Title
Does ACTH mediate the corticosteroid response to 15 ml/kg hemorrhage in conscious dogs?

Author
Wood, Charles Evans

Publication Date
1980

Peer reviewed|Thesis/dissertation

Does ACTH Mediate the Corticosteroid Response to 15 ml/kg Hemorrhage in Conscious Dogs?

by

Charles Evans Wood

A.B. Univ. of Calif., Berkeley, 1974

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Endocrinology

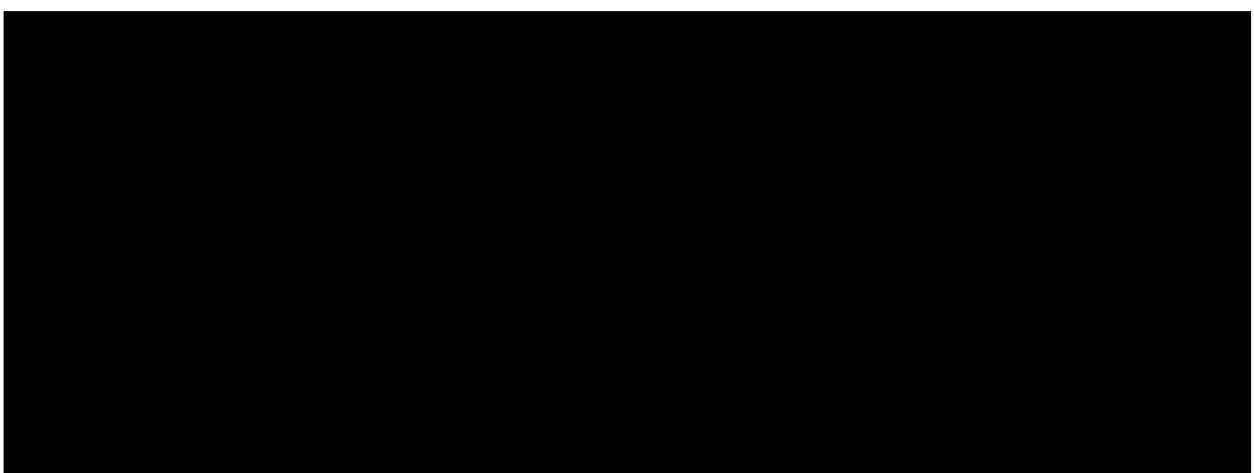
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



Date University Librarian

Degree Conferred: JUN 2 9 1980

DISSERTATION ABSTRACT

Fifteen conscious dogs with chronically maintained arterial catheters were subjected to rapid (<3 min) 10, 12-13, and 15 ml/kg hemorrhage and 30 min of hypovolemia. Hemorrhage of 15 ml/kg, but not 10 ml/kg caused hypotension and increases in heart rate and plasma ACTH and corticosteroid concentrations ($p<.001$). In 8 experiments on 5 dogs, the ACTH responses to 15 ml/kg hemorrhage were not reproducible.

In 20 experiments, dogs with elevated rectal temperature at the time of the experiment had higher prehemorrhage heart rate, ACTH, corticosteroids, vasopressin, and renin activity and responded to hemorrhage with a greater fall in mean arterial blood pressure and larger heart rate, vasopressin, ACTH, and corticosteroid responses than normothermic dogs. Thus, preselection of dogs for study on the basis of rectal temperature at the time of the experiment improves the reproducibility of cardiovascular and hormonal responses to 15 ml/kg hemorrhage.

Nine normothermic dogs responded to 15 ml/kg hemorrhage with small, slow ACTH responses (peak increase 11 pg/ml at 25 min) that were apparently dissociated from

the large, faster corticosteroid response (peak increase 2.7 ug/dl at 20 min). Hemorrhage decreased dichloromethane-extractable tritium counts 30% from steady-state levels during infusion of 3H-(1,2)-cortisol ($p<.001$), indicating that the increase in adrenal secretion of corticosteroids after hemorhage was underestimated by measurement of changes in peripheral plasma levels.

The dynamics and magnitudes of the adrenal response to increases in plasma ACTH concentration, were measured using intravenous infusions of synthetic a1-24 ACTH. In 13 of 16 experimental periods in which dogs were infused with saline, plasma ACTH concentration was not constant, and in 9 of 16 experimental periods, plasma corticosteroids were not related to plasma ACTH. Infusion of 10, 30, and 150 ng ACTH/min for 30 min elevated plasma corticosteroids to levels which were linearly related to the logarithm of the ACTH infusion rate and steady-state plasma ACTH concentration. Dogs responded as well to 3 pulses of 100 or 300 ng ACTH in 30 min as to the same total amount given as constant infusions. In all experiments, the lag between the first elevation in plasma ACTH and corticosteroid concentrations was 3 min or longer. Overall data were used to calculate estimates of ACTH half-disappearance time (1.4-3.8 min), total clearance rate (20.4-40.7 ml/kg/min) and volume of dis-

tribution (110-113 ml/kg).

Two experimental designs were used to test for hemorrhage-induced shifts in adrenal sensitivity to ACTH. In the first, dexamethasone-pretreated dogs were subjected to four 5 min steps of ACTH infusion, from 38 to 380 ng/min (in a preliminary set of experiments, dexamethasone was shown not to change adrenal sensitivity to ACTH). Plasma corticosteroids were elevated to levels linearly related to the logarithm of the ACTH infusion rate and plasma ACTH concentration. Corticosteroid responses were not changed by hemorrhage. In a second series of experiments, dexamethasone pretreated dogs were subjected to steady-state ACTH infusions at 5, 10, and 20 ng/min (N=4 at each dose). Corticosteroid responses were linearly related to the logarithm of the ACTH infusion rate ($r^2 = .77$), and not changed by hemorrhage. Hemorrhage did not change the rate of disappearance of dichloromethane-extractable tritium counts 35-65 min after injection of 3H-(1,2)-cortisol), indicating that adrenal gain was not changed by the hemorrhage.

Five ng/min infusion of ACTH for 30 min elevated plasma corticosteroids about 3 ug/dl, while theoretically increasing plasma ACTH 6-12 pg/ml (similar to increases during 15 ml/kg hypovolemia), and the first rise in ACTH causing the first rise in corticosteroids

was about 3.5 pg/ml. Because of this remarkable sensitivity of the adrenal to ACTH, it is likely that increases in plasma corticosteroid during 15 ml/kg hypovolemia are caused by undetectable increases in plasma ACTH.

ACKNOWLEDGEMENTS

First, I would like to thank Mary Dallman for her wisdom and persistent patience, guidance, and support.

Thanks to John Baxter and Pentti Siiteri for their time and energies as committee members.

Thanks to Ian Reid and John Urquhart for their comments.

Thanks to Jeannette Shinsako, Shirley Chi, Brian Matsumura, Helen Hughes, Barbara Moffat, and Lanny Keil for their expertise in hormone assay.

Thanks to David Ramsay, who introduced me to conscious dogs. Thanks to David Ramsay, Roy Shackelford, and Colin Rudolph for teaching me the art of catheter implantation.

CONTENTS

FIGURES

TABLES

CHAPTER ONE

INTRODUCTION

Uncompensated hemorrhage would lead to decreased mean circulatory pressure, decreased cardiac output, and a fall in arterial blood pressure. However, compensatory mechanisms restore blood pressure on the short term by increasing peripheral resistance and redistributing blood flow (to defend perfusion pressure to the heart and brain), and on the long term by restoring blood volume and thus cardiac output and normal distribution of blood flow throughout the body.

After hemorrhage, arterial pressure is increased by increases in heart rate, contractility, and peripheral resistance. Heart rate and contractility are increased by an increase in sympathetic tone to the heart, and by an increase in circulating levels of catecholamines. Heart rate is also increased by a decrease in vagal tone to the heart. Peripheral resistance is increased by increases in sympathetic tone to the afferent arterioles, and increases in circulating levels of angiotensin, vasopressin, and catecholamines. Intracapillary pressure was initially reduced by the fall in mean circulatory pressure at the time of hemorrhage, and is further reduced by afferent arteriolar constriction. The low intracapillary pressure favors the movement of

interstitial fluid into the vasculature. The colloid osmotic pressure of the plasma is maintained by the restoration of a normal concentration of plasma proteins, a process dependent upon the hemorrhage-induced secretion of corticosteroids. Blood volume is conserved by an increase in plasma vasopressin concentration, which decreases renal free water clearance, and an increase in plasma aldosterone concentration, which increases the rate of renal sodium reabsorption. Finally, hemorrhage stimulates thirst; drinking replenishes the fluid volume lost during the hemorrhage.

Cardiovascular afferent fibers from the carotid sinuses and aortic arch on the high pressure side of the circulation and from the cardiac atria on the low pressure side of the circulation mediate the ACTH, corticosteroid, vasopressin, and renin responses to hemorrhage. If the volume of hemorrhage is small, the reflex vasoconstriction and increased cardiac contractility will maintain normal or near-normal mean arterial blood pressure. However, if the volume of hemorrhage is large, the short term compensation will be inadequate to return the arterial pressure to prehemorrhage levels. Therefore, after small hemorrhage, the error signal from the low pressure side of the circulation will be large relative to that from the high pressure side of the circulation. Theoretically, therefore, the low pressure recep-

tors play a dominant role in the mediation of the endocrine response to nonhypotensive hemorrhage.

Not much is known quantitatively about the various endocrine responses to nonhypotensive or mildly hypotensive hemorrhage in conscious animals, and not much is known about factors which modulate the endocrine responses. For example: 1) it might be assumed that the ACTH and vasopressin responses to nonhypotensive hemorrhage are temporally similar, since, theoretically, both responses are mediated by afferent fibers from the atrial volume receptors; but this has not been previously tested by measuring both ACTH and vasopressin during hypovolemia; 2) endocrine responses to hemorrhage in conscious dogs have been described as variable, but no attempts have been made to explain the variability (Share, 1967); 3) investigators have assumed that ACTH responses to hemorrhage can be adequately inferred from the adrenal venous corticosteroid response to hemorrhage; but as described in Chapter 4, increases in plasma corticosteroids during 15 ml/kg hypovolemia are not always accompanied or preceeded by measurable increases in plasma ACTH. The experiments reported in Chapter 3 were designed to quantitate the blood pressure, heart rate, ACTH, corticosteroid, vasopressin, and renin responses to 15 ml/kg hemorrhage in conscious dogs, and to examine the effect of fever (or factors

associated with or contributing to fever) on the hemodynamic and hormonal responses to hemorrhage. The experiments reported in Chapter 4 were designed to determine the threshold volume of hemorrhage required for stimulation of ACTH and corticosteroid secretion, and to describe the apparent dissociation of plasma ACTH and corticosteroid concentrations during the response to 15 ml/kg hemorrhage in conscious dogs. In Chapters 5 and 6, the temporal and dose-response relationships of plasma ACTH and corticosteroid concentrations are examined, and the hypothesis that adrenal sensitivity is increased by hemorrhage is tested. The experiments in Chapters 5 and 6 demonstrate that adrenals are normally sensitive to small increments in plasma ACTH, that adrenal responses can be initiated by unmeasurable increases in plasma ACTH concentration. The experimental results are preceeded by a Historical Background (Chapter 2), which provides an historical perspective of the progression of ideas and experiments that defined hemorrhage as a stimulus to vasopressin, renin, and ACTH secretion, and that elucidated the mechanisms by which hemorrhage acts as a stimulus. The Historical Background ends with a review of the current knowledge of the quantitative relationship of plasma ACTH and corticosteroid concentrations in perpipheral plasma. Following the experimental results is a General Discussion (Chapter 7).

CHAPTER TWO

HISTORICAL BACKGROUND

DISCOVERY OF VOLUME RECEPTORS:

TYPE B ATRIAL RECEPTORS

In 1915, Bainbridge reported that rapid injection of saline (200-400 ml in 1.5-4 min) into the venous circulation of anesthetized dogs elicited a cardioacceleration, which could be abolished by vagotomy. He suspected that the initiating stimulus was an increase in venous pressure, because if the saline injection did not raise the diastolic pressure and dilate the heart, acceleration did not occur. Sassa and Miyazaki (1920) found that they could initiate Bainbridge's reflex by inflating a small rubber balloon (actually a rubber membrane covering a small hole in one end of a metal tube) in the superior vena cava near its junction with the right atrium. Inflation of the balloon in either atrium was also an effective stimulus, independent of changes in arterial or venous pressure. Inflating the balloon in the superior or inferior vena cavae at some distance from the heart had no effect on heart rate.

Anatomical definition of (what appeared to be) stretch receptors in the cardiac atria was reported by Nonidez (1937). He found dense innervation and what he interpreted as sensory endings at the junction of the vena cavae and the right atrium, and at the junction of the pulmonary veins and the left atrium. He reasoned that the (sensory) endings appeared similar to those in the carotid sinuses. Because these endings were found in the same areas found by Sassa and Miyazaki (1920) to be sensitive to balloon inflation, he proposed that the initial stimulus in Bainbridge's reflex was a stretching of the sensory endings by increased venous pressure.

Recordings from atrial fibers were first made by Amman and Schaefer (1943), and by Walsh and Whitteridge (1944). Both groups describe fibers which fire at the time of the "a" wave of the venous pulse. According to Whitteridge (1948), the fibers "show activity corresponding to the cardiac rhythm." These fibers originate in the cardiac atria, from receptors which were later named the "Type A atrial receptors" by Paintal (1953).

Walsh and Whitteridge (1944) and Whitteridge (1948) first reported cervical vagal fibers which "are believed to arise from endings on the small vessels of the lung" (Whitteridge, 1948), termed "pulmonary vascular receptors." The activity of these fibers was increased

during inspiration and after venous injection of saline. Support for the notion that these fibers originated in receptors in the pulmonary vasculature was advanced by Pearce and Whitteridge (1951), who demonstrated that firing rates in these fibers correlated linearly with the pulmonary artery pulse pressure, when cats were subjected to positive and negative pressure breathing. Paintal (1953), however, found that these fibers actually originated in the cardiac atria. By constricting the vasculature at various places and measuring firing rates, he deduced that the receptors were located at the junction of the vena cavae with the right atrium, and at the junction of the pulmonary veins with the left atrium. The rate of firing in these fibers was linearly related to the degree of filling (tested in the arrested heart). He named these receptors the "Type B atrial receptors."

HEMORRHAGE AS A STIMULUS TO VASOPRESSIN (ANTIDIURETIC HORMONE) SECRETION

Reduction of circulating blood volume has been shown to increase circulating levels of antidiuretic hormone in rats (Ginsburg and Heller, 1953), dogs (Baratz and Ingraham, 1959, 1960; Share, 1961, 1962;

Claybaugh and Share, 1972), and cats (Beleslin, et al, 1967). The original hypothesis that antidiuretic hormone secretion was influenced by changes in blood volume came from the work of Rydin and Verney (1938), who bled conscious dogs approximately 5 ml/kg and found a "profound and long-lasting inhibition of urine flow, the course of which is quite unparalleled by the blood-pressure trace." The investigators speculated that the "agent" responsible for the antidiuresis "is humorally mediated to the kidney and is not adrenaline." Because Rydin and Verney (1938) had shown that injection of posterior pituitary extract into conscious dogs reduced urine flow, they further speculated that hemorrhage stimulated the release of antidiuretic hormone from the posterior pituitary.

In 1953, Ginsburg and Heller tested Rydin and Verney's hypothesis by measuring plasma antidiuretic hormone activity (by bioassay employing the rate of urine flow in water-loaded, ethanol-anesthetized rats) in anesthetized rats subjected to a progressive hemorrhage. They found that increasing degrees of hemorrhage produced increasingly elevated plasma antidiuretic activity. They proposed that the rise in plasma antidiuretic activity was due to increased secretion of the hormone from the posterior pituitary, because the antidiuretic activity in the external jugular vein blood

was greater than that in the common carotid artery blood. Baratz and Ingraham (1959, 1960) employed bioassay of plasma antidiuretic activity (changes in specific gravity of urine in water-loaded, ethanol-anesthetized rats) to demonstrate that bleeding anesthetized dogs 25% of blood volume provokes a rise in plasma antidiuretic activity. These results were largely confirmed by Weinstein, et al (1960), who bled anesthetized dogs to 50 Torr and measured pressor activity in the blood draining the head.

Share (1961, 1962) substantially improved the bioassay of antidiuretic hormone, combining the extraction method of Weinstein, et al (1960) in their relatively insensitive pressor assay, and the continuous measurement of urine conductivity in water-loaded, ethanol-anesthetized rats. Whereas Ginsburg and Heller (1953) found unstimulated levels of "less than 0.1 mU" of antidiuretic activity per ml of rat plasma, and Baratz and Ingraham (1959, 1960) found about 100 uU antidiuretic activity per ml of dog plasma, Share (1962) measured approximately 4 uU antidiuretic activity per ml of dog plasma before a volume stimulus was applied. This represented a substantial improvement in experimental technique (improving bioassay sensitivity and avoiding the high "resting" levels of antidiuretic activity caused by extensive surgery) and therefore was probably

the best estimate of unstimulated plasma antidiuretic activity at the time. Using his improved techniques, Share (1961) demonstrated that reduction of extracellular fluid volume by peritoneal lavage with hypertonic glucose solution elevated plasma antidiuretic activity. He went on to show (Share, 1962) that the increase in plasma antidiuretic activity after peritoneal lavage was due to reduction in the circulating blood volume.

Volume control of plasma antidiuretic hormone cncentration is not as well characterized in man, because of obvious ethical limitations in experimental design. Goetz, et al (1974) bioassayed plasma antidiuretic activity in blood from normal subjects before and after they donated one unit of blood. In 24 subjects, the mean decrease of 9.9% of estimated blood volume did not change resting (2.1 $\pm$ Ø.2 uU/ml) levels of antidiuretic activity. Robertson and Mahr (1972) used radioimmunoassay to demonstrate that 5% hemorrhage does not elevate plasma antidiuretic hormone concentration in man.

Another approach to the study of volume control of antidiuretic hormone levels in man has been the study of postural changes on bioassayable (Segar and Moore, 1968; Share, et al, 1972) and radioimmunoassayable (Robertson and Athar, 1976) antidiuretic hormone. Segar and Moore (1968) found that plasma antidiuretic activity was

increased when subjects were changed from supine (Ø.4 ± Ø.6 uU/ml) to quiet sitting (1.4 ± Ø.7 uU/ml) to quiet standing (3.1 ± 1.5 uU/ml). In these experiments, the subjects were kept as quiet as possible, to minimize the effects of muscular exercise on venous return. Share, et al (1972), on the other hand, bioassayed plasma antidiuretic activity in people who were either recumbent or ambulatory, and found that standing and ambulation did not increase plasma antidiuretic activity.

Reduction of the circulating blood volume profoundly increases plasma antidiuretic hormone concentration, especially when the hemorrhage causes hypotension (Baratz and Ingraham, 1959, 1960; Share, 1961), and to a lesser degree when the blood pressure is normal (Share, 1962). Dunn, et al (1973) have shown that the increase in plasma antidiuretic hormone concentration (measured by radioimmunoassay) after hemorrhage is linearly related to the logarithm of the hemorrhage volume. The threshold of this response is probably somewhat greater than 10% of the blood volume in humans (Robertson, 1978; Share, et al, 1972), but probably somewhat less than 10% of the blood volume in dogs (Share, 1967a).

Stimulation of antidiuretic hormone by hypovolemia almost always elevates plasma concentrations above those neccessary to produce maximum urine concentration (Morton, et al, 1975; Robertson, 1976), reaching levels

which have been shown to be pressor (Rocha e Silva and Rosenberg, 1969). Because antidiuretic hormone might be involved in the regulation of blood pressure in severely hypotensive animals (Laycock, et al, 1979), this reflex could be considered a defense of blood volume and blood pressure after substantial (Goetz, et al, 1974; Robertson and Mahr, 1972) reduction in circulating blood volume.

Afferent mechanisms controlling the ADH response to hemorrhage. Control of vasopressin secretion by atrial stretch receptors was discovered by investigators studying hemodynamic responses to changes in blood volume distribution. In 1947, Drury, Henry, and Goodman were interested in the then well-known phenomenon that positive pressure breathing eventually led to cardiovascular collapse. In attempts to facilitate higher altitude flight, physiologists experiementally raised alveolar oxygen tension by having the pilots breathe a mixture of gas (which was mostly oxygen) at a higher-than-ambient pressure. However, this "continuous pressure breathing" or positive-pressure breathing often led to syncope and collapse, an effect attributed to "circulatory stress" (Drury, Henry and Goodman, 1947). These authors sought to "assay the impairment of the circulation induced by pressure breathing" by measuring urine flow and urea clearance during positive-pressure breathing. In four

subjects, positive-pressure breathing above 20 Torr decreased both urine flow and urea clearance without changing the arterial blood pressure. The effects of positive pressure breathing persisted for 20-30 min after the subjects began breathing at ambient pressure again. The investigators suspected that the long latency of the off-response was due to hormonal mediation of the renal effects, because at the time it was known that injections of posterior pituitary extracts ("pituitrin") could decrease the urine/blood urea ratio (Addis and Drury, 1923).

In 1935, J.P. Peters reasoned (from clinical data) that "it may well be the fulness of the blood stream which provokes the diuretic response on the part of the kidneys." Supporting this notion, Henry and Gauer (1951) demonstrated that a change in blood volume could change urine flow without concomitant changes in arterial pressure. In morphine-treated dogs, 20% hemorrhage halved urine flow, while 20% volume expansion doubled urine flow. They hypothesized that changes in circulating blood volume could be sensed by neural stretch receptors in the vascular tree. If this were true, they hypothesized, the decrease in urine flow caused by positive-pressure breathing (Drury, Henry, and Goodman, 1947) should be reversed by negative-pressure breathing (breathing a mixture of gas at a pressure lower than the

ambient pressure). In a study involving 70 experiments in 40 chloralose-morphine anesthetized dogs, negative-pressure breathing at -8 and -5 cm water increased urine flow by 100-200% (Gauer, Henry, Sieker, and Wendt, 1954); the opposite effect was caused by periods of positive-pressure breathing. During the period of negative-pressure breathing, central venous pressure was decreased and heart rate was increased, although arterial blood pressure did not change. Again, the induced changes in urine flow were slow in onset and slow to return after switching to ambient-pressure breathing. These findings were confirmed in the human being (Sieker, Gauer, and Henry, 1954), and extended to show that negative-pressure breathing does not change sodium or potassium excretion. The decreases in urine flow coupled with no change in sodium or potassium excretion raised the suspicion that the effector was antidiuretic hormone.

In an attempt to localize the cardiovascular afferents involved in this apparent reflex, Henry, et al (1953) found that the increase in urine flow during negative-pressure breathing could be blocked by vagotomy, but not by thoracic sympathectomy. They could increase urine flow by pulmonary artery obstruction (constriction or balloon obstruction) or by partial constriction of the pulmonary veins. They concluded that

the receptors are in the pulmonary veins and/or capillary bed and that the afferent fibers travel to the CNS via the vagus nerve. Since Paintal (1953) had described the type B atrial receptors, it became evident that the same receptors could be responsible for changes in urine flow. Paintal (1953) had described the atrial B receptors in cats, but his manipulations were limited to mechanical stimulation of the atria and volume changes in the arrested heart. Henry and Pearce (1956) extended these findings, demonstrating atrial B receptors in anesthetized dogs, showing that negative-pressure breathing increased the activity of these receptors, and that decreasing and increasing blood volume reduced and elevated their activity, respectively. Finally, Henry, Gauer, and Reeves (1956) demonstrated that mitral obstruction and therefore elevated left atrial pressure increased urine flow in anesthetized dogs.

Since the afferent limb of this diuretic reflex was now well characterized, increasing attention was being given to the identification of the efferent limb. Working on the hypothesis that the effector of the renal effects was antidiuretic hormone, Baisset and Montrasuc (1957) assayed plasma antidiuretic activity in chloralose-anesthetized dogs subjected to left atrial distention. They found that the atrial distention decreased circulating antidiuretic activity while

increasing urine flow. The authors refined the measurement of the lags involved: the diuresis began 2-4 min after balloon inflation, and ended 5-8 min after balloon deflation. These results were later confirmed by Share (1965). A problem with both of these studies is that initial plasma antidiuretic activity was quite high. Initial levels of 400 uU/ml (Baisset and Montrasuc, 1957) and 100 uU/ml (Share, 1965) were probably higher than what one would find in the dog not subjected to surgical trauma (Share, 1961). Indeed, these initial levels of antidiuretic hormone were probably high enough to produce maximum urine concentration (Morton, et al, 1975; Robertson, 1974). The other major problem in these studies was that the increases in left atrial pressure caused by balloon inflation were probably not physiological. Baisset and Montrasuc (1957) elevated atrial pressure 20-30 cm water and Share (1965) elevated atrial pressure about 15 cm water. Johnson, et al (1969) reported that when both of these problems are eliminated (i.e., resting antidiuretic activity is "low", and atrial pressure was elevated a relatively small amount), atrial distention could still be shown to decrease antidiuretic activity and increase the rate of urine flow. Using pentobarbital-anesthetized dogs, they demonstrated that a 4.4 cm water elevation in atrial pressure decreased plasma antidiuretic activity from 6.3 to 4.4 uU/ml, while increasing urine flow from 0.82 to

Ø.92 ml/min. The changes in plasma antidiuretic activity were linearly related to the changes in urine flow. Brennan, et al (1971) later confirmed these results, using a 5 cm water increment in left atrial pressure. These authors went on to show that increases in left, but not right, atrial pressure decreased plasma ADH concentration.

It seems, therefore, that one mechanism mediating volume control of antidiuretic hormone secretion, is the changing firing pattern of the Type B atrial receptors. The attempts to use a "pure" stimulus to the atrial receptors (atrial balloons to stimulate atrial B activity) were often confounded by decreases in mean arterial pressure (Henry and Pearce, 1956; Henry, Gauer, and Reeves, 1956), later shown to be itself a stimulus to antidiuretic hormone secretion (Share, 1965). However, with improvement of techniques, Johnson, et al (1969) demonstrated the effectiveness of raising atrial pressure, uncomplicated by decreases in arterial pressure, in lowering the antidiuretic activity of circulating plasma. Indeed, stimulation of the atrial B receptors was shown to lower both stimulated (Baisset and Montrasuc, 1957; Share, 1965) and "unstimulated" (Johnson, et al, 1969) plasma antidiuretic activity.

However, the problem of whether the original observation of diuresis during negative-pressure breathing

(Gauer, et al, 1954) or atrial balloon inflation (Henry and Pearce, 1956) could be explained by a decrease in the circulating concentration of antidiuretic hormone remains unsolved. In the work of Baisset and Montrasuc (1957), atrial distention decreased circulating antidiuretic activity and increased the rate of urine flow, although initial levels of antidiuretic activity were very high, and fell to levels (60 uU/ml) which were still probably saturating the antidiuretic effect at the kidney (Morton, et al, 1975; Robertson, 1976). Ledsome, Linden, and O'Connor (1961) repeated the experiments of Henry, Gauer, and Reeves (1956) in anesthetized dogs, and confirmed the finding that atrial distention causes a diuresis. However, they tried to inhibit the diuresis with an infusion of antidiuretic hormone (25 and 100 uU per minute) that could inhibit the diuresis after water loading conscious dogs (Ledsome, Linden, and O'Connor, 1961). They found that the vasopressin infusion did not reduce the diuresis caused by atrial distention. However, their infusion of antidiuretic hormone also did not alter the unstimulated rate of urine flow, indicating that in their experiments as well, the initial levels of antidiuretic activity were high enough to saturate the antidiuretic response of the kidneys. Lydtin and Hamilton (1964), however, used hydrated and chloralose-anesthetized dogs (allowed to recover from surgery for at least a week) to demonstrate that

increases in left atrial pressure could increase the rate of urine flow, and that the diuresis could be partially inhibited by an infusion of 25 uU/min/kg antidiuretic hormone. In contrast to the results of Ledsome, et al (1961), the infusion did lower the initial rate of urine flow (probably owing to the lower initial plasma antidiuretic hormone concentration). Ledsome and Mason (1972) demonstrated that in chloralose-anesthetized dogs, infusion of antidiuretic hormone in doses of 25, 100, and 1000 uU/kg/min inhibited the increase in free water clearance during atrial distention, although the increase in urine flow was not completely inhibited. They speculated that "there may be two agents affecting the kidney to produce a diuretic response, a decrease in circulating levels of antidiuretic hormone and an unknown agent, possibly a haemodynamic change producing an increased osmolal clearance."

The antidiuretic hormone response to hypotensive hemorrhage is mediated by afferent nerves from the atria of the heart and from the carotid sinuses. Carotid occlusion in anesthetized dogs does not, by itself, increase the plasma ADH concentration (Share and Levy, 1962). However, vagotomy (which itself increases the basal level of ADH) converts the carotid occlusion into a potent stimulus to ADH secretion (Share and Levy, 1962). These results suggested the existence of two

pathways, a vagally mediated pathway (from the cardiac atria and possibly from the aortic arch) and a pathway from the carotid sinuses. In the dogs with intact vagi, the reflex increase in systemic blood pressure during carotid occlusion increased the impulse activity in the vagally mediated pathway (by increasing pressure in the aortic arch and possibly an increase in left atrial pressure). Share and Levy occluded the carotid arteries both above and below the thyrocarotid arterial junction finding no difference between the sites in the ability to stimulate ADH secretion. They concluded that "receptors at the thyrocarotid junction are not involved to any appreciable extent" in the ADH response to carotid occlusion (Share and Levy, 1962).

Pulse pressure within the carotid sinus, independent of changes in carotid sinus mean pressure, is a determinant of plasma ADH concentration. Share and Levy (1966) perfused the carotid sinuses of vagotomized dogs with femoral arterial blood, introducing and controlling a pulse pressure with an automatic pipetting machine. Transition from pulsatile to nonpulsatile carotid sinus pressure (with no change in the mean pressure) elevated both the systemic arterial pressure and the blood ADH concentration.

Overall, most or all of the ADH response to hemorrhage is due to changes in vagal activity and changes in

carotid sinus pressure. Share (1967a) demonstrated that vagotomized dogs with perfused carotid sinuses (at constant mean and pulse pressures) do not respond to graded hemorrhage (to 50% of blood volume) with appreciable increases in plasma ADH concentration.

HEMORRHAGE AS A STIMULUS TO ACTH AND CORTICOSTEROID SECRETION

The first attempt at measuring adrenal steroid production was made by Vogt (1943) who, in several species (dog, cat, goat, rabbit, and pig), collected adrenal venous blood for the bioassay of "cortical hormone."* In these experiments, no standard stimulus was applied, but the blood pressure ranged from 25 to 132 Torr, probably because of the large volumes of blood (30 ml) required for each sample in the bioassay. Vogt found no correlation between blood pressure and adrenal steroid production.

In 1945, Sayers, Sayers, Liang, and Long reported that hemorrhage decreased adrenal cholesterol and ascorbic acid content in unanesthetized rats. The first

* Vogt used the bioassay technique of Selye and Schenker (1938) in which young adrenalectomized rats were subjected to a cold environment and injected with standard or unknown amounts of adrenal cortical extract. The mean survival time of the rats was linearly related to the logarithm of the dose of extract.

formal test of increased cortisol secretion after hemorrhage was reported by Hume and Nelson (1955) who bled pentobarbital-anesthetized dogs to shock levels, and collected adrenal venous blood samples before and during the period of hypovolemia. They found that adrenal secretion of corticosteroids was increased by hypotensive hemorrhage (to 25-45 Torr), and that the response was abolished by hypophysectomy indicating that a pituitary factor (probably ACTH) was responsible for the stimulation of corticosteroid secretion. Other investigators confirmed the importance of the pituitary in that hypophysectomy lowered resting cortisol and corticosterone secretion in dogs (Bartter, et al, 1961; Slater, et al, 1963), and that hypophysectomy (Davis, et al, 1961; Farrell, Rosnagle, and Rauschkolb, 1956), or decapitation (Davis, et al, 1961) abolished the cortisol (Farrell, Rosnagle, and Rauschkolb, 1956), corticosterone (Davis, et al, 1961) and 17OHCS (Ganong and Mulrow, 1961) response to hemorrhage.

Gann and Cryer (1973) have demonstrated that the magnitude of the adrenal venous corticosteroid response to hemorrhage is linearly related to the logarithm of the hemorrhage volume. According to this work, the threshold volume is 2-3 ml/kg, and the saturating volume is 20-30 ml/kg. The magnitude of the response is dependent upon the rate as well as the volume of hemorrhage

(Gann, 1969). Thus, in anesthetized dogs, 10 ml/kg hemorrhage to isovolemia (that is, bleeding from hypervolemia to isovolemia) in 1.5 min increased adrenal venous cortisol secretion by 6 ug/min and the same hemorrhage in 3 min increased adrenal venous cortisol secretion by 2 ug/min.

The first test of hemorrhage as a stimulus to ACTH secretion was performed by Sydnor and Sayers (1954), who demonstrated that rats which were ether anesthetized and rapidly exsanguinated elevated blood ACTH activity from undetectable levels (less than 0.5 mU/100ml) to 2-3 mU/100ml, as measured by bioassay (adrenal ascorbic acid depletion test). These investigators did not attempt to separate the effects of ether anesthesia from those of hemorrhage. Redgate (1968), using the same bioassay method, showed that hemorrhage to a mean arterial blood pressure of 35 Torr for 1-4 min in pentobarbital-anesthetized cats elevated plasma ACTH activity from less than 1.0 mU/100ml to greater than 5 mU/100ml. Prolongation of the period of hypotension increased plasma ACTH activity to over 7 mU/100ml. More recently, Engeland, et al (1979) have shown that radioimmunoassayable plasma ACTH concentration increased from 30 $\pm$ 6 pg/ml to 57 $\pm$ 13 pg/ml 30 min after the onset of 10 ml/kg hemorrhage in conscious dogs with chronically maintained adrenal venous catheters.

Afferent mechanisms controlling the ACTH and corticosteroid responses to hemorrhage. Egdahl (1961) first demonstrated that partial constriction of the inferior vena cava (to raise venous pressure "below" the constriction 10 cm water) increased the rate of adrenal 17OHCS secretion in pentobarbital-anesthetized dogs with adrenal venous catheters. This maneuver decreased the mean arterial pressure by 20 to 40 Torr, and would be expected to decrease the pressure in both cardiac atria. Gann, Gould, Morley, and Mumma (1964) hypothesized that the decreased mean arterial pressure, sensed by the cardiovascular afferents in the carotid sinuses, stimulated 17OHCS secretion. Bilateral carotid constriction (until mean femoral arterial pressure rose 20 Torr) was not an effective stimulus to 17OHCS secretion in pentobarbital-anesthetized dogs, unless the cervical vagi had been cut prior to the carotid constriction. These investigators hypothesized that in dogs with intact vagi, carotid constriction acted as a stimulus to 17OHCS secretion, but that this stimulus was counteracted by inhibitory afferent signals in the vagus (probably coming from the cardiac atria and/or aortic arch).

Ganong and his colleagues (1967) found that alpha-ethyltyramine, a monoamine oxidase inhibitor, inhibited the 17OHCS response to laparotomy when infused at a dose

sufficient to raise mean arterial blood pressure 20 Torr. The effect of the drug could be abolished by maintaining the blood pressure at preinfusion levels (by simultaneous hemorrhage), indicating that activation of afferent fibers from the high pressure side of the cardiovascular system inhibits the release of 17OHCS. Location of these "high pressure" afferents was investigated by Gann (1966). He found that bilateral denervation of the carotid sinuses attenuated (approximately 50%) the corticosteroid response to carotid occlusion in the vagotomized dog. Bilateral denervation of the thyrocarotid arterial junctions, on the other hand, did not significantly attenuate the response. Combined denervation of carotid sinuses and thyrocarotid arterial junctions completely inhibited the response to carotid constriction.

The fact that carotid constriction in the intact dog does not elevate adrenal corticosteroid secretion, and that carotid constriction in the vagotomized dog is a potent stimulus to adrenal corticosteroid secretion suggests that the vagus nerve carries afferent signals from the cardiovascular system, tonically inhibiting corticosteroid secretion. Hypothetically, it should therefore be possible to selectively stimulate these afferent nerves and thereby change the rate of corticosteroid secretion without changing mean arterial pres-

sure. Gann and Egdahl (1964, 1965) found that infusion of the ganglion-blocking drug trimethaphan camphorsulfonate into pentobarbital-anesthetized dogs at a rate sufficient to lower mean arterial blood pressure to 60 Torr increased adrenal venous corticosteroid output to maximal levels, as did hypovolemic hypotension to 60 Torr. When l-norepinephrine was infused with the trimethaphan camphorsulfonate to maintain mean arterial blood pressure at preinfusion levels, the rate of 17OHCS secretion from the adrenal was not increased. Infusion of l-norepinephrine alone raised mean arterial blood pressure 20 Torr, but did not significantly change the rate of 17OHCS secretion. Hemorrhage of 400-500 ml (body weights of the dogs ranged from 12 to 20 kg), combined with an infusion of l-norepinephrine to maintain mean arterial blood pressure at prehemorrhage levels raised 17OHCS output maximally. These experiments therefore proved that the stimulatory effect of hemorrhage on adrenal corticosteroid production could be dissociated from the accompanying hypotension (suggesting that the inhibitory vagal afferent fibers originated in the venous circulation).

Vagal afferent fibers involved in the control of the cardiovascular system are known to originate in the aortic arch, pulmonary artery, cardiac atria, and cardiac ventricles (Paintal, 1978). To distinguish between

these sites, Cryer and Gann (1970, 1973) placed an inflatable cuff around the root of the aorta in anesthetized dogs. The aortic constriction, when leading to a fall in mean arterial blood pressure of less than 20 Torr, decreased adrenal corticosteroid secretion, unless the dog had been vagotomized prior to the constriction, in which case adrenal corticosteroid secretion was increased. Carotid constriction (Gann, Gould, Morley, and Mumma, 1964; Gann, 1966; Gann, 1971) would lower mean blood pressure within the carotid sinuses but increase the mean blood pressure in the aortic arch and cardiac atria. Constriction of the aorta at its root (Cryer and Gann, 1970, 1973), on the other hand, would be expected to lower mean blood pressure in both carotid sinuses and aortic arch, and increase the mean pressure in both carotid sinuses and aortic arch, and increase the mean pressure upstream of the constriction (atria, ventricles, and pulmonary artery). To differentiate these possible sites of origin of inhibitory vagal fibers upstream from the root of the aorta, Cryer and Gann (1971) constricted the pulmonary artery at its root and decreased the rate of adrenal cortisol secretion. This maneuver decreased mean arterial blood pressure by less than 20 Torr, and (theoretically) increased the pressure upstream from the constriction. From the results of these experiments, Gann and his colleagues hypothesized that inhibitory vagal afferent fibers

originate in the right atrium.

To test this hypothesis directly, Cryer and Gann (1974) bled dogs 5 ml/kg (within 3 min) with or without inflation of a small balloon in the right atrial appendage. The dogs were anesthetized with pentobarbital and prepared with adrenal venous catheters for the measurement of adrenal corticosteroid secretion. With the balloon uninflated, the hemorrhage elevated cortisol secretion 4 ug/min. With the balloon inflated, the hemorrhage elevated cortisol secretion only 1 ug/min. In a direct study of these fibers, Baertschi, Ward, and Gann (1976) recorded from single vagal fibers while delivering repetitive 1 ml volume pulses from a piston pump to either the right or left atrium. The experiments were performed on chloralose-urethane-anesthetized cats. The investigators found single fibers originating in right and left Type A and B atrial receptors. Fibers from Type B receptors exhibited lower firing rates after 5 ml/kg hemorrhage. They continuously measured plasma ACTH concentration and corticosteroid secretion rate (from adrenal venous catheters) while pulsing either atrium, and correlated the changes in plasma ACTH concentration and adrenal corticosteroid secretion to changes in atrial pulsing activity. They concluded that decreased activity of right atrial B fibers (turning off the atrial pulsations) was associated with an increase

in the rate of corticosteroid secretion. Decreases in the left atrial B fiber activity were associated with increased plasma ACTH concentration and corticosteroid secretion only if the right atrial B fiber activity was also decreased. That is, it appeared that the fibers coming from the right atrium were the dominant low pressure receptors controlling ACTH and corticosteroid secretion.

Many of the studies of hemorrhage as a stimulus to the adrenocortical system have been based on the assumption that the rate of adrenal secretion of corticosteroids is an accurate reflection of plasma ACTH concentration (i.e., Gann, 1969; Gann and Cryer, 1973). The assumption is justified by the observation (Nelson and Hume, 1955; Urquhart, 1965) that the rate of adrenal corticosteroid secretion is linearly related to the logarithm of the adrenal presentation rate (blood concentration of ACTH multiplied by the adrenal blood flow). As described earlier, Redgate (1968) found that hypotensive hemorrhage increased bioassayable plasma ACTH in the pentobarbital-anesthetized cat. In an attempt to determine the afferent mechanisms of volume control of ACTH, he found that bilateral carotid occlusion elevated plasma ACTH activity (unlike the anesthetized dog: Gann, Gould, Morley, and Mumma, 1964), that vagosympathectomy prior to the experiment made the carotid occlusion a

more effective stimulus. Denervation of the carotid sinuses, thyrocarotid arterial junctions, and aortic arch decreased the response to hemorrhage by approximately 50%. The remaining ACTH response was further decreased by removal of the superior cervical and nodose ganglia. The ACTH response to hypotensive hemorrhage in the carotid-aortic denervated, ganglionectomized cats was abolished by nephrectomy. Recently, Gann and his colleagues directly measured plasma ACTH by radioimmunoassay while changing the activity of Type B atrial receptors (Gann, et al, 1977; Gann, 1978). In chloralose-urethane-anesthetized cats, Gann (1978) demonstrated that caval constriction (during the period of caval constriction blood was pumped from the inferior vena cava upstream from the constriction to the aorta, to maintain pre-constriction arterial pressure) increased plasma ACTH, and that repetitive atrial pulsation (Baertschi, Ward, and Gann, 1976; Baertschi and Gann, 1977) reversed the increase. It therefore appears that cardiovascular stimuli known to increase adrenal secretion of corticosteroids are also effective in increasing plasma ACTH concentration.

