RELEASE AND RENAL ACTIONS OF THE AVIAN ANTIDIURETIC HORMONE, ARGININE VASOTOCIN

by

John Nicholas Stallone

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PHYSIOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by John Nicholas Stallone

entitled Release and Renal Actions of the Avian Antidiuretic Hormone, Arginine Vasotocin

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Richard L. Stauffer	May 16, 1984 Date
William H. Dantzler	May 16, 1984 Date
Mac E. Nadler	May 16, 1984 Date
J. F. Burke	May 16, 1984 Date
W. A. Sawyer	May 16, 1984 Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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Eldon J. Braun	25 May 1984
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SIGNED: John Nicholas Stallone

To Adrienne Michelle, who renewed
my sense of hope and optimism
for the future.

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ABSTRACT

Recently developed radioimmunoassay (RIA) techniques were employed in a quantitative investigation of the release and renal actions of the avian antidiuretic hormone, arginine vasotocin (AVT) in the conscious domestic fowl. The investigation was composed of a series of three studies with goals to: 1) define the functional characteristics of the avian hypothalamo-neurohypophysial system (HNS) in terms of the extracellular osmotic and volemic stimuli that elicit release of AVT; 2) characterize responses of the HNS to the natural challenge of negative fluid balance; and 3) quantify the relative contributions of AVT-induced glomerular and tubular antidiuresis to the conservation of water by the avian kidney.

Characterization of AVT secretion revealed that plasma osmolality (P_{OSM}) is a primary determinant of AVT secretion by the HNS of the domestic fowl. Highly correlated and significant relationships between P_{OSM} and plasma AVT (P_{AVT}) exist both above and below the observed basal P_{OSM} of normally hydrated birds. Analysis of the present data also suggested that the HNS is insensitive to changes in blood volume of 10% or less, since neither isotonic expansion nor reduction of blood volume altered P_{AVT}.

The results of the fluid deprivation experiments indicate that AVT secretion is closely linked to the state of hydration

during negative fluid balance in the domestic fowl. Analysis of the data indicated that increases in P_{AVT} that occur with dehydration are mediated primarily by extracellular hyperosmolality and that the HNS is relatively insensitive to the simultaneous hypovolemia incurred with fluid deprivation.

Comparison of the dose-response relationships between P_{AVT} and glomerular and tubular mechanisms of antidiuresis over the entire range of physiological P_{AVT} levels in the domestic fowl revealed that tubular mechanisms are of primary importance and glomerular mechanisms of secondary importance in the conservation of water by the avian kidney. The greatest proportion of the total AVT-induced reduction in renal water excretion occurred at low physiological P_{AVT} levels and appeared to be the exclusive result of tubular mechanisms of antidiuresis. At high P_{AVT} levels, glomerular and tubular mechanisms overlapped and their effects on water conservation could not be separated; however, only minor additional amounts of water were conserved by the combined actions of glomerular and tubular mechanisms. Thus glomerular mechanisms appear to have only a minor, secondary effect on water conserving ability of the avian kidney.

CHAPTER 1

INTRODUCTION TO THE PROBLEM

As terrestrial homeothermic vertebrates, birds encounter problems in osmoregulation qualitatively and quantitatively similar to those of mammals. However, birds have developed several mechanisms important in the regulation of extracellular fluid osmolality and volume which are qualitatively and quantitatively different from those of mammals. The overall strategy for extracellular fluid osmoregulation in birds involves a coordinated effort between the kidney, cloaca/hindgut, and in those species which possess it, the nasal salt gland. Intimately related to this strategy is the excretion of uric acid as the major end product of nitrogen metabolism.

The role of the avian kidney in the regulation of extracellular volume appears to be regulated primarily by arginine vasotocin (AVT), the naturally occurring antidiuretic principle of the avian neurohypophysis. AVT is known to act on the kidney to produce antidiuresis and regulate water excretion by its actions to increase tubular permeability to water and decrease glomerular filtration rate (GFR). Although AVT has been identified as the avian antidiuretic hormone and its actions on the kidney partially characterized, very little is known about the physiological regulation of AVT secretion or the relative importance of tubular and glomerular mechanisms in the regulation of water excretion.

The early studies of Heller (1941) were the first to demonstrate clearly significant differences in the biological properties of posterior pituitary extracts of birds (and other non-mammalian vertebrates) as compared to like extracts of the mammalian posterior pituitary. Subsequent studies by a variety of investigators resulted in complete biological and chemical characterization of the avian antidiuretic principle, which included estimates of the hormone content of the neurohypophysis (DeLawder, Tarr, and Geiling 1954, Pickering and Heller 1959, Munsick, Sawyer, and van Dyke 1959, 1960). These pioneering studies were followed by extensive efforts during the 1960's to characterize the release and renal actions of AVT as the putative avian antidiuretic hormone. Several investigators demonstrated depletion of neurohypophysial content in response to osmotic stress (dehydration or salt loading); such depletion was often assumed to represent secretion of the antidiuretic principle. However, measurements of plasma levels of AVT and efforts to characterize AVT secretion were very rare, probably because of the difficulty involved in bioassay of AVT in plasma.

The dual mechanisms of action of antidiuretic principles in the avian kidney were reported as early as 1933 by Burgess and associates (Burgess, Harvey, and Marshall 1933) who observed tubular and glomerular antidiuresis in the domestic fowl in response to injections of Pitressin (an aqueous preparation of the mammalian antidiuretic principle, arginine vasopressin, AVP). In numerous subsequent studies, mammalian, and later, avian neurohypophysial preparations were used to investigate the mechanisms of antidiuresis

and regulation of GFR in the avian kidney. These studies demonstrated that administration of exogenous neurohypophysial principles mimicked the renal antidiuretic responses to dehydration or salt loading (increased tubular permeability and decreased GFR). The relative contributions of these two mechanisms to the overall antidiuretic responses of the avian kidney remained uncertain, however, because the observed reductions in GFR were highly variable. Much of this variability in glomerular antidiuresis was probably due to differences in experimental design, particularly the dosages and routes of administration of hormone preparations. Additional possible causes of the observed variability include the use of anesthetics and the administration of intravenous infusions (saline or mannitol), as well as real interspecific differences among the species studied (most often the domestic fowl, duck, or quail).

Considerations of Experimental Design and Techniques

Previous studies designed to characterize the release and renal actions of AVT in the avian osmoregulatory system were of limited success because of the inability to measure plasma AVT levels. The use of recently developed radioimmunoassay (RIA) techniques facilitates the measurement of plasma AVT concentrations and permits the use of experimental designs more appropriate for characterization of AVT secretion and definition of tubular and glomerular mechanisms of antidiuretic response.

Characterization of AVT Secretion

The use of RIA techniques for measurements of plasma AVT concentration avoids the necessity of large blood samples for bioassay of AVT. The ability to measure AVT in small samples permits frequent blood sampling (without significant alteration of fluid balance of experimental animals) and therefore, accurate assessment of AVT secretion in response to experimental manipulations. Thus, experiments can be designed to characterize AVT secretion in conscious animals in response to acute individual osmoregulatory stimuli, and in response to prolonged complex stimuli such as dehydration.

Definition of Tubular and Glomerular Antidiuretic Responses

The accurate characterization of AVT secretion in response to osmoregulatory stimuli will permit precise assessment of the relative contributions of tubular and glomerular mechanisms to the antidiuretic response. Previous experiments commonly employed pulse injections of AVT; however, this technique resulted in continuously declining (often initially pharmacological) plasma levels of AVT which were associated with transient renal responses and therefore, inaccurate assessments of tubular and glomerular contributions to antidiuresis. The use of constant infusion techniques to maintain stable, physiological plasma levels of AVT will avoid the problems common to previous studies and allow accurate assessment of renal responses under steady state conditions.

Statement of the Hypothesis

The secretion of AVT from the avian neurohypophysis is precisely regulated by a negative feedback control system which is composed of sensors which monitor extracellular osmolality and volume and stimulate secretion in response to extracellular hyperosmolality or hypovolemia. Plasma-borne AVT acts on the effector (the kidney) to stimulate reabsorption of water and restore extracellular osmolality and/or volume to normal, which inhibits AVT secretion to complete the negative feedback loop. The magnitude of AVT secretion (and resultant plasma concentration) is directly proportional to the magnitude of extracellular hyperosmolality and/or hypovolemia. The antidiuretic action of AVT on the avian kidney is the result of AVT-stimulated increases in tubule water reabsorption and reductions in glomerular filtration rate. The tubular mechanism is of primary importance in the reabsorption of water; however, renal water conservation is enhanced substantially through the reduction of glomerular filtration rate.

CHAPTER 2

REVIEW OF THE LITERATURE

The regulation of AVT secretion and the actions of this hormone on the avian renal osmoregulatory system are the subjects of this dissertation research. Prior to the review of these subjects, basic descriptions of avian renal anatomy and physiology will be presented as essential background information.

Avian Renal Anatomy and Physiology

Avian Renal Anatomy

Structurally and functionally, the avian kidney represents a transition in vertebrate evolution between non-mammalian terrestrial vertebrates and mammals. In external morphology, it most closely resembles the kidneys of chelonian and saurian reptiles. However, unlike these kidneys, the avian kidney typically consists of three divisions: A large posterior division, a small middle division, and an intermediately sized anterior division, each with its own arterial blood supply (Johnson 1968). The renal artery, branching directly from the aorta, supplies the anterior division, while branches of the ischiatic artery supply the middle and posterior divisions (Akester 1967, Siller and Hindle 1969). The kidney is also supplied with afferent venous blood by a renal portal system (Sperber 1948). Both

arterial and venous blood supplies are conducted from the kidney by a common efferent renal vein.

The internal morphology of the avian kidney is truly transitional in its possession of amphibian and reptilian characteristics in the cortical region and mammalian characteristics in the medullary region. The cortex is composed of cylindrical units (lobules) which form radiating patterns over the entire surface of the kidney. Each lobule consists of a central efferent vein, about the long axis of which, radiate simple nephrons without loops of Henle which empty at right angles into collecting ducts (Fig. 1; Braun and Dantzler 1972). These simple, superficial cortical nephrons are referred to as reptilian-type (RT) nephrons because of their resemblence to those found in reptilian kidneys (Huber 1917, Siller 1971). Deep to the central points where groups of radiating lobules meet, are found the large, more complex nephrons that possess loops of Henle. Because these nephrons closely resemble those found in mammalian kidneys, they are referred to as mammalian-type (MT) nephrons (Huber 1917, Siller 1971). The glomeruli of the RT nephrons are much smaller than those of the MT nephrons, and tend to decrease in size from the proximal to the more distal regions of the intralobular arteries from which the glomeruli radiate (Braun 1980). The avian renal medulla is composed of the loops of Henle, vasa recta (from MT nephron efferent arterioles), and collecting ducts of both RT and MT nephrons, all of which are arranged in parallel to form connective tissue-sheathed medullary cones (Poulson 1965, Braun and Dantzler 1972). The tip of each medullary cone is formed by one large collecting duct which

Figure 1. A three-dimensional drawing of a section of the avian kidney that shows the types of nephrons present, their relative positions in the kidney, and their relationships to other renal structures (from Braun and Dantzler, 1972).

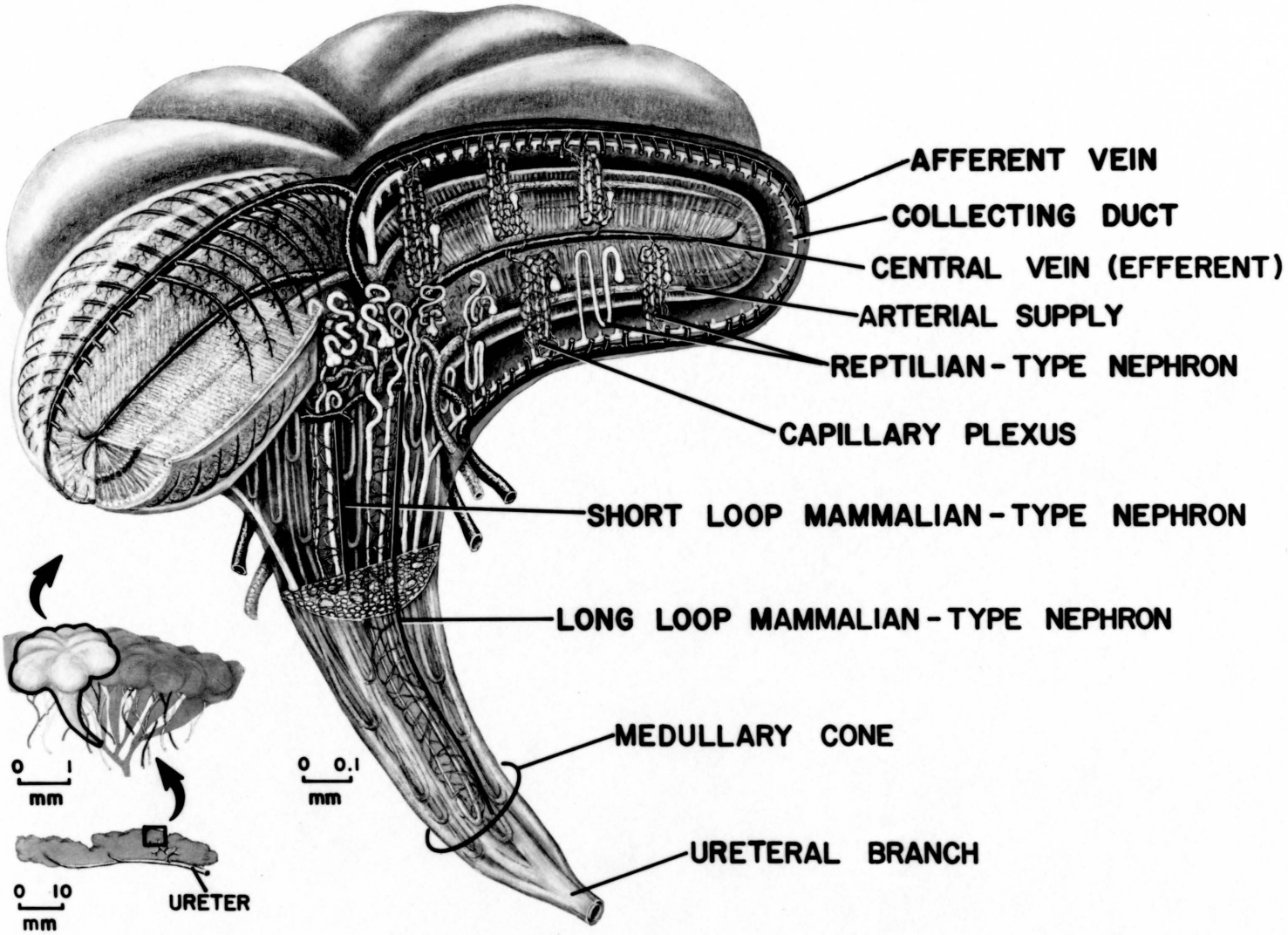
AFFERENT VEIN
COLLECTING DUCT
CENTRAL VEIN (EFFERENT)
ARTERIAL SUPPLY
REPTILIAN-TYPE NEPHRON
CAPILLARY PLEXUS
SHORT LOOP MAMMALIAN-TYPE NEPHRON
LONG LOOP MAMMALIAN-TYPE NEPHRON
MEDULLARY CONE
URETERAL BRANCH
0 1 mm
0 0.1 mm
0 10 mm
URETER

Figure 1.

terminates as a branch of the ureter. The afferent venous blood supply of the avian kidney (the renal portal system) perfuses the peritubular spaces of the RT and MT nephrons (exclusive of the medullary cones) in combination with blood from the efferent arterioles (Wideman, Braun, and Anderson 1981). Thus, the peritubular surfaces of the nephrons are bathed in a mixture of arterial and venous blood.

Avian Renal Function

The ability of the avian kidney to produce urine hypertonic to the plasma appears to depend on the anatomical arrangement of the medullary cones, which permits the operation of a countercurrent multiplier system, similar to that of the mammalian kidney (Skadhauge and Schmidt-Nielsen 1967a, Emery, Poulson, and Kinter 1972). However, there are several interesting differences in the operation of the system in birds as compared to mammals. In the avian kidney, the interstitial concentration gradient (present in the medullary cones) is composed primarily of sodium chloride and does not contain urea (Skadhauge and Schmidt-Nielsen 1967a). The maximum concentrating ability is modest by mammalian standards, usually achieving urine osmolalities 2-3 times that of plasma (Skadhauge 1973, 1974, 1981). Furthermore, concentrating ability appears to be directly proportional to the relative amount of medullary tissue and/or the number of medullary cones (Johnson and Muggas 1970, Johnson and Ohmart 1973a, 1973b, Skadhauge 1976), rather than the length of the individual cones (Johnson 1974). These correlations appear to reflect the importance

of numbers of MT nephrons present rather than length of the counter-current multiplier, as in the mammalian kidney.

A particularly interesting aspect of water conservation by the avian kidney concerns the changes in glomerular function that occur with changes in state of hydration. As early as 1938 in the classical studies of Pitts (1938) and Shannon (1938) on the domestic fowl, total kidney GFR was consistently correlated with urine flow rate. Korr (1939) was probably the first investigator to demonstrate that GFR in the domestic fowl varied inversely with hydration, based on measurements made in normally hydrated, dehydrated, and water-loaded birds. In later studies on the domestic fowl (Dantzler 1966, Skadhauge and Schmidt-Nielsen 1967b) and several other species, including the budgerigar (Krag and Skadhauge 1972), Pekin duck (Holmes, Fletcher, and Stewart 1968), and Gambel's quail (Braun 1976), investigators confirmed these initial observations and demonstrated that GFR was reduced in response to osmotic stress (dehydration or salt loading) and/or augmented in response to hydration. In these studies, GFR decreased proportionately as dehydration increased, although the exact mechanism of this glomerular antidiuresis was unclear. In more recent studies it was demonstrated that dehydration-induced reductions in GFR are the result of vascular antidiuresis, due primarily to reductions in the numbers of filtering RT nephrons (Braun and Dantzler 1972, 1974, 1975). Although changes in single nephron glomerular filtration rate (SNGFR) do occur, the on-off shutdown of RT nephrons is now considered to be the primary mechanism in the regulation of urine osmolality and renal water

excretion by the avian kidney (Dantzler 1978, 1980a, 1980b, Dantzler and Braun 1980). The shutdown of RT nephrons appears to conserve water at the expense of ionic and nitrogenous waste excretion, and enhances function of the renal medullary concentrating mechanism by reduction of volume flow through the collecting ducts.

Hormonal Regulation of Renal Water Excretion

The excretion of water by the avian kidney is regulated primarily by the hormone arginine vasotocin (AVT) which was established as the naturally occurring antidiuretic principle of birds by Munsick and associates in the early 1960's (Munsick et al. 1960, Munsick 1964). AVT appears to regulate renal water excretion by its actions on tubule water permeability and glomerular filtration rate. The classical action of antidiuretic hormone (ADH) to increase water permeability of the collecting duct was demonstrated in the domestic fowl by Skadhauge (1963, 1964), although direct measurement of AVT action on tubule permeability has yet to be accomplished. More recent studies suggest that AVT produces glomerular antidiuresis by its action on intrarenal vascular smooth muscle. This action of AVT has been described in domestic fowl (Ames, Steven, and Skadhauge 1971) and Gambel's quail (Braun and Dantzler 1974); in both studies, total kidney GFR was reduced in response to pulse (intravenous) injections of AVT. Subsequent studies by Braun (1976) strongly suggested that the reductions in GFR were the result of reduced numbers of filtering RT nephrons (i.e., vascular-glomerular antidiuresis) due to AVT-induced vasoconstriction of the afferent arterioles. Although AVT has been identified as the avian ADH and

its actions on the avian kidney partially characterized, very little is known about the physiological regulation of AVT secretion or the relative contributions of glomerular and tubular mechanisms to AVT-induced antidiuresis. The regulation of AVT secretion and its role in avian osmoregulation will be reviewed presently.

The Role of AVT in Avian Osmoregulation

Arginine vasotocin is one of ten neurohypophysial peptides identified in vertebrates (for reviews, see Sawyer 1977, Bentley 1980, Pang, Furspan, and Sawyer 1983). These principles are all octapeptides with molecular weights of approximately 1,000 and a molecular structure that consists of a five amino acid ring stabilized by a disulfide bridge with cystine and a three amino acid side chain (Table 1). The ten peptides are divided into two groups: The vasopressor-antidiuretic peptides with basic amino acids in the 8 position (arginine vasopressin, lysine vasopressin, arginine vasotocin, and phenypressin) and the neutral (oxytocin-like) peptides (oxytocin, mesotocin, isotocin, glumitocin, valitocin, and aspartocin). The neutral group exhibits considerably more evolutionary variation, but all six peptides have rather similar chemical and pharmacological properties (Sawyer 1977).

The typical vertebrate neurohypophysis contains two active principles; a basic peptide involved with osmoregulation, and a neutral peptide involved with a variety of as yet ill-defined functions. Arginine vasotocin is the most ubiquitous of the ten peptides, and has been chemically and/or pharmacologically identified

TABLE 1. The chemical structure and distribution of neurohypophysial peptides in vertebrates.

Common Structure: Cys-(2)-(3)-(4)-Asn-Cys-Pro-(8)-Gly(NH_2)

Peptide	Variable Amino Acids in Positions 2	3	4	8	Distribution
Vasopressor Group					
Arginine Vasopressin	Tyr	Phe	Gln	Arg	Most mammals
Lysine Vasopressin	Tyr	Phe	Gln	Lys	Pigs and some marsupials
Phenypressin	Phe	Phe	Gln	Arg	Some marsupials
Arginine Vasotocin*	Tyr	Ile	Gln	Arg	Possibly all vertebrates
Neutral Group					
Aspartocin	Tyr	Ile	Asn	Leu	Sharks
Glumitocin	Tyr	Ile	Ser	Leu	Rays
Isotocin	Tyr	Ile	Ser	Ile	Actinopterygians
Mesotocin	Tyr	Ile	Gln	Ile	Lungfish, amphibians, reptiles, birds, and marsupials
Oxytocin	Tyr	Ile	Gln	Leu	Mammals and holocephalens
Valitocin	Tyr	Ile	Gln	Val	Sharks

*Note the hybrid nature of arginine vasotocin (AVT), which is composed of the ring component of oxytocin and tail component of arginine vasopressin.

in every major vertebrate group (Sawyer 1977, Bentley 1980, Pang et al. 1983). Because of its common occurrence among the vertebrates, Sawyer (1977) has suggested that AVT is probably the ancestral neurohypophysial peptide.

The Avian Hypothalamo-Neurohypophysial System

The anatomical organization of the avian hypothalamo-neurohypophysial system is similar to that of reptilian and mammalian systems (Valtin, Stewart, and Sokol 1974). The magnocellular neurosecretory neurons of the hypothalamus are distributed in cluster-like aggregations to form the primary neurosecretory nuclei of the anterior hypothalamus (paraventricular and supraoptic nuclei); additional minor aggregations occur in scattered distributions in the anterior, lateral, and ventral hypothalamus. This generalized pattern has been described for a number of avian species, including the collared dove, Pekin duck, zebra finch, domestic fowl, pigeon, and Japanese quail, and has been summarized and reviewed recently (Oksche and Farner 1974, Goosens et al. 1977, Oksche and Hartwig 1980). The neurosecretory axons from the primary nuclei project in two major bundles to form neurohemal terminations in the median eminence and neurohypophysis. Recent studies have employed immunocytochemical techniques to demonstrate that avian neurohypophysial hormones (AVT and mesotocin) are synthesized in separate neurons (Goosens et al. 1977, Bons 1980), in confirmation of the "one neuron-one hormone" hypothesis of Valtin, Stewart, and Sokol (1974). After synthesis, AVT and mesotocin are transported down the neurosecretory axons in association with carrier

proteins (similar to the mammalian neurophysins; Peek and Watkins 1979) for storage in the median eminence and neurohypophysis. Hormone release from the neurohypophysis occurs primarily in response to osmoregulatory stimuli. Release from the median eminence appears to be related to photoperiod and gonadotropin secretion (George 1980), although the target organ(s) and actions of the median eminence secretions remain unknown.

Identification of AVT in the Avian Brain

P.T. Herring (1908, 1913) was the first to study extracts of non-mammalian vertebrate pituitaries; he observed that posterior pituitary extracts of birds (domestic fowl) and bony fishes injected intravenously into cats produced responses (increased blood pressure, kidney volume, and "urine secretion") very similar to those produced by posterior pituitary extracts of mammals. It was not until 1941 that Heller demonstrated clearly significant differences in the biological properties of mammalian posterior pituitary extracts as compared to like extracts of teleost fish, amphibians, reptiles, and birds (Heller 1941). In these experiments, acid extracts from the posterior pituitaries of codfish or pigeons were injected into frogs and produced positive water balance responses (increased uptake of water) of the same magnitude as did extracts from frog posterior pituitaries. In comparison, injections of mammalian pituitary extracts produced only slight water balance responses (less than 1% of frog extracts). Based on these experiments, Heller concluded that the posterior lobe of non-mammalian vertebrates is the site of formation

and/or storage of the "amphibian water balance principle", and that this compound is chemically different from either known posterior pituitary principle of mammals (i.e., arginine vasopressin and oxytocin). Following these early studies, investigators sought to identify the posterior pituitary principles of all classes of vertebrates. For birds, DeLawder, Tarr, and Geiling (1954) were the first to report distinct antidiuretic and oxytocic activities (by bioassay) in the acid extracts of the domestic fowl neurohypophysis. Pickering and Heller (1959) and Munsick et al. (1959) provided the first independently confirmed identification of two active principles in the avian neurohypophysis, based on chemical (chromatographic) and pharmacological (bioassay) data. At that time, Sawyer and associates had proposed that AVT (previously synthesized by Katsoyannis and duVigneaud (1958) as a chemical analog of oxytocin) was, in fact, the natural principle of the non-mammalian vertebrate neurohypophysis, based on the similarity of its bioassay properties with those of the amphibian "water balance principle" (Sawyer, Munsick, and van Dyke 1959). Using a variety of chemical and pharmacological means, Munsick et al. (1960) provided the first conclusive identification of AVT as the active principle of the chicken neurohypophysis. They reported that AVT occurred in combination with oxytocin (or a related compound) in a ratio of approximately 10:1 (AVT:oxytocin). This tentative identification received immediate chemical and pharmacological confirmation from the laboratories of Chauvet, Lenci, and Acher (1960), Heller and Pickering (1961), and Munsick (1964). An important concept that arose from these bioassay studies is that target tissues

show the greatest affinities (and sensitivities) for their endogenous neurohypophysial principles. Although similar peptides will elicit the same tissue response (e.g., AVP vs AVT in the frog water balance assay), the endogenous peptide (AVT) will exhibit substantially greater potency. More recent pharmacological and chemical evidence indicates that AVT is present in a wide phyletic distribution among birds, and that the neutral oxytocin-like principle that occurs with AVT is mesotocin (Sawyer 1968, Acher, Chauvet, and Chauvet 1970).

Physiological Actions of AVT in Birds

Neurohypophysial hormones exert a variety of actions in birds and other vertebrates; these actions can be classified into three major groups, based on the target or effector tissues involved (Heller 1969): Epithelial membranes, vascular and oviducal smooth muscle, and (in mammals) mammary myoepithelium. In birds, AVT and other neurohypophysial peptides have been shown to influence the function of several epithelial and smooth muscle tissues and metabolic pathways including: Renal tubular and vascular tissues (water and electrolyte excretion), nasal salt gland (electrolyte excretion), oviducal smooth muscle (oviposition), metabolic pathways (plasma glucose and fatty acids), and vascular smooth muscle (systemic blood pressure). Apart from the actions of AVT on primary target tissues of the kidney, the physiological relevance of its actions on other target tissues remains uncertain. The data regarding these secondary target tissues will be reviewed and summarized briefly.

Nasal Salt Gland. The nasal salt gland of birds functions as an extrarenal avenue for salt excretion and can secrete hypertonic sodium chloride solutions in response to the administration of sodium chloride loads (Schmidt-Nielsen 1960, Schmidt-Nielsen et al. 1963). Although secretion is mediated primarily by parasympathetic innervation to the gland, several investigators have studied the effects of neurohypophysial peptides and other osmoregulatory hormones on salt gland function. Initial studies which utilized mammalian neurohypophysial preparations yielded conflicting data; AVP injection was reported to reduce secretion rate and sodium concentration of secreted fluid in salt-loaded ducks and geese (Hughes 1962, Gill and Burford 1969, cited by Harvey and Phillips 1982), whereas oxytocin injection was reported to initiate a transient secretion in the water-loaded duck (Holmes and Adams 1963). In a later study that utilized AVT, intravenous and intra-carotid injections were observed to prolong secretion and reduce sodium concentration of secreted fluid in salt-loaded geese, and initiate low level secretion in the absence of salt loads (Peaker 1971). Although the observed effects on salt gland secretion are elicited by AVT at much lower doses than either AVP or oxytocin, the doses of AVT required (10-40 μg i.v.) are pharmacological in nature. In addition, salt gland function in ducks is not altered in response to neurohypophysectomy, despite the appearance of diabetes insipidus in such birds (Wright et al. 1967, Bradley, Holmes, and Wright 1971, cited by Harvey and Phillips 1982). From these experiments, it has been concluded that AVT may have a minor permissive role in the regulation of salt gland secretion possibly

through modulation of primary nervous control (Peaker 1979).

Oviducal Smooth Muscle. Several studies have implicated AVT in the regulation of avian oviposition; however the available data regarding a possible ecbolic function of AVT are equivocal. In early studies, injections of mammalian neurohypophysial preparations were shown to accelerate oviposition in domestic fowl, doves and pigeons (Riddle 1921, Burrows and Byerly 1942, Burrows and Fraps 1942). In later studies, workers sought to establish a relationship between endogenous avian peptides and oviposition. Pituitary levels of AVT activity (in bioassay) were observed to decline significantly at oviposition (Tanaka and Nakajo 1962). Subsequently, several investigators reported very large transient increases (40-130x) in plasma AVT activity (in bioassay) during oviposition in domestic fowl (Douglas and Sturkie 1964, Sturkie and Lin 1966, Niezgoda, Rzasa, and Ewy 1973). In each of these studies, the ovipositional surge of plasma AVT activity was preceeded by a slight increase in AVT activity (5-10x) at 5-10 minutes before ovulation. The ovipositional surge was followed by a precipitous reduction in plasma AVT activity to pre-ovulatory levels (5-10x basal level) within 10 minutes. The possible ecbolic role of AVT suggested by these studies is uncertain however, since neurohypophysectomy does not alter normal oviposition in domestic fowl (Shirley and Nalbandov 1956, Opel 1965). Although release of AVT from other hypothalamic sources (such as the median eminence) was not excluded in these studies, the appearance of diabetes insipidus in neurohypophysectomized birds (Shirley and Nalbandov 1956) strongly suggests the absence of circulating AVT.

These conflicting data suggest that AVT is not responsible for oviposition in birds. The increases in plasma AVT activity observed during oviposition may instead reflect a response to oviposition.

Metabolic Pathways. Hyperglycemic and hyperlipemic actions of neurohypophysial peptides in birds have been reported by several investigators. Intravenous injections of oxytocin (20-80 mU/kg) in fasted domestic fowl resulted in transient, dose-dependent increases in plasma glucose and fatty acid levels that peaked 10-20 minutes after injection and returned to normal by 60-90 minutes (Kook, Cho, and Yun 1964). The metabolic effects of oxytocin injection were accompanied by transient hypotension at the higher doses. Similar results were reported by Bentley (1966) for fasted domestic fowl injected intravenously with AVT or oxytocin (0.1 nmole/kg); plasma glucose levels increased by a maximum of 13% or 18% in response to oxytocin or AVT, respectively, and the hyperglycemic action of AVT persisted for a longer period of time than that of oxytocin. Neither the underlying metabolic mechanisms of these actions or their physiological significance are understood. Since the doses of AVT used in these studies are pharmacological, the resultant metabolic actions are probably not physiologically relevant.

Vascular Smooth Muscle. The vascular actions of neurohypophysial principles were the first effects of these peptides to be studied in birds. Paton and Watson (1912) were the first to report that extracts of the mammalian neurohypophysis produced vasodepression in anesthetized ducks with intact or sectioned vagi. The reduction of arterial blood pressure was accompanied by arteriolar dilation and an

increase in cardiac contractility. Similar responses were observed by Hogben and Schlapp (1924) in anesthetized domestic fowl and ducks, as part of the first comparative study of vasomotor actions of mammalian neurohypophysial extracts in mammals, birds, reptiles, and amphibians. A subsequent complimentary study established that pituitary extracts from all classes of non-mammalian vertebrates produce the same qualitative effects (mammalian oxytocic and avian vasodepressor activities) as do mammalian pituitary extracts (Hogben and deBeer 1925). Using separated active mammalian principles (arginine vasopressin and oxytocin) Gaddum (1928) demonstrated that avian vasodepressor responses to mammalian pituitary extracts were principally due to the oxytocin present in such extracts. This characteristic response was used to develop a standard bioassay for oxytocin (Coon 1939).

The vasodepressor response observed in birds is the result of peripheral vasodilation, first observed *in vivo* by Paton and Watson (1912) and later studied in detail by Woolley and Waring (1958) using the isolated perfused leg of the domestic fowl. This response is the exact opposite of that observed in mammals: Vasopressin elicits a vasopressor response due to peripheral vasoconstriction, whereas oxytocin has little or no effect (Nakano 1974). The endogenous avian peptide (AVT) also elicits a vasodepressor response, but at only 60% the efficacy of oxytocin in the avian vasodepressor bioassay (Munsick *et al.* 1960). This is most likely the result of the "hybrid" molecular structure of AVT (i.e., the ring structure of oxytocin and tail structure of vasopressin). The vasodepressor actions of oxytocin

and vasopressin were studied in avian species as phylogenetically diverse as the cormorant, domestic fowl, duck, emu, penguin, and pigeon (Strahan and Waring 1954, Waring, Morris, and Stephens 1956, Woolley 1959, Boissonnas et al. 1961, Holmes and Adams 1963, Chan 1977). Oxytocin uniformly elicited vasodepression in all species studied, whereas vasopressin elicited vasodepressor or biphasic vasopressor and vasodepressor responses in highly variable species-specific patterns. The physiological significance of avian vascular responses to neurohypophysial peptides remains questionable (Bentley 1974). The vasodepressor responses to AVT could enhance antidiuresis through hypotensive reduction of GFR; however, the ability of AVT to elicit vasodepression at physiological plasma levels remains unknown.

Metabolism of AVT

The metabolism of AVT has been studied only briefly, and the available data are limited to measurements of plasma hormone half-life and clearance rate. Hasan and Heller (1968) and Heller and Hasan (1968) studied AVT metabolism in cockerels, laying hens, and non-laying hens of the domestic fowl. In these experiments, intravenous injection of a pharmacological dose AVT (5 U/kg) resulted in a two-component exponential decay curve for P_{AVT} concentration that consisted of a rapid initial phase (attributed to hormone mixing) and a slower secondary phase. The decay curves were similar among the three experimental groups and yielded half-lives of 18-24 minutes for the secondary phase, and 12-14 minutes for the overall straight line

relationship between P_{AVT} and time. These data were re-analyzed by Lauson (1974) who calculated an initial phase half-life of 7-8 minutes, and suggested that the actual plasma half-life of AVT is "considerably shorter" than 15-20 minutes when P_{AVT} concentration is in the physiological range. Using the constant infusion method, Simon and associates derived plasma clearance rates of 25.7 ml/min • kg (Mohring et al. 1980) and 25.1 ml/min • kg (Gray and Simon 1983) in the conscious Pekin duck. Based on the earlier data of Mohring et al. (1980), the plasma half-life of AVT in the Pekin duck was estimated to be approximately 7 minutes (Simon-Oppermann and Simon 1982).

The enzymatic degradation of AVT in birds has not been studied. However, the mechanisms for inactivation and degradation of neurohypophysial peptides in mammals have been studied extensively; these data have been reviewed and summarized by Lauson (1974). There appear to be two primary mechanisms for hormone inactivation and both involve covalent bond cleavage: a) the initial reductive breakage of the disulfide bridge and subsequent non-specific aminopeptidase scission of peptide bonds, and b) cleavage of the bond between peptide residues 8 and 9 in the tripeptide "tail" sequence of the molecule. These inactivation mechanisms appear to be located in the tissues of the kidney, liver, and other organs.

