in sodium retaining cirrhotics (Abstract), Am Soc Nephr 20th Ann. Meeting, p. 125A, 1987.

Levens, N., Peach, M., Carey, R. Role of the intrarenal renin-angiotensin system in the control of renal function. Circ Res 48(2):157-167, 1981.

Levinsky, N., Lalone, R. Sodium excretion during acute saline loading in dogs with vena caval obstruction. J Clin Invest 44:565-?, 1965.

Levy, M. Effects of acute volume expansion and altered hemodynamics on renal tubular function in chronic caval dogs. J Clin Invest 51(4):922-938, 1972.

Levy, M. Renal function in dogs with acute selective hepatic venous outflow block. Am J Physiol 227:1074-1083, 1974.

Levy, M. Sodium retention and ascites formation in dogs with experimental portal cirrhosis. Am J Physiol 233:F572-F585, 1977a.

Levy, M. Sodium retention in dogs with cirrhosis and ascites: efferent mechanisms. Am J Physiol 233(6):P586-

F592, 1977b.

Levy, M. Increased sympathetic activity in cirrhosis. New Engl J Med 308:1031, 1983.

Levy, M., Allotey, J. Temporal relationships between urinary salt retention and altered systemic hemodynamics in dogs with experimental cirrhosis. J Lab Clin Med 92:560-569, 1978.

Levy, M., Wexler, M. Fechner, C. Renal perfusion in dogs with experimental hepatic cirrhosis: role of prostaglandins. Am J Physiol 245:F521-F529, 1983.

Levy, M., Wexler, M., McCaffrey, C. Sodium retention in dogs with experimental cirrhosis following removal of ascites by continuous peritoneovenous shunting. J Lab Clin Med 94:933-946, 1979.

Levy, M., Wexler, M. Salt and water balance in liver disease. Hospital Practice, July:57-71, 1984.

Liang, C. The influence of hepatic portal circulation on urine flow. J Physiol 214:571-581, 1971.

Lillienfield, L., Maganzini, H., Bauer, M. Blood flow in the renal medulla. Circ Res 9:614-617, 1961.

Linden, R., Mary, D., Weatherill, D. The nature of the atrial receptors responsible for a reflex decrease in the activity of renal nerves in the dog. J Physiol 300:31-40, 1980.

Lindheimer, M., Lalone, R. Levinsky, N. Evidence that an acute increase in glomerular filtration has little effect

on sodium excretion in the dog unless extracellular volume is expanded. J Clin Invest 46:256-265, 1967.

Lopez-Novoa, J., Rengel, M., Hernando, L. Dynamics of ascites formation in rats with experimental cirrhosis. Am J Physiol 238:F353-F357, 1980.

Luft, F., Lang, R., Aronoff, G., Ruskaoho, H., Toth, M., Ganten, D., Sterzel, R., Unger, T. Atriopeptidn III kinetics and pharmacodynamics in normal and anephric rats. J Exp Therap 236:416-418, 1986.

Maack, T., Camargo, J., Kleinert, M., Laragh, J., Atlas, S. Atrial natriuretic factor: structure and functional properties. Kid Int 27:607-615, 1985.

Maack, T., Marion, D., Camargo, M., Kleinert, H., Laragh, J., Vaughan, E., Atlas, S. Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function and the renin-aldosterone system in dogs. Am J Med 77:1069-1075, 1984.

Maack, T., Suzuki, M., Almeida, F., Nussenzveig, D., Scarborough, R., McEnroe, G., Lewicki, J. Physiological role of silent receptors of atrial natriuretic factor. Science 2238:675-678, 1987.

Mancia, G., Romero, J., Shepherd, J. Continuous inhibition of renin release in dogs by vagally innervated receptors in the cardiopulmonary region. Circ Res 36:529-535, 1975.

Marin-Grez, M., Fleming, J., Steinhausen, M. Atrial

natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. Nature 473-476, 1986.

Mendez, R., Dunn, B., Troy, J., Brenner, B. Modulation of the natriuretic response to atrial natriuretic peptide by alterations in peritubular Starling forces in the rat. Circ Res 59(6):605-611, 1986.