Neural pathways within the central nervous system mediating volume control of ACTH are, at present, only partially elucidated. Baertschi, et al (1975) have found neurons in the medullas of chloralose-urethane-

anesthetized cats which are responsive to increases and decreases in Type B receptor activity. About half of these neurons were activated by antidromic stimulation from the dorsal rostral pons. Cells which were both responsive to atrial pulsations and capable of antidromic activation from the dorsal rostral pons were found ventral and lateral to the solitary tract and in the medial nucleus intercalatus and in the medial extent of the dorsal motor nucleus of the vagus. Sites in the pons from which stimulation antidromically activated medullary cells responsive to atrial stretch were found near to or in the tegmental nuclei of Gudden, the parabrachial nucleus, and the locus subcoeruleus (Ward, Baertschi, and Gann, 1977). Stimulation of cells near the nucleus of the tractus solitarius in the medulla increased or decreased plasma ACTH in the anesthetized cat (Ward and Gann, 1976). Stimulation in the pons (Ward, Grizzle, and Gann, 1976) increased and decreased plasma ACTH when the stimulation sites were in or near the principal locus coeruleus, tegmental nuclei of Gudden, locus subcoeruleus, periventricular gray, and periaqueductal gray. Above the pons, the work to date has focused on electrical stimulation and measuring subsequent changes in ACTH (Grizzle, et al, 1974; Ward, Bolton, and Gann, 1978; Maran, et al, 1978). Full discussion of these studies is beyond the scope of this review. Several areas within the midbrain and

hypothalamus were found that, when stimulated, increased or decreased plasma ACTH concentration. Likely, not all of these areas are involved in volume control of ACTH secretion. Further elucidation of the pathway will require more information on neuronal responses to atrial stretch, recorded from the midbrain and hypothalamus.

Gann and Cryer (1973) first demonstrated the importance of the kidneys in the adrenocortical response to hemorrhage by showing that nephrectomy and dexamethasone pretreatment acted synergistically in shifting the dose-response relationship of hemorrhage volume to adrenal secretion of corticosteroids to the right in anesthetized dogs. Redgate (1968) proved that the small ACTH response to hypotensive hemorrhage in aortic-carotid denervated, superior cervical- and nodose- ganglionectomized (anesthetized) cats could be completely abolished by nephrectomy.

Angiotensin has been postulated to affect the adrenocortical axis at all levels. The best studied of these effects is the action of angiotensin on the adrenal cortex to stimulate the release of cortisol and corticosterone. Angiotensin is only a weak agonist to cortisol and corticosterone secretion, and its effect is only elicited at pharmacologic doses, as will be discussed in Chapter 4. Stimulation of ACTH secretion by angiotensin has been proposed by Maran and Yates (1977).

They found that intrapituitary infusions of angiotensin in doses too small to cause a response when infused intravenously, caused an increase in the rate of adrenal corticosteroid secretion. Infusion of the same dose into the third ventricle caused a similar response, but the response was delayed 5-10 min compared to the direct intrapituitary infusion. Angiotensin stimulation of CRF secretion has been proposed by Gann and Cryer (1973), who localized an effect of angiotensin with ablation experiments. They infused angiotensin at a rate of 1-2 ug/min, causing an increase in the rate of adrenal corticosteroid secretion. The response was inhibited by hypophysectomy, indicating that the pituitary was neccessary for the response. Isolation of median eminence and pituitary did not abolish the response, but isolation of pituitary alone did, indicating that both median eminence and pituitary were neccessary. Their conclusion was that circulating angiotensin modified CRF secretion.

It is clear that the cardiovascular receptors mediating the ACTH and corticosteroid responses to hemorrhage are located in the atria (mainly right atrium), the carotid sinuses, and the thyrocarotid arterial junction. In some conditions, the kidneys contribute to the response. Gann and his coworkers believe that the atrial receptors are the most important for mediating

the adrenocortical response to small hemorrhage, because in the anesthetized dog with intact vagi, carotid constriction in ineffective in elevating adrenal corticosteroid secretion (Gann, Gould, Morley, and Mumma, 1966), and because denervation of the carotid sinuses actually potentiates the adrenal venous corticosteroid response to 5 ml/kg hemorrhage (Gann and Cryer, 1973). Receptors on the high pressure side of the circulation, on the other hand, are probably more important in the response to large hemorrhage, because carotid sinus denervation reduces the adrenocortical response to 35 ml/kg hemorrhage in anesthetized dogs (Gann and Cryer, 1973), and because carotid-aortic denervation decreases the ACTH response to hypotensive hemorrhage in the anesthetized cat (Redgate, 1968).

HEMORRHAGE AS A STIMULUS TO RENIN AND ALDOSTERONE SECRETION

In 1941, Sapirstein, Ogden, and Southard reported that hypovolemia elevated blood renin levels in conscious dogs. They hypothesized that if decreased pulse pressure in the isolated perfused kidney increased the rate of renin secretion, as demonstrated by Kohlstaedt and Page (1940), " a similar fall in the pulse pressure

in the intact animal would affect the kidney similarly." The renin bioactivity of blood samples taken immediately before and 24 hours after 28 ml/kg hemorrhage in 5 dogs was measured as the degree of contraction of guinea pig ileum. The tissue was only mildly stimulated, if at all, by blood samples taken before the hemorrhage. Blood samples taken 24 hours after hemorrhage caused clear contractions. Huidobro and Braun-Mendez (1942) demonstrated that anesthetized dogs respond to progressive hemorrhage (1% steps to 4% of total body weight) with an increase in arterial blood pressor activity. The source of this pressor substance was the kidney, because nephrectomy before hemorrhage blocked the response. Dexter, et al (1943) confirmed and extended these findings, demonstrating that hypovolemia and hypotension in conscious dogs decreased the circulating levels of "hypertensinogen" as the circulating levels of renin were increased. The homeostatic role of renin in maintaining blood pressure during hypovolemia was suggested by Hamilton and Collins (1942), who found that 34-43 ml/kg hemorrhage caused a renal arteriovenous difference in pressor activity (bioassayed by injection of blood from the bled dog into a smaller recipient dog). Because of the nonspecificity of the bioassay method, they went on to prove that epinephrine did not contribute to the pressor activity by showing that adrenalectomy did not abolish the response while

adrenalectomy plus nephrectomy did.

In 1966, Regoli and Vane substantially improved the bioassay of circulating pressor substances, using three types of tissue (rat colon, rat stomach, and chick colon) in a blood-bathed superfusion system to continuously measure pressor activity in anesthetized dogs. This assay system offered the advantage that the tissue strips are differentially sensitive to catecholamines and angiotensin. They found that hemorrhage to 20 Torr below control increased angiotensin but not epinephrine. A further reduction of the blood pressure of 40 Torr was needed to elevate epinephrine as well. Using the same assay technique, Hodge, Lowe, and Vane (1966b) found that 8-26 ml/kg hemorrhages in anesthetized dogs increased blood angiotensin concentration. They also found that volume expansion (10-30 ml/kg) with either Dextran or Krebs solution lowered blood angiotensin concentration. Furthermore, they confirmed the findings of Huidobro and Braun-Mendez (1942) and Hamilton and Collins (1942) by showing that the increase in blood angiotensin concentration is due to increased production, not decreased clearance, and that the kidneys are essential to the response (the response is prevented by clamping the renal pedicles).

As discussed earlier, the first observation suggesting elevated adrenal secretion of corticosteroids

during hypovolemia was made by Sayers, Sayers, Liang, and Long (1945). These investigators bled unanesthetized rats to both shock and non-shock levels of hypovolemia and found in both instances a striking decrease in adrenal ascorbic acid content. Farrell, Rosnagle, and Rauschkolb (1956) reported the first formal test of the hypothesis that hemorrhage stimulates aldosterone secretion. Using pentobarbital-anesthetized dogs with cannulated lumboadrenal veins (for the direct measurement of adrenal steroid secretion), they demonstrated that progressive bleeding caused a progressive rise in aldosterone secretion.

By the late 1950's, it was known that aldosterone was the main salt-retaining steroid of the adrenal cortex (Simpson, et al, 1953; Simpson and Tait, 1952), and because of the work of Farrell, Rosnagle, and Rauschkolb (1956), it was known that changes in blood volume influenced plasma aldosterone concentration. Years earlier, Sapirstein, Ogden, and Southard (1941) had discovered volume control of blood renin levels, and Hamilton and Collins (1942) had proposed that renin (its pressor activity) played a homeostatic role in the maintenance of blood pressure during hypovolemia. In 1951, Deane and Masson reported that repeated injections of renin into rats widened the zona glomerulosa of the adrenal cortex. They interpreted their findings to mean that

the renin was increasing the "activity" of that zone of the adrenal. It was known that reduction of renal blood flow by placing a clip on one or two renal arteries caused a sustained increase in mean arterial blood pressure (Goldblatt, Lynch, Hanzal, and Summerville, 1933). Reduction of renal blood flow was known to increase renin secretion (Kohlstaedt and Page, 1940). In 1958, Pasqualino and Bourne reported that clamping one renal artery in rats increased the width of the zona glomerulosa. Putting these observations together, Gross (1960) hypothesized that the renin-angiotensin system controlled the rate of aldosterone secretion.

Gross' hypothesis was supported by the observation of Laragh and coworkers (1960) that angiotensin infusion into human subjects increased circulating levels of aldosterone. This finding was supported by Genest, et al (1961) in humans, and by Bartter, et al (1961), Slater, et al (1963), and Carpenter, et al (1961) in dogs with adrenal venous catheters. Similarly, infusion of renin into dogs with adrenal venous cannulae caused an increase in aldosterone secretion rate.

Mulrow and Ganong (1960) first tested the hypothesis that hemorrhage-induced aldosterone secretion was not dependent on pituitary ACTH secretion. They found that acutely hypophysectomized, pentobarbital-anesthetized dogs responded to 15 ml/kg hemorrhage with

elevated adrenal aldosterone secretion. The rate of 17OHCS secretion was not increased by the hemorrhage (Ganong and Mulrow, 1961), but was increased by injection of one unit of ACTH. Ligation of the renal vessels in the hypophysectomized dogs reduced the resting aldosterone secretion rate (Ganong and Mulrow, 1961, 1962). Removal of the kidneys inhibited the aldosterone secretion after hemorrhage in hypophysectomized dogs, and saline extracts of the removed kidneys elevated the aldosterone secretion rate (Ganong and Mulrow, 1961, 1962). Saline alone or saline extracts of spleen did not change the aldosterone secretion rate.

Similar experiments were performed by Davis, et al (1961), who demonstrated that hypophysectomy plus nephrectomy, but neither hypophysectomy alone nor hypophysectomy plus hepatectomy eliminated the aldosterone response to (approximately 15 ml/kg) hemorrhage in anesthetized dogs. In fact, they demonstrated that totally decapitated dogs with intact kidneys responded to hemorrhage with elevated aldosterone secretion, ruling out the possibility that the CNS is required for the elaboration of response. Like Ganong and Mulrow, they also demonstrated that saline extracts of kidneys elevated the rate of aldosterone secretion. Bartter, et al (1961) reported that temporary removal of the kidneys from the circulation of anesthetized dogs (by transplan-

tation of the kidneys to another dog, and crossperfusion) decreased aldosterone, but not 17OHCS secretion. Slater, et al (1963) found that hypophysectomized dogs, allowed to recover from anesthesia and studied conscious, secreted less cortisol and corticosterone than intact dogs. They also found that kidney extracts from animals on low salt, but not high salt diets, infused into dogs elevated aldosterone secretion. ACTH (2.4 mU/min) infusion (for 4 min) increased cortisol secretion seven times, but did not change aldosterone secretion. ACTH infusions of 1.2 to 3.4 units per minute for 4 min increased aldosterone secretion rate by almost 140% (maximal stimulation by angiotensin increased aldosterone secretion by 300%).

Afferent mechanisms controlling the Renin-Angiotensin-Aldosterone response to hemorrhage. Renin secretion can be stimulated by decreases in central venous pressure, carotid arterial pressure, or renal perfusion pressure. After small, nonhypotensive hemorrhage, elevations in plasma renin concentration are mediated mostly by vagal afferents, probably originating at the atrial volume receptors. Hypotensive hemorrhage stimulates renin secretion probably by activating the carotid and intrarenal receptors, as well as the atrial receptors.

The direct effect of decreased renal perfusion pressure on renin secretion was first demonstrated by

Goldblatt, Lynch, Hanzal, and Summerville, (1933), who produced hypertension in dogs by placing clips on the renal arteries, which reduced the blood flow through the kidneys. These investigators proposed that the mechanism of this increased renin secretion was renal ischemia, which was produced by the decreased flow. Huidobro and Braun-Mendez (1942), however, found that hemorrhage and hypotension stimulated the secretion of renin, even when renal venous oxygen content was not lowered by the reduction in flow. Skinner, McCubbin, and Page (1963) found that reduction of renal perfusion pressure, without lowering the renal venous oxygen content, increased the renal venous pressor activity. This response to decreased renal perfusion pressure could be elicited, even when renal blood flow was not reduced. This led these investigators (Skinner, McCubbin and Page, 1963) to propose that an intrarenal baroreceptor controls renin secretion. An alternative hypothesis was proposed by Vander (1964), who found that decreases in the filtered load of sodium, independent of changes in renal blood flow or perfusion pressure, could increase the rate of renin secretion. According to this hypothesis ("macula densa theory"), factors which decrease renal flow or perfusion pressure stimulate renin secretion by decreasing the rate at which sodium is filtered past the macula densa.

During 15 ml/kg hypovolemia, however, increases in the rate of renin secretion are probably not attributable to direct hemodynamic effects on the kidney. The reduction in blood volume is sensed elsewhere, and the signal to increase renin secretion is conveyed to the kidney via the renal nerves. Hodge, Lowe, and Vane (1965) found that nonhypotensive hemorrhage (15 ml/kg) in chloralose-anesthetized dogs elevated renal renin secretion. Infiltration of the renal pedicle with lidocaine blocked this response. Bunag, Page, and McCubbin (1966) confirmed these results by demonstrating that ganglion blockade with tetraethylammonium chloride (TEAC) inhibited the renin response to 15 ml/kg hemorrhage in 6 of 7 pentobarbital-anesthetized dogs, and in the seventh, reduced the renin response to about half the normal response. In another 3 dogs, the renal venous renin concentration during 15 ml/kg hypovolemia could be returned to prehemorrhage levels by infiltrating the renal pedicle with lidocaine.

Carotid occlusion is an effective stimulus to renin secretion (Bunag, Page, and McCubbin, 1966; McPhee and Lakey, 1971; Reid and Jones, 1976; Hodge, Lowe, and Vane, 1966a), and aldosterone (Bartter, Mills, and Gann, 1960; Gann, Mills, and Bartter, 1960; Bartter and Gann, 1960; Biglieri and Ganong, 1961; Gann and Travis, 1964). The aldosterone response was abolished by stripping the

carotid arteries, but not denervation of the carotid sinuses alone. Selective denervation of the thyrocarotid arterial junctions was sufficient to completely inhibit the aldosterone response (Bartter, Mills, and Gann, 1960).

Constriction of the vena cava (Davis, et al, 1957; Mills, Casper, and Bartter, 1958; Gann, Mills, and Bartter, 1960; Gann and Travis, 1964) or constriction of the pulmonary artery (Davis, et al, 1957) stimulates aldosterone secretion. Infusion of blood after caval constriction in anesthetized dogs lowers the aldosterone secretion rate. The effect of caval constriction, however, was not purely attributable to changes in pressure on the low pressure side of the circulation, because denervation of the thyrocarotid arterial junction reduced the aldosterone response to caval constriction (Bartter, Mills, and Gann, 1960), while the effect of vagotomy was to prolong the duration of the aldosterone response to caval constriction (Mills, Casper, and Bartter, 1958). Tonic inhibition of the activity of the renin-angiotensin system is exerted via the vagus nerve, however. Section or cold block of the cervical vagi increased plasma angiotensin (Hodge, Lowe, Ng, and Vane, 1969) concentration and the rate of renin secretion (Mancia, Romero, and Shepard, 1975). With the carotid sinus pressure maintained at 40 Torr, cooling the cervi-

cal vagi caused an even greater increase in the rate of renin secretion (Mancia, Romero, and Shepard, 1975).

At least some of the vagal fibers influencing the activity of the renin-angiotensin system are from the atrial voume receptors. Brennan, et al (1971) inflated balloons in the right and left atria of chloralose-anesthetized dogs to demonstrate that right atrial distention, but not left, decreased plasma renin concentration. Anderson, McCally, and Farrell (1959) examined the effects of atrial volume on aldosterone secretion by stretching the right or left atrium with sutures which extended from the atria to the chest wall. This method allowed manipulation of atrial volume without the use of balloons which impede blood flow. They found that right, but not left, atrial stretch decreased the rate of aldosterone secretion.

DISTRIBUTION OF CARDIOVASCULAR AFFERENTS

In summary, cardiovascular stretch receptors mediating the hormonal responses to hemorrhage are located in the cardiac atria (at the junctions of the great veins and the atria), the carotid sinuses, the thyrocarotid arterial junctions, and in the renal vascula-

ture. On the low pressure side of the circulation, right atrial stretch receptors influence the secretion of renin (Brennan, et al, 1971), aldosterone (Anderson, McCally, and Farrell, 1959), ACTH (Baertschi, Ward, and Gann, 1976), and cortisol (Cryer and Gann, 1974; Baertschi, Ward, and Gann, 1976), whereas left atrial stretch receptors influence the secretion of vasopressin (Brennan, et al, 1971). On the high pressure side of the circulation, stretch receptors at the thyrocarotid arterial junctions influence the secretion of corticosteroids (Gann, 1966) and aldosterone (Bartter, Mills, and Gann, 1960), and stretch receptors in the carotid sinuses influence the secretion of corticosteroids (Gann, Gould, Morley, and Mumma, 1964) and vasopressin (Share and Levy, 1962). Decreased renal perfusion pressure (Davis and Freeman, 1976) and/or decreased renal filtered sodium load (Vander, 1964) directly influence(s) renin secretion. Renin, secreted in response to hypotensive hemorrhage, contributes to the ACTH (Redgate, 1968) and corticosteroid (Gann and Cryer, 1973) responses to the hemorrhage. Finally, ventricular receptors have been proposed as controllers of renin (Thames, 1977) and vasopressin (Thames and Schmid, 1980) secretion, although the physiologic significance of this mechanism is not yet clear.

DYNAMICS OF PLASMA ACTH AND CORTICOSTEROID CONCENTRATIONS AND THE ADRENAL RESPONSE TO ACTH

Recently, several investigators have become interested in the dynamics of plasma ACTH and corticsteroid concentrations. In rats, (Rees, et al, 1971) and in humans (Krieger, et al, 1971) a circadian rhythm of ACTH has been demonstrated. Superimposed on this rhythm is a much higher frequency ultradian rhythm, demonstrated in human beings (Berson and Yalow, 1968; Krieger, et al, 1971; Krieger and Allen, 1972) and in sheep (Jones, 1979). It is thought that the increasing and decreasing activity of the ultradian rhythm summates to produce the circadian rhythm (Krieger, 1979). These changes in plasma ACTH concentration are generally followed by changes in plasma corticosteroid concentration. A circadian rhythm in corticosteroids has been demonstrated in several species, including human beings (Krieger, et al, 1971; Krieger and Allen, 1972; Hellman, et al, 1970; Weitzman, et al, 1971), and rats (Guillemin, Dear, and Liebelt, 1959). However, a clear circadian rhythm has not yet been demonstrated in dogs. As expected from the temporal pattern of plasma ACTH concentration, plasma corticosteroid concentration has been shown to undergo an ultradian rhythm in human beings

(Krieger, et al, 1971; Hellman, et al, 1970; Weitzman, et al, 1971) and in sheep (Fulkerson and Tang, 1979; Jones, 1979). It is thought that the episodic secretion of corticosteroids is directly attributable to the fluctuations of plasma ACTH concentration (Krieger, 1979). Krieger (1979) observes that in the human being, there appears to be six to nine episodes of ACTH and corticosteroid secretion per day, and that the "episodic fluctuations of cortisol and ACTH concentrations seem to be less frequent than those reported for luteinizing hormone secretion." This conclusion was based on studies employing measurement of plasma ACTH every 30 min (Krieger, et al, 1971) and measurement of cortisol every 20 min (Hellman, et al, 1970; Weitzman, et al, 1971). Hellman, et al (1970) demonstrated that increases in plasma cortisol concentration are due to "secretory episodes", during which the adrenal secretes cortisol in an "all or none fashion" and between which the adrenal is inactive. Weitzman, et al (1971) claimed that the data "seriously challenge the concept that a 'steady-state' or 'basal level' of cortisol is present during any extended time compartment of the 24 hour cycle." Krieger and Allen (1975), employing a more frequent (5 min) sampling interval, found that there was no strict quantitative relationship of plasma corticosteroid concentration to plasma ACTH concentration. According to Krieger (1979), "the basis for such a finding is unclear

at present." Krieger and Allen found a good correlation between bioassayable and immunoassayable plasma ACTH concentration, ruling out the possibility that the rapid fluctuations in plasma ACTH concentration were biologically inactive. Hume (1955), and later Miller and coworkers (1976), demonstrated that the basal secretion of corticosteroids by the adrenals of dogs is not constant. Hume commented that "when repeated measurements of adrenal secretion in the resting state were made throughout the day in a given animal a succession of very low values might be found, and then, all at once, an elevated value, flanked on both sides by values of very low range. It was as though a little 'puff' of ACTH were being secreted at periodic intervals to maintain adrenal sensitivity, and to produce a daily secretion of adrenal steroid adequate to maintain the minimum requirements of the resting state."

The magnitude of the adrenal response to ACTH is dependent upon the concentration of ACTH in the blood perfusing the adrenal, and the rate of blood flow through the adrenal (Urquhart, 1965; Urquhart and Li, 1969; L'Age, Gonzalez-Luque, and Yates, 1970). Porter and Klaiber (1964) demonstrated that restriction of venous outflow from the adrenal decreased the adrenal secretion of corticosterone in chloralose-anesthetized rats. Urquhart (1965) used the in situ perfused adrenal

in anesthetized dogs to show that reduction of adrenal blood flow be decreasing perfusate flow reduced the rate of adrenal cortisol secretion. In 1970, L'Age, Gonzalez-Luque, and Yates reported that in conscious dexamethasone-pretreated dogs, increases in adrenal blood flow (by injection of histamine or methacholine) in the presence of constant plasma ACTH concentration increased the rate of adrenal cortisol secretion, as long as the plasma ACTH concentration used was not causing a maximal response to start with. Thus, the adrenal secretion rate is determined by the adrenal presentation rate of ACTH (Urquhart, 1965) which is defined as the concentration of ACTH in arterial blood multiplied by the flow rate through the adrenal.

Urquhart and Li (1968, 1969) have shown that the adrenal response to step increases in plasma ACTH cocentration is characterized by an initial overshoot and settling to plateau levels of output. This phenomenon is observed when the input concentration is less than 10 uU/ml. Miller, et al (1976) found overshooting responses when intravenous infusion of ACTH into conscious dogs (body weights 18-30 kg) was 8 mU/min or less. Urquhart and Li (1968) found a 2 min delay between the step increase in perfusate ACTH concentration and the adrenal venous corticosteroid output. Lake and Gann (1972), however, using anesthetized dogs with cannulated

adrenal veins, found the delay to be less than 1 minute. They concluded that the *in situ* perfused adrenal is dynamically less responsive than the intact gland.

The overall magnitude of the adrenal response to infused or injected ACTH is linearly related to the logarithm of the ACTH presentation rate. This relationship holds as long as the conditions of the experiment (steady-state response or constant time interval between stimulus and measured response) are constant. Thus, Nelson and Hume (1955) demonstrated that the adrenal venous corticosteroid output 3-13 min after intravenous injection of ACTH into anesthetized, hypophysectomized dogs was linearly related to the logarithm of the dose of ACTH. Hypophysectomized rats responded to injections of ACTH with increases in adrenal corticosterone concentration (5 min after injection) that were linearly related to the logarithm of the ACTH dose (Vernikos-Danellis, 1969). Conscious dogs responded to constant infusions of ACTH with elevations in adrenal cortisol secretion that were linearly related to the logarithm of the ACTH infusion rate (Miller, *et al*, 1976). This log dose-response relationship has been demonstrated in dispersed adrenal cells (Seelig and Sayers, 1973; Kolanowski and Crabbe, 1976). The smallest dose of ACTH used to stimulate corticosteroid secretion *in vivo* is 1 uU/ml (about 10 pg/ml; Urquhart, 1965), and the smallest

CHAPTER THREE

Heterogeneity of Hemodynamic and Hormonal Responses to 15 ml/kg Hemorrhage in Conscious Dogs.

ABSTRACT

The ACTH, corticosteroid, vasopressin, and renin responses to hemorrhage are involved in the maintenance of blood pressure and the restitution of blood volume during hypovolemia. However, not much quantitative information is known about the responses of these hormones to hemorrhage in conscious animals, and not much is known about factors which modulate the responses. These experiments were designed to quantitate the blood pressure, heart rate, ACTH, corticosteroid, vasopressin, and renin responses to 15 ml/kg hemorrhage in conscious dogs, and to examine the effect of fever (or factors associated with or contributing to fever) on the hemodynamic and hormonal responses to hemorrhage.

Five conscious dogs were bled 15 ml/kg within 3 min (N=8 experiments) from chronically maintained femoral arterial catheters. The ACTH responses were not reproducible from dog to dog or from experiment to experiment

when the same dog was studied more than once.

To define a criterion for a priori reduction of variability of hormone response magnitude, I performed an additional 20 experiments on 9 conscious dogs, measuring the dog's rectal temperature at the time of the experiment. I found that rectal temperature was a significant covariate of prehemorrhage heart rate, ACTH, corticosteroids, vasopressin, and renin activity, and of the magnitudes of the mean arterial pressure, ACTH, corticosteroid, and vasopressin responses to hemorrhage.

In dogs with elevated rectal temperatures, hemorrhage caused a greater fall in mean arterial blood pressure than in normothermic dogs. This exaggerated fall in blood pressure was probably responsible for the larger vasopressin, ACTH, and corticosteroid responses and the faster onset of ACTH and corticosteroid responses in dogs with elevated rectal temperatures. In febrile dogs, the renin response was small in the presence of the markedly elevated plasma vasopressin levels, suggesting that augmented vasopressin responses may inhibit the renin responses to hemorrhage.

I conclude that the reproducibility of the magnitudes of cardiovascular and hormonal responses to 15 ml/kg hemorrhage can be greatly improved by preselection of dogs for study on the basis of rectal temperature at

the time of the experiment.

INTRODUCTION

Hemorrhage initiates a series of homeostatic endocrine responses including increased circulating levels of ACTH (Redgate, 1968), adrenocortical steroids (Farrell, Rosnagle, and Rauschkolb, 1956; Gann, 1969), vasopressin (Ginsburg and Heller, 1953), and plasma renin activity (Sapirstein, Ogden, and Southard, 1941). In the pentobarbital-anesthetized dog, Gann (1969) has shown that the magnitude of the adrenal venous corticosteroid response to hemorrhage is proportional to the logarithm of the volume of the blood removed (threshold dose 2–3 ml/kg), and is suppressed by prior administration of dexamethasone (when the hemorrhage volume is less than 20 ml/kg). Based on the results of these studies, I chose to apply a 15 ml/kg hemorrhage in the conscious dog to study cortisol feedback on stimulus-induced ACTH secretion.

In 8 preliminary experiments, I bled 5 conscious dogs 15 ml/kg within 3 min with or without a cortisol infusion at various times before and during hemorrhage. I found that 15 ml/kg hemorrhage induced highly variable

cardiovascular and hormonal responses which could not be explained by the time or dose of cortisol infusion. I therefore studied the variability in the magnitudes of the cardiovascular and hormonal responses to hemorrhage, and defined a measured covariate of response magnitude.

MATERIALS AND METHODS

I studied 14 mongrel dogs of either sex weighing between 15 and 30 kg. They were housed in individual cages and given free access to food and water. Before surgery, the dogs were accustomed to standing in a loose cloth sling (Alice King Chatham Medical Arts, Inc.).

Surgery: Dogs were anesthetized with sodium pentobarbital (30 mg/kg,iv), and under sterile conditions a catheter was inserted into a femoral artery, and the tip advanced to the common iliac artery. Catheter material was either Medical Grade Silastic tubing (Dow Corning, Inc., .078"ID, .125"OD) or Tygon R-3603 polyvinylchloride tubing (Norton Plastics, Inc., .094"ID, .156"OD). The cannula was routed subcutaneously from a femoral triangle to the back where the free end emerged through the skin. The catheter was filled with sodium heparin (1000 units/ml) and the end was wrapped in a

clean gauze packet which was held in place by a zippered jacket (Alice King Chatham Medical Arts, Inc.). At least 2 days elapsed between surgery and experimentation.

Experiments: All experiments were started between 0800 and 1000h to minimize between-dog variation due to possible circadian variations in resting hormone levels and hormone responses to hemorrhage. On the morning of an experiment, a dog was brought from the animal quarters to the laboratory, placed in the sling, and its rectal temperature was measured using a clinical mercury rectal thermometer, inserted to a depth of 6 cm and held in place for at least 2 min. The temperature was read to the nearest 0.1 degree. Next, a superficial leg vein was catheterized (Angiocath, 18g, Deseret Pharmaceutical Co.).

Infusions: In 9 experiments, a constant intravenous infusion (17 ug/min) of cortisol was administered. In 6 experiments, the cortisol was infused from 120 to 60 min before the start of hemorrhage; in 1 experiment, cortisol was infused from 30 to 0 min before hemorrhage; and in 2 experiments, cortisol was infused from 2 min before to 30 min after the onset of hemorrhage. In 15 experiments, either a saline infusion (from 120 to 60 min before hemorrhage) or no infusion was administered. All saline used in these experiments was sterile, pyrogen-free normal saline (Travenol Laboratories,

Inc.). All dogs not infused received venipuncture at least 40 min before hemorrhage.

Hemorrhage and Sampling: Blood was pumped from the femoral arterial catheter using a roller pump at a rate sufficient to achieve a 15 ml/kg hemorrhage within 3 min. The hemorrhage volume was pumped into a sterile Donor-Pack (Fenwal Co.) which contained 1000 units of sodium heparin. Thirty minutes after the onset of hemorrhage, the shed blood was returned to the dog over a period of 5 min. Twelve blood samples (8-12 ml) were collected for hormone analysis. Heart rate and femoral arterial blood pressure were recorded using a Statham pressure transducer and a Grass polygraph. At the end of all experiments, the red blood cells from blood samples were resuspended in sterile, pyrogen-free normal saline and returned to the dog.

Hormone Assay: Blood samples were placed on ice, centrifuged at 4 C, and plasma was assayed for ACTH, corticosteroids, vasopressin, and renin activity. Plasma vasopressin was measured by radioimmunoassay (Keil and Severs, 1977), as was ACTH (Dallman, DeManicor, and Shinsako, 1974; Rees, et al , 1971; Sato, et al , 1975). All ACTH samples from a single experiment on one animal were run in a single extraction and incubation. The reproducibility of the assay in the range of ACTH values of interest was tested by running in one assay

multiplicate samples of a plasma pool using both 1.0 ml aliquots and 0.5 ml aliquots diluted to 1.0 ml in ACTH-free plasma. Mean values for 5 samples of 1.0 ml were 65 + 4.9 (SD), and for 4 samples of 0.5 ml were 31.0 + 2.9 (SD) pg ACTH /sample. Thus, at these levels, the intraassay coefficient of variation of the assay is 8-9%. Over a period of 3 months, the interassay coefficient of variation was 19% for a plasma pool that contained an average of 78 pg ACTH/ml (N=46 extractions and assays).

Plasma renin activity was measured using a radioimmunoassay for angiotensin I generated during a 3 hour incubation in vitro (Reid, et al , 1972; Stockigt, Collins, and Biglieri, 1971). Plasma corticosteroids were measured using a competitive protein binding assay (Murphy, 1967), employing human transcortin as the binding protein.

Plasma sodium concentration was measured using an Instrument Laboratories flame photometer. Plasma osmolality was measured using an Advanced Osmometer.

Statistical Analyses: Data were analyzed using paired "t" test, linear regression analysis, and one way analysis of variance for repeated measures.

RESULTS

In 8 preliminary experiments in 5 conscious dogs (Figure 1, right and left) 15 ml/kg hemorrhage and 30 minutes of the resultant hypovolemia evoked highly variable responses in plasma ACTH concentration (but also in mean arterial blood pressure, heart rate, and plasma vasopressin and corticosteroid concentrations). The hormonal and cardiovascular responses to hemorrhage were quite variable from dog to dog, but also from experiment to experiment in those dogs studied more than once. In Figure 1 (right), one dog was infused with cortisol (17 ug/min) from 120 to 60 min before hemorrhage, one dog from 30 to 0 min before hemorrhage, and two dogs from 2 min before to 30 min after the onset of hemorrhage. It was not possible to detect a reproducible effect of the cortisol infusions due to the overall nonreproducibility of the ACTH responses.

I hypothesized that one source of variability in these experiments could be the development of fevers after catheter implantation, although the dogs at the time of the experiment did not, generally, appear unhealthy or "depressed". I therefore performed 20 hemorrhage experiments on 9 conscious dogs, measuring the rectal temperature of the dog at the time of the

experiment. Rectal temperatures ranged from 38.2 to 41 C; the range of rectal temperatures of non-febrile dogs is 38-39 C (Altman and Dittmer, 1973). Almost all dogs with fevers appeared normal and healthy (cold nose, bright eyes, and wagging tail); the only way of reliably detecting the fever was to measure the dog's rectal temperature.

In Figure 2 are shown linear regressions of integrated cardiovascular and hormonal responses to hemorrhage on the rectal temperature at the time of the experiment. The ACTH, corticosteroid, vasopressin, and mean arterial pressure responses to hemorrhage were linearly related to rectal temperature. In addition to the elevated responses, dogs with elevated rectal temperatures tended to have elevated resting ACTH, corticosteroid, and vasopressin concentrations, elevated resting plasma renin activity, and elevated resting heart rate (Table 1).

TABLE 1. Correlations of baseline values with temp.

Correlated variable	r^2	p	N
ACTH	.29	<.005	20
11OHCS	.49	<.001	20
Vasopressin	.22	<.05	20
PRA	.17	<.05	20
MAP	.07	NS	20
HR	.19	<.05	20

Based on the results of the linear regression analyses (Figure 2), I divided the data into three groups according to rectal temperature (38-38.9 C, 39-39.9 C, and

40-41 C) at the time of the experiment. Table 2 shows the results of one-way analyses of variance in each temperature range for mean arterial blood pressure, heart rate, plasma ACTH, corticosteroid, and vasopressin concentrations, and plasma renin activity. The fall in blood pressure after hemorrhage was pronounced in the highest temperature range (Figure 3). Prehemorrhage heart rates were progressively increased in the higher temperature ranges (Table 1); in none of the ranges did mean heart rate increase above 140 beats per min. Thus in the 40-41 C range, the initial heart rate was relatively rapid and did not increase after hemorrhage (Table 2). Probably as a result of this, blood pressure fell markedly in this group of dogs.

The plasma ACTH and corticosteroid responses to hemorrhage in dogs in the three temperature ranges are shown in Figure 4. It is clear that the responses were quicker in onset and of greater magnitude in dogs in the higher temperature ranges.

The plasma vasopressin responses to hemorrhage (Figure 5, left) were increased in the higher temperature ranges (see also Figure 2). The peak vasopressin concentration was found in the first sample collected after hemorrhage in each temperature range. In contrast to the other endocrine responses, the response in plasma renin activity (especially in the first 15 min

after hemorrhage) appeared smaller in the higher temperature ranges (Figure 5, right).

In 5 dogs whose rectal temperatures increased from 38 ± Ø.2 C to 4Ø.7 ± Ø.2 C between the first and second experiment, prehemorrhage plasma sodium concentration fell from 143 ± Ø.2 mEq/l to 137 ± Ø.6 mEq/l ($p<.ØØ1$ by paired "t" test). There was no coincident change in the dogs' body weights (change in body weight = -Ø.3 ± Ø.4 kg; p=NS by paired "t" test). In 3 of these 5 dogs, plasma osmolality was measured and decreased from 292 ± 1 mOsm/l to 279 ± 2 mOsm/l as the dogs became febrile.

TABLE 2. One-Way Analyses of Variance (-1Ø to +6Ø min)

	38-38.9 C (N=7)		39-39.9C (N=7)		4Ø-41 C (N=6)	
VAR.	F	p	F	p	F	p
MAP	4.3	<.ØØ1	11.9	<.ØØ1	9.3	<.ØØ1
HR	3.2	<.ØØ1	4.6	<.ØØ1	Ø.8	NS
ACTH	3.1	<.ØØ1	8.5	<.ØØ1	12.9	<.ØØ1
11OHCS	1Ø.6	<.ØØ1	11.6	<.ØØ1	18.4	<.ØØ1
AVP	2.8	<.Ø5	3.3	<.Ø5	5.6	<.ØØ1
PRA	5.9	<.ØØ1	3.5	<.Ø5	2.5	<.Ø5

Figure 1

Individual plasma ACTH responses to 15 ml/kg hemorrhage in 8 experiments (5 dogs) whose rectal temperature had not been measured. Dogs were infused with saline (A, left) from 120 to 60 minutes before hemorrhage or with 17 ug/min cortisol (B, right) from 120 to 60 minutes (N=1; open circles) or from 60 to 0 minutes (N=1; triangles), or from 2 minutes before to 30 minutes after hemorrhage (N=2; filled circles).

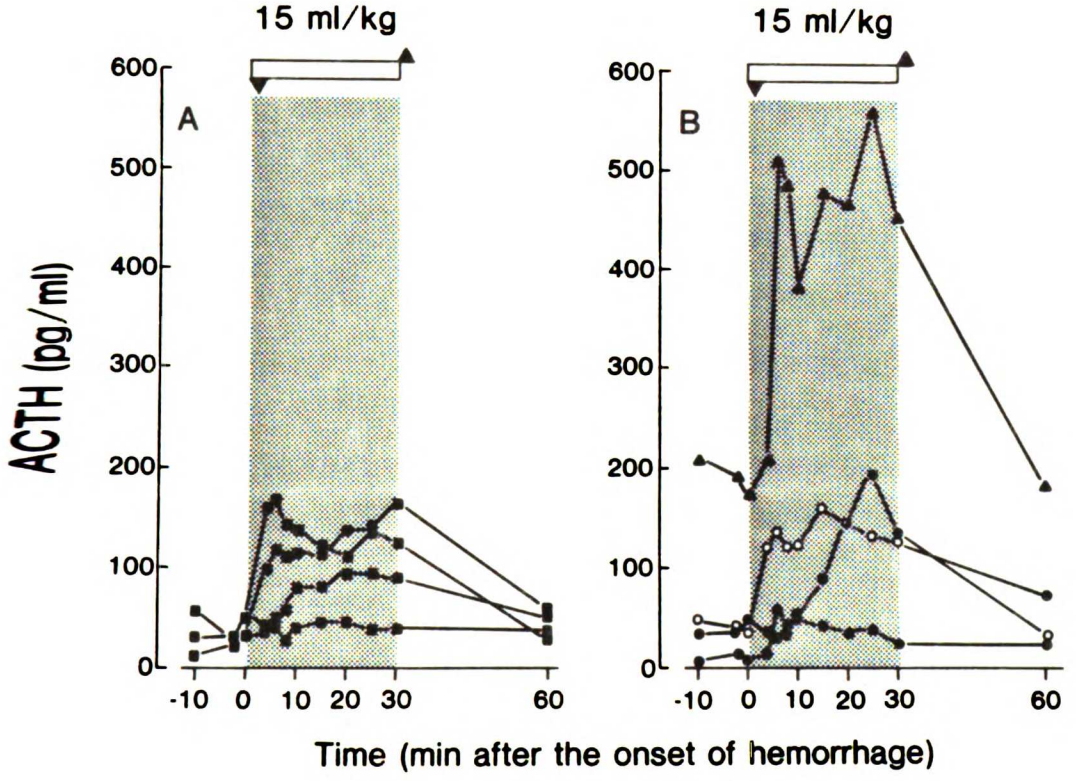

15 ml/kg
15 ml/kg
A
B
600
500
400
300
200
100
0
ACTH (pg/ml)
-10
0
10
20
30
60
Time (min after the onset of hemorrhage)

Figure 2

Correlation of integrated hormonal and cardiovascular responses to hemorrhage with rectal temperature at the time of the experiment. The integrated response (ACTH, A; corticosteroids, B; vasopressin, C; plasma renin activity, D; heart rate, E; and mean femoral arterial blood pressure, F) represents the area under the entire curve obtained over 70 minutes minus the area over 70 minutes of the mean value for prehemorrhage samples.