In mammals, biologically active neurohypophysial peptides are probably cleared from the plasma by several simultaneous mechanisms, including renal excretion and tissue inactivation and degradation. Qualitatively similar mechanisms are likely involved in the clearance of AVT from the plasma of birds.

Confirmation of AVT as the Avian Antidiuretic Hormone

The pioneering work of Chauvet, Heller, Munsick, Sawyer, and their colleagues that led to the identification of AVT as the amphibian water balance principle and the major neurohypophysial principle of the avian neurohypophysis, was followed by extensive efforts to characterize the release and renal actions of AVT as the putative avian antidiuretic hormone. A variety of techniques have been employed to establish AVT as the major principle of avian water balance, including: histochemistry, ablation-replacement therapy, administration of exogenous AVT, and most recently, radioimmunoassay measurements of plasma levels of AVT in response to changes in state of hydration.

Histochemistry. Numerous early studies utilized histochemical staining and/or bioassay of neurohypophysial and hypothalamic (median eminence) tissues of birds to determine the responsiveness of the hypothalamo-neurohypophysial system to alterations in state of hydration. Depletion of neurohypophysial neurosecretory material (NSM) in response to dehydration was first observed in the domestic fowl (Legait and Legait 1955) and white-crowned sparrow (Oksche et al. 1959). In subsequent studies which utilized aldehyde-fuchsin staining (specific for NSM) and tissue bioassay techniques, similar responses were observed in the pigeon (Ishii, Hirano, and Kobayashi 1962), zebra finch (Oksche et al. 1963), domestic fowl (Graber and Nalbandov 1965), Japanese quail (Follett and Farner 1966) and silver-bill finches (Kripalani, Ghosh, and Rahman 1967). In these studies, dehydration or saline loading resulted in significant but variable levels of

depletion of stainable and/or bioassay active NSM from the neurohypophysis and to a lesser degree, from the median eminence. Oksche and associates observed substantial activation of the neurosecretory nuclei of the white-crowned sparrow (Oksche et al. 1959) and the zebra finch (Oksche et al. 1963), as evidenced by the increased size and granularity of the neurosecretory cells. An exception to the consistent response demonstrated in these studies was reported by Uemura (1964), who observed no significant changes in the amount of stainable NSM or in the size of neurosecretory cells in parakeets after chronic dehydration. However, the maintenance of a constant body mass by these birds suggests that the dehydration regime utilized may not have been of sufficient magnitude to activate the neurosecretory system and/or deplete NSM. More recently Blahser and Simon (1978) used sophisticated immunocytochemical techniques to study cytological responses of vasotocinergic and mesotocinergic neurosecretory neurons of the Pekin duck to osmotic stress (hypertonic saline drinking solutions). They observed substantial increases in the number and size (accumulation of NSM) of vasotocin-positive stained neurons, whereas mesotocin-positive stained neurons increased in number only.

Ablation-Replacement Therapy. Several investigators sought to demonstrate the importance of neurohypophysial peptides in avian water balance through removal of the neurohypophysis or lesioning of the hypothalamic neurosecretory nuclei. Neurohypophysectomy performed on the domestic fowl (Shirley and Nalbandov 1956) and Pekin duck (Bradley et al. 1971) resulted in immediate polydipsia (2-3x control) and

polyuria. In addition, osmolar and sodium excretion rates increased by 50% in the Pekin duck. Intramuscular injection of Pitressin, AVP, or AVT produced dose-dependent antidiuretic effects which reversed the polydipsia and polyuria of both species, and restored osmolar and sodium excretion rates to normal in the Pekin duck. The specificity of avian receptors for AVT was reflected by the ten-fold greater potency of AVT in the antidiuretic response (decreased urine volume, increased urine osmolality) as compared to AVP. Braun (1975) performed neurohypophysectomy on desert quail and observed a significant increase in the percentage of filtering RT nephrons (71% to 100%) concomitant with a significant reduction in SNGFR of both RT and MT nephrons. These data provide evidence, at a more mechanistic level, for the importance of AVT in the regulation of water excretion by the avian kidney. These data also suggest that at basal plasma levels, AVT acts by vascular means to regulate RT nephron function and presumably, water excretion.

Similar although more variable data were obtained from hypothalamic lesion experiments. Ralph (1960) electrically lesioned the anterior hypothalamus of domestic fowl, in or near the supraoptic nucleus, and observed variable degrees of polydipsia which were temporarily reversible with intramuscular injection of Pitressin. Similar results were obtained by Koike and Lepkovsky (1967) after hypothalamic lesioning in domestic fowl; polyuria (markedly hypo-osmotic in nature) and in some instances polydipsia were produced, although plasma osmolality and sodium concentration remained normal. Bioassay of posterior pituitary antidiuretic

activity revealed drastic reduction of neurohypophysial peptide production (0.5% of normal), as a result of the lesions. Lepkovsky and Yasuda (1967) also lesioned the anterior hypothalamus in domestic fowl, but produced adipsic birds with no other symptoms. These lesions probably damaged or destroyed different hypothalamic nuclei, not directly involved with production and/or secretion of AVT.

Administration of Exogenous AVT. In numerous studies, exogenous neurohypophysial principles have been administered to birds to characterize their effects on renal function, and more recently, to determine the underlying mechanism(s) of action by which AVT alters renal function. These studies have demonstrated that administration of exogenous neurohypophysial principles mimics the antidiuretic responses to osmotic stress (decreased urine volume and increased urine concentration) observed in several species of birds, including the budgerigar (Krag and Skadhauge 1972), domestic fowl (Korr 1939, Dantzler 1966, Dicker and Haslam 1966, Skadhauge and Schmidt-Nielsen 1967b), Pekin duck (Holmes et al. 1968), and Gambel's quail (Braun 1976). Prior to the identification of AVT in birds, Burgess and his colleagues described the antidiuretic effects of Pitressin in conscious domestic fowl (Burgess et al. 1933); intramuscular injections (0.1-1.0 U/kg) resulted in reductions in urine flow rate (50-90%) and GFR (10-50%). Korr (1939) reported increased urine concentration (from 85 to 465 mosm/l) and decreased urine flow rate (from 0.20 to 0.02 ml/min) in a conscious domestic fowl, following injection of Pitressin (1.0 U). Similar responses were observed in conscious Pekin ducks injected with Pitressin or Pitocin

(0.1-5.0 U/animal, Holmes and Adams 1963). Skadhauge (1963, 1964) was the first investigator to characterize responses of the avian renal tubule to infusion of AVT. He infused synthetic AVT (7-50 ng/animal intravenously over 15 min period) into the renal portal circulation of anesthetized domestic fowl and observed significant simultaneous increases in urine osmolality (up to 56%) and decreases in urine flow rate (up to 43%). Infusion into the renal portal circulation (the Sperber technique; Sperber 1946, 1948) resulted in the direct exposure of peritubular surfaces to the AVT; thus Skadhauge was able to demonstrate that AVT increased water permeability of the renal tubule through its actions on the peritubular surfaces. This classic study was followed by more detailed studies on the renal actions of AVT completed by Skadhauge and his colleagues (Ames et al. 1971). With intravenous injections of synthetic AVT (5-200 ng/kg) into anesthetized hens and roosters (domestic fowl), these investigators observed dose-independent increases in urine osmolality (up to 335%) and decreases in urine volume (up to 77%), and dose-dependent decreases in GFR (up to 41%). These antidiuretic responses persisted for 25-30 minutes, and were associated with increases in the osmotic U/P ratio (from 0.33 to 1.11) and in the fractional reabsorption of water (from 88 to 97%) at maximum antidiuresis. Very similar observations were reported by Braun and Dantzler (1974) from their study of the effects of AVT on GFR in Gambel's quail. Following intravenous injections of synthetic AVT (10, 50, or 200 ng/kg), they observed dose-dependent decreases in urine flow rate (up to 50%) and total kidney GFR (up to 40%). The reductions in total kidney GFR

were associated with decreased numbers of filtering RT nephrons, and with changes in single nephron GFR of MT nephrons. Rzasa and associates (1974) administered intravenous injections of synthetic AVT (100 ng/kg) to domestic fowl cockerels and observed significant increases in blood volume that averaged 10% at 90 minutes after injection, presumably the result of AVT-induced increases in negative free water clearance by the kidney. More recently Mohring et al. (1980) studied the renal responses to intravenous infusions of synthetic AVT (0.008-2.0 ng/min) in conscious water-loaded Pekin ducks. They observed dose-dependent linear decreases in urine flow rate and plasma osmolality, and log-linear increases in urine osmolality.

Evidence from several of these studies suggests that AVT also may influence renal electrolyte excretion. Holmes and Adams (1963) observed that intramuscular injections of AVP and oxytocin (Pitressin and Pitocin, 0.1-5.0 U/animal) decreased urinary potassium excretion, while oxytocin increased sodium excretion. Rzasa and Niezgoda (1969) gave intravenous injections of AVT and oxytocin (100-400 ng/kg) to conscious female domestic fowl and observed increases in blood (not plasma) sodium concentration and decreases in blood potassium levels that were inversely dose-dependent. The studies of Skadhauge and associates (Ames et al. 1971) revealed that synthetic AVT-induced antidiuresis caused a major decrease (30%) in fractional excretion of sodium and chloride (i.e., increased tubular reabsorption), and a biphasic decrease/increase in fractional excretion of potassium. It is unclear whether these effects of neurohypophysial peptides on renal

electrolyte excretion have any physiological significance (Bentley 1974).

Radioimmunoassay of Plasma AVT. The combined data obtained from the various experimental techniques provided strong evidence for the role of AVT as the avian antidiuretic hormone. However, the advent of sensitive, specific radioimmunoassays (RIA) for measurement of plasma AVT (P_{AVT}) levels provided the means for true experimental verification of the role of AVT in avian renal physiology, since measurements of P_{AVT} could be made concomitant with alterations in the state of hydration of experimental animals. Prior to the development of RIA for AVT, two groups of investigators reported measurements of P_{AVT} in the domestic fowl, pigeon, and Japanese quail using the frog bladder bioassay. In a brief report, Douglas and Sturkie (1964) stated that acute hemorrhage by withdrawal of 20 ml of blood failed to evoke an increase in P_{AVT} levels of domestic fowl. Sturkie and Lin (1966) observed an average three-fold increase in P_{AVT} levels of normally hydrated domestic fowl in response to intracarotid injection of hypertonic saline (7 ± 2 to 23 ± 12 pg/ml, mean $\pm$ S.E.; data converted by Skadhauge 1981); although the increase was not statistically significant because of substantial variability in the data. They also observed that intraperitoneal injection of hypertonic glucose had no effects on P_{AVT} levels. Niezgoda and Rzasa (1971) reported P_{AVT} levels of 661 ± 92, 262 ± 16, and 288 ± 17 pg/ml (mean $\pm$ S.E.) respectively, for normally hydrated male domestic fowl, pigeons, and Japanese quail. In a subsequent study, Niezgoda (1975) measured P_{AVT} levels of domestic fowl hens and cocks during

normal hydration (310-697 pg/ml) and in response to hypertonic saline injection (1,003-1,280 pg/ml), oral hypertonic saline loading (958-1,055 pg/ml), and dehydration (1,680-3,305 pg/ml). Although a large discrepancy exists between the bioassay values of the two laboratories, both groups reported three- to eight-fold increases in P_{AVT} levels in response to osmotic stress.

The first RIA measurements of P_{AVT} in birds were made by Koike and associates in conscious, normally hydrated and dehydrated female domestic fowl (Koike et al. 1977). They reported a basal P_{AVT} concentration of 0.7 μU/ml (vasopressor activity, equivalent to 6.25 pg/μU) that increased in parallel with plasma osmolality (P_{OSM}) to 3.9 μU/ml in response to 72 hours of dehydration. The changes in P_{AVT} and P_{OSM} were weakly correlated ($r^2 = 0.24$) and were described by the equation: P_{AVT} (μU/ml) = 0.048 (P_{OSM} - 293), which provided evidence (albeit weak) for a relationship between P_{OSM} and AVT secretion similar to that observed in mammals for P_{OSM} and AVP secretion (Dunn et al. 1973, Hayward et al. 1976, Robertson, Shelton, and Athar 1976, Reaves et al. 1981). (From studies of the mammalian renal-osmoregulatory system, it was determined that the relationship between P_{OSM} and P_{AVP} could be described by linear regression functions: The slope and x-intercept correspond to the sensitivity and set-point, respectively, of the osmoreceptor-AVP secretory system (Robertson et al. 1973, 1976).) Because dehydration results in hypovolemia as well as hyperosmolality of the extracellular fluid, Koike's studies could not separate possible osmotic and volemic effects on AVT release. Subsequent

studies by Koike (Koike, Pryor, and Neldon 1979, 1980) focused on independent osmotic and volemic regulation of AVT secretion. In those experiments, intravenous infusion of conscious female domestic fowl with hypertonic saline (1.0 M) resulted in parallel increases in P_{OSM} and P_{AVT} in the absence of hypovolemia. P_{AVT} increased from a basal level of 4.7 μU/ml to a maximum of 21.9 μU/ml after 30 minutes of saline infusion. The changes in P_{AVT} and P_{OSM} were related by the equation: P_{AVT} (μU/ml) = 0.23 (P_{OSM} - 296) and were weakly correlated (r^2 = 0.58), but provided evidence for an appropriate relationship between P_{OSM} and release of an antidiuretic hormone (AVT). In separate experiments, acute rapid hemorrhage (20 and 30% of estimated blood volume) of conscious female domestic fowl did not result in any significant changes in P_{AVT} concentration, despite reductions in blood pressure. It was concluded that reductions in vascular volume do not stimulate the release of AVT in the domestic fowl. Following these studies, Koike and his associates studied the possible influence of cardiac volume receptors in the regulation of AVT secretion (Koike et al. 1981). Domestic fowl were acutely or chronically cardiac denervated and the effects of hypertonic saline infusion on AVT secretion evaluated. Both acute and chronic denervation groups exhibited a two-fold increase in the osmotic sensitivity of AVT secretion, as compared to their respective sham-operated control groups (as reflected by the regression slopes of the P_{OSM} - P_{AVT} relationships). These results suggested that atrial and/or ventricular cardiac mechanoreceptors exert, via afferent pathways, a tonic inhibitory influence on the AVT system similar to

that on the AVP system observed in mammals (Share 1974). These data were contradicted however, by those obtained from chronically denervated birds which received electrical stimulation of the middle cardiac nerve (central end). In response to stimulation (expected to inhibit AVT release), these birds exhibited reductions in mean arterial blood pressure and three- to eight-fold increases in jugular P_{AVT} concentration. The authors were unable to explain the paradoxical nature of their data.

The regulation of AVT secretion in the domestic fowl has also been studied by Skadhauge and associates who reported briefly on the effects of dietary NaCl load on P_{OSM} and P_{AVT} (Arnason, Rice, and Skadhauge 1982). They observed that animals adapted to high dietary levels of NaCl exhibited elevated P_{AVT} levels that correlated positively with P_{OSM} and decreased osmotic sensitivity of AVT release, as compared to animals adapted to low levels of NaCl. Dehydration of low and high salt groups resulted in hyperosmolality and hypovolemia that stimulated AVT release maximally and increased the osmotic sensitivity of AVT secretion. From these data, the authors implied that the osmotic sensitivity of AVT secretion is modified in response to alterations in extracellular volume, in a manner similar to the volume-mediated changes in AVP secretion observed in mammals (Robertson et al. 1976). However, the data from these experiments provide only indirect evidence for an association between osmolality and volume in the regulation of AVT secretion in the domestic fowl.

The elegant studies of Simon and associates (Mohring et al. 1980, Simon-Oppermann et al. 1980) on conscious Pekin ducks provided additional data on the regulation of AVT secretion in birds. From experiments on ducks chronically adapted to either hypertonic saline (600 mosm/kg H_2O) or tap water as drinking fluid, a highly significant correlation (r^2=0.79) was established between P_{OSM} and P_{AVT}, described by the equation (rearranged for comparative purposes): AVT_{serum} (fmol/ml) = 0.39 ($osmolality_{serum}$ - 280), (sensitivity conversion: 1.052 pg/fmol). In addition, acutely over-hydrated ducks (intravenously infused with hypotonic glucose solutions) exhibited minimal or undetectable levels of AVT in their blood (Mohring et al. 1980). In subsequent studies, vagal blockade was shown to induce a three-fold increase in basal P_{AVT} (2.6 to 7.1 fmol/ml; 1.052 pg/fmol) which suggested volume mediated release of AVT, normally inhibited by tonic afferent vagal input. Also, cerebral osmotic stimulation (by intracarotid or intracerebroventricular infusions of hypertonic saline) was shown to result in clear antidiuretic actions concomitant with enhanced secretion of AVT, in confirmation of the concept of intracerebral osmoreception as a mechanism for control of AVT secretion in birds (Simon-Oppermann et al. 1980). Intracerebral osmoreception and the osmotic and volemic control of diuresis were studied in subsequent experiments on conscious Pekin ducks. The investigators observed a symmetrical relationship between alterations in carotid blood osmolality and resultant changes in renal water excretion. The antidiuretic effect of increasing the osmolality of blood supplied to the brain was

mediated by enhanced release of AVT, directly demonstrated by RIA of P_{AVT} levels in peripheral blood. The diuretic effects of decreasing carotid blood osmolality or of isosmotic volume expansion were ascribed to inhibition of AVT release, although no direct measurements of P_{AVT} were made (Simon-Oppermann and Simon 1982). Thus, the investigators provided evidence (albeit indirect) for volemic regulation of AVT secretion. A comparative study of the responses of avian and mammalian ADH systems to dehydration was reported by Gray and Simon (1983). Normally hydrated dogs and Pekin ducks exhibited identical basal P_{OSM} (298 mosm/kg H_2O) and similar basal P_{AVP} (2.4 pg/ml in dog) and P_{AVT} (5.8 pg/ml in ducks). In response to 24 hours of dehydration, ADH systems of dogs and ducks exhibited similar sensitivities to changes in P_{OSM}, described by the regression functions: P_{AVP} (pg/ml) = 0.24 (P_{OSM} - 284), r^2 = 0.55 for the dog, and P_{AVT} (pg/ml) = 0.39 (P_{OSM} - 283), r^2 = 0.85 for the duck.

The RIA studies completed to date provide preliminary, sometimes conflicting data on the factors important in the regulation of P_{AVT} in birds. The studies summarized suggest that changes in extracellular (plasma) osmolality are a primary stimulus in the regulation of AVT secretion. Changes in extracellular volume also appear to influence AVT secretion; conflicts in the data concerning this mechanism appear to be related to species differences in the integration of osmoregulatory mechanisms and the presence or absence of functional nasal salt glands.

Mechanisms of Action of AVT in the Avian Kidney

Early studies on avian renal function demonstrated alterations in urine volume and concentration in response to experimental alterations in state of hydration. These changes in urine volume were accompanied by concomitant changes in renal tubule water reabsorption and GFR, although the relative contributions of these two mechanisms and the role of AVT in their regulation have been sources of some disagreement in the literature. Prior to the identification of AVT in birds, Burgess et al. (1933) and Korr (1939) observed that intramuscular injections of Pitressin in domestic fowl mimicked the antidiuretic responses to dehydration: Increased renal tubule water reabsorption and reduction of GFR. Although several subsequent studies demonstrated similar antidiuretic responses to injection of AVT, Skadhauge (1963, 1964) was the first to carefully analyze the effects of AVT on the avian kidney. He reported that intravenous infusion of AVT in the domestic fowl produced antidiuresis that resulted solely from increases in water reabsorption by the renal tubules. The use of renal portal infusion (the Sperber technique, Sperber 1946, 1948) facilitated initial delivery of AVT to the renal peritubular surfaces and enabled Skadhauge to demonstrate that AVT acts via the peritubular surfaces of the renal tubule cells. The lack of consistent changes in GFR led Skadhauge to conclude that the previously reported glomerular antidiuretic actions of AVT were pharmacological in nature. However, re-examination of Skadhauge's GFR data (rearranged to reflect increasing doses of AVT) does suggest a dose-response relationship between AVT and percent decrease in GFR, at

the lower doses of AVT infused. Subsequent studies in which several species of birds (budgerigar, domestic fowl, Pekin duck, Gambel's quail) were either dehydrated or salt loaded (now known to be potent stimuli for AVT release) demonstrated that GFR decreased approximately 16-70%, although tubular reabsorption of water still appeared important in reduction of urine volume (Dantzler 1966, Skadhauge and Schmidt-Nielsen 1967b, Holmes et al. 1968, Krag and Skadhauge 1972, Braun 1976).

Although Skadhauge clearly demonstrated that AVT increases renal tubule water permeability by its actions on the peritubular surfaces, subsequent studies failed to identify the mechanism involved in glomerular antidiuresis and to quantify the relative contributions of glomerular and tubular mechanisms to the production of antidiuresis in the avian kidney. Burgess et al. (1933) and Heller (1942) were the first investigators to suggest that AVT could act as a vasoconstrictor of the renal vasculature (at the glomerulus or other sites) to produce glomerular antidiuresis. Although Dantzler (1966) was able to demonstrate that glomerular intermittency was responsible for a 60% reduction in the GFR of conscious domestic fowl infused with hypertonic saline, he was unable to reproduce the effect with injections of AVT. More recently Ames and associates (1971) were able to demonstrate a dose-dependent reduction in GFR of anesthetized domestic fowl after intravenous injection of AVT (5-200 ng/kg). GFR was observed to decrease by as much as 41% after the highest doses of AVT; although depression of GFR occurred only during the first 10 minutes of the 30 minute antidiuresis, and was accompanied by

transient dose-dependent reductions in mean arterial blood pressure. The investigators concluded that initial AVT-induced reductions in GFR were probably vascular in origin, because of the simultaneous reductions in mean arterial blood pressure and GFR early in the antidiuresis. Braun and Dantzler (1972, 1974) studied the effects of hypertonic saline infusion and AVT injection on the regulation of total kidney and single nephron GFR in anesthetized Gambel's quail. Infusion of hypertonic saline (40 meq/kg) resulted in a substantial (80%) decrease in total kidney GFR and tubular maximum for para-aminohippurate (PAH), suggesting that GFR decreased with the number of functioning nephrons (Braun and Dantzler 1972). Histological data confirmed this relationship and showed that 55% of the RT nephron proximal convoluted tubule lumena had closed after the 40 meq/kg salt load; suggesting that it is the RT nephrons which shutdown during salt loading to conserve body water. SNGFR measurements confirmed the histological data, and showed that with salt loading no RT nephrons were functioning, as reflected by the absence of Prussian Blue (used in SNGFR measurements) in the proximal convoluted tubule lumena of RT nephrons. The presence of Prussian Blue in the peritubular vasculature of such nephrons suggested the presence of glomerular bypass vessels which would shunt blood past the RT nephron glomeruli and into the peritubular vasculature. In subsequent studies, pulse intravenous injections of AVT in anesthetized quail (10, 50, or 200 ng/kg) resulted in dose-dependent decreases in total kidney GFR, urine flow, and fraction of filtering RT nephrons, even at low doses of AVT. At 200 ng/kg AVT, mean

arterial blood pressure exhibited varied short term increases, decreases, or biphasic responses (Braun and Dantzler 1974). The effects of NaCl loading appear to mimic the actions of AVT, although following higher doses of AVT, there were more marked decreases in the numbers of filtering RT nephrons. Braun and Dantzler suggested that the shutdown of RT nephrons could contribute to water conservation in two ways: a) shutdown of nephrons unable to form urine hypertonic to the plasma, and b) reduction of volume flow through the medullary collecting ducts, which could enhance urine concentrating ability. Subsequently, Braun (1976) used a silicone rubber casting compound to demonstrate that during hypertonic saline loading in quail, shutdown of the RT nephrons occurred due to vasoconstriction at the afferent arteriole. A similar inverse correlation between GFR and P_{AVT} levels was reported recently by Simon and associates (Kaul et al. 1983) in their studies of the conscious salt-adapted Pekin duck. These investigators observed that rapid intravenous injection of synthetic AVT (10 ng/kg) that doubled P_{AVT} (19 to 38 pg/ml) resulted in a 40-90% reduction in GFR, while reduction of P_{AVT} by intravenous injection of anti-AVT antibodies increased GFR. Alteration of endogenous AVT secretion through perfusion of the third ventricle resulted in similar changes in GFR.

In summary, these studies provide strong evidence in favor of a vascular role for AVT in the regulation of avian renal function. Although GFR may be more sensitive to state of hydration in desert quail (Braun and Dantzler 1972), the studies summarized suggest that glomerular intermittency is the primary mechanism of dehydration-

induced reductions in GFR, and that such intermittency is regulated by AVT. However, the studies to date have failed to quantify the relative contributions of glomerular and tubular mechanisms to the production of antiduresis in the avian kidney.

Unresolved Aspects of AVT and Avian Renal Function

Although the criteria established for the physiological significance of neurohypophysial hormones (Heller 1969) have been met for the role of AVT as the avian antidiuretic hormone, several crucial aspects of the secretion and intrarenal vascular mechanism of action of AVT remain unresolved.

Regulation of Secretion. The available data demonstrate that increases in P_{OSM} are a primary stimulus for the release of AVT, although a complete characterization of the osmotic and volemic regulation of AVT secretion has yet to be accomplished. The studies performed by Koike (Koike et al. 1977, 1979) provided a partial characterization of osmotic regulation of P_{AVT} in the domestic fowl, but these studies were deficient in several respects: a) the successive infusion of two different osmotically active compounds (mannitol followed by hypertonic saline) which may have adversely altered hypothalamic osmoreceptor function and AVT release; b) incomplete definition of the AVT secretion threshold and secretion characteristics under hypo-osmolar conditions; c) weak correlations for the P_{OSM}-P_{AVT} relationships described; and d) extreme variability in RIA measurements. The elegant studies of Simon and associates (Mohring et al. 1980, Simon-Oppermann et al. 1980)

partially characterized osmotic regulation of AVT secretion and established its relationship with intracerebral osmoreception in the Pekin duck, but these characterization studies were also incomplete and may have obscured P_{OSM}-P_{AVT} relationships by the use of hypertonic saline-adapted ducks. Their data suggest that salt adaptation in birds with functional salt glands may alter osmotic regulation of AVT secretion, since a P_{OSM}-P_{AVT} relationship could not be established in salt-adapted ducks. Indeed, the basic characteristics of the mechanisms that regulate AVT secretion may differ among species, related to the presence or absence of functional nasal salt glands.

The available data on volemic regulation of P_{AVT} are incomplete and appear to be in direct conflict. The studies of Simon and associates (Simon-Oppermann et al. 1980, Simon-Oppermann and Simon 1982) are very suggestive of a vagally-mediated tonic inhibitory input of extracellular (blood) volume on the release of AVT, similar to that described in mammals (Share 1974, Robertson et al. 1976). In this system, reduction of blood volume (sensed by cardiac mechanoreceptors) reduces vagal inhibitory input to the AVP osmoreceptor-secretory system, which results in direct hypovolemic release of AVP, as well as enhanced sensitivity to osmotic stimuli. Conversely, expansion of blood volume inhibits the release of AVP and decreases osmotic sensitivity of the system. Although the effects of extracellular volume expansion on P_{AVT} levels were not studied directly by Simon and his colleagues, the renal diuretic responses observed (which suggest inhibition of AVT secretion) strongly suggest that

extracellular (or blood) volume is important in the regulation of P_{AVT} in the Pekin duck. The conflicting studies of Koike and associates provide evidence for and against volemic regulation of P_{AVT} in the domestic fowl. In initial studies, reductions in blood volume (20-30% hemorrhage) did not alter P_{AVT} levels (Koike et al. 1980). However, in subsequent studies, cardiac denervation enhanced osmotic sensitivity of AVT release, whereas electrical stimulation of these presumably inhibitory afferent nerves paradoxically increased AVT release. The reasons for the observed discrepancies concerning volemic regulation of P_{AVT} in birds remain uncertain; however, it is possible that true physiological differences exist among species related to the presence or absence of functional nasal salt glands.

Mechanisms of Action of AVT in the Avian Kidney. The available data clearly demonstrate that antidiuresis in the avian kidney is the result of increases in renal tubule water permeability and decreases in GFR, and that both of these mechanisms are probably mediated by the avian antidiuretic hormone, AVT. However, the relative contributions of these two mechanisms to antidiuresis remain uncertain. Previous studies in which birds were dehydrated, salt-loaded, or given exogenous AVT reported highly variable reductions in GFR and therefore variable contributions by glomerular antidiuresis to the overall antidiuretic responses of the avian kidney (Burgess et al. 1933, Korr 1939, Holmes and Adams 1963, Skadhauge 1964, Dantzler 1966, Skadhauge and Schmidt-Nielsen 1967b, Holmes et al. 1968, Ames et al. 1971, Krag and Skadhauge 1972, Braun and Dantzler 1972, 1974, Braun 1976, Kaul et al. 1983). Much of this variability is likely the

result of differences in experimental techniques used including: a) the use of anesthetics, which alter blood pressure and possibly, renal function; b) the doses of AVT administered, which varied from low physiological to high pharmacological levels; and c) the routes by which AVT was administered; this profoundly affects the resultant peak and average blood levels of the hormone, and therefore, the duration and intensity of observed hormone actions. The use of intravenous injection was frequently associated with transient reductions in arterial blood pressure which likely contributed to reductions in GFR by non-specific means.

The major flaw common to virtually all previous studies of AVT and glomerular antidiuresis is the lack of knowledge concerning physiological plasma levels of AVT and whether the observed responses to salt loading or AVT injection were physiological or pharmacological in nature. To accurately assess the relative contributions of tubular and glomerular mechanisms to antidiuresis, the dose-response characteristics of these mechanisms must be determined at physiological plasma concentrations of AVT.

CHAPTER 3

SUMMARY OF THE PRESENT STUDIES

In the present studies, recently developed radioimmunoassay techniques were employed in a quantitative investigation of the avian antidiuretic hormone, arginine vasotocin, in the domestic fowl. Specifically, the studies described involved characterization of AVT secretion by the avian hypothalamo-neurohypophysial system, and elucidation of the relative contributions of AVT-induced glomerular and tubular mechanisms of antidiuresis to the conservation of water by the avian kidney.

Purpose

The present investigation is composed of a series of three studies; the purposes of these studies are: 1) to define the functional characteristics of the osmoreceptor system that regulates AVT secretion, in terms of the extracellular stimuli that elicit release of AVT; 2) to characterize responses of the osmoreceptor system to the natural osmoregulatory challenge of negative fluid balance; and 3) to quantify the relative contributions of AVT-induced tubular and glomerular antidiuresis to the conservation of water by the kidney.

Experimental Rationale

The experiments described utilized male domestic fowl (*Gallus gallus* v. *domesticus*) of the Rhode Island Red strain as the

experimental animal. This bird was selected primarily because its common use in studies of avian renal function and osmoregulation provided abundant and valuable background data and technical information. Furthermore, the absence of functional nasal salt glands in this species makes it an excellent experimental model for the study of avian antidiuretic hormone secretion and renal actions without potential complication by extrarenal mechanisms that regulate extracellular fluid composition and volume. In all experiments, birds were studied in the conscious state to avoid potential alterations in normal physiological responses with the use of general anesthetics.

Functional characteristics of the Osmoreceptor System. In the first study, the functional characteristics of the osmoreceptor were evaluated in two separate experiments in which plasma AVT concentrations were measured in response to independent experimental manipulation of extracellular osmolality or volume. Simultaneous measurements of plasma AVT and either plasma osmolality or volume in these experiments permitted determination of osmoreceptor sensitivity to independent osmotic or volemic stimuli, and "set-point" of the AVT secretory system under conditions of normal hydration.

Responses of the Osmoreceptor System To A Natural Osmoregulatory Challenge. In the second study, birds were subjected to chronic, progressive dehydration (total fluid deprivation) to characterize responses of the osmoreceptor system to the natural osmoregulatory challenge of negative fluid balance. During these experiments, repetitive measurements of plasma AVT and plasma osmolality were made in individual animals to assess both the

renal-osmoregulatory abilities of the birds, and the responses of the osmoreceptor system. Importantly, the plasma AVT measurements made during these experiments provided valuable information on physiological levels of the hormone maintained during normal hydration and in response to dehydration.

Relative Contributions of Glomerular and Tubular Antidiuresis. In the third study, dose-response characteristics of glomerular and tubular mechanisms of antidiuresis were determined, based on the plasma AVT levels measured in response to water deprivation. Renal water conservation was assessed through dose-response measurements of GFR, ureteral urine flow rate and osmolality, osmotic urine-to-plasma (U/P) ratio, and free water clearance. These experiments permitted comparison of the renal water conservation achieved through tubular and glomerular mechanisms at physiological AVT levels, and thus, determination of the relative contributions of these two mechanisms to water conservation by the avian kidney.

CHAPTER 4

OSMOTIC AND VOLEMIC REGULATION OF PLASMA ARGININE VASOTOCIN IN CONSCIOUS DOMESTIC FOWL

Introduction

Arginine vasotocin (AVT) is one of ten neurohypophysial peptides that have been identified in vertebrates (Sawyer 1977, Bentley 1980, Pang, Furspan, and Sawyer 1983); it has been found in species of every major vertebrate group, and thus is the most ubiquitous of the neurohypophysial peptides (Sawyer 1977, Pang et al. 1983). In all non-mammalian tetrapod vertebrates studied, AVT appears to function primarily as an antidiuretic hormone (ADH; Bentley 1974, 1980, Pang et al. 1983). It was established as the naturally occurring ADH of birds through the extensive chemical and pharmacological analyses of Munsick, Sawyer and van Dyke (1960) and Munsick (1964). Avian ADH appears to regulate water excretion by its actions on tubule water permeability and glomerular filtration rate (Dantzler 1978, 1980a, 1980b, Dantzler and Braun 1980, Skadhauge 1981).

Although AVT has been identified as the avian ADH and its actions on the kidney partially characterized, very little is known about the physiological regulation of AVT secretion in birds. Early studies were hampered by the inability to accurately measure plasma AVT (P_{AVT}) concentrations in experimental animals. Several investigators employed the frog bladder bioassay to measure P_{AVT}

in domestic fowl in response to osmotic and volemic stress; however, the relative inaccuracy and insensitivity of this method resulted in highly variable data. Thus, injection of hypertonic glucose or saline, dehydration, or hemorrhage either failed to alter P_{AVT} levels significantly (Douglas and Sturkie 1964, Sturkie and Lin 1966), or increased them three- to eight-fold (Niezgoda 1975). More recently, radioimmunoassay techniques have been utilized to characterize osmotic and volemic regulation of P_{AVT} in the domestic fowl and Pekin duck. Dehydration, saline adaptation, or saline infusion of these species resulted in positive linear correlations between plasma osmolality (P_{OSM}) and P_{AVT} over a wide range of plasma osmolalities (Koike et al. 1977, 1979, Mohring et al. 1980, Gray and Simon 1983). Extracellular volume expansion or vagal blockade in the Pekin duck provided consistent evidence for volemic regulation of P_{AVT} (Simon-Oppermann et al. 1980, Simon-Oppermann and Simon 1982); whereas hemorrhage and cardiac afferent denervation in the domestic fowl provided conflicting evidence for such a mechanism (Koike et al. 1980, 1981).

The available data demonstrate that increases in P_{OSM} are a primary stimulus for the release of AVT, although a complete characterization of the osmotic regulation of P_{AVT} has yet to be accomplished. Furthermore, the data on volemic regulation of P_{AVT} are incomplete and appear to conflict. The purpose of the present investigation was to define the functional characteristics of the AVT secretory system in the domestic fowl. The osmotic regulation of

P_{AVT} was studied in response to hyperosmotic and hypo-osmotic stimuli to obtain a complete characterization of osmotically-mediated AVT secretion. The volemic regulation of P_{AVT} was studied in response to volume expansion and hemorrhage to characterize the secretion of AVT in response to opposite volemic stimuli.

Materials and Methods

Animals and General Experimental Procedures

All studies utilized Rhode Island Red cockerels, which were obtained from a commercial supplier (Red Wing Hatchery, Los Angeles, CA) at one week of age and raised in flocks at University of Arizona Facilities (Department of Animal Resources) in an environmentally controlled room (ambient temperature 26±1°C) with a 12:12 light-dark cycle. The birds were maintained in screen-bottomed cages, and provided with a commercial diet (chick starter mash and mixed scratch grain, Arizona Feeds, Inc.) and tap water *ad libitum*. When the birds attained 9-10 weeks of age (1200-1300g body weight) they were transferred to individual cages for at least one week prior to any surgical or experimental procedures.