Mendez, R., Dunn, B., Troy, J., Brenner, B. Atrial natriuretic peptide and furosemide effects on hydraulic pressure in renal papilla. Kidney Inter 34:36-42, 1988.

Metzler, C., Lee, M., Thrasher, T., Ramsay, D. Increased right or left atrial pressure stimulates release of atrial natriuretic peptides in conscious dogs. Endocrinology 119:2396-2398, 1986.

Miki, K., Hajduczok, G., Klocke, M., Krasney, J., Hong, S., De Bold, A. Atrial natriuretic factor and renal function during head-out water immersion in conscious dogs. Am J Physiol 251:R1000-R1008, 1986.

Miki, K., Shiraki, K., Sagawa, S., De Bold, A., Hong, S. Atrial natriuretic factor during head-out immersion at night. Am J Physiol 254:R235-R241, 1988.

Mills, I., Osbaldiston, G. The effect of stretch of the right atrium on arterial pressure and renal function in the dog. J Physiol 197:40-41, 1968.

Misono, K., Fukumi, H., Grammer, R., Inagami, T. Rat atrial natriuretic factor: complete amino acid sequence

and disulfide linkage essential to biological activity. Biochem Biophys Res Comm 119:524-529, 1984.

Miyata, A., Kangawa, K., Toshimori, T., Hatoh, T., Matsuo, H. Molecular forms of atrial natriuretic polypeptides in mammalian tissues and plasma. Biochem Biophys Res Comm 129:248-255, 1985.

Murphy, K., McLaughlin, L., Michener, M., Needleman, P. Autoradiographic localization of atriopeptin III receptors in rat kidney. Eur J Pharmacol 111:291-292, 1985.

Murthy, K., Thibault, G., Garcia, R., Gutkowska, J., Cenest, J., Cantin, M. Degradation of atrial natriuretic factor in the rat. Biochem J 240:461-469, 1986.

Murthy, K., Thibault, G., Schiffrin, et al. Peptides 7:241-246, 1986.

Myers, J., Godley, J. Cardiovascular and renal function during total body water immersion of dogs. J App Phys 22(3):573-579, 1967.

Nakao, K., Katsura, G., Morii, N., Itoh, H., Shiono, S., Yamada, T., Sugawara, A., Sakamoto, M., Saito, Y., Eigyo, M., Matsushita, A., Imura, H. Inhibitory effect of centrally administered atrial natriuretic polypeptide on the brain dopaminergic system in rats. Eur J Pharmacol 131:171-177, 1986.

Nakao, K., Sugawara, A., Morii, N., Sakamoto, M., Yamada, T., Itoh, H., Shiono, S., Saito, Y., Nishimura, K., Ban,

T., Kangawa, K., Matsuo, H., Imura, H. The pharmacokinetics of α-human atrial natriuretic polypeptide in healthy subjects. Eur J Clin Pharm 31:101-103, 1986.

Niijima, A. Baroreceptor effects on renal and adrenal nerve activity. Am J Physiol 230:1733-1736, 1976.

Niijima, A. Afferent discharges from venous pressoreceptors in liver. Am J Physiol 232:C76-C81, 1977.

Nonoguschi, H., Knepper, M.A., Manganiello, V.C. Effects of atrial natriuretic factor on cyclic guanosine monophosphate and cyclic adenosine monophosphate accumulation in microdissected nephron segments from rats. J Clin Invest 79:500-507, 1987.

Obana, K., Naruse, M., Inagami, T., Brown, A.B., Naruse, K., Kurimoto, F., Sakurai, H., Demura, H., Shizume, K. Atrial natriuretic factor inhibits vasopressin secretion from rat posterior pituitary. Biochem Biophys Res Commun 132:1088-1094, 1985.

Obana, K., Naruse, M., Naruse, K. Synthetic rat atrial natriuretic factor inhibits in vitro and in vivo renin secretion in rats. Endocrinology 117(3):1282-1284, 1985.

Ogihara, T., Shima, J., Hara, H., Kumahara, Y., Kangawa, K., Matsuo, H. Changes in human plasma atrial natriuretic polypeptide concentration in normal subjects during passive leg raising and whole-body tilting. Clin

Sci 71:147-150, 1986.