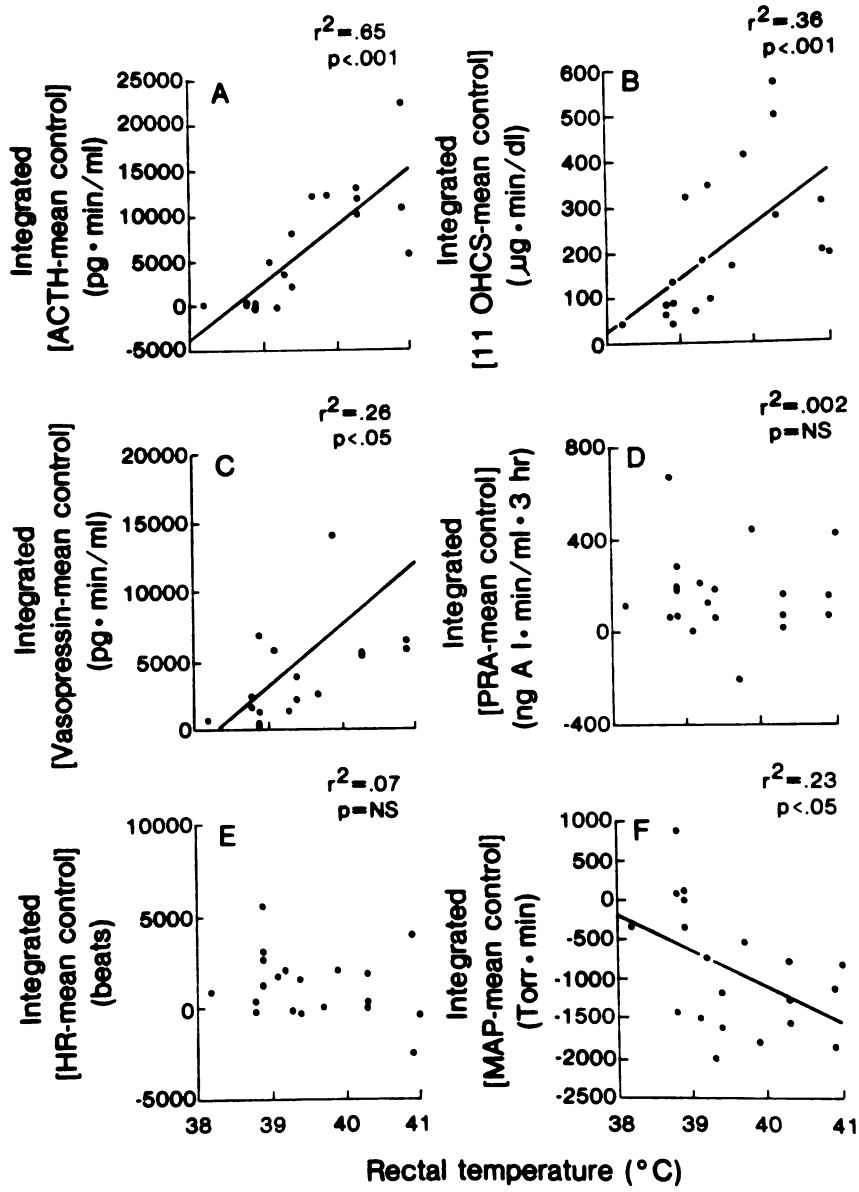
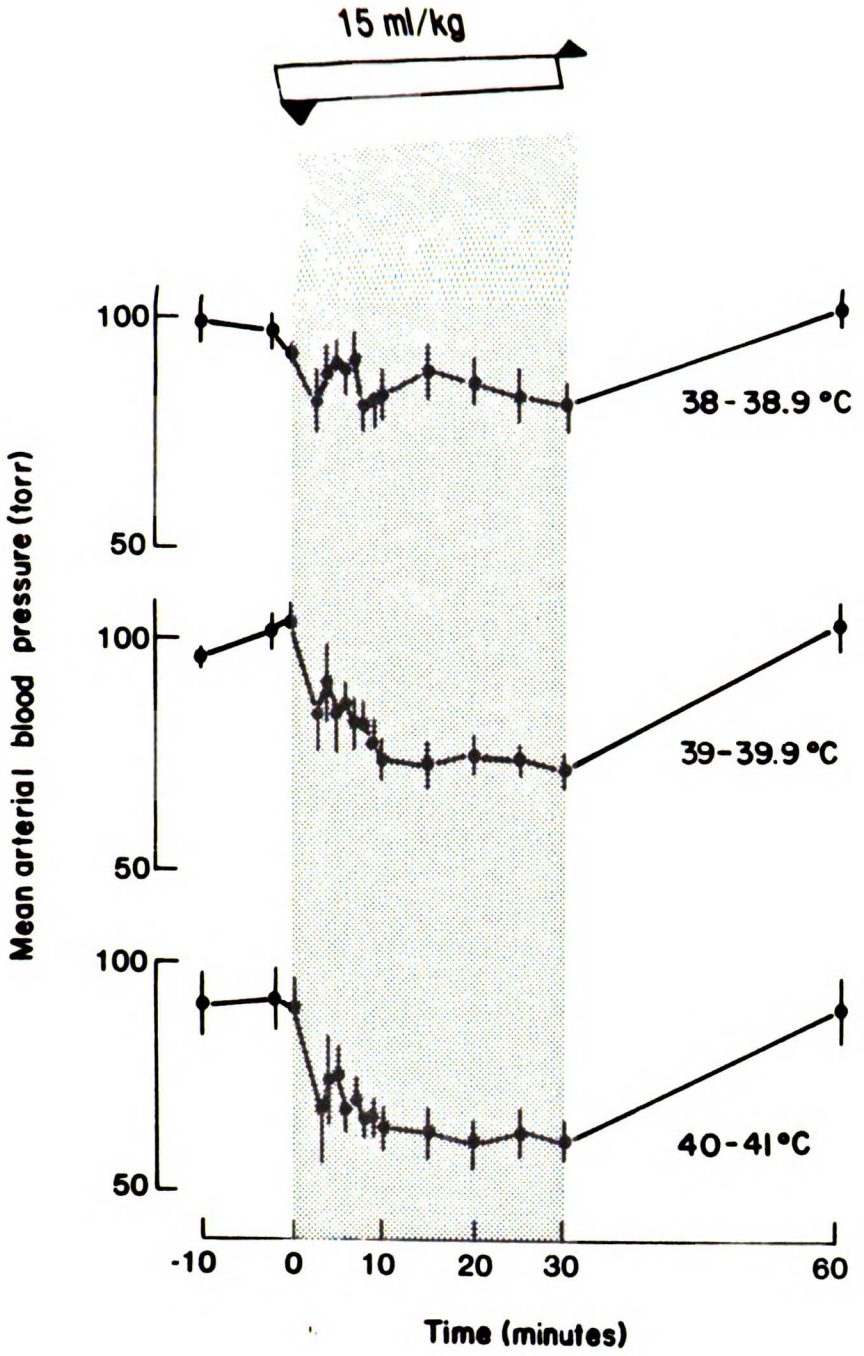

A
r^2=.65
p<.001
Integrated [ACTH-mean control] (pg • min/ml)
25000
20000
15000
10000
5000
0
-5000
B
r^2=.36
p<.001
Integrated [11 OHCS-mean control] (μg • min/dl)
600
500
400
300
200
100
0
C
r^2=.26
p<.05
Integrated [Vasopressin-mean control] (pg • min/ml)
20000
15000
10000
5000
0
D
r^2=.002
p=NS
Integrated [PRA-mean control] (ng A I • min/ml • 3 hr)
800
400
0
-400
E
r^2=.07
p=NS
Integrated [HR-mean control] (beats)
10000
5000
0
-5000
F
r^2=.23
p<.05
Integrated [MAP-mean control] (Torr • min)
1000
500
0
-500
-1000
-1500
-2000
-2500
38
39
40
41
Rectal temperature (°C)

Figure 3

Mean femoral arterial blood pressure (left) and heart rate (right) responses to 15 ml/kg hemorrhage as a function of 3 ranges in rectal temperature. The results in the 38-38.9 C and the 39-39.9 C ranges are each from 7 experiments. The 40-41 C range represents results from 6 experiments. The mean values, shown as circles, are accompanied by vertical lines representing one SEM.


15 ml/kg
Mean arterial blood pressure (torr)
100
50
38 - 38.9 °C
39 - 39.9 °C
40 - 41 °C
-10
0
10
20
30
60
Time (minutes)

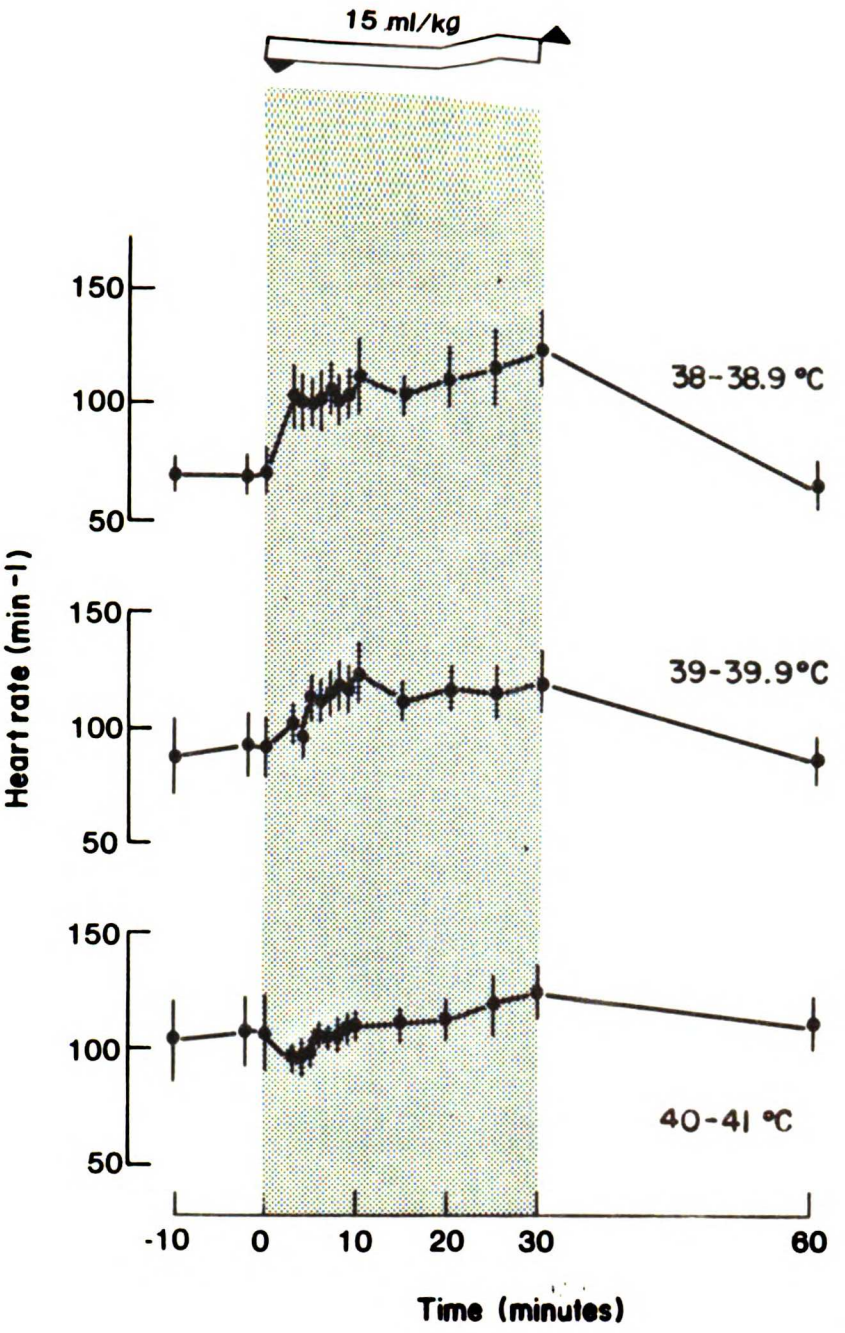

15 ml/kg
Heart rate (min -1)
150
100
50
38 - 38.9 °C
39 - 39.9 °C
40 - 41 °C
-10
0
10
20
30
60
Time (minutes)

Figure 4

Plasma ACTH (left) and corticosteroid (right) responses to 15 ml/kg hemorrhage divided into 3 temperature ranges. The numbers of experiments and symbols are as in Figure 3.

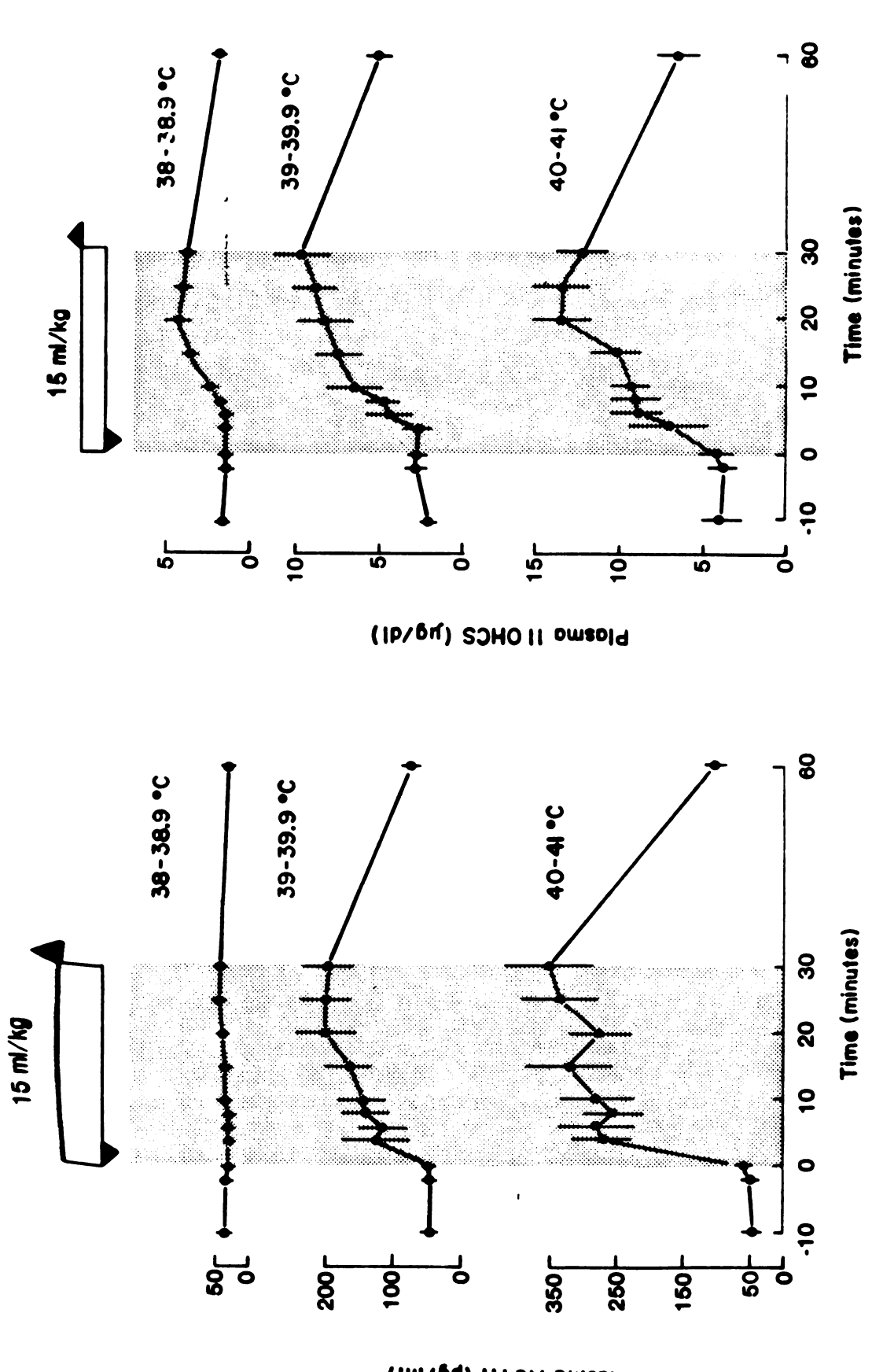
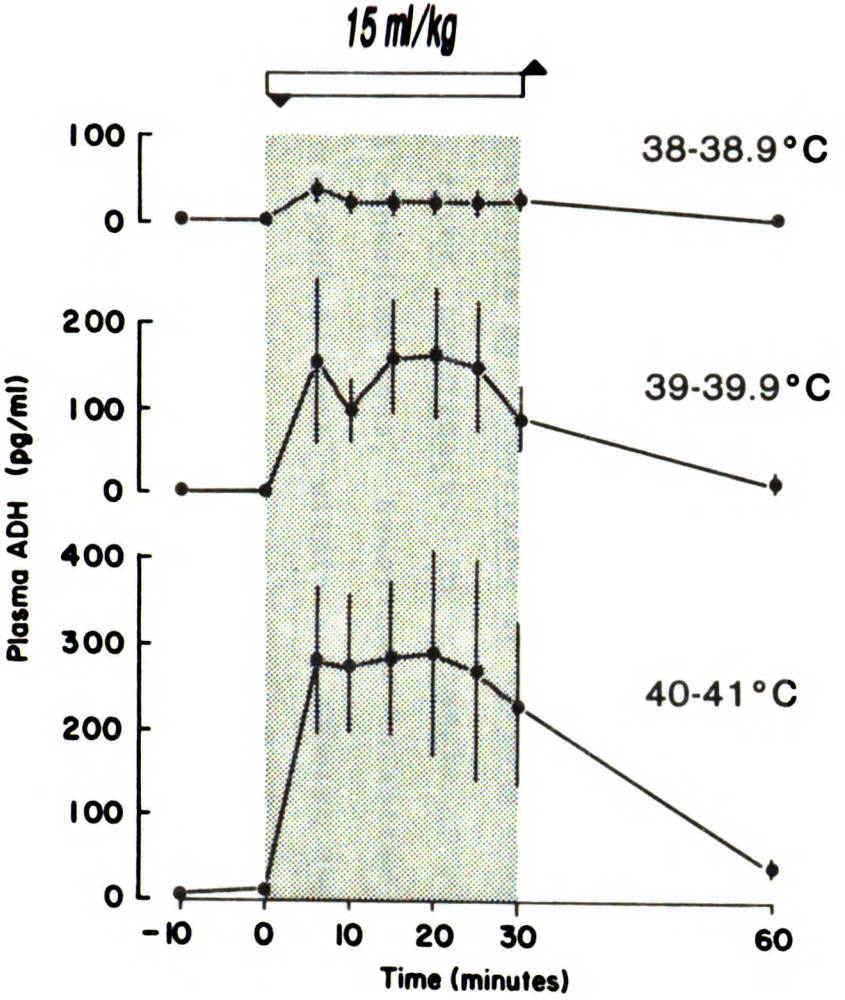
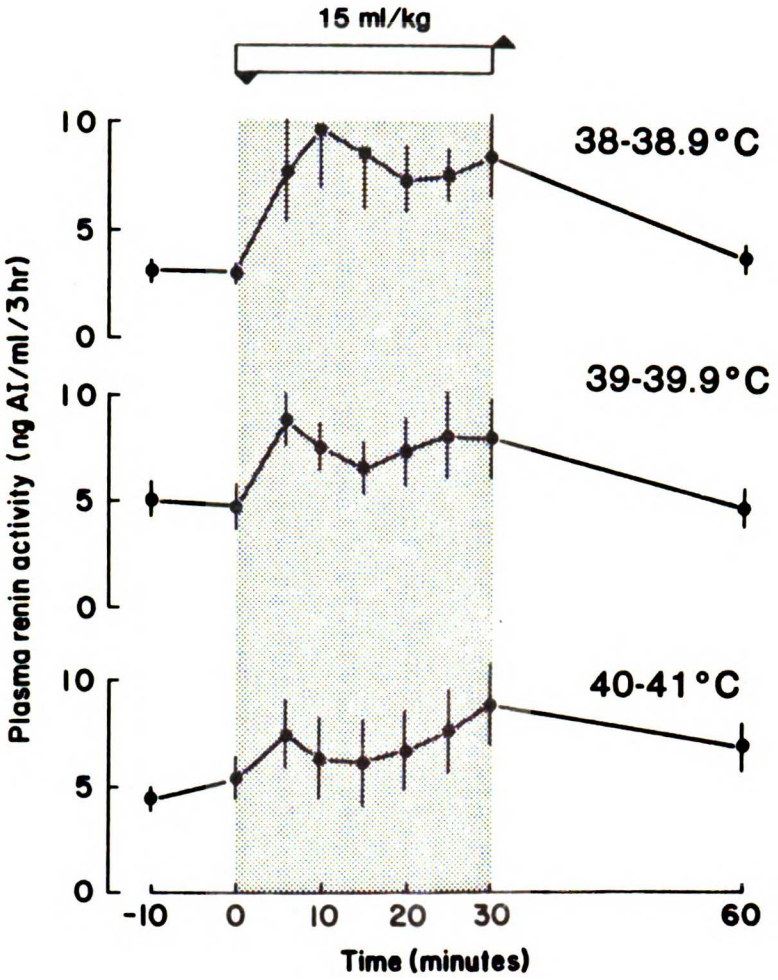

15 ml/kg
38-38.9 °C
39-39.9 °C
40-41 °C
Plasma 11 OHCS (µg/dl)
Time (minutes)
15 ml/kg
38-38.9 °C
39-39.9 °C
40-41 °C
Plasma ACTH (pg/ml)
Time (minutes)

Figure 5

Plasma vasopressin (left) and renin activity (right) responses to 15 ml/kg hemorrhage divided into 3 temperature ranges. The results of 15 experiments are shown for vasopressin (38-38.9 C, N=5; 39-39.9 C, N=4; 40-41 C, N=6) because of a change in the sampling frequency after the first 5 experiments which were therefore excluded from the analysis. The results of 20 experiments (numbers of experiments equal to those in Figure 4) are shown for plasma renin activity. The symbols are as in Figures 3 and 4.


15 ml/kg
38-38.9°C
39-39.9°C
40-41°C
Plasma ADH (pg/ml)
100
0
200
100
0
400
300
200
100
0
-10
0
10
20
30
60
Time (minutes)


15 ml/kg
38-38.9°C
39-39.9°C
40-41°C
Plasma renin activity (ng AI/ml/3hr)
10
5
0
-10
0
10
20
30
60
Time (minutes)

DISCUSSION

In this study, I have 1) characterized the ACTH, corticosteroid, vasopressin, and plasma renin responses to 15 ml/kg hemorrhage in conscious dogs, 2) described variability in the magnitudes of hormone responses to hemorrhage when the dogs are randomly selected for study, and 3) described a measured covariate (rectal temperature) of the magnitudes of the hormonal responses to hemorrhage thereby defining a reliable *a priori* criterion for reducing variability in hormone responses to hemorrhage. The adrenocortical response to hemorrhage in the normothermic, conscious dog is discussed elsewhere (Chapter 4). This discussion will be limited to the variability in hormone responses to hemorrhage in conscious dogs.

Rectal temperature as a covariate of hormone response magnitude after hemorrhage. In a sample of 8 experiments on 5 conscious dogs, hemorrhage elicited highly variable responses both within and between dogs. In a subsequent study of 20 experiments in 9 conscious dogs, I found that much of the variability in the hormonal and hemodynamic responses could be attributed to differences in the dogs' rectal temperatures at the time of hemorrhage. Thus, dogs with fevers (probably of different

etiologies) responded to hemorrhage predictably: the magnitudes of the blood pressure, ACTH, corticosteroid, and vasopressin responses were linearly related to rectal temperature. Variability in the hormonal responses to hemorrhage have been reported (Share, 1967b), but I have found no other published studies of the hormonal responses to hemorrhage in either conscious or anesthetized dogs in which the animals' rectal temperatures were reported.

Most dogs in this study, regardless of rectal temperature, appeared normal and displayed no outward signs of sickness at the time that they were studied. Since I have begun routinely measuring rectal temperatures of dogs received at our animal care facility from pounds elsewhere, I have found that at least 30% of them arrive at the facility with rectal temperatures above 39 C. Other animals develop respiratory infections and fever after they have been at the facility for 1 to 2 weeks. Some dogs which were not febrile before surgery developed fevers after implantation of the arterial catheter. I believe that at least some of the dogs expressed latent viral infections after surgery. My subsequent experience has been that if I keep each dog in the animal care facility for at least 4 weeks and "condition" it by treating illnesses that develop during this period, it does not develop an elevated rectal tem-

perature after surgery.

Endocrine responses to hemorrhage in dogs with elevated rectal temperatures. In dogs with elevated rectal temperatures, 1) the resting levels of some hormones were increased (Table 1), and 2) 15 ml/kg hemorrhage became a more effective stimulus to ACTH, corticosteroid, and vasopressin secretion (Figures 2, 4, and 5). Plasma ACTH, corticosteroid, renin, and vasopressin concentrations before hemorrhage correlated positively with the dogs' rectal temperatures. The elevated prehemorrhage plasma vasopressin concentration and renin activity may have caused the marked reduction in plasma sodium and osmolality observed in the dogs with very high rectal temperatures.

The ACTH, corticosteroid, and vasopressin responses to hemorrhage were larger at the higher rectal temperatures (Figure 2). The vasopressin and ACTH responses to hemorrhage are thought to be mediated by cardiovascular afferents from the cardiac atria (Henry, Gauer, and Reeves, 1956; Gann, 1971) and carotid sinuses (Share and Levy, 1966; Gann, Gould, Morley, and Mumma, 1964). It is therefore not surprising that in the 40-41 C range, a larger fall in mean blood pressure caused larger increases in vasopressin and ACTH. However, in the normothermic dogs, plasma vasopressin concentration was maximal in the first sample (6 min) after hemorrhage,

whereas plasma ACTH did not peak until 25 min and certainly did not appear to have increased between 4 and 10 min after hemorrhage. Because the hemorrhage-induced ACTH and vasopressin responses were temporally separated, the afferent pathways leading to secretion of these hormones may not be identical.

The relationship between the plasma renin activity response to hemorrhage and body temperature was unanticipated. Because of the large pressure drop in the 40-41 C range (Figure 3), one would expect the plasma renin response to be greatest in these experiments (Davis and Freeman, 1976). However, the overall renin response was not increased at elevated rectal temperatures (Figure 5, right); in the first 15 min after hemorrhage, the response appeared diminished in the higher temperature ranges. This apparently contradictory finding may be explained by the marked elevations in vasopressin levels after hemorrhage in dogs in the intermediate and high temperature ranges (Figure 5, left). Vasopressin has been shown to inhibit renin secretion in the sodium depleted (Tagawa, et al , 1971) and sodium replete dog (Malayan, et al , 1978). Therefore, the apparent negative relationship between body temperature and the plasma renin response to the first 15 min of hypovolemia may reflect an inhibition of renin release by vasopressin at elevated rectal temperatures.

Increased resting- and stimulated- hormone levels in febrile dogs might be explained by decreased blood volume. ACTH, corticosteroids, renin, and vasopressin act directly or indirectly to increase blood volume and therefore mean arterial pressure. Levels of these hormones might be expected to correlate negatively with the dog's blood volume. The nominal stimulus of 15 ml/kg hemorrhage would be effectively greater at elevated rectal temperatures if blood volume decreases with the development of fever in our dogs. To date, however, I have found no evidence of a decrease in body weight, increase in hematocrit, or changes in any other variable which would suggest a progressive dehydration with the development of fever in these animals. In conclusion, the results of these experiments suggest that fever or factors contributing to or associated with fever (decreased blood volume?) were responsible for altering the endocrine and hemodynamic responses to hypovolemia.

CHAPTER FOUR

Discrepant ACTH and Corticosteroid Responses to Hemorrhage in Conscious Dogs.

ABSTRACT

The results of experiments reported in Chapter 3 demonstrate that conscious dogs with elevated rectal temperatures respond to 15 ml/kg hemorrhage with larger heart rate, blood pressure, ACTH, corticosteroid, and vasopressin responses than normothermic dogs. The experiments reported in this chapter were designed to: 1) identify the threshold volume of hemorrhage required for stimulation of ACTH and corticosteroid secretion; and 2) examine the relationship of plasma corticosteroid and ACTH concentrations during 15 ml/kg hypovolemia in normothermic dogs.

To identify the threshold volume of hemorrhage for stimulation of ACTH and corticosteroid secretion, 15 conscious dogs were rapidly bled 10, 12-13, and 15 ml/kg from a previously implanted femoral arterial catheter. Hypovolemia was maintained for 30 min before the shed blood was reinfused. Arterial plasma levels of ACTH,

corticosteroids, and renin activity were measured before, during, and after the period of hypovolemia. Hemorrhage of 15 ml/kg, but not 10 ml/kg caused hypotension and increases in heart rate and plasma ACTH and corticosteroid concentrations ($p<.001$). Fifteen ml/kg hemorrhage caused a small, slow ACTH response (peak increase 33% at 25 min) that did not explain the large, faster corticosteroid response (peak increase 186% at 20 min). The correlation of renin and corticosteroids was higher ($r^2=.39$; $p<.001$) than that of ACTH and corticosteroids ($r^2 =.06$; $p<.05$). To ascertain that adrenal secretion of corticosteroids was increased during 15 ml/kg hypovolemia, changes in clearance or distribution volume of cortisol were estimated by counting tritium extracted from plasma of 5 dogs infused with 3H-(1,2)-cortisol to steady-state levels before and during hypovolemia. The stimulus resulted in a 30% reduction from steady-state levels of dichloromethane-extractable tritium counts ($p<.001$). Combined with the observed increase in plasma corticosteroid levels, these results show that the increase in adrenal secretion of corticosteroids after hemorrhage was underestimated by measurement of changes in peripheral plasma levels. The data suggest that factors in addition to circulating ACTH levels may drive adrenal corticosteroid secretion in the conscious dog.

INTRODUCTION

Hemorrhage stimulates adrenocortical secretion (Farrell, Rosnagle, and Rauschkolb, 1956; Gann, 1969) via cardiovascular stretch receptors located in the atria (Cryer and Gann, 1973) and carotid sinuses (Gann, Gould, Morley, and Mumma, 1964). In earlier studies, investigators have assumed that increases in plasma ACTH concentration mediate the steroid response to hemorrhage (Gann, 1969). Based on this assumption, studies on the control of plasma ACTH concentration during hypovolemia have been designed such that the only measured variable in the adrenocortical system is the rate of adrenal corticosteroid secretion (i.e., Gann, 1969; Cryer and Gann, 1973).

This is a detailed investigation of changes in plasma ACTH and corticosteroid concentrations in the conscious dog bled 15 ml/kg. I provide evidence that plasma ACTH concentration cannot always be inferred from plasma corticosteroid concentration in conscious dogs. Some of these data have been reported in abstract (Wood, et al , 1978), and in Chapter 3.

MATERIALS AND METHODS

Mongrel dogs of either sex (17 to 28 kg) were housed in individual cages with free access to food and water. Four dogs were surgically prepared with carotid skin loops (Ramsay, et al , 1978) at least 4 weeks prior to the first experiment. Twelve dogs were surgically prepared with chronically maintained femoral arterial catheters (see Chapter 3) at least 2 days prior to experiments. Dogs prepared with carotid skin loops were bled 10 and 12-13 ml/kg (see below), and dogs prepared with femoral arterial catheters were bled 15 ml/kg (see below). Before experiments, each dog was accustomed to quiet standing in a loose canvas sling (Alice King Chatham Medical Arts, Inc.).

For each dog, at least 2 days were allowed between experiments. On the morning of an experiment, a dog was brought from the animal quarters to the laboratory, placed in the sling, and (for all dogs with femoral arterial catheters) its rectal temperature was measured using a clinical rectal mercury thermometer, inserted to a depth of 6 cm and held in place for at least 2 min. The temperature was read to the nearest 0.1 degree.

Nineteen experiments were started between 0800 and 1000 h to minimize between-dog variations in resting and

hemorrhage- stimulated hormone levels. After the dog was placed in the sling and its rectal temperature measured, a superficial leg vein was catheterized with an Angiocath (Deseret Pharmaceutical Co., 18g) or an Intracath (Deseret Pharmaceutical Co., 17g needle and 19g catheter), and at least 40 min were allowed before the first blood sample was taken. In dogs with carotid skin loops, one carotid artery was catheterized with an Angiocath (18g) at least 40 min before the first blood sample was taken.

In 2 (15 ml/kg) experiments, a constant intravenous infusion of cortisol (17 ug/min) was started and continued for one hour. After completion of the infusion, another hour was allowed before the onset of hemorrhage. In 14 (10, 12-13, and 15 ml/kg) experiments, either a saline infusion (iv) or no infusion was given before the onset of hemorrhage. In 5 (15 ml/kg) experiments, 3H-(1,2)-cortisol was injected (5 uCi, iv) followed by an infusion of 3H-(1,2)-cortisol (0.0855 uCi/min, iv) for the rest of the experiment. Hemorrhage was performed 100 min after the pulse of 3H-(1,2)-cortisol.

At the time of hemorrhage, dogs were bled 10 or 12-13 ml/kg within 5 min from the carotid catheter, or 15 ml/kg within 3 min from the femoral catheter. In dogs bled 10 and 12-13 ml/kg, blood was drawn into heparinized 60 ml plastic syringes. In dogs bled 15

ml/kg, blood was pumped (with a roller pump) at a constant rate from the femoral arterial catheter into a sterile Donor-Pack (Fenwal Co.) containing 1000 units of sodium heparin. In all cases, 30 min after the onset of hemorrhage, the shed blood was returned to the dog (over a period of 5 min). In dogs infused with 3H-(1,2)-cortisol, 14 femoral arterial blood samples (5 ml each) were taken for extraction of tritium counts with dichloromethane (see below) and for assay of ACTH and corticosteroids. In the other experiments, 12 femoral or carotid arterial blood samples (8-12 ml each) were taken for assay of ACTH, corticosteroids, renin activity, and vasopressin. When not being used for blood sampling or for removal or return of the hemorrhage volume, the arterial catheter was connected to a Statham pressure transducer and a Grass polygraph for the measurement of blood pressure and heart rate. At the end of all experiments, the red blood cells from blood samples were resuspended in pyrogen-free, sterile normal saline and returned to the dog.

Blood samples were placed on ice, centrifuged at 4 C and plasma was assayed for ACTH, corticosteroids, and renin activity. Plasma ACTH was measured by radioimmunoassay (Dallman, DeManicor, and Shinsako, 1974; Rees, et al , 1971; Sato, et al , 1975). Plasma corticosteroids were measured using a competitive protein bind-

ing assay (Murphy, 1967), employing human transcortin. The added tritium counts from the infused labelled cortisol in the 5 experiments described above accounted for less than 1% of the counts in the corticosteroid assay and correction was not made for this. Plasma renin activity was measured using a radioimmunoassay for angiotensin I generated during a 3 hour incubation _in vitro_ (Reid, _et al_, 1972; Stockigt, Collins, and Biglieri, 1971).

3H-(1,2)-cortisol was extracted from triplicate aliquots of Ø.5 ml plasma with 5 ml dichloromethane. Because more than 9Ø% of the extractable tritium counts were found to be extracted in the first 5 ml volume of dichloromethane, a single extraction was used for 3 of the 5 experiments. The dichloromethane extracts were dried under an air stream, redissolved in Omnifluor scintillation fluid (New England Nuclear, Inc.), and counted in a Packard Tricarb scintillation counter. The coefficient of variation of the triplicate samples was 8.Ø%.

The data were analyzed by one-way analysis of variance for repeated measures and by linear regression analysis.

RESULTS

Conscious dogs were bled 10 ml/kg (Figure 6, left; N=4), 12-13 ml/kg (N=3), and 15 ml/kg (Figure 6, right; N=9). Hemorrhage did not stimulate a significant increase in plasma ACTH concentration unless the hemorrhage volume was 15 ml/kg ($p<.001$ by ANOVA). Ten ml/kg hemorrhage did not stimulate changes in blood pressure or heart rate, whereas 15 ml/kg hemorrhage caused a significant fall in blood pressure and a significant increase in heart rate (Chapter 3).

Adrenocortical response to 15 ml/kg hemorrhage. Nine normothermic, conscious dogs responded to 15 ml/kg hemorrhage (Figure 6, right) with an 11 pg/ml increment in plasma ACTH concentration (peak at 25 min after the onset of hemorrhage) and a 2.7 ug/dl increment in plasma corticosteroid concentration (peak at 20 min after the onset of hemorrhage). The data from the 9 dogs are presented individually in Figure 7. In each of these dogs, there was a clear rise in corticosteroids beginning within 10 min after the onset of hemorrhage, and a return to near prehemorrhage levels 30 min after the hemorrhage volume was reinfused. Plasma ACTH did not always increase before the rise in corticosteroids. In fact, it appears that there was very little relationship

of ACTH to corticosteroids in any of the 9 dogs. In 6 of the 9 dogs, corticosteroids were elevated before ACTH rose above its highest control level. In 2 dogs, ACTH rose above its highest control level only after peak corticosteroid concentrations were achieved. In 8 of 9 experiments, ACTH did not rise more than 20 pg/ml above control, and in 5 of 9 experiments, the increase in ACTH was 12 pg/ml or less. Correlation of plasma ACTH and corticosteroid concentrations at each time point in the 9 dogs demonstrated a significant ($p<.05$), although very slight ($r^2=.06$) relationship between the two variables.

Changes in plasma renin activity in 7 of the 9 normothermic dogs bled 15 ml/kg are also shown in Figure 7. In 6 of the 7 experiments, plasma renin activity increased before corticosteroid levels rise, and in the seventh (10/31), plasma renin activity rose concomitantly with the increase in corticosteroids. In all dogs except 8/10 and 10/31, the corticosteroid response to hemorrhage appeared more closely related to the renin response than to the ACTH response. In 7 dogs, plasma corticosteroid concentrations were linearly related to the plasma renin activities ten minutes earlier (Figure 8; $r^2=.39$; $p<.001$).

Adrenal secretion occurs. To test whether 15 ml/kg hemorrhage-induced elevations in plasma corticosteroid concentrations were due to increases in adrenal

secretion, I infused 5 dogs with 3H-(1,2)-cortisol to steady-state levels of tritium counts extractable from plasma with dichloromethane. A 15 ml/kg hemorrhage was performed 100 min after the start of the infusion. Increases in the clearance rate and/or distribution volume of cortisol would be reflected by a decrease from plateau levels of dichloromethane-extractable counts, and a decrease in the clearance rate and/or distribution volume would be reflected by an increase in counts.

Three dogs with rectal temperatures of 38.3, 38.6, and 39.2 C responded to the hemorrhage with small changes in plasma ACTH concentration that did not seem to account for the clearly discernable rises in plasma corticosteroid concentration (Figure 9, left). Two dogs with rectal temperatures of 39.5 and 39.7 C responded to hemorrhage with relatively large increments in plasma ACTH and corticosteroid concentrations (Figure 9, right), as predicted from the results of a previous study (Chapter 3 and Wood, et al , 1978). In none of the 5 dogs did the clearance rate and/or distribution volume of cortisol decrease during the period of 15 ml/kg hypovolemia. In the 3 dogs with rectal temperatures less than or equal to 39.2 C, the tritium counts extracted from plasma with dichloromethane decreased by an average of 36% ($p<.001$ by ANOVA), whereas in the 2 dogs with rectal temperatures of 39.5 and 39.7 C, tri-

tium counts did not appear to change from control levels during hypovolemia. Clearly the rise in peripheral corticosteroid concentration in the normothermic dogs after hemorrhage was not due to decreased clearance, and therefore must have resulted from increased adrenal cortical secretion.

Figure 6

Plasma ACTH and corticosteroid (11OHCS) concentrations before and during a 3Ø min period of 1Ø ml/kg (left, N=4) and 15 ml/kg (right, N=9) hypovolemia. Dogs were bled within 3 min starting at time Ø. All dogs bled 15 ml/kg were known to be normothermic at the time of the experiment. Means are represented as closed circles (ACTH) and as open circles (11OHCS). Standard errors are represented as vertical bars.

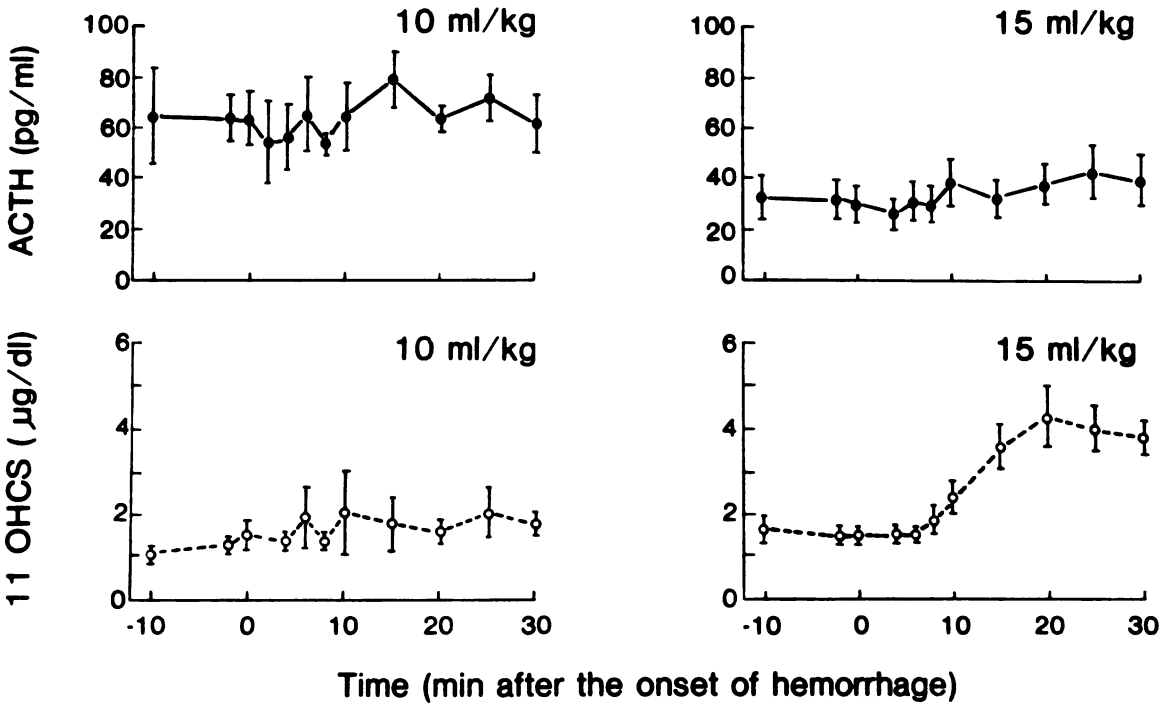

10 ml/kg
15 ml/kg
ACTH (pg/ml)
100
80
60
40
20
0
10 ml/kg
15 ml/kg
11 OHCS (μg/dl)
6
4
2
0
-10
0
10
20
30
Time (min after the onset of hemorrhage)

Figure 7

Individual plasma ACTH, corticosteroid (11OHCS), and renin activity (PRA) levels in 7-9 dogs rectal temperatures 38.2-38.9 C) subjected to 15 ml/kg hemorrhage at Ø time, and 3Ø min of the resultant hypovolemia.