All experiments were performed during daylight hours at an ambient temperature of 22-25°C. Quiet conditions were maintained in the laboratory during all experimentation to avoid excitation of conscious animals. In all experiments, baseline blood samples were taken at the beginning of each experiment which allowed each animal to serve as its own control for the experimental manipulations that followed.

Surgical Preparations

Chronic Catheterization. To avoid undue excitement or surgical stress during measurements of AVT secretion and to facilitate intravenous (i.v.) infusion and blood sample collection, birds were chronically catheterized. Animals were fasted overnight and under ketamine hydrochloride pre-anesthetic (20 mg/kg) and methoxyflurane inhalation anesthesia, surgical grade silastic catheters (0.940 mm OD x 0.508 mm ID) were placed under aseptic conditions in the right brachial artery and basilic vein with the tips of the catheters in or near the common carotid artery and cranial vena cava, respectively. The potential compromise in blood flow to the distal wing caused by chronic arterial and venous catheterization is readily overcome by the abundant collateral circulation present in the wing of the domestic fowl (Baumel 1979). The catheters were occluded with metal stylets, tunneled subcutaneously, and externalized on the neck 2-3 cm below the comb. The ends of the catheters were secured to the skin with a wound clip to prevent the birds from pecking at the free ends. During experiments, extensions were connected to the catheters to facilitate infusion and sample collection. The animals were given 5-6 days to recover and during that time, were treated prophylactically with oxytetracycline. During the recovery period, the birds were acclimated to handling (to reduce excitement during experiments) and the catheters rinsed with heparinized saline (40 U/ml) on alternate days to maintain their patency. The inhalation anesthesia was administered by a specially designed anesthetic device which utilized a unidirectional flow mask and enabled precise control of anesthetic

dosage (Stallone and Braun, manuscript in preparation). Usually, animals recovered completely without ill effects from anesthesia or surgery, and the catheters remained patent throughout the experimental period.

Acute Catheterization. As part of the studies involving plasma AVT and blood volume, a group of birds was acutely catheterized for use in the ^{51}Cr-RBC experiments (vide infra) to determine blood volumes and validate the use of hematocrit as an endogenous indicator of changes in blood volume. Immediately prior to each experiment, an animal was lightly restrained and a cloth hood placed over the head to prevent visual stimulation. Under local anesthesia (2% lidocaine), the right brachial artery and basilic vein were catheterized with PE-50 tubing. The skin was closed with wound clips and the bird allowed to recover for one hour before the experiment was started.

Osmotic Regulation of Plasma AVT

To characterize the osmotic regulation of P_{AVT}, chronically catheterized birds (vide supra) were administered i.v. saline infusions to acutely alter plasma osmolality. Animals were divided into three experimental groups: a control group which received a NaCl infusion isotonic to the plasma (308 mosm/kg H_2O), a hypertonic group which received 1.0 M NaCl, and a hypotonic group which received double distilled water. The experimental protocol was as follows: A conscious animal was suspended upright in a rip-stop nylon restraining sling that allowed free movement of the head and feet but prevented the animal from struggling. A cloth hood was placed over the head to

prevent visual stimulation, and the bird allowed to acclimate to ambient conditions for one hour. At the end of the acclimation period, an arterial blood sample was taken (for plasma analyses and hematocrit measurement) and i.v. infusion at 0.4 ml/kg • min started, using a Sage infusion pump (model 355, Orion Res., Inc.). Additional arterial blood samples were taken at 15, 30, and 60 min of infusion. Blood sample volume (1.2 ml) was replaced with isotonic saline after each sample was taken. At the end of the experiment, the animal was sacrificed with an i.v. overdose of sodium pentobarbital. All blood samples were centrifuged at 4°C immediately after collection, hematocrit measured, and the resultant plasma removed, aliquoted, and analyzed for AVT, electrolytes, osmolality, and protein using the techniques described in detail below.

Body weights of the animals used in these experiments averaged 1229±52.9, 1166±32.4, and 1387±35.9g, respectively, for control, hypertonic, and hypotonic groups.

Volemic Regulation of Plasma AVT

Effects of Volume Alteration on Plasma AVT. To characterize the volemic regulation of P_{AVT}, chronically catheterized birds (vide supra) were subjected to i.v. infusion of isotonic saline or to acute arterial hemorrhage to produce small, quantifiable isosmotic expansions or reductions, respectively, in blood volume. Details of the experimental protocol for i.v. infusion were described in the previous section (see osmotic regulation of P_{AVT}, control group). In the hemorrhage studies, animals were divided into three

experimental groups: Two volume depletion groups hemorrhaged 10% or 20% of blood volume (56.5 ml/kg as determined by the ^{51}Cr-RBC method, vide infra), and a non-hemorrhaged group which served as a time and blood sampling control. The experimental protocol was as follows: A conscious animal was suspended upright in a restraining sling, hooded to prevent visual stimulation, and allowed to acclimate to ambient conditions for one hour. At the end of the acclimation period, the bird was heparinized with 200 U/kg sodium heparin, an arterial blood sample was taken (for plasma analyses and hematocrit measurement), and the bird subjected to acute arterial hemorrhage by withdrawl of blood at 1.0 ml/kg • min into a heparinized (200 U) 20 cc syringe using an infusion/withdrawal pump (model 906, Harvard Apparatus Co.). Additional arterial blood samples were taken at 2.5 and 5.0 min post-hemorrhage; the collected blood was then reinfused i.v. (1.0 ml/kg • min), and additional arterial blood samples taken at 10, 20, and 40 min post-reinfusion. Erythrocytes were resuspended to blood sample volume (1.2 ml) in isotonic saline and reinjected after each sample. Arterial blood pressure and heart rate were recorded before and after hemorrhage and reinfusion periods using a Gould-Statham pressure transducer (model p23dB) and a Gould chart recorder (model 2400S, Gould Inst., Inc.). At the end of the experiment, the animal was sacrificed with an i.v. overdose of sodium pentobarbital. All blood samples were centrifuged at 4°C immediately after collection, hematocrit measured, and the resultant plasma removed, aliquoted, and analyzed for AVT, electrolytes, osmolality, and protein using the techniques described in detail below.

Body weights of the birds used in the hemorrhage experiments averaged 1363±35.8, 1443±70.1, and 1340±44.1g, respectively, for control, 10%, and 20% HEM groups.

Validation of Blood Volume Markers. Characterization of the volemic regulation of P_{AVT} required an accurate measurement of normal blood volume in the experimental animals and validation of hematocrit as an endogenous indicator of changes in blood volume. The ^{51}chromium technique was used to isotopically label red blood cells (^{51}Cr-RBC method; Gray and Sterling 1950, anonymous 1973) to determine total RBC volume, and in combination with hematocrit, total blood volume. This technique has been used successfully in Japanese quail (Nirmalan and Robinson 1972) and in chickens (New Hampshire cocks; Rzasa, Niezgoda, and Kahl 1974). The ^{51}Cr-RBC label was prepared under sterile conditions as follows: 6.0 ml of blood obtained by venipuncture of a donor bird was heparinized and centrifuged at 2500 x g for 15 minutes and the resultant plasma removed and retained. Twenty-five microcuries of ^{51}Cr (as chromate in sterile isotonic saline, sp. act. 1.0 mCi/ml, 424 mCi/mg, New England Nuclear) were added to the packed erythrocytes, which were swirled gently, and incubated in a water bath for 1 hr at 41°C with gentle swirling every 15-20 minutes. After incubation, the packed cells were washed in 10 ml isotonic saline, centrifuged at 2500 x g for 15 minutes, and the supernatant discarded. After three saline washes, the packed cells were resuspended in the original plasma, the hematocrit measured (by microhematocrit technique), and the suspension stored at 4°C until use. The experimental protocol for the blood

volume and validation measurements was as follows: Acutely catheterized birds (vide supra) were hooded and placed upright in a restraining sling and injected i.v. with 1.0 ml of the ^{51}Cr-RBC suspension. The exact volume of label injected was calculated by weighing the syringe before and after injection and correcting the difference for density of whole blood (assumed to be 1.0448 g/ml; value averaged from the data of Medway and Kare (1959) for appropriate age and hematocrit). Small (0.50 ml) arterial blood samples (for ^{51}Cr analysis and hematocrit measurement) were taken at 10, 20, 30, 60, and 90 min after label injection, centrifuged, hematocrit measured, and the resultant plasma discarded. A 100 µl sample of the packed cells was pipetted into a counting tube containing 1.0 ml distilled water and 50 µl saponin solution (hemolytic agent) and ^{51}Cr counted in an automatic well-type gamma counter (Model 1185, Searle Analytic). A 0.50 ml sample of the ^{51}Cr-RBC suspension (the standard) was treated and counted in an identical manner. At the end of the experiment, the animal was sacrificed with an i.v. overdose of sodium pentobarbital. The total RBC volume (RBCV) was calculated from the formula presented by the International Committee for Standardization in Hematology (anonymous 1973):

$$\text{RBCV (ml)} = \frac{I}{C} = \frac{SVH_V}{B}$$

where I = total RBC radioactivity injected (cpm)

C = Radioactivity in systemically-mixed RBC's (cpm/ml RBC)

S = counting rate of standard (cpm/ml)

V = volume of labelled RBC suspension injected (ml)

H_V = packed cell volume (hematocrit) of injected RBC suspension

B = counting rate of collected blood sample (cpm/ml RBC)

The total blood volume (BV) is:

$$BV(ml) = \frac{RBCV}{HCT}$$

where HCT = hematocrit ($\frac{\%\ PCV}{100}$) of collected blood sample

After the temporal stability of the ^{51}Cr-RBC label was confirmed for blood volume measurements, the protocol was modified to validate the use of hematocrit as an endogenous indicator of changes in blood volume. Changes in hematocrit are used to estimate changes in blood volume (expressed as percent of initial value) by the proportion $(BV_2/BV_1) = (HCT_1/HCT_2)$ which assumes constancy of circulating erythrocyte volume. In these experiments, birds were prepared and injected with the ^{51}Cr-RBC suspension, and blood sampled at 10 and 20 min post-injection to determine baseline blood volume and hematocrit. Following the baseline measurements, the birds were volume expanded by i.v. infusion of isotonic saline at 0.8 ml/kg · min (Sage infusion pump, model 355, Orion Res., Inc.) and arterial blood samples (0.50 ml) taken at 15, 30, and 60 min of infusion. These blood samples provided simultaneous measurements of

blood volume and hematocrit during volume expansion and thus permitted correlation between increases in blood volume and reductions in hematocrit. For all of these experiments, it is important to emphasize that calculation of total blood volume from a measurement of either total RBCV or plasma volume and hematocrit is less reliable than independent measurements of each volume (anonymous 1973). Although the actual blood volumes measured may be in slight error, the stability and reliability of the ^{51}Cr-RBC method permit highly accurate measurements of changes in blood volume.

Radioimmunoassay of AVT

The radioimmunoassay of AVT in these studies utilized the R-70 antiserum developed by Fisher and associates at the UCLA School of Medicine (Rosenbloom and Fisher 1974, Skowsky, Rosenbloom, and Fisher 1974). This antiserum cross-reacts completely with AVT and AVP (arginine vasopressin, the mammalian antidiuretic hormone) and very poorly with mesotocin (W.H. Sawyer, pers. comm.) and oxytocin (Rosenbloom and Fisher 1974, Skowsky et al. 1974), the corresponding neutral neurohypophysial hormones. As part of this research, this RIA system was established in the laboratory of Dr. Eldon J. Braun, University of Arizona, for the measurement of AVT in avian plasma. This RIA was validated in terms of the following generally accepted criteria for radioimmunoassay of neurohypophysial hormones:
i) cross-reactivity relationships between AVT, AVP, mesotocin and oxytocin which are identical with previously reported descriptions for the R-70 antiserum (Rosenbloom and Fisher 1974, Skowsky et al. 1974,

W.H. Sawyer, pers. comm.); ii) a zero blank value is obtained for extracted and assayed distilled water (there are no known AVT deficient birds for a true blank plasma, although a zero blank value is obtained for plasma of acutely water loaded domestic fowl); iii) increasing amounts of AVT added to plasma prior to extraction and subsequently assayed yield a linear relationship with a regression line passing through zero; iv) serial dilutions of several extracted plasma pools yield linear relationships between AVT measured and the dilution factors, with regression lines passing through zero; and v) changes in plasma AVT concentration are measured which correspond with hydration/dehydration of experimental animals. As employed in these studies, the least detectable concentration of AVT (that which yields a binding response two standard deviations from the zero dose response) is 0.75 μU/ml (3.6 pg/ml) or the equivalent of 0.36 pg per assay tube. The intra-assay and inter-assay coefficients of variation average (mean±S.E.) 7.2±0.7% and 8.0±0.9%, respectively. Cross-reactivity relationships between ^{125}I-AVT and AVT, AVP, mesotocin, or oxytocin are shown in Figure 2. The relative cross-reactivities of AVP, oxytocin, and mesotocin were computed against ^{125}I-AVT and AVT at 50% displacement and amounted to 133.3, 11.1, and 1.1%, respectively, for AVP, oxytocin and mesotocin. Figure 3 depicts a logit-log transformation (Rodbard and Lewald 1970) of the AVT standard curve from 15 assays. The regression coefficient of this linear transformation is r^2=0.994. Superimposed on the standard curve is the linear transformation for assayed, serially diluted domestic fowl plasma, which demonstrates immunoreactive parallelism between AVT of

Figure 2. A typical standard curve of the AVT radioimmunoassay that shows immunoreactivities of arginine vasopressin (AVP), arginine vasotocin (AVT), mesotocin (MT), and oxytocin (OXY) with the R-70 antiserum. The curves were obtained under disequillibrium assay conditions using ^{125}I-AVT as the tracer (see text for details of the RIA procedures).

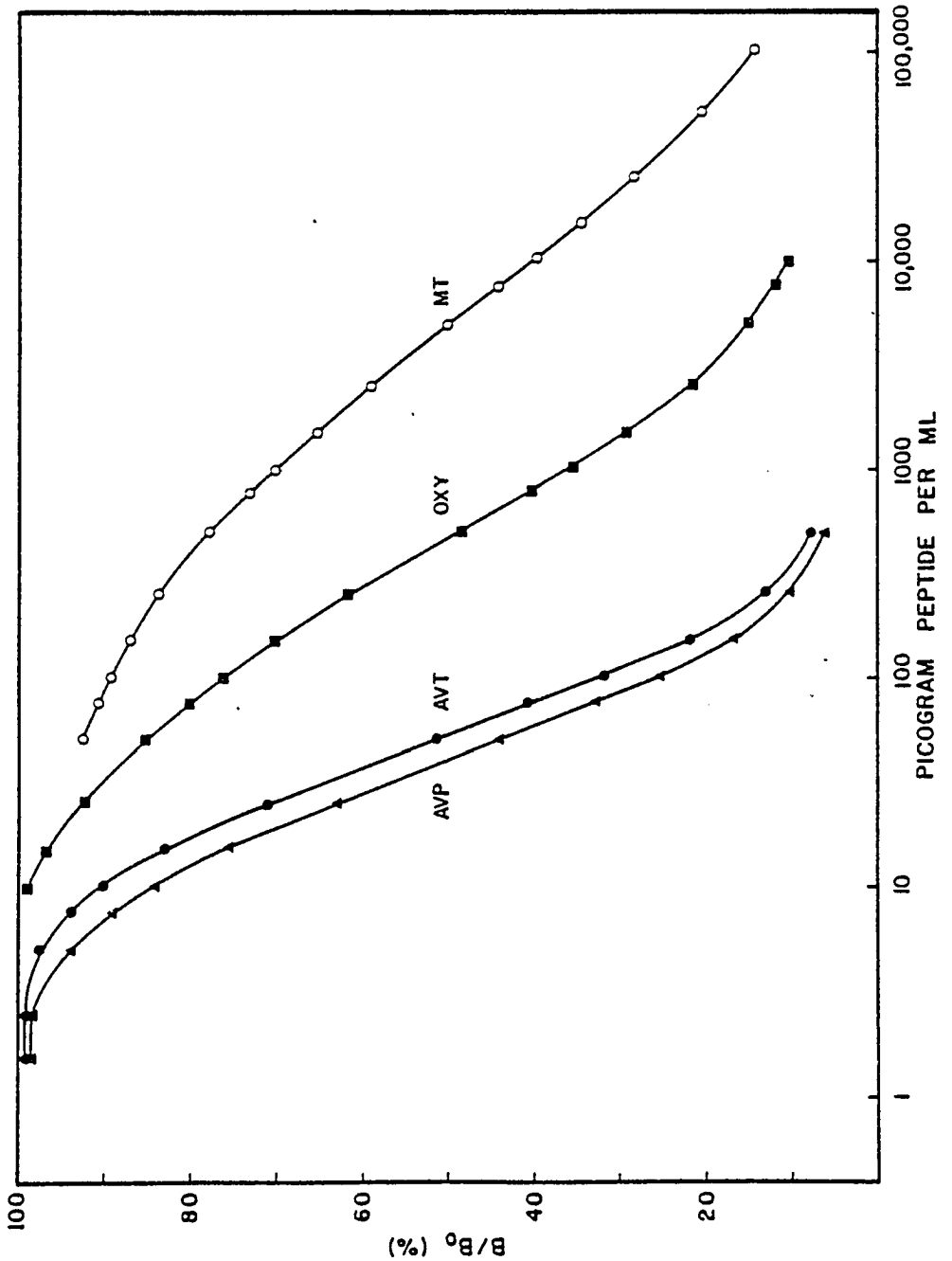
100
80
60
40
20
B/Bo (%)
1
10
100
1000
10,000
100,000
PICOGRAM PEPTIDE PER ML
MT
OXY
AVT
AVP

Figure 2.

Figure 3. Logit-log transformation of the AVT standard curve averaged from 15 assays. Each point represents the mean $\pm$ one standard error. Superimposed on the standard curve is the linear transformation for assayed, serially diluted domestic fowl plasma, which demonstrates immunoreactive parallelism between the AVT of standards and unknowns.

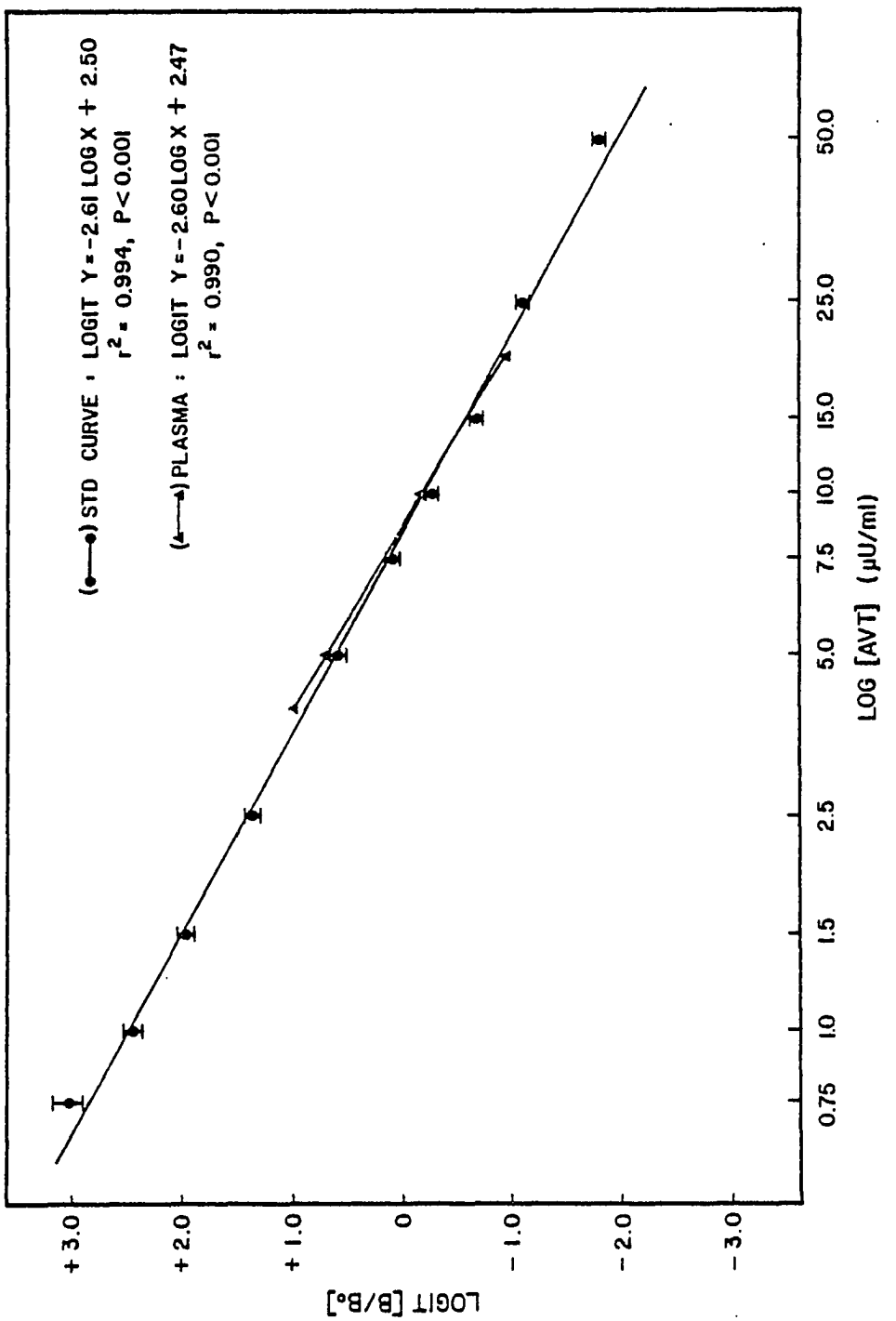
(●—●) STD CURVE : LOGIT Y=-2.61 LOG X + 2.50
r² = 0.994, P<0.001
(▲—▲) PLASMA : LOGIT Y=-2.60 LOG X + 2.47
r² = 0.990, P<0.001
LOGIT [B/Bo]
+3.0
+2.0
+1.0
0
-1.0
-2.0
-3.0
0.75
1.0
1.5
2.5
5.0
7.5
10.0
15.0
25.0
50.0
LOG [AVT] (µU/ml)

Figure 3.

standards and unknowns. Detailed descriptions of plasma collection and handling, plasma extraction, assay, and label iodination procedures will follow.

Collection and Handling of Plasma. Blood was collected in chilled, lithium-heparinized 1.5 ml polyethylene tubes and centrifuged at 4°C for 5 min to separate plasma from cells. Aliquots (500 or 750 µl) of each plasma sample were pipetted into 12 x 75 mm borosilicate glass tubes, sealed with Parafilm (American Can Co.), and stored at -20°C for subsequent RIA of AVT. Plasma samples stored in this manner remained immunoreactively stable for at least 30 days (personal observation), although most samples were extracted and assayed within seven days of collection.

Extraction of Plasma. AVT was extracted from plasma by adsorption onto octadecasilyl-silica (Bennett et al. 1977, LaRochelle, North, and Stern 1980, Crofton et al. 1980) contained in pre-packed cartridges (Sep-Pak C_{18}, Waters Assoc.) according to the techniques of Crofton et al. (1980) and W.H. Sawyer (pers. comm.). Prior to each use, cartridges were primed by sequential washing with 5 ml methanol, 5 ml 8M urea, and 10 ml double distilled water. Then 500 or 750 µl of frozen plasma (thawed and acidified to pH 2-3 with 1 N HCl) was slowly (over 1 min) passed through the cartridge. The plasma sample tube was rinsed with 2 ml double distilled water that was passed through the cartridge. The plasma-loaded cartridge was then washed sequentially with 10 ml double-distilled water and 10 ml 4% glacial acetic acid (v/v). The AVT was eluted with 10 ml of a 40% ethanol-4% glacial acetic acid mixture (v/v). Each cartridge was used for no more than

four samples. The eluate was dried in a vacuum-evaporator (Evapo-mix, Buchler Inst. Co.), and the dried extracted samples sealed with Parafilm and stored at -20°C until RIA, usually within one week. Extracted plasma samples stored in this manner were immunoreactively stable for at least 30 days (personal observation). Recovery of synthetic AVT added to pooled plasma samples (5 or 10 µU) averaged 92.8±2.0% (mean±S.E.).

Radioimmunoassay. The RIA was performed in a final volume of 0.500 ml and utilized the R-70 antiserum at a final dilution of 1:500,000 in a sequential incubation (disequillibrium) system (D.A. Fisher, pers. comm.). Synthetic AVT (Bachem, Inc., lot no. R2750), bioassayed by W.H. Sawyer (210 U/mg, rat pressor activity), was used as the standard. It was dissolved in 0.25% acetic acid - 0.5% chlorobutanol (to 75 µg/ml), sealed in glass vials, and stored in the dark at 4°C. To facilitate serial dilution for the RIA standard curve, an aliquot of the AVT standard was diluted in assay buffer to make a "working standard" of 2 U/ml. A fresh working standard was made up monthly and stored identically as the primary standard. The assay was performed in 12 x 75 mm borosilicate glass tubes as follows: 50 µl di-sodium EDTA (0.1 M, pH 7.2) and 200 µl assay buffer (0.15 M phosphate buffer with 0.1% sodium azide (w/v) and 0.25% normal rabbit serum (v/v), pH 7.2) were added to duplicate standard and unknown tubes. Then 100 µl aliquots of either AVT standards (0.50 - 50.0 µU/ml) or previously extracted unknowns (resuspended in assay buffer) were added to the appropriate tubes. The antiserum was diluted in assay buffer (1:50,000), a 50 µl aliquot was added to each

tube, and after 24 hr preincubation at 4°C, a 100 µl aliquot of ^{125}I-AVT (2,000-2,200 cpm) was added and the tubes incubated for 72 hr at 4°C. Additional tubes were prepared to determine non-specific binding (no antiserum) and zero-dose binding (no standard). Free and bound hormone were separated using a modification (D.A. Fisher, pers. comm.) of the polyethylene glycol method (Desbuquois and Aurbach 1971). Fifty microliters of burro anti-rabbit gamma globulin (Immunocorp.) followed by 100 µl of 25% PEG solution (w/v) were added to each tube, and after thorough mixing, the tubes were centrifuged at 2,000 x g for 45 min at 4°C. The resulting supernatant was removed by aspiration and the precipitate (bound fraction) counted to 1% error in an automatic well-type gamma counter (Model 1185, Searle Analytic).

Radioiodination of AVT. The labelling of AVT with ^{125}iodine was performed by the North et al. (1976) modification of the lactoperoxidase method of Thorell and Johanssen (1971), adapted for AVT by W.H. Sawyer (pers. comm.). Synthetic AVT (3.9 µg, sp. act. 210 U/mg; Bachem, Inc., lot no. R2750) was mixed with lactoperoxidase (244 mU, sp. act. 122 IU/mg; Calbiochem-Behring) in 20 µl of 0.05 M sodium phosphate buffer, pH 7.0. To this mixture was added 1.0 mCi (10 µl) of carrier-free [^{125}I] NaI solution (sp. act. 100 mCi/ml, 11-17 mCi/mg; Amersham Radiochemicals), and to initiate the reaction, 10 µl of 0.003% aqueous solution of H_2O_2. The reaction mixture was stirred immediately and allowed to proceed at room temperature (22-24°C) for 5 min. The reaction was stopped by dilution of the reaction mixture with 500 µl of phosphate buffer. [^{125}I] monoiodo-AVT was isolated according to the W.H. Sawyer (pers. comm.)

modification of the North et al. (1976) method by subjecting the reaction products to anion exchange chromatography (0.7 x 1.0 cm column of AG2-X8, acetate form; Biorad. Labs.) to remove free iodide, and to gel filtration chromatography (0.9 x 60 cm column of Sephadex G-25, Pharmacia, Inc.) equilibrated with a 0.125% solution (w/v) of bovine serum albumin in 0.05 M acetic acid. The peak and first trailing fractions of the third peak ([^{125}I] monoiodo-AVT) were collected for use in RIA, diluted in assay buffer to 1 x 10^6 cpm/ml, and stored at -70°C. Undamaged [^{125}I] monoiodo-AVT produced by this technique has a specific activity of 1,400-1,900 µCi/nmol (1,500-2,000 µCi/µg peptide) and was identified by its ability to bind excess antibody at $\geq$ 95%, and RIA-diluted antibody (1:500,000) at 35-40% (W.H. Sawyer, pers. comm.).

Additional Analyses of Plasma

Aliquots of plasma from each experiment were stored either at 4°C (for rapid analysis of osmolality) or at -20°C (for later analyses of electrolytes and plasma protein). Plasma osmolalities were measured by vapor pressure osmometry using a Wescor osmometer (model 5100B; Wescor, Inc.) with a sample size of 8.0 µl. The variation of any one sample (3-5 replicate measurements) was $\pm$0.5% (standard error). All measurements were made on fresh samples of plasma kept at 4°C and not previously frozen. Plasma electrolytes (Na^+ and K^+) were measured by flame photometry with an internal lithium standard (model 143, Instrumentation Laboratories, Inc.) using a sample size of 10 µl. Plasma protein concentrations were determined

spectrophotometrically using the biuret protein assay modified for micro-samples (10 µl, Itzhaki and Gill 1964). The assay was read at 545 nm using a Spinco Spectro-Colorimeter (model 151, Beckman Inst., Inc.). Hematocrit (percent packed cell volume) of arterial blood samples was determined by the microhematocrit method. Duplicate heparinized microhematocrit tubes were filled directly from the arterial catheter and immediately sealed and centrifuged at 13,000 x g for 5 min using a microhematocrit centrifuge (model MB, International Eqpmt. Co.). The tubes were read to the nearest 0.25 mm, the hematocrits calculated, and duplicate values averaged.

Statistical Analyses

All data are presented as the mean ± one standard error. Data groups were subjected to one-way analysis of variance (ANOVA) to detect significant differences, followed by the Student Newman-Keuls test to distinguish significant differences among the means of a data group (Zar 1974). Linear regression analysis was performed by the least-squares method (Zar 1974). All statistical analyses were performed on a programmable desk-top calculator (model HP-97, Hewlett-Packard, Inc.) programmed from the Hewlett-Packard Statistical Package.

Results

Osmotic Regulation of Plasma AVT

The effects of saline infusion on plasma osmolality (P_{OSM}) are shown in Figure 4. Pre-infusion P_{OSM} of the three experimental groups were nearly identical and averaged 308.3±1.3,

Figure 4. Changes in plasma osmolality of domestic fowl in response to saline infusion. Each point represents the mean $\pm$ one standard error (n = sample size). $^{a-f}$Within each experimental group, mean values without common script are significantly different ($P < 0.01$). *Mean values of the control infusion group are not significantly different ($P > 0.05$).

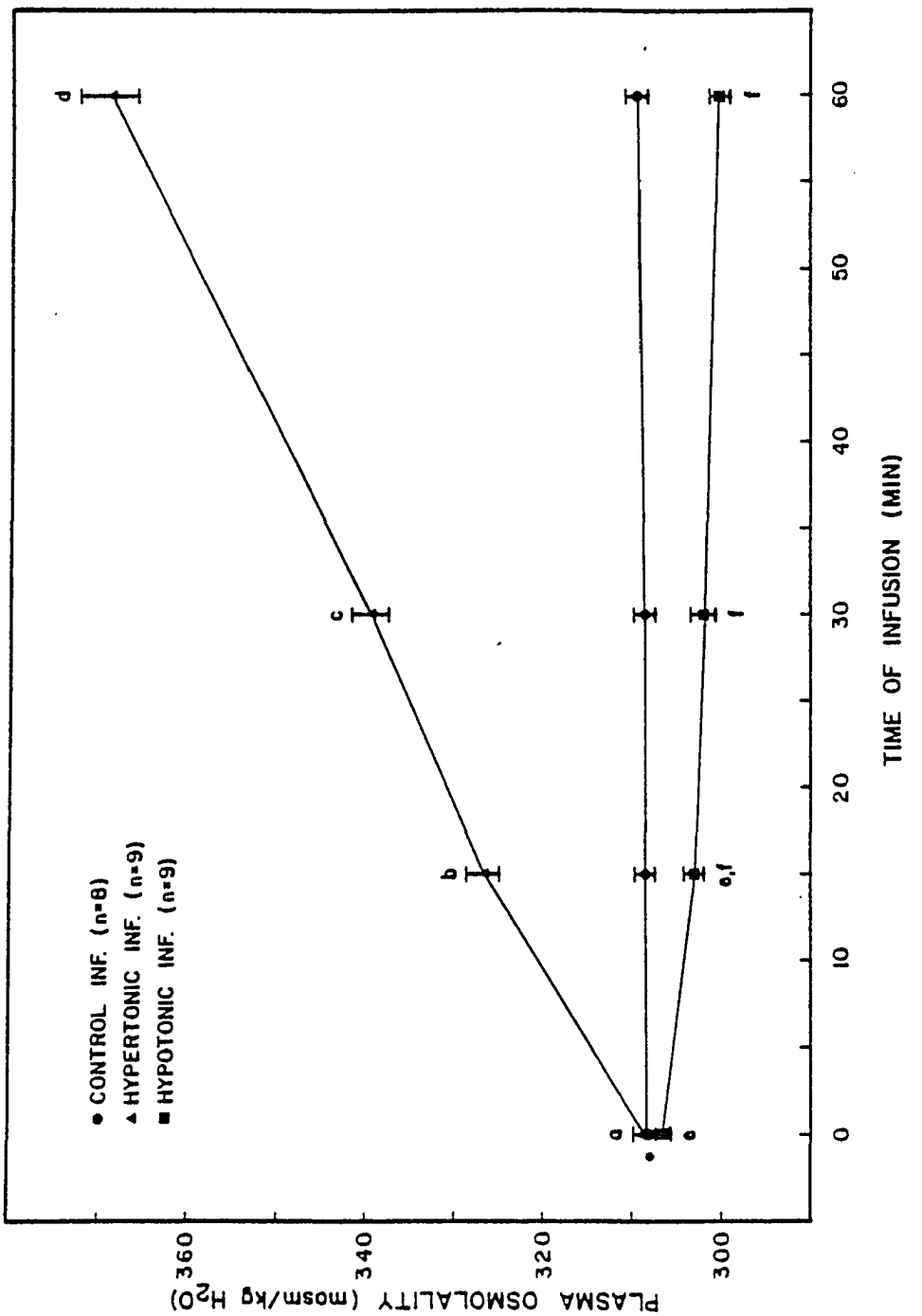
PLASMA OSMOLALITY (mosm/kg H_2O)
300
320
340
360
0
10
20
30
40
50
60
TIME OF INFUSION (MIN)
● CONTROL INF. (n=8)
▲ HYPERTONIC INF. (n=9)
■ HYPOTONIC INF. (n=9)
a
b
c
d
e
e,f
f
f
f

Figure 4.

308.9±1.0, and 307.1±0.7 mosm/kg H_2O, respectively, for control, hypertonic, and hypotonic group birds. Infusion of isotonic saline (control group) had no effect on P_{OSM} at 15, 30, or 60 min ($P > 0.05$). Hypertonic group birds exhibited a significant ($P < 0.01$) linear increase in P_{OSM} that averaged nearly 20% at 60 min of infusion. In response to distilled water infusion, P_{OSM} of hypotonic group birds declined gradually but significantly at 30 and 60 min to a mean minimum of 300.5±1.0 mosm/kg H_2O at 60 min ($P < 0.01$).

Changes in plasma AVT concentrations (P_{AVT}) of the birds in response to saline infusion paralleled the observed changes in P_{OSM}, as shown in Figure 5. Baseline P_{AVT} levels of the birds were quite similar and averaged 3.4±0.6, 2.8±0.3, and 2.4±0.3 μU/ml, respectively, for control, hypertonic, and hypotonic groups (16.2±2.9, 13.3±1.4 and 11.4±1.4 pg/ml using the specific conversion factor of 4.76 pg/μU). Isotonic saline infusion had no significant effects ($P > 0.05$) on P_{AVT} of control birds. Infusion of hypertonic saline resulted in a dramatic, linear increase in P_{AVT} that exceeded baseline by more than seventeen-fold at 60 min ($P < 0.01$). Hypotonic group birds displayed a gradual decrease in P_{AVT} that was significant at 30 and 60 min of infusion ($P < 0.01$). Plasma AVT levels of several birds in this group were barely detectable (0.50-0.75 μU/ml) after 60 min of infusion.

