Title
Changes in plasma adrenocorticotropic hormone in dogs subjected to dexamethasone and multiple laparotomies

Author
Coulton, Lesley Dean

Publication Date
1974

Peer reviewed|Thesis/dissertation

CHANGES IN PLASMA ADRENOCORTICOTROPIC HORMONE IN DOGS SUBJECTED TO DEXAMETHASONE AND MULTIPLE LAPAROTOMIES

by

Lesley Dean Coulton
B.A., Wellesley College, 1971

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF ARTS

in

PHYSIOLOGY

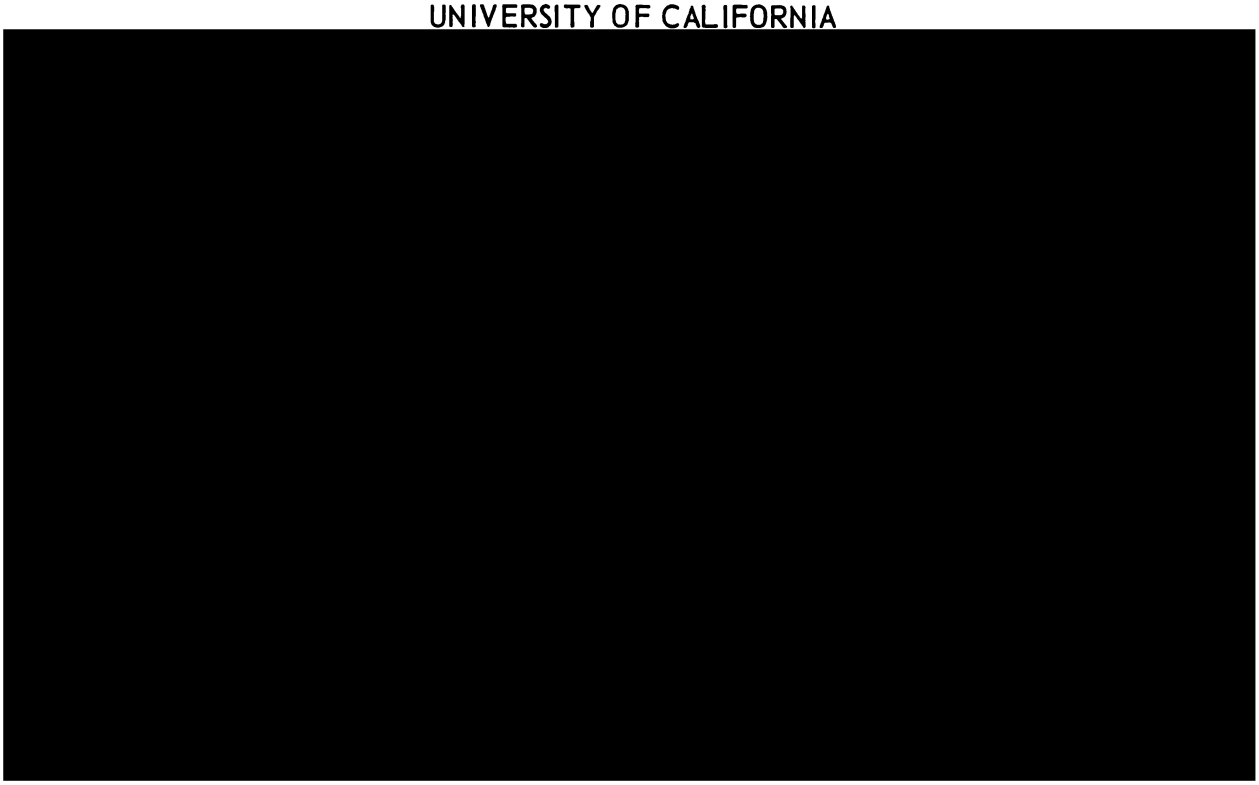
in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA



With special thanks to Drs. W. F. Ganong and M. F. Dallman for their patience, guidance, and encouragement and R. Shackleford for invaluable aid at the surgical table.

TABLE OF CONTENTS

LIST OF CHARTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION TO THE HYPOTHALAMIC-PITUITARY-ADRENAL SYSTEM.