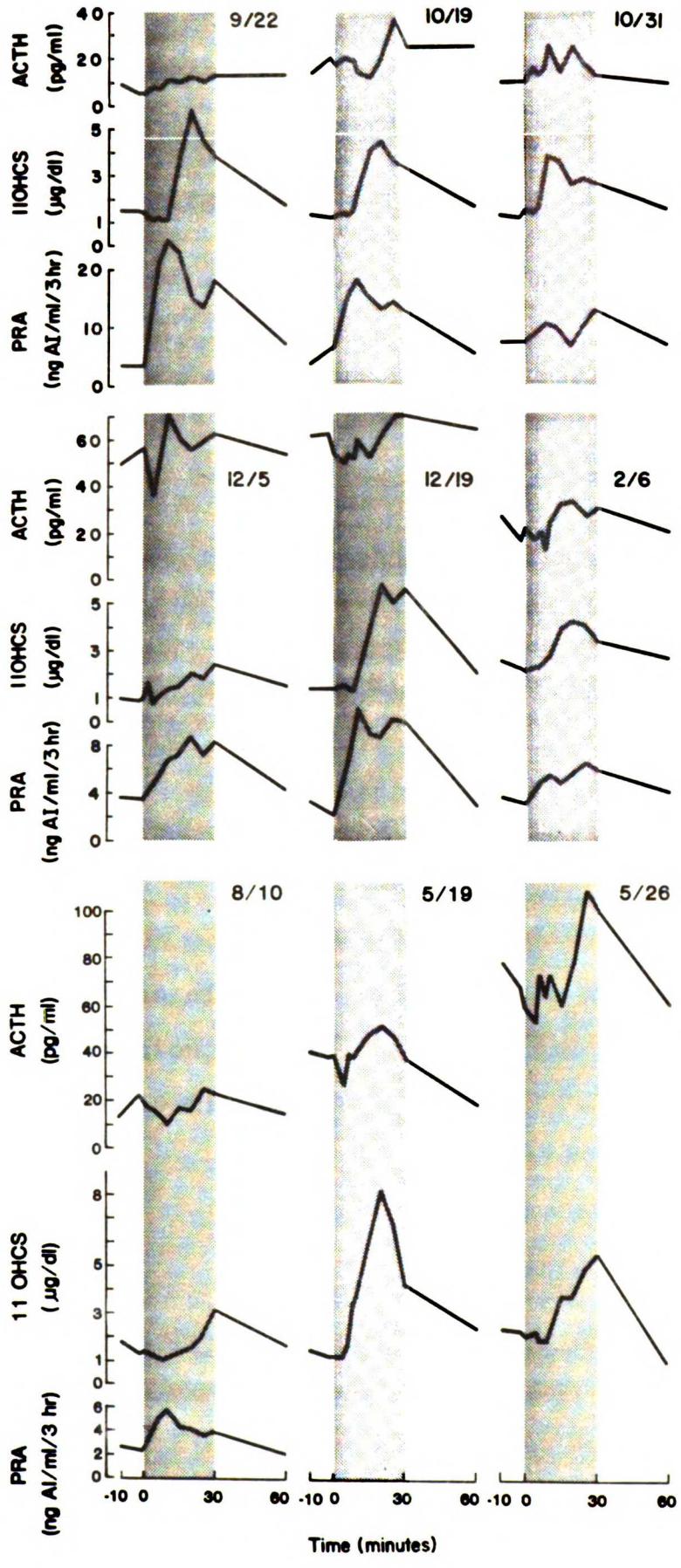

9/22
10/19
10/31
12/5
12/19
2/6
8/10
5/19
5/26
ACTH (pg/ml)
11OHCS (µg/dl)
11 OHCS (µg/dl)
PRA (ng AI/ml/3hr)
PRA (ng AI/ml/3 hr)
-10 0 30 60
Time (minutes)

Figure 8

Correlation of plasma corticosteroid (11OHCS) concentration to plasma renin activity (PRA) measured 10 min earlier.

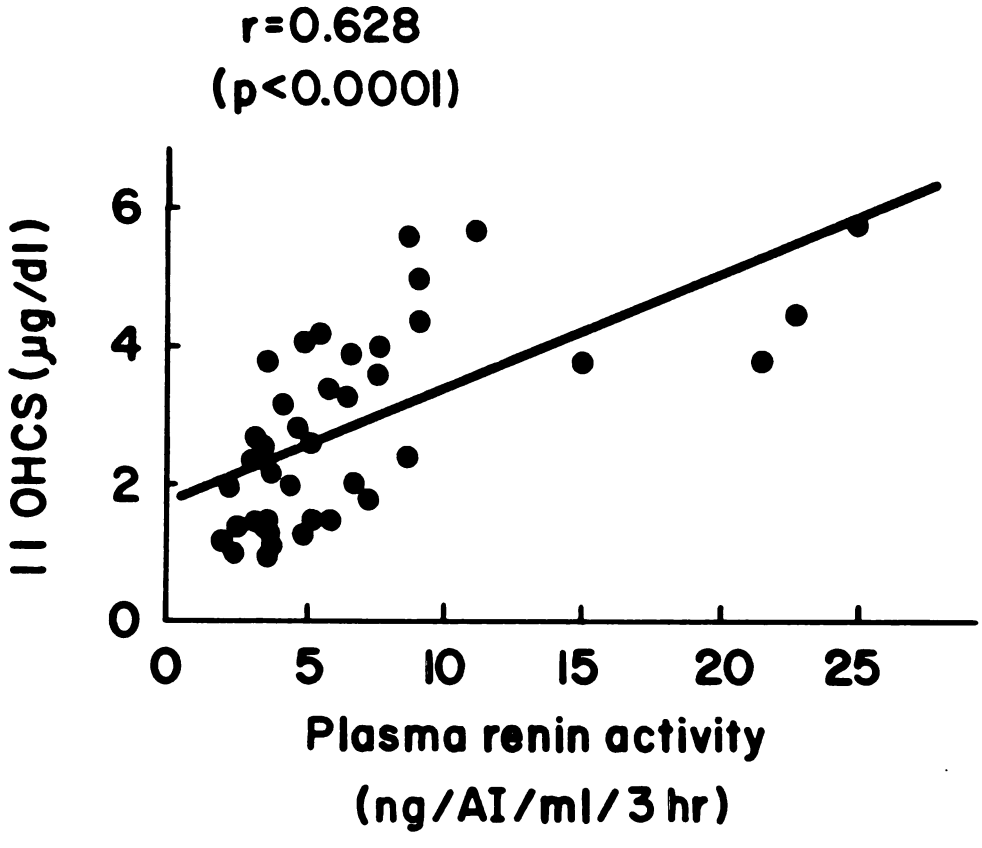
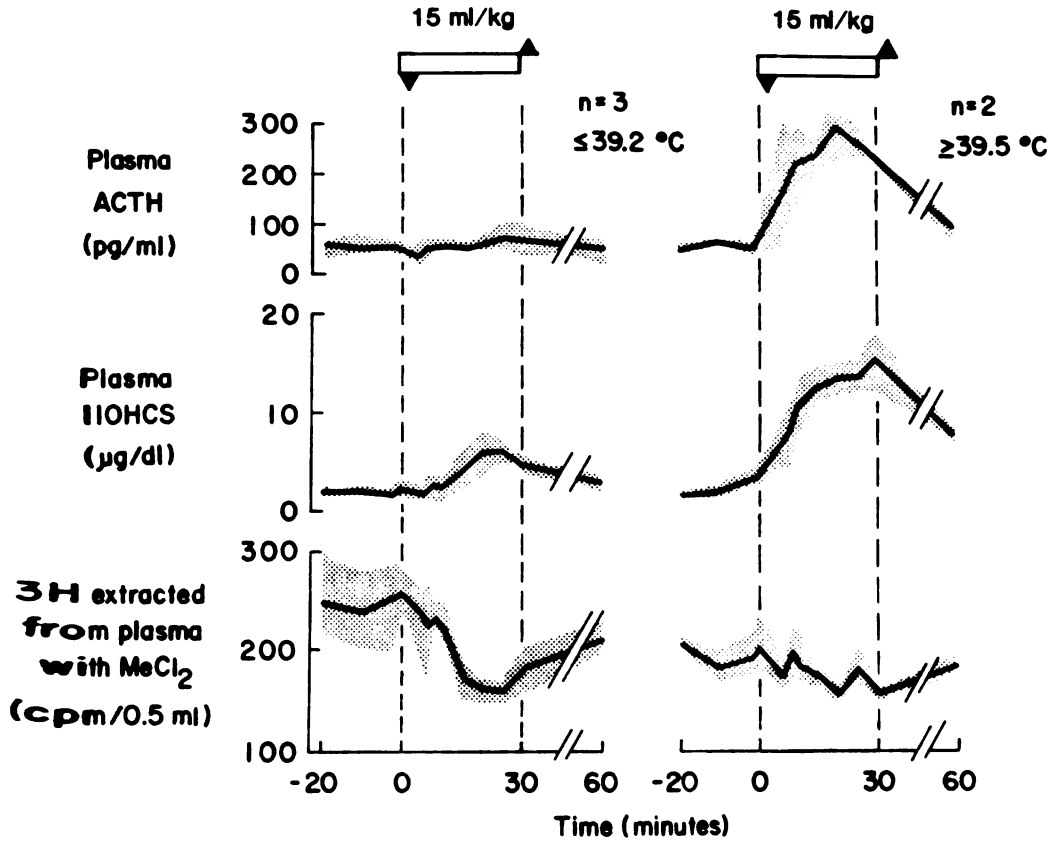

r=0.628
(p<0.0001)
11 OHCS (µg/dl)
0
2
4
6
0
5
10
15
20
25
Plasma renin activity
(ng/AI/ml/3 hr)

Figure 9

Changes in plasma ACTH, corticosteroid levels, and tritium counts extracted from plasma with dichloromethane before, during, and after 30 min of 15 ml/kg hypovolemia in 3 dogs with small ACTH responses (left), and 2 dogs with large ACTH responses (right). 3H-(1,2)-cortisol was infused starting 100 min before hemorrhage. Data are plotted as means (solid lines) accompanied by the range of values (shaded area).


15 ml/kg
15 ml/kg
n=3
≤39.2 °C
n=2
≥39.5 °C
Plasma ACTH (pg/ml)
300
200
100
0
Plasma 11OHCS (µg/dl)
20
10
0
3H extracted from plasma with MeCl2 (cpm/0.5 ml)
300
200
100
-20
0
30
60
-20
0
30
60
Time (minutes)

DISCUSSION

The results of these studies suggest that 15 ml/kg hemorrhage in normothermic conscious dogs causes an increase in peripheral plasma corticosteroid concentration without concomitant increases in plasma ACTH concentration (Figure 7). The results also show that the threshold for the detection of the corticosteroid response to hemorrhage in the conscious dog is between 10 and 15 ml/kg (Figure 6).

Stimulation of adrenal secretion. I chose to measure changes in peripheral plasma corticosteroid concentration rather than the more sensitive and direct measure of adrenal venous corticosteroid concentration for two reasons. First, studies in rats have shown that manipulation of the adrenal pedicle results in neurally mediated changes in the contralateral adrenal (Dallman, et al , 1976), and it seemed possible that the act of "choking" the adrenal vein to collect blood might in itself alter the cortisol secretory rate. Second, increases in arterial blood corticosteroid concentrations are a physiologically meaningful measure. In the absence of a convincing relationship between plasma ACTH and corticosteroid concentrations after 15 ml/kg hemorrhage I determined that the increases in plasma cor-

ticosteroid concentration were attributable to increased adrenal secretion (Figure 9).

The finding of a highly significant decrease in tritium counts and the normal increase in corticosteroid concentration after hemorrhage shows that the increase in peripheral plasma corticosteroid concentration underestimated the increase in adrenal corticosteroid secretion rate. The decrease in dichloromethane-extractable tritium counts during hypovolemia could be explained by 1) an increase in the distribution volume of cortisol, possibly as a result of fluid shifts from the intracellular fluid compartment (Skillman, et al , 1968), or 2) increased utilization of cortisol (Sayers and Sayers, 1947), or 3) an increase in the rate of cortisol clearance. I cannot distinguish among these possibilities with the current data.

In these experiments the threshold volume of hemorrhage for the detection of increases in peripheral plasma ACTH and corticosteroid concentrations is approximately 15 ml/kg. Dogs bled 10 ml/kg (Figure 6, left) and 12-13 ml/kg did not respond with significant increases in ACTH. After 10 ml/kg hemorrhage, the overall mean of arterial plasma corticosteroid concentration increased 1 ug/dl, although the increase is not significant. Nevertheless, it is possible that 10 ml/kg hemorrhage in these experiments increased adrenal

corticosteroid secretion if there is an increase in cortisol clearance or distribution volume of 30%, similar to that measured after 15 ml/kg hemorrhage, and if there is an increase in peripheral corticosteroid levels of 1 ug/dl. Using these assumptions and the data of others for metabolic clearance rate of cortisol (McCormick, et al , 1974), it can be calculated that adrenal venous secretion of cortisol would have increased by 5.7-7.4 ug/min. Gann has reported increased cortisol secretion in the absence of changes in ACTH after 10 ml/kg hemorrhage in one conscious dog (Gann, 1979). In other studies , 10 ml/kg hemorrhage in conscious dogs resulted in a small increase in plasma ACTH concentration that peaked at 30 min with a 4ug/dl increase in peripheral cortisol levels (Engeland, et al, 1979). The adrenal venous corticosteroid secretion rate was increased 8.5 ug/min. The time course of the response resembled that observed in this study. However, this corticosteroid response to 10 ml/kg hemorrhage was greater than the corticosteroid response to 15 ml/kg hemorrhage reported here. The difference might result from the more extensive surgical preparation used by Gann (1979) and Engeland, et al (1979) for catheterization of the adrenal vein.

Apparent dissociation of ACTH and corticosteroids. The results of these experiments suggest that during 10

ml/kg and 15 ml/kg hypovolemia in conscious dogs, increases in adrenal secretion of corticosteroids were not associated with measurable increases in plasma ACTH. This could be explained by 1) inadequacy of the 2 and 5 min sampling intervals used during hypovolemia to characterize changes in plasma ACTH responsible for the stimulation of adrenal secretion, or 2) the possibility that a non-ACTH factor was increasing adrenal sensitivity to ACTH or increasing adrenal secretion by an action independent of ACTH.

The half-disappearance time of ACTH is 1-8 min in man (Wolf, et al, 1965; Besser, et al, 1971), rat (Greensapan, Li, and Evans, 1950; Sydnor and Sayers, 1953), and dog (Urquhart, 1967; Wood, Shinsako, and Dallman, 1980). If large pulses of ACTH are released in the dog, assuming that each pulse is homogeneously distributed in its distribution volume, the 2 min sampling frequency should be adequate to detect fluctuations in plasma ACTH. However, it is possible that small pulses of ACTH were secreted during hemorrhage, small enough to not appreciably raise the plasma ACTH concentration after its distribution, but large enough to raise the plasma ACTH concentration of the arterial blood perfusing the adrenals on its first pass around the circulation. The rate of fall of plasma ACTH concentration after a small, undistributed pulse of ACTH would not be

limited by the half-disappearance time of ACTH. It is conceivable that small pulses of ACTH, released during hypovolemia, stimulate adrenal secretion of corticosteroids. One possible method which could be used to test this hypothesis is to continuously sample at a rate sufficient to allow one ACTH measurement per minute, before and during hypovolemia. Continuous measurement of ACTH in arterial plasma would allow a true measurement of the amount of ACTH reaching the adrenal per unit time (assuming constant adrenal blood flow, as demonstrated by Engeland, et al , 1979). Additionally, the high resolution of measurement should allow us to better define the fluctuations in plasma ACTH with time.

The literature contains several reports of increased adrenal secretion of corticosteroids during hypovolemia in anesthetized dogs (i.e., Farrell, Rosnagle, and Rauschkolb, 1956; Gann, 1969; Gann, 1971; Gann, Gould, Morley, and Mumma, 1964; Hume and Nelson, 1955; Cryer and Gann, 1977). Prior hypophysectomy, without maintaining the adrenals with injections of ACTH prevented this response. However, when hypophysectomized dogs were maintained with injections of ACTH before hemorrhage and during the period of hypovolemia, increased rates of corticosteroid production were demonstrated (Hume and Nelson, 1955). These data therefore indicate that ACTH is neccessary for corticosteroid

secretion during hypovolemia in the anesthetized dog, but also that a second factor (not of pituitary origin, but requiring ACTH in a more passive role) contributes to this response. Our data might demonstrate a similar phenomenon in the conscious dog.

The data (Figure 7) suggest that plasma renin (angiotensin II) might be the second factor. Pharmacologic doses of angiotensin II have been shown to stimulate the output of corticosteroids as well as aldosterone from bovine adrenal slices (Kaplan, 1965; Kaplan and Bartter, 1962; Peytremann, et al , 1973), in the rat in vivo (Dufau and Kliman, 1968), and in anesthetized dogs (Ganong, Mulrow, Boryczka, and Cera, 1962; Bravo, Khosla, and Bumpus, 1975). Espiner and coworkers (Espiner, Lun, and Hart, 1978) used conscious sheep with adrenal glands transplanted to the neck to demonstrate that high doses of angiotensin II infused directly into an adrenal artery could increase adrenal cortical secretion. However, when angiotensin II was infused into conscious beagles in doses ranging from 3 to 24 ng/kg/min, plasma cortisol concentrations were not increased (Nicholls, et al , 1978). To date, therefore, physiologic levels of angiotensin II have not been shown to stimulate the release of cortisol from the adrenal cortex in quantities sufficient to elevate systemic corticosteroid levels. If, in the conscious dog bled 15

ml/kg, increases in plasma renin activity were responsible for increases in plasma corticosteroid concentration, the sensitivity of the adrenal to angiotensin II must have been increased. I think that it is more likely that the stimulus of hemorrhage affected plasma renin activity and corticosteroids through parallel inputs.

Other explanations of the data might be that the radioimmunoassay for ACTH is not accurately measuring biologically active ACTH, or that it is insensitive to (and therefore underestimating) stimulus-induced increments in plasma ACTH. Although the correlations of plasma ACTH with corticosteroids in conscious dogs bled 10 and 15 ml/kg were slight (r^2=.10, p<.05; and r^2=.06, p<.05, respectively), correlation of these two hormones in other experiments using the same assays (r^2=.58; Ramsay, Keil, Sharpe, and Shinsako, 1978) was greater. In dogs bled 10, 12-13, and 15 ml/kg, plasma ACTH changed less than 40 pg/ml (except 5/26, Figure 7). However, in other experiments using the same assay (M. Wood, Shinsako, and Dallman, 1980) conscious dogs subjected to injection of 0.25 units insulin per kilogram body weight increased plasma ACTH to levels greater than 300 pg/ml. Therefore, it appears unlikely that possible discrepancies between biologically active and radioimmunoassayable ACTH were due to a failure of the assay, or that

the assay is insensitive to stimulated levels of plasma ACTH. It is possible, however, that if the adrenals were very sensitive to ACTH during hypovolemia, small increases in plasma ACTH concentration, too small to be detected by the assay, could have been responsible for the corticosteroid response.

The results of these experiments question the concept that adrenal secretion of corticosteroids always reflect the level of ACTH under physiologic conditions. The results demonstrate that plasma ACTH cannot always be inferred by indirect means, and suggest that under certain circumstances ACTH may act permissively to allow other factors to cause corticosteroid secretion.

CHAPTER FIVE

Arterial Plasma Corticosteroid Responses to Exogenous ACTH in Conscious Dogs

ABSTRACT

In experiments reported in Chapter 4, conscious dogs responded to rapid 15 ml/kg hemorrhage with small increases in ACTH (peak increase 11 pg/ml at 25 min) that were apparently unrelated to relatively rapid and large increases in plasma corticosteroids (peak increase 2.7 ug/dl at 20 min). To understand the dynamics and magnitudes of the corticosteroid response to increases in plasma ACTH, plasma concentrations of ACTH and corticosteroids were measured in dogs infused with saline or ACTH. In 13 of 16 experimental periods in which dogs were infused with saline, plasma ACTH was not constant over time. In 9 of these 13 experimental periods, plasma corticosteroid concentrations were not related to plasma ACTH concentrations.

Infusion of 10 ng ACTH/min for 30 min elevated plasma ACTH concentration approximately 15-20 pg/ml and elevated plasma corticosteroid concentration approximately 4-5 ug/dl. Infusion of the same amount of ACTH

in 30 min as 3 short (12 sec) infusions of 100 ng ACTH increased plasma corticosteroid concentration equivalently. Infusion of 150 ng ACTH/min for 30 min elevated plasma ACTH approximately 300 pg/ml and plasma corticosteroids approximately 10 ug/dl. The lag between the first elevation in plasma ACTH and corticosteroid concentrations was 3 min or longer. Overall data from ACTH infusions were used to calculate estimates of ACTH half-disappearance time (1.4-3.8 min), total clearance rate (20.4-47.5 ml/kg/min) and volume of distribution (99-113 ml/kg).

I conclude that: 1) the adrenal glands of dogs are exquisitely sensitive to small increments in plasma ACTH concentration; 2) the magnitude of the adrenal response to infused ACTH is determined by the total dose of ACTH, rather that the pattern of administration; and 3) the lag between elevated arterial plasma ACTH and corticosteroid concentrations is at least 3 min.

INTRODUCTION

Conscious dogs bled 15 ml/kg within 3 min increased arterial plasma ACTH and corticosteroid concentrations 11 pg/ml and 2.7 ug/dl, respectively (Chapter 4). The

increases in plasma corticosteroids were not consistently preceeded by measurable increases in plasma ACTH. To better understand the dynamics and magnitudes of the arterial plasma corticosteroid responses to elevated arterial plasma ACTH concentration, plasma ACTH and corticosteroid concentrations were measured before, during, and after infusions of ACTH into conscious dogs. The results of these experiments demonstrate that endogenous plasma ACTH concentration is not constant, and that the adrenals of conscious dogs are normally quite sensitive to small elevations in mean plasma ACTH concentration, and that a finite time lag of not less than 3 min, but not greater than 5 min, separates the first rise in plasma ACTH concentration from the resultant rise in plasma corticosteroids. Some of these data have been presented in abstract form (Wood, Shinsako, and Dallman, 1980a).

MATERIALS AND METHODS

Seven dogs, 14-25 kg, were housed in individual cages and allowed free access to food and water. Each dog was accustomed to standing in a loose cloth sling (Alice King Chatham Medical Arts, Inc.). At least 2 days before the first experiment, dogs were sedated with

Acepromazine (Ayerst, Inc., Ø.5 mg/kg, sc) and anesthetized with sodium pentobarbital (15 mg/kg, iv). Under sterile conditions, a polyvinylchloride catheter (Tygon R-36Ø3, .Ø94"ID, .156"OD; or, Tygon S-54-HL, .Ø5Ø"ID, .Ø9Ø"OD) was inserted into a femoral or brachial artery of each dog. Details of the surgical procedure and maintenance of the catheters are described in Chapter 3. All dogs were studied while conscious.

Protocols. All experiments were started at approximately 11ØØ h, to minimize between-dog differences due to possible circadian variation in adrenal responsiveness to ACTH (Engeland, et al, 1977). Dogs were weighed and placed in the sling between Ø93Ø and 1ØØØ h, and a superficial leg vein was catheterized with an Intracath (Deseret Pharmaceutical Co., 17g needle and 19g catheter). The venipuncture was completed at least 45 min before the start of the experiment. Venous catheters were used for administration of ACTH.

Each experiment consisted of one or two 32 min periods of study. During the first 7 min of each experimental period, one 2.5 ml arterial blood sample was taken every 1 min. During the remaining 25 min, one 2.5 ml blood sample was taken every 2.5 min. During each 32 min period, therefore, 18 blood samples (totalling 47 ml of blood) were drawn. Within 45 min after the end of each period, the red blood cells from blood samples were

resuspended in sterile, pyrogen-free normal saline and returned to the dog through the venous catheter.

ACTH used in infusions was synthetic al-24 ACTH (Cortrosyn, Organon, Inc.).

1) Eight saline infusion experiments were performed on the seven dogs. Each experiment consisted of two 32 min periods of saline administration and blood sampling, the first period starting at 1100 h and the second period starting at 1300 h. During one of these periods, the dog received one constant saline infusion (0.76 ml/min for 30 min) and during the other period, the dog received three short saline infusions (38.2 ml/min for 12 sec each) beginning 2, 12, and 22 min after the first blood sample was drawn. In 5 experiments, 5 dogs received the 3 short saline infusions in the first period and the constant infusion in the second period. In 3 experiments in 3 dogs, the order was reversed.

2) Eight 300 ng/30 min ACTH infusion experiments were performed on four dogs. Each experiment consisted of two 32 min periods of ACTH administration and blood sampling, the first period starting at 1100 h and the second period starting at 1300 h. During one of these periods, the dog received a constant infusion of ACTH in saline (10 ng/min, 0.76 ml/min for 30 min) and

during the other period, the dog received three short infusions of ACTH in saline (100 ng per short infusion of 38.2 ml/min for 12 sec) at 2, 12, and 22 min after the start of the period. In 4 experiments on 4 dogs, the constant infusion of ACTH was administered in the first period, and the 3 short infusions of ACTH were administered in the second period. In 4 more experiments on the same 4 dogs, the protocol was reversed. Two dogs were subjected to the constant infusion in the first period of the first experiment and the 3 short ACTH infusions in the first period of the second experiment. In the other 2 dogs, the order was reversed.

3) Three 900 ng/30 min ACTH infusion experiments were performed on 3 dogs. As above, each experiment consisted of two 32 min experimental periods. During one period, the dog received a constant infusion of ACTH in saline (30 ng/min, 0.76 ml/min for 30 min) and during the other period, the dog received three short infusions of ACTH in saline (300 ng per short infusion of 38.2 ml/min for 12 sec). In 2 experiments, 2 dogs received the short infusions in the first period; in 1 experiment, 1 dog received the constant infusion in the first period.

4) Five 4500 ng/30 min (150 ng/min) ACTH infusion experiments were performed in five dogs. Each experiment consisted of one 32 min period of ACTH

infusion and blood sampling starting at 1100 h. Two minutes after the first blood sample was taken, the infusion was started (150 ng ACTH/min, 0.76 ml/min for 30 min).

Hormone analysis. Blood samples were placed on ice, centrifuged at 4 C and plasma was assayed for ACTH and corticosteroids. Plasma ACTH was measured by radioimmunoassay (Rees, et al , 1971; Sato, et al , 1975; Dallman, DeManicor, and Shinsako, 1974; Chapters 3 and 4). The 18 blood samples from each experimental period were run in a single extraction and incubation. The intraassay coefficient of variation of the ACTH radioimmunoassay was measured by running in one assay 18 samples of a plasma pool (1.0 ml each). The ACTH concentration of the plasma pool was 44 $\pm$ 6 (SD) pg ACTH per sample. Thus, at these levels, the intraassay coefficient of variation of the ACTH assay was 14%. Over a period of 3 months, the interassay coefficient of variation was 19% for a plasma pool that contained an average of 78 pg ACTH/ml (N=46 extractions and assays). Plasma corticosteroid concentration was measured using a competitive protein binding assay (Murphy, 1967), employing human plasma as the source of transcortin.

Statistical Analyses. Data were analyzed using one and two way analysis of variance for repeated measures, and the Lowentin "F" test (Winer, 1971). Correlation between

variables was tested with linear regression analysis.

RESULTS

Fluctutation of plasma ACTH concentration in saline-treated dogs. In 13 of 16 experimental periods in which 7 dogs were not treated with exogenous ACTH (Figure 10), plasma ACTH concentration was not constant over time ($p<.05$ by Lowentin "F" test). The coefficents of variation of plasma ACTH concentration in these 13 experimental periods ranged from 27% to 53% (Table 3), and were significantly greater than the intraassay coefficient of variation of the ACTH radioimmunoassay (14%, see METHODS). In 4 experimental periods, changes in plasma corticosteroid concentration were significantly ($p<.05$ by linear regression) related to changes in plasma ACTH concentration (Table 3). In 9 of 13 experimental periods in which plasma ACTH was not constant, plasma corticosteroids did not follow endogenous changes in plasma ACTH concentration. Over all 16 experimental periods (Figure 11), there was no change in mean plasma corticosteroid concentrations ($p=NS$ by ANOVA), although mean plasma ACTH concentration decreased slightly ($p<.05$ by ANOVA).

TABLE 3. ACTH and 11OHCS in Saline-Treated Dogs.

FIRST EXPERIMENTAL PERIOD: 1100-1130h				
	Plasma ACTH		ACTH vs 11OHCS	
Dog	C.V.(%)	p	r^2	p
Sally	30	<.005	.114	NS
Booboo	28	<.005	.017	NS
Linus	32	<.001	.002	NS
Booboo	39	<.001	.142	NS
Homer	15	NS	.001	NS
Piglet	27	<.005	.002	NS
Plain Jane	32	<.005	.216	NS
Piglet	35	<.001	.335	<.05

SECOND EXPERIMENTAL PERIOD: 1300-1330h				
	Plasma ACTH		ACTH vs 11OHCS	
Dog	C.V.(%)	p	r	p
Sally	31	<.001	.336	<.05
Booboo	31	<.001	.013	NS
Linus	15	NS	.001	NS
Booboo	53	<.001	.017	NS
Homer	29	<.001	.591	<.001
Piglet	16	NS	.001	NS
Plain Jane	49	<.001	.007	NS
Piglet	33	<.001	.513	<.001

Adrenal responses to constant infusions of ACTH. In 8 experimental periods in 4 dogs, infusions of 10 ng ACTH/min (Figure 12) elevated plasma ACTH concentration from a mean control of 27 pg/ml to between 40 and 50 pg/ml. Plasma corticosteroids were elevated from 1.5 ug/dl to between 4.8 and 5.8 ug/dl.

In 3 experimental periods in 3 dogs, infusions of 30 ng ACTH/min (Figure 13) elevated plasma ACTH approximately 30 pg/ml while increasing plasma corticosteroids approximately 5-6 ug/dl.

In 5 experimental periods in 5 dogs, infusions of 150 ng/min ACTH (Figure 14) elevated plasma ACTH concentration approximately 300 pg/ml, increasing plasma corticosteroids approximately 10 ug/dl.

Plateau concentrations of ACTH and corticosteroids were calculated as the mean of 7 measurements from 15 to 30 min after the start of 10, 30, and 150 ng/min infusions. Overall, there was a significant linear relationship between plateau plasma ACTH and plateau plasma corticosteroids (r^2=.56, p<.001; Figure 15).

Adrenal responses to 3 short infusions of ACTH. Short (12 sec) infusions of 100 ng (8 experimental periods in 4 dogs; Figure 16) or 300 ng (3 experimental periods in 3 dogs; Figure 17) ACTH, one per 10 min over 30 min, significantly (p<.001 by ANOVA) elevated both plasma ACTH and corticosteroid concentrations. After the first short infusion of ACTH, arterial blood samples were taken every min, allowing characterization of the falling phase of plasma ACTH concentration after the infusion was turned off. During the second and third short infusions of ACTH, blood samples were taken every 2.5 min. One min after the first short infusion of 100 ng ACTH, plasma ACTH was increased an average of 37 pg/ml and 2.5 min after the second and third short infusions, plasma ACTH was increased 20 and 19 pg/ml, respectively (Figure 16). One min after the first short infusion of

300 ng ACTH, plasma ACTH was increased an average of 93 pg/ml, and 2.5 min after the second and third short infusions, plasma ACTH was increased 36 and 45 pg/ml, respectively (Figure 17).

In all short infusion experiments, the first elevated corticosteroid value was 3 min after the first pulse of ACTH. Overall, the first short infusion of 100 ng ACTH elevated corticosteroids from 1.8 ug/dl to 5.5 ug/dl, the second short infusion to 6.3 ug/dl, and the third short infusion to 6.6 ug/dl (Figure 16). After each peak the plasma corticosteroid concentration fell until at least 3 min after the next ACTH infusion. The first short infusion of 300 ng ACTH elevated plasma corticosteroids from 1.4 to 6 ug/dl, the second infusion to 6.4 ug/dl, and the third infusion to 7 ug/dl (Figure 17). The plasma corticosteroid response to 30 min of 10 ng/min infusion, measured as the area under the corticosteroid response curve above control, was not different than the corticosteroid response to 3 pulses of 100 ng ACTH in 30 min (81.8 $\pm$ 16.0 vs. 99.2 $\pm$ 21.0 ug*min/dl, p=NS by paired "t" test). Similarly, the plasma corticosteroid response to 30 min of 30 ng/min infusion was not different than the corticosteroid response to 3 pulses of 300 ng ACTH in 30 min (101 $\pm$ 27 vs. 97 $\pm$ 33 ug*min/dl, p=NS by paired "t" test).

Time lag between increased ACTH and increased corticosteroids. The lag between elevations in arterial plasma concentrations of ACTH and corticosteroids was 3 or more minutes. Plasma corticosteroid concentration was always elevated 3 min after the beginning of the first short infusion of 100 or 300 ng ACTH; corticosteroids began to rise in the second blood sample (5 min) after the second and third short infusions of ACTH. Similarly, corticosteroids began to rise 3 min after the beginning of the 150 ng/min infusions. During the 10 and 30 ng/min infusions, the lag between elevations in ACTH and corticosteroids was 3-5.5 min. The first rise in ACTH was measurable 2 min after the onset of infusion, and the first significantly elevated corticosteroid concentration was measured 7.5 min after the onset of infusion, although corticosteroids apparently began to rise in the 5 min sample. In none of the experiments was the lag between ACTH and corticosteroid response less than 3 min.

ACTH distribution and metabolism. ACTH half-disappearance time ($T_{1/2}$), volume of distribution V_d, and total clearance rate (TCR) were estimated by calculating nonlinear fits of single exponential equations to measured ACTH values during and after infusions of ACTH (Figure 18). Mean ACTH values for 7 plasma samples drawn 1-10 min after the first 100 or 300 ng short infu-

sion were used to estimate parameters B_1, B_2, B_3 in the single exponential equation (1)

$$[A] = B_1 e^{-B_2 t} + B_3$$

where "A" is the plasma ACTH concentration at time "t". Mean ACTH values for 7 plasma samples drawn 0-7.5 min after the onset of 10 and 150 ng/min infusions (8 experiments on 4 dogs, and 5 experiments on 5 dogs, respectively) were used to estimate parameters B_1,B_2,B_3 in the single exponential equation (2)

$$[A] = B_1(1-e^{-B_2 t}) + B_3$$

For both equations, the best-fit half disappearance time was calculated as

$$\frac{\ln(2)}{B_2}$$

From equation (1), the volume of distribution was calculated as

$$V_d = \frac{I}{B_1}$$

and the total clearance rate was calculated

$$TCR = \left(\frac{B_1}{B_2}\right) * I$$

where I = total injected pg ACTH. From equation (2), the total clearance rate was calculated as

$$TCR = \frac{R}{B_1}$$

where "R" is the infusion rate. The volume of distribution of ACTH was calculated as

$$V_d = \frac{TCR}{B_2}$$

Half-disappearance times, volumes of distribution, and clearance rates of ACTH calculated from data obtained from the 3 types of experiments are shown in Table 4.

TABLE 4. Distribution and Metabolism of ACTH.

Infusion	Half Life (min)	TCR (ml/kg/min)	V_d (ml/kg)
10 ng/min	3.8	20.4	113
100 ng/12 sec	1.8	40.1	104
300 ng/12 sec	1.4	47.5	99
150 ng/min	2.5	31.1	112
Mean(SEM)	2.4(0.5)	34.8(5.9)	107(3)

Figure 10

Arterial plasma ACTH (filled circles) and corticosteroids (11OHCS; open circles) concentrations in 4 individual experimental periods in 4 dogs infused with saline. The coefficients of variation of plasma ACTH concentration in panels A, C, and D were significantly greater than the intraassay coefficient of variation. In none of the panels are plasma corticosteroid concentrations related plasma ACTH concentrations (p=NS by linear regression analysis).

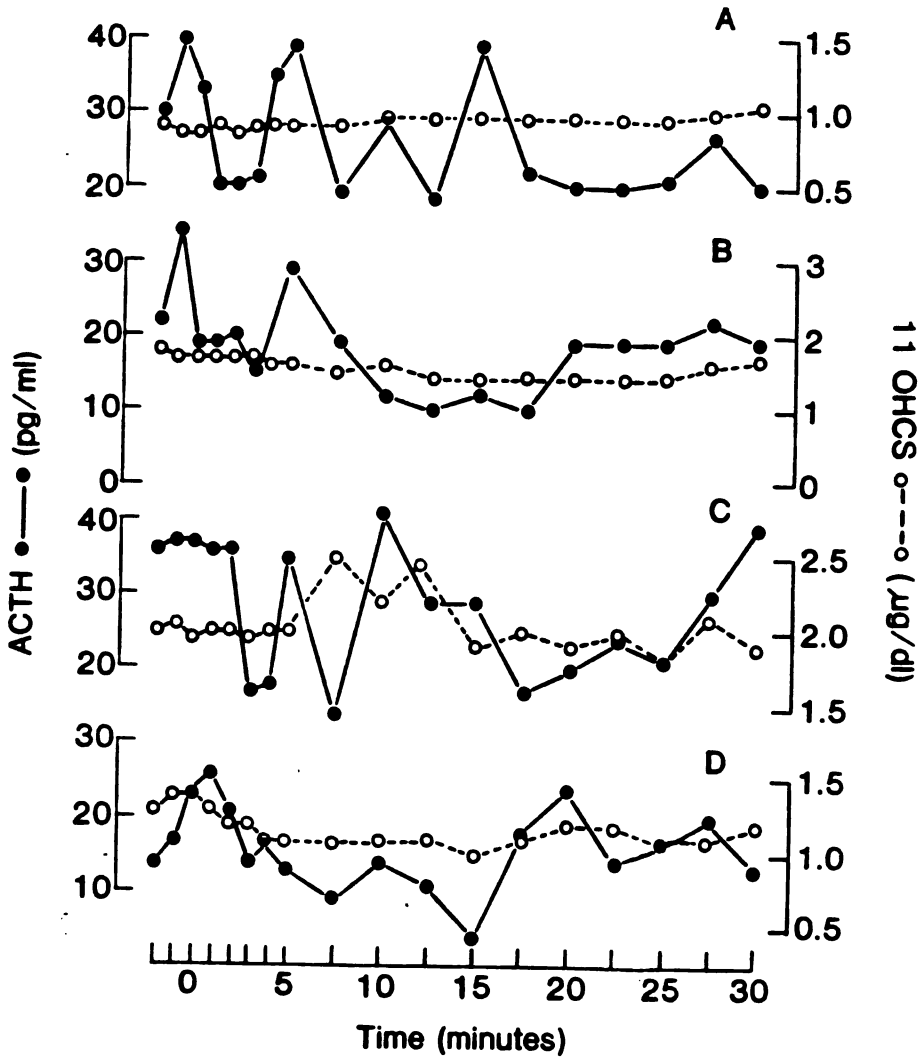

A
B
C
D
40
30
20
1.5
1.0
0.5
30
20
10
0
3
2
1
0
40
30
20
2.5
2.0
1.5
30
20
10
1.5
1.0
0.5
0
5
10
15
20
25
30
Time (minutes)
ACTH ●—● (pg/ml)
11 OHCS ○---○ (μg/dl)

Figure 11

Mean arterial plasma ACTH and corticosteroid concentrations in 16 experimental periods in 7 dogs infused with saline. Means are represented as filled (ACTH) or open (11OHCS) circles, and the standard error of the mean is represented as vertical lines.

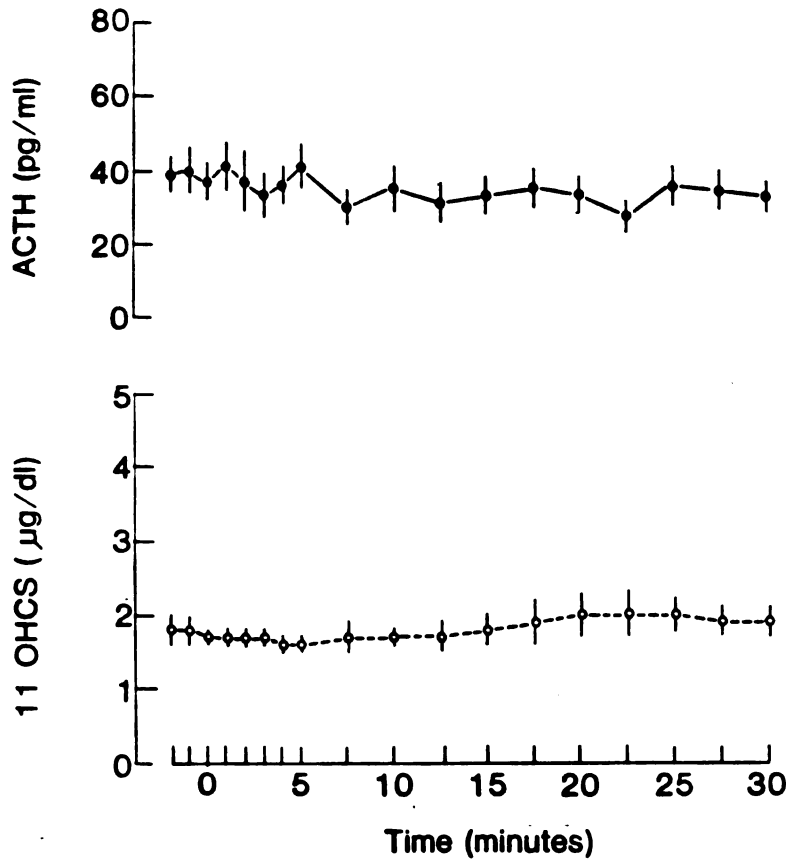

ACTH (pg/ml)
80
60
40
20
0
11 OHCS (µg/dl)
5
4
3
2
1
0
0
5
10
15
20
25
30
Time (minutes)

Figure 12

Mean arterial plasma ACTH and corticosteroid concentrations in 8 experimental periods in 4 dogs infused with 10 ng ACTH/min from 0 to 30 min. Symbols and abbreviations as in Figure 11.