Plasma AVT was significantly correlated with P_{OSM} in hypertonic and hypotonic groups. Because the isotonic (control) infusion data indicated that P_{AVT} did not vary significantly at

Figure 5. Changes in plasma AVT concentration of domestic fowl in response to saline infusion. Each point represents the mean ± one standard error (n = sample size). $^{a-f}$Within each experimental group, mean values without common script are significantly different ($P < 0.01$). *Mean values of the control infusion group are not significantly different ($P > 0.05$).

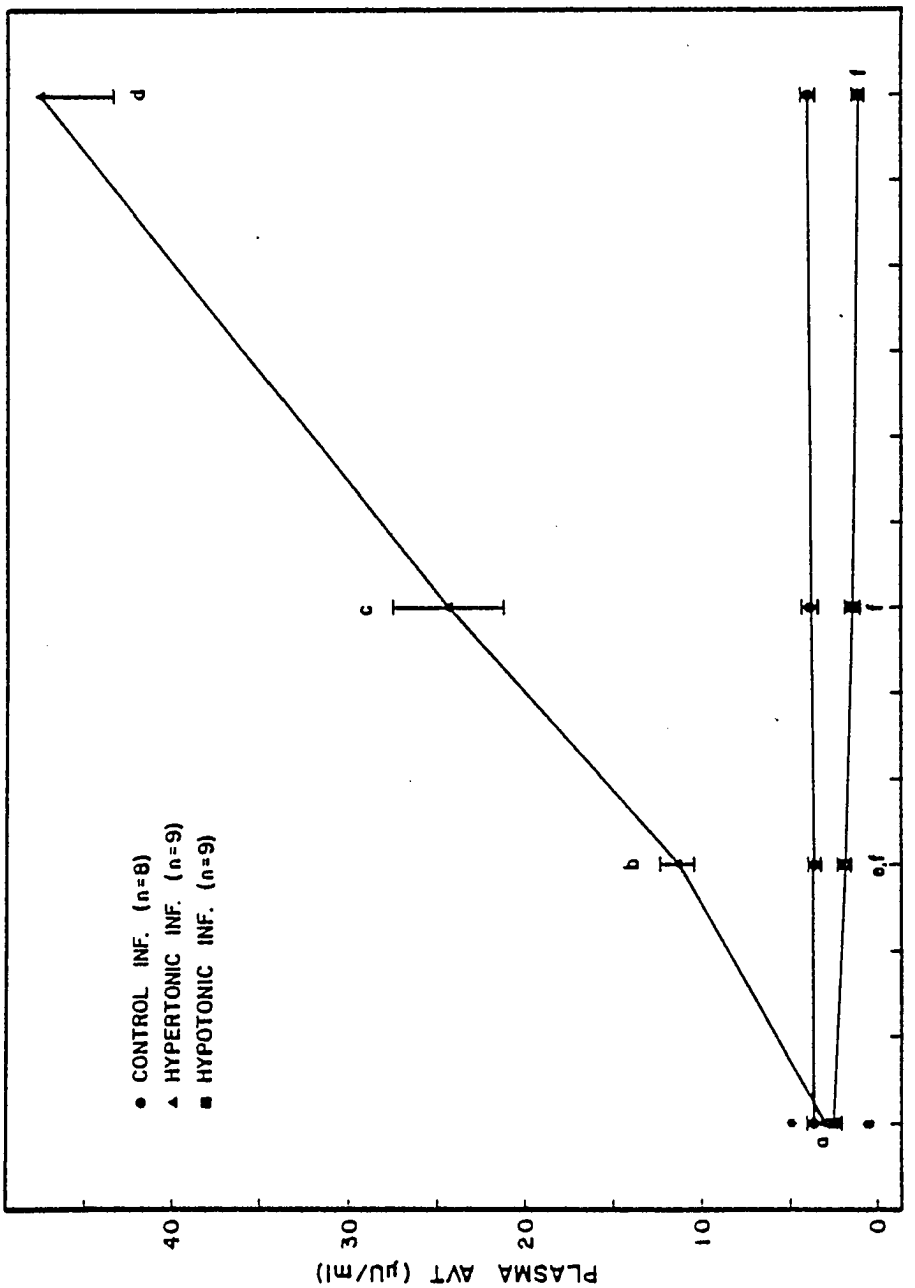
PLASMA AVT (μU/ml)
0
10
20
30
40
0
10
20
30
40
50
60
TIME OF INFUSION (MIN)
● CONTROL INF. (n=8)
▲ HYPERTONIC INF. (n=9)
■ HYPOTONIC INF. (n=9)
a
b
c
d
e,f
f
f

Figure 5.

the baseline P_{OSM} of 308 mosm/kg H_2O, it was decided a priori to analyze the P_{OSM}-P_{AVT} relationships of the hypertonic and hypotonic birds independently; these relationships are summarized in Figure 6. Hypertonic group birds with P_{OSM} of > 308 mosm/kg H_2O exhibit a highly correlated, significant relationship between P_{OSM} and P_{AVT} (r^2=0.889, $P < 0.001$) described by the linear regression equation: $P_{AVT}(\mu U/ml) = 0.77\ (P_{OSM} - 308.1)$. Hypotonic group birds with P_{OSM} < 308 mosm/kg H_2O exhibit a less highly correlated but nonetheless significant relationship between P_{OSM} and P_{AVT} ($r^2 = 0.334$, $P < 0.001$) described by the equation: $P_{AVT}\ (\mu U/ml) = 0.12(P_{OSM} - 288.8)$. The latter equation indicates an osmolal threshold for AVT secretion at 288.8 mosm/kg H_2O.

The effects of saline infusion on hematocrit and plasma protein and electrolyte concentrations are summarized in Table 2. Initial hematocrits (HCT) of the experiment animals were quite similar and did not differ significantly ($P > 0.05$). With infusion, HCT tended to decline in all birds; however, this trend was significant only in hypertonic and isotonic group birds ($P < 0.01$), and averaged, respectively, 29.3±2.5 or 13.3±0.6% at 60 min of infusion. Plasma protein concentrations (P_{PROT}) were similar at baseline and did not differ significantly ($P > 0.05$). As with HCT, P_{PROT} tended to decline with infusion; however, this trend was significant only in hypertonic group birds and averaged 32.7% at 60 min of infusion. Plasma sodium concentrations ($P_{Na}+$) of the three experimental groups were nearly identical prior to infusion ($P > 0.05$) and exhibited patterns identical to those of P_{OSM} in

Figure 6. The relationship between plasma osmolality and plasma AVT concentration in normally hydrated and saline infused domestic fowl. The P_{OSM} - P_{AVT} data of hypotonic birds (289 - 308 mosm/kg H_2O) and hypertonic birds (308 - 395 mosm/kg H_2O) were analyzed separately.

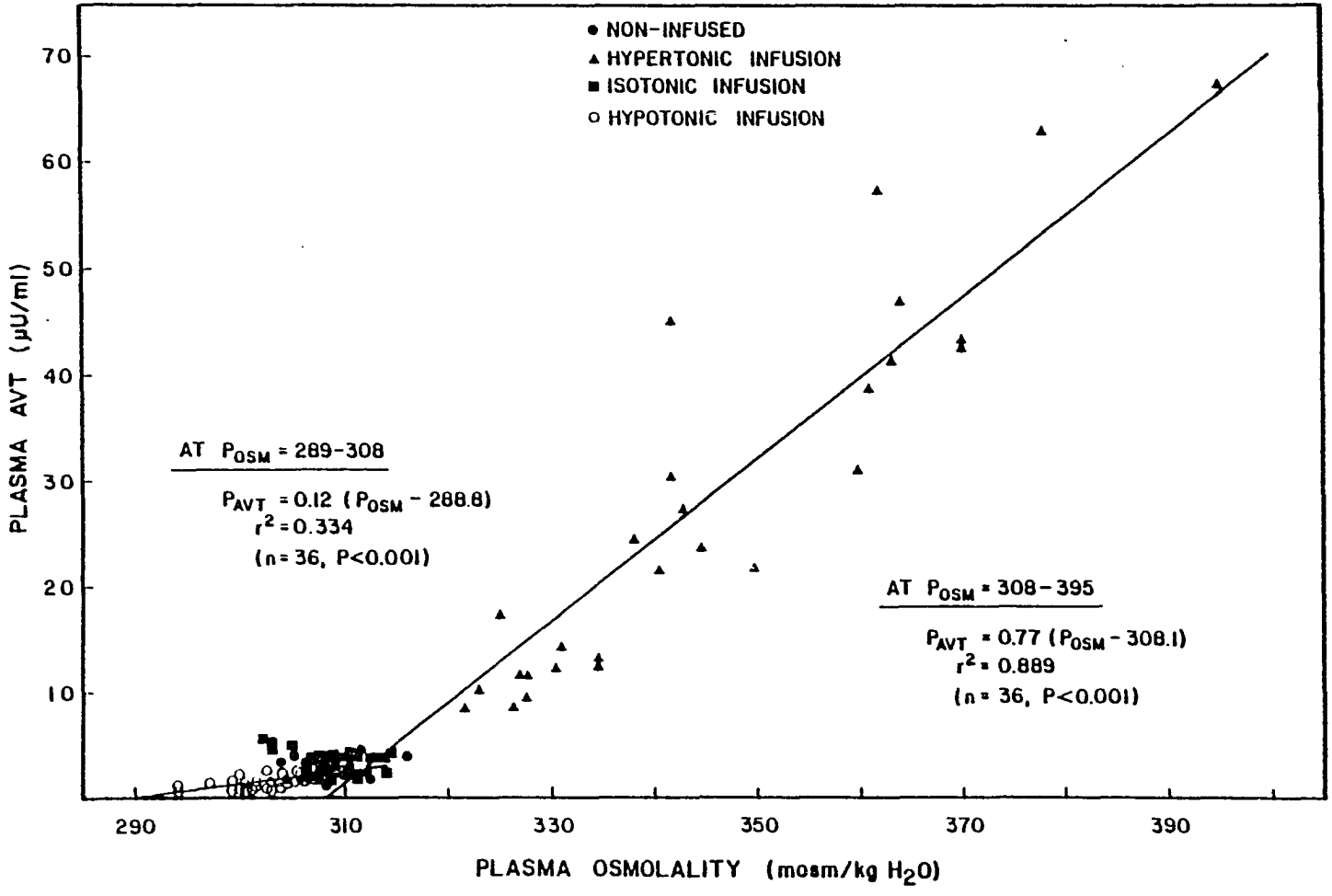
• NON-INFUSED
▲ HYPERTONIC INFUSION
■ ISOTONIC INFUSION
○ HYPOTONIC INFUSION
AT P_{OSM} = 289-308
P_{AVT} = 0.12 (P_{OSM} - 288.8)
r^2 = 0.334
(n = 36, P<0.001)
AT P_{OSM} = 308-395
P_{AVT} = 0.77 (P_{OSM} - 308.1)
r^2 = 0.889
(n = 36, P<0.001)
PLASMA AVT (μU/ml)
PLASMA OSMOLALITY (mosm/kg H_2O)
10
20
30
40
50
60
70
290
310
330
350
370
390

Figure 6.

Table 2. Hematocrit and plasma concentrations of protein, sodium, and potassium in saline infused domestic fowl.[a]

Variable	Experimental Group	Time of Infusion (min) 0	15	30	60
Hematocrit (% PCV)	Control (n=8)	25.9±0.6[b]	24.6±0.5[b,c]	23.5±0.6[b,c]	22.4±0.6[c]
	Hypertonic (n=9)	26.0±1.0[d]	22.0±1.0[e]	20.3±1.0[e]	18.5±0.8[e]
	Hypotonic (n=9)	26.7±0.3*	26.2±0.4	26.0±0.3	25.7±0.4
Plasma Protein (g/dl)	Control (n=8)	4.16±0.12*	4.22±0.13	4.05±0.14	4.11±0.21
	Hypertonic (n=9)	4.90±0.40[f]	3.90±0.22[g]	3.63±0.15[g]	3.30±0.14[g]
	Hypotonic (n=9)	4.95±0.16*	4.85±0.14	4.69±0.16	4.57±0.16
Plasma Na^+ (meq/l)	Control (n=8)	145.6±0.9*	146.7±0.7	146.3±0.9	146.1±0.9
	Hypertonic (n=9)	147.0±0.5[h]	157.9±1.6[i]	164.2±2.0[j]	179.1±1.8[k]
	Hypotonic (n=9)	145.8±0.7*	144.1±0.7	142.7±0.9	142.5±0.8
Plasma K^+ (meq/l)	Control (n=8)	4.2±0.09*	4.2±0.08	4.1±0.09	4.2±0.18
	Hypertonic (n=9)	4.2±0.06[l]	3.9±0.08[l,m]	3.7±0.11[m,n]	3.4±0.08[n]
	Hypotonic (n=9)	4.2±0.03*	4.1±0.04	4.1±0.06	4.1±0.05

[a]All values are mean ± S.E. (n = sample size)

[b-n]Within a row, mean values without common superscript are significantly different ($P < 0.01$).

*Within this row, mean values are not significantly different ($P > 0.05$).

response to saline infusion. Baseline plasma potassium concentrations (P_K+) were identical among the three groups ($P > 0.05$) and were not significantly altered by isotonic or hypotonic infusion ($P > 0.05$). Plasma potassium of hypertonic group birds declined progressively with infusion and was significantly reduced by an average of 19.0% at 60 min of infusion ($P < 0.01$).

Volemic Regulation of Plasma AVT

Blood Volume and Marker Validation. As determined by the ^{51}Cr-RBC method, mean blood volume (BV) of Rhode Island Red cockerels (1237±35.8g body weight) is 56.5±2.0 ml/kg (5.65±0.20% body weight) at a corresponding mean HCT of 25.6±0.5% (n=13). In these animals, volume expansion resulted in a highly correlated and significant relationship between percent change in BV and percent change in HCT ($r^2 = 0.900$, $P < 0.001$) described by the linear regression equation: $y = 0.82x + 1.37$, and illustrated in Figure 7. These data indicate that changes in HCT can be used reliably to estimate changes in blood volume during acute experimentation.

Effects of Volume on Plasma AVT. The effects of isosmotic volume expansion on P_{AVT} were summarized in the previous section (see "control group" under osmotic regulation of P_{AVT}). In those experiments, infusion of isotonic saline did not alter P_{AVT} significantly ($P > 0.05$), despite significant ($P < 0.01$) isosmotic blood volume expansion that averaged 13.3% (based on HCT data) after 60 min of infusion (see Figs. 4 and 5, Table 2).

Figure 7. The relationship between increases in blood volume and decreases in hematocrit in domestic fowl intravenously infused with isotonic saline.

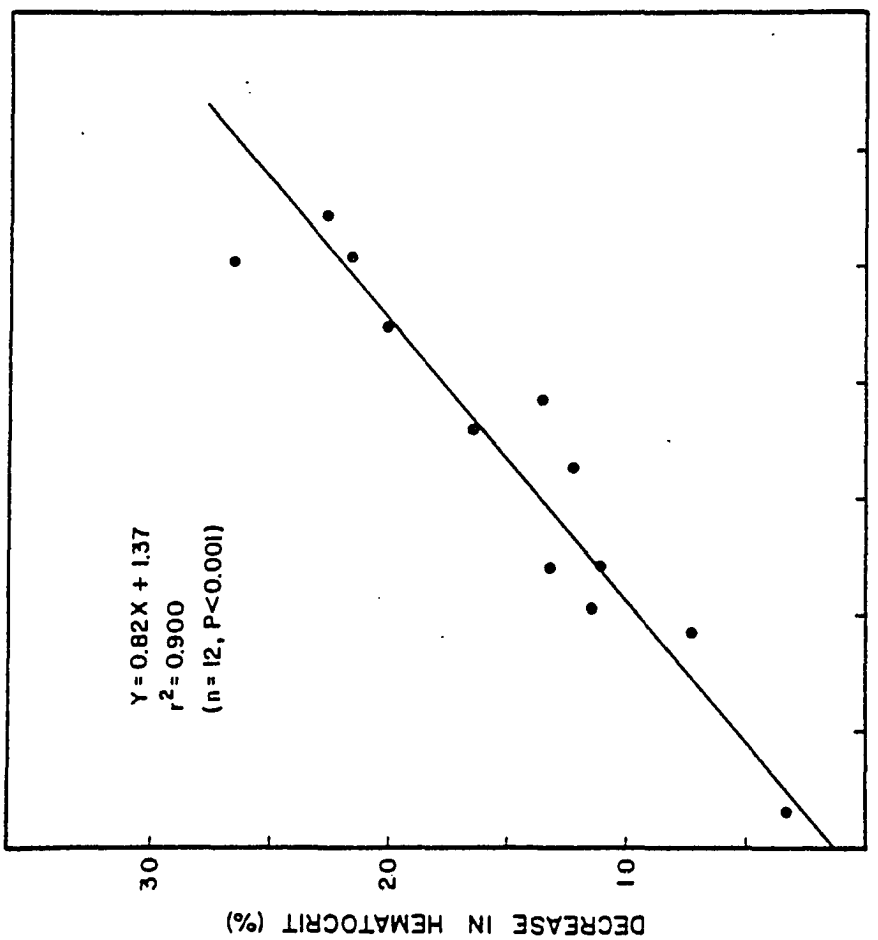
Y = 0.82X + 1.37
r² = 0.900
(n = 12, P<0.001)
DECREASE IN HEMATOCRIT (%)
INCREASE IN BLOOD VOLUME (%)
10
20
30
10
20
30

Figure 7.

The effects of isosmotic volume reduction on AVT secretion were assessed through acute arterial hemorrhage (HEM). The effects of HEM on HCT are summarized in Figure 8. Pre-HEM HCT of the three experimental groups were similar and averaged 27.5±0.8, 28.0±0.7 and 26.4±0.6%, respectively, for time-control, 10%, and 20% HEM birds. Hematocrit of time-control birds tended to decrease slightly with time, although the decline was not significant ($P > 0.05$). In response to HEM, HCT of both 10% and 20% HEM group birds decreased progressively at 2.5 and 5.0 min post-HEM, followed by a gradual but incomplete return to pre-HEM levels during the post-reinfusion (RIF) period. Because of significant animal-to-animal variability in these data, the observed changes in mean HCT are not significant ($P > 0.05$). However, when HCT data from individual birds are used to compute changes in HCT (as percent of initial pre-HEM HCT), the observed progressive decreases in HCT at 2.5 and 5.0 min post-HEM are significant, when compared to the corresponding data of the time-control birds ($P < 0.01$, Table 3). Because of the HCT-BV relationship established in these studies (see Fig. 7), the observed reductions in HCT also reflect increases in BV of equivalent magnitude. Thus, within 5.0 min post-HEM, more than 50% of the blood volume removed during HEM had been replenished in both 10% and 20% HEM group birds (see Table 3).

The effects of HEM on heart rate (HR) and mean arterial blood pressure (MAP) are summarized in Figure 9. Pre-HEM HR were very similar among all birds, and averaged 349±8.7, 360±5.9, and 351±7.3

Figure 8. Changes in hematocrit of domestic fowl in response to acute arterial hemorrhage (HEM) and subsequent reinfusion (RIF) of shed blood. Each point represents the mean $\pm$ one standard error (n = sample size). *Within each experimental group, mean values are not significantly different ($P > 0.05$).

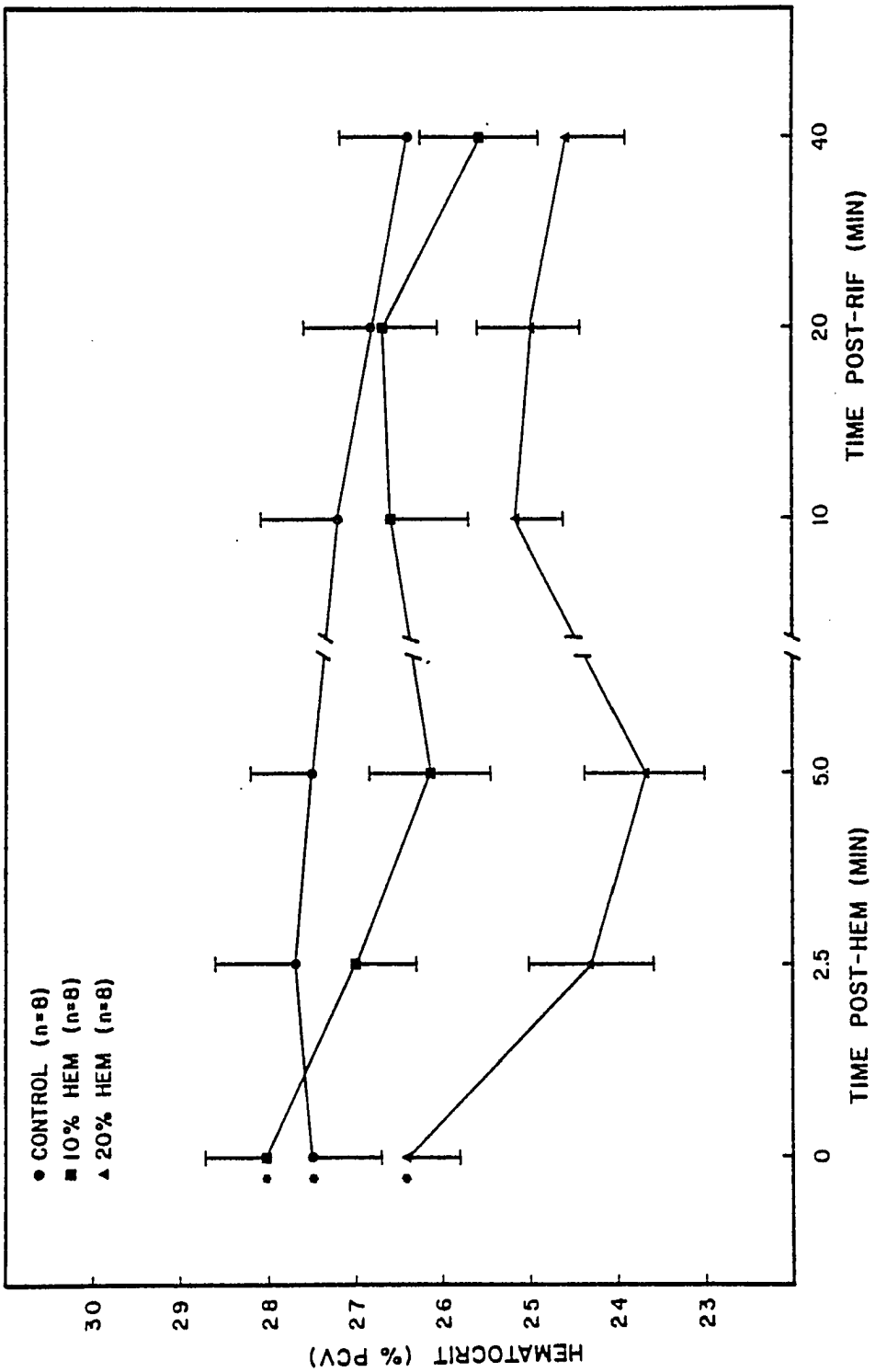
● CONTROL (n=8)
■ 10% HEM (n=8)
▲ 20% HEM (n=8)
HEMATOCRIT (% PCV)
30
29
28
27
26
25
24
23
0
2.5
5.0
10
20
40
TIME POST-HEM (MIN)
TIME POST-RIF (MIN)

Figure 8.

Table 3. Decreases in hematocrit (as percent of initial pre-hemorrhage hematocrit) in hemorrhaged and subsequently reinfused domestic fowl.[a]

Experimental Group	Time After Hemorrhage (min)			Time After Reinfusion (min)		
	0	2.5	5.0	10	20	40
Control (n=8)	---	+0.35±1.20	0.28±1.34	1.50±1.33	2.93±1.55	4.31±1.16
10% HEM (n=8)	---	3.77±0.64*	6.92±0.95*	5.45±1.62	5.24±1.53	8.29±1.14
20% HEM (n=8)	---	8.88±1.56*	11.70±1.68*	4.93±1.22	5.40±1.35	7.85±1.81

[a]All values are mean ± S.E. (n = sample size). (+) signifies an increase in hematocrit.

*Within a column, mean HEM values are significantly different from mean control value ($P < 0.01$).

Figure 9. Changes in heart rate (upper figure) and mean arterial blood pressure (lower figure) of domestic fowl in response to acute arterial hemorrhage (HEM) and subsequent reinfusion (RIF) of shed blood. Each point represents the mean $\pm$ one standard error (n = sample size). $^{a-d}$ Within each experimental group, mean values without common script are significantly different ($0.05 > P > 0.01$). *Within each experimental group, mean values are not significantly different ($P > 0.05$).

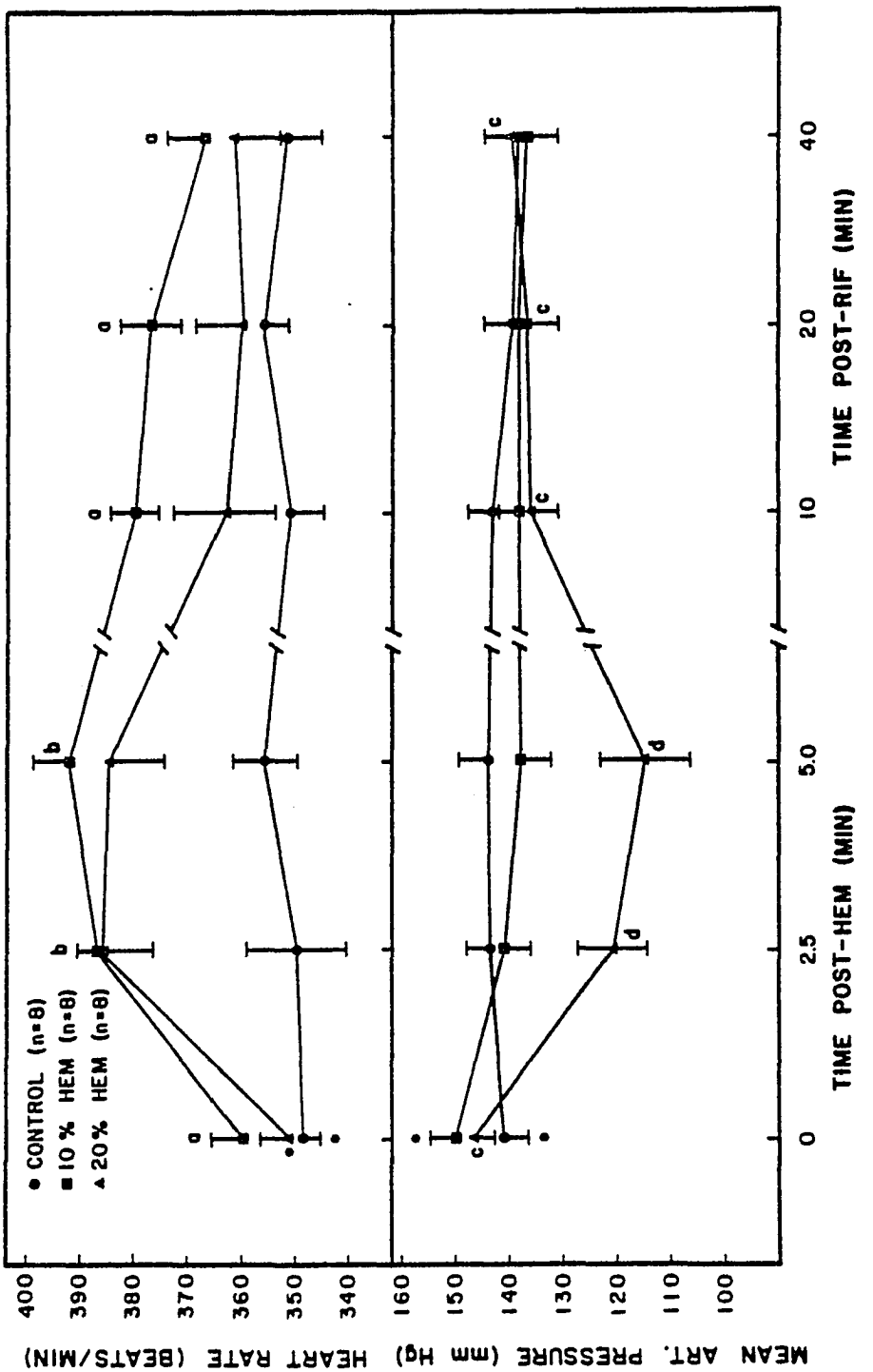
● CONTROL (n=8)
■ 10 % HEM (n=8)
▲ 20 % HEM (n=8)
HEART RATE (BEATS/MIN)
MEAN ART. PRESSURE (mm Hg)
TIME POST-HEM (MIN)
TIME POST-RIF (MIN)
0
2.5
5.0
10
20
40
400
390
380
370
360
350
340
160
150
140
130
120
110
100
a
b
c
d

Figure 9.

beats per min, respectively, for control, 10%, and 20% HEM groups. Mean HR of control birds did not vary by more than 7 beats per min over the course of the experiment. In response to HEM, mean HR of 10% and 20% HEM group birds increased sharply at 2.5 min post-HEM, and remained elevated at 5.0 min post-HEM. Following RIF, mean HR declined to pre-HEM levels in both HEM groups; the decline was more gradual in the 10% HEM group. The observed increases in HR during post-HEM were significant ($P < 0.05$) only in the 10% HEM group. HR data of the 20% HEM group tended to be more variable as a result of two birds that exhibited only slight increases in HR in response to HEM. Pre-HEM MAP of control, 10%, and 20% HEM group birds did not differ significantly ($P > 0.05$) and averaged 141 ± 4.8, 150 ± 4.5, and 147 ± 4.1 mmHg, respectively (see Fig. 9). MAP of control birds exhibited only slight, insignificant variation over the time course of the experiment ($P > 0.05$). In 10% HEM group birds, MAP declined slightly but insignificantly ($P > 0.05$) at 2.5 and 5.0 min post-HEM, whereas MAP of 20% HEM groups birds exhibited a pronounced and progressive reduction at 2.5 and 5.0 min post-HEM that was significant ($P < 0.05$). Following RIF, MAP of 10% HEM birds remained at the 5.0 min post-HEM level, whereas MAP of 20% HEM birds returned to pre-HEM levels.

Acute HEM was ineffective in altering secretion of AVT; this is clearly shown by the lack of significant changes in P_{AVT}, summarized in Figure 10. Pre-HEM P_{AVT} averaged 1.6 ± 0.3, 2.8 ± 0.4, and 2.9 ± 0.5 μU/ml, respectively, in control, 10%, and 20% HEM group birds. Mean P_{AVT} of control birds varied slightly but insignificantly

Figure 10. Changes in plasma AVT concentration of domestic fowl in response to acute arterial hemorrhage (HEM) and subsequent reinfusion (RIF) of shed blood. Each point represents the mean ± one standard error (n = sample size). *Within each experimental group, mean values are not significantly different ($P > 0.05$).

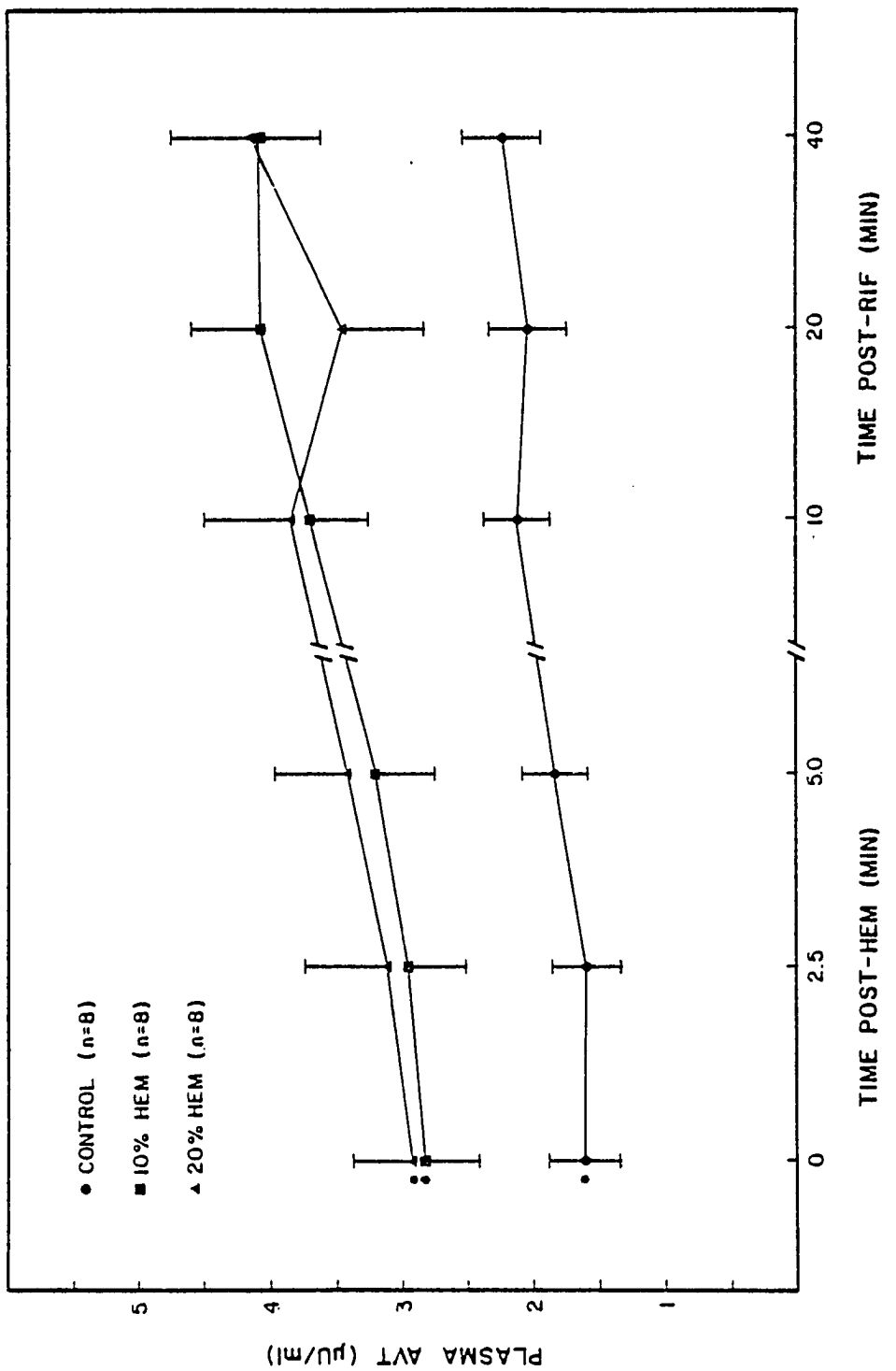
PLASMA AVT (μU/ml)
1
2
3
4
5
● CONTROL (n=8)
■ 10% HEM (n=8)
▲ 20% HEM (n=8)
0
2.5
5.0
10
20
40
TIME POST-HEM (MIN)
TIME POST-RIF (MIN)

Figure 10.

over the time course of the experiment ($P > 0.05$). Mean P_{AVT} of 10% and 20% HEM birds exhibited similar insignificant variation in response to HEM and RIF ($P > 0.05$). To control for possible errors in the AVT blood sampling protocol that would prevent detection of HEM-related changes in P_{AVT}, additional blood samples were taken at 10 min post-HEM from several birds in the 20% HEM group. Mean P_{AVT} of these birds at 10 min post-HEM (5.0 ± 1.1 μU/ml) did not differ significantly from P_{AVT} at 5.0 min post-HEM (5.2 ± 0.9 μU/ml; n=3, $P > 0.05$). The consistently lower mean P_{AVT} levels of the time-control group were the result of several birds that were randomly assigned *a priori* to the control group which exhibited lower than average baseline P_{AVT} levels.