Ogihara, T., Shima, J., Hara, H., Tabuchi, Y., Hashizume, K., Nagano, M., Katahira, K., Kangawa, K., Matsuo, H., Kumahara, Y. Significant increase in plasma immunoreactive atrial natriuretic polypeptide concentration during head-out water immersion. Life Sci 38:2413-2418, 1986.

Ohlstein, E., Berkowitz, B. cGMP mediates vascular relaxation induced by ANF. Hypertension 7(2):306-310, 1985.

Oikawa, S., Imai, M., Inuzuka, C., Tawaragi, Y., Nakazato, H., Matsuo, H. Structure of dog and rabbit precursors of atrial polypeptides deduced from nucleotide sequence of cloned cDNA. Biochem Biophys Res Comm 132:892-899, 1985.

Oikawa, S., Imai, M., Ueno, A., Tanaka, S., Noguchi, T., Nakazato, H., Kangawa, K., Fukuda, A., Matsuo, H. Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. Nature 309:724-726, 1984.

Olins, G., Spear, K., Siegel, N., Zurcher-Neely, H. Inactivation of atrial natriuretic factor by the renal brush border. Biochemica Biophys Acta 901:L97-100, 1987.

Olivera, A., Gutkowska, J., Rodriguez-Puyol, D., Fernandez-Cruz, A., Lopez-Nova, J. Atrial natriuretic peptide in rats with experimental cirrhosis of the liver without

ascites. Endocrinology 122:840-846, 1988.

Oshima, T., Currie, M., Geller, D., Needleman, P. An atrial peptide is a potent renal vasodilator substance. Circ Res 54:612-616, 1984.

Paganelli, W., Cant, J., Pintal, R., Kifor, I., Barger, A., Dzau, V. Plasma atrial natriuretic factor during chronic thoracic inferior vena caval constriction. Circ Res 62:279-285, 1988.

Page, E., Goings, G., Power, B., Upshaw-Earley, J. Ultrastructural features of atrial peptide secretion. Am J Physiol 251:H340-H348, 1986.

Paintal, A. A study of right and left atrial receptors. J Physiol 120:596-610, 1953.

Peterson, L., DeRouffignac, C., Sonnenberg, H., Levine, D. Thick ascending limb response to dDNAP and ANF in vivo. Am J Physiol 252:F374-F381, 1987.

Pollack, D., Arendshorst, W. Effect of atrial natriuretic factor on renal hemodynamics in the rat. Am J Physiol 251(5):F795-F801, 1986.

Porush, J., Cacciaguida, R., Pablo, N., Basilio, R., Kaloyanides, G., Salsman, K. Urinary concentrating operation in caval dogs. Am J Physiol 220(5):1547-1552, 1971.

Porush, J., Kaloyanides, G., Cacciaguida, R., Rosen, S. The effects of angiotension II on renal water and electrolyte excretion in normal and caval dogs. J Clin

Invest 46(12):2109-2122, 1967.

Priebe, H., Heimann, J., Hedley-Whyte, Effects of renal and hepatic venous congestion on renal function in the presence of low and normal cardiac output in dogs. Circ Res 47:883-890, 1980.

Rankin, A., Wilson, N., Ledsome, J. Influence of isoproterenol on plasma immunoreactive atrial natriuretic peptide and plasama vasopressin in the anesthetized rabbit. Pflugers Arch 408:124-128, 1987.

Reineck, H., Stein, J. Renal regulation of extracellular fluid volume. Brenner, B., Stein, J. (eds):Sodium and water homeostasis. Contemporary Issues in Nephrology. Churchill Livingstone, New York, pp. 24-50, 1978.

Richards, A., Ikram, H., Yandle, T., Nicholls, M., Webster, M., Espiner, E. Renal hemodynamic andhormonal effects of human alpha natriuretic peptide in healthy volunteers. Lancet I:545-549, 1985.

Richardson, A., Ikram, H., Yandle, T., Nicholls, M., Webster, M., Espiner, E.A. Renal, haemodynamic, and hormonal effects of human α-atrial natriuretic peptide in healthy volunteers. Lancet 1:545-548, 1985.