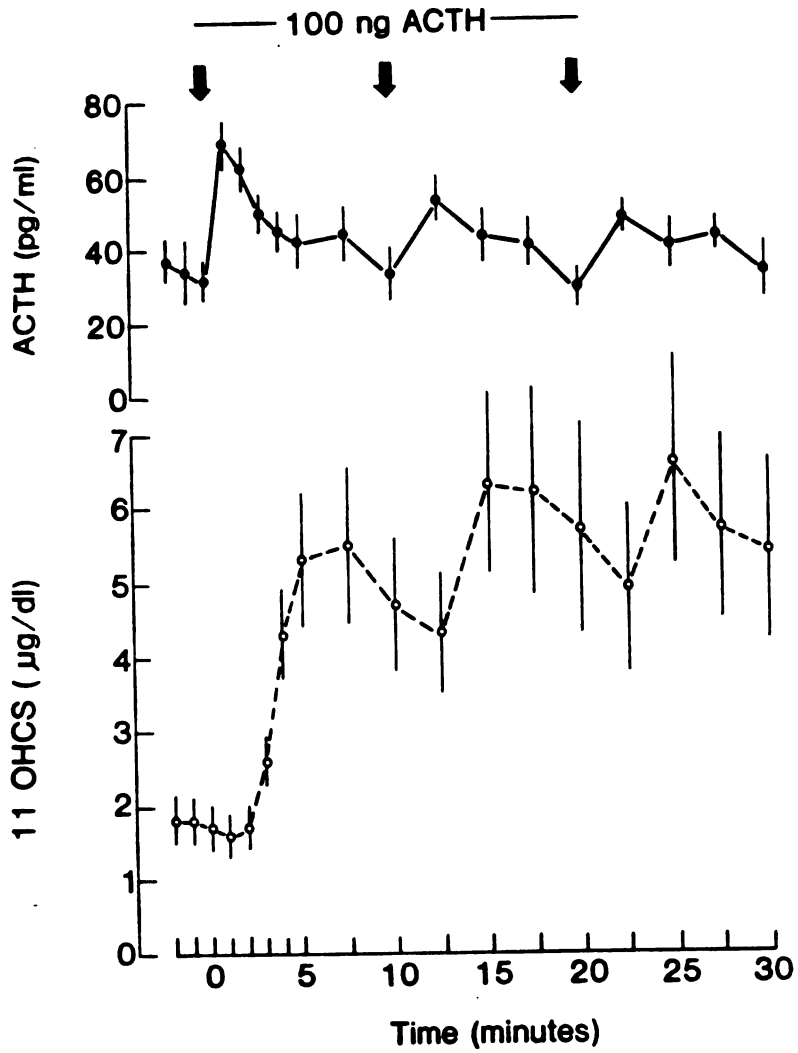
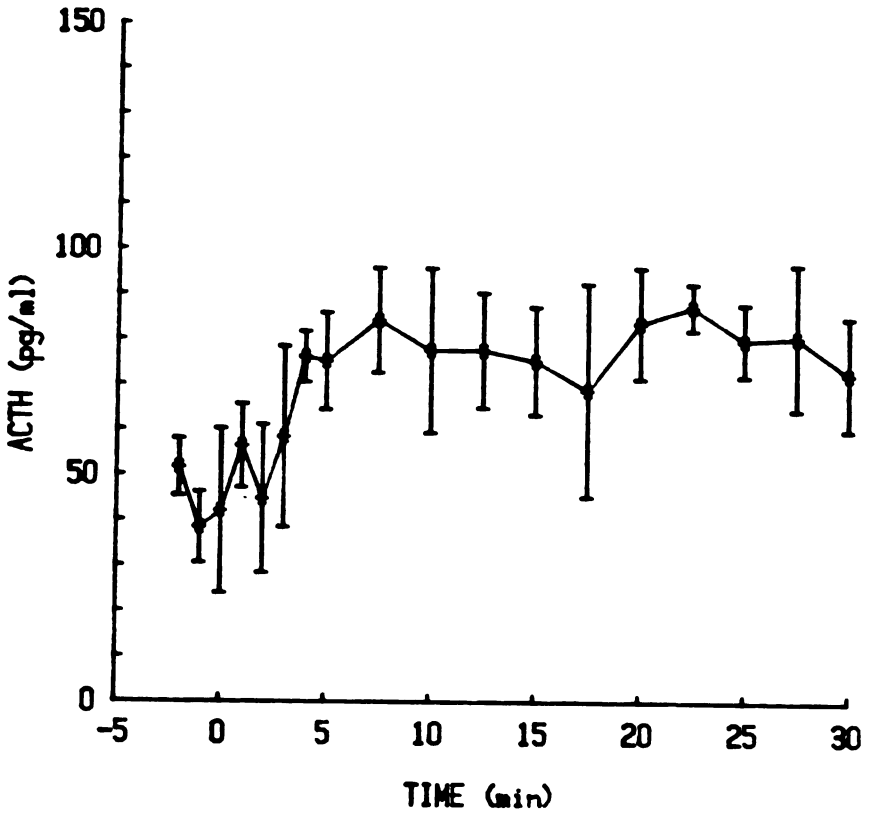

100 ng ACTH
ACTH (pg/ml)
80
60
40
20
0
11 OHCS (µg/dl)
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
Time (minutes)

Figure 17

Mean arterial plasma ACTH and corticosteroid concentrations in 3 experimental periods in 3 dogs given 3 short (12 sec) infusions of 300 ng ACTH at 0, 10, and 20 min. Symbols and abbreviations are as in Figure 11.


150
100
50
0
ACTH (pg/ml)
-5
0
5
10
15
20
25
30
TIME (min)

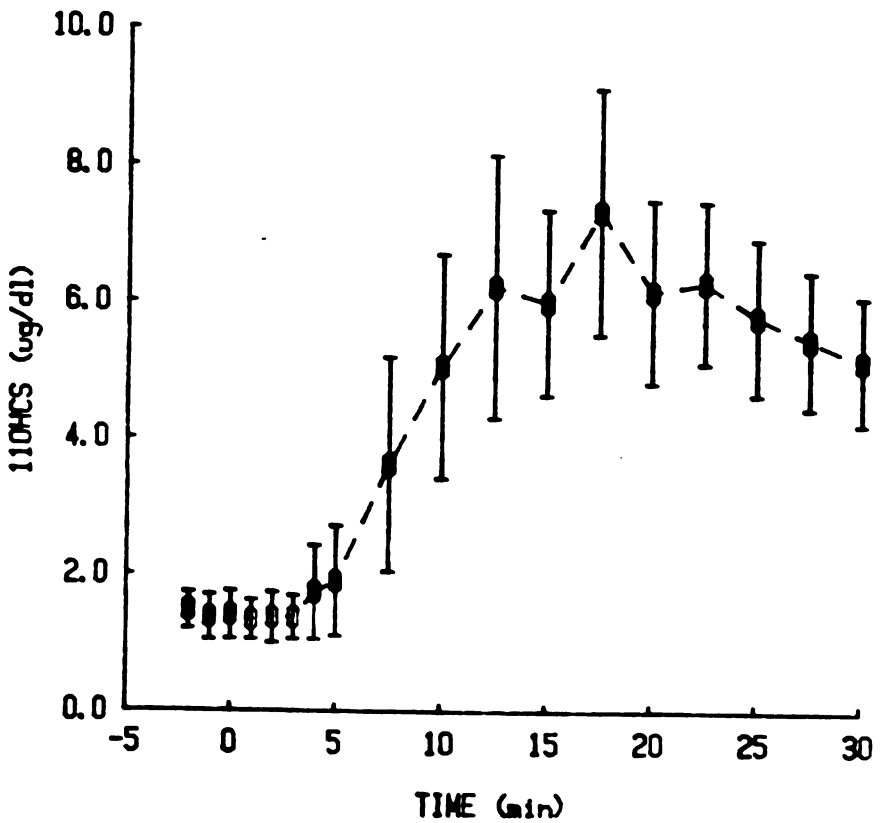

10.0
8.0
6.0
4.0
2.0
0.0
11DHCS (ug/dl)
-5
0
5
10
15
20
25
30
TIME (min)

Figure 14

Mean arterial plasma ACTH and corticosteroid concentrations in 5 experimental periods in 5 dogs infused with 150 ng ACTH/min from 0 to 30 min. Symbols and abbreviations as in Figure 11.

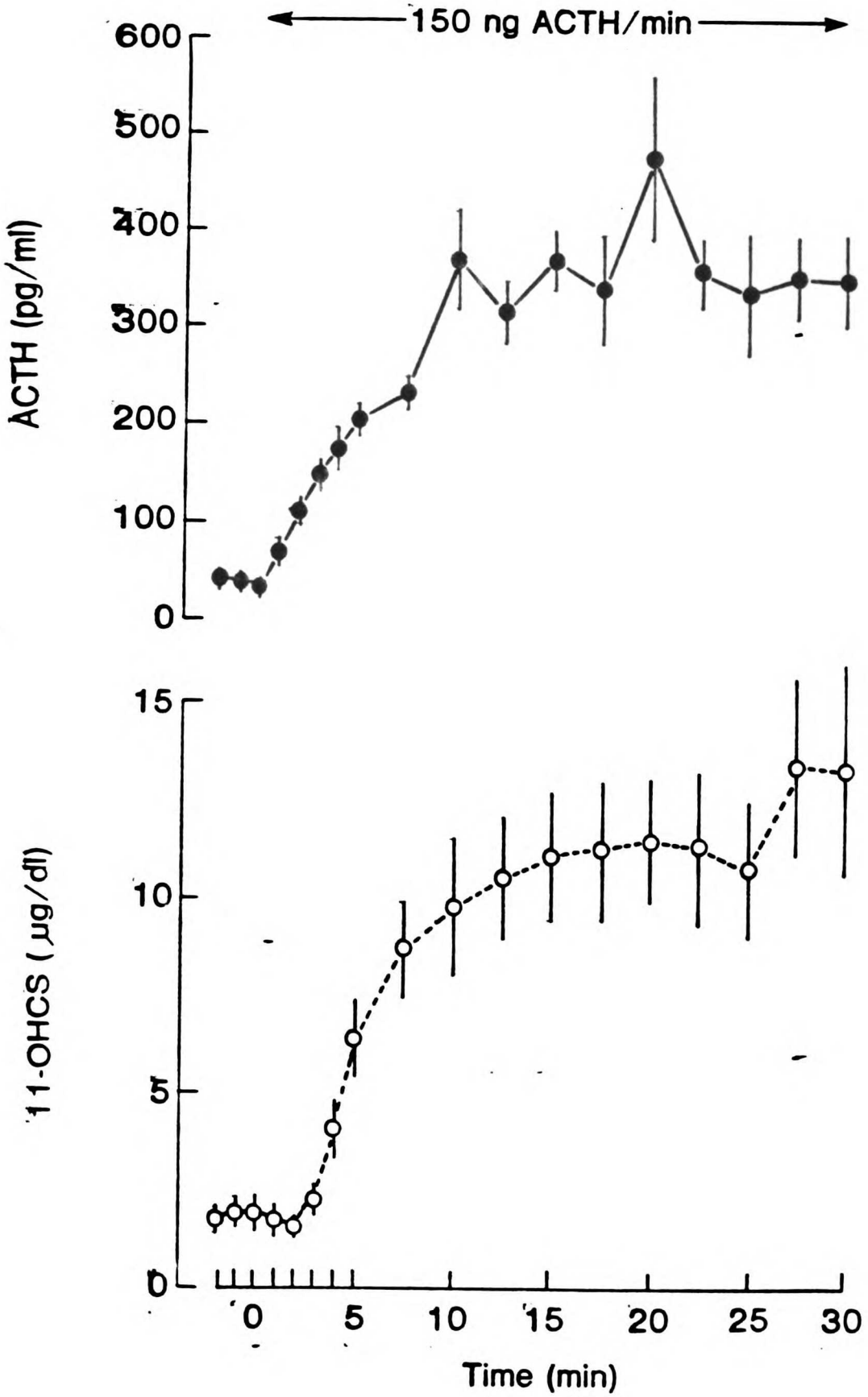
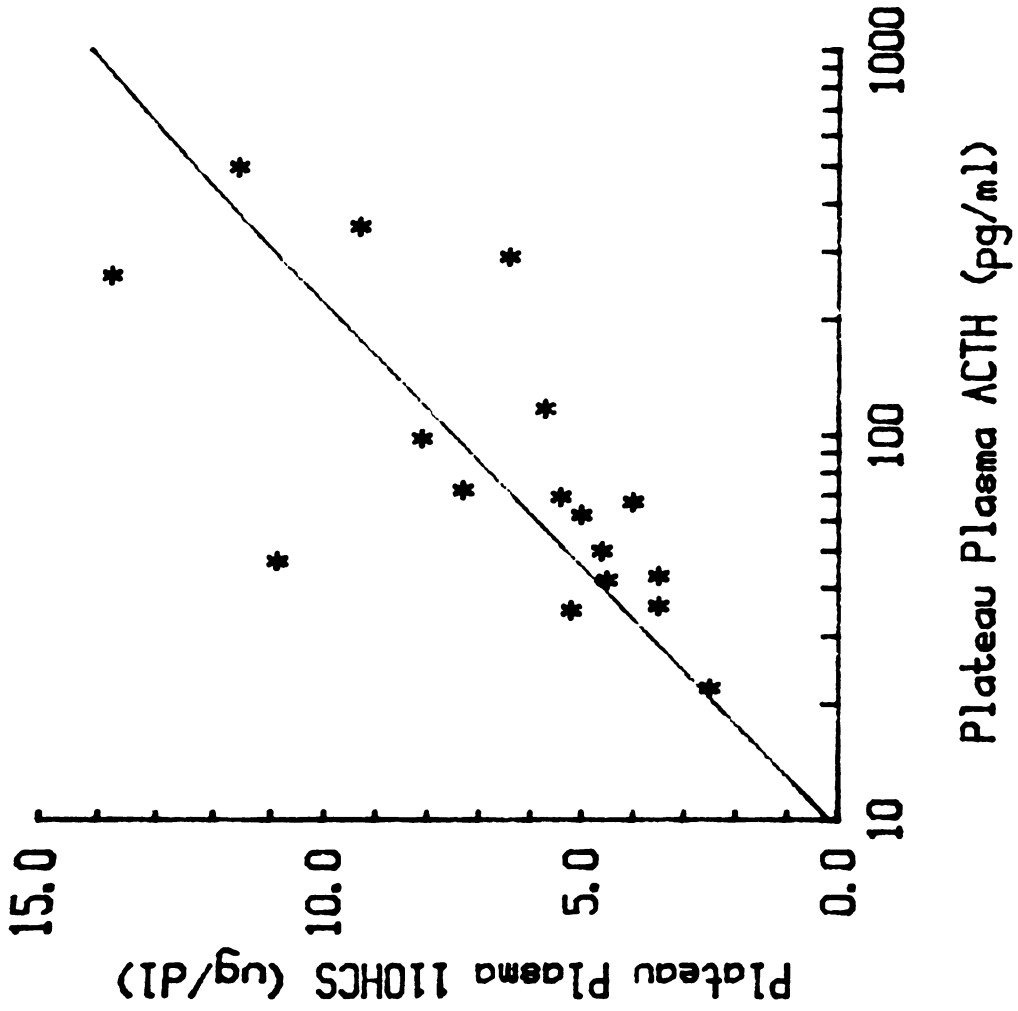
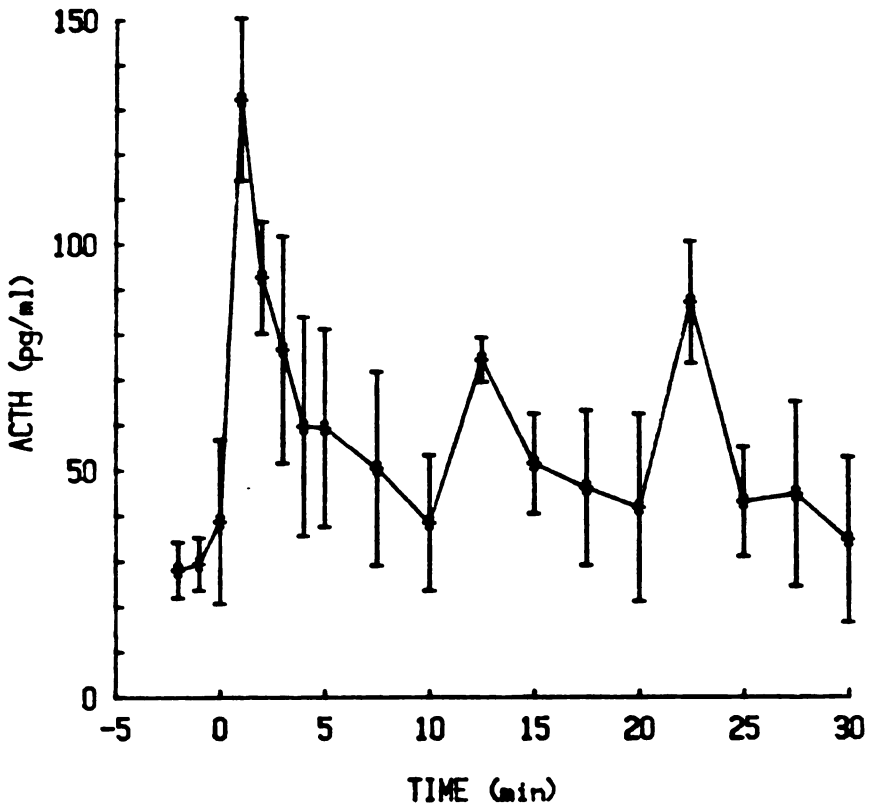

150 ng ACTH/min
ACTH (pg/ml)
600
500
400
300
200
100
0
11-OHCS (µg/dl)
15
10
5
0
0
5
10
15
20
25
30
Time (min)

Figure 15

Correlation of mean plateau ACTH and mean plateau corticosteroid (11ØHCS) concentrations during 1Ø, 3Ø, and 15Ø ng/min infusions of ACTH (r^2=.56, p<.ØØ1). Plateau concentrations were calculated as the mean of 7 ACTH or corticosteroid concentrations from 15 to 3Ø min after the start of the infusion.


Plateau Plasma 11OHCS (ug/dl)
15.0
10.0
5.0
0.0
10
100
1000
Plateau Plasma ACTH (pg/ml)


150
100
50
0
ACTH (pg/ml)
-5
0
5
10
15
20
25
30
TIME (min)

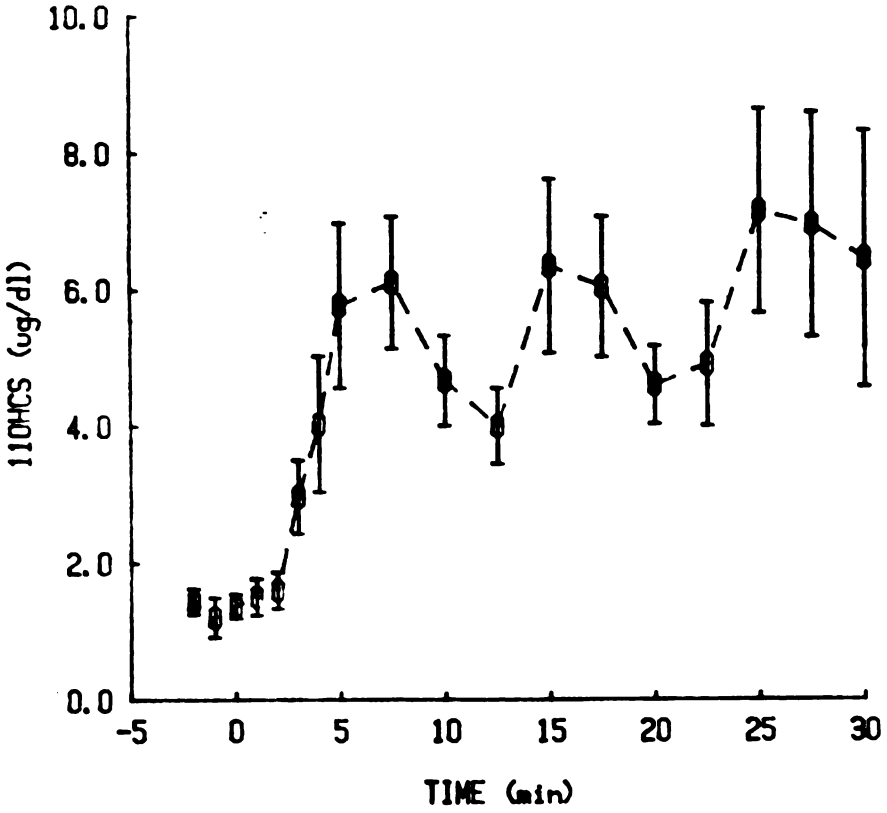
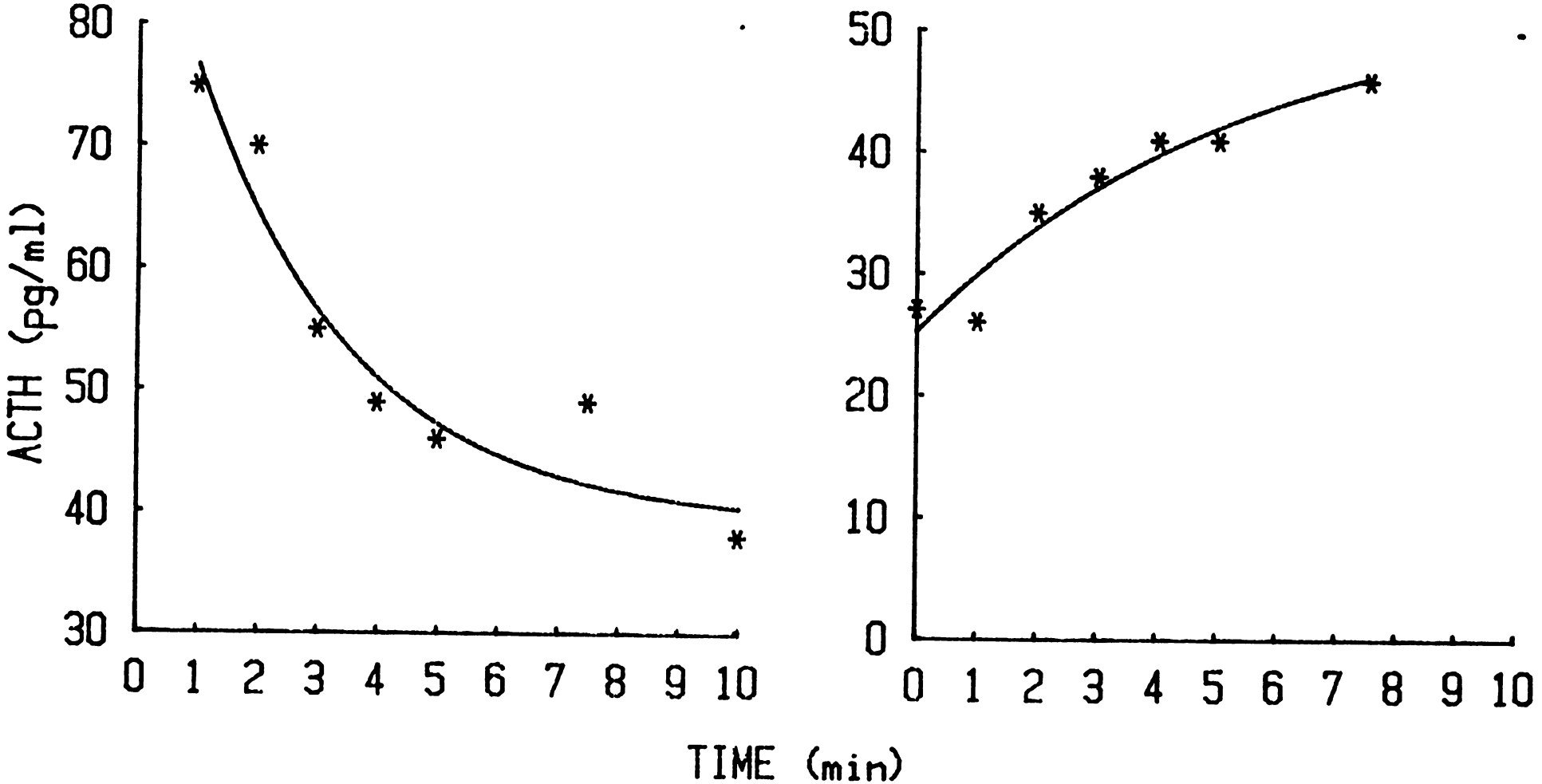

10.0
8.0
6.0
4.0
2.0
0.0
11DHCS (ug/dl)
-5
0
5
10
15
20
25
30
TIME (min)

Figure 18

Nonlinear fits of single exponential functions to mean plasma ACTH concentrations after the first 100 ng pulse of ACTH in 8 experimental periods (left), and during 10 ng/min infusion of ACTH in 8 experimental periods (right).


80
70
60
50
40
30
ACTH (pg/ml)
0 1 2 3 4 5 6 7 8 9 10
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
TIME (min)

DISCUSSION

The results of these experiments demonstrate that 1) in conscious dogs, plasma ACTH concentration fluctuates (probably reflecting pulsatile secretion), 2) the adrenals of conscious dogs are exquisitely sensitive to small increments in plasma ACTH concentration, 3) the magnitude of the adrenal corticosteroid response to ACTH appears to be related to the total dose of ACTH, rather than the minute-to-minute plasma ACTH concentration, 4) there is a finite lag of 3 min between a rise in arterial plasma ACTH concentration and the induced rise in arterial plasma corticosteroid concentration, and 5) the half-disappearance time of ACTH is less than 5 min and the distribution volume of ACTH is approximately equal to blood volume in dogs.

Endogenous fluctuations in plasma ACTH concentration. Results of experiments presented in Figure 1Ø and Table 3 demonstrate that in dogs, as in human beings (Berson and Yalow, 1968; Krieger, Allen, Rizzo, and Krieger, 1971; Krieger and Allen, 1975), plasma ACTH concentration is not constant. It is an apparent paradox that endogenous plasma ACTH can vary by as much as 2Ø pg/ml without concomitant changes in plasma corticosteroids (Figure 1Ø and Table 3), but that longer term

changes in plasma ACTH by as little as 15 pg/ml (Figure 12) can more than triple plasma corticosteroid concentration. One possible explanation is that not all the measured immunoreactive ACTH is biologically active. Fluctuating plasma ACTH concentration with (Berson and Yalow, 1968; Krieger, Allen, Rizzo, and Krieger, 1971) and without (Krieger and Allen, 1975) changes in plasma corticosteroids has been reported in human subjects. Krieger and Allen (1975) found that changes in radioimmunoassayable plasma ACTH concentration were significantly correlated to changes in bioassayable plasma ACTH concentration. By analogy, it seems possible that the same relationship is true in my experiments.

It is, in fact, likely that the rate of corticosteroid secretion directly follows the changes in endogenous plasma ACTH. I have demonstrated that 3 short infusions of 100 or 300 ng ACTH, one every 10 min for 30 min (Figures 16 and 17) are as effective in raising plasma corticosteroid concentration as one 30 min infusion of 10 or 30 ng ACTH/min (Figures 12 and 13). Equal quantities of ACTH administered in 2 different patterns yielded equivalent corticosteroid responses, suggesting that the total amount of ACTH reaching the adrenal over time ("adrenal presentation rate" as suggested by Urquhart, 1965), rather than the rate of change or level of plasma ACTH at any one time deter-

mines the magnitude of the corticosteroid response. The endogenous fluctuations in plasma ACTH probably stimulate short duration increases in plasma corticosteroid secretion. Collectively, the total amount of corticosteroid secreted over time would be the amount that, when averaged, maintain a plasma corticosteroid concentration of 1-2 ug/dl (Figure 11). The endogenous "pulses" of secreted corticosteroid become mixed into the blood volume, and because of this mixing, the femoral arterial plasma corticosteroid concentration is constant. This hypothesis is supported by the observations of Hume (1955) and Miller and coworkers (1976) that adrenal venous corticosteroid concentration is not constant over time. In fact, Hume (1955) speculated that these endogenous peaks of corticosteroid secretion might reflect a fluctuating endogenous plasma ACTH concentration.

Arterial plasma corticosteroid responses to infused ACTH. My objectives in choosing the 150 ng/min infusion rate were 1) to elevate plasma ACTH concentration to high (but not supraphysiologic) levels, and 2) to define a maximal or near-maximal arterial plasma corticosteroid response to elevated plasma ACTH concentration. The infusion elevated plasma ACTH approximately 300 pg/ml, and plasma corticosteroids approximately 10 ug/dl. Both of these concentrations are similar to those achieved

during (0.25 U/kg) insulin-induced hypoglycemia in conscious dogs (M. Wood, Shinsako, and Dallman, 1980).

My next objective was to reduce the infusion rate to a level which was hoped to cause a plasma corticosteroid response similar to that caused by 15 ml/kg hemorrhage. The 10 ng/min infusion elevated plasma ACTH 15-20 pg/ml, and plasma corticosteroids approxmately 4-5 ug/dl (approximately twice the magnitude of the response to 15 ml/kg hemorrhage, Chapter 4). Thus, elevation of plasma ACTH 15-20 pg/ml increased plasma corticosteroids to about half the level achieved in response to a 300 pg/ml rise in ACTH. A lower dose of ACTH will be needed to increase plasma corticosteroids to levels achieved during 15 ml/kg hypovolemia (see Chapter 6).

If the plasma corticosteroid response to 150 ng ACTH/min represented a maximal plasma corticosteroid response to increased plasma ACTH, the log dose-response relationship of ACTH infusion rate to plateau plasma corticosteroid concentration should not be linear to 150 ng/min. Using this logic, I designed the 30 ng/min infusions to answer the question of whether the responses to 150 ng ACTH/min were truly maximal. The 30 ng/min infusions elevated plasma ACTH approximately 30 pg/ml, while increasing plasma corticosteroids 5-6 ug/dl. Over all experiments, plateau plasma corticosteroid concentration was linearly related to both the

logarithm of the ACTH infusion rate (r^2=.49, p<.ØØ1) and the logarithm of plateau plasma ACTH concentration (r^2=.56, p<.ØØ1; Figure 14), suggesting that the corticosteroid response to 15Ø ng/min was not, after all, a maximum response.

Assuming that the metabolic clearance rate of cortisol in the dog is 18 ml/kg/min (McCormick, et al , 1974), the 1Ø ng ACTH/min infusion elevated the rate of cortisol secretion 12-23 ug/min (assuming dog body weights of 14-25 kg), and the 15Ø ng ACTH/min infusion elevated the rate of cortisol secretion 24-46 ug/min. Miller and coworkers (1976) have found that in conscious dogs (18-3Ø kg) with cannulated adrenal veins, intravenous infusions of 1 mU ACTH/min (equivalent to 1Ø ng/min infusion rate in these experiments) elevated adrenal cortisol secretion rate 5 ug/min/adrenal. In the same dogs, infusion of 15 mU ACTH/min (equivalent to 15Ø ng/min infusion rate in these experiments) increased adrenal cortisol secretion 2Ø-25 ug/min/adrenal (Miller, et al , 1976). The adrenal sensitivity to ACTH in experiments reported here is therefore similar to that reported in other experiments employing conscious dogs with adrenal venous catheters (Miller, et al , 1976).

ACTH distribution and metabolism. The ACTH half-disappearance time of 1.4-3.8 min reported here agrees with other values reported for the dog (3-5 min,

Urquhart, 1967) and rat (5 min, Greenspan, Li, and Evans, 1950; 1 min, Sydnor and Sayers, 1953), but is less than values reported for man (7 min, Wolf, et al , 1965; 7-8 min, Besser, et al , 1971; and 22-30 min, Berson and Yalow, 1968). The ACTH volume of distribution reported here (104-113 ml/kg) is greater than values reported for the dog ("plasma volume", Urquhart, 1967; 5.1, 6.5, and 7.2 % of body weight in 3 dogs reported by Cowan, 1974) and rat (6% of body weight, Cowan, 1974), but is smaller than that reported for man (43% of body weight, Wolf, et al , 1965). The data support the concept of plasma binding of ACTH (Urquhart, 1974), because the calculated volume of distribution (approximately twice plasma volume) suggests that most of the infused ACTH is being held in the plasma compartment. The ACTH clearance rate of 20.4-47.5 ml/kg/min is greater than clearance rates previously reported for the dog (9.6 ml/kg/min, Cowan, Davis, and Layberry, 1974; and approximately 10 ml/kg/min calculated from the data of Urquhart, 1967); but is closer to hepatic blood flow in conscious dogs (21.8 $\pm$ 8.5 ml/kg/min; Hopkinson and Schenk, 1968). The values for half-disappearance time, volume of distribution, and total clearance rate calculated from the parameters B_1, B_2, B_3 calculated from nonlinear fits of single exponential equations (1) and (2) should be considered as estimates of the true physiological values for two reasons. First, the analysis

was based on the assumption that changes in plasma ACTH over time were adequately described by first order kinetics. This assumption is not strictly valid, because it ignores ACTH distribution and it ignores the possibility that ACTH might be bound by plasma proteins (Urquhart, 1974). Second, included in the mean ACTH data used to fit the single exponential equations is the "noise" (Figure 10) caused by endogenous secretion of ACTH. The endogenous fluctuation in plasma ACTH concentration reduces the accuracy of calculation and eliminates the possibility of mathematically distinguishing best-fit first, second, or third order kinetics.

Conclusions. Thus, it appears from these data that in the dog, as in man, the secretion of ACTH is pulsatile, and that the short term pulses of ACTH cause short term pulses of adrenal corticosteroid secretion. The pulses of corticosteroid secretion are probably too small to individually elevate the peripheral plasma corticosteroid concentration, but collectively contribute to the relatively constant levels of plasma corticosteroids. The data also reveal an exquisite sensitivity of the adrenals of conscious dogs to ACTH, such that an elevation in plasma ACTH concentration of 15-20 pg/ml increases plasma corticosteroids to about half the concentration acheived during a 300 pg/ml increment in plasma ACTH concentration.

CHAPTER SIX

Adrenal Sensitivity to Exogenously Administered ACTH is not Altered by 15 ml/kg Hemorrhage in Conscious Dexamethasone-Treated Dogs

ABSTRACT

In a previous study, conscious dogs responded to 15 ml/kg hemorrhage with increases in corticosteroids that were unaccompanied by measurable increases in plasma ACTH (Chapter 4). The experiments reported in this chapter were designed to test adrenal sensitivity to low rates of ACTH infusion, and to test for a hemorrhage-induced shift in adrenal sensitivity to ACTH.

Hemorrhage-induced changes in adrenal responsiveness to ACTH were tested in two ways. First, dexamethasone-pretreated dogs were subjected to five 5 min steps of ACTH infusion, increasing in rate from 38 to 380 ng/min. The increasing rates of ACTH infusion elevated plasma ACTH to levels that were linearly related to the ACTH infusion rate, and elevated plasma corticosteroids to levels that were linearly related to

the logarithm of the ACTH infusion rate and plasma ACTH concentration. The magnitude of the corticosteroid response to the ACTH step-infusion was not changed by simultaneous hemorrhage or by step-infusion or hemorrhage 1.5-2 hours earlier. Second, dexamethasone-pretreated dogs were subjected to 30 min infusions of ACTH at rates of 5, 10, or 20 ng/min. The infusions elevated plasma corticosteroids to levels that were linearly related to the logarithm of the ACTH infusion rate (r^2=.77). The dose-response relationship of ACTH infusion rate and corticosteroid response was not altered by hemorrhage. Cortisol distribution and/or clearance did not change during the period of hypovolemia, indicating that adrenal gain was not changed. The steady-state infusion experiments demonstrate that the adrenals are normally exquisitely sensitive to small increases in plasma ACTH. Overall, 5 ng ACTH/min for 30 min elevated plasma corticosteroids 3 ug/dl. This rate of ACTH infusion was calculated to increase plasma ACTH 6-12 pg/ml in the steady-state. These increases in ACTH and corticosteroids are similar in magnitude to those observed during 15 ml/kg hypovolemia.

I conclude that the adrenals of conscious dogs are normally very sensitive to small changes in plasma ACTH concentration, and that this sensitivity is not changed during 15 ml/kg hypovolemia. The adrenal sensitivity to

ACTH is normally high enough to account for the plasma corticosteroid response to 15 ml/kg hemorrhage.

INTRODUCTION

In Chapter 4, I reported that 15 ml/kg hemorrhage in conscious dogs elevated plasma corticosteroid concentration 2.7 ug/dl and increased plasma ACTH concentration 11 pg/ml. Overall, however, there was no obvious relationship between plasma ACTH and corticosteroid concentrations (r^2=.Ø6). In several of the dogs, the first rise in plasma corticosteroids was observed before the first rise in plasma ACTH above control.

The adrenals of conscious dogs are normally quite sensitive to small increases in plasma ACTH (Chapter 5). Steady-state increases in plasma ACTH concentration of 15-2Ø pg/ml increased plasma corticosteroids 4-5 ug/dl (to about half the level caused by increases in plasma ACTH to more than 3ØØ pg/ml). Because of this high sensitivity of adrenals to ACTH, it seemed possible that very small increases in plasma ACTH might mediate the plasma corticosteroid response to 15 ml/kg hemorrhage. The experiments reported in this chapter were designed to further quantitate the dose-response relationship of infused ACTH to plasma corticosteroids, and to test for a shift in adrenal sensitivity to ACTH during

hypovolemia. Some of these data have been presented in abstract form (Wood, Shinsako, and Dallman, 1979, 1980b).

MATERIALS AND METHODS

Fifty-eight experiments were performed on 19 conscious mongrel dogs of either sex. The dogs were housed in individual cages and allowed free access to food and water. All dogs were accustomed to quiet standing in a loose cloth sling, as described in Chapter 3.

At least 2 days before the first experiment, a dog was sedated and anesthetized as described in Chapter 3. Under sterile conditions, a polyvinylchloride catheter (Tygon R-3603 .094"ID, .156"OD; or Tygon S-54-HL .050"ID, .090"OD) was implanted into a femoral or brachial artery and was maintained for a period of 1-4 weeks. Details of the surgery and catheter maintenance have been described in previous chapters. Catheters were removed from some dogs and, after a four week period of recovery, a new catheter was implanted into another (femoral or brachial) artery. No dog carried more than one arterial catheter at one time.

Experimental Protocols. On the morning of an experiment, a dog was brought to the laboratory, weighed, and placed in the sling. At least 45 min before the first blood sample was taken, one or two superficial leg veins were catheterized with Intracaths (Deseret Pharmaceutical Co., 17g needle and 19g catheter). The venous catheter was used for intravenous infusion of ACTH or saline, and in dogs without arterial catheters (see below), a second venous catheter was used for blood sampling. The arterial catheter was used for blood sampling and for measurement of blood pressure and heart rate. All ACTH used in infusions was synthetic al-24 ACTH (Cortrosyn, Organon, Inc.).

1) Step-Infusions of ACTH.

a) Effect of dexamethasone on adrenal responses to infused ACTH. Nine preliminary experiments were performed on 8 dogs (without arterial catheters) to test for possible changes in adrenal sensitivity after dexamethasone pretreatment. In 5 experiments, 5 dogs were pretreated with dexamethasone (4 mg, sc) 12 and 2 hours before the start of the experiment. In 4 experiments, 4 dogs were not pretreated with dexamethasone. In all 9 experiments, ACTH was infused intravenously in three 5 min steps of increasing rate (7.6, 19.1, and 38 ng/min). Venous blood samples were drawn at the start of the infusion and at the end of the 7.6 and 19.1

ng/min steps, and 5 min after the end of the 38 ng/min step. In the one dog studied twice, one week was allowed between experiments.

b) Effect of 15 ml/kg hemorrhage on adrenal response to infused ACTH. Eighteen experiments were performed on 9 dogs (13-25 kg). Each dog was pretreated with dexamethasone (4 mg, sc, 12 and 2 hours before the experiment). Each experiment consisted of two periods of study, the first starting between 1030 h and 1130 h, and the second exactly 2 hours later. In each experimental period of 10 experiments on 5 dogs, ACTH was infused intravenously in a stepwise fashion. ACTH was infused in four steps of 38, 76, 150, and 380 ng/min. Four or five blood samples were taken in 5 min intervals before the start of ACTH infusion, and then one blood sample was taken at the end of each 5 min step. In the first experimental period, 9 blood samples were taken, and in the second experimental period, 8 blood samples were taken. All blood samples were 5 ml. Between experimental periods and at the end of the second experimental period, red blood cells from blood samples were resuspended in sterile pyrogen-free normal saline and reinfused intravenously. In 5 experiments, 5 dogs were bled 15 ml/kg (within 3 min, as described in Chapter 3) starting 15 min before the start of the ACTH infusion in the first experimental period. In another 5 experi-

ments, the same 5 dogs were not bled. Three of these 5 dogs experienced the hemorrhage in their first experiment, and two of the 5 dogs experienced the hemorrhage in their second experiment, thus reducing the overall effects of any possible bias exerted by the first experiment on the results of the second experiment. Two experiments on any one dog were separated by one week.

In 8 experiments on 5 dogs, the same general protocol was followed, except that saline alone, without ACTH, was infused. In 4 experiments, 4 dogs were bled 15 ml/kg, as above, during the first experimental period. In 4 experiments, 4 dogs were not bled. As above, any two experiments on any one dog were separated by one week.

2) Steady-state infusions of ACTH. Thirty-two experiments were performed on 11 dogs (14-32 kg). Each dog was treated with dexamethasone (4 mg) once, 4 hours before the start of the experiment. The dogs were injected with dexamethasone via the arterial catheter, except dogs without chronically maintained arterial catheters (see below) which were injected subcutaneously. All experiments started between 1100 h and 1115 h. In each experiment, ACTH was infused intravenously (infusate flow rate = 0.76 ml/min) for 30 min. Eight or nine blood samples (10-12 ml each) were drawn at 5 min intervals before and during the ACTH infusion. Each dog

was studied twice at any particular dose of ACTH. At the end of the first experiment, one unit of ACTH was injected subcutaneously (to maintain adrenal sensitivity to ACTH). The second experiment was performed on the fourth day after the first.

a) Effect of a first experiment on the results of a second experiment. The possibility that one dexamethasone pretreatment and steady-state infusion of ACTH affects the corticosteroid response during a similar experiment 4 days later was directly tested in 4 dogs without arterial catheters. As explained above, each dog was subjected to two experiments, 4 days apart. In each experiment, ACTH was infused at a rate of 10 ng/min, and the plasma corticosteroid response to the infusion was measured in samples of venous blood.

b) Effect of 15 ml/kg hemorrhage on adrenal response to infused ACTH. In each of 11 dogs with chronically maintained arterial catheters, ACTH was infused in one of 3 doses (5, 10, or 20 ng/min), and the plasma corticosteroid response to the infusion was measured in samples of arterial blood. As explained above, each dog was studied twice at any particular dose. In one experiment, the dog was bled 15 ml/kg within 3 min starting at the time the infusion pump was turned on. In the other experiment (either 4 days before or after the experiment in which the dog was bled) the dog was

not bled. In one-half of the experiment pairs, the hemorrhage was performed in the first experiment, and in the other half, the hemorrhage was performed in the second experiment.

c) Distribution and metabolism of cortisol. In previous experiments (Chapter 4), I found that hemorrhage increased the volume of distribution and/or rate of clearance of cortisol. Those experiments employed a steady-state infusion of 3H-(1,2)-cortisol. In the present experiments, I decided to reexamine the phenomenon, this time using a single pulse of tritiated cortisol, monitering changes in distribution volume and/or clearance by measuring the disappearance of dichloromethane-extractable tritium counts from plasma over time. A preliminary experiment was performed to determine the length of time required for distribution of cortisol. In this experiment, I found that 30 min was ample time to allow for distribution. Five dogs that were otherwise taking part in the steady-state ACTH infusion experiments were injected with 20 uCi of 3H-(1,2)-cortisol 30 min before the start of the experiment. As above, each dog underwent two experiments, one in which the dog was bled 15 ml/kg, and one in which the dog was not bled. Therefore, 10 experiments were performed (eight 10 ng/min and two 20 ng/min).

Measurements. All blood samples were placed on ice, centrifuged at 4 C, and the plasma stored frozen until analyzed. All plasma samples were assayed for plasma ACTH and corticosteroid concentrations as described in previous chapters. In the steady-state infusion experiments in which 3H-(1,2)-cortisol was injected, 3H-(1,2)-cortisol was extracted from triplicate aliquots of Ø.5 ml plasma with 5 ml dichloromethane (see Chapter 4). During all experiments employing dogs with chronically maintained arterial catheters, mean arterial blood pressure and heart rate were monitered with a Statham pressure transducer and Grass polygraph.