A. Measurements

Measurement of function of the pituitary-adrenal system until recently has depended on such indices as adrenal ascorbic acid depletion (Sayers et al., 1948), eosinopenia (Steenberg et al., 1955) adrenal vein corticoid output, or peripheral plasma corticoid levels (Porter et al., 1950; Silber et al., 1954). The finding that the adrenal ascorbic acid depleting activity of an ACTH preparation may not always be directly related to the ability of ACTH to cause release of adrenal steroids emphasized the importance of direct measurements of adrenocorticotropin activity (Little et al., 1954). One could assess CRF or ACTH activity only indirectly with the end point being either the resulting corticoid output or plasma concentration reached at a given time following administration of the test preparation. Amounts of CRF and ACTH above those which maximally stimulated corticoid secretion could not be distinguished one from another. There have been many studies of sequential changes in peripheral plasma corticoids and adrenal venous corticoid output in stressed dogs (Nelson et al., 1955), rats (Hodges et al., 1959; Dallman et al., 1973c) and other laboratory animals. These studies have been supplemented by occasional analyses of plasma ACTH by bioassay. Recent advances made in radioimmunoassay by Berson et al. (1956, 1962, 1968) with modifications by Landon (1968), Landon et al. (1968), Rees et al. (1971), Dallman et al. (1973b) and others have now made direct measurement of ACTH possible in the human, dog and rat.

Because human, bovine, ovine, porcine, ACTH are identical in the first 24 N-terminal amino acids immunization with this portion of the molecule,permits one to obtain an antibody with which one can assay for ACTH in all these species (Wey et al. 1964). Although the entire chemical structure of ACTH has not been ascertained in the rat or dog, it is presumed that the structures are similar enough to permit good cross-reactivity. Indeed, good cross-reactivity has been documented for rat and dog ACTH by Rees et al (1971) and Dallman et al (1973b). Furthermore, since the N-terminal 1-24 amino acid portion of the ACTH molecule is the steroidogenic component, one is more likely to be assessing biological activity of ACTH using an antibody directed against the N-terminal 1-24 amino acids than when using one directed against the C-terminal portion (Besser et al., 1971).

Whereas previous assays for ACTH were: 1) cumbersome and costly, requiring many hypophysectomized assay animals (Nelson et al., 1955; Sayers et al., 1948), large volumes of blood and, 2) were not very sensitive, the radioimmunoassay for ^{ACTH}, 1) permits measurement in as little as one milliliter of blood depending on concentration of ACTH in the plasma; 2) allows frequent sampling in the same animal; 3) does not require additional assay animals; 4) yields values of ACTH which are reproducible from assay to assay, thus not involving assay animal variation, and 5) is more sensitive, measuring down to 20-40 picograms ACTH/milliliter plasma assayed.

The new tool of the radioimmunoassay for ACTH makes

possible direct studies of the rapid fluctuations in plasma ACTH concentration during response to stress, hormonal feedback, and circadian periodicity.

This thesis describes the rapid changes in plasma ACTH concentration in the dog in response to one or more laparotomy stresses and correlates changes in plasma ACTH concentration with changes in adrenal venous 17-hydroxycorticoid output and with changes in peripheral corticoid concentrations. It also describes suppression of the ACTH secretory response to stress by negative feedback of dexamethasone administered intravenously in the dog. However, this these does not deal with the canine ACTH circadian rhythm.

B. Corticotropic Releasing Factor (CRF)

Corticotropin releasing factor (CRF) which is secreted from the median eminence region of the hypothalamus (probably from the post peduncular eminence and stalk and not from the postchiasmatic region nor from the posterior pituitary) (Porter et al., 1970) reaches its site of action, the anterior pituitary, by means of the hypothalamic-pituitary portal blood system. Wislocki and King (1936) first discerned the direction of blood flow through these vessels; their discovery led Friedgood (1970) to originate the theory of neurovascular control of the pituitary, whereby hormones (releasing and inhibiting factors) secreted by neurons with endings in the hypothalamus are carried to their site of action at the pituitary via the portal system. CRF functions primarily by