Riegger, A., Kromer, E., Kochsiek, K. Human atrial natriuretic peptide: plasma levels and hemodynamic, hormonal, and renal effects in patients with heart failure. In: Brenner, B., Laragh, J., Biologically Active Atrial Peptides. Vol 1:527-530, 1987

Ring-Larsen, H., Henriksen, J., Christensen, N. Increased sympathetic activity in cirrhosis. New Engl J Med 308:1029-1030, 1983.

Rosenbaum, J., Papper, S., Aschley, M. Variations in renal excretion of sodium independent of change in adrenal cortical hormone dosage in patients with Addison's disease. J Clin Endo Metab 15:1459-1474, 1955.

Roy, D. Effect of synthetic ANP on renal and loop of Henle functions in the young rat. Am J Physiol 251:F220-225, 1986.

Sagnella, G., Markandu, N., Shore, A., MacGregor, G. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. Lancet 2:1208-1210, 1985.

Sagnella, G., Markandu, N., Shore, A., MacGregor, G. Plasma immunoreactive atrial natriuretic peptide and changes in dietary sodium intake in man. Life Sci 40(2):139-143, 1986.

Sagnella, G.A., Markandu, N.D., Shore, A.C.,Forsling, M.L., MacGregor, G.A. Plasma atrial natriuretic peptide: its relationship to changes in sodium intake, plasma renin activity and aldosterone in man. Clin Sci 72:25-30, 1987.

Salazar, F., Fiksen-Olsen, M., Opgenorth, T., Granger, J., Burnett, J., Romero, J. Renal effects of ANP without changes in glomerular filtration rate and blood pressure.

Am J Physiol 251:F532-F536, 1986.

Salazar, F., Romero, J., Burnett, J., Schryver, S., Granger, J. Atrial natriuretic peptide levels during acute and chronic saline loading in conscious dogs. Am J Physiol 251:R499-R503, 1986.

Salerno, F., Badalamenti, S., Incerti, P., Capozza, L., Mainardi, L. Renal response to atrial natriuretic peptide in patients with advanced liver cirrhosis. Hepatology 8(1):21-26, 1988.

Samson, W. Atrial natriuretic factor inhibits dehydration and hemorrhage-induced vasopressin release. Neuroendocrinology 40:277-279, 1985.

Sawchenko, P., Friedman, M. Sensory functions of the liver - a review. Am J Physiol 236:R8-R20, 1979.

Schrier, R,. Humphreys, M. Factors involved in the antinatriuretic effects of acute constriction of the thoracic and abdominal inferior vena cava. Circ Res 24:479-489, 1971.

Sealey, J., Laragh, J. A proposed cybernetic system for sodium and potassium homeostasis: coordination of aldosterone and intrarenal physical factors. Kid Int 6:281-290, 1974.

Smith, J., Lincoln, T. Angiotensin decreases cyclic GMP accumulation produced by atrial natriuretic factor. Am J Physiol 253:C147-C150, 1987.

Schnermann, J., Marin-Grez, M., Briggs, J. Filtration

pressure response to infusion of atrial natriuretic peptide. Pflugers Arch 406(2):237-239, 1986.

Schroeder, E., Anderson, G., Goldman, S., Streeten, D. Effect of blockage of angiotensin II on blood pressure, renin and aldosterone in cirrhosis. Kidney Inter 9:511-519, 1976.

Shutz, H., Fater, D., Sundet, W., Geer, P., Goetz, K. Reflexes elicited by acute stretch of atrial vs pulmonary receptors in concious dogs. Am J Physiol 242:H1065-H1076, 1982.

Schwab, T., Edwards, B., Heublein, D., Burnett, J. Role of atrial natriuretic peptide in volume-expansion natriuresis. Am J Physiol 251:R310-R313, 1986.

Schwartz, D., Geller, D., Manning, P., Siegal, N., Fox, K., Smith, S., Needleman, P. Ser-leu-arg-arg-atriopeptin III: The major circulating form of atrial peptide. Science 229:397-400, 1985.