Statistical Analyses. The data were analyzed by one-, two-, and three-way analysis of variance for repeated measures, by linear regression analysis, and when appropriate, paired "t" test.

RESULTS

Effect of dexamethasone on adrenal responsiveness to ACTH. Dexamethasone pretreatment (4 mg, sc) 12 and 2 hours before the start of the experiment did not significantly alter the venous plasma corticosteroid response to 3 steps of intravenous ACTH infusion (p=NS by two-way

ANOVA), although the dexamethasone pretreatment decreased preinfusion plasma corticosteroid concentration ($p<.05$ by two-way ANOVA). The venous plasma corticosteroid responses to this step-infusion are shown in Table 5.

TABLE 5. Effect of Dexamethasone on Adrenal Sensitivity.

Time (min)	ACTH Inf Rate (ng/min)	Plasma 11OHCS (ug/dl)	
		+Dex	-Dex
0	0	0.3(0.1)	1.3(0.2)
5	7.6	1.0(0.6)	1.4(0.1)
10	19	1.4(0.6)	2.1(0.2)
20	38	2.1(0.7)	2.6(0.2)

*Values are represented as means(SEM).

Dexamethasone inhibits the adrenocortical response to 15 ml/kg hemorrhage. In control experiments for the ACTH step-infusion study, the effect of dexamethasone on the adrenocortical response to hemorrhage was tested in 5 dogs. The dogs were pretreated with 4 mg dexamethasone, 12 and 2 hours before the start of the experiment. In 4 experments, 4 dogs were bled 15 ml/kg in the first period of study, and in another 4 experiments, 4 dogs were not bled. Each dog was subjected to step-infusion of saline at the same rates used in the ACTH step-infusion experiments. Overall, the dogs had low or undetectable plasma ACTH and corticosteroid concentrations, and did not respond to hemorrhage with increases in either (Table 6).

TABLE 6. Step-Infusions: Saline Controls (N=4).

		-HEM		+HEM	
TIME (min)	I.R.	ACTH (pg/ml)	11OHCS (ug/dl)	ACTH (pg/ml)	11OHCS (ug/dl)
Ø	Ø	11(2)	Ø.7(Ø.2)	2Ø(5)	Ø.7(Ø.1)
Ø	Ø	11(2)	Ø.7(Ø.2)	2Ø(5)	Ø.7(Ø.1)
5	Ø	11(2)	Ø.7(Ø.2)	25(9)	Ø.7(Ø.2)
1Ø	Ø	11(3)	Ø.7(Ø.1)	17(5)	Ø.7(Ø.1)
15	Ø	13(5)	Ø.8(Ø.2)	17(5)	Ø.8(Ø.1)
2Ø	Ø	9(Ø)	Ø.7(Ø.2)	17(6)	Ø.7(Ø.1)
25	Ø	16(6)	Ø.8(Ø.2)	14(2)	Ø.7(Ø.2)
3Ø	Ø	17(4)	Ø.7(Ø.2)	16(5)	Ø.7(Ø.2
35	Ø	9(1)	Ø.7(Ø.2)	12(1)	Ø.7(Ø.2)
12Ø	Ø	12(3)	Ø.6(Ø.2)	14(3)	Ø.6(Ø.1)
125	Ø	13(3)	Ø.7(Ø.2)	17(1)	Ø.7(Ø.1)
13Ø	Ø	1Ø(1)	Ø.8(Ø.2)	17(1)	Ø.7(Ø.1)
135	Ø	1Ø(1)	Ø.8(Ø.2)	13(3)	Ø.6(Ø.1)
14Ø	Ø	12(2)	Ø.7(Ø.2)	14(4)	Ø.7(Ø.2)
145	Ø	16(3)	Ø.7(Ø.2)	15(5)	Ø.7(Ø.2)
15Ø	Ø	15(1)	Ø.7(Ø.2)	12(3)	Ø.7(Ø.2)
155	Ø	17(3)	Ø.8(Ø.2)	13(4)	Ø.7(Ø.2)

*Values are represented as means(SEM).
**(N=3)
I.R. = ACTH Infusion Rate (ng/min)

Effect of 15 ml/kg hemorrhage on plasma corticosteroid responses to ACTH step-infusions. The results of the ACTH step-infusion experiments demonstrate that the adrenals of conscious dogs are normally very sensitive to small increases in plasma ACTH, and that the sensitivity is not increased during hypovolemia. In the first experimental period of 1Ø experiments on 5 dexamethasone-pretreated dogs (5 with hemorrhage and 5 without hemorrhage), the first step of the ACTH step-infusion significantly elevated both plasma ACTH and corticosteroid concentrations. Thus, a 5 min infusion of 38 ng ACTH/min elevated plasma ACTH from unmeasurable levels to 28 $\pm$ 4 pg/ml in dogs that were bled and to 21 $\pm$ 5 pg/ml in dogs that were not bled (Table 7). These

TABLE 7. Step-Infusions: ACTH (N=5)*.

		-HEM		+HEM	
TIME (min)	I.R.	ACTH (pg/ml)	11OHCS (ug/dl)	ACTH (pg/ml)	11OHCS (ug/dl)
-5	Ø	15(3)	Ø.5(Ø.1)	13(4)	Ø.5(Ø.1)
Ø	Ø	13(3)	Ø.5(Ø.1)	15(2)	Ø.5(Ø.1)
5	Ø	13(3)	Ø.5(Ø.1)	13(4)	Ø.5(Ø.1)
1Ø	Ø	13(2)	Ø.5(Ø.1)	2Ø(4)	Ø.4(Ø.1)
15	Ø	14(3)	Ø.6(Ø.1)	19(4)	Ø.5(Ø.1)
2Ø	38	21(5)	1.6(Ø.8)	28(4)	2.3(Ø.9)
25	76	48(6)	2.6(Ø.7)	59(4)	3.7(Ø.9)
3Ø	15Ø	92(9)	3.3(Ø.4)	119(6)	4.6(Ø.9)
35	38Ø	268(3Ø)	4.6(Ø.8)	382(5Ø)	4.9(1.Ø)
12Ø	Ø	16(7)	1.9(Ø.6)	19(4)	1.4(Ø.2)
125	Ø	2Ø(8)	1.8(Ø.6)	18(3)	1.3(Ø.2)
13Ø	Ø	14(1)	1.5(Ø.4)	12(1)	2.Ø(1.1)
135	Ø	17(2)	1.4(Ø.4)	18(4)	1.5(Ø.7)
14Ø	38	25(7)	2.5(Ø.9)	29(4)	2.2(Ø.8)
145	76	52(8)	4.5(1.3)	59(6)	4.Ø(1.Ø)
15Ø	15Ø	1Ø3(14)	5.6(1.3)	122(12)	4.5(Ø.8)
155	38Ø	25Ø(53)	5.8(1.Ø)	277(27)	5.2(Ø.9)

*Values are represented as means(SEM).
I.R. = ACTH Infusion Rate (ng/min)

increases in plasma ACTH initiated the adrenal response to the step-infusion, increasing plasma corticosteroids from unmeasurable levels to 2.3 ± 1.Ø ug/dl and 1.6 ± Ø.8 ug/dl in dogs that were and were not bled, respectively. Plasma ACTH and corticosteroid concentrations in two dogs are illustrated in Figure 19.

The effect of hemorrhage on the adrenal response to ACTH step-infusion was tested in 5 dexamethasone-pretreated dogs (4 mg, 12 and 2 hours before the start of the experiment). The ACTH step-infusion elevated arterial plasma ACTH concentration to levels linearly related to the infusion rate (r^2=.99, Figure 2Ø). Superimposition of 15 ml/kg hypovolemia on the step-infusion resulted in higher measured plasma ACTH

trations, probably due to the lowered blood volume ($p<.01$ by two-way ANOVA, Figure 20). However, this effect was restricted to the ACTH concentration measured after the highest rate of ACTH infusion ($p<.05$ by Newman-Keuls multiple range test). In each experimental period, the arterial plasma corticosteroid concentration was linearly related to the logarithm of the ACTH infusion rate (Figure 20). The plasma corticosteroid response to the ACTH step-infusion was affected by neither simultaneous 15 ml/kg hypovolemia nor prior hemorrhage nor prior step-infusion (p=NS by two-way ANOVA, Table 7).

In experimental periods in which dogs were not bled mean arterial blood pressure and heart rate did not change (p=NS by ANOVA). However, in experimental periods in which dogs were bled 15 ml/kg, mean arterial blood pressure decreased and heart rate increased (both $p<.001$ by one- and two-way ANOVA, Table 8). The effect of hemorrhage on blood pressure and heart rate was not altered by ACTH administration (p=NS by two-way ANOVA). Hematocrit was measured in all experiments, and was not affected by blood sampling or by 15 ml/kg hemorrhage (Table 8).

Steady-state infusions of ACTH.

a) Preliminary experiments. The design of

TABLE 8. Step-Infusions: Hemodynamic data (N=9).

	-HEM			+HEM		
TIME (min)	MAP (Torr)	HR (bpm)	Hct (%)	MAP (Torr)	HR (bpm)	Hct (%)
-5	107(4)	73(6)	30(2)	97(3)	70(4)	33(2)
0	102(4)	78(5)	30(2)	102(4)	65(4)	32(2)
2	103(4)	73(5)		89(5)	129(13)	
4	102(3)	76(5)		90(3)	120(7)	
5			29(2)			33(2)
6	102(3)	69(6)		92(3)	119(8)	
8	105(4)	71(5)		93(2)	116(11)	
10	106(5)	73(4)	30(2)	93(4)	116(10)	33(2)
15	105(3)	73(6)	29(2)	91(3)	116(10)	33(2)
20	105(3)	71(6)	30(2)	92(2)	110(10)	33(1)
25	106(4)	76(6)	29(2)	87(3)	111(10)	32(2)
30	104(5)	71(6)	29(2)	95(3)	107(9)	32(2)
35	102(5)	74(6)	29(2)	89(4)	113(11)	31(2)
120	110(5)	79(5)	30(2)	101(3)	63(6)	31(2)
122	110(7)	76(8)		101(4)	70(5)	
124	106(4)	77(8)		99(3)	66(4)	
125			30(2)			31(1)
126	109(4)	73(8)		100(4)	66(4)	
128	105(6)	74(7)		100(4)	68(5)	
130	103(3)	79(12)	30(2)	100(6)	66(3)	31(2)
135	108(5)	77(8)	31(2)	99(5)	69(3)	31(2)
140	102(6)	82(13)	31(2)	100(6)	70(3)	31(2)
145	109(6)	75(8)	31(2)	97(3)	68(3)	31(2)
150	105(7)	76(10)	32(2)	97(5)	72(5)	30(2)
155	105(6)	84(14)	30(2)	95(6)	71(6)	30(2)

Values are represented as Means(SEM).
*(N=8)
**(N=6)

the steady-state infusion experiments (each dog at each dose was studied twice, once with and once without hemorrhage) required the assumption that the dexamethasone pretreatment and ACTH infusion in the first experiment did not affect the adrenal response to the ACTH infusion in the second experiment. Therefore, as a preliminary series of experiments, 4 dogs were treated with dexamethasone (4 mg, sc, 4 hours before the start of the experiment) and given a steady-state 10 ng/min infusion of ACTH. Four days later, the experiment was

repeated. As shown in Figure 21, the venous plasma corticosteroid responses to the two infusions were indistinguishable (p=NS by two-way ANOVA), indicating that the performance of the first experiment did not bias the results of the second experiment.

b) Effect of 15 ml/kg hemorrhage on plasma corticosteroid responses to steady-state infusions of ACTH. ACTH was infused intravenously at 5, 10, and 20 ng/min into 4 dexamethasone-pretreated dogs at each dose. The steady-state infusions of ACTH increased arterial plasma corticosteroid concentration to levels that were related to the ACTH infusion rate ($p<.001$ by three-way ANOVA, Figure 22). The magnitudes of the plasma corticosteroid responses to the 3 doses of ACTH infusion were not changed by simultaneous 15 ml/kg hypovolemia (p=NS by three-way ANOVA, Figure 22). Because of variation in dog body weights, random assignment of the dogs to infusion dose groups produced a continuous distribution of infusion doses calculated as ng ACTH/min per kilogram body weight. As shown in Figure 23, the integrated corticosteroid response to ACTH infusion (calculated as the area under the corticosteroid response curve above mean control) was linearly related to the ACTH infusion rate in normovolemic ($r^2=.77$, $p<.001$ by linear regression analysis) and hypovolemic ($r^2=.54$, $p<.05$ by linear regression analysis) dogs. The

slope of the regression line was not changed by hemorrhage (p=NS) again indicating that 15 ml/kg hemorrhage did not alter adrenal sensitivity to ACTH in (dexamethasone pretreated) conscious dogs.

In the steady-state ACTH infusion experiments, 15 ml/kg hemorrhage significantly (p<.001 by two-way ANOVA) elevated heart rate, but did not change mean arterial blood pressure (p=NS by two-way ANOVA, Table 9). Hematocrit was measured in these experiments, and was not affected by blood sampling or by hemorrhage (p=NS by one- and two-way ANOVA, Table 9).

TABLE 9. Steady-state infusions: Hemodynamic data (N=11).

	-HEM			+HEM		
TIME (min)	MAP (Torr)	HR (bpm)	Hct (%)	MAP (Torr)	HR (bpm)	Hct (%)
-5	103(7)	73(5)	40(1)*	101(6)	70(6)	39(1)
0	100(5)	72(7)	40(1)	104(6)	73(6)	39(1)
5	103(5)	68(5)	40(1)	98(5)	103(10)	41(1)
10	98(6)	63(4)	39(1)	97(4)	102(8)	40(1)
15	103(5)	66(5)	39(1)	100(5)	106(9)	40(1)
20	99(7)	75(7)	39(1)	96(4)	115(11)	40(1)
25	109(9)	80(8)	40(1)	96(5)	107(6)	40(1)
30	101(4)	80(8)	40(1)	93(5)	111(7)	39(1)

*(N=10)

c) Distribution and metabolism of cortisol. In 10 steady-state infusion experiments, (eight 10 ng/min and two 20 ng/min) on 5 dogs, the effect of hemorrhage on the distribution and metabolism of cortisol was tested. Twenty uCi of 3H-(1,2)-cortisol were injected through the arterial catheter 30 min before the start of the experiment, and tritium counts were extracted from plasma samples taken during the regular infusion

protocol. Plasma cortisol half-disappearance time, volume of distribution, and total clearance rate were calculated from parameters B_1 and B_2 obtained by non-linear fit of the function

$$[A] = B_1 e^{-B_2 t}$$

to the data in each experiment. Cortisol half-disappearance time was calculated as $T_{1/2} = \ln 2/B_2$, volume of distribution calculated as $V_d = I/B_1$ (where I is the total cpm injected), and the total clearance rate calculated as TCR = B*V . In the calculation of volume of distribution, the total activity of the injectate was taken to be $2*10^7$ cpm. The results of this analysis are shown in Table 10 Hemorrhage did not

TABLE 10. Effect of Hemorrhage on Cortisol DBM.

	-HEM		
DOG	Half-Life (min)	Vol. Dist. (ml/kg)	TCR (ml/kg/min)
King	25.5	490	13.3
Ferdinand	20.2	540	18.5
Frances	13.6	270	13.8
Frieda	17.3	270	10.8
Max	19.6	426	15.0
Mean(SEM)	19.2(1.9)	399(56)	14.3(1.3)

	+HEM		
DOG	Half-Life (min)	Vol. Dist (ml/kg)	TCR (ml/kg/min)
King	11.0	560	35.3
Ferdinand	17.4	250	10.0
Frances	16.2	480	20.5
Frieda	13.6	190	19.7
Max	19.6	434	17.3
Mean(SEM)	15.6(1.5)	383(70)	18.6(4.7)

significantly affect the value of any calculated

variable (p=NS by paired "t" test). When the logarithm of dichloromethane-extractable tritium counts are plotted with time, the disappearance of tritiated cortisol is log-linear with time in all experiments (Figure 24).

Figure 19

Arterial plasma ACTH and corticosteroid (11OHCS) concentrations during the first experimental period of ACTH step-infusion experiments in 2 dogs. Dog A (left) was bled 15 ml/kg within 3 min at time Ø; Dog B (right) was not bled. ACTH and corticosteroid values at -5, Ø, 5, 1Ø, 15, 2Ø, 25, and 3Ø min are connected by solid and broken lines, respectively.

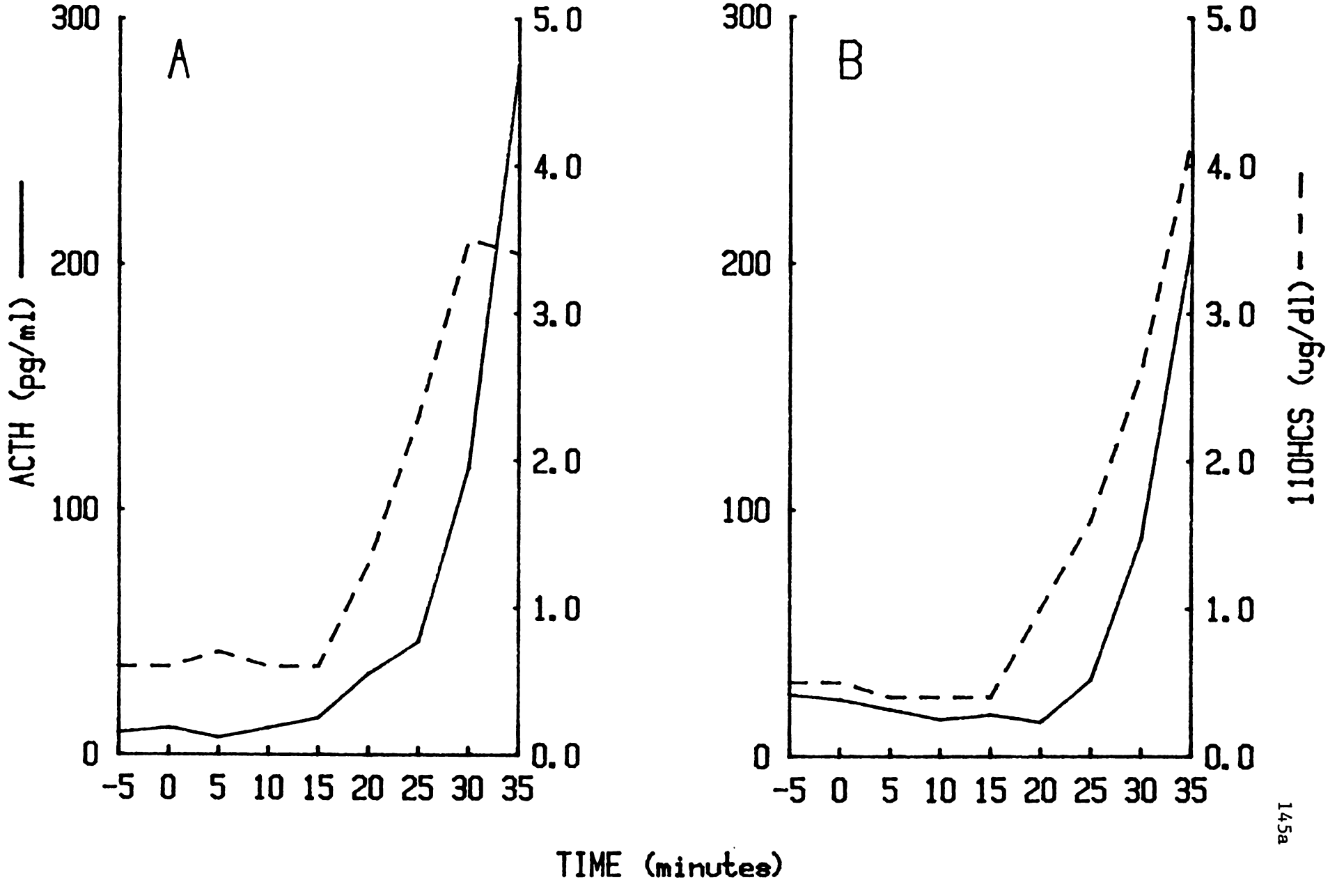

A
B
300
200
100
0
5.0
4.0
3.0
2.0
1.0
0.0
-5 0 5 10 15 20 25 30 35
ACTH (pg/ml) ——
11OHCS (ug/dl) - - -
TIME (minutes)

Figure 2Ø

Arterial plasma ACTH and corticosteroid (11OHCS) concentrations during the first experimental period of ACTH step-infusion experiments in which 5 dogs were bled 15 ml/kg (B, right) or in which the same 5 dogs were not bled (A, left). ACTH and corticosteroid concentrations are plotted as functions of the rate of ACTH infusion in the immediately preceeding 5 min step. Mean ACTH values are shown as (*) connected by solid lines, and mean corticosteroid values are shown as (o) connected by broken lines. Vertical bars represent SEM.

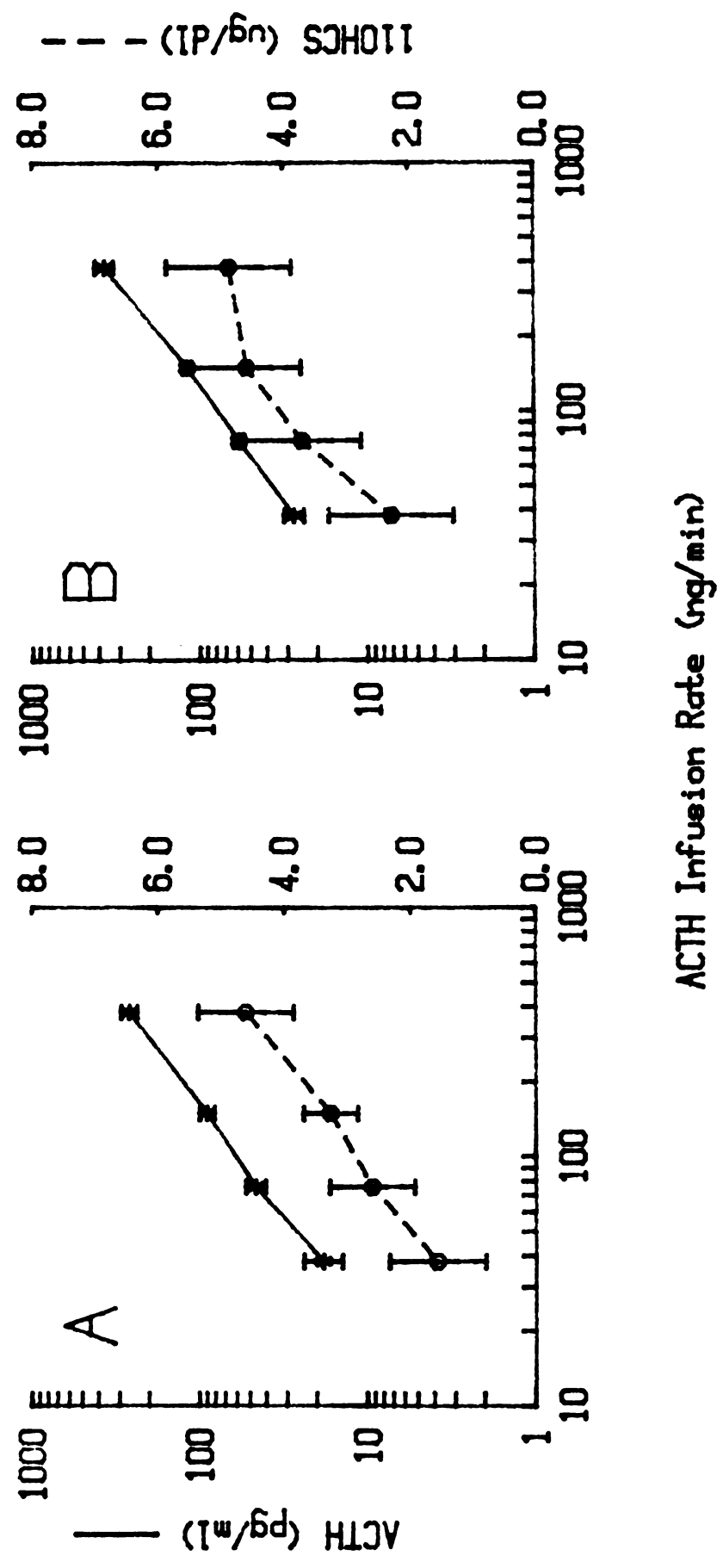

11OHCS (ug/dl) - - -
8.0
6.0
4.0
2.0
0.0
1000
100
10
1
B
A
ACTH (pg/ml) ——
ACTH Infusion Rate (ng/min)

Figure 21

Venous plasma corticosteroid (11OHCS) responses to two 1Ø ng/min infusions of ACTH, four days apart. Means are plotted as (*) for the first infusion and as (o) for the second infusion. Standard errors are shown as vertical bars.

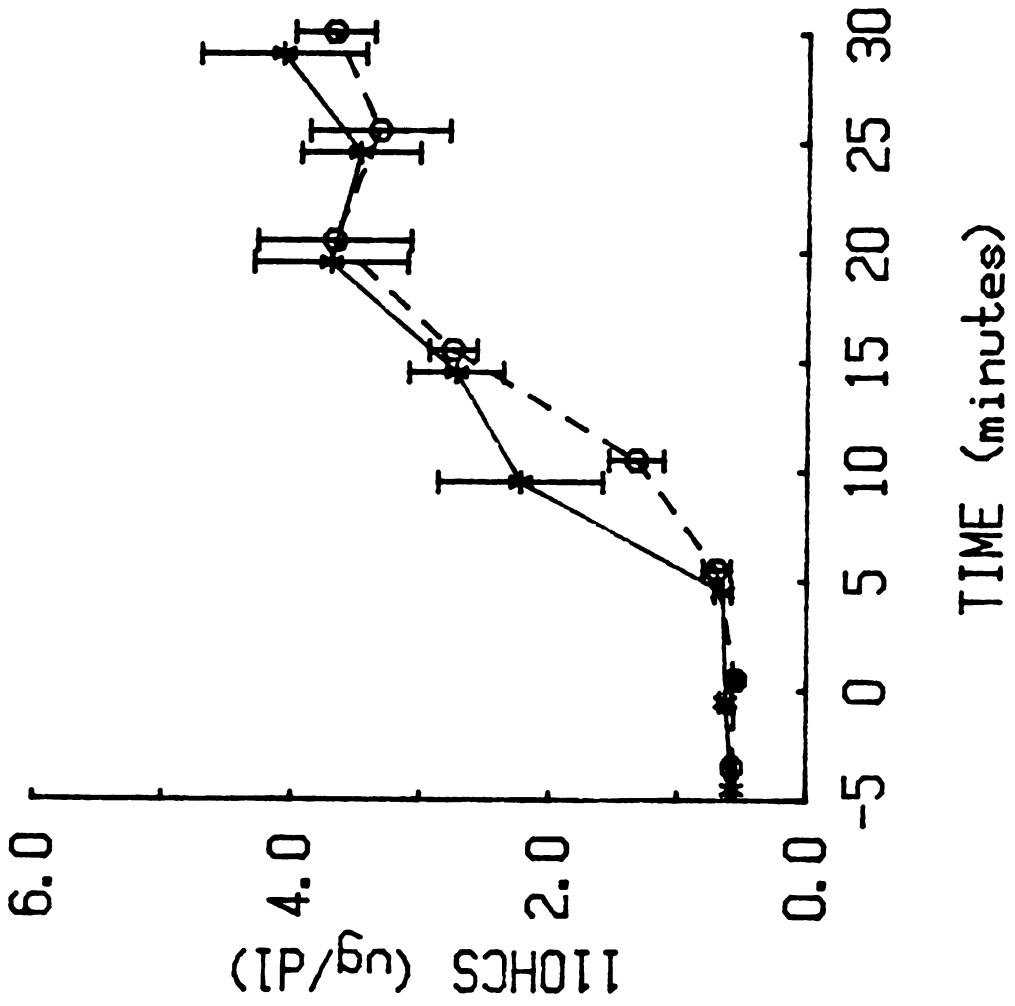

11OHCS (ug/dl)
6.0
4.0
2.0
0.0
-5
0
5
10
15
20
25
30
TIME (minutes)

Figure 22

Arterial plasma corticosteroid (11OHCS) responses to 5 (A, top), 1Ø (B, middle), and 2Ø (C, bottom) ng/min infusions of ACTH (Ø to 3Ø mins). In each panel, the overall responses of 4 dogs are shown as mean values connected with solid lines. The responses to infusion in the 4 dogs bled 15 ml/kg (at time Ø) are shown as mean values connected with broken lines. Standard errors are shown as vertical bars.

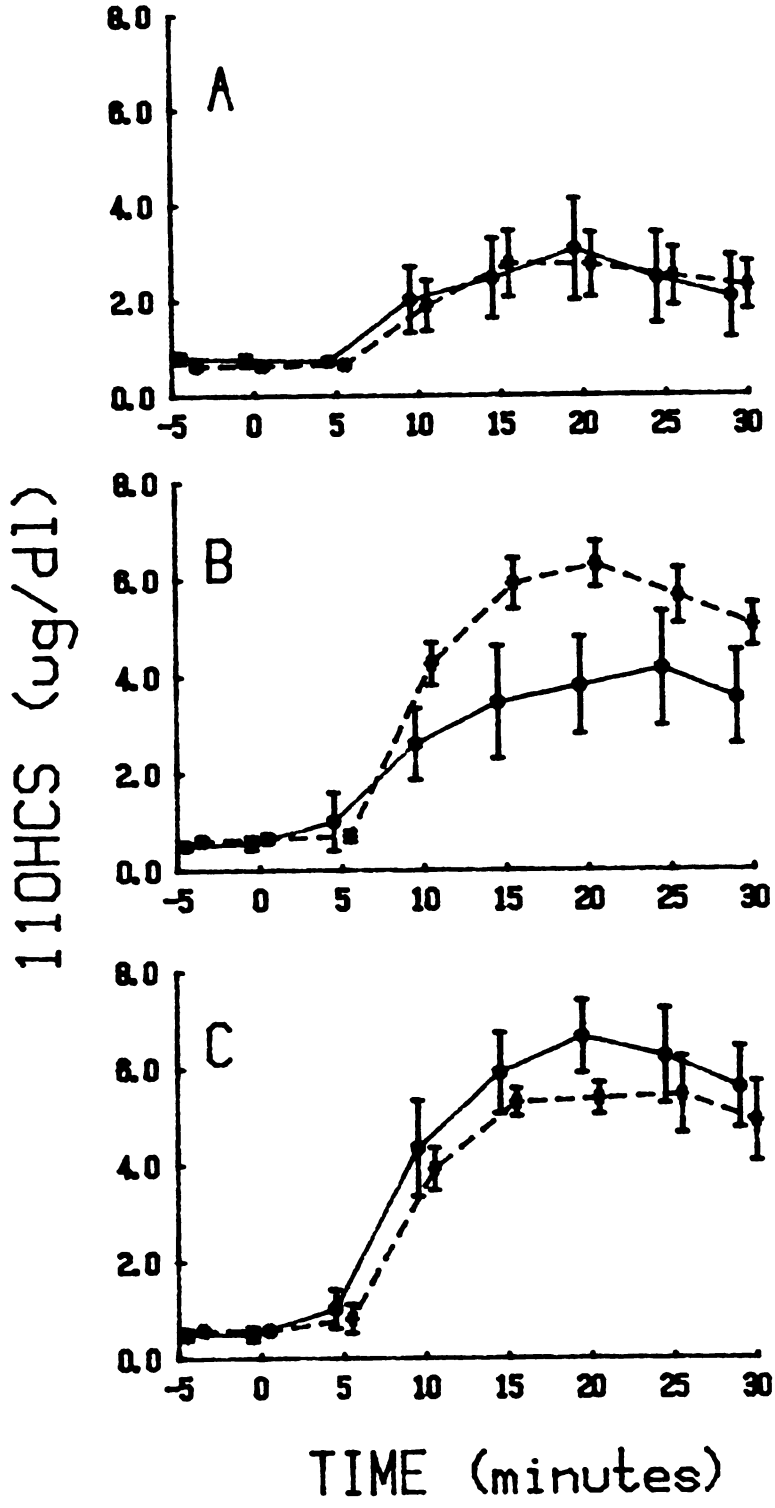

A
B
C
8.0
6.0
4.0
2.0
0.0
-5
0
5
10
15
20
25
30
11OHCS (ug/dl)
TIME (minutes)

Figure 23

Correlation of integrated arterial plasma corticosteroid responses (to 5, 10, and 20 ng/min infusions of ACTH in dexamethasone-pretreated dogs) with ACTH infusion rate normalized to body weight. The correlation was significant for dogs not bled (A, left; $r^2 = .77$, $p < .001$) and for dogs bled 15 ml/kg at the onset of infusion (B, right; $r^2 = .54$, $p < .05$). The slopes of the regression lines were not different (p=NS). Integrated response is calculated as the area under the corticosteroid response curve above mean control from -5 to 30 min.

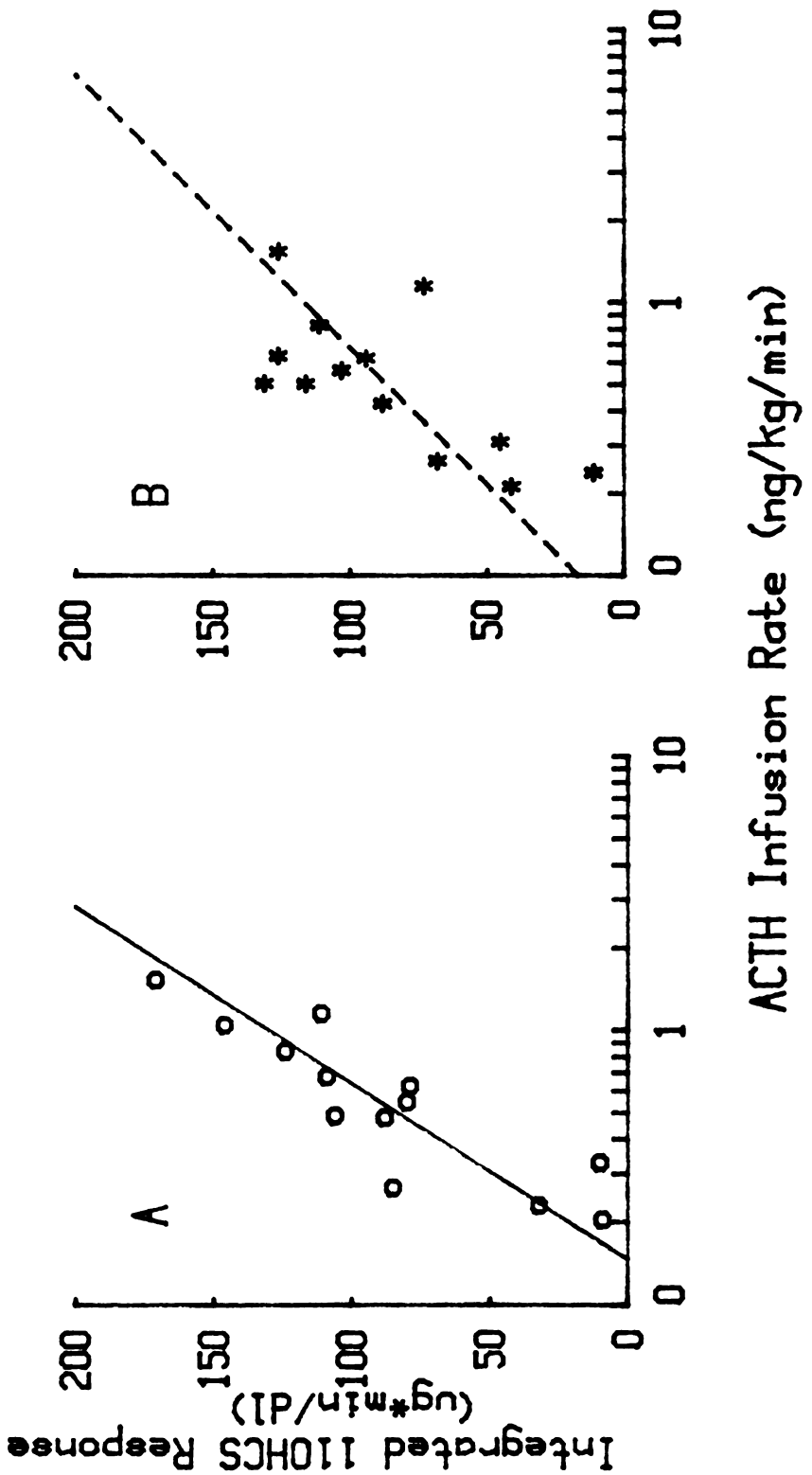
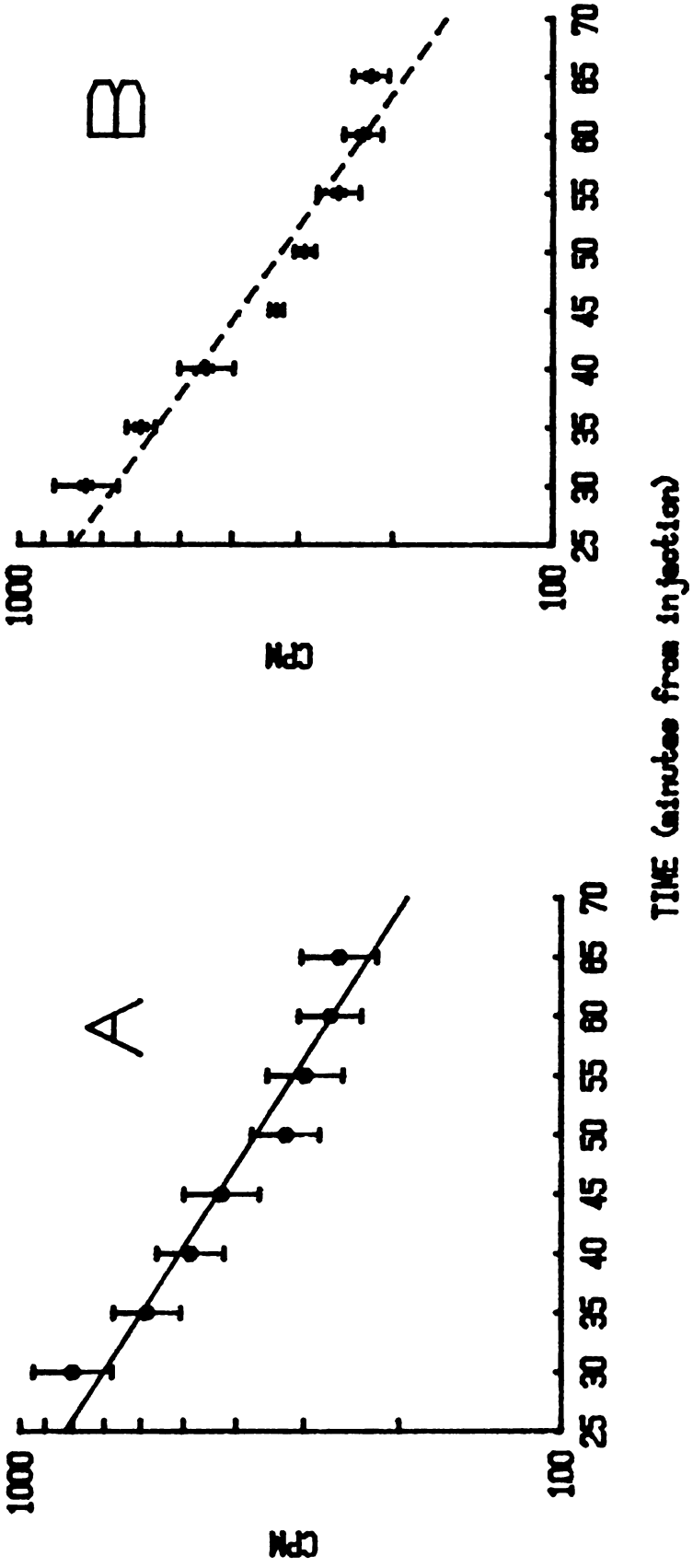

Integrated 11OHCS Response
(ug*min/dl)
200
150
100
50
0
A
0
1
10
B
ACTH Infusion Rate (ng/kg/min)

Figure 24

Disappearance of dichloromethane-extractable tritium counts after injection of 20 uCi 3H-(1,2)-cortisol in 5 dogs that were not bled (A) and in the same dogs bled 15 ml/kg at 30 min (B). Means are accompanied by vertical bars representing SEM. Best-fit regression lines are shown as a solid and a broken line in A and B, respectively.


B
A
CPM
1000
100
25 30 35 40 45 50 55 60 65 70
TIME (minutes from injection)

DISCUSSION

These studies collectively demonstrate that the adrenals of conscious dogs are normally very sensitive to small increments in plasma ACTH concentration, and that adrenal sensitivity to ACTH is not increased during or 1.5-2 hours after a 30 min period of hypovolemia.

Effect of dexamethasone on adrenal sensitivity to ACTH. Because 15 ml/kg hypovolemia is a stimulus to ACTH and corticosteroid secretion (Chapters 3 and 4), tests of the effect of hypovolemia on adrenal responsiveness to infused ACTH required, as a part of the experimental design, suppression of endogenous ACTH secretion. Miller and coworkers (1976) have reported that daily injections of dexamethasone decrease the sensitivity of the adrenals to ACTH. Preliminary to the ACTH step-infusions, therefore, I tested the effect of the dose of dexamethasone used in those experiments (4 mg, 12 and 2 hours before the experiment) on the adrenal response to 3 steps of ACTH infusion. As shown in Table 5, the response to this ACTH signal was not reduced by dexamethasone. Since experimental design required that each dog be studied more than once (once with hemorrhage and once without hemorrhage), the order of the experiments performed was randomized, so that there would be

no overall effect of a possible cumulative effect of dexamethasone on adrenal responsiveness. However, any possible cumulative effect of dexamethasone would slightly reduce the likelihood of detecting a hemorrhage-induced shift in adrenal responsiveness to infused ACTH because it would add a new source of variability to the corticosteroid response magnitude. Before the steady-state infusions, therefore, I tested for a cumulative effect of dexamethasone (4 mg, 4 hours before the experiment) on the corticosteroid response to ACTH infusion. As shown in Figure 21, the dexamethasone pretreatment in the first experiment did not change the adrenal response to the 10 ng/min infusion during a second experiment 4 days later. As further evidence that this dose of dexamethasone did not reduce adrenal responsiveness, the arterial plasma corticosteroid response to infusion of 10 ng ACTH/min was the same in these experiments as in experiments in non-dexamethasone-pretreated dogs reported in Chapter 5 (plateau levels not different, p=NS by two-way ANOVA).