increasing the rate of release of adrenocorticotropic hormone (ACTH) from the pituitary. The exact cell type in the pituitary responsible for elaborating ACTH is uncertain; the basophil (Bloom and Fawcett, 1966), acidophil and chromophobe (Ohtsuka et al., 1972) have all been implicated. Since, in pituitaries deprived of CRF by stalk section, both content of ACTH and capacity to release ACTH are decreased, CRF must also function by increasing ACTH synthesis (Vernikos-Danellis, 1965). Whether ACTH release is dependent upon *de novo* synthesis is unclear; Vernikos-Danellis et al., claimed that ethionine, an amino acid analog and inhibitor of protein synthesis prevented CRF-induced release *in vivo*, while Estep et al. (1967) and Greer et al. (1967) both found no effect of protein inhibitors on ACTH release.

The structure of CRF is unknown although it is believed to be a polypeptide (Schally et al., 1960; Guillemin et al., 1957) and distinct from vasopressin, oxytocin, histamine, acetylcholine, norepinephrine, epinephrine, and serotonin (Guillemin et al., 1957).

By what mechanism of action does CRF effect a response at the pituitary level? Many studies on effects of ionic environment carried out on pituitary tissue cultures have led to the "stimulus secretion coupling hypothesis" (McCann, 1971). It has been demonstrated that an elevated potassium concentration, which effectively depolarizes the cells, leads to increased release of luteinizing hormone (LH) (Salmi et al., 1958; Wakabayashi et al., 1968, 1969), thyroid stimulating

hormone (TSH) (Vale et al., 1967), follicle stimulating hormone (FSH) (Wakabayashi et al., 1966; Jutisz et al., 1970), growth hormone (GH) (MacLeod et al., 1970) and ACTH (Draicer et al., 1969). This effect of potassium is reversible for both luteinizing hormone (LH) and ACTH. Removal of calcium (Ca^{++}) or addition of excess magnesium can block both potassium-induced or releasing factor stimulated release of FSH and LH. These and related observations led to the hypothesis that releasing factors alter cell permeability, allowing influx of calcium which somehow activates release of the hormone. Elevated external potassium, which depolarizes the cell, effectively modifies its permeability and thus may stimulate or mediate the effects of releasing factors.

A second possible mechanism of action of releasing factors involves the second messenger, cyclic adenosine monophosphate (cyclic AMP), which has already been shown to play a role in protein-containing granules (McCann, 1971). Presumably, specificity lies in a receptor protein in the pituitary cell membrane which responds only to the appropriate releasing or inhibiting hormone. Hormone-receptor interaction leads to an increase in cyclic AMP which somehow leads to hormone release. Several lines of evidence implicate cyclic AMP in ACTH release: (1) phosphodiesterase inhibitor aminophyline increases release of ACTH in response to vasopressin (Fleischer et al., 1969); (2) cyclic AMP or dibutyl cyclic AMP, both of which may enter cells, increase release of ACTH (Fleischer et al., 1969; (3) crude ovine hypothalamic extract administered to pituitaries

or pituitary homogenates increases adenyl cyclase and cyclic AMP levels without changing phosphodiesterase activity, whereas, cortical extract did not cause a similar increase (Zor et al., 1970). Furthermore this effect of hypothalamic extract on cyclic AMP levels was specific for pituitary cyclic AMP since no effect on adrenal, thyroid, pineal, or posterior pituitary cyclic AMP levels were observed.

Rasmussen and Tennhouse (1968) have combined the two theories, suggesting that the binding of the releasing factor to the receptor activates adenyl cyclase which increases cyclic AMP formation from adenosine triphosphate (ATP). The cyclic AMP thus formed acts at the membrane to alter cell permeability, thus permitting an influx of calcium which is necessary for granule extrusion. Furthermore, if, as in melanocyte secretion (Green 1968), a microtubular system "guides" the granules to the membrane for secretion, one might anticipate a role for calcium in activating a myosin AMP-ase on the contractile tubule (Lacy et al., 1968). In addition, cyclic AMP could have a second role in increasing hormone synthesis by activating protein synthesis as has been postulated in the adrenal cortex (Garren et al., 1966). Note that the mechanism of action of an inhibiting factor would operate through inhibition of adenyl cyclase and cyclic AMP, stabilizing the cell membrane permeability and state of depolarization (McCann 1971).