Seely, J., Levy, M. Control of extracellular fluid volume. In: Brenner, B., Rector, F., (eds.). The Kidney. W.B. Saunders Co., Philadelphia, 1:371-407, 1981.

Seldin, D. Sodium balance and fluid volume. In: Bricker, N.(ed): The Sea Within Us. Searle and Co., San Juan, Puerto Rico, pp. 4-13, 1975.

Seymour, A., Blaine, E., Mazack, E., Smith, S., Stabilito, I., Haley, A., Napier, M., Whinnery, M., Nutt, R. Renal and systemic effects of synthetic atrial natriuretic

factor. Life Sci 36:33-44, 1985.

Seymour, A.A., Marsh, E.A., Mazack, E.K., Stabilito, I.I., Blaine, E.H. Synthetic atrial natriuretic factor in conscious normotensive and hpertensive rats. Hypertension 7, Suppl. I:I-35-I-42, 1985.

Seymour, A., Smith, S., Mazack, E. Effects of renal perfusion pressure on the natriuresis induced by atrial natriuretic factor. Am J Physiol 253:F234-F238, 1987.

Seymour, A., Smith, S., Mazack, E., Blaine, E. A comparison of synthetic rat and human atrial natriuretic factor in conscious dogs. Hypertension 8:211-216, 1986.

Shasha, S., Better, O., Chaimovitz, C., Doman, J., Kishon, Y. Hemodynamic studies in dogs with chronic bile-duct ligation. Clin Sci Mol Med 50:533-537, 1976.

Shenker, Y., Sider, R., Ostafin, E. Grekin, R.J. Plasma levels of immunoreactive atrial natriuretic factor in healthy subjects and in patients with edema. J Clin Invest 76:1684-1687, 1985.

Shepherd, J. Modern medical physiology. Intrathoracic baroreflexes. Mayo Clin. Proc. 48:426-437, 1973.

Showalter, C., Zimmerman, R., Schwab, T., Edwards, B., Opgenorth, T., Burnett, J. Renal response to atrial natriuretic factor is modulated by intrarenal angiotensin II. Am J Physiol 254:R453-R456, 1988.

Slick, G., Aguilera, A., Zambraski, E., DiBona, G., Kaloyanides, G. Renal neuroadrenergic transmission. Am

J Physiol 229(1):60-65, 1975.

Slick, G., DiBona, G., Kaloyanides, G. Renal sympathetic nerve activity in sodium retention of acute caval constriction. Am J Physiol 226(4):925-932, 1974.

Solomon, L., Atherton, J., Bobinski, H., Green, R. Effect of posture on plasma immunoreactive atrial natriuretic peptide concentrations in man. Clin Sci 71:299-305, 1986.

Sonnenberg, H. Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Comm 124(2):443-449, 1984.

Sonnenberg, H., Cupples, W., De Bold, A., Veress, A. Intrarenal localization of the natriuretic effect of cardiac atrial extract. Can J Phys Pharm 60(9):1149-1152, 1982.

Sonnenberg, H., Honrath, U., Chang, C., Wilson, D. Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. Am J Physiol 250:F963-F966, 1986.

Sonnenberg, H., Veress, A.T. Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Com 124:443-449, 1984.

Sosa, R., Volpe, M., Marion, D., Atlas, S., Laragh, J., Vaughan, E., Maack, T. Relationships betweeen renal hemodynamic and natriuretic effects of atrial natriuretic factor. Am J Physiol 250:F520-F524, 1986.

Steigerwalt, S., Carretero, O., Beierwaltes, W. A stop-

flow study of intrarenal effects of atrial natriuretic factor. Proc Soc Exp Biol Med 182(1):88-94, 1986.

Stephenson, S., Kenny, A. The hydrolysis of α-human atrial natriuretic peptide by kidney microvillar membranes is initiated by endopeptidase-24.11. Biochem J 243:183-187, 1987.

Stokes, T., McConkey, C., Martin, K. Atriopeptin II increases cGMP in glomeruli but not in proximal tubules of dog kidney. Am J Physiol 250:F27-F31, 1986.