The effects of HEM on plasma osmolality, electrolytes and protein are summarized in Table 4. Baseline P_{OSM} of the three groups were nearly identical prior to HEM and did not differ significantly ($P > 0.05$). Mean P_{OSM} of the control group varied slightly but insignificantly over the time course of the experiment ($P > 0.05$). P_{OSM} of 10% and 20% HEM birds exhibited similar insignificant variation in response to HEM or RIF ($P > 0.05$). Plasma sodium and P_{K^+} data displayed a similar lack of variability and did not differ significantly in response to HEM or RIF ($P > 0.05$). Plasma protein of the three experimental groups were similar prior to HEM, and exhibited patterns similar to those of the HCT data in response to HEM and RIF (see Fig. 8). Because of similar animal-to-animal variability in the P_{PROT} data, the observed changes in mean P_{PROT}

Table 4. Plasma osmolality and plasma concentrations of sodium, potassium, and protein in hemorrhaged and subsequently reinfused domestic fowl. [a,b]

Variable	Experimental Group	Time After Hemorrhage(min)			Time After Reinfusion(min)		
		0	2.5	5.0	10	20	40
Plasma Osmolality (mosm/kg H_2O)	Control (n=8)	309.2±0.7	308.4±1.0	308.2±0.9	308.0±0.8	309.2±0.9	307.9±0.5
	10% HEM (n=8)	309.8±0.9	310.4±0.9	308.8±0.6	310.3±0.8	309.2±0.7	310.2±0.7
	20% HEM (n=8)	310.5±0.9	310.6±0.9	310.9±1.0	308.5±0.9	310.2±1.0	310.5±0.9
Plasma Na+ (meq/l)	Control (n=8)	150.9±0.6	151.1±0.4	151.4±0.7	151.6±0.6	151.9±0.5	152.2±0.3
	10% HEM (n=8)	148.8±0.5	148.6±0.2	149.7±0.6	148.4±0.8	149.4±0.5	148.7±0.6
	20% HEM (n=8)	149.2±0.6	148.8±0.6	149.3±1.1	150.3±0.6	149.6±0.7	150.2±0.7
Plasma K+ (meq/l)	Control (n=8)	4.2±0.06	4.0±0.04	3.9±0.02	4.1±0.07	4.0±0.06	4.0±0.06
	10% HEM (n=8)	4.1±0.10	4.1±0.10	4.2±0.14	4.1±0.06	4.0±0.08	4.0±0.05
	20% HEM (n=8)	4.2±0.07	4.0±0.10	4.0±0.11	4.0±0.08	4.0±0.06	4.3±0.18
Plasma Protein (g/dl)	Control (n=8)	5.69±0.13	5.56±0.13	5.56±0.13	5.37±0.13	5.32±0.12	5.37±0.15
	10% HEM (n=8)	4.98±0.14	4.80±0.15	4.66±0.16	4.77±0.19	4.77±0.13	4.70±0.15
	20% HEM (n=8)	4.62±0.24	4.35±0.22	4.07±0.29	4.33±0.23	4.44±0.22	4.41±0.18

[a] All values are mean ± S.E. (n = sample size).

[b] For all variables, means within each experimental group do not differ significantly ($P > 0.05$).

are not significant ($P > 0.05$), although computed changes in post-HEM P_{PROT} are quite similar to those calculated for HCT (see Table 3).

Discussion

In the present studies, recently developed radioimmunoassay (RIA) techniques were employed to quantitatively characterize the secretion of AVT by the avian hypothalamo-neurohypophysial system. The use of i.v. saline infusion and acute arterial hemorrhage permitted independent characterization of osmotic and volemic regulation of AVT secretion.

Osmotic Regulation of Plasma AVT

The basal plasma AVT (P_{AVT}) of domestic fowl in the present study is comparable to previously reported RIA measurements of P_{AVT} in conscious, normally hydrated domestic fowl and Pekin ducks (Table 5). From inspection of these data, it is apparent that basal P_{AVT} levels are similar among various breeds and species of birds, but vary with the plasma osmolality (P_{OSM}) maintained during normal hydration.

In the present study, the results of the saline infusion experiments provide conclusive evidence that P_{OSM} is a primary determinant of AVT secretion by the hypothalamo-neurohypophysial system of the domestic fowl. The primary functional characteristics of this system, sensitivity and secretion threshold, can be quantitatively defined by linear regression analysis of the relationship between P_{OSM} and P_{AVT} concentration established during experimental manipulation of P_{OSM}. Thus, the slope and x-intercept of the regression line that relates P_{OSM} and P_{AVT}

Table 5. Radioimmunoassay measurements of basal plasma AVT concentrations and corresponding plasma osmolalities of conscious, normally hydrated birds as reported in past and present studies.[a]

Bird	Plasma AVT (pg/ml)	Plasma Osmolality (mosm/kg H_2O)	Reference
Domestic Fowl (White Leghorn)	6.6±1.4[b]	315.0±3.6	Koike et al. 1977
Domestic Fowl (White Leghorn)	25.5±6.9[b] 36.3±15.4[b]	314.0±2.3 320.0±6.7	Koike et al. 1979
Pekin Duck	5.4±1.5[c,e] 23.9±3.2[c,f]	293.7±2.2 330.0±7.6	Mohring et al. 1980
Pekin Duck	5.8±0.4[c,e]	297.6±1.2	Gray and Simon 1983
Domestic Fowl (Rhode Island Red)	12.9±1.0[d,g]	308.1±0.6	Present Study

[a]All values are mean ± S.E. For purposes of comparison, AVT concentrations have been converted to pg/ml and when necessary, corrected for recovery, as reported by the authors.

[b-d]Specific conversion factors for AVT concentrations: [b]0.160 µU/pg, [c]1.052 pg/fmol, [d]0.210 µU/pg.

[e]Tap water-adapted animals.

[f]Saline-adapted animals.

[g]Mean of the three experimental groups in saline infusion experiments.

indicate, respectively, the sensitivity and threshold (or secretion set-point) of the osmoreceptor system that regulates AVT secretion. This type of analysis was first proposed by Robertson and his colleagues (1973), and has since been used by a variety of investigators to characterize the AVP secretion systems of several mammalian species (for reviews see Robertson et al. 1976, Schrier, Berl, and Anderson 1979).

In the present studies, neither P_{OSM} nor P_{AVT} of domestic fowl were altered in response to isotonic (control) infusion, thus it can be concluded that the i.v. infusion protocol had no effects per se on AVT secretion (Figs. 4,5,6). Conversely, the infusion of hypertonic saline or distilled water significantly altered P_{OSM} and P_{AVT} (Figs. 4,5) and resulted in highly correlated and significant relationships between these two variables both above and below the observed basal P_{OSM} of normally hydrated birds (Fig. 6). The a priori decision to apply independent linear regression analysis to the P_{OSM}-P_{AVT} relationship of hypertonic and hypotonic birds resulted in clear and precise definition of the sensitivity and secretion threshold of the osmoreceptor system that regulates AVT secretion in the domestic fowl. At $P_{OSM} > 308$ mosm/kg H_2O, AVT secretion is stimulated and P_{AVT} increases linearly with a sensitivity of 0.77 μU/ml per mosm/kg H_2O (3.67 pg/ml per mosm/kg H_2O, Fig. 6). The x-intercept of the linear regression line relating P_{OSM} and P_{AVT} at $P_{OSM} > 308$ mosm/kg H_2O is 308.1 mosm/kg H_2O; however, this does not represent a true secretion threshold, since P_{AVT} (and

therefore AVT secretion) is measurable and is not constant at P_{OSM} < 308 mosm/kg H_2O. Below this basal P_{OSM}, AVT secretion is reduced and P_{AVT} decreases linearly, but with a significantly lower sensitivity of 0.12 μU/ml per mosm/kg H_2O (0.57 pg/ml per mosm/kg H_2O, Fig. 6). The x-intercept of this linear regression line is 288.8 mosm/kg H_2O, which appears to represent a true AVT secretion threshold, since P_{AVT} was undetectable in animals that exhibited a $P_{OSM} \leq$ 290 mosm/kg H_2O. The correlation between P_{OSM} and P_{AVT} is much lower in the hypo-osmolal region (r^2 = 0.334) than it is in the hyperosmolal region (r^2 = 0.889) because of the greater scatter in P_{AVT} data at P_{OSM} < 308 mosm/kg H_2O. This variability can be attributed to several factors, including: The normal variability in basal P_{AVT} levels (which ranged 1.2-5.3 μU/ml prior to infusion), the relatively minor changes in P_{AVT} that occur when P_{OSM} is reduced below basal P_{OSM}, and the increased variability of RIA measurements at low P_{AVT} concentrations. However, since each animal utilized in the hypotonic experiments exhibited a progressive decline in P_{AVT} as P_{OSM} decreased, the hypo-osmolal P_{OSM}-P_{AVT} relationship described by linear regression analysis likely represents a real physiological phenomenon.

The combined hypo-osmolal and hyperosmolal relationships described above strongly suggest that secretion of AVT by the avian hypothalamo-neurohypophysial system conforms to the exponential model, first proposed by Weitzman and Fisher (1977) for the secretion of AVP in sheep, rather than the threshold model proposed

by Robertson and associates from their studies on AVP secretion in humans (Robertson et al. 1973) and laboratory rats (Dunn et al. 1973). There is continued controversy in regard to the relative accuracy of the two models in the description of AVP secretion by the mammalian osmoreceptor system, related in part to differences in analysis of hyperosmolal and hypo-osmolal P_{OSM}-P_{AVP} relationships (Rodbard and Munson 1978, Schrier et al. 1979). Although the separate linear relationships of the present hypo-osmolal and hyperosmolal P_{OSM}-P_{AVT} data might also be described by a single exponential or power function relationship, it is clear that AVT secretion (P_{AVT}) increases gradually (in a rheostat-like manner) with P_{OSM}, rather than abruptly at a specific P_{OSM}. Thus AVT secretion in the domestic fowl conforms better to the exponential model. These data may, in fact, represent a sigmoidal P_{OSM}-P_{AVT} relationship that would result from recruitment of AVT secretory units, as suggested by the early studies of O'Connor (1962) on the mammalian neurohypophysis.

The osmotic regulation of P_{AVT} in birds has been studied by few other investigators, and RIA measurements of P_{AVT} have been limited to studies of the domestic fowl and Pekin duck. These studies have focused primarily on the regulation of P_{AVT} in the hyperosmolal region of P_{OSM} and have not fully characterized the avian AVT secretion system. Koike and associates (Koike et al. 1977, 1979) studied White Leghorn domestic fowl and reported weakly correlated but significant P_{OSM}-P_{AVT} relationships in response to water deprivation (P_{AVT} (μU/ml) = 0.048 (P_{OSM} - 293), r^2 = 0.240)

and hypertonic saline infusion (P_{AVT} = 0.23 (P_{OSM} - 296), r^2 = 0.578; sensitivity conversion to pg/ml = 6.25 pg/μU). Similar P_{OSM}-P_{AVT} correlations were obtained by Mohring et al. (1980) in their studies of Pekin ducks, which normally exhibit a wide range of P_{OSM} when adapted to tap water or hypertonic saline. P_{OSM} and P_{AVT} of tap water-adapted ducks were highly correlated (P_{AVT}(pg/ml) = 0.55 (P_{OSM} - 284.5), r^2 = 0.792); although P_{OSM} and P_{AVT} of salt-adapted ducks were not significantly correlated, the combined data of salt- and water-adapted birds were highly correlated (P_{AVT} (pg/ml) = 0.39 (P_{OSM} - 280.2), r^2 = 0.792; data converted from fmol/ml units reported by authors). These investigators also reported that P_{AVT} was undetectable in 4 out of 5 tap water-adapted ducks acutely over-hydrated with intravenous infusions of hypotonic glucose solution. In a subsequent study, these investigators (Gray and Simon 1983) reported a highly significant P_{OSM}-P_{AVT} correlation in dehydrated tap water-adapted Pekin ducks (P_{AVT} (pg/ml) = 0.39 (P_{OSM} - 282.7), r^2 = 0.846).

These data are similar to those of the present study with the exception of two important observations. First, the x-intercepts of the P_{OSM}-P_{AVT} relationships of domestic fowl (288.8-296 mosm/kg H_2O) appear to be uniformly greater than those of the Pekin duck (280.2-284.5). This difference in AVT secretion threshold may be related to osmoregulatory adaptation and nasal salt gland function in the Pekin duck; however, since hypo-osmolal P_{OSM}-P_{AVT} relationships were not investigated in previous studies, the significance of

this difference is uncertain. Second, it is apparent that AVT secretion sensitivity is much greater in saline infused birds (Koike et al. 1979 and present study) than it is in dehydrated or salt- or tap water-adapted birds (Koike et al. 1977, Mohring et al. 1980, Gray and Simon 1983). This difference may be related to the rate of change of P_{OSM}; the relatively rapid alteration of P_{OSM} by saline infusion (> 2%/hr, as compared to water deprivation) is associated with increased osmotic sensitivity for AVP secretion in mammals (Robertson et al. 1976, 1977). Thus, P_{OSM}-P_{AVT} data obtained by saline infusion are probably not comparable to that obtained by adaptation or dehydration. The saline infusion-derived sensitivity reported by Koike et al. (1979) is lower than that reported in the present study (0.23 vs. 0.77 μU/ml per mosm/kg H_2O). This variation may be due to several factors in the former study, including: The experimental protocol used (birds were infused with mannitol before saline), the low recovery and high variability of AVT RIA data, and genetic differences between the breeds of domestic fowl studied (note the significantly higher P_{OSM} of normally hydrated White Leghorn domestic fowl).

The existence of a central osmoreceptor in birds was first proposed by Schmidt-Nielsen (1960) in analogy to the mammalian mechanism identified by Verney (1947) and later localized to the anterior hypothalamus (Jewell and Verney, 1957). A role for cerebral osmoreception in the regulation of AVT secretion in birds has been confirmed in the recent studies of the Pekin duck by Simon and associates (Deutsch and Simon 1980, Simon-Oppermann et al. 1980,

Simon-Oppermann and Simon 1982). In the present studies, infusion of saline (or distilled water) resulted in parallel changes in P_{OSM} and plasma sodium ($P_{Na}+$), which indicates that changes in P_{OSM} were primarily due to infusion-induced alterations in $P_{Na}+$ (Fig. 4, Table 2). These data do not permit confirmation of central osmoreception in the domestic fowl, or characterization of the mechanism in terms of sensitivity to ionic or osmotic changes in the extracellular fluid. However, since AVT secretion is similarly regulated by extracellular osmolality in both the domestic fowl and duck, it seems likely that cerebral osmoreception is involved in the regulation of AVT secretion in domestic fowl as well.

Inspection of the plasma potassium ($P_{K}+$), hematocrit (HCT), and plasma protein (P_{PROT}) data from the present studies reveals that i.v. infusion resulted in significant expansion of vascular volume in hypertonic (29%) and isotonic (13%) group birds (Table 2). Thus, the hyperosmolal P_{OSM}-P_{AVT} relationship established in the present study may be influenced by the simultaneous effects of vascular (and interstial) extracellular volume expansion. Several investigators using either bioassay or RIA have observed significant reduction in basal P_{AVP} and in the osmotic sensitivity of AVP secretion in mammals following acute isosmotic expansion of vascular or extracellular volume by 10-25% (Zehr, Johnson, and Moore 1969, Johnson, Zehr, and Moore 1970, Szczepanska-Sadowska 1972a, Billman et al. 1983). However, the inability of isosmotic saline infusion to alter P_{AVT} in the present study strongly suggests that vascular volume expansion (up to 13% based on changes in HCT) is ineffective

in alteration of basal P_{AVT} or osmotic sensitivity of AVT release in the domestic fowl (see further discussion below).

The data of the present study, in combination with that of previous studies reflect the primary role of plasma (or extracellular) osmolality in the regulation of AVT secretion by the avian hypothalamo-neurohypophysial system. This relationship is at least qualitatively similar to that described in other tetrapod vertebrate groups, including amphibians, reptiles, and mammals. In amphibians, P_{AVT} of conscious, normally hydrated frogs (genus Rana) averages about 6.1 pg/ml (measured by RIA, recovery corrected). P_{AVT} has been observed to increase approximately three- to eighteen-fold in response to dehydration (3-24 hr) or immersion in hypertonic mannitol (Rosenbloom and Fisher 1974, Sawyer and Pang 1975, Pang 1977, Nouwen and Kuhn 1983). Interestingly, i.v. infusion of hypertonic saline (5%) increased P_{OSM} but failed to alter P_{AVT} in R. catesbiana (Sawyer and Pang 1975, Pang 1977). Although P_{OSM}-P_{AVT} relationships were not quantified in these studies, inspection of the data suggests that while basal P_{AVT} levels are relatively low when compared to birds, AVT secretion sensitivity to osmotic stimuli is relatively high and ranges 0.7-5.7 pg/ml per mosm/kg H_2O in these water-dependent, dehydration-sensitive animals (sensitivities calculated from mean data). In reptiles, the relationship between P_{OSM} and P_{AVT} has been quantified in a single species of desert-adapted lizard, the sand goanna (Varanus gouldii, Rice 1982). Although basal P_{AVT} was not reported, P_{AVT} of water loaded lizards averaged 2.2 ± 0.6 pg/ml (recovery corrected) at a P_{OSM} of

274±10.5 mosm/kg H_2O. Prolonged water deprivation or salt loading resulted in a significant correlation between P_{OSM} and P_{AVT}, described by the equation: P_{AVT} (pg/ml) = 0.085 (P_{OSM} - 276), r^2 = 0.602. Based on these data, both the osmotic sensitivity and threshold for AVT secretion in reptiles are low in comparison with either birds or amphibians. In mammals, the P_{OSM}-P_{AVP} relationship has been studied in a variety of species, including the cat (Reaves et al. 1981), dog (Gray and Simon 1983), goat (Weitzman and Fisher 1977), human (Robertson et al. 1973), monkey (Hayward et al. 1976), and rat (Dunn et al. 1973). In conscious, normally hydrated mammals, P_{AVP} ranges from 2.3±0.2 pg/ml at a P_{OSM} of 293.6±0.4 mosm/kg H_2O in the rat to 9.8±2.0 pg/ml at a P_{OSM} of 320±2 mosm/kg H_2O in the cat (data recovery corrected). Salt loading or water deprivation of mammals result in significant, highly correlated P_{OSM}-P_{AVP} relationships; osmotic sensitivity varies from 0.24 (dog) to 4.04 pg/ml per mosm/kg H_2O (cat), while secretion threshold varies from 279.3 (goat) to 314 mosm/kg H_2O (cat). Based on these data, basal P_{OSM} and P_{AVP} levels of mammals are low in comparison with birds, whereas osmotic sensitivity and secretion threshold values of both groups are quite similar. At these levels of sensitivity, changes in P_{OSM} of as little as 1% would significantly alter plasma levels of AVP or AVT, and in turn, renal water excretion.

Volemic Regulation of Plasma AVT

Mean blood volume (BV) of Rhode Island Red cockerels (56.5±2.0 ml/kg) as determined by the ^{51}Cr-RBC technique in the present study

is quite similar to previously reported BV of White Leghorn domestic fowl (57-63 ml/kg) determined by the radio-iodinated serum albumin technique (Burton, Sahara, and Smith 1967, Wyse and Nickerson 1971). The results of the BV measurements and HCT-BV validation experiments (Fig. 7) indicate that HCT is an accurate and reliable indicator of acute isotonic changes in blood volume in the domestic fowl.

The results of the blood volume experiments provide direct evidence that acute changes in blood (extracellular) volume are unimportant in the regulation of AVT secretion in the domestic fowl. This conclusion is based on the observations that neither acute isotonic expansion or reduction of blood volume alter P_{AVT} levels. The i.v. infusion of isotonic saline (saline infusion control, vide supra) expanded BV by 13.3% (based on HCT data) but did not alter P_{OSM} or P_{Na^+} (Fig. 4, Table 2). Despite this significant isotonic expansion of blood volume, P_{AVT} remained unchanged (Fig. 5). Similarly, acute arterial hemorrhage (HEM) that reduced blood volume 10 or 20% did not alter P_{AVT} (Fig. 10), despite significant reductions in mean arterial pressure and increases in heart rate (Fig. 9). The reductions in HCT that occurred in response to HEM illustrate the marked ability of these birds to restore vascular volume; more than 50% of the volume removed during HEM was replenished by 5 min post-HEM (Table 3). The phenomenon of rapid hemodilution has been described in several species of birds (domestic fowl, duck, and pigeon) and appears to involve rapid influx of protein-poor interstitial fluid to restore vascular volume (Djojosugito, Folkow, and Kovach 1968, Kovach and Balint 1969, Wyse and Nickerson 1971,

Cornelius, Klugman, and Hattingh 1982). The absence of significant changes in P_{OSM}, $P_{Na}+$, or $P_{K}+$ during the post-HEM period of the present experiments (Table 4) strongly suggests that the observed hemodilution involved movement of interstitial fluid. The reinfusion of the shed blood restored heart rate and blood pressure to pre-HEM levels in both 10% and 20% HEM groups (Fig. 9), but did not fully restore HCT because of the pronounced hemodilution that occurred during the post-HEM period.

The rapid restoration of blood volume in these birds suggests that acute HEM may not be an appropriate technique for the study of volume-mediated AVT release, since the volume depletion stimulus was partially counteracted by rapid compensatory mechanisms. However, blood volume of 20% HEM group birds remained substantially depleted (approximately 10%) at 5 and 10 min post-HEM, yet P_{AVT} remained unchanged even at 10 min post-HEM. Thus it can be concluded that either the AVT secretory system is insensitive to volume depletion of 10% or less, or that the blood sampling protocol employed failed to detect resultant changes in P_{AVT}. The latter possibility seems unlikely however, since maximum HEM-stimulated increases in P_{AVP} occur within 5-10 min post-HEM in most mammalian species studied (cf. Share 1974).

Previous studies of volemic regulation of P_{AVT} in birds have utilized both direct (RIA of P_{AVT}) and indirect means (measurements of renal water excretion) to characterize volume-mediated AVT release in the domestic fowl and Pekin duck. In these studies, investigators have assumed that extracellular volume in birds is sensed and

regulated by mechanisms similar to those described in mammals. In the mammalian system vagally-mediated input from cardiac (left atrial) volume receptors and arterial baroreceptors normally exerts tonic inhibitory influence on AVP secretion. In response to hypovolemia (e.g. acute hemorrhage), the inhibitory influence of these receptors is reduced, which results in both direct volume-mediated release of AVP and in elevation of the osmotic sensitivity of AVP release (i.e., increased slope of the P_{OSM}-P_{AVP} relationship) in direct proportion to the magnitude of the volume deficit. Conversely, with hypervolemia, inhibitory influence is increased, which results in the reduction of both basal P_{AVP} and the osmotic sensitivity for AVP secretion (for reviews, see Share 1974, Robertson et al. 1976, 1977, Schrier et al. 1979).

Previous studies on the domestic fowl resulted in conflicting data on volemic regulation of P_{AVT}. Acute arterial hemorrhage of 20 or 30% of blood volume decreased blood pressure and increased heart rate, but did not alter P_{AVT} (Koike et al. 1980). Conversely, acute or chronic cardiac denervation (that presumably removed inhibitory influence of volume receptors) resulted in two-fold increases in the osmotic sensitivity of AVT secretion (Koike et al. 1981). In these same studies, electrical stimulation of the central end of the middle cardiac nerve (which might be expected to inhibit AVT release) paradoxically increased jugular P_{AVT} levels three- to eight-fold in acutely denervated birds.

Previous studies on the Pekin duck have consistently provided evidence (much of it indirect) for volemic regulation of P_{AVT}.

Bilateral vagal blockade resulted in a three-fold increase in P_{AVT} that was correlated with antidiuresis in tap water-adapted Pekin ducks; removal of the blockade reduced P_{AVT} to control levels and reestablished diuresis (Simon-Oppermann et al. 1980). These investigators also provided evidence that i.v. infusion (volume expansion) reduced the osmotic sensitivity of AVT secretion in ducks from 0.39 to 0.28 fmol/ml per mosm/kg H_2O. In subsequent studies, these investigators indirectly demonstrated a non-osmotic systemic effect of volume expansion on AVT secretion. Intracarotid or intravenous infusions of isotonic saline that expanded extracellular volume as little as 1% resulted in a significant diuresis that was attributed to non-osmotic inhibition of AVT release (Simon-Oppermann and Simon 1982). These investigators also observed that i.v. infusion of whole blood (that primarily expands vascular volume) elicited less of a diuretic response than did i.v. infusion of an equal volume of isotonic saline (that expands vascular and interstitial volume); and thus concluded that changes in interstitial volume are more important in the regulation of renal water excretion. Since P_{AVT} was not measured in any of these experiments, the possibility that diuresis resulted from non-endocrine responses to volume expansion cannot be excluded.

The comparison of previous data with that of the present study fails to provide consistent or conclusive evidence for volemic regulation of P_{AVT} in birds. Direct manipulation of extracellular volume by i.v. infusion or hemorrhage does not appear to measureably alter P_{AVT}, but is associated with alteration of the osmotic

sensitivity of AVT secretion. Conversely, interruption of nervous pathways that presumably convey inhibitory input from volume receptors results in the elevation of both basal P_{AVT} levels and the osmotic sensitivity of AVT release. If interstitial volume is important in the domestic fowl, then reductions in vascular volume by hemorrhage may not affect P_{AVT} per se. However, the rapid and pronounced hemodilution that occurred in response to hemorrhage in the present studies (presumably from the influx of interstitial fluid) should have reduced interstitial volume significantly, yet P_{AVT} was unaffected. Part of the discrepancy in the data may be the result of interspecific differences in the osmoregulatory mechanisms employed, particularly as related to the absence or presence of nasal salt glands in the species studied. On the contrary, the possibility that extracellular volume is not involved in the regulation of renal water excretion (by endocrine and/or nervous pathways) seems highly unlikely. A complete resolution to this problem will require further experimentation that combines simultaneous efforts to alter extracellular volume and monitor endocrine and renal responses with interruption of input from the presumed afferent nervous pathways.

Previous studies in other vertebrate groups have provided evidence for volemic regulation of antidiuretic hormone release. In amphibians (bullfrogs, R. catesbiana) P_{AVT} (measured by RIA) is observed to increase markedly in response to experimental procedures that reduce extracellular volume (dehydration, hemorrhage, or immersion in hypertonic mannitol; Sawyer and Pang 1975, Pang 1977).

Since i.v. infusions of hypertonic saline increase P_{OSM} but do not alter P_{AVT} levels, it has been concluded that extracellular volume is of primary importance in the regulation of AVT release in the bullfrog (Pang, 1977). In mammals, extracellular volume is clearly important in the regulation of P_{AVP}. Although the AVP system is more sensitive to increases in extracellular osmolality, reductions in blood volume are much more potent stimuli for AVP secretion, and often increase P_{AVP} ten- to thirty-fold (Szczepanska-Sadowska 1972a, Larsson, Olsson, and Fyhrquist 1978, Wood et al. 1981). Reduction of blood volume by either hemorrhage or hypertonic peritoneal dialysis increases P_{AVP} in direct proportion to the volume reduction, as shown by bioassay (reviewed by Share 1974) and more recently, by RIA of P_{AVP} (Dunn et al. 1973, Arnauld et al. 1977). Volume reduction also results in elevation of the osmotic sensitivity of AVP release (Dunn et al. 1973, Robertson et al. 1976). Conversely, blood (or extracellular) volume expansion decreases both basal P_{AVP} (Zehr et al. 1969, Johnson et al. 1970, Szczepanska-Sadowska 1972a, Billman et al. 1983) and the osmotic sensitivity of AVP secretion (Szczepanska-Sadowska 1972a, Robertson et al. 1976). The volemic effects on AVP release appear to be mediated by inhibitory vagal input from cardiac (left atrial) volume receptors and arterial baroreceptors, since interruption of vagal pathways abolishes the effects of volume alteration on AVP release (Szczepanska-Sadowska 1972b, Share 1974).

In summary, the data of the present study provide direct evidence that P_{OSM} is the primary determinant of AVT release in

the domestic fowl. Although a role for cerebral osmoreception in the regulation of AVT secretion in the domestic fowl was not established in this study, the sensitivity of the AVT secretion system to changes in P_{OSM} ($\leq 1\%$) and the similarity of the present data with that of previous studies strongly suggest that the osmotic regulation of P_{AVT} is governed by a central osmoreceptor system. Analysis of the present data also suggests that the AVT secretion system of the domestic fowl is insensitive to changes in blood volume of 10% or less. Comparison of these data with that of previous studies fails to provide consistent or conclusive evidence for volemic regulation of P_{AVT} in birds. When past and present avian data are compared with that of other tetrapod vertebrates, it is apparent that the osmotic regulation of antidiuretic hormone secretion is qualitatively similar among reptiles, birds, and mammals, but that secretion sensitivity is substantially greater in the latter two (homeothermic) groups.

CHAPTER 5

REGULATION OF PLASMA ARGININE VASOTOCIN IN CONSCIOUS WATER DEPRIVED DOMESTIC FOWL

Introduction

Arginine vasotocin (AVT) was established as the naturally occurring antidiuretic hormone (ADH) of birds by Munsick and associates in the early 1960's (Munsick et al. 1960, Munsick 1964). Avian ADH appears to regulate renal water excretion by its dual actions on tubule water permeability and glomerular filtration rate (for reviews, see Dantzler 1978, 1980a, 1980b, Dantzler and Braun 1980, Skadhauge 1981).

Although AVT has been identified as the avian ADH and its actions on the kidney partially characterized, very little is known about the physiological regulation of AVT secretion in birds, primarily because of the inability to accurately measure plasma AVT (P_{AVT}) concentrations. In early studies, the frog bladder bioassay was employed to measure P_{AVT} in domestic fowl in an attempt to characterize AVT secretion in response to osmotic and volemic stress (Douglas and Sturkie 1964, Sturkie and Lin 1966, Niezgoda 1975). However, the relatively low sensitivity and high variability inherent to this technique resulted in highly variable and inconclusive data.

The recent development of sensitive and specific radioimmunoassay techniques for the measurement of P_{AVT} now has enabled

investigators to begin to characterize AVT secretion in birds in response to osmotic and volemic stimuli. Hypertonic saline infusion of domestic fowl (Koike et al. 1979) and saline or tap water adaptation of Pekin ducks (Mohring et al. 1980) resulted in positive linear correlation between plasma osmolality (P_{OSM}) and P_{AVT} over a wide range of P_{OSM}. Extracellular volume depletion (hemorrhage) or cardiac denervation in the domestic fowl (Koike et al. 1980, 1981) and extracellular volume expansion or vagal blockade in the Pekin duck (Simon-Oppermann et al. 1980, Simon-Oppermann and Simon 1982) resulted in conflicting evidence for volemic regulation of P_{AVT} in birds. The combined effects of extracellular hyperosmolality and hypovolemia on the regulation of P_{AVT} have been studied by water deprivation experiments. Increases in P_{OSM} and P_{AVT} are weakly correlated and highly variable in the domestic fowl (Koike et al. 1977), but highly correlated and uniform in the Pekin duck (Gray and Simon 1983). The substantial variability observed in the former study may be attributable to marked variability in RIA measurements by these investigators (Koike et al. 1977, 1979).

The available data demonstrate that increases in extracellular (plasma) osmolality are a primary stimulus for the release of AVT in birds, but the role of extracellular volume in the regulation of P_{AVT} remains uncertain. The purpose of the present investigation was to characterize the responses of the AVT secretion system of the domestic fowl to the natural osmoregulatory challenge of fluid deprivation. In addition, an attempt was made to separate the osmotic and volemic components of dehydration-stimulated AVT release.

Materials and Methods

Animals and General Experimental Procedures

All studies utilized Rhode Island Red cockerels, which were obtained from a commercial supplier (Red Wing Hatchery, Los Angeles, CA) at one week of age and raised in flocks at University of Arizona Facilities (Department of Animal Resources) in an environmentally controlled room (ambient temperature 26±1°C) with a 12:12 light-dark cycle. The birds were maintained in screen-bottomed cages, and provided with a commercial diet (chick starter mash and mixed scratch grain, Arizona Feeds, Inc.) and tap water ad libitum. When the birds attained 9-10 weeks of age (1200-1300g body weight) they were transferred to individual cages for at least one week prior to any surgical or experimental procedures.

All experiments were performed during daylight hours at an ambient temperature of 22-25°C. Quiet conditions were maintained in the laboratory during all experimentation to avoid excitation of conscious animals. In all experiments, baseline blood samples were taken at the beginning of each experiment which allowed each animal to serve as its own control for the experimental manipulations that followed.

Surgical Preparation

To avoid undue excitement or surgical stress during measurements of AVT secretion and to facilitate intravenous (i.v.) infusion and blood sample collection, birds were chronically catheterized. Animals were fasted overnight and under ketamine

hydrochloride pre-anesthetic (20 mg/kg) and methoxyflurane inhalation anesthesia, surgical grade silastic catheters (0.940 mm OD x 0.508 mm ID) were placed under aseptic conditions in the right brachial artery and basilic vein with the tips of the catheters in or near the common carotid artery and cranial vena cava, respectively. The potential compromise in blood flow to the distal wing caused by chronic arterial and venous catheterization is readily overcome by the abundant collateral circulation present in the wing of the domestic fowl (Baumel 1979). The catheters were occluded with metal stylets, tunneled subcutaneously, and externalized on the neck 2-3 cm below the comb. The ends of the catheters were secured to the skin with a wound clip to prevent the birds from pecking at the free ends. During experiments, extensions were connected to the catheters to facilitate infusion and sample collection. The animals were given 5-6 days to recover and during that time, were treated prophylactically with oxytetracycline. During the recovery period, the birds were acclimated to handling (to reduce excitement during experiments) and the catheters rinsed with heparinized saline (40 U/ml) on alternate days to maintain their patency. The inhalation anesthesia was administered by a specially designed anesthetic device which utilized a unidirectional flow mask and enabled precise control of anesthetic dosage (Stallone and Braun, manuscript in preparation). Usually, animals recovered completely without ill effects from anesthesia or surgery, and the catheters remained patent throughout the experimental period.

Fluid Deprivation - Osmotic and Volemic Regulation of Plasma AVT

To characterize the effects of combined osmotic and volemic stimuli on the regulation of P_{AVT}, chronically catheterized birds were subjected to total fluid deprivation. Prolonged dehydration results in a progressive increase in plasma osmolality and a concomitant decrease in plasma volume. Although this procedure results in chronic exposure of the osmoreceptor to combined osmotic and volemic stimuli, it is the only technically feasible method to follow the responses of individual birds over a time course. Moreover, this method provides a realistic representation of the osmoreceptor system response to combined osmotic and volemic stimuli as they actually occur under conditions of negative fluid balance.

Fluid Deprivation. Chronically catheterized birds were maintained on normal ad libitum food and tap water during the surgical recovery period. The experimental protocol was as follows: On the first day of the experiment, a conscious animal was weighed and then suspended upright in a rip-stop nylon restraining sling that allowed free movement of the head and feet but prevented the animal from struggling. A cloth hood was placed over the head to prevent visual stimulation, and the bird allowed to acclimate to ambient conditions for 30 min. At the end of the acclimation period an arterial blood sample (for plasma analyses and hematocrit measurement) was taken.

After this control (normal hydration) blood sample, the animal was returned to its cage and drinking water (but not food) was withheld. At 24, 48, 72, and 96 hours of dehydration, the animal was

weighed, returned to the restraining sling, allowed to acclimate for 30 min, and an arterial blood sample taken. Erythrocytes were resuspended to blood sample volume (1.2 ml) in isotonic saline and reinjected after each sample. Arterial blood pressure and heart rate were recorded after each acclimation period using a Gould-Statham transducer (model p23dB) and a Gould chart recorder (model 2400S, Gould Inst., Inc.). All blood samples were centrifuged at $4^{\circ}C$ immediately after collection, hematocrit measured and the resultant plasma removed, aliquoted, and analyzed for AVT, electrolytes, and osmolality using the techniques described in detail below.

Volume Repletion. An attempt was made to separate and quantify the contributions of plasma hyperosmolality and hypovolemia to the regulation of P_{AVT} during fluid deprivation. The technique involved rapid repletion of plasma volume lost during dehydration (modified from Wade, Keil, and Ramsay 1983) and was achieved by i.v. infusion of plasma from donor birds. The experimental protocol was as follows: Immediately after completion of the experimental procedures at 96 hr of dehydration, the animal was retained in the restraining sling for further experimentation. Plasma volume deficit of the bird was estimated from the change in hematocrit between normal hydration and 96 hr samples, using the proportion $(BV_2/BV_1) = (HCT_1/HCT_2)$ and assuming an initial blood volume of 56.5 ml/kg (previously determined in this breed using the ^{51}Cr-RBC technique; see Chapter 4). The calculated volume deficit was then replaced by i.v. infusion of plasma (0.4 ml/kg · min, Sage infusion pump, model 355, Orion Res., Inc.) obtained from normally hydrated donor birds. Arterial blood

samples were taken at 0, 30, and 60 min post-infusion and treated as above, and arterial blood pressure was recorded throughout the experiment. At the end of the experiment, the bird was sacrificed with an i.v. overdose of sodium pentobarbital.