C. Adrenocorticotropic Hormone (ACTH)

ACTH is a single straight chain polypeptide of 39 amino

acids and has been synthesized. Biologic activity resides in the 24 N-terminal amino acids. Some mammalian ACTH molecules studied resemble each other precisely in the N-terminal portion but vary slightly from amino acids #25 to 39 (Chart 1).

Chart 1.

Structural differences among ACTH's isolated from the pituitaries of various species*

Structure of human ACTH: (Straight chain containing 39 amino acids.)

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

Pro-Asp-Ala-Gly-Glu-Asp-Gln-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

Species	Amino Acid Residue in Position: 25 26 27 28 29 30 31 32 33
Pig	-Asp-Gly-Ala-Glu-Asp-Gln-Leu-Ala-Glu-
Sheep	-Ala-Gly-Glu-Asp-Asp-Glu-Ala-Ser-Gln-
Beef	-Asp-Gly-Glu-Ala-Glu-Asp-Ser-Ala-Gln-

*From Ganong, W. F. Review of Medical Physiology. Lange Medical Publishers, Los Altos, California, 1971.

ACTH is released from the pituitary into the portal blood system which drains through the cavernous sinus back to the general circulation. Thus ACTH reaches its site of action, the adrenal cortex, via the systemic circulation. Alpha 1-24 ACTH-I^{131} administered intravenously (50-70 nanograms) to guinea pigs showed preferential concentration in the kidney followed by its appearance in the urine within 2 minutes (Golder et al., 1971a). Liver, lung, spleen, adrenal, plasma and heart all had similar concentrations (cpm/gm), although less than that observed in the kidney. Dexamethasone did not alter this

distribution in the guinea pig (Golder et al., 1971b). Earlier studies using crude ACTH preparations showed preferential uptake into the kidney (Richards et al., 1951) or both kidney and liver (Sonnenberg et al., 1951; Cats et al., 1957). Gold et al. (1963) showed that no significant amounts of ACTH were released from the kidneys of dogs into the circulation during either surgical stress or hemorrhage. The major source of ACTH under stress appears to be the pituitary.

In one study in 7 humans using I^{131} β-ACTH, ACTH distribution volume in terms of body weight ranged from 38-56 percent, averaging 43 percent. Distribution is a function of the size of the molecule, its charge, a binding protein, and amount present (Samuels, 1966). The latter determinant is itself a function of rate of secretion of ACTH and rate of metabolism or excretion. Voight et al. (1971) have made preliminary observations suggesting presence of an ACTH binding factor in plasma, although the physiological function of such a compound was not discussed. It is difficult to estimate the rate of ACTH secretion *in vivo* although concentration of ACTH in blood from the cavernous sinus (McFarland et al., 1960) was found to be twice as high as that from the recurrent tarsal vein in unanesthetized sheep. One would have to know the blood flow through the cavernous sinus and the fraction of that originating from the pituitary in order to estimate ACTH secretion rate in this case. Pituitary incubation studies are useful in assessing ACTH secretion rate in response to CRF or hypothalamic extracts; however, information thus acquired does not necessarily

reflect secretion rates *in vivo* in response to various stresses.

The half life of ACTH varies among species, possibly related to size and metabolic activity of the animal (Matsuyama et al., 1971) (See Chart 2). Exogenous porcine ACTH administered and studied in man has a longer half life than does synthetic human ACTH; therefore, some specificity in degradation also exists as might be expected (Besser et al., 1971).

ACTH has two important functions at the adrenal level: (1) ACTH elicits a rise in corticoid output within 2 minutes of reaching the adrenal in the circulation of the dog (Nelson & Hume, 1955; Rauschkolb et al., 1954) with maximal output occurring 4 minutes after injection of ACTH into hypophysectomized dogs (Nelson & Hume, 1955). Peripheral corticoid levels rise maximally within 15-20 minutes in the rat (Hodges & Jones, 1959) and within about 10 minutes in the dog (Salcman et al., 1970). Maximum output and peripheral concentration reached depend upon the dose of ACTH reaching the adrenal (Nelson & Hume, 1955; Ganong 1971). Duration of increased corticoid output depends on maintained high levels of ACTH (Nelson & Hume, 1955; Yates and Brennan 1968); (2) ACTH also maintains adrenal sensitivity to further ACTH. Hypophysectomized dogs respond to a single dose of ACTH with a smaller rise in corticoid output than do intact animals and the rise in hypophysectomized dogs decreases with time after hypophysectomy up until 24 hours (Ganong 1971). This trophic effect of ACTH on the adrenals does not appear to be mediated by cyclic AMP. Effects of ACTH on steroidogenesis and secretion however, do

Chart 2.