Adrenals are very sensitive to small changes in plasma ACTH concentration. In Chapter 5, I demonstrated that a steady-state increase in plasma ACTH of 15-20 pg/ml increased plasma corticosteroids approximately 4-5 ug/dl in the steady-state. This result suggested that increments in plasma ACTH concentration of far less than 15-

20 pg/ml were required to initiate the corticosteroid response. In the step-infusions reported in this chapter, the first step of ACTH infusion (38 ng/min) elevated plasma ACTH concentration from unmeasurable levels to between 20 and 30 pg/ml. These increases in plasma ACTH elevated plasma corticosteroid concentration from unmeasurable levels to between 1.5 and 2.5 ug/dl (Figure 19). Because the step is 5 min long, and because the lag between first increases in ACTH and corticosteroids is 3 min (Chapter 5), the increment in plasma ACTH caused by that step is probably larger than the smallest increment in ACTH needed to increase (or initiate) adrenal secretion of corticosteroids.

The steady-state infusions of ACTH presented in this chapter were designed to be a true test of adrenal sensitivity to ACTH, when sensitivity is defined as steady-state response to a steady-state signal. As shown in Figure 21, the magnitude of the adrenal response to infused ACTH was linearly related to the logarithm of the ACTH infusion rate. All infusions increased plasma corticosteroids, even when the infusion rate was as low as 200 pg/kg/min. Using the distribution and metabolism parameters calculated in Chapter 5, this low rate of ACTH infusion would be expected to raise plasma ACTH concentration 5-10 pg/ml in the steady-state. The first increment in circulating plasma

ACTH concentration causing the first increment in plasma corticosteroids must be even smaller than this.

Adrenal sensitivity to ACTH is not increased during 15 ml/kg hypovolemia. The normally high sensitivity of the adrenals to elevated plasma ACTH concentration is not increased during 15 ml/kg hypovolemia. The first elevation in corticosteroids in the step-infusion experiments was observed after the first step (38 ng/min) of ACTH infusion. In dogs not bled 15 ml/kg, that step increased plasma ACTH from unmeasurable levels to 21 $\pm$ 5 pg/ml and increased plasma corticosteroids from unmeasurable levels to 1.6 $\pm$ Ø.8 ug/dl. In the same dogs bled 15 ml/kg, plasma ACTH was increased to 28 $\pm$ 4 pg/ml and plasma corticosteroids to 2.3 $\pm$ Ø.9 ug/dl. The hemorrhage did not significantly change the corticosteroid response to this first step of ACTH (p=NS by paired "t" test). In fact, the corticosteroid response to all steps of ACTH infusion was not affected by hemorrhage (p=NS by two-way ANOVA, Figure 2Ø). In the step-infusion experiments, however, the adrenocortical system is biased to steadily increasing activity. At low rates of ACTH infusion, comparable to the first 4 steps of the ACTH step-infusion, the adrenal response to increases in ACTH presentation is characterized by an overshoot and subsequent settling to steady-state response (Urquhart, Krall, and Li, 1968; Miller, et al, 1976). The 5 min

steps of ACTH infusion used in these experiments therefore focus more on the acute phase of the adrenal response to increased ACTH, and never allows the adrenal response to reach steady-state. It is possible that a subtle change in sensitivity would be masked by the dynamic nature of the adrenal response to increased plasma ACTH. For this reason, I decided to qunatitate adrenal sensitivity to steady-state increases in plasma ACTH concentration.

In the steady-state ACTH infusion experiments (Figure 22 and Table 6), the corticosteroid response to ACTH infusions were linearly related to the logarithm of the ACTH dose (Figure 23). In these experiments, 15 ml/kg hemorrhage simultaneous with the onset of the ACTH infusion did not alter the dose-response relationship (Figure 23). Data were analyzed in two ways, by three-way analysis of variance for repeated measures, and by linear correlation of the integrated corticosteroid responses to the logarithm of the ACTH infusion dose: neither method demonstrated a significant effect of hemorrhage.

Effect of hemorrhage on cortisol distribution and metabolism. Corticosteroid distribution and metabolism is best described by a two-compartment model in dogs (McCormick, et al, 1974) and in human beings (Tait, et al, 1961). The model requires the assumption that

cortisol is distributed into two compartments, which can transfer cortisol between them, and that cortisol metabolism occurs from only one of the compartments (Tait, et al, 1961). Therefore, the rate at which cortisol disappears from plasma would be determined by the rate of metabolism (for the purposes of this discussion consider this to be metabolism and excretion) and the rate at which cortisol is transferred between compartments. The measured plasma cortisol concentration would also be affected by changes in its distribution volume, in either of the two compartments. Barring any dynamic changes in cortisol clearance or distribution volume, a plot of the logarithm of the cortisol concentration in plasma against time should reveal two components, with different slopes.

Because of the requirement of uniform design throughout all steady-state infusion experiments, it was neccessary to restrict the period of blood sampling to 35 min. The experiments were designed so that 20 uCi of 3H-(1,2)-cortisol were injected 30 min before the start of the experiment. The period of blood sampling therefore covered 30-65 min after the injection of the labelled steroid, and focused on the second component of the two-component disappearance curve. Because observations were restricted to this time frame, the cortisol distribution and metabolism parameters calculated and

reported in Table 8 must be interpreted with the following restrictions. The rate of cortisol disappearance is the result of two processes, metabolism of cortisol from the metabolizable pool, and transfer of cortisol between the two pools. Thus, if the 3H-(1,2)-cortisol was evenly distributed among the two pools at some time before the first blood sample was drawn, the rate of cortisol disappearance would be slower that that defined by metabolism alone, because of net transfer of cortisol from the nonmetabolizable pool to the metabolizable pool. For the same reason, the calculated clearace rate slightly overestimates the true clearance rate, and the calculated volume of distribution does not correspond to either compartment of the two-compartment system.

The experimental design, therefore, does not allow exact measurement of volumes of distribution in either of the two hypothetical compartments, nor does it allow exact measurement of the rate of cortisol clearance or transfer between compartments. The results of experiments reported in Chapter 4, however, suggest that during 15 ml/kg hypovolemia, the increase in clearance rate and/or distribution volume of cortisol in dogs untreated with dexamethasone was transient, and that the perturbation did not reach steady-state. Because a new steady-state was not achieved, the ideal goal of exact measurement of all parameters with and without 15 ml/kg hemor-

rhage is therefore unattainable. The experiments were thus designed to detect a change in cortisol distribution or metabolism or transfer between compartments. In Figure 24, the disappearance of dichloromethane-extractable tritium counts from 30-65 min after the pulse of 3H-(1,2)-cortisol was linear when plotted as the logarithm of the extracted counts. Neither the rate of disappearance of cortisol nor the linearity of the curves was changed by hemorrhage, indicating that dexamethasone pretreatment abolished the change in cortisol clearance and/or distribution volume normally caused by 15 ml/kg hemorrhage (Chapter 4).

It is possible that the hemorrhage-induced change in cortisol distribution and/or metabolism was dependent upon the blood pressure response to hemorrhage. The dogs in these experiments did not respond to the hemorrhage with a decrease in mean arterial pressure. The inability to demonstrate statistical significance in these experiments might be due to the small absolute change in blood pressure caused by 15 ml/kg hemorrhage (Table 8), and the relatively few observations in the steady-state infusion experiments. It seems unlikely, however, that the increase in distribution volume and/or rate of clearance of cortisol observed in Chapter 4 is a simple function of mean arterial pressure because 2 dogs with elevated rectal temperatures responded to 15

ml/kg hemorrhage with large decreases in mean arterial pressure and no change in cortisol distribution and/or clearance (Chapter 4).

Conclusions. It is possible that the corticosteroid response to 15 ml/kg hemorrhage (Chapter 4), often initiated before any measured increases in circulating ACTH, can be completely accounted for by small increases in plasma ACTH concentration. This hypothesis is supported by the following facts. 1) The adrenal corticosteroid response to hemorrhage was blocked by dexamethasone, indicating that ACTH or a non-ACTH but dexamethasone-suppressible factor was essential for the elaboration of the response. 2) The adrenals of conscious dogs are exquisitely sensitive to small increases in plasma ACTH concentration. Thirty minute infusions of 5 ng ACTH/min elevated plasma corticosteroids 3 ug/dl in the steady-state, while theoretically increasing plasma ACTH to a plateau level of 6-12 pg/ml. These increases in ACTH and corticosteroids were similar in magnitude to those achieved during 30 min of 15 ml/kg hypovolemia (Chapter 4). It is logical to assume that if a steady-state increase in plasma ACTH of 6-12 pg/ml causes a steady-state increase of 3 ug/dl in corticosteroids, the first increment in plasma ACTH responsible for the initiation of the corticosteroid response must be smaller. Assuming that ACTH distribution and meta-

bolism is adequately described by a single compartment model (Chapter 5) and that the lag between elevated ACTH and elevated corticosteroids is 3 min, the size of this increment can be estimated. Using parameters calculated in Chapter 5, infusion of 5 ng ACTH/min for 2 min should increase plasma ACTH about 3.5 pg/ml. 3) In dogs not treated with dexamethasone, plasma ACTH fluctuated by as much as 20 pg/ml (Chapter 5) without causing an overall change in arterial plasma corticosteroid concentration. These fluctuations in plasma ACTH might have masked the small (perhaps undetectable) hemorrhage-induced increases in plasma ACTH concentration that increased arterial plasma corticosteroid concentration. In conclusion, therefore, because of the exquisite sensitivity of adrenals to small changes in plasma ACTH concentration, there is no reason to propose that non-ACTH factors mediate or contribute to the adrenal response to 15 ml/kg hemorrhage. All of the data presented in this dissertation are consistent with this conclusion.

CHAPTER SEVEN

GENERAL DISCUSSION

When I started these experiments, I intended to use 15 ml/kg hemorrhage in a study of the feedback effects of corticosteroids on ACTH secretion. I chose this stimulus because it was reported to be a reliable and reproducible stimulus to ACTH secretion (Gann, 1969; Gann and Cryer, 1973). However, the finding that normothermic dogs respond to the hemorrhage with a tripling in plasma corticosteroids and with a seemingly unrelated ACTH response changed the direction of the project. At the time, it seemed highly unlikely that the corticosteroid response could be directly attributable to changes in plasma ACTH. In the individual dogs, the first rise in ACTH above control was often preceeded by the first rise in corticosteroids. Overall, there was only a slight relationship between ACTH and corticosteroids (r^2=.Ø6). The total mean increase in plasma ACTH was only 11 pg/ml, which was very small when compared to other stimuli in dogs (3ØØ pg/ml in response to Ø.25 U/kg insulin injection; M. Wood, Shinsako, and Dallman, 198Ø) and rats (1ØØ-25Ø pg/ml in response to intraperitoneal injection of normal saline; Engeland, et al, 1977). Because of the lack of an obvious relationship between ACTH and corticosteroids, and because of

the small size of the ACTH response, it seemed likely that other factors were involved in the corticosteroid response to hemorrhage.

The results of the ACTH infusion experiments reported in Chapters 5 and 6, however, demonstrate that the sensitivity of adrenals to ACTH is high enough to account for the apparent dissociation of ACTH and corticosteroids. In dogs not infused with ACTH, plasma ACTH concentration fluctuated by as much as 20 pg/ml without causing an overall change in plasma corticosteroid concentration. However, increasing the mean arterial plasma ACTH concentration by only 15-20 pg/ml increased arterial plasma corticosteroid concentration to levels higher than those caused by 15 ml/kg hemorrhage. A lower rate of ACTH infusion, calculated to increase plasma ACTH 6-12 pg/ml in the steady-state (therefore matching the ACTH increase during 15 ml/kg hypovolemia), increased plasma corticosteroids to the same level achieved during 15 ml/kg hypovolemia. Assuming that the lag between the first increases in arterial plasma concentrations of ACTH and corticosteroids is 3 min, it can be calculated that the first rise in ACTH causing the first rise in corticosteroids is about 3.5 pg/ml, or about $1.5*10^{-12}$ M. This small increment in plasma ACTH concentration cannot be measured by the assay. Because of this limitation, I cannot directly

prove that the corticosteroid response to 15 ml/kg hemorrhage is entirely attributable to small increments in plasma ACTH concentration. If, however, small increases in ACTH do mediate the corticosteroid response to 15 ml/kg hemorrhage, the 3-4 pg/ml increase in plasma ACTH concentration would be obscured by the much greater 20 pg/ml fluctuations in background ACTH. Because of the extraordinary sensitivity of the adrenal to small increases in mean ACTH concentration, one could predict that in this situation, the first increases in plasma corticosteroids would not be consistently preceeded by increases in plasma ACTH, and that plasma ACTH need never increase above "control." Therefore, although it cannot be directly proven that small increases in ACTH mediate the corticosteroid response to 15 ml/kg hemorrhage, the adrenals are sensitive enough to account for such a mechanism.

Calculation of adrenal sensitivity to ACTH. Because dexamethasone pretreatment does not reduce adrenal sensitivity to infused ACTH, it is possible to combine the data from Chapters 5 and 6 to define the log dose-response relationship of ACTH to corticosteroid response. Nelson and Hume (1955) demonstrated that the adrenal venous corticosteroid response to injections of ACTH is linearly related to the logarithm of the ACTH dose. In the experiments reported in Chapters 5 and 6,

there is an overall linear relationship between the logarithm of the ACTH infusion rate (in ng/kg/min) and the integrated arterial plasma corticosteroid response (Figure 25). This is probably the most unbiased method of expressing these data, because the calculation of the integrated corticosteroid response requires no assumptions about achievement of steady-state. The linearity of the relationship demonstrates that none of the doses of ACTH used were causing maximal corticosteroid responses. However, this method of analysis does not allow comparison of the data to the results of experiments reported by others. Most studies of adrenal sensitivity to ACTH have employed direct measurement of adrenal corticosteroid output; adrenal sensitivity has been expressed as the relationship of the adrenal secretory response to intravenous or intraadrenal arterial ACTH infusion rate.

Because the magnitude of the adrenal response is linearly related to the logarithm of the ACTH dose (Nelson and Hume, 1955; and Figure 25), it is possible to define the steady-state adrenal response to a steady-state ACTH signal as

$$C = k \log A$$

where C is the steady-state adrenal corticosteroid secretion rate in response to the steady-state ACTH

presentation rate, A. For the experiments reported in Chapters 5 and 6, the steady-state adrenal secretion rates can be calculated if the total clearance rate of cortisol is assumed to be 18 ml/kg/min (McCormick, et al, 1974). Thus, for each dog, the corticosteroid secretion rate is calculated as

$$C = 18*BW*P$$

where C is the steady-state secretion rate, BW is the dog's weight in kilograms, and P is the steady-state (plateau) corticosteroid concentration. For each experiment, the plateau corticosteroid concentration is calculated as the mean of 7 corticosteroid values from 15 to 30 min after the start of the ACTH infusion. As shown in Figure 26, the mean corticosteroid secretion rate at each rate of ACTH infusion (in ng/min) is linearly related to the logarithm of the ACTH infusion rate (r^2=.99; $p<.001$). The slope of the best-fit regression line, k, is 18.8 ug/min/log(ng/min). This slope, a measure of adrenal sensitivity to ACTH is similar to the slope of 18 calculated by Miller and coworkers (1976), but is substantially higher than that calculated from the data of Urquhart (approximately 4; Urquhart, 1965).

Miller, et al (1976) reported that the maximum adrenal secretion rate is approximately 45

ug/min/adrenal, or 90 ug/min total. In contrast, Urquhart (1965) reported that the maximum secretion rate is approximately 8 ug/min/adrenal, or 16 ug/min total. In the experiments reported in Chapter 5, 150 ng/min ACTH infusion increased the calculated adrenal corticosteroid secretion rate to 45 ug/min, which is not a maximum response (as explained in Chapter 6).

The calculation of the steady-state adrenal sensitivity is dependent upon the value of total clearance rate of cortisol used in these calculations. The value of the regression coefficient, k, can be expressed as

$$k = \frac{TCR*BW*P}{\log(A)}$$

where TCR is the total clearance rate, expressed as ml/kg/min, BW is the dog's body weight in kilograms, P is the plateau plasma corticosteroid concenration, and A is the ACTH infusion rate. Thus, if I used the value of 3.8 ml/kg/min for the total clearance rate, the calculated corticosteroid secretion rates and adrenal sensitivity would agree with the data of Urquhart (1965). However, the value of 18 ml/kg/min (McCormick, et al, 1974) agrees, generally, with the value of 13-15 ml/kg/min measured by Yates and his associates (personal communication), and also agrees with the value of 16 ml/kg/min calculated in Chapter 6 from a one-compartment model.

Direct measurement of corticosteroid secretion rate (Gann and Cryer, 1973; Urquhart, 1965, Miller, et al, 1976; Nelson and Hume, 1955) allows a direct assessment of adrenal sensitivity, as well as definition of the dynamics of the adrenal response to changes in the ACTH input signal (Urquhart, 1965, Urquhart and Li, 1969). However, experimental technique employed in such experiments including the use of general anesthesia (Gann and Cryer, 1973; Nelson and Hume, 1955; Urquhart, 1965), in situ perfusion technique (Urquhart, 1965), the use of adrenal vein catheters and chokers (Gann and Cryer, 1973; Lake and Gann, 1972; Nelson and Hume, 1955; Miller, et al, 1976; Urquhart, 1965) might change the characteristics of the system's response to stimuli, and might introduce a variable amount of error leading to disagreement between labs. For example, Lake and Gann (1972) suggest that the intact adrenal (with cannulated adrenal vein) is dynamically more responsive than the in situ perfused adrenal (Urquhart, 1965). On the other hand, calculation of adrenal secretion rates from steady-state corticosteroid concentrations requires the assumption of independence of clearance rate from the overall level of plasma corticosteroids (which is not neccessarily a valid assumption, as discussed by McCormick, et al, 1974).

In studies of the contribution of adrenal sensi-

tivity to the overall adrenocortical response to stimuli (as in Chapters 5 and 6), the relationship of steady-state arterial plasma corticosteroid response to steady-state arterial plasma ACTH concentration is probably more meaningful (Figure 15 in Chapter 5), because the glucocorticoid-sensitive tissues throughout the body presumably respond to changes in arterial plasma corticosteroid concentration. Because the steady-state arterial plasma ACTH concentrations from the 5, 10, and 20 ng/min infusion experiments reported in Chapter 6 are not available, all steady-state infusion data from Chapters 5 and 6 can be combined by plotting the relationship of plateau plasma corticosteroid response to the ACTH infusion rate (in ng/kg/min). Overall, the plateau plasma corticosteroid response is linearly related to the ACTH infusion rate (r^2=.60, $p<.001$; Figure 25). As explained earlier, the results of these experiments do not demonstrate a maximum corticosteroid response. The maximum can, however, be estimated, assuming that the maximum corticosteroid secretion rate is 90 ug/min (Miller, et al, 1976) and assuming that the cortisol clearance rate is 18 ml/kg/min (McCormick, et al, 1974). If the average dog body weight is 30 kg, the calculated maximum arterial plasma corticosteroid concentration is approximately 17 ug/dl. Thus, infusion of 10 ng ACTH/min elevates plasma corticosteroids to about quarter-maximal, and infusion of 50 ng ACTH/min elevates

plasma corticosteroids to about half-maximal (in dogs whose body weights average about 20 kg, as in Chapters 5 and 6).

The lowest rate of ACTH infusion (5 ng/min) consistently stimulated corticosteroid secretion. Assuming a lag of 3 min, as discussed above, the first rise in ACTH causing a first rise in corticosteroids can be calculated to be about 3.5 pg/ml. This is a smaller increment than previously shown in vivo, but approaches the smallest threshold shown in vitro. Urquhart (1965) demonstrated that adrenal corticosteroid output was increased significantly when arterial plasma ACTH concentration was increased from 0 to 1 uU/ml (equivalent to 10 pg/ml in these experiments). However, he did not attempt to use a lower dose. Seelig and Sayers (1973) showed that dispersed rat adrenal cells respond to 10 pg ACTH/ml incubation medium with an increase in corticosterone production. Kolanowski and Crabbe (1976) have demonstrated an even higher sensitivity of dispersed adrenal cells to ACTH. In their experiments, dispersed human adrenal cells responded to 1 pg ACTH/ml incubation medium with an increase in cortisol production.

Adrenal sensitivity to ACTH during hypovolemia, revisited. The definition of the normal adrenal sensitivity to ACTH, expressed as the relationship of plateau plasma corticosteroids to plateau plasma ACTH (Figure

15) allows a reexamination of the ACTH and corticosteroid responses to hemorrhage in normothermic and febrile dogs reported in Chapters 3 and 4. Assuming that the response to hemorrhage is a constant autoinfusion of ACTH, it is possible to to calculate "plateau" ACTH and corticosteroid concentrations between 15 and 30 min after hemorrhage. In Figure 27 is shown a correlation of "plateau" plasma corticosteroids with the logarithm of the "plateau" plasma ACTH during hypovolemia in 25 experiments on dogs with rectal temperatures between 38.2 and 41 C. Also shown in the figure is the best-fit regression line and 95% confidence limits from the relationship of plateau corticosteroids to log plateau ACTH during infusions of ACTH in normothermic, normovolemic dogs (Figure 15). It is clear that although the adrenocortical response was exaggerated in the febrile dogs, the relationship of corticosteroids to ACTH was normal (none of the points fall outside the 95% confidence limits). This agrees with the results of experiments reported in Chapter 6 demonstrating that 15 ml/kg hemorrhage does not increase adrenal sensitivity to ACTH. Because hemorrhage or increased body temperature does not change the relationship of corticosteroids to ACTH, the data from the hemorrhage experiments (Figure 27) and the data from the ACTH infusion experments (Figure 15) can be combined in an overall correlation of plateau corticosteroid response to log plateau ACTH (Figure 28).

This overall correlation yields a more confident estimate of the slope and the degree of scatter (r^2=.60) defining the relationship of plateau corticosteroid and plateau ACTH concentrations.

Adrenal amplification of the ACTH signal. The very high sensitivity of the adrenals to small (even unmeasurable) increases in plasma ACTH suggests that information transfer across the adrenal is efficient, that the adrenal greatly amplifies the ACTH signal. Assuming a total clearance rate of ACTH of 35 ml/kg/min (Chapter 5), and assuming an average dog body weight of 20 kg, a 5 ng/min infusion of ACTH raises plasma ACTH concentration about 7 pg/ml. Assuming a total adrenal blood flow of 10 ml/min (for both adrenals), the total amount of ACTH reaching the adrenal (adrenal presentation rate) is about 70 pg/min, or $3*10^{-14}$ moles/min. At this rate of ACTH infusion, the corticosteroid secretion rate is calculated to be about 10 ug/min, or about $3*10^{-8}$ moles/min. The adrenal therefore amplifies the ACTH signal about 10^6 times. The same reasoning can be applied to calculate the adrenal amplification at the 150 ng/min ACTH infusion rate. At this rate, the adrenal amplifies the ACTH input signal about 10^4 times. Therefore, quadrupling the magnitude of the corticosteroid response is effected at the expense of a factor of 100 in the amplification of the ACTH signal. Put

another way, to increase the rate of corticosteroid output by a factor of 4, the ACTH input has to be increased by a factor of 30. Clearly, the transfer of information across the adrenal is more efficient at low plasma ACTH concentrations.

Does ACTH convey multiple messages? Most of the stimuli to adrenocortical secretion that have been studied in rats, dogs, and man increase plasma ACTH levels above 100 pg/ml. On the other hand, 15 ml/kg hemorrhage stimulates a very small increase in ACTH and a tripling of plasma corticosteroids. Injection of 0.01 U/kg insulin (which lowers plasma glucose from a fasting level of 80 mg/dl to 60 mg/dl; M. Wood, Shinsako, and Dallman, 1980) causes a response similar in magnitude to that caused by hemorrhage. Is the dog a special case, or have most of the stimuli studied actually overestimated the intensity of stimuli encountered in daily life? To what levels do environmental stimuli, such as acute exposure to heat or cold, stimulate adrenocortical secretion? Hodges, Jones, and Stockham (1962) have shown that the anxiety of university students undergoing oral examination can raise plasma ACTH and corticosteroids to levels ordinarily seen in Cushing's Disease or during surgery. If human adrenals respond to ACTH with the same high sensitivity that dog adrenals do (and they probably do, Kolanowski and Crabbe, 1976), why are

such high levels of ACTH and corticosteroids important? Do extraadrenal actions of ACTH (Bohus and DeKloet, 1979; Urquhart, 1974) play a role in the individual's response to these stimuli? Vasopressin, in the range of Ø-1Ø pg/ml decreases renal free water clearance, and in higher concentrations causes vasoconstriction. Angiotensin II stimulates both aldosterone secretion and vasoconstriction. Both actions each of vasopressin and angiotensin are important for successful recovery from hemorrhage. Is ACTH analagous?

Figure 25

Correlation of integrated arterial plasma corticosteroid responses (A, left) and plateau arterial plasma corticosteroid concentrations (B, right) with steady-state ACTH infusion rate in 12 experiments in which dogs were pretreated with dexamethasone and in 18 experiments in which dogs were not pretreated with dexamethasone.

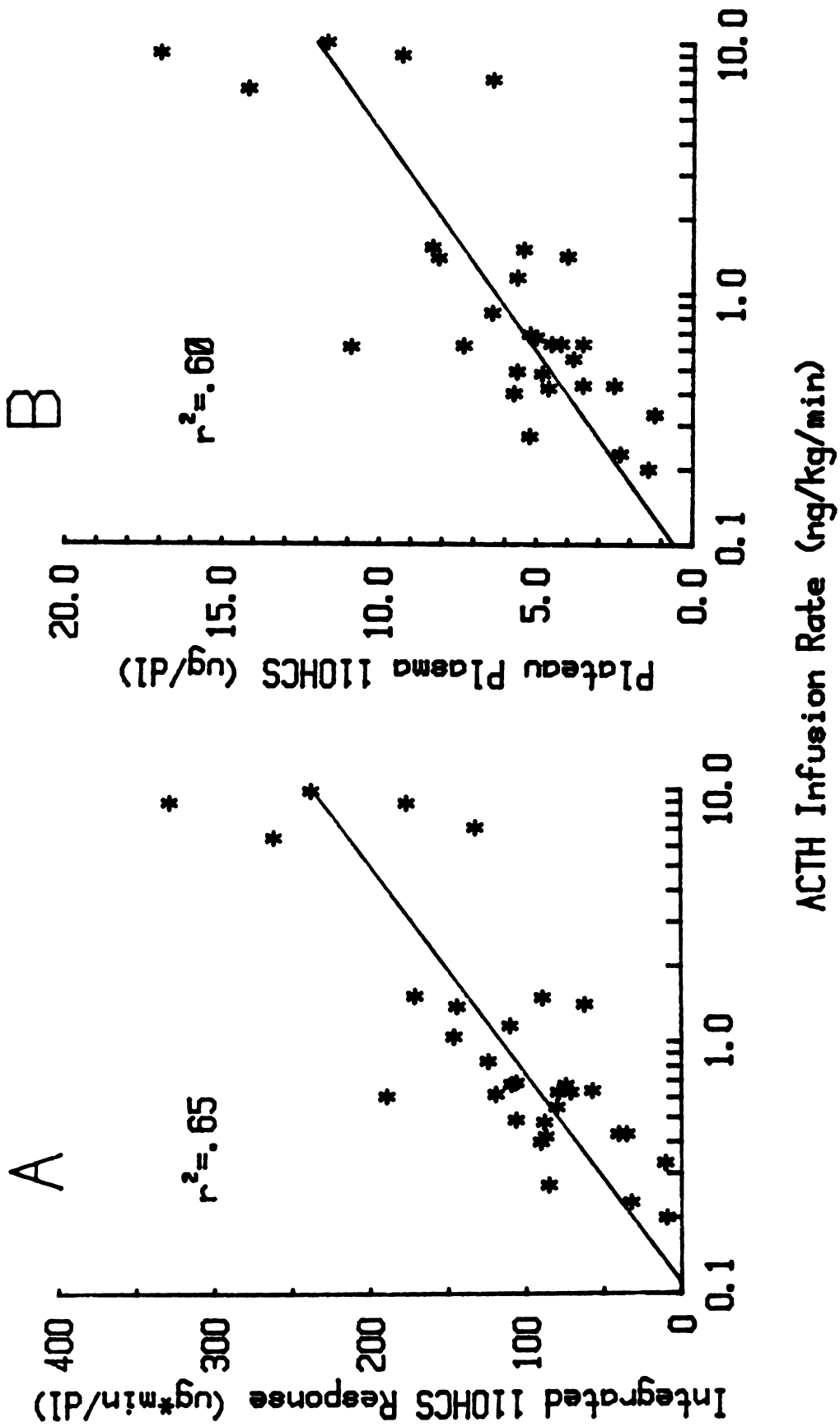

A
r^2=.65
Integrated 11OHCS Response (ug*min/dl)
400
300
200
100
0
0.1
1.0
10.0
B
r^2=.60
Plateau Plasma 11OHCS (ug/dl)
20.0
15.0
10.0
5.0
0.0
0.1
1.0
10.0
ACTH Infusion Rate (ng/kg/min)

Figure 26

Mean calculated corticosteroid secretion rates during 5, 10, and 20 ng/min infusion of ACTH in dexamethasone-pretreated dogs and during 10, 30, and 150 ng/min infusion of ACTH in unpretreated dogs.

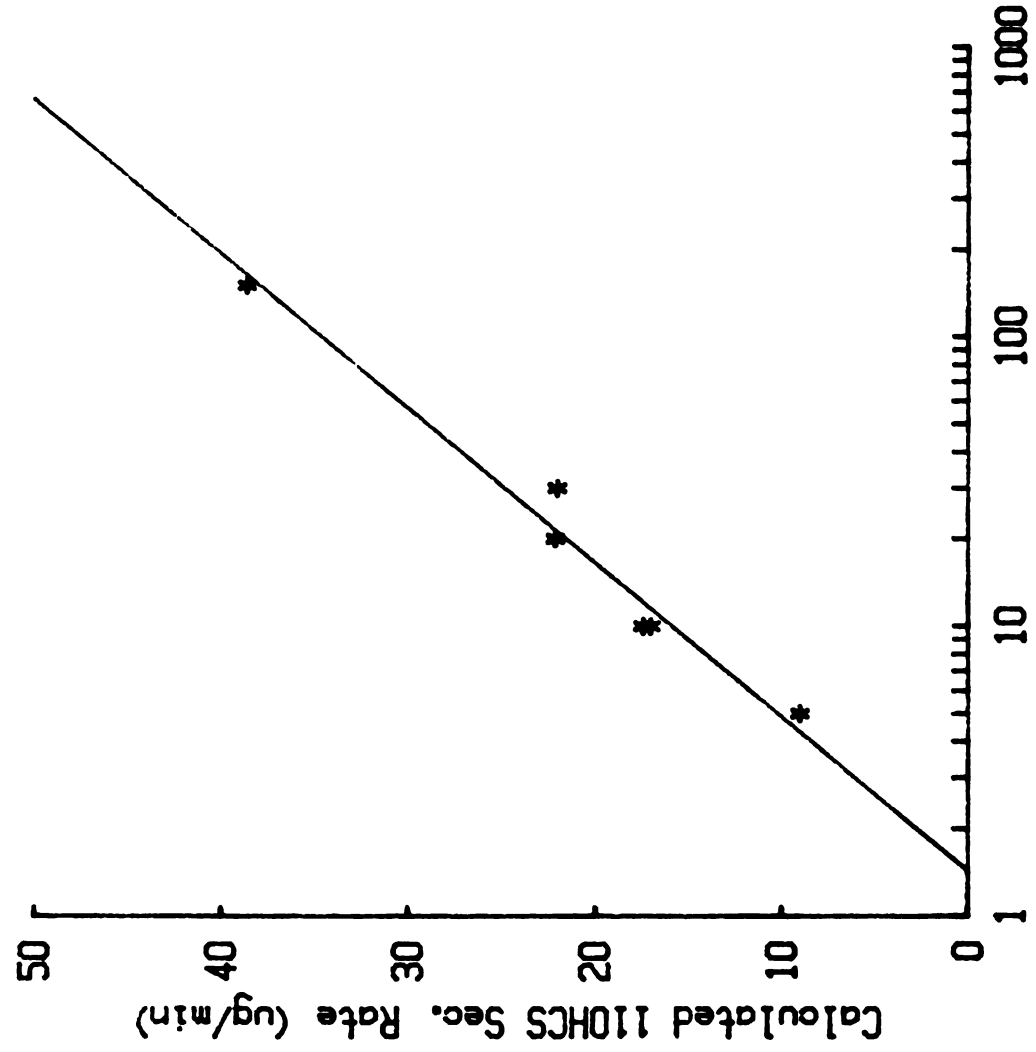

Calculated 11DHCS Sec. Rate (ug/min)
0
10
20
30
40
50
1
10
100
1000
ACTH Infusion Rate (ng/min)

Figure 27

Plateau plasma corticosteroid and plateau plasma ACTH concentrations in 25 experiments in which dogs (whose rectal temperatures ranged from 38.2 to 41 C) were bled 15 ml/kg. The solid and broken lines are the best-fit regression line and 95% confidence limits from the correlation of the two variables in 18 experiments in which normothermic, normovolemic dogs were infused with 10, 30, and 150 ng ACTH/min.

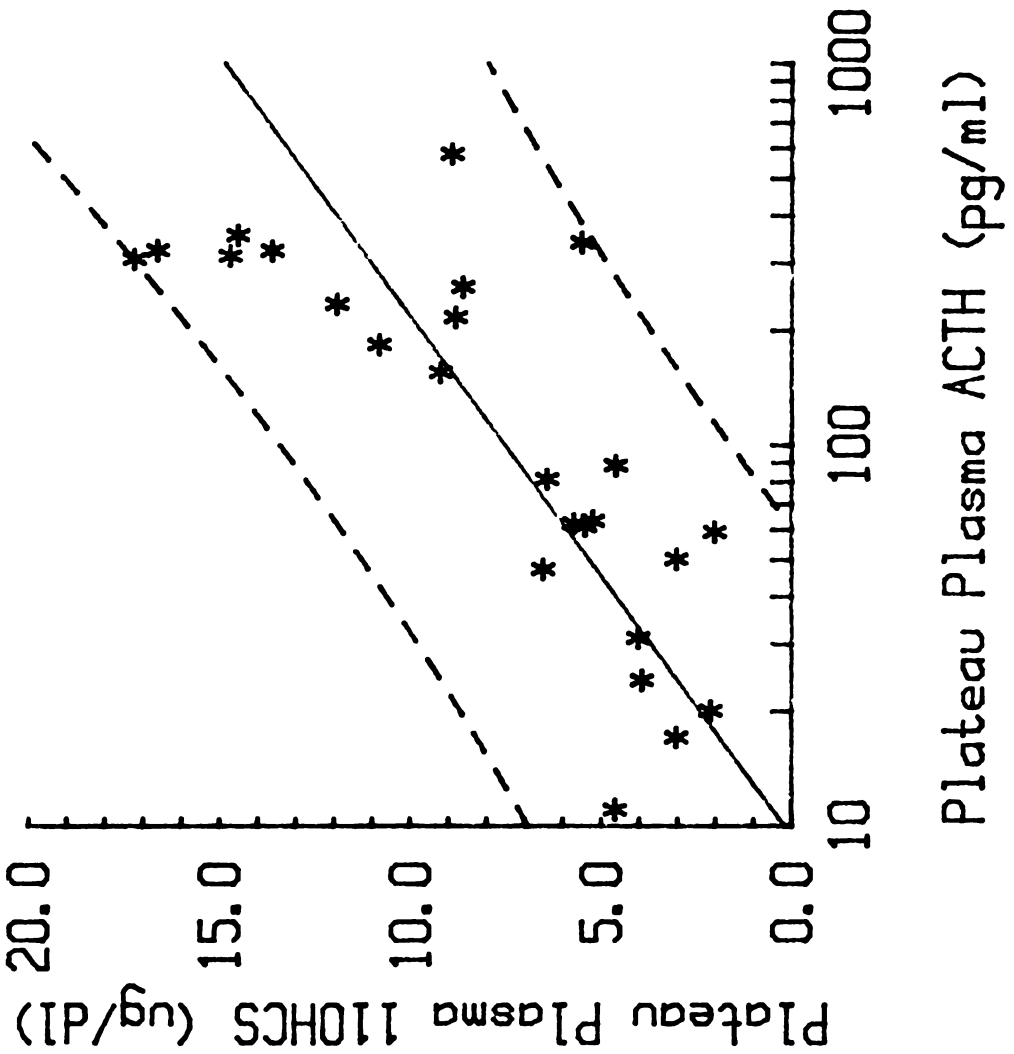
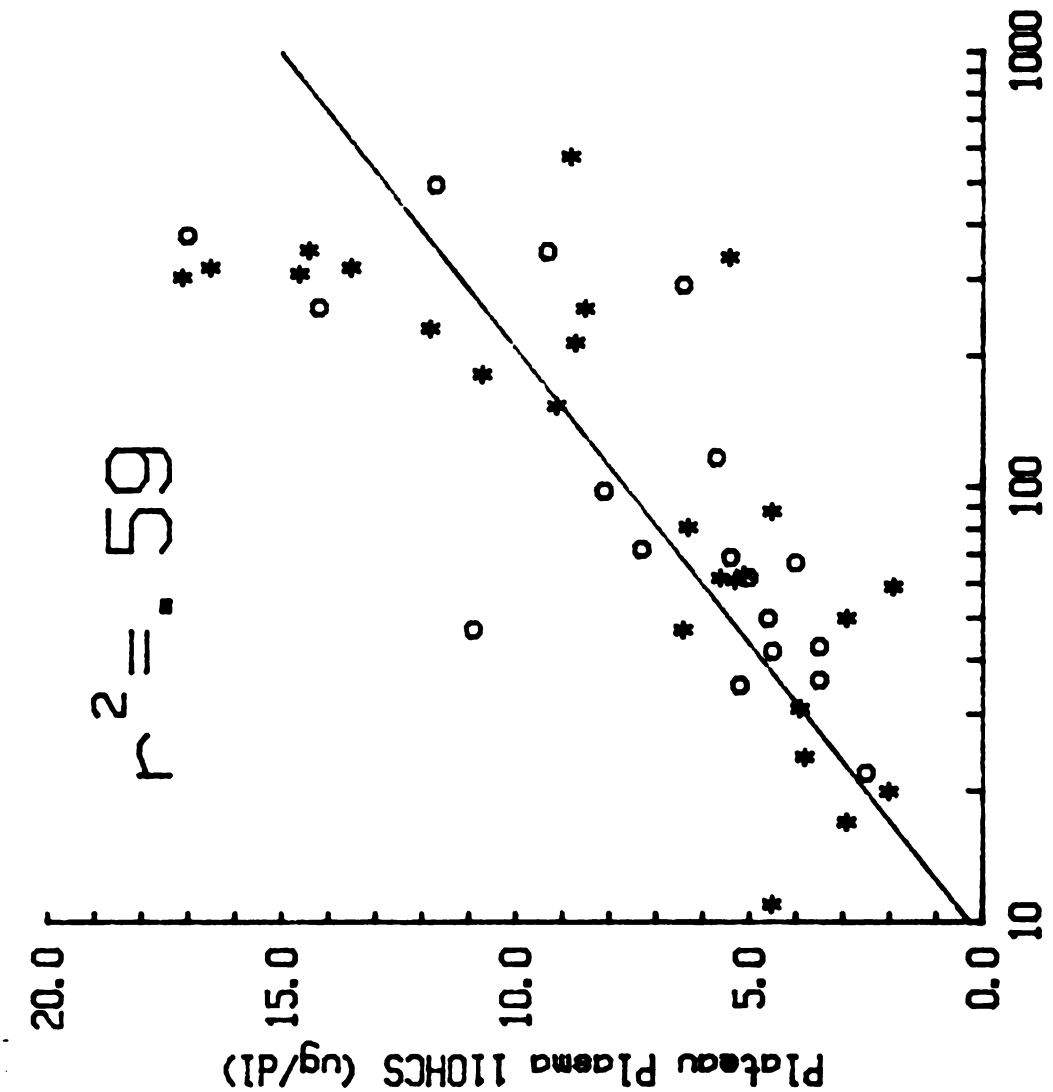

Plateau Plasma 11OHCS (ug/dl)
20.0
15.0
10.0
5.0
0.0
10
100
1000
Plateau Plasma ACTH (pg/ml)

Figure 28

Correlation of plateau plasma corticosteroid and plateau plasma ACTH concentrations in 25 experiments in which dogs (rectal temperatures 38.2-41 C) were bled 15 ml/kg and in 18 experiments in which normovolemic, normothermic dogs were infused with 10, 30, and 150 ng ACTH/min.


$r^2 = .59$
Plateau Plasma 11OHCS (ug/dl)
20.0
15.0
10.0
5.0
0.0
10
100
1000
Plateau Plasma ACTH (pg/ml)

REFERENCES

[1] Addis, T., and D.R. Drury. The rate of urea excretion VII. The effect of various other factors than blood urea concentration on the rate of urea excretion. J. Biol. Chem. 55: 629-638, 1923.

[2] Altman, P.L., and D.S. Dittmer (eds.), Biology Data Book, vol. II, ed. 2, Federation of American Societies for Experimental Biology, Bethesda, 1973, p.865.

[3] Amann, A., and H. Schaefer. Uber sensible impulse im herznerven. Pflugers Archiv 246: 757-787, 1943.

[4] Anderson, C.H., M. McCally, and G.L. Farrell. The effects of atrial stretch on aldosterone secretion. Endocrinology 64: 202-207, 1959.

[5] Baertschi, A.J., and D.S. Gann. Responses of atrial mechanoreceptors to pulsation of atrial volume. J. Physiol., Lond. 273: 1-21, 1977.

[6] Baertschi, A.J., R.F. Munzner, D.G. Ward, R.N. Johnson, and D.S. Gann. Right and left atrial B-fiber input to the medulla of the cat. Brain Research 98: 189-193, 1975.

[7] Baertschi, A.J., D.G. Ward, and D.S. Gann. Role of atrial receptors in the control of ACTH. Am. J. Physiol. 231: 692-699, 1976.

[8] Bainbridge, F.A. The influence of venous filling upon the rate of the heart. J. Physiol. 50: 65-84, 1915.

[9] Baisset, A., and P. Montrasuc. Polyurie par distention auriclaire chez la chien: role de l'hormone antidiuretique. J. Physiol, Paris 49: 33-36, 1957.

[10] Baratz, R.A., and R.C. Ingraham. Sensitive bioassay method for measuring antidiuretic hormone in mammalian plasma. Proc. Soc. Exp. Biol. Med. 100: 296-299, 1959.