Radioimmunoassay of AVT

The radioimmunoassay of AVT in these studies utilized the R-70 antiserum developed by Fisher and associates at the UCLA School of Medicine (Rosenbloom and Fisher 1974, Skowsky et al. 1974). The RIA was performed in a final volume of 0.500 ml and utilized the R-70 antiserum at a final dilution of 1:500,000 in a sequential incubation (disequillibrium) system (D.A. Fisher, pers. comm.). Synthetic AVT (Bachem, Inc., lot no. R2750), bioassayed by W.H. Sawyer (210 U/mg, rat pressor activity), was used as the standard. Detailed descriptions of the assay characteristics, collection and extraction of plasma, and radioiodination of AVT have been described previously (Chapter 4, Materials and Methods). As employed in these studies, the least detectable concentration of AVT (that which yields a binding response two standard deviations from the zero dose response) is 0.75 μU/ml (3.6 pg/ml) or the equivalent of 0.36 pg per assay tube. The intra-assay and inter-assay coefficients of variation average (mean±S.E.) 7.2±0.7% and 8.0±0.9%, respectively.

Additional Analyses of Plasma

Aliquots of plasma from each experiment were stored either at 4°C (for rapid analysis of osmolality) or at -20°C (for later analyses of electrolytes). Plasma osmolalities were measured by vapor

pressure osmometry using a Wescor osmometer (model 5100B; Wescor, Inc.) with a sample size of 8.0 µl. The variation of any one sample (3-5 replicate measurements) was ±0.5% (standard error). All measurements were made on fresh samples of plasma kept at 4°C and not previously frozen. Plasma electrolytes (Na^+ and K^+) were measured by flame photometry with an internal lithium standard (model 143, Instrumentation Laboratories, Inc.) using a sample size of 10 µl. Hematocrit (percent packed cell volume) of arterial blood samples was determined by the microhematocrit method. Duplicate heparinized microhematocrit tubes were filled directly from the arterial catheter and immediately sealed and centrifuged at 13,000 x g for 5 min using a microhematocrit centrifuge (model MB, International Eqpmt. Co.). The tubes were read to the nearest 0.25 mm, the hematocrits calculated, and duplicate values averaged.

Statistical Analyses

All data are presented as the mean ± one standard error. Data groups were subjected to one-way analysis of variance (ANOVA) to detect significant differences, followed by the Student Newman-Keuls test to distinguish significant differences among the means of a data group (Zar 1974). Linear regression analysis was performed by the least-squares method (Zar 1974). All statistical analyses were performed on a programmable desk-top calculator (model HP-97, Hewlett-Packard, Inc.) programmed from the Hewlett-Packard Statistical Package.

Results

Fluid Deprivation

The effects of fluid deprivation on plasma osmolality (P_{OSM}) are shown in Figure 11. During normal hydration, mean P_{OSM} was 309.3±0.7 mosm/kg H_2O; with dehydration, mean P_{OSM} increased in curvilinear fashion to a maximum of 346.6±2.0 mosm/kg H_2O at 96 hr. The maximum increase in P_{OSM} with dehydration occurred during the first 24 hr; thereafter, P_{OSM} increased more gradually, but continually with further dehydration.

Changes in plasma AVT concentrations (P_{AVT}) of the birds in response to fluid deprivation paralleled the observed changes in P_{OSM}, as shown in Figure 12. Baseline P_{AVT} averaged 2.2±0.3 μU/ml, and increased by six-fold at maximum levels, which occurred at 48 hr. Despite further significant increases in P_{OSM} from 48 to 96 hr, P_{AVT} levels did not increase significantly after 48 hr, but exhibited minor, insignificant oscillations at 72 and 96 hr. As illustrated in Figure 13, P_{AVT} was significantly correlated with P_{OSM} during fluid deprivation ($r^2 = 0.781$, $P < 0.001$); the relationship is described by the linear regression equation:

$$P_{AVT}\ (\mu U/ml) = 0.28(P_{OSM} - 296.0).$$

The effects of fluid deprivation on body weight, hematocrit, plasma electrolytes, blood pressure, and heart rate are summarized in Table 6. Body weight (BW) of the experimental birds declined continuously in response to fluid deprivation, with the largest loss of weight occurring during the first 24 hr period (5.4% of initial

Figure 11. Changes in plasma osmolality of domestic fowl in response to water deprivation. Each point represents the mean $\pm$ one standard error of measurements from eleven birds. $^{a-d}$Mean values without common script are significantly different ($P < 0.01$).

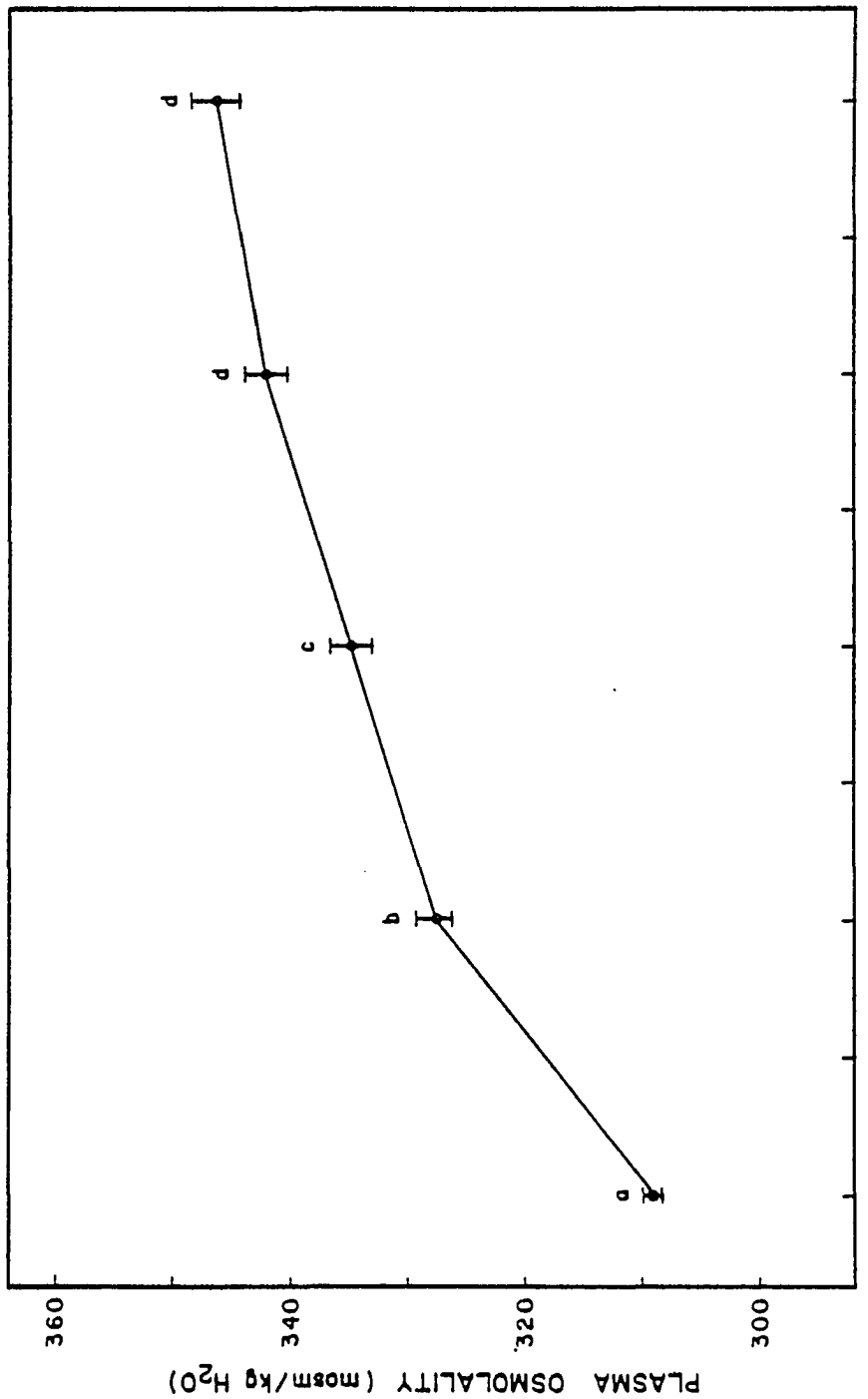
PLASMA OSMOLALITY (mosm/kg H_2O)
360
340
320
300
0
24
48
72
96
TIME OF DEHYDRATION (HR)
a
b
c
d
d
d

Figure 11.

Figure 12. Changes in plasma AVT concentration of domestic fowl in response to water deprivation. Each point represents the mean ± one standard error of measurements from eleven birds. $^{a-b}$Mean values without common script are significantly different ($P < 0.01$).

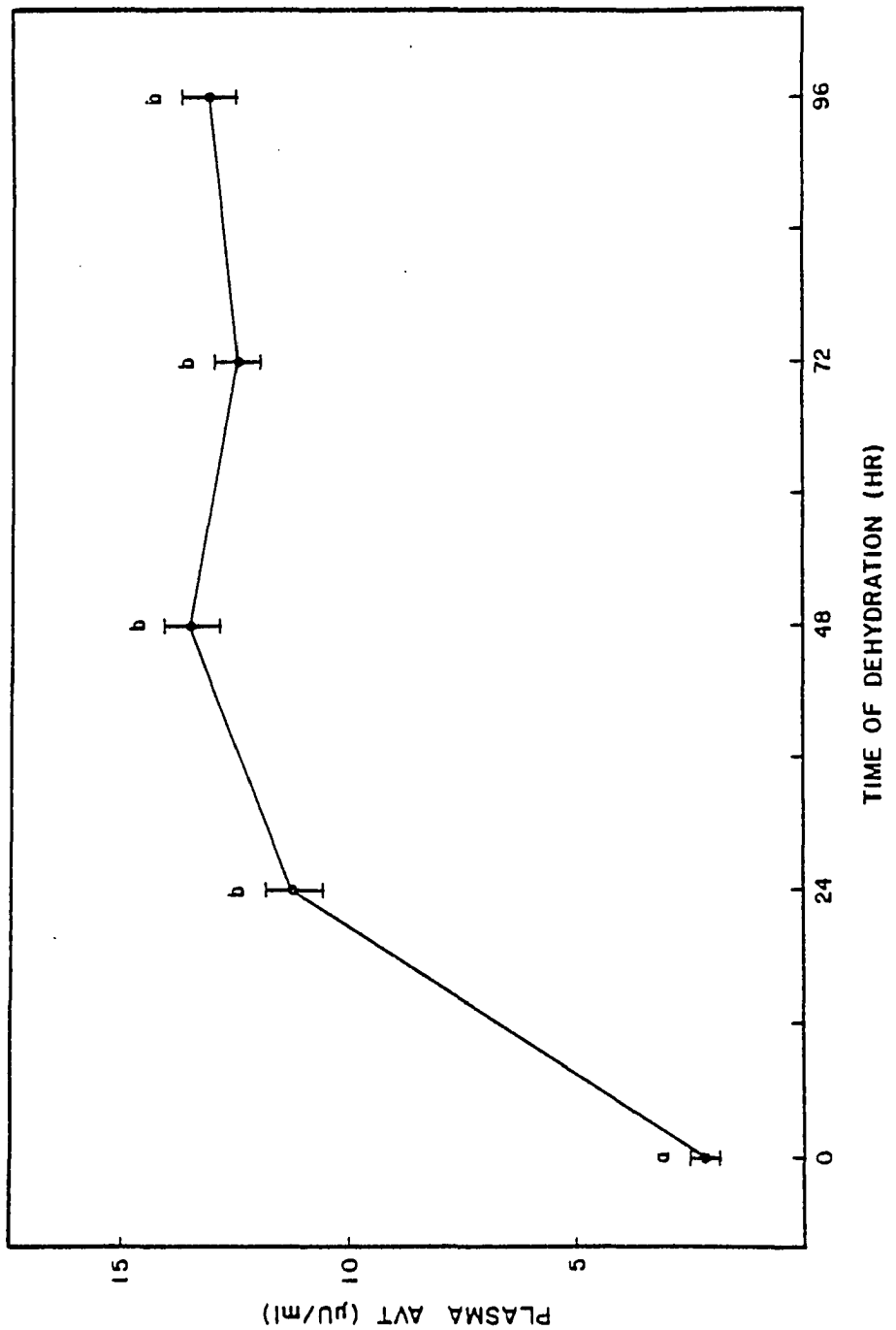
PLASMA AVT (μU/ml)
15
10
5
0
24
48
72
96
TIME OF DEHYDRATION (HR)
a
b
b
b
b

Figure 12.

Figure 13. The relationship between plasma osmolality and plasma AVT concentration in normally hydrated and water deprived domestic fowl.

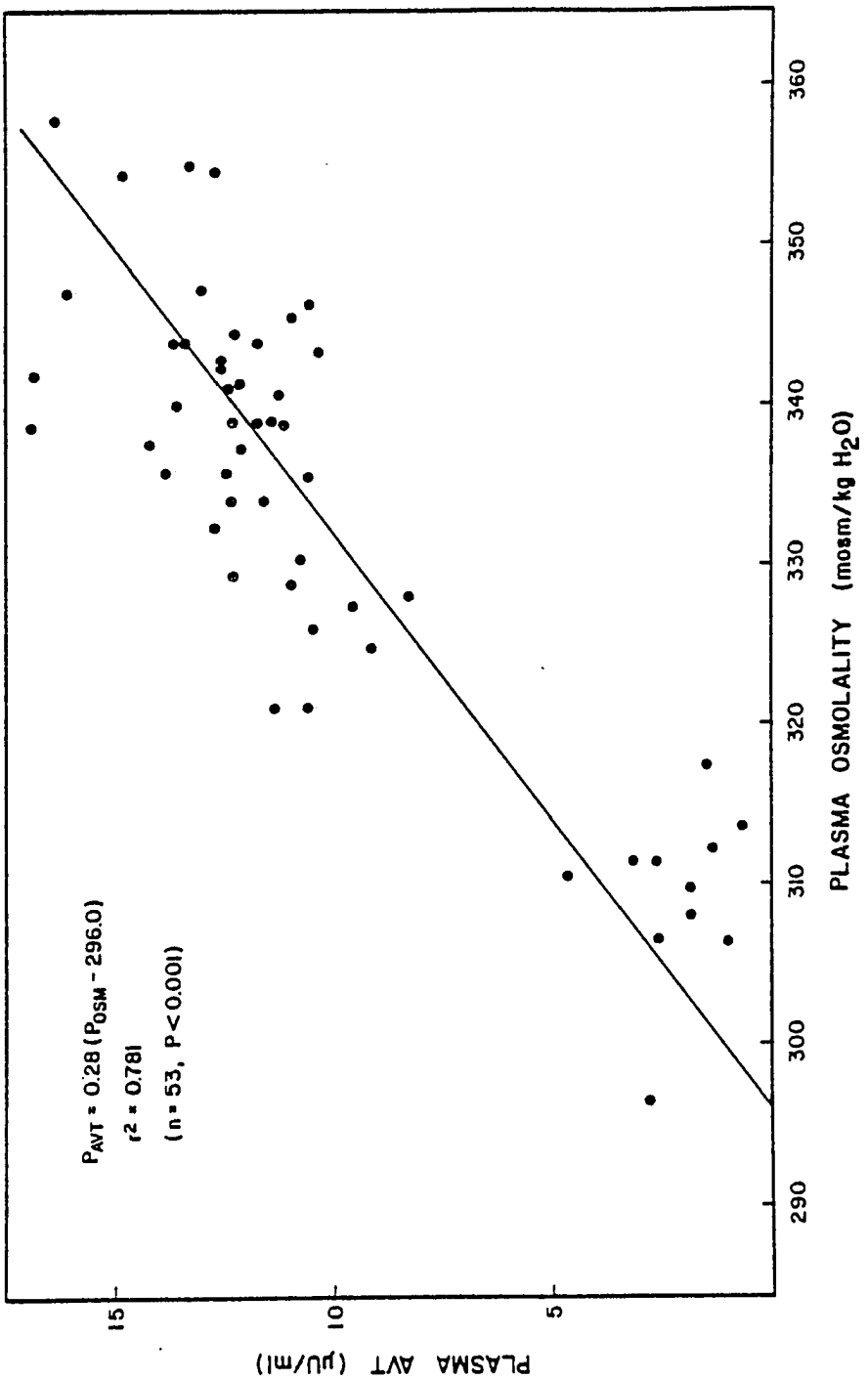
PLASMA AVT (μU/ml)
15
10
5
290
300
310
320
330
340
350
360
PLASMA OSMOLALITY (mosm/kg H2O)
PAVT = 0.28 (POSM - 296.0)
r2 = 0.781
(n = 53, P<0.001)

Figure 13.

Table 6. Body weight, hematocrit, plasma sodium and potassium concentrations, mean arterial blood pressure, and heart rate of normal and water deprived domestic fowl.[a]

Variable		Time of Dehydration (hr)				
		0	24	48	72	96
Body Weight (g)	(n=11)	1287±36.6[b]	1217±35.6[b,c]	1173±34.8[b,c]	1134±34.4[c]	1095±33.2[c]
Hematocrit (% PCV)	(n=11)	26.1±0.5[d]	27.3±0.4[d,e]	28.8±0.5[e,f]	30.3±0.6[f]	31.9±0.7[f]
Plasma Na^+ (meq/l)	(n=11)	149.1±0.5[g]	159.4±0.9[h]	163.7±0.6[i]	167.8±0.9[j]	170.9±0.9[k]
Plasma K^+ (meq/l)	(n=11)	3.8±0.10*	3.7±0.11	3.6±0.12	3.8±0.10	3.7±0.11
Mean Arterial Blood Pressure	(n=11) (mmHg)	143±3.1*	139±2.2	139±2.2	142±3.2	142±2.7
Heart Rate (beats/min)	(n=11)	343±5.9[l]	325±6.0[l,m]	311±6.5[m]	302±5.3[m,n]	282±8.7[n]

[a]All values are mean ± S.E. (n = sample size).

[b-n]Within each row, mean values without common superscript are significantly different ($^{b-c}P < 0.05$, $^{d-n}P < 0.01$).

*Within this row, mean values are not significantly different ($P > 0.05$).

BW). During each subsequent 24 hr period, the birds lost an average of approximately 3% of their initial BW. Hematocrit (HCT) of fluid deprived birds increased continuously in a linear fashion during the 96 hr experiment. The observed changes in HCT can be used to estimate changes in blood volume (expressed as percent of initial value) with the proportion: $(BV_2/BV_1) = (HCT_1/HCT_2)$, which assumes constancy of the circulating erythrocyte mass. Thus, by 96 hr of fluid deprivation, the birds lost an average of 18.2% of their initial blood volume. Plasma sodium concentration ($P_{Na}+$) increased continuously during dehydration in a pattern very similar to that of P_{OSM}; thus $P_{Na}+$ exhibited its greatest increase during the initial 24 hr of fluid deprivation, with more gradual increases during the subsequent 72 hr that totaled 14.6% at 96 hr. Plasma potassium was relatively constant during the experiment and exhibited no significant changes in response to fluid deprivation ($P > 0.05$).

Mean arterial blood pressure (MAP) remained nearly constant throughout the experiment and exhibited only minor variation among the experimental birds, as evidenced by the small standard errors of the means (Table 6). MAP averaged 143 ± 3.1 mmHg initially and decreased insignificantly by no more than 4 mmHg during the entire experiment ($P > 0.05$). Mean heart rate exhibited a gradual decline during fluid deprivation that first became significant at 48 hr of dehydration ($P < 0.01$). By 96 hr, heart rate decreased by an average of 17.8% from the initial pre-dehydration value of 343 ± 5.9 beats/min.

Volume Repletion

The effects of volume repletion on HCT, P_{OSM}, and P_{AVT} of 96 hr fluid-deprived birds are summarized in Table 7. Intravenous infusion of homologous plasma reduced the 96 hr HCT of all birds at 0, 30, and 60 min after infusion. Differences in mean HCT before and after infusion were not significant ($P > 0.05$) primarily because of animal-to-animal variability in HCT and the small sample size. However, when individual reductions in HCT are expressed as a percentage of the 96 hr HCT, the observed reductions were quite uniform, and averaged $10.1 \pm 0.6\%$; this reduction did not vary significantly at 30 or 60 min after infusion ($P > 0.05$). For purposes of comparison, it should be noted that HCT of the four animals used in this experiment increased by an average of 20.5% after 96 hr of fluid deprivation; thus, it would appear that approximately half of the blood volume lost during dehydration was replaced by the plasma infusion. P_{OSM} of 96 hr fluid deprived birds remained nearly constant in response to homologous plasma infusion and did not differ significantly ($P > 0.05$). Osmolality of the infused plasma averaged 312 mosm/kg H_2O.

Mean P_{AVT} concentration of the experimental birds was not significantly altered in response to volume repletion ($P > 0.05$). The 96 hr P_{AVT} values of individual birds did not vary beyond average intra-assay variation ($7.2 \pm 0.7\%$) at 0, 30 or 60 min after infusion, with the exception of one bird, whose P_{AVT} decreased progressively

Table 7. Hematocrit, plasma osmolality, and plasma AVT concentration of 96 hr fluid deprived and subsequently volume repleted domestic fowl.[a,b]

Variable		96 hr Fluid Deprivation	Time After Volume Repletion (min) 0	30	60
Hematocrit (% PCV)	(n=4)	30.9±1.0	27.7±0.7	28.2±1.0	27.7±0.9
Plasma Osmolality (mosm/kg H_2O)	(n=4)	342.2±2.3	343.3±2.9	341.7±2.7	342.5±3.1
Plasma AVT (μU/ml)	(n=4)	12.6±1.4	12.3±1.7	11.9±1.5	12.3±1.7

[a]All values are mean ± S.E. (n = sample size).

[b]Within each row, mean values are not significantly different ($P > 0.05$).

to 85% of its 96 hr value at 60 min after infusion. MAP of the birds used in this experiment averaged 142±6.6 mmHg and did not vary significantly in response to the infusion procedure ($P > 0.05$).

Discussion

In the present investigation, recently developed radioimmunoassay (RIA) methods were employed to quantitatively characterize AVT secretion in the domestic fowl in response to chronic dehydration. Water deprivation permitted characterization of AVT secretion in response to the extracellular hyperosmolality and hypovolemia incurred during negative fluid balance; the subsequent repletion of extracellular volume permitted separation of potential osmotic and volemic factors involved in the regulation of AVT secretion.

Fluid Deprivation

Plasma AVT of conscious, normally hydrated domestic fowl averaged 2.2±0.3 μU/ml (10.5±1.4 pg/ml) at the corresponding mean plasma osmolality (P_{OSM}) of 309.3±0.7 mosm/kg H_2O. These data are comparable to previously reported RIA measurements of basal P_{AVT} in conscious, normally hydrated domestic fowl (Koike *et al.* 1977, 1979) and Pekin ducks (Mohring *et al.* 1980, Gray and Simon 1983; see Table 5, Chapter 4 for comparisons).

The results of the fluid deprivation experiments indicate that AVT secretion is closely linked to the state of hydration during negative fluid balance in the domestic fowl. Dehydration of the birds (as reflected by the increases in P_{OSM} and decreases in body weight)

was most rapid during the initial 24 hr of fluid deprivation; the rate of dehydration was increasingly more gradual during subsequent 24 hr intervals (Fig. 11, Table 6). Plasma AVT paralleled P_{OSM} in response to dehydration and increased to a maximum of 13.5±0.6 μU/ml (64.3±2.9 pg/ml) at 48 hr (Fig. 12). Although P_{OSM} continued to increase gradually from 48 to 96 hr, P_{AVT} remained constant during further water deprivation. These data suggest that maximum sustained AVT secretion was achieved at 48 hr, since continued dehydration at 72 and 96 hr resulted in only minor, insignificant oscillations in P_{AVT}. These data also suggest that AVT-stimulated renal water conservation attained maximum levels at 24-48 hr, since further fluid deprivation resulted in continued dehydration at a significantly lower, constant rate. Despite the apparent uncoupling of P_{OSM} and P_{AVT} after prolonged dehydration, fluid deprivation resulted in a highly correlated and significant relationship between these two variables, quantitatively defined by linear regression analysis (Fig. 13). The slope and x-intercept of the regression line that relates P_{OSM} and P_{AVT} indicate, respectively, the osmotic sensitivity (0.28 μU/ml per mosm/kg H_2O or 1.33 pg/ml per mosm/kg H_2O) and threshold (or secretion set-point, 296.0 mosm/kg H_2O) of the osmoreceptor system that regulates AVT secretion. This type of analysis was first proposed by Robertson et al. (1973, 1976) to study the osmotic regulation of arginine vasopressin (AVP) secretion in man, and has since been used by numerous investigators to characterize the hypothalamo-neurohypophysial systems of birds, mammals, and reptiles (vide infra).

The regulation of P_{AVT} in water deprived birds has been studied previously in the domestic fowl and Pekin duck. Koike et al. (1977) subjected White Leghorn domestic fowl to chronic dehydration (96 hr) and reported a weak but significant correlation between P_{OSM} and P_{AVT} (P_{AVT}(pg/ml) = 0.30 (P_{OSM} - 296), r^2 = 0.240). Gray and Simon (1983) reported that P_{OSM} and P_{AVT} were highly correlated in acutely dehydrated (24 hr) tap water adapted-Pekin ducks (P_{AVT}(pg/ml) = 0.39 (P_{OSM} - 282.7), r^2 = 0.846). The P_{OSM}-P_{AVT} relationships reported in these studies are qualitatively similar to the relationship described in the present study. The loose P_{OSM}-P_{AVT} relationship and markedly low osmotic sensitivity of AVT secretion in White Leghorn domestic fowl reported by Koike et al. (1977) may be the artifactual result of the low recovery and high variability of the RIA measurements in this study. The quantitative differences in osmotic sensitivity and AVT secretion threshold of domestic fowl in the present study and Pekin ducks in the previous study may be related to osmoregulatory adaptation and nasal salt gland function in the latter species. Nonetheless, it is apparent that AVT secretion is closely linked to the state of hydration in both species.

Inspection of the plasma sodium ($P_{Na}+$) and hematocrit (HCT) data from the present study (Table 6) reveals that fluid deprivation resulted in significant elevation of $P_{Na}+$ and significant reduction of vascular volume. The close relationship between P_{OSM} and $P_{Na}+$ in these experiments strongly suggests that dehydration-induced elevations of P_{OSM} are primarily attributable to the increased concentrations of sodium and its attendant anions. A similar

relationship between P_{OSM} and $P_{Na}+$ has been described by Koike et al. (1977, 1983) in water deprived White Leghorn domestic fowl.

The increases in HCT observed in the present study suggest that vascular volume was reduced substantially during water deprivation. The changes in HCT were used to estimate changes in blood volume (assuming constancy of circulating erythrocyte mass). Although the effects of plasma hyperosmolality on erythrocyte volume (and HCT) may result in underestimation of blood volume changes, these data indicate that birds lost an average of 18.2% of their initial blood volume by 96 hr of water deprivation. Thus, the P_{OSM}-P_{AVT} relationship described in the present study may be influenced by the simultaneous reductions of vascular volume.

The data of the present study, in combination with that of previous studies reflect the close linkage between the state of hydration and the secretion of AVT by the avian hypothalamo-neuro-hypophysial system. Similar relationships have been described in other vertebrate groups, including amphibians, reptiles, and mammals. P_{AVT} increases three- to eighteen-fold in frogs (genus Rana) in response to dehydration (3-24 hr, Rosenbloom and Fisher 1974, Sawyer and Pang 1975, Pang 1977, Nouwen and Kuhn 1983). Although P_{OSM}-P_{AVT} relationships were not quantified in these studies, inspection of the data suggests that the osmotic sensitivity of AVT secretion in amphibians is quite high and ranges 0.7 to 5.7 pg/ml per mosm/kg H_2O (calculated from mean data). In reptiles, the regulation of P_{AVT} has been studied in a single species, the sand goanna (Varanus gouldii, Rice 1982). Prolonged water deprivation or

salt loading resulted in a significant correlation between P_{OSM} and P_{AVT}, described by the equation: P_{AVT}(pg/ml) = 0.085 (P_{OSM} - 276), r^2 = 0.602. These data suggest that both the osmotic sensitivity and threshold for AVT secretion in reptiles are low in comparison with either amphibians or birds. In mammals, water deprivation resulted in significant, highly correlated P_{OSM}-P_{AVP} relationships in a variety of species including the cat (Reaves et al. 1981), dog (Gray and Simon 1983), man (Robertson et al. 1973), and monkey (Hayward et al. 1976). Osmotic sensitivity varies from 0.24 (dog) to 4.04 pg/ml per mosm/kg H_2O (cat), while secretion threshold varies from 280 (man) to 314 mosm/kg H_2O (cat). Based on these data, osmotic sensitivity and secretion threshold values of birds and mammals are quite similar.

Volume Repletion

The results of the volume repletion experiments provide direct evidence that reductions in blood volume are unimportant in the regulation of P_{AVT} in water deprived domestic fowl. This conclusion is based on the observation that acute repletion of blood volume with homologous plasma in water deprived birds does not alter the P_{AVT} concentrations maintained during chronic dehydration (Table 7). Although only 50% of the blood volume lost during dehydration was apparently replaced by this technique (based on the observed changes in HCT during dehydration and volume repletion), this represented a substantial reduction in the hypovolemia incurred during water deprivation. The validity of the volume repletion technique was

demonstrated recently in water deprived dogs, in the study of Wade et al. (1983). Total repletion of extracellular volume lost during 24 hr of water deprivation by i.v. infusion of isotonic saline reduced P_{AVP} from 5.3 to 4.4 pg/ml. It was concluded that the hypovolemia incurred during dehydration contributed to 33% of the increase in P_{AVP} observed during water deprivation. In recent studies of the Pekin duck, Simon-Oppermann and Simon (1982) observed that i.v. infusion of isotonic saline or whole blood that expanded extracellular volume as little as 1% resulted in a significant diuresis that was attributed (indirectly) to non-osmotic inhibition of AVT secretion. Although i.v. infusion of isotonic saline resulted in greater diuretic responses, the i.v. infusions of plasma in the present study expanded extracellular volume to a substantially greater degree, yet P_{AVT} remained unaltered. Thus, it can be concluded that hypovolemia is apparently unimportant in the regulation of P_{AVT} in water deprived domestic fowl.

In summary, the data of the present study provide direct evidence that AVT secretion is closely coupled to the state of hydration in domestic fowl during water deprivation. The data also suggest that increases in P_{AVT} that occur with dehydration are mediated primarily by extracellular hyperosmolality and that the AVT secretion system is relatively insensitive to the simultaneous hypovolemia incurred with fluid deprivation. When past and present avian data are compared with that of other tetrapod vertebrates, it is apparent that the osmotic sensitivity of AVT secretion during fluid deprivation is quantitatively similar in birds and mammals.

CHAPTER 6

RELATIVE CONTRIBUTIONS OF GLOMERULAR AND TUBULAR MECHANISMS TO AVT-INDUCED ANTIDIURESIS IN CONSCIOUS DOMESTIC FOWL

Introduction

The excretion of water by the avian kidney is regulated primarily by the peptide hormone arginine vasotocin (AVT) which was established as the naturally occurring antidiuretic principle of birds by Munsick and associates (Munsick et al. 1960, Munsick 1964). Arginine vasotocin appears to regulate renal water excretion by its actions on tubule water permeability and glomerular filtration rate (GFR). The dual mechanisms of action of antidiuretic principles in the avian kidney were reported as early as 1933 by Burgess et al. (1933) who described tubular and glomerular antidiuresis in domestic fowl injected with Pitressin. In subsequent studies, mammalian, and later avian neurohypophysial principles were used to study the mechanisms of antidiuresis in the avian kidney. Exogenous administration of these hormones consistently mimicked the renal antidiuretic responses to dehydration or salt loading (increased tubule water reabsorption and decreased GFR) observed in a variety of species, including the budgerigar, domestic fowl, Pekin duck, and Gambel's quail (for reviews, see Bentley 1974, Dantzler 1978, 1980a, 1980b, Dantzler and Braun 1981, Skadhauge 1981). The classical action

of antidiuretic hormone (ADH) to increase water permeability of the renal collecting duct was demonstrated in the domestic fowl by Skadhauge (1963, 1964), although direct measurement of AVT action on tubule permeability has yet to be accomplished. The glomerular action of AVT has been described in the domestic fowl (Ames et al. 1971), Gambel's quail (Braun and Dantzler 1974), and Pekin duck (Kaul et al. 1983).

Although AVT has been identified as the avian ADH and its actions on the kidney partially characterized, very little is known about the relative contributions of glomerular and tubular mechanisms to AVT-induced antidiuresis. Previous studies in which birds were dehydrated, salt-loaded, or given exogenous AVT reported highly variable reductions in GFR and therefore variable contributions by glomerular mechanisms to the overall antidiuretic responses of the kidney. The major flaw common to virtually all previous studies is the lack of knowledge concerning plasma AVT (P_{AVT}) levels, and whether the observed responses to salt loading or AVT injection were physiological or pharmacological in nature. To accurately assess the relative contributions of glomerular and tubular mechanisms to antidiuresis, the dose-response characteristics of these mechanisms must be determined at physiological plasma concentrations of AVT.

The recent development of sensitive and specific radioimmunoassay (RIA) techniques for the measurement of AVT (Rosenbloom and Fisher 1974, Mohring et al.. 1980) has permitted accurate measurement of P_{AVT} in normally hydrated and dehydrated birds (Koike et al. 1977, Gray and Simon 1983, present study, see Chapter 5).

The purpose of the present investigation was to determine the dose-response characteristics of glomerular and tubular mechanisms of antidiuresis in the domestic fowl. Constant infusion techniques were used to produce P_{AVT} concentrations similar to those measured in dehydrated domestic fowl.

Materials and Methods

Animals and General Experimental Procedures

All studies utilized Rhode Island Red cockerels, which were obtained from a commercial supplier (Red Wing Hatchery, Los Angeles, CA) at one week of age and raised in flocks at University of Arizona Facilities (Department of Animal Resources) in an environmentally controlled room (ambient temperature 26±1°C) with a 12:12 light-dark cycle. The birds were maintained in screen-bottomed cages, and provided with a commercial diet (chick starter mash and mixed scratch grain, Arizona Feeds, Inc.) and tap water *ad libitum*. When the birds attained 9-10 weeks of age (1200-1300g body weight) they were transferred to individual cages for at least one week prior to any surgical or experimental procedures.

All experiments were performed during daylight hours at an ambient temperature of 22-25°C. Quiet conditions were maintained in the laboratory during all experimentation to avoid excitation of conscious animals. In all experiments, baseline blood samples were taken at the beginning of each experiment which allowed each animal to serve as its own control for the experimental manipulations that followed.

Surgical Preparation

To avoid undue excitement or surgical stress during measurements of AVT secretion and to facilitate intravenous (i.v.) infusion and blood sample collection, birds were chronically catheterized. Animals were fasted overnight and under ketamine hydrochloride pre-anesthetic (20 mg/kg) and methoxyflurane inhalation anesthesia, surgical grade silastic catheters (0.940 mm OD x 0.508 mm ID) were placed under aseptic conditions in the right brachial artery and basilic vein with the tips of the catheters in or near the common carotid artery and cranial vena cava, respectively. The potential compromise in blood flow to the distal wing caused by chronic arterial and venous catheterization is readily overcome by the abundant collateral circulation present in the wing of the domestic fowl (Baumel 1979). The catheters were occluded with metal stylets, tunneled subcutaneously, and externalized on the neck 2-3 cm below the comb. The ends of the catheters were secured to the skin with a wound clip to prevent the birds from pecking at the free ends. During experiments, extensions were connected to the catheters to facilitate infusion and sample collection. The animals were given 5-6 days to recover and during that time, were treated prophylactically with oxytetracycline. During the recovery period, the birds were acclimated to handling (to reduce excitement during experiments) and the catheters rinsed with heparinized saline (40 U/ml) on alternate days to maintain their patency. The inhalation anesthesia was administered by a specially designed anesthetic device which utilized a unidirectional flow mask and enabled precise control of anesthetic

dosage (Stallone and Braun, manuscript in preparation). Usually, animals recovered completely without ill effects from anesthesia or surgery, and the catheters remained patent throughout the experimental period.