Suzuki, M., Almeida, F., Nussenzveig, D., Sawyer, D., Maack, T. Binding and functional effects of atrial natriuretic factor in isolated rat kideny. Am J Physiol 253(22):F917-F928, 1987.

Sweet, W., Freeman, R., Davis, J., Vari, R. Ganglionic blockade in conscious dogs with chronic caval constriction. Am J Physiol, 249:H1038-1044, 1985.

Synhorst, D., Gutkowska, J. Atrial distension of isolated rabbit hearts and release of atrial natriuretic factor. Am J Physiol 255:R232-R236, 1988.

Takezawa, K., Cowley, A., Skelton, M., Roman, R. Atriopeptin III alters renal medullary hemodynamics and the pressure-diuresis response in rat. Am J Physiol 252:F992-F1002, 1987.

Tanaka, I., Misono, K., Inagami, T. Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay. Biochem

Biophys Res Comm 124:663-668, 1984.

Tang, J., Fei, H., Xie, C., Suen, M., Han, J., Webber, R., Chang, D., Chang, J. Characterization and localization of atriopeptin in rat atrium. Peptides 5:1173-1177, 1984.

Thibault, G., Garcia R., Carrier, F., Seidah, N., Lazure, C., Chretien, M., Cantin, M., Genest, J. Strucutre-activity relationships of atrial natriuretic factor (ANF). I. Natriuretic activity and relaxation of intestinal smooth msucle. Biochem Biophys Res Commun 125:938-946, 1984.

Thibault G., Garcia, R., Gutkowska, J. Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. The propeptide $Asn^{1}Tyr^{-126}$ is the storage form of rat atrial natriuretic factor. Biochem J 241:265-272, 1987.

Thibault, G., Lazure, C., Schiffrin, E., Gutkowska, J., Chartier, L., Garcia, R., Seidah, N., Chretien, M., Genest, J., Cantin, M. Identification of a biologically active circulating form of rat atrial natriuretic factor. Biochem Biophys Res Comm 130:981-986, 1985.

Tikkanen, I., Metsarinne, K., Fyhrquist, F., Leidenius, R. Plasma atrial natriuretic peptide on cardiac disease and during infusion in healthy volunteers. Lancet. July 13: 66-69, 1985.

Tremblay, J., Gerzer, R., Vinay, P., Pang, S.C., Beliveau,

R., Hamet, P. The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. FEBS Lett 181:17-22, 1985.

Tsunoda, K., Mendelsohn, A., Sexton, P., Chai, S., Hodsman, G., Johnston, C. Decreased atrial natriuretic peptide binding in renal medulla in rats with chronic heart failure. Circ Res 62:155-161, 1988.

Unikowsky, B., Wexler, M., Levy, M. Dogs with experimental cirrhosis of the liver but without intrahepatic hypertension do not retain sodium or form ascites. J Clin Invest 72:1594-1604, 1983.

Vakil, N., Gutkowska, J., Abu-alfa, A., Mujais, S. Am J Hem No. 5031, p. 135A, 1988.

Verburg, K., Freeman, R., Davis, J., Villarreal, D., Vari, R. Control of atrial natriuretic factor release in conscious dogs. Am J Physiol 251:R947-R956, 1986.

Veress, A., Milojevic, S., Yip, C., Flynn, T., Sonnenberg, H. In vitro secretion of atrial natriuretic factor: receptor-mediated release of prohormone. Am J Physiol 254:R809-R814, 1988.

Veress, A., Sonnenberg, H. Right atrial appendectomy reduces the renal response to acute hypervolemia in the rat. Am J Physiol 247:R610-R613, 1984.

Villarreal, D., Freeman, R., Davis, J., Verburg, K., Vari, R. Atrial natriuretic factor secretion in dogs with experimental high-output heart failure. Am J Physiol

252:H692-H696, 1987.

Volpe, M., Odell, G., Kleinert, H., Muller, F., Camargo, M., Laragh, J., Maack, T., Vaughan, E., Atlas, S. Effect of atrial natriuretic factor on blood pressure, renin and aldosterone in Goldblatt hypertension. Hypertension 7(Supp I):43-48, 1985.