[11] Baratz, R.A., and R.C. Ingraham. Renal hemodynamics and antidiuretic hormone release associated with volume regulation. Am. J. Physiol. 198: 565-570, 1960.

[12] Bartter, F.C., A.G.T. Casper, C.S. Delea, and J.D.H. Slater. On the role of the kidney in control of adrenal steroid production. Metabolism 10: 1006-1020, 1961.

[13] Bartter, F.C., and D.S. Gann. On the hemodynamic regulation of the secretion of aldosterone. Circulation 21: 1016-1026, 1960

[14] Bartter, F.C., I.H. Mills, and D.S. Gann. Increase in aldosterone secretion by carotid artery constriction in the dog and its prevention by thyrocarotid arterial junction denervation. J. Clin. Invest. 39: 1330-1336, 1960.

[15] Beleslin, D., G.W. Bisset, J. Haldar, and R.L. Polak. The release of vasopressin and oxytocin in response to hemorrhage. Proc. Soc. Exp. Biol. Med. 166: 443-458, 1967.

[16] Berson, S.A., and R.S. Yalow. Radioimmunoassay of ACTH in plasma. J. Clin. Invest. 47: 2725-2751, 1968.

[17] Besser, G.M., D.N. Orth, W.E. Nicholson, R.L. Byyny, K. Abe, and J.P. Woodham. Dissociation of the disappearance of bioactive and radioimmunoactive ACTH from plasma of man. J. Clin. Endocr. Metab. 32: 595-603, 1971.

[18] Biglieri, E.G., and W.F. Ganong. Effect of hypophysectomy on adrenocortical response to bilateral carotid constriction. Proc. Soc. Exp. Biol. Med. 106: 806-809, 1961.

[19] Bohus, B., and E.R. DeKloet. Behavioral effects of neuropeptides (Endorphins, Enkephalins, ACTH fragments) and corticosteroids. In: Interaction within the Brain-Pituitary-Adrenocortical System. M.T. Jones, B. Gillham, M.F. Dallman, and S Chattopadhyay (eds), 1979, pp. 7-16.

[20] Bravo, E.L., M.C. Khosla, and F.M. Bumpus. Vascular and adrenocortical responses to a specific antagonist of angiotensin II. Am. J. Physiol. 228: 110-114, 1975.

[21] Brennan, L.A., R.L. Malvin, K.E. Jochim, and D.E. Roberts. Influence of right and left atrial receptors on plasma concentrations of ADH and renin. Am. J. Physiol. 221: 273-278, 1971.

[22] Bunag, R.D., I.H. Page, and J.W. McCubbin. Neural stimulation of release of renin. Circ. Res. 19: 851-858, 1966.

[23] Carpenter, C.C.J., J.O. Davis, and C.R. Ayers. Relation of renin, angiotensin II, and experimental renal hypertension to aldosterone secretion. J. Clin. Invest. 40: 2026-2042, 1961.

[24] Claybaugh, J.R., and L. Share. Role of the renin-angiotensin system in the vasopressin response to hemorrhage. Endocrinology 90: 453-460, 1972.

[25] Cowan, J.S. Adrenocorticotropin secretion rates following histamine injection in adult and newborn dogs. Can. J. Physiol. Pharmacol. 53: 592-602, 1974.

[26] Cowan, J.S., A.E. Davis, and R.A. Layberry. Constancy and linearity of the metabolic clearance of adrenocorticotropin in dogs. Can. J. Physiol. Pharmacol. 52: 8-13, 1974.

[27] Cryer, G.L., and D.S. Gann. Mechanism of the vagus-mediated response of secretion of cortisol to hemodynamic changes. Fed. Proc. 29: 831, 1970.

[28] Cryer, G.L., and D.S. Gann. Evidence that receptors in the right heart mediate changes in secretion of cortisol. Fed Proc. 30: 311, 1971.

[29] Cryer, G.L., and D.S. Gann. Location of vagal receptors controlling adrenal corticosteroid secretion. Am. J. Physiol. 225: 1346-1350, 1973

[30] Cryer, G.L., and D.S. Gann. Right atrial receptots mediate the adrenocortical response to small hemorrhage. Am. J. Physiol. 227: 325-328, 1974.

[31] Dallman, M.F., D. DeManicor, and J. Shinsako. Diminished corticotrope capacity to release ACTH during sustained stimulation: the twenty-four hours after bilateral adrenalectomy in the rat. Endocrinology 95: 65-73, 1974.

[32] Dallman, M.F., W.C. Engeland, and J. Shinsako. Compensatory adrenal growth: a neurally mediated reflex. Am. J. Physiol. 231: 408-414, 1976.

[33] Davis, J.O., C.C.J. Carpenter, C.R. Ayers, J.E. Holman, and R.C. Bahn. Evidence for secretion of an aldosterone-stimulating hormone by the kidney. J. Clin. Invest. 40: 684-696, 1961.

[34] Davis, J.O., and R.H. Freeman. Mechanisms regulating renin release. Physiol. Rev. 56: 1-56, 1976.

[35] Davis, J.O., M.M. Pechet, W.C. Ball, and M.J. Goodkind. Increased aldosterone secretion in dogs with right-sided congestive heart failure and in dogs with thoracic inferior vena cava constriction. J. Clin. Invest. 36: 689-694, 1957.

[36] Deane, H.W., and G.M.C. Masson. Adrenal cortical changes in rats with various types of experimental hypertension. J. Clin. Endocrinol. 11: 193-208, 1951.

[37] Dexter, L., H.A. Frank, F.W. Haynes, and M.D. Altshule. Traumatic shock. VI. The effect of hemorrhagiic shock on the concentration of renin and hypertensinogen in the plasma in unanesthetized dogs. J. Clin. Invest. 22: 847-852, 1943.

[38] Drury, D.R., J.P. Henry, and J. Goodman. The effects of continuous pressure breathing on kidney function. J. Clin. Invest. 26: 945-951, 1947.

[39] Dufau, M.L. and B. Kliman. Pharmacologic effects of angiotensin II-amide on aldosterone and corticosterone secretion by the intact anesthetized rat. Endocrinology 82: 29-36, 1968.

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

[41] Egdahl, R.H. Corticosteroid secretion following caval constriction in dogs with isolated pituitaries. Endocrinology 68: 226-231, 1961.

[42] Engeland, W.C., G.J. Byrnes, K. Presnell, H. Santana, and D.S. Gann. Changes in ACTH and cortisol secretion after hemorrhage: Quantitative and temporal considerations. Endocrinology 104: 627A, 1979.

[43] Engeland, W.C., J. Shinsako, C.M. Winget, J. Vernikos-Danellis, and M.F. Dallman. Circadian patterns of stress-induced ACTH secretion are modified by corticosterone responses. Endocrinology 100: 138-147, 1977.

[44] Espiner, E.A., S. Lun, and D.S. Hart. Failure of angiotensin II to inhibit corticotropin-stimulated cortisol secretion. Endorinology 102: 1362-1365, 1978.

[45] Farrell, G.L., R.S. Rosnagle, and E.W. Rauschkolb. Increased aldosterone secretion in response to blood loss. Circ. Res. 4: 606-611, 1956.

[46] Fulkerson, W.J., and B.Y. Tang. Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. J. Endocrinol. 81: 135-141, 1979.

[47] Gann, D.S. Carotid vascular receptors and control of adrenal corticosteroid secretion. Am. J. Physiol. 211: 193-197, 1966.

[48] Gann, D.S. Parameters of the stimulus initiating the adrenocortical response to hemorrhage. Ann. N. Y. Acad. Sci. 156: 740-755, 1969.

[49] Gann, D.S. Vagus-mediated control of adrenal corticosteroid secretion in the dog. Am. J. Physiol. 221: 1004-1008, 1971.

[50] Gann, D.S., cortisol secretion after hemorrhage: multiple mechanisms. Nephron 23: 119-124, 1979.

[51] Gann, D.S., and G.L. Cryer. Feedback control of ACTH secretion by cortisol. In: Brain-Pituitary-Adrenal Interrelationships, E. Redgate and A. Brodish, (eds). Karger, Basel, 1973. pp 197-223.

[52] Gann, D.S., G.L. Cryer, and J.C. Pirkle. Physiological inhibition and facilitation of adrenocortical response to hemorrhage. Am. J. Physiol. 232: R5-R9, 1977.

[53] Gann, D.S., and R.H. Egdahl. Mechanisms of hemodynamic stimulation of 17-hydroxycorticoid secretion. J. Clin. Invest. 43: 1289, 1964.

[54] Gann, D.S., and R.H. Egdahl. Responses of adrenal corticosteroid secretion to hypotension and hypovolemia. J. Clin. Invest. 44: 1-7, 1965.

[55] Gann, D.S., K.L. Gould, J.E. Morley, and J.V. Mumma. Effects of vagotomy and or carotid constriction on corticosteroid secretion in the dog. Proc. Soc. Exp. Biol. Med. 115: 944-947, 1964.

[56] Gann, D.S., I.H. Mills, and F.C. Bartter. On the hemodynamic parameter mediating increases in aldosterone secretion in the dog. Fed. Proc. 19: 605-610, 1960.

[57] Gann, D.S., and R.H. Travis. Mechanisms of hemodynamic control of secretion of aldosterone in the dog. Am. J. Physiol. 207: 1095-1101, 1964.

[58] Gann, D.S., D.G. Ward, and D.E. Carlson. Neural control of ACTH: a homeostatic reflex. Rec. Prog. Horm. Res. 34: 357-396, 1978.

[59] Ganong, W.F., A.T. Boryczka, L.C. Lorenzen, and A.S. Egge. Lack of effect of alpha-ethyltryptamine on ACTH secretion when blood pressure is held constant. Proc. Soc. Exp. Biol. Med. 124: 558-559, 1967.

[60] Ganong, W.F., and P.J. Mulrow. Evidence of secretion of an aldosterone-stimulating substance by the kidney. Nature 190: 1115-1116, 1961.

[61] Ganong, W.F., and P.J. Mulrow. Role of the kidney in adrenocortical response to hemorrhage in hypophysectomized dogs. Endocrinology 70: 182-188, 1962.

[62] Ganong, W.F., P.J. Mulrow, A. Boryczka, and G. Cera. Evidence for a direct effect of angiotensin II on adrenal cortex of the dog. Proc. Soc. Exp. Biol. Med. 109: 381-384, 1962.

[63] Gauer, O.H., J.P. Henry, H.O. Sieker, and W.E. Wendt. The effect of negative-pressure breathing on urine flow. J. Clin. Invest. 33: 287-296, 1954.

[64] Genest, J., P. Biron, E. Kiow, W. Nowaczynski, P. Bouchier, and M. Chretien. Studies of the pathogenesis of human hypertension. The adrenal cortex and renal pressor mechanism. Ann. Int. Med. 55: 12-28, 1961.

[65] Ginsburg, M., and H. Heller. Antidiuretic activity in blood obtained from various parts of the cardiovascular system. J. Endocrinol. 9: 274-282, 1953.

[66] Goetz,K.L., G.C. Bond, and W.E. Smith. Effect of moderate hemorrhage in humans on plasma ADH and renin. Proc. Soc. Exp. Biol. Med. 145: 277-280, 1974.

[67] Goldblatt, H., J. Lynch, R.F. Hanzal, and W. Summerville. Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J. Exptl. Med. 59: 347-379, 1934.

[68] Greenspan, F.S., C.H. Li, and H.M. Evans. Disappearance rate of adrenocorticotropic hormone from rat's plasma after intravenous injection. Endocrinology 46: 261-264, 1950.

[69] Grizzle, W.E., M.F. Dallman, L.P. Schramm, and D.S. Gann. Inhibitory and facilitatory hypothalamic areas mediating ACTH release in the cat. Endocrinology 95: 1450-1461, 1974.

[70] Gross, F. Adrenocortical function and renal pressor mechanisms in experimental hypertension. In Bock, K.D. and P.T. Cottier, eds., Essential Hypertension, and International Symposium (Ciba Limited). Springer-Verlaug, Berlin, 1960. pp. 92-111.

[71] Guillemin, R.W., W.E. Dear, and R.A. Liebelt. Nycthemeral variations in plasma free corticosteroid levels of the rat. Proc. Soc. Exp. Biol. Med. 101: 394-395, 1959.

[72] Hamilton, A.S., and D.A. Collins. The homeostatic role of a renal humoral mechanism in homorrhage and shock. Am. J. Physiol. 136: 275-284, 1942.

[73] Hellman, L., F. Nakada, J. Curti, E.D. Weitzman, J. Kream, H. Roffward, S. Ellman, D.K. Fukushima, and T.F. Gallagher. Cortisol is secreted episodically by normal man. J. Clin. Endocr. Metab. 30: 411-422, 1970.

[74] Henry, J.P., and O.H. Gauer. Certain hemodynamic factors concerned with control of blood volume. Fed. Proc.10: 62, 1951.

[75] Henry, J.P., O.H. Gauer, and J.L. Reeves. Evidence of the atrial location of receptors influencing urine flow. Circ. Res. 4: 85-90, 1956.

[76] Henry, J.P., O.H. Gauer, H.O. Sieker, W.E. Wendt, J.L. Reeves, and M.F. Lee. Effects of obstruction of pulmonary vascular bed and interference with thoracic vago-sympathetics upon urine flow. Proc. XIX Int. Physiol. Congr. 1953, pp 453-454.

[77] Henry, J.P., and J.W. Pearce. The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. J. Physiol. 131: 572-585, 1956.

[78] Hodge, R.L., R.D. Lowe, K.K.F. Ng, and J.R. Vane. Role of the vagus nerve in the control of the concentration of angiotensin II in the circulation. Nature 221: 177-179, 1969.

[79] Hodge, R.L., R.D. Lowe, and J.R. Vane. The effect of acute changes in blood volume on the concentration of circulating angiotensin in dogs. J. Physiol., Lond. 181: 59-60P, 1965.

[80] Hodge, R.L., R.D. Lowe, and J.R. Vane. Increased Angiotensin formation in response to carotid occlusion in the dog. Nature 211: 491-493, 1966a.

[81] Hodge, R.L., R.D. Lowe, and J.R. Vane. The effects of alteration of blood volume on the concentration of circulating angiotensin in anesthetized dogs. J. Physiol, Lond. 185: 613-626, 1966b.

[82] Hodges, J.R., M.T. Jones, and M.A. Stockham. Effect of emotion on blood corticotrophin and cortisol concentrations in man. Nature 193: 1187-1188, 1962.

[83] Hopkinson, B.R., and W.G. Schenk. The electromagnetic measurement of liver blood flow and cardiac output in conscious dogs during feeding and exercise. Surgery 63: 970-975, 1968.

[84] Huidobro, F., and E. Braun-Mendez. The secretion of renin by the intact kidney. Am. J. Physiol. 137: 47-55, 1942.

[85] Hume, D.M. The method of hypothalamic regulation of pituitary and adrenal secretion in response to trauma. In: Pathophysiologia Diencephalica. Symposium Internationale Milano, 1956. S.B. Curri and L. Martini (eds). Springer-Verlaug, Wien, 1958.

[86] Hume, D.M., and D.H. Nelson. Adrenal cortical function in surgical shock. Surg. Forum, Proc. Congr. Am. Coll. Surgeons, 40th, 1954. Philadelphia, Saunders, 1955.

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

[88] Jones, C.T. Normal fluctuations in the concentration of corticosteroid and adrenocorticotrophin in the plasma of foetal and pregnant sheep. Horm. Metab. Res. 11: 237-241, 1979.

[89] Kaplan, N.M. The biosynthesis of adrenal steroids: effects of angiotensin II, adrenocorticotropin, and potassium. J. Clin. Invest. 44: 2029-2039, 1965.

[90] Kaplan, N.M., and F.C. Bartter. The effect of ACTH, renin, angiotensin II and various precursors of biosynthesis of aldosterone by adrenal slices. J. Clin. Invest. 41: 715-724, 1962.

[91] Keil, L.C., and W.B. Severs. Reduction in plasma vasopressin levels of dehydrated rats following acute stress. Endocrinology 100: 30-38, 1977.

[92] Kohlstaedt, K.G., and I.H. Page. The liberation of renin by perfusion of kidneys following reduction of pulse pressure. J. Exp. Med. 72: 201-216, 1940.

[93] Kolanowski, J., and J. Crabbe. Characteristics of the response of human adrenocortical cells to ACTH. Molecular and Cellular Endocrinology 5: 255-267, 1976.

[94] Krieger, D.T. Rhythms in CRF, ACTH, and corticosteroids. In: Endocrine Rhythms, D.T. Krieger (ed) Raven Press, New York, 1979. pp 123-142.

[95] Krieger, D.T., and W. Allen. Relationship of bioassayable and immunoassayable plasma ACTH and cortisol concentretions in normal subjects and in patients with Cushing's Disease. J. Clin. Endocr. Metab. 10: 675-687, 1975.

[96] Krieger, D.T., W. Allen, F. Rizzo, and H.P. Krieger. Characterization of the normal patterns of plasma corticosteroid levels. J. Clin. Endocr. Metab. 32: 266-284, 1971.

[97] L'Age, M., A. Gonzalez-Luque, and F.E. Yates. Adrenal blood flow dependence of cortisol secretion rate in unanesthetized dogs. Am. J. Physiol. 219: 281-287, 1970.

[98] Lake, R.B., and D.S. Gann. Dynamic response of the intact canine adrenal to infused ACTH. Annals of Biomedical Engineering 1: 56-68, 1972.

[99] Laragh, J.H., M. Angers, W.G. Kelley, and S. Lieberman. Hypotensive agents and pressor substances. J. Am. Med. Assoc. 174: 234-240, 1960.

[100] Laycock, J.F., W. Penn, D.G. Shirley, and S.J. Walter. The role of vasopressin in blood pressure regulation immediately following acute haemorrhage in the rat. J. Physiol., Lond., 296: 267-275, 1979.

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

[102] Ledsome, J.R., and J.M. Mason. The effects of vasopressin on the diuretic response to left atrial distension. J. Physiol, Lond. 221: 427-440, 1972.

[103] Lydtin, H., and W.F. Hamilton. Effect of acute changes in left atrial pressure on urine flow in unanesthetized dogs. Am. J. Physiol. 207: 530-536, 1964.

[104] Malayan, S.A., D.J. Ramsay, L.C. Keil, and I.A. Reid. Effects of vasopressin on plasma renin activity, blood pressure, heart rate, and plasma 17-0H corticosteroid concentration in conscious dogs. Physiologist 21: 75, 1978.

[105] Mancia, G., J.C. Romero, and J.T. Shepard. Continuous inhibition of renin release in dogs by vagally mediated receptors in the cardiopulmonary region. Circ. Res. 36: 529-535, 1975.

[106] Maran, J.W., D.E. Carlson, W.E. Grizzle, D.G. Ward, and D.S. Gann. Organization of the medial hypothalamus for control of adrenocorticotropin in the cat. Endocrinology 103: 957-970, 1978.

[107] Maran, J.W., and F.E. Yates. Cortisol secretion during intrapituitary infusion of angiotensin II in conscious dogs. Am. J. Physiol. 233: E273-E285, 1977.

[108] McCormick, J.R., A.H. Herman, W.M. Lien, and R.H. Egdahl. Hydrocortisone metabolism in the adrenalectomized dog: the quantitative significance of each organ system in the total metabolic clearance of hydrocortisone. Endocrinology 94: 17-26, 1974.

[109] McPhee, M.S., and W.H. Lakey. Neurogenic release of renin in mongrel dogs. Can. J. Surg. 14: 142-147, 1971.

[110] Miller, R.E., J.W. Maran, G.D. Meier, and F.E. Yates. Static gain and dynamics of cortisol secretory response to intravenous corticotropin (ACTH) in unanesthetized dogs. Annals of Biomedical Engineering 4: 364-409, 1976.

[111] Mills, I.H., A. Casper, and F.C. Bartter. On the role of the vagus in the control of aldosterone secretion. Science 128: 1140-1141, 1958.

[112] Morton, J.J., P.L. Padfield, and M.L. Forsling. A radioimmunoassay for plasma arginine-vasopressin in man and dog. J. Endocr. 65: 411-424, 1975.

[113] Mulrow, P.J., and W.F. Ganong. Aldosterone secretion following hemorrhage in hypophysectomized dogs. Fed. Proc. 19; 152, 1960.

[114] Murphy, B.E.P. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurements of various steroids in body fluids by competitive protein-binding radioassay. J. Clin. Endocrinol. Metab. 27: 973-990, 1973.

[115] Nelson, D.H., and D.M. Hume. Corticosteroid secretion in the adrenal venous blood of the hypophysectomized dog as an assay for ACTH. Endocrinology 57: 184-192, 1955.

[116] Nicholls, M.G., M. Tree, J.J. Brown, B.H. Douglas, R. Fraser, G.D. Hay, A.F. Lever, J.J. Morton, and J.I.S. Robertson. Angiotensin II/aldosterone dose-response curves in the dog: effect of changes in sodium balance. Endocrinology 102: 485-493, 1978.

[117] Nonidez, J.F. Identification of the receptor areas in the venae cavae and pulmonary veins which initiate reflex cardiac acceleration (Bainbridge's Reflex). Am. J. Anat. 61: 203-232, 1937.

[118] Paintal, A.S. A study of right and left atrial receptors. J. Physiol., Lond. 120: 596-610, 1953.

[119] Pearce, J.W., and D. Whitteridge. The relation of pulmonary arterial pressure variations to the activity of afferent pulmonary vascular fibres. Quart. J. Exp. Physiol. 36: 177-188, 1951.

[120] Peters, J.P. Body Water: The Exchange of Fluids in Man. Charles C. Thomas, Springfield, 1935. p. 287.

[121] Peytremann, A., W.E. Nicholson, R.D. Brown, G.W. Liddle, and J.G. Hardman. Comparative effects of angiotensin and ACTH on cyclic AMP and steroidogenesis in isolated bovine adrenal cells. J. Clin. Invest. 52: 835-842, 1973.

[122] Porter, J.C., and M.S. Klaiber. Relationship of input of ACTH to secretion of corticosterone in rats. Am. J. Physiol. 207: 789-792, 1964.

[123] Ramsay, D.J., L.C. Keil, M.C. Sharpe, and J. Shinsako. Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. Am. J. Physiol. 234: R66-R71, 1978.

[124] Redgate, E.S. Role of the baroreceptor reflexes and vasoactive polypeptides in the corticotropin release evoked by hypotension. Endocrinology 82: 704-720, 1968.

[125] Rees, L.H., D.M. Cook, J.W. Kendall, C.F. Allen, R.M. Kramer, J.G. Ratcliff, and R.A. Knight. A radioimmunoassay for rat plasma ACTH. Enodrinology 89: 254-261, 1971.

[126] Regoli, D., and J.R. Vane. The continuous estimation of angiotensin formed in the circulation of the dog. J. Physiol., Lond. 183: 513-531, 1966.

[127] Reid, I.A., and A. Jones. Effects of carotid occlusion and clonidine on renin secretion in anesthetized dogs. Clin. Sci. and Mol. Med. 51: 109s-111s, 1976.

[128] Reid, I.A., J.R. Stockigt, A. Goldfien, and W.F. Ganong. Stimulation of renin secretion in dogs by theophylline. European J. Pharmacol. 17: 325-332, 1972.

[129] Richards, J.B., and G. Sayers. Fate and excretion of adrenocorticotrophic hormone. Proc. Soc. Exp. Biol. Med. 77: 87-93, 1951.

[130] Robertson, G.L. Vasopressin in osmotic regulation in man. Ann. Rev. Med. 25: 315-322, 1974.

[131] Robertson, G.L. The regulation of vasopressin function in health and disease. Rec. Prog. Horm. Res. 33: 333-385, 1977.

[1

[1

[1

[1

[1

[1

[1

[1

[1

[14

[132] Robertson, G.L., and S. Athar. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. J. Clin. Endocr. Metab. 42: 613-620, 1976.

[133] Robertson, G., and E. Mahr. The importance of plasma osmolality in regulating antidiuretic hormone secretion in man. J. Clin. Invest. 51: 79a, 1972.

[134] Rocha e Silva, M., and M. Rosenberg. The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. J. Physiol., Lond. 202: 535-557, 1969.

[135] Rydin, H., and E.B. Verney. The inhibition of water-diuresis by emotional stress and muscular exercise. Quart. J. Exp. Physiol. 27: 343-374, 1938.

[136] Sapirstein, L.A., E. Ogden, and F.D. Southard, Jr. Renin-like substance in blood after hemorrhage. Proc. Soc. Exp. Biol. Med. 48: 505-508, 1941.

[137] Sassa, K., and H. Miyazaki. The influence of venous pressure upon heart-rate. J. Physiol., Lond. 54: 203-212, 1920.

[138] Sato, T., M. Sato, J. Shinsako, and M.F. Dallman. Corticosterone-induced changes in hypothalamic CRF content after stress. Endocrinology 97: 265-274, 1975.

[139] Sayers, G. and M. Sayers. Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress. Endocrinology 40: 265-273, 1947.

[140] Sayers, G., M.A. Sayers, T.Y. Liang, and C.N.H. Long. The cholesterol and ascorbic acid content of the adrenal, liver, brain, and plasma following hemorrhage. Endocrinology 37: 96-110, 1945.

[141] Seelig, S., and G. Sayers. Isolated adrenal cortex cells: ACTH agonists, partial agonists, cyclic AMP, and corticosterone production. Archives of Biochemistry and Biophysics 154: 230-239, 1973.

[142] Segar, W.E., and W.W. Moore. The regulation of antidiuretic hormone release in man. J. Clin. Invest. 47: 2143-2151, 1968.

[143] Selye, H. and Schenker. A rapid and sensitive method for bioassay of the adrenal cortical hormone. Proc. Soc. Exp. Biol. Med. 39: 518-522, 1938.

[144] Share, L. Acute reduction in extracellular fluid volume and the concentration of antidiuretic hormone in blood. Endocrinology 69: 925-933, 1961.

[145] Share, L. Vascular volume and blood level of antidiuretic hormone. Am. J. Physiol. 202: 791-794, 1962.

[146] Share, L. Effects of carotid occlusion and left atrial distention on plasma vasopressin titer. Am. J. Physio. 208: 219-223, 1965.

[147] Share, L. Role of peripheral receptors in the increased release of vasopressin in response to hemorrhage. Endocrinology 81: 1140-1146, 1967a.

[148] Share, L. Vasopressin, its bioassay and the physiological control of its release. Am. J. Med. 42: 701-712, 1967b.

[149] Share, L., J.R. Claybaugh, F.E. Hatch, J.G. Johnson, S. Lee, F.E. Muirhead, and P.Shan. Effects of change in posture and of sodium depletion on plasma levels of vasopressin and renin in normal humna subjects. J. Clin. Endocrinol. Metab. 35: 171-174, 1972.

[150] Share, L., and M.N. Levy. Cardiovascular receptors and blood titer of antidiuretic hormone. Am. J. Physiol. 203: 425-428, 1962.

[151] Share, L., and M.N. Levy. Carotid sinus pressure, a determinant of plasma antidiuretic hormone activity. Am. J. Physiol. 211: 721-724, 1966.

[152] Sieker, H.O., O.H. Gauer, and J.P. Henry. The effect of continuous negative pressure breathing on water and electrolyte excretion by the human kidney. J. Clin. Invest. 33: 572-577, 1954.

[153] Simpson, S.A., and J.F. Tait. A quantitative method for the bioassay of the effect of adrenal cortical steroids on mineral metabolism. Endocrinology 50: 150-161, 1952.

[154] Simpson, S.A., J.F. Tait, A. Wettstein, R. Neher, J.v.Euw, and T. Reichstein. Isolation from adrenals of a new crystalline hormone with extremely high effect on the metabolism of minerals. Experientia 9: 333-335, 1953.

[155] Skillman, J.J., E.K. Eltringham, R.H. Goldensen, and F.D. Moore. Transcapillary refilling after hemorrhage in the splenectomized dog. J. Surg. Res. 8: 57-67, 1968.

[156] Skinner, S.L., J.W. McCubbin, and I.R. Page. Renal baroreceptor control of renin release. Science 141: 814-816, 1963.

[157] Slater, J.D.H., B.H. Barbour, H.H. Henderson, A.G.T. Casper, and F.C. Bartter. Influence of the pituitary and the renin-angiotensin system on the secretion of aldosterone, cortisol, and corticosterone. J. Clin. Invest. 42: 1504-1520, 1963.

[158] Steenburg, R.W., and W.F. Ganong. Observations on the influence of extraadrenal factors on circulating 17-hydroxycorticoids in the surgically stressed, adrenalectomized dog. Surgery 38: 92-104, 1955.

[159] Stockigt, J.R., R.D. Collins, and E.G. Biglieri. Determination of plasma renin concentration by angiotensin I immunoassay. Circ. Res. 28(Suppl 2): 175-189, 1971.

[160] Sydnor, K.L., and G. Sayers. Biological half-life of endogenous ACTH. Proc. Soc. Exp. Biol. Med. 83: 729-733, 1953.

[161] Sydnor, K.L., and G. Sayers. Blood and pituitary ACTH in intact and adrenalectomized rats after stress. Endocrinology 55: 621-636, 1954.

[162] Tagawa, H., A.J. Vander, J.P. Bonjour, and R.L. Malvin. Inhibition of renin secretion by vasopressin in anesthetized sodium-deprived dogs. Am. J. Physiol. 220: 949-951, 1971.

[163] Tait, J.F., S.A.S. Tait, B. Little, and K.R. Laumas. The disappearance of 7-3H-d-Aldosterone in the plasma of normal subjects. J. Clin. Invest. 40: 72-80, 1961.

[164] Thames, M.D. Reflex suppression of renin release by ventricular receptors with vagal afferents. Am. J. Physiol. 233: H181-H184, 1977.

[165] Thames, M.D., and P.G. Schmid. Ventricular receptors with vagal afferents inhibit ADH secretion in dogs. Fed. Proc. 39: 840, 1980.

[166] Urquhart, J. Adrenal blood flow and the adrenocortical response to corticotropin. Am. J. Physiol. 209: 1162-1168, 1965.

[167] Urquhart, J. Dynamics of ACTH in blood. Fed. Proc. 26: 586, 1967.

[168] Urquhart, J. Physiological actions of adrenocorticotrophic hormone. In: Handbook of Physiology, Section 7, vol. IV, The Pituitary Gland and its Neuroendocrine Control. R.O. Greep and E.B. Astwood (eds). The American Physiological Society, Washington, 1974.

[169] Urquhart, J., R.L. Krall, and C.C. Li. Analysis of the Koritz-Hall hypothesis for the regulation of steroidogenesis by ACTH. Endocrinology 83: 390-394, 1968.

[170] Urquhart, J., and C.C. Li. The dynamics of adrenocortical secretion. Am. J. Physiol. 214: 73-85, 1968.

[171] Urquhart, J., and C.C. Li. Dynamic testing and modeling of adrenocortical secretory function. Ann. N. Y. Acad. Sci. 156: 756-778, 1969.

[172] Vander, A.J., and R. Miller. Control of renin secretion in the anesthetized dog. Am. J. Physiol. 207: 537-546, 1964.

[173] Vernikos-Danellis, J., Sensitivity of the adrenal corticosterone response to ACTH as a function of time after hypophysectomy. Endocrinology 84: 1507-1508, 1969.

[174] Vogt, M. The output of cortical hormone by the mammalian suprarenal. J. Physiol., Lond. 102: 341-356, 1943.

[175] Walsh, E.G., and D. Whitteridge. Vagal activity and the tachypnea produced by multiple pulmonary emboli. J. Physiol., Lond. 103: 37-38P, 1944.

[176] Ward, D.G., A.J. Baertschi, and D.S. Gann. Neurons in medullary areas controlling ACTH: atrial input and rostral projections. Am. J. Physiol. 233: R116-R126, 1977.

[177] Ward, D.G., M.G. Bolton, and D.S. Gann. Inhibitory and facilitatory areas of the ventral midbrain mediating release of corticotropin in the cat. Endocrinology 102: 1147-1154, 1978.

[178] Ward, D.G., and D.S. Gann. Inhibitory and facilitatory areas of the dorsal medulla mediating ACTH release in the cat. Endocrinology 99: 1213-1219, 1976.

[179] Ward, D.G., W.E. Grizzle, and D.S. Gann. Inhibitory and facilitatory areas of the rostral pons mediating ACTH release in the cat. Endocrinology 99: 1220-1228, 1976.

[180] Weinstein, H., R.M. Berne, and H. Sachs. Vasopressin in Blood: Effect of hemorrhage. Endocrinology 66: 712-718, 1960.

[181] Weitzman, E.D., D. Fukushima, C. Nogiere, H. Roffwag, T.G. Gallagher, and L. Hellman. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J. Clin. Endocr. Metab. 33: 14-22, 1971.

[182] Whitteridge, D. Afferent nerve fibers from the heart and lungs in the cervical vagus. J. Physiol., Lond. 107: 496-512, 1948.

[183] Winer, B.J. Statistical Principles in Experimental Design. McGraw-Hill, New York, 1971.

[184] Wolf, R.L., M. Mendlowitz, L.J. Soffer, J. Roboz, and S.E. Gitlow. Metabolism of corticotropin in man. Proc. Soc. Exp. Biol. Med. 119: 244-248, 1965.

[185] Wood, C.E., J. Shinsako, L.C. Keil, D.J. Ramsay, and M.F. Dallman. Hemorrhage-induced increase in peripheral 11OHCS levels in the absence of increased ACTH. Fed. Proc. 37: 811, 1978.

[186] Wood, C.E., J. Shinsako, and M.F. Dallman. Peripheral corticosteroid (11OHCS) responses to 5 min steps of 6 doses ACTH infused with or without hemorrhage in the conscious dexamethasone-pretreated dog. Physiologist 22: 135, 1979.

[187] Wood, C.E., J. Shinsako, and M.F. Dallman. ACTH and corticosteroids (11OHCS) in peripheral plasma from conscious dogs. Fed. Proc. 39: 1072, 1980a.

[188] Wood, C.E., J. Shinsako, and M.F. Dallman. Adrenal responses are initiated by unmeasurable increases in ACTH. Physiologist (in press), 1980b.

[189] Wood, M.K., J. Shinsako, and M.F. Dallman. ACTH and corticosteroid (11ØHCS) responses to hypoglycemia in conscious dogs. Physiologist (in press), 1980.

[190] Yalow, R.S., S.M. Glick, J. Roth, and S.A. Berson. Radioimmunoassay of human plasma ACTH. J. Clin. Endocrinol. Metab. 24: 1219-1225, 1964.

Does ACTH Mediate the Corticosteroid Response to 15 ml/kg Hemorrhage in Conscious Dogs?

by Charles Evans Wood

The hypothalamus-pituitary-adrenal axis plays a role in homeostatic responses to a number of stimuli. In hemorrhage, the increased secretion of corticosteroids (11OHCS) is required for restoration of normal blood volume. However, not much is known about the functioning of this neuroendocrine reflex in conscious animals. These studies were designed to: 1) evaluate the dose-response relationships of hemorrhage volume to the ACTH-11OHCS response; 2) identify factors which modulate the response; and 3) explain the relationship of ACTH and 11OHCS during the response to 15 ml/kg hemorrhage.

To determine the threshhold ACTH and 11OHCS response to hemorrhage, conscious dogs were rapidly (<3 min) bled 15 ml/kg from previously implanted femoral arterial catheters, or 10 ml/kg from carotid skin loops. Hemorrhage of 15 ml/kg, but not 10 ml/kg, decreased blood pressure and increased heart rate and circulating ACTH and 11OHCS concentrations. However, dogs with

elevated rectal temperatures (39-41 C) responded to 15 ml/kg hemorrhage with greater decreases in blood pressure and greater increases in heart rate and plasma ACTH, 11OHCS, and vasopressin concentrations than normothermic (38-39 C) dogs. These results suggest that fever or factors contributing to or associated with fever modify the response to hemorrhage.

Normothermic dogs responded to 15 ml/kg hemorrhage with small, slow ACTH responses (peak increase 11 pg/ml at 25 min) that were apparently dissociated from the large, faster 11OHCS responses (peak increase 2.7 ug/dl at 20 min). In the individual dogs, the first rise in ACTH above control was often preceeded by the first rise in 11OHCS. The clearance and/or distribution volume of 11OHCS was increased during hypovolemia in normothermic dogs, suggesting that the increase in plasma 11OHCS concentration underestimated the increase in the adrenal 11OHCS secretion rate.

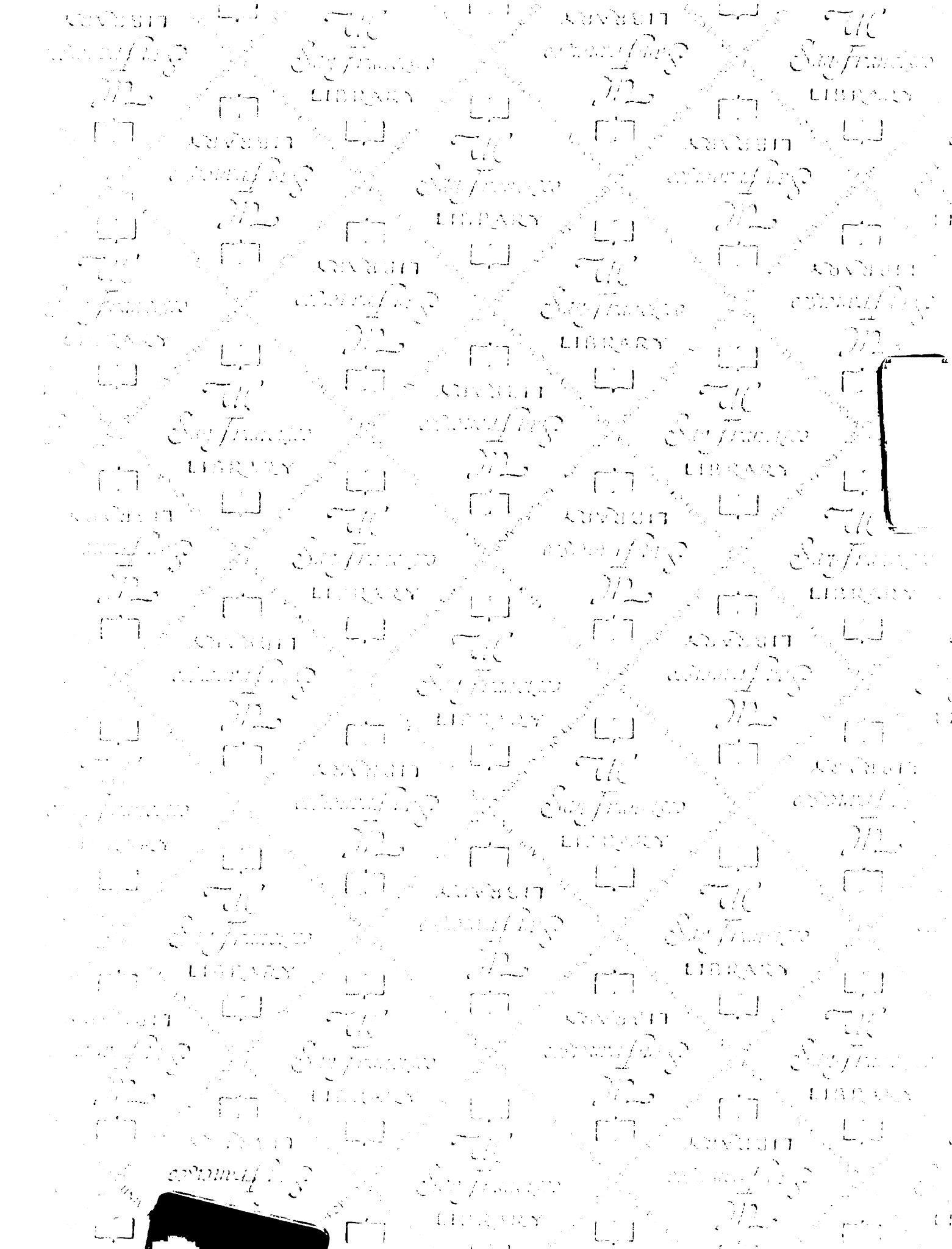
To understand the relationship between plasma ACTH and 11OHCS, plasma ACTH and 11OHCS concentrations were measured during infusions of ACTH and saline. In dogs infused with saline, plasma ACTH fluctuated by as much as 20 pg/ml, suggesting that ACTH is secreted episodically in dogs as it is in man. In these dogs, plasma 11OHCS usually did not change. Infusion of 10, 30, and 150 ng ACTH/min elevated plasma 11OHCS to levels

linearly related to the logarithm of the plasma ACTH concentration. Plasma 11OHCS responses to 3 pulses of 100 or 300 ng ACTH in 30 min were equal to responses to the same total amounts of ACTH given as constant infusions, suggesting that the magnitude of the adrenal response is determined by the total dose of ACTH rather than the pattern of administration. In all experiments, the lag between first elevations in ACTH and 11OHCS was 3-5 min. Overall data were also used to calculate estimates of ACTH half-disappearance time (1.4-3.8 min), total clearance rate (20.4-40.7 ml/kg/min), and volume of distribution. (110-113 ml/kg).

Two experimental designs were used to test whether 15 ml/kg hemorrhage increased adrenal sensitivity to ACTH in dexamethasone-pretreated dogs. Dexamethasone pretreatment (4 mg, given either s.c., 12 and 2 hours or i.a., 4 hours before the experiment) was shown not to acutely alter adrenal responsiveness to ACTH. Hemorrhage did not change the nonsteady-state 11OHCS responses to four 5 min steps of ACTH infusion (38-380 ng/min) or the steady-state responses to constant 30 min infusions of 5, 10, and 20 ng ACTH/min. Hemorrhage did not change the clearance rate and/or distribution volume of cortisol in these experiments, suggesting that dexamethasone pretreatment inhibited the hemorrhage-induced increases in cortisol clearance/distribution observed

previously. Because hemorrhage altered neither the plasma 11OHCS response to infused ACTH nor the rate of cortisol clearance, hemorrhage did not change adrenal sensitivity to ACTH. Five ng/min infusions of ACTH elevated plasma 11OHCS 3 ug/dl, while theoretically increasing ACTH 6-12 pg/ml. The first rise in ACTH causing the first rise in 11OHCS (assuming a 3 min lag) would have been 3.5 pg/ml. This small increase in plasma ACTH cannot be measured using our radioimmunoassay. Therefore, it is possible that the 11OHCS response to 15 ml/kg hemorrhage was mediated by ACTH, but that the small increases in ACTH initiating the 11OHCS responses were obscured by fluctuations in plasma ACTH.

Mary J. Dallman


San Francisco
LIBRARY