Effects of AVT Infusion on Glomerular Filtration Rate

To quantify the contribution of glomerular antidiuresis to renal water conservation, chronically catheterized birds were infused with AVT to obtain a dose-response relationship between physiological levels of plasma AVT and GFR. Glomerular filtration rate was measured by the clearance of [carboxyl-^{14}C]-inulin (sp. act. 2.6 mCi/g, lot no. 1496-149, New England Nuclear). The animals were fasted, with drinking water available ad libitum, for 24 hours prior to the start of the experiment to keep the cloaca free of feces during collection of urine. The experimental protocol was as follows: Twenty minutes prior to the start of an experiment, an animal was given a water load (20 ml/kg distilled water) by stomach tube to insure adequate hydration. The bird was suspended upright in a rip-stop nylon restraining sling that allowed free movement of the head and feet but prevented the bird from struggling. A cloth hood was placed over the head to prevent visual stimulation. The cloaca was cannulated with a specially modified 1.5 ml conical polyethylene centrifuge tube which permitted direct collection of ureteral urine as it was discharged into the cloaca and alleviated dead space errors. The cannula was secured in place by three 2-0 silk sutures that had been tied into the skin around the cloacal vent at the time of surgery. Urine was

collected into tared, 12 x 75 mm plastic culture tubes and volume determined gravimetrically with an accuracy of ± 0.01 ml. At the start of the experiment, the bird was given an i.v. priming dose of ^{14}C-inulin (5.0 μCi/kg) and hypotonic saline (4.0 ml/kg, 50 mosm/kg H_2O), and an i.v. maintenance infusion started (0.4 ml/kg · min, Sage infusion pump, model 355, Orion Res., Inc.) with hypotonic saline containing ^{14}C-inulin (0.20 μCi/ml) for 1 hr or more to obtain a stable control diuresis. When urine flow rate approximated the infusion rate, 3-4 consecutive urine samples were collected quantitatively for 5 min each to obtain control clearance values. A small arterial blood sample (400 μl) was taken at the midpoint of each clearance period for measurements of plasma inulin, electrolytes, and osmolality, and one large sample (1.0 ml) was taken during the middle clearance period for plasma AVT analysis.

AVT Infusions. Upon completion of the control clearance periods, synthetic AVT (sp. act. 210 U/mg, Bachem., Inc., lot no. R2750) was added to the maintenance infusion to deliver 0.125, 0.250, 0.500, or 1.00 ng/kg·min. Prior to the start of the AVT infusion, the bird was given a small i.v. priming dose (0.45-1.80 ng/kg) calculated to produce an instantaneous concentration (in the vascular space) equivalent to the steady state plasma AVT concentration achieved at the particular infusion rate. The AVT maintenance infusion was continued for 30 min to achieve a steady plasma AVT level, and then 3 consecutive clearance measurements (10 min each) were made. The longer clearance periods were necessitated by the reduced urine flow rates during AVT infusions. Arterial blood samples were taken at the

midpoint of each clearance period as described above. Each animal received two consecutive AVT infusions. The doses were overlapped between individual birds (i.e., 0.125 → 0.250, 0.250 → 0.500 ng/kg · min, etc.) to control for possible renal tachyphylaxis to AVT. Arterial blood pressure was recorded continuously during the experiment using a Gould-Statham pressure transducer (model p23dB) and a Gould chart recorder (model 2400S, Gould Inst., Inc.). At the end of the experiment, the bird was sacrificed with an i.v. overdose of sodium pentobarbital. All blood samples were centrifuged at 4°C immediately after collection, and the resultant plasma removed, aliquoted, and analyzed for inulin, osmolality, and AVT using the techniques described in detail below. Urine samples were centrifuged (2500 x g) for 5 min to precipitate urates and the supernatant urine removed, aliquoted, and analyzed for inulin and osmolality as described in detail below.

Radioimmunoassay of AVT

The radioimmunoassay of AVT in these studies utilized the R-70 antiserum developed by Fisher and associates at the UCLA School of Medicine (Rosenbloom and Fisher 1974, Skowsky et al. 1974). The RIA was performed in a final volume of 0.500 ml and utilized the R-70 antiserum at a final dilution of 1:500,000 in a sequential incubation (disequillibrium) system (D.A. Fisher, pers. comm.). Synthetic AVT (Bachem, Inc., lot no. R2750), bioassayed by W.H. Sawyer (210 U/mg, rat pressor activity), was used as the standard. Detailed descriptions of the assay characteristics, collection and extraction of

plasma, and radioiodination of AVT have been described previously (Chapter 4, Materials and Methods). As employed in these studies, the least detectable concentration of AVT (that which yields a binding response two standard deviations from the zero dose response) is 0.75 μU/ml (3.6 pg/ml) or the equivalent of 0.36 pg per assay tube. The intra-assay and inter-assay coefficients of variation average (mean±S.E.) 7.2±0.7% and 8.0±0.9%, respectively.

Additional Analyses of Plasma and Urine

Aliquots of plasma and urine from each experiment were stored at 4°C for rapid analysis of osmolality and ^{14}C-inulin. Plasma and urine osmolalities were measured by vapor pressure osmometry using a Wescor osmometer (model 5100B; Wescor, Inc.) with a sample size of 8.0 μl. The variation of any one sample (3-5 replicate measurements) was ± 0.5% (standard error). All measurements were made on fresh samples of plasma and urine kept at 4°C and not previously frozen. Activities of ^{14}C-inulin in plasma and urine were determined by liquid scintillation spectrometry using a Searle scintillation counter (Analytic 81, Searle Analytic). To avoid the potential problem of inulin precipitation, plasma and urine samples were prepared and counted immediately after each experiment using a clear, rigid gel-type scintillation cocktail (BetaPhase, WestChem, Inc.). Plasma (100 μl + 900 μl distilled water) and urine (50 μl + 950 μl distilled water) samples were added to 3.0 ml scintillation cocktail in plastic mini-vials and counted to 1% error.

Statistical Analyses

All data are presented as the mean ± one standard error. Data groups were subjected to one-way analysis of variance (ANOVA) to detect significant differences, followed by the Student Newman-Keuls test to distinguish significant differences among the means of a data group (Zar 1974), using a programmable desk-top calculator (model HP-97, Hewlett-Packard, Inc.) programmed from the Hewlett-Packard Statistical Package. Regression analyses were performed by the least-squares method (Zar 1974) using a desk-top computer (Apple II Plus, Apple Computers, Inc.) and an interactive software package (Curve Fitter, Interactive Microware, Inc.).

Results

AVT Infusion

The effects of AVT infusion on plasma AVT (P_{AVT}) concentration are summarized in Figure 14. In experimental birds infused i.v. with hypotonic saline (50 mosm/kg H_2O) and undergoing a control diuresis, mean P_{AVT} was 1.0±0.1 μU/ml (4.8±0.5 pg/ml). The infusion of AVT resulted in a linear increase in P_{AVT} to an average of 15.7±0.4 μU/ml at the highest dose of AVT; the nearly perfect linear relationship between P_{AVT} (y) and AVT dose (x) is described by the linear regression equation: $y = 14.67x + 0.90$ ($r^2 = 0.978$, $n=67$, $P < 0.001$).

Figure 14. The relationship between plasma AVT concentration and AVT infusion rate in domestic fowl. Each point represents the mean $\pm$ one standard error.

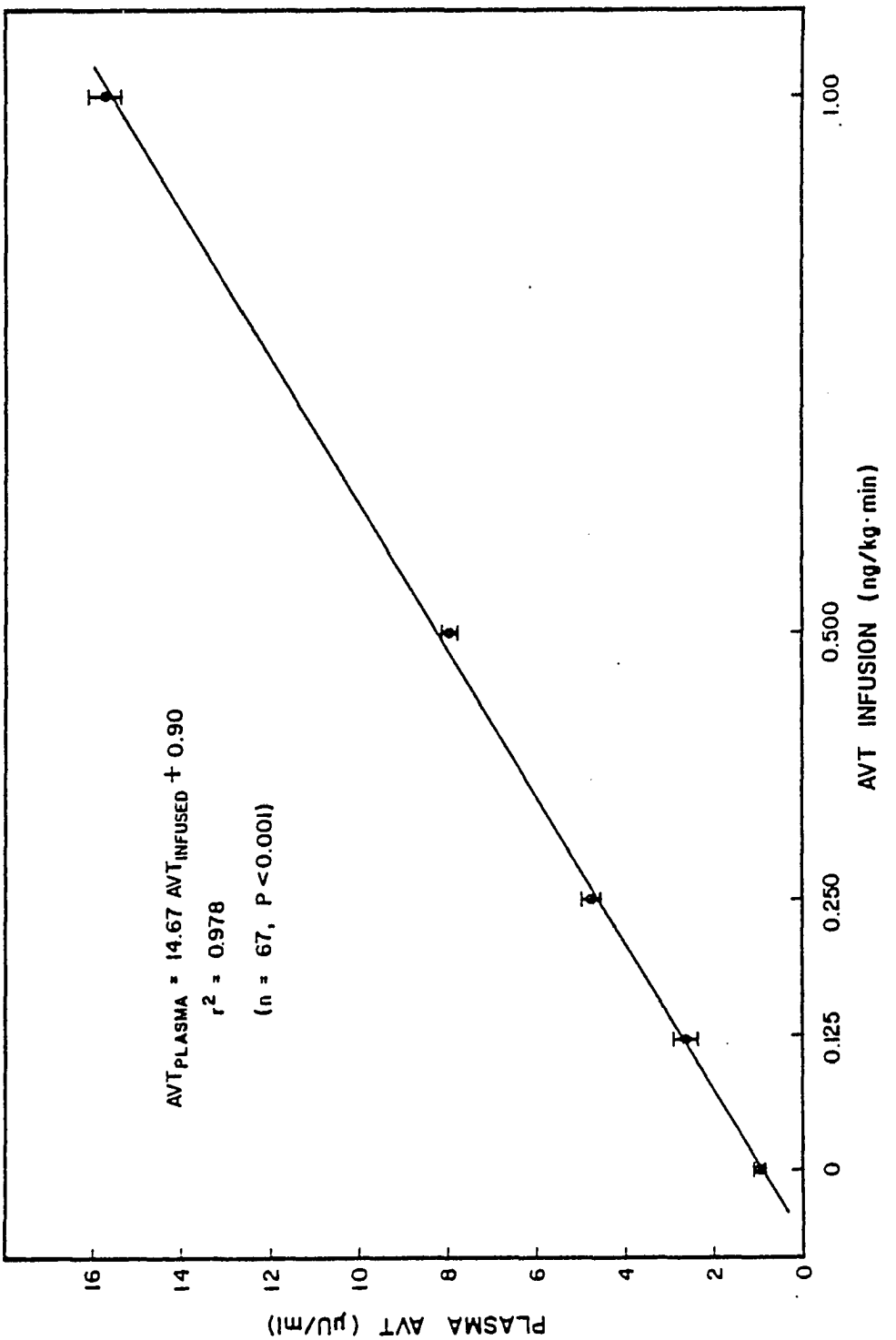
PLASMA AVT (μU/ml)
0
2
4
6
8
10
12
14
16
0
0.125
0.250
0.500
1.00
AVT INFUSION (ng/kg·min)
AVT_PLASMA = 14.67 AVT_INFUSED + 0.90
r² = 0.978
(n = 67, P<0.001)

Figure 14.

Glomerular Filtration Rate

The effects of AVT infusion on glomerular filtration rate (GFR) of conscious, intravenously infused domestic fowl are shown in Figure 15. Prior to the administration of AVT (control period), GFR averaged 2.85±0.13 ml/kg · min; with AVT infusion, GFR decreased in a dose-dependent curvilinear manner to an average of 72.2±2.10% of the initial (control) GFR at the highest dose of AVT (1.00 ng/kg · min). Changes in GFR of individual birds were computed as percentages of their initial (control) GFR to improve resolution of the responses to AVT that might otherwise be obscured by the significant individual variation in GFR commonly observed in birds. The highly correlated and significant curvilinear dose-response relationship between P_{AVT} (x) and GFR (y) is described by the second order polynomial equation: $y = 99.82-1.33(\log x)-17.87(\log x)^2$, ($r^2 = 0.834$, n=67, $P < 0.001$).

Mean arterial blood pressure (MAP) of the experimental birds averaged 136±3.3 mmHg (n=20) during the control diuresis, and did not vary by more than one standard error (± 3.3 mmHg) in response to AVT infusion at all doses studied. The average MAP did not vary systematically with the dose of AVT, and was not significantly different from the average MAP obtained during the control period at all doses studied ($P > 0.05$). Furthermore, MAP of individual birds remained constant or varied only slightly in response to either priming injection or constant infusion of AVT at all doses studied.

Figure 15. The dose-response relationship between plasma AVT concentration and glomerular filtration rate (GFR) in AVT-infused domestic fowl. Each point represents the mean $\pm$ one standard error (n = sample size).

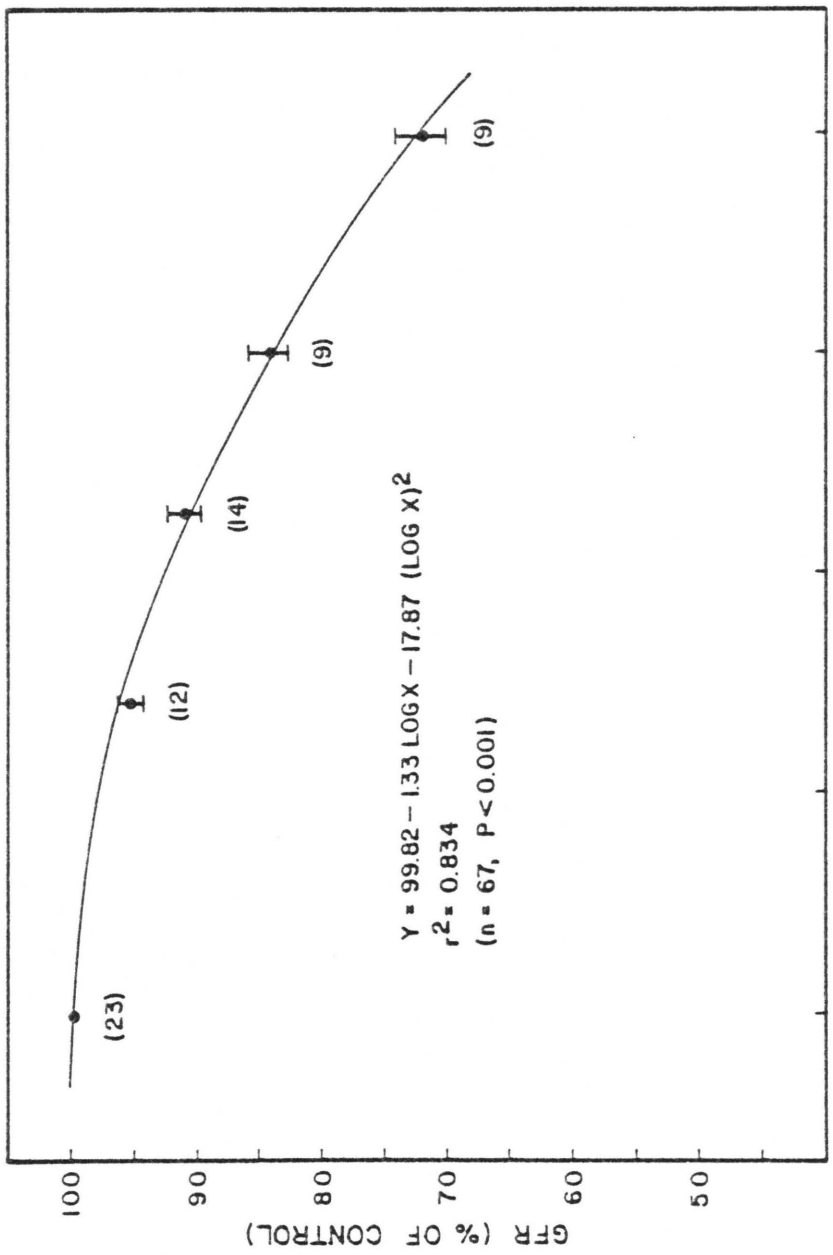
GFR (% OF CONTROL)
100
90
80
70
60
50
1.0
2.0
4.0
8.0
16.0
PLASMA AVT (μU/ml)
(23)
(12)
(14)
(9)
(9)
Y = 99.82 − 1.33 LOG X − 17.87 (LOG X)²
r² = 0.834
(n = 67, P < 0.001)

Figure 15.

Tubular Function

Renal water excretion was profoundly affected by the infusion of AVT, as reflected by the striking curvilinear dose-response relationships between P_{AVT} and both the osmotic urine to plasma (U/P) ratio (Fig. 16) and free water clearance (C_{H_2O}, Fig. 17). During the control diuresis, the osmotic U/P ratio averaged 0.275±0.009; with the administration of AVT, the U/P ratio increased in a dose-dependent fashion to a maximum of 1.690±0.035 at 1.0 ng/kg · min AVT. Thus, the U/P ratio increased by slightly more than six-fold in response to the maximum dose of AVT. The osmotic U/P ratio increased markedly at the two lower doses of AVT (0.125 and 0.250 ng/kg·min) and approached 1.0 at the lowest dose of AVT; at the two higher doses, the U/P ratio increased much more gradually. The significant ($P < 0.001$) curvilinear relationship between the osmotic U/P (y) and P_{AVT} (x) can be described by the third order polynomial equation: $y = 0.299+1.121(\log x)+1.257(\log x)^2-1.021(\log x)^3$, ($r^2 = 0.699$, $n=67$, $P < 0.001$). Free water clearance of the experimental birds averaged 0.272±0.021 ml/kg · min during the control diuresis; with AVT infusion, C_{H_2O} decreased curvilinearly to a low of - 0.013±0.002 ml/kg · min at the highest dose of AVT. C_{H_2O} decreased sharply at the lower doses of AVT (0.125 and 0.250 ng/kg· min) and approached 0 at 0.250 ng/kg · min AVT; thereafter C_{H_2O} decreased much more gradually. The relationship between C_{H_2O} (y) and P_{AVT} (x) is significant ($P < 0.001$) and can be described by the second order polynomial equation:
$y = 0.260-0.550(\log x)+0.269(\log x)^2$, ($r^2 = 0.684$, $n = 67$).

Figure 16. The dose-response relationship between plasma AVT concentration and osmotic urine to plasma (U/P) ratio in AVT-infused domestic fowl. Each point represents the mean $\pm$ one standard error (n = sample size).

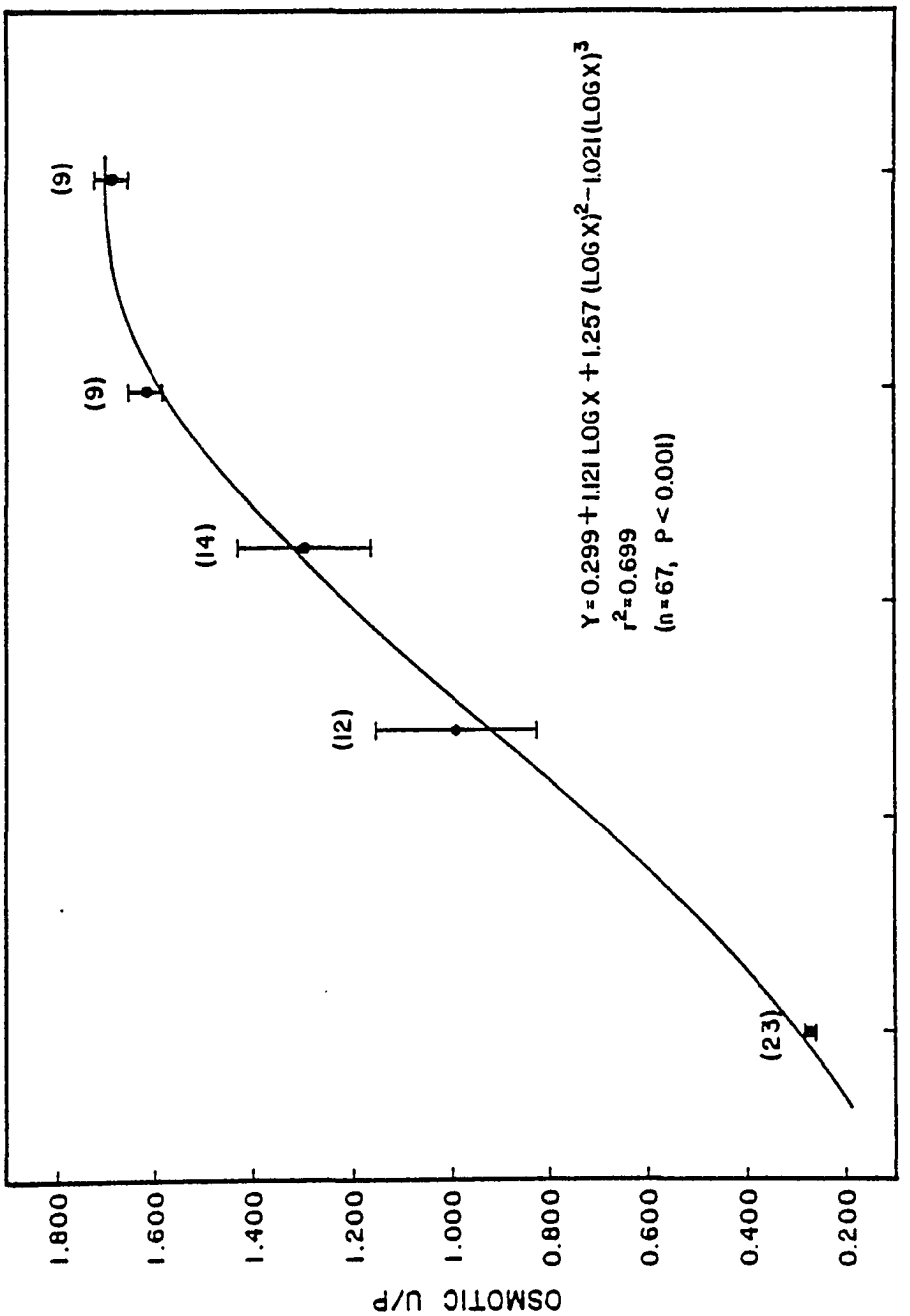
OSMOTIC U/P
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
1.0
2.0
4.0
8.0
16.0
PLASMA AVT (μU/ml)
(9)
(9)
(14)
(12)
(23)
Y=0.299+1.121 LOG X + 1.257 (LOG X)²−1.021(LOG X)³
r²=0.699
(n=67, P<0.001)

Figure 16.

Figure 17. The dose-response relationship between plasma AVT concentration and free water clearance (C_{H_2O}) in AVT-infused domestic fowl. Each point represents the mean $\pm$ one standard error (n = sample size).

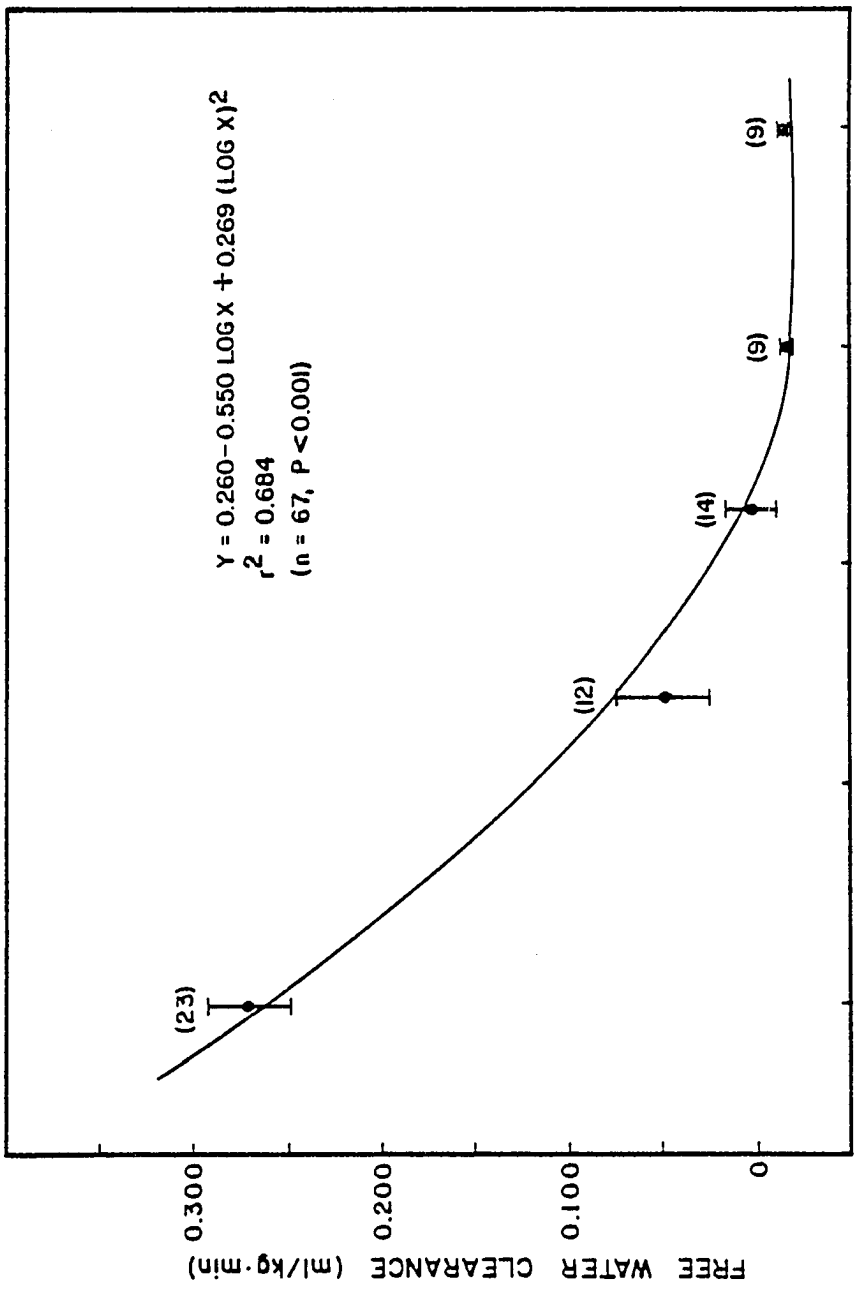
Y = 0.260−0.550 LOG X + 0.269 (LOG X)²
r² = 0.684
(n = 67, P<0.001)
(23)
(12)
(14)
(9)
(9)
FREE WATER CLEARANCE (ml/kg·min)
0.300
0.200
0.100
0
1.0
2.0
4.0
8.0
16.0
PLASMA AVT (μU/ml)

Figure 17.

The effects of AVT infusion on urine flow rate($\dot{V}$), inulin urine to plasma (U/P) ratio, urine osmolality (U_{OSM}), and plasma osmolality (P_{OSM}) are summarized in Table 8. In response to AVT infusion, $\dot{V}$ decreased significantly ($P < 0.01$) in a curvilinear manner similar to that of C_{H_2O} (Fig. 16). The greatest reduction in $\dot{V}$ occurred at the lowest dose of AVT when $\dot{V}$ fell to 32.9% of the control diuresis value. At higher doses of AVT, $\dot{V}$ declined more gradually to a minimum of 4.8% of the control value at 1.00 ng/kg • min AVT. The inulin U/P ratio increased progressively with the dose of AVT infused in a curvilinear pattern similar to that of the osmotic U/P ratio (Fig. 15). In response to the highest dose of AVT administered, the inulin U/P ratio increased by thirteen-fold over the basal value during the control diuresis. A similar curvilinear response was observed in U_{OSM} in response to AVT infusion. Thus, the greatest increase in U_{OSM} (nearly four-fold) occurred in response to the lowest dose of AVT (0.125 ng/kg • min); at higher doses of AVT, U_{OSM} increased more gradually to a maximum value nearly six-fold greater than basal U_{OSM} during the control diuresis. P_{OSM} exhibited a gradual linear decline in response to AVT administration that was significant ($P < 0.01$) at AVT doses of 0.250 ng/kg • min and higher.

Statistical comparisons of all renal function data indicate that renal responses of the birds at any given dose level of AVT do not differ significantly ($P > 0.05$) regardless of whether a given AVT dose preceeded or was followed by another dose during the experimental protocol.

Table 8. Urine flow rate, inulin U/P ratio, and urine and plasma osmolalities of AVT-infused domestic fowl.[a]

Variable	AVT Infusion (ng/kg · min)				
	0	0.125	0.250	0.500	1.00
Urine Flow Rate (ml/kg · min)	0.374±0.029[b] (n=23)	0.123±0.030[c] (n=12)	0.062±0.013[c] (n=14)	0.024±0.002[d] (n=9)	0.018±0.002[d] (n=9)
Inulin U/P	8.6±0.6[e] (n=23)	48.6±11.1[f] (n=12)	66.4±9.7[f] (n=14)	104.7±7.9[g] (n=9)	112.6±3.7[g] (n=9)
Urine Osmolality (mosm/kg H_2O)	82.1±2.5[h] (n=23)	293.8±49.4[i] (n=12)	375.9±39.6[i] (n=14)	478.4±12.8[j] (n=9)	486.6±9.8[j] (n=9)
Plasma Osmolality (mosm/kg H_2O)	298.4±1.3[k] (n=23)	293.3±2.7[k,l] (n=12)	289.7±2.8[l] (n=14)	294.4±1.8[k,l] (n=9)	287.9±1.5[l] (n=9)

[a]All values are mean ± S.E. (n = sample size).

[b-l]Within each row, mean values without common superscript are significantly different ($P < 0.01$).

Discussion

In the present investigation, the dose-response characteristics of glomerular and tubular mechanisms of AVT-induced antidiuresis were determined in the domestic fowl. Constant infusion techniques were utilized to produce plasma AVT (P_{AVT}) concentrations similar to those measured by RIA in water deprived domestic fowl.

AVT Infusion

Plasma AVT (1.0±0.1 μU/ml or 4.8±0.5 pg/ml) and plasma osmolality (P_{OSM}, 298.4±1.3 mosm/kg H_2O) of conscious domestic fowl infused i.v. with hypotonic saline in the present study are nearly identical to the measurements in conscious, hypo-osmotic water-loaded domestic fowl reported in the studies of osmotic regulation of AVT secretion (see Chapter 4). Together, these studies illustrate that reductions in P_{OSM} result in inhibition of AVT secretion (decreased P_{AVT}) and concomitant diuresis. The infusion of AVT resulted in a linear increase in P_{AVT} (Fig. 14), and was accompanied by dose-dependent antidiuretic responses (vide infra). The maximum P_{AVT} concentrations achieved by constant infusion (15.7±0.4 μU/ml) are similar to the maximum concentrations measured in water deprived domestic fowl (13.5±0.6 μU/ml, range 11.8 - 17.0 μU/ml; see Chapter 5).

Glomerular Filtration Rate

The basal glomerular filtration rate (GFR) of conscious birds in the present study (2.85±0.13 ml/kg · min) is quite similar to previously reported GFR measurements in both anesthetized and

conscious domestic fowl and Pekin ducks, hydrated by oral water load or i.v. hypotonic infusion (range 2.12-3.18 ml/kg · min; Korr 1939, Skadhauge 1964, Dantzler 1966, Skadhauge and Schmidt-Nielsen 1967b, Holmes et al. 1968, Ames et al. 1971, Kaul et al. 1983). Thus it can be assumed that renal function of the birds in the present study (prior to the administration of AVT) was comparable to that of birds in previous studies, during comparable states of hydration.

The results of the AVT infusion experiments in the present study indicate that avian antidiuretic hormone is a primary factor in the regulation of GFR in the domestic fowl. Infusions of exogenous AVT that result in P_{AVT} levels comparable to those measured during chronic water deprivation result in dose-dependent curvilinear reductions in GFR which average nearly 30% at maximum physiological P_{AVT} levels (Fig. 15).

Previous studies in which birds were administered exogenous AVT to mimic the renal antidiuretic responses to osmotic stress reported highly variable reductions in GFR, which ranged from 0 to 90% (Skadhauge 1964, Dantzler 1966, Ames et al. 1971, Braun and Dantzler 1974, Kaul et al. 1983). Thus, the contribution by glomerular mechanisms to the overall antidiuretic responses of the avian kidney appeared to be quite variable and uncertain. Much of this variability is likely the result of differences in experimental techniques used including: a) the use of anesthetics which alter blood pressure and possibly, renal function; b) the doses of AVT administered, which would be expected to result in low physiological to high pharmacological plasma levels; and c) the administration of AVT by

i.v. injection which results in highly variable peak and average plasma levels of the hormone, and therefore, highly variable duration and intensity of observed hormone actions. Pulse intravenous injection of high doses of AVT (> 50 ng/kg) was frequently associated with transient reductions in arterial blood pressure which likely contributed to reductions in GFR by non-specific means.

In the present studies, constant i.v. infusion techniques permitted accurate assessment of the dose-response relationship between P_{AVT} and GFR in conscious birds during steady-state, physiological P_{AVT} levels. Since glomerular (and tubular) responses at any given dose of AVT were not significantly different ($P > 0.05$) whether a given dose was preceeded or followed by a subsequent dose, it can be concluded that renal tachyphylaxis to AVT did not occur during these constant infusion experiments. Furthermore, the lack of effect of AVT on arterial blood pressure in these experiments indicates that AVT does not exert systemic vasodepressor activity at physiological plasma levels. Thus, the observed reductions in GFR were likely the result of specific vasoconstrictor action of AVT on the renal vasculature, probably at the level of the afferent arteriole to induce intermittency of reptilian-type nephrons, as described previously by Dantzler (1966), Braun and Dantzler (1972, 1974), and Braun (1976).

Tubular Function

In the present study, infusions of AVT profoundly affected renal water excretion. During the control diuresis when P_{AVT}

concentration was minimal (Fig. 14), renal water excretion attained maximal levels; this is reflected by the high $\dot{V}$ (0.375 ml/kg · min) and C_{H_2O} (0.272 ml/kg · min). The close approximation of $\dot{V}$ (0.375 ml/kg · min) to the i.v. infusion rate (0.4 ml/kg · min) during diuresis suggests that the birds were in input-output balance prior to the administration of AVT. Infusions of exogenous AVT result in dose-dependent reductions in renal water excretion, as reflected by the striking curvilinear relationships between P_{AVT} and $\dot{V}$ and C_{H_2O} (Table 8, Fig. 17). The pronounced reductions in renal water excretion effected by AVT are also reflected by the dose-dependent reductions in P_{OSM} (Table 8).

The maximal responses to AVT uniformly occurred at the lower doses of AVT (0.125 and 0.250 ng/kg · min); at the higher doses, little additional change in tubular responses occurred, as evidenced by the asymptotic nature of the dose-response relationships (Figs. 16, 17, Table 8). These data strongly suggest that maximal renal tubular responses to AVT occur at or near the highest dose used in this study (1.0 ng/kg · min), which results in a P_{AVT} level (15.7 μU/ml) quite comparable to the maximum measured during chronic water deprivation (13.5 μU/ml, range 11.8–17.0 μU/ml, see Chapter 5). In two birds that received AVT infusions of 2.0 ng/kg · min (double the highest dose used in this study), renal tubular responses to AVT were not significantly altered ($P > 0.05$) beyond the apparent maxima observed at 1.0 ng/kg · min. Thus, it can be suggested that maximal renal tubular responses to AVT in the domestic fowl occur at P_{AVT} levels that correspond to the maximum measured during chronic water deprivation.

Previous studies in which birds were administered exogenous AVT reported renal tubular responses that were qualitatively similar to the responses observed in the present study. Intravenous injections of up to 200 ng/kg AVT in domestic fowl (Ames et al. 1971) and Gambel's quail (Braun and Dantzler 1974) resulted in pronounced tubular antidiuretic responses (decreased $\dot{V}$, C_{H_2O}, increased U_{OSM} and osmotic U/P ratio). However, dose-dependency of the tubular responses was not clearly established in either of these studies, and was likely obscured by the transient peak antidiuretic responses that result from pulse i.v. injections of the hormone. In a more recent study in which Pekin ducks were given constant i.v. infusions of AVT (.008-2.0 ng/min; Mohring et al. 1980), clearly defined dose-response relationships were established between physiological serum AVT concentrations and $\dot{V}$ and U_{OSM}. These dose-response relationships are qualitatively and quantitatively similar to those established in the present study, which suggests that renal tubular mechanisms of the domestic fowl and Pekin duck exhibit similar sensitivity to physiological P_{AVT} levels.