Waldman, S., Rapoport, R., Murad, F. J Biol Chem 259:14332, 1984.

Warren, J., Stead, E. Fluid dynamics in chronic congestive heart failure: an interpretation of the mechanisms producing edema, increased plasma volume and elevated venous pressure in certain patients with prolonged congestive heart failure. Arch Internal Med 73:138, 1944.

Watkins, L., Burton, J., Huber, E., Cant, J., Smith, F., Barger, A. The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. J Clin Invest 57:1606-1617, 1976.

Waugh, W. Angiotensin II: local renal effects of physiological increments in concentration. Can J Physiol Pharm 50:711-716, 1972.

Weaver, L. Cardiopulmonary sympathetic afferent influences on renal nerve activity. Am J Physiol 233(5):H592-H599, 1977.

Weil, J., Lang, R., Suttmann, H., Rampf, U., Bidling-Maier, F., Gerzer, R. Concomitant increase in plasma atrial

natriuretic peptide and cyclic GMP during volume loading. Klin Wochenschr 63:1265-1268, 1985.

Wernze, H., Speck, H., Muller, G. Studies on the activity of the renin-angiotensin-aldosterone system in patients with cirrhosis of the liver. Klin Wochenchr 56:389-397, 1978.

Weselcouch, E., Humphrey, W., Aiken, J. Effect of pulmonary and renal circulations on activtity of atrial natriuretic factor. Am J Physiol 249:R595-R602, 1985.

Wilkinson, S., Williams, R. Renin-angiotensin-aldosterone system in cirrhosis. Gut 21:545-554, 1980.

Wilson, M., Ninomiya, I., Franz, G., Judy, W. Hypothalamic stimulation and baroreceptor reflex interaction and renal nerve activity. Am J Physiol 221:1768-1773, 1971.

Winaver, J., Hoffmann, A., Burnett, J., Haramati, A. Hormonal determinants of sodium excretion in rats with experimental high-output heart failure. Am J Physiol 254:R776-R784, 1988.

Winquist, R., Faison, E., Waldman, S., Schwartz, K., Murad, F., Rapoport, R.M. Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. Proc Natl Acad Sci USA 81:5946-5950, 1985.

Wright, F., Briggs, J. Feedback control of glomerular blood flow, pressure, and filtration rate. Physiol Rev 59(4):958-1006, 1979.

Yamaji, T., Ishibashi, M., Takaku, F. Atrial natriuretic factor in human blood. J Clin Invest 76(4):1705-1709, 1985.

Yamanaka, M., Greenberg, B., Johnson, L., Seilhamer, J., Brewer, M., Friedeman, T., Miller, J., Atlas, S., Laragh, J., Lewicki, J., Fiddes, J. Cloning and sequence analysis of cDNS encoding for the rat atrial natriuretic factor precursor. Nature 309:719-722, 1984.

Yandle, T., Espiner, E., Nicholls, M., Duff, H. Radioimmunoassay and characterization of atrial natriuretic peptide in human plasma. J Clin Endo Metab 63:72-79, 1986.

Zambraski, E., Dunn, M. Importance of renal prostaglandins in control of renal function after chronic ligation of the common bile duct in dogs. J Lab Clin Med 103:549-559, 1984.

Zamir, N., Haas, M., Dave, J., Zukowska-Grojec, Z. Anterior pituitary gland modulates the release of ANP from cardiac atria. Proc Nat Acad Sci 84(2):541-545, 1987.

Zehr, J., Hasbargen, J., Kurz, K. Reflex suppressionof renin secretion during distension of cardiopulmonary receptors in dogs. Circ Res 38:232-239, 1976.

Zeidel, M., Seifter, J., Lear, S., Brenner, B., Silva, P. Atrial peptides inhibit oxygen consumption in kidney medullary collecting duct cells. Am J Physiol 251:F379-

F383, 1986

Zimmerman, R., Schirger, J., Edwards, B., Schwab, T., Heublein, D., Burnett, J. Cardio-renal-endocrine dynamics during stepwise infusion of physiologic and pharmacologic concentrations of atrial natriuretic factor in the dog. Cir Res 60:63-69, 1987.