Relative Contributions of Glomerular and Tubular Mechanisms to Antidiuresis

Comparison of the dose-response relationships between P_{AVT} and GFR (Fig. 15), osmotic U/P ratio (Fig. 16), C_{H_2O} (Fig. 17), and $\dot{V}$ (Table 8) in the present study indicates that tubular mechanisms are of primary importance and glomerular mechanisms of secondary importance in the conservation of water by the domestic fowl. This conclusion is based on the observation that the greatest proportion of

the total AVT-induced reduction in renal water excretion occurs at the lower doses of AVT (P_{AVT} < 5μU/ml), prior to any significant reduction in GFR. Comparison of mean glomerular and tubular responses at the second lowest dose of AVT (0.250 ng/kg · min) vividly illustrates this point. At this dose level (mean P_{AVT} = 4.8±0.2 μU/ml, Fig. 14), $\dot{V}$ and C_{H_2O} decreased by 88% and 94% respectively, of the maximum response, and the osmotic U/P ratio and U_{OSM} increased by 72% and 73% respectively, of the maximum response. In contrast, the observed reduction in GFR (to 91.1±1.4% of control) first becomes significant ($P < 0.01$) at this dose of AVT. Thus, the greatest AVT-induced reductions in water excretion occur at P_{AVT} < 4.8 μU/ml and appear to be the exclusive result of tubular mechanisms of antidiuresis.

At higher doses of AVT (high physiological P_{AVT} levels), glomerular and tubular mechanisms of antidiuresis overlap and their effects on water conservation cannot be separated. Although GFR is reduced by nearly 30% at the highest dose of AVT, only minor additional amounts of water are conserved by the combined actions of glomerular and tubular mechanisms. In previous studies on the effects of AVT on GFR in Gambel's quail (Lophortyx gambelii), Braun and Dantzler (1974) observed that AVT-induced reductions in GFR were quantitatively accounted for by reductions in the numbers of filtering reptilian-type (RT) nephrons. They suggested that reductions in volume flow through the medullary collecting ducts brought about by such reductions in GFR, might be more important than AVT-induced changes in tubular permeability to water in enhancing the concentrating

ability of the avian kidney. However, in the present study, the reduction in volume flow through the medullary collecting ducts that occurs with a 30% reduction in GFR appears to have very little effect on the concentrating ability of the domestic fowl kidney. Since RT nephrons do not function in concert to produce urine hyperosmotic to the plasma, reduction of the number of filtering nephrons would help to conserve water at the expense of excreting waste. Although reductions in the number of filtering nephrons appear to occur in the domestic fowl in response to osmotic stress (Dantzler 1966), the data of the present study indicate that at physiological P_{AVT} levels, tubular mechanisms of antidiuresis are of primary importance in the conservation of water.

In summary, the data of the present study provide strong evidence that tubular mechanisms of antidiuresis are of primary importance in the conservation of water by the avian kidney. This conclusion is based on the dose-response relationships established between P_{AVT} and glomerular and tubular mechanisms of antidiuresis over the entire range of physiological P_{AVT} concentrations in the domestic fowl. These data also indicate that reductions in GFR of approximately 30% that occur at maximum physiological P_{AVT} levels have only a minor, secondary effect on water conserving ability of the avian kidney.

REFERENCES CITED

Acher, R., Chauvet, J., and Chauvet, M.-T. 1970. Phylogeny of the neurohypophysial hormones - the avian active peptides. Eur. J. Biochem., 17: 509-513.

Akester, A.R. 1967. Renal portal shunts in the kidney of the domestic fowl. J. Anat., 101: 569-594.

Ames, E., Steven, K., and Skadhauge, E. 1971. Effects of arginine vasotocin on renal excretion of Na^+, K^+, Cl^-, and urea in the hydrated chicken. Am. J. Physiol., 221: 1223-1228.

Anonymous. 1973. Standard techniques for the measurement of red-cell and plasma volume. Br. J. Haematol., 25: 801-814.

Arnason, S.S., Rice, G.E., and Skadhauge, E. 1982. The effects of dietary NaCl and dehydration on plasma levels of aldosterone, corticosterone, and arginine vasotocin in the domestic fowl. Acta Physiol. Scand., 116: Suppl. 508: 34.

Arnauld, E., Czernichow, P., Fumoux, F., and Vincent, J.-D. 1977. The effects of hypotension and hypovolaemia on the liberation of vasopressin during haemorrhage in the unanaesthetized monkey (Macaca mulatta). Pflugers Arch., 371: 193-200.

Baumel, J.J. 1979. Systema cardiovasculare, pp. 343-407 in: Nomina Anatomica Avium, Baumel, J.J., ed., New York, Academic Press.

Bennett, H.P.J., Hudson, A.M., McMartin, C., and Purdon, G.E. 1977. Use of octadecasilyl-silica for the extraction and purification of peptides in biological samples. Biochem. J., 168: 9-13.

Bentley, P.J. 1966. Hyperglycaemic effect of neurohypophysial hormones in the chicken, Gallus domesticus. J. Endocrinol., 34: 527-528.

Bentley, P.J. 1974. Actions of neurohypophysial peptides in amphibians, reptiles, and birds, pp. 545-563 in: Handbook of Physiology, Sec. 7: Endocrinology, Vol. IV, The Pituitary Gland and Its Neuroendocrine Control, Part 1, Knobil, E., and Sawyer, W.H., eds., Washington, D.C., Amer. Physiol. Soc.

Bentley, P.J. 1980. Evolution of neurohypophysial peptide functions, pp. 95-106 in: Evolution of Vertebrate Endocrine Systems, Pang, P.K.T., and Epple, A. eds., Lubbock, TX, Texas Tech. Univ.

Billman, G.E., Keyl, M.J., Dickey, D.T., Kem, D.C., Keil, L.C., and Stone, H.L. 1983. Hormonal and renal response to plasma volume expansion in the primate Macaca mulatta. Am. J. Physiol., 244: H201-H205.

Blahser, S., and Simon, E. 1978. Immunocytochemical studies on vasotocinergic and mesotocinergic neurons in the hypothalamus of osmotically stressed domestic mallards. Gen. Comp. Endocrinol., 34: 67.

Boissonnas, R.A., Guttmann, St., Berde, B., and Konzett, H. 1961. Relationships between the chemical structures and the biological properties of the posterior pituitary hormones and their synthetic analogues. Experientia, 17: 377-390.

Bons, N. 1980. The topography of mesotocin and vasotocin systems in the brain of the domestic mallard and Japanese quail: Immunocytochemical identification. Cell Tissue Res., 213: 37-51.

Bradley, E.L., Holmes, W.N., and Wright, A. 1971. The effects of neurohypophysectomy on the pattern of renal excretion in the duck (Anas platyrhynchos). J. Endocrinol., 51: 57-65.

Braun, E.J. 1975. Effects of neurohypophysectomy on single nephron glomerular filtration rates (SNGFR) in the desert quail. Physiologist, 18: 150.

Braun, E.J. 1976. Intrarenal blood flow distribution in the desert quail following salt loading. Am. J. Physiol., 231: 1111-1118.

Braun, E.J. 1980. Qualitative and quantitative description of avian glomeruli and the functional significance of this analysis. Proc. 28th Int. Union Physiol. Sci., Budapest, 1980, 24: 337.

Braun, E.J., and Dantzler, W.H. 1972. Function of mammalian-type and reptilian-type nephrons in kidney of desert quail. Am. J. Physiol., 222: 617-629.

Braun, E.J., and Dantzler, W.H. 1974. Effects of ADH on single-nephron glomerular filtration rates in the avian kidney. Am. J. Physiol., 226: 1-8.

Braun, E.J., and Dantzler, W.H. 1975. Effects of water load on renal glomerular and tubular function in desert quail. Am. J. Physiol., 229: 222-228.

Burgess, W.W., Harvey, A.M., and Marshall, E.K., Jr. 1933. The site of the antidiuretic action of pituitary extract. J. Pharmacol. Exp. Ther., 49: 237-249.

Burrows, W.H., and Byerly, T.C. 1942. Premature expulsion of eggs by hens following injection of whole posterior pituitary preparations. Poult. Sci., 21: 416-421.

Burrows, W.H., and Fraps, R.M. 1942. Action of vasopressin and oxytocin in causing premature oviposition by domestic fowl. Endocrinology, 30: 702-705.

Burton, R.R., Sahara, R., and Smith, A.H. 1967. The use of human radio-iodinated (I^{131}) serum albumin in determining whole body blood volumes in the chicken. Poult. Sci., 46: 1395-1397.

Chan, D.K.O. 1977. Comparative physiology of the vasomotor effects of neurohypophysial peptides in the vertebrates. Am. Zool., 17: 751-762.

Chauvet, J., Lenci, M.-T., and Acher, R. 1960. Presence de deux vasopressines dans la neurohypophyse du poulet. Biochim. Biophys. Acta, 38: 571-573.

Coon, J.M. 1939. A new method for the assay of posterior pituitary extracts. Arch. Int. Pharmacodyn. Ther., 62: 79-99.

Cornelius, S.T., Klugman, K.P., and Hattingh, J. 1982. Effects of haemorrhage on colloid osmotic pressure in the pigeon. Comp. Biochem. Physiol. (A), 71: 337-339.

Crofton, J.T., Share, L., Wang, B.C., and Shade, R.E. 1980. Pressor responsiveness to vasopressin in the rat with DOC-salt hypertension. Hypertension, 2: 424-431.

Dantzler, W.H. 1966. Renal response of chickens to infusion of hyperosmotic sodium chloride solution. Am. J. Physiol., 210: 640-646.

Dantzler, W.H. 1978. Some renal glomerular and tubular mechanisms involved in osmotic and volume regulation in reptiles and birds, pp. 187-201 in: Osmotic and Volume Regulation, Alfred Benzon Symposium XI, Jorgensen, C.B., and Skadhauge, E., eds., Copenhagen, Munksgaard.

Dantzler, W.H. 1980a. Renal mechanisms for osmoregulation in reptiles and birds, pp. 91-110 in: Animals and Environmental Fitness, Gilles, R., ed., Oxford, Pergamon Press.

Dantzler, W.H. 1980b. Significance of comparative studies for renal physiology. Am. J. Physiol., 238: F437-F444.

Dantzler, W.H., and Braun, E.J. 1980. Comparative nephron function in reptiles, birds, and mammals. Am. J. Physiol., 239: R197-R213.

DeLawder, A.M., Tarr, L., and Geiling, E.M.K. 1954. The distribution in the chicken's hypophysis of the so-called posterior lobe principles. J. Pharmacol., 51: 142-143.

Desbuquois, B., and Aurbach, G.D. 1971. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J. Clin. Endocrinol. Metab., 33: 732-738.

Deutsch, H., and Simon, E. 1980. Intracerebroventricular osmosensitivity in the Pekin duck. Properties and functions in salt and water balance. Pflugers Arch., 387: 1-8.

Dicker, S.E., and Haslam, J. 1966. Water diuresis in the domestic fowl. J. Physiol. (Lond.), 183: 225-235.

Djojosugito, A.M., Folkow, B., and Kovach, A.G.B. 1968. The mechanisms behind rapid blood volume restoration after hemorrhage in birds. Acta Physiol. Scand., 74: 114-122.

Douglas, D.S., and Sturkie, P.D. 1964. Plasma levels of antidiuretic hormone during oviposition in the hen. Fed. Proc., 23: 150.

Dunn, F.L., Brennan, T.J., Nelson, A.E., and Robertson, G.L. 1973. The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J. Clin. Invest., 52: 3212-3219.

Emery, N., Poulson, T.L., and Kinter, W.B. 1972. Production of concentrated urine by avian kidneys. Am. J. Physiol., 223: 180-187.

Follett, B.K., and Farner, D.S. 1966. The effects of the daily photoperiod on gonadal growth, neurohypophysial hormone content, and neurosecretion in the hypothalamo-hypophysial system of the Japanese quail (Coturnix coturnix japonica). Gen. Comp. Endocrinol., 7: 111-124.

Gaddum, J.H. 1928. Some properties of the separated active principles of the pituitary (posterior lobe). J. Physiol. (Lond.), 65: 434-440.

George, J.C. 1980. Structure and physiology of posterior lobe hormones, pp. 85-115 in: Avian Endocrinology, Epple, A., and Stetson, M.H., eds., New York, Academic Press.

Gill, J.B., and Burford, H.J. 1969. The effect of antidiuretic hormone on avian salt gland function. Tissue Cell, 1: 497-501.

Goossens, N., Blahser, S., Oksche, A., Vandesande, F., and Dierickx, K. 1977. Immunocytochemical investigation of the hypothalamo-neurohypophysial system in birds. Cell Tissue Res., 184: 1-13.

Graber, J.W., and Nalbandov, A.V. 1965. Neurosecretion in the white leghorn cockerel. Gen. Comp. Endocrinol., 5: 485-492.

Gray, D.A., and Simon, E. 1983. Mammalian and avian antidiuretic hormone: Studies related to possible species variation in osmoregulatory systems. J. Comp. Physiol. (B), 151: 241-246.

Gray, S.J., and Sterling, K. 1950. The tagging of red cells and plasma proteins with radioactive chromium. J. Clin. Invest., 29: 1604-1613.

Harvey, S., and Phillips, J.G. 1982. Endocrinology of salt gland function. Comp. Biochem. Physiol. (A), 71: 537-546.

Hasan, S.H., and Heller, H. 1968. The clearance of neurohypophysial hormones from the circulation of non-mammalian vertebrates. Br. J. Pharmacol., 33: 523-530.

Hayward, J.N., Pavasuthipaisit, K., Perez-Lopez, F.R., and Sofroniew, M.V. 1976. Radioimmunoassay of arginine vasopressin in rhesus monkey plasma. Endocrinology, 98: 975-981.

Heller, H. 1941. Differentiation of an (amphibian) water balance principle from the antidiuretic principle of the posterior pituitary gland. J. Physiol. (Lond.), 100: 125-141.

Heller, H. 1942. The posterior pituitary principles of a species of reptile (Tropidonotus natrix) with some remarks on the comparative physiology of the posterior pituitary gland generally. J. Physiol. (Lond.), 101: 317-326.

Heller, H. 1969. Class and species specific actions of neurohypophysial hormones. Colloques Int. du Cent. Natl. Rech. Sci. (C.N.R.S.), 177: 35-43.

Heller, H., and Hasan, S.H. 1968. The fate of vasotocin and oxytocin in the domestic fowl. Arch. Anat. Histol. Embryol., 51: 315-319.

Heller, H., and Pickering, B.T. 1961. Neurohypophysial hormones of non-mammalian vertebrates. J. Physiol. (Lond.), 155: 98-114.

Herring, P.T. 1908. A contribution to the comparative physiology of the pituitary body. Q. J. Exp. Physiol., 1: 261-280.

Herring, P.T. 1913. Further observations upon the comparative anatomy and physiology of the pituitary body. Q. J. Exp. Physiol., 6: 73-108.

Hogben, L.T., and de Beer, G.R. 1925. Studies on the pituitary-VI. Localisation and phyletic distribution of active materials. Q. J. Exp. Physiol., 15: 163-176.

Hogben, L.T., and Schlapp, W. 1924. Studies on the pituitary-III. The vasomotor activity of pituitary extracts throughout the vertebrate series. Q. J. Exp. Physiol., 14: 229-258.

Holmes, W.N., and Adams, B.M. 1963. Effects of adrenocortical and neurohypophysial hormones on the renal excretory pattern in the water-loaded duck (Anas platyrhynchos). Endocrinology, 73: 5-10.

Holmes, W.N., Fletcher, G.L., and Stewart, D.J. 1968. The patterns of renal electrolyte excretion in the duck (Anas platyrhynchos) maintained on freshwater and on hypertonic saline. J. Exp. Biol., 48: 487-508.

Huber, G.C. 1917. On the morphology of the renal tubules of vertebrates. Anat. Rec., 13: 305-339.

Hughes, M.R. 1962. Studies on renal and extrarenal salt excretion in gulls and terns. Ph.D. Thesis, Duke University, North Carolina.

Ishii, S., Hirano, T., and Kobayashi, H. 1962. Neurohypophyseal hormones in the avian median eminence and pars nervosa. Gen. Comp. Endocrinol., 2: 433-440.

Itzhaki, R.F., and Gill, D.M. 1964. A micro-biuret method for estimating proteins. Anac. Biochem., 9: 401-410.

Jewell, P.A., and Verney, E.B. 1957. An experimental attempt to determine the site of the neurohypophysial osmoreceptors in the dog. Philos. Trans. R. Soc. Lond. (Biol.), 240: 197-324.

Johnson, J.A., Zehr, J.E., and Moore, W.W. 1970. Effects of separate and concurrent osmotic and volume stimuli on plasma ADH in sheep. Am. J. Physiol., 218: 1273-1280.

Johnson, O.W. 1968. Some morphological features of avian kidneys. Auk, 85: 216-228.

Johnson, O.W. 1974. Relative thickness of the renal medulla in birds. J. Morphol., 142: 277-284.

Johnson, O.W., and Mugaas, J.N. 1970. Quantitative and organizational features of the avian renal medulla. Condor, 72: 288-292.

Johnson, O.W., and Ohmart, R.D. 1973a. Some features of water economy and kidney microstructure in the large-billed savannah sparrow (Passerculus sandwichensis rostratus). Physiol. Zool., 46: 276-284.

Johnson, O.W., and Ohmart, R.D. 1973b. The renal medulla and water economy in vesper sparrows (Pooecetes graminens). Comp. Biochem. Physiol. (A), 44: 655-661.

Katsoyannis, P.G., and duVigneaud, V. 1958. Arginine vasotocin, a synthetic analogue of the posterior pituitary hormones containing the ring of oxytocin and the side chain of vasopressin. J. Biol. Chem., 233: 1352-1354.

Kaul, R., Gerstberger, R., Gray, D.A., and Simon, E. 1983. Altering plasma concentration of arginine vasotocin changes glomerular filtration rate in saline-adapted Pekin ducks. Physiologist, 26: A-58.

Koike, T.I., Estavillo, J.A., Pryor, L.R., and Neldon, H.L. 1981. Vasotocin secretion in the domestic fowl (Gallus domesticus). 9th Intl. Symp. Comp. Endocrinol., Hong Kong.

Koike, T., and Lepkovsky, S. 1967. Hypothalamic lesions producing polyuria in chickens. Gen. Comp. Endocrinol., 8: 397-402.

Koike, T.I., Pryor, L.R., and Neldon, H.L. 1979. Effect of saline infusion on plasma immunoreactive vasotocin in conscious chickens (Gallus domesticus). Gen. Comp. Endocrinol., 37: 451-458.

Koike, T.I., Pryor, L.R., and Neldon, H.L. 1980. Effect of hemorrhage on plasma vasotocin levels in conscious chickens. Physiologist, 23: 106.

Koike, T.I., Pryor, L.R., and Neldon, H.L. 1983. Plasma volume and electrolytes during progressive water deprivation in chickens (Gallus domesticus). Comp. Biochem. Physiol. (A), 74: 83-87.

Koike, T.I., Pryor, L.R., Neldon, H.L., and Venable, R.S. 1977. Effect of water deprivation on plasma radioimmunoassayable arginine vasotocin in conscious chickens (Gallus domesticus). Gen. Comp. Endocrinol., 33: 359-364.

Kook, Y., Cho, K.B., and Yun, K.O. 1964. Metabolic effects of oxytocin in the chicken. Nature, 204: 385-386.

Korr, I.M. 1939. The osmotic function of the chicken kidney. J. Cell. Physiol., 13: 175-193.

Kovach, A.G.B., and Balint, T. 1969. Comparative study on haemodilution after haemorrhage in the pigeon and the rat. Acta Physiol. Acad. Sci. Hung., 35: 231-243.

Krag, B., and Skadhauge, E. 1972. Renal salt and water excretion in the budgerygah (Melopsittacus undulatus). Comp. Biochem. Physiol. (A), 41: 667-683.

Kripalani, K., Ghosh, A., and Rahman, H. 1967. Hypothalamic neurosecretion in relation to water deprivation in ploceids of arid and swampy zones. J. Morphol., 123: 35-42.

LaRochelle, F.T., Jr., North, W.G., and Stern, P. 1980. A new extraction of arginine vasopressin from blood: the use of octadecasilyl - silica. Pflugers Arch., 387: 79-81.

Larsson, B., Olsson, K., and Fyhrquist, F. 1978. Vasopressin release induced by hemorrhage in the goat. Acta Physiol. Scand., 104: 309-317.

Lauson, H.D. 1974. Metabolism of the neurohypophysial hormones, pp. 287-393 in: Handbook of Physiology, Sec. 7: Endocrinology, Vol IV, The Pituitary Gland and Its Neuroendocrine Control, Part 1, Knobil, E., and Sawyer, W.H., eds., Washington, D.C., Amer. Physiol. Soc.

Legait, H., and Legait, E. 1955. Nouvelles recherches sur les modifications de structure du lobe distal de l'hypophyse au cours de divers etats physiologiques et experimentaux chez la poule Rhode Island. C. R. Assoc. Anat. (Paris), 91: 902-907.

Lepkovsky, S., and Yasuda, M. 1967. Adipsia in chickens. Physiol. Behav., 2: 45-47.

Medway, W., and Kare, M.R. 1959. Blood and plasma volume, hematocrit, blood specific gravity and serum protein electrophoresis of the chicken. Poult. Sci., 38: 624-631.

Mohring, J., Schoun, J., Simon-Oppermann, C., and Simon, E. 1980. Radioimmunoassay for arginine vasotocin (AVT) in serum of Pekin ducks: AVT concentrations after adaptation to fresh water and salt water. Pflugers Arch., 387: 91-97.

Munsick, R.A. 1964. Neurohypophysial hormones of chickens and turkeys. Endocrinology, 75: 104-112.

Munsick, R.A., Sawyer, W.H., and van Dyke, H.B. 1959. Hormonal characteristics of avian neurohypophysis. Fed. Proc., 18: 110.

Munsick, R.A., Sawyer, W.H., and van Dyke, H.B. 1960. Avian neurohypophysial hormones: Pharmacological properties and tentative identification. Endocrinology, 66: 860-871.

Nakano, J. 1974. Cardiovascular responses to neurohypophysial hormones, pp. 395-442 in: Handbook of Physiology, Sec. 7: Endocrinology, Vol. IV The Pituitary Gland and Its Neuroendocrine Control, Part 1, Knobil, E., and Sawyer, W.H., eds., Washington, D.C., Amer. Physiol. Soc.

Niezgoda, J. 1975. Changes in blood arginine vasotocin level after osmotic stimuli in domestic fowl. Acta Physiol. Pol., 26: 591-597.

Niezgoda, J., and Rzasa, J. 1971. Blood levels of vasotocin in birds. Bull. Acad. Pol. Sci. (Biol., Cl. II), 19: 359-361.

Niezgoda, J., Rzasa, J., and Ewy, Z. 1973. Changes in blood vasotocin activity during oviposition in the hen. J. Reprod. Fertil., 35: 505-509.

Nirmalan, G.P., and Robinson, G.A. 1972. Effect of age, sex, and egg laying on the total erythrocyte volume ($^{51}CrO_4$ label) and the plasma volume (^{125}I-serum albumin label) of Japanese quail. Can. J. Physiol. Pharmacol., 50: 6-10.

North, W.G., LaRochelle, F.T., Jr., Haldar, J., Sawyer, W.H., and Valtin, H. 1978. Characterization of an antiserum used in a radioimmunoassay for arginine-vasopressin: Implications for reference standards. Endocrinology, 103: 1976-1984.

Nouwen, E.J., and Kuhn, E.R. 1983. Radioimmunoassay of arginine vasotocin and mesotocin in serum of the frog Rana ridibunda. Gen. Comp. Endocrinol., 50: 242-251.

O'Connor, W.J. 1962. Release of antidiuretic hormone from the neurohypophysis, pp. 50-63 in: Renal Function, O'Connor, W.J., London, Arnold.

Oksche, A., and Farner, D.S. 1974. Neurohistological studies of the hypothalamo-hypophysial system of Zonotrichia leucophrys gambelii. With special attention to its role in the control of reproduction. Adv. Anat. Embryol. Cell Biol., 48: 1-136.

Oksche, A., Farner, D.S., Serventy, D.L., Wolff, F., and Nicholls, C.A. 1963. The hypothalamo-hypophysial neurosecretory system of the zebra finch, Taeniopygia castanotis. Z. Zell. Mikrosk. Anat., 58: 846-914.

Oksche, A., and Hartwig, H.-G. 1980. Structural principles of central neuroendocrine systems, pp. 75-84 in: Avian Endocrinology, Epple, A., and Stetson, M.H., eds., New York, Academic Press.

Oksche, A., Laws, D.F., Kamemoto, F.I., and Farner, D.S. 1959. The hypothalamo-hypophysial neurosecretory system of the white-crowned sparrow, Zonotrichia leuclophrys gambelii. Z. Zell. Mikrosk. Anat., 51: 1-42.

Opel, H. 1965. Oviposition in chickens after removal of the posterior lobe of the pituitary by an improved method. Endocrinology, 76: 673-677.

Pang, P.K.T. 1977. Osmoregulatory functions of neurohypophysial hormones in fishes and amphibians. Am. Zool., 17: 739-749.

Pang, P.K.T., Furspan, P.B., and Sawyer, W.H. 1983. Evolution of neurohypophyseal hormone actions in vertebrates. Am. Zool., 23: 655-662.

Paton, D.N., and Watson, A. 1912. The actions of pituitrin, adrenalin, and barium on the circulation of the bird. J. Physiol. (Lond.), 44: 413-424.

Peaker, M. 1979. Control mechanisms in birds, pp. 323-348 in: Mechanisms of Osmoregulation in Animals, Gilles, R., ed., New York, Wiley.

Peaker, M. 1971. Effects of arginine vasotocin on the salt gland of the goose, Anser anser. J. Endocrinol., 49: xxvi-xxvii.

Peek, J.C., and Watkins, W.B. 1979. Identification of neurophysin-like proteins in the posterior pituitary gland of the chicken (Gallus Domesticus). Neuroendocrinology, 28: 52-63.

Pickering, B.T., and Heller, H. 1959. Chromatographic and biological characteristics of fish and frog neurohypophysial extracts. Nature, 184: 1463-1464.

Pitts, R.F. 1938. The excretion of phenol red by the chicken. J. Cell. Physiol., 11: 99-115.

Poulson, T.L. 1965. Countercurrent multipliers in avian kidneys. Science, 148: 389-391.

Ralph, C.L. 1960. Polydipsia in the hen following lesions in the supraoptic hypothalamus. Am. J. Physiol., 198: 528-530.

Reaves, T.A., Jr., Liu, H.-M., Qasim, M.M., and Hayward, J.N. 1981. Osmotic regulation of vasopressin in the cat. Am. J. Physiol., 240: E108-E111.

Rice, G.E. 1982. Plasma arginine vasotocin concentrations in the lizard Varanus gouldii (Gray) following water loading, salt loading, and dehydration. Gen. Comp. Endocrinol., 47: 1-6.

Riddle, O. 1921. A simple method of obtaining premature eggs from birds. Science, 54: 664-666.

Robertson, G.L., Athar, S., and Shelton, R.L. 1977. Osmotic control of vasopressin function, pp. 125-148 in: Disturbances in Body Fluid Osmolality, Andreoli, T.E., Grantham, J.J., and Rector, F.C., eds., Washington, D.C., Amer. Physiol. Soc.

Robertson, G.L., Mahr, E.A., Aṭhar, S., and Sinha, T. 1973. Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J. Clin. Invest., 52: 2340-2352.

Robertson, G.L., Shelton, R.L., and Athar, S. 1976. The osmoregulation of vasopressin. Kidney Int., 10: 25-37.

Rodbard, D., and Lewald, J.E. 1970. Computer analysis of radioligand assay and radioimmunoassay data. Acta Endocrinol. (Copenh.), 64: Suppl. 147: 79-103.

Rodbard, D., and Munson, P.J. 1978. Is there an osmotic threshold for vasopressin release? Am. J. Physiol., 234: E339-E342.

Rosenbloom, A.A., and Fisher, D.A. 1974. Radioimmunoassay of arginine vasotocin. Endocrinology, 95: 1726-1732.

Rzasa, J., and Niezgoda, J. 1969. Effects of the neurohypophysial hormones on sodium and potassium level in the hen's blood. Bull. Acad. Pol. Sci. (Biol., Cl. II), 17: 585-588.

Rzasa, J., Niezgoda, J. and Kahl, S. 1974. The effect of arginine vasotocin on blood volume, plasma protein and electrolyte concentrations in the cockerel, Gallus domesticus. Br. Poult. Sci., 15: 261-265.

Sawyer, W.H. 1968. Phylogenetic aspects of the neurohypophysial hormones, pp. 717-747 in: Handbuch der Experimentellen Pharmakologie. Neurohypophysial Hormones and Similar Peptides, V. XXIII, Berde, B., ed., Berlin, Springer-Verlag.

Sawyer, W.H. 1977. Evolution of active neurohypophysial principles among the vertebrates. Am. Zool., 17: 727-738.

Sawyer, W.H., Munsick, R.A., and van Dyke, H.B. 1959. Pharmacological evidence for the presence of arginine vasotocin and oxytocin in neurohypophysial extracts from cold-blooded vertebrates. Nature, 184: 1464-1465.

Sawyer, W.H., and Pang, P.K.T. 1975. Endocrine adaptation to osmotic requirements of the environment: Endocrine factors in osmoregulation by lungfishes and amphibians. Gen. Comp. Endocrinol., 25: 224-229.

Schmidt-Nielsen, K. 1960. The salt secreting glands in marine birds. Circulation, 21: 955-967.

Schmidt-Nielsen, K., Borut, A., Lee, P., and Crawford, E., Jr. 1963. Nasal salt excretion and the possible function of the cloaca in water conservation. Science, 142: 1300-1301.

Schrier, R.W., Berl, T., and Anderson, R.J. 1979. Osmotic and non-osmotic control of vasopressin release. Am. J. Physiol., 236: F321-F332.

Shannon, J.A. 1938. The excretion of exogenous creatinine by the chicken. J. Cell. Physiol., 11: 123-134.

Share, L. 1974. Blood pressure, blood volume, and the release of vasopressin, pp. 243-255 in: Handbook of Physiology, Sec. 7: Endocrinology, Vol. IV The Pituitary Gland and Its Neuroendocrine Control, Part 1, Knobil, E., and Sawyer, W.H., eds., Washington, D.C., Amer. Physiol. Soc.

Shirley, H.V., and Nalbandov, A.V. 1956. Effects of neurohypophysectomy in domestic chickens. Endocrinology, 58: 477-483.

Siller, W.G. 1971. Structure of the kidney, pp. 197-232 in: Physiology and Biochemistry of the Domestic Fowl, Vol. I, Bell, D.J., and Freeman, B.M., eds., New York, Academic Press.

Siller, W.G., and Hindle, R.M. 1969. The arterial blood supply to the kidney of the fowl. J. Anat., 104: 117-135.

Simon-Oppermann, C., and Simon, E. 1982. Osmotic and volume control of diuresis in conscious ducks (Anas platyrhynchos). J. Comp. Physiol., (B), 146: 17-25.

Simon-Oppermann, C., Simon, E., Deutsch, H., Mohring, J., and Schoun, J. 1980. Serum arginine vasotocin (AVT) and afferent and central control of osmoregulation in conscious Pekin ducks. Pflugers Arch., 387: 99-106.

Skadhauge, E. 1963. Does antidiuretic hormone act on kidney cells from the blood side or from the urine side? Acta Physiol. Scand., 59: Suppl. 213: 143.

Skadhauge, E. 1964. Effects of unilateral infusion of arginine-vasotocin into the portal circulation of the avian kidney. Acta Endocrinol. (Copenh.), 47: 321-330.

Skadhauge, E. 1973. Renal and cloacal salt and water transport in the fowl (Gallus domesticus). Dan. Med. Bull., 20: Suppl. I: 1-82.

Skadhauge, E. 1974. Renal concentrating ability in selected West Australian birds. J. Exp. Biol., 61: 269-276.

Skadhauge, E. 1976. Water conservation in xerophilic birds. Isr. J. Med. Sci., 12: 732-739.

Skadhauge, E. 1981 Osmoregulation In Birds, Berlin, Springer-Verlag.

Skadhauge, E., and Schmidt-Nielsen, B. 1967a. Renal medullary electrolyte and urea gradient in chickens and turkeys. Am. J. Physiol., 212: 1313-1318.

Skadhauge, E., and Schmidt-Nielsen, B. 1967b. Renal function in domestic fowl. Am. J. Physiol., 212: 793-798.

Skowsky, W.R., Rosenbloom, A.A., and Fisher, D.A. 1974. Radioimmunoassay measurement of arginine vasopressin in serum: Development and application. J Clin. Endocrinol. Metab., 38: 278-287.

Sperber, I. 1946. A new method for the study of renal tubular excretion in birds. Nature, 158: 131.

Sperber, I. 1948. Investigations on the circulatory system of the avian kidney. Zool. Bidr. Fran. Upps., 27: 429-448.

Strahan, R., and Waring, H. 1954. The effect of pituitary posterior lobe extracts on the blood pressure of the fowl. Aust. J. Exp. Biol. Med. Sci., 32: 193-206.

Sturkie, P.D., and Lin, Y.-C. 1966. Release of vasotocin and oviposition in the hen. J. Endocrinol., 35: 325-326.

Szczepanska-Sadowska, E. 1972a. The activity of the hypothalamo-hypophysial antidiuretic system in conscious dogs. I. The influence of isoosmotic blood volume changes. Pflugers Arch., 335: 139-146.

Szczepanska-Sadowska, E. 1972b. The activity of the hypothalamo-hypophysial antidiuretic system in conscious dogs. II. Role of the left vagosympathetic trunk. Pflugers Arch., 335: 147-152.

Tanaka, K., and Nakajo, S. 1962. Participation of neurohypophysial hormone in oviposition in the hen. Endocrinology, 70: 453-458.

Thorell, J.I., and Johansson, B.G. 1971. Enzymatic iodination of polypeptides with ^{125}I to high specific activity. Biochim. Biophys. Acta, 251: 363-369.

Uemura, H. 1964. Effects of water deprivation on the hypothalmo-hypophysial neurosecretory system of the grass parakeet, Melopsittacus undulatus. Gen. Comp. Endocrinol., 4: 193-198.

Valtin, H., Stewart, J., and Sokol, H.W. 1974. Genetic control of the production of posterior pituitary principles, pp. 131-171 in: Handbook of Physiology, Sec. 7: Endocrinology, Vol. IV The Pituitary Gland and Its Neuroendocrine Control, Part 1, Knobil, E., and Sawyer, W.H., eds., Washington, D.C., Amer. Physiol. Soc.

Verney, E.B. 1947. The antidiuretic hormone and the factors which determine its release. Proc. R. Soc. Lond. (Biol.), 135: 25-106.

Wade, C.E., Keil, L.C., and Ramsay, D.J. 1983. Role of volume and osmolality in the control of plasma vasopressin in dehydrated dogs. Neuroendocrinology, 37: 349-353.

Waring, H., Morris, L., and Stephens, G. 1956. The effect of pituitary posterior lobe extracts on the blood pressure of the pigeon. Aust. J. Exp. Biol. Med. Sci., 34: 235-238.

Weitzman, R.E., and Fisher, D.A. 1977. Log linear relationship between plasma arginine vasopressin and plasma osmolality. Am. J. Physiol., 233: E37-E40.

Wideman, R.F., Jr., Braun, E.J., and Anderson, G.L. 1981. Microanatomy of the renal cortex in the domestic fowl. J. Morphol., 168: 249-267.

Wood, C.E., Shinsako, J., Keil, L.C., Ramsay, D.J., and Dallman, M.F. 1981. Hormonal and hemodynamic responses to 15 ml/kg hemorrhage in conscious dogs: Responses correlate to body temperature. Proc. Soc. Exp. Biol. Med., 167: 15-19.

Woolley, P. 1959. The effect of posterior lobe pituitary extracts on blood pressure in several vertebrate classes. J. Exp. Biol., 36: 453-458.

Woolley, P., and Waring, H. 1958. Responses of the perfused fowl leg to posterior lobe pituitary extracts. Aust. J. Exp. Biol. Med. Sci., 36: 447-456.

Wright, A., Phillips, J.G., Peaker, M., and Peaker, S.J. 1967. Some aspects of the endocrine control of water and salt-electrolytes in the duck (Anas platyrhynchos). Proc. Third Asia Oceania Cong. Endocrinol., Manila, pp. 322-327.

Wyse, D.G., and Nickerson, M. 1971. Studies on hemorrhagic hypotension in domestic fowl. Can. J. Physiol. Pharmacol., 49: 919-926.

Zar, J.H. 1974. Biostatistical Analysis, Englewood Cliffs, Prentice-Hall.

Zehr, J.E., Johnson, J.A., and Moore, W.W. 1969. Left atrial pressure, plasma osmolality, and ADH levels in the unanesthetized ewe. Am. J. Physiol., 217: 1672-1680.