Estimates of halflife of ACTH

	Halflife (minutes)	Measured In:	ACTH Measured:	Nature of Assay:	Ref.
1.	5-10	Human	β 1-24 ^{131}I-ACTH	Decay rate	Wolf et al., 1965
2.	4-18	Human	Porcine	Bioassay	Meakin et al., 1959
3.	10-25	Human	Endog-enous	RIA*	Berson et al., 1968
4.	4-18 10-25	Human Human		Bioassay RIA	Matsukura et al, 1967
5.	20-90	Human	Porcine	Bioassay	Sayers et al., 1949
6.	7-28	Pig	Porcine	RIA	Murphy et al., 1969
7.	7	Dog	Human	RIA	Dallman (unpublished)
8.	1/2-5 1/2	Rat	Rat	Bioassay	Greenspan et al., 1950
9.	1/2-5 1/2	Rat	Rat & Porcine	Bioassay	Gemzell et al., 1951
10.	1/2-5 1/2	Rat	Endog-enous	Bioassay	Sydnor et al., 1953
11.	1/2-5 1/2	Rat	Endog-enous	Bioassay	Estep et al., 1967
12.	3	Rat	Rat	Bioassay	Richards et al, 1951
13.	4	Rat	^{131}I Porcine	Decay Rate	Sonenberg et al., 1951
14.	5-10	Rat	^{131}I Rat	Decay Rate	Cats et al., 1959
15.	2.9 4.1	Rat Rat	alpha ACTH (Li)	Bioassay RIA	Matsuyama et al., 1972

RIA* = Radioimmunoassay

appear to be mediated by cyclic AMP (Robinson et al., 1971). The action of cyclic AMP appears to be at a point beyond RNA synthesis since its effects cannot be blocked by actinomycin D (Ney et al., 1966). Specifically cyclic AMP and NADPH are necessary for conversion of cholesterol to pregnenolone, the rate-limiting step in steroid biosynthesis (Robinson et al., 1971). Although evidence for inactivation of ACTH at the receptor site is sparse, Birmingham and Kurlents (1958) found that contact with adrenal tissue *in vitro* caused a decrease and eventual disappearance of ACTH activity in Krebs-Ringer-bicarbonate medium.

ACTH is one of the few known physiologic stimuli to adrenal steroidogenesis. Direct neural stimulation of the adrenal does not stimulate glucocorticoid secretion since neither adrenal denervation (Colfer, 1950; Vogt, 1952), adrenal medullectomy (Vogt, 1952; Gordon, 1950) nor complete sympathectomy (Recant, 1950; Hume, 1950, 1952) interfered with response of the pituitary-adrenal axis to various stresses as indicated by adrenal ascorbic acid depletion assays or eosinopenia. Furthermore, epinephrine was found to be a weak stimulus to the adrenocortical system in the rat (Coutinho, 1953) and while epinephrine effected a rise in plasma 17-hydroxycorticoids in the dog 1/2 the magnitude of the rise seen after ACTH administration (Harwood, 1956), these effects were probably mediated via the central nervous system and pituitary ACTH, especially since Mulrow et al. (1962) found that infusions of norepinephrine into hypophysectomized, nephrectomized dogs had no effect on

adrenocortical secretion despite marked blood pressor responses. Besides ACTH, other stimuli to adrenocortical secretion of 17-hydroxycorticoids include angiotensin II (Ganong et al., 1968).

D. Adrenal Glucocorticoids

The major corticoid secreted depends upon the species. Man, cows, sheep, cats, monkeys secrete primarily cortisol, while rats, birds, and mice secrete primarily corticosterone (Ganong, 1971). Dogs, unlike the above species, secrete both cortisol and corticosterone in a ratio of 1:1 (Bush, 1953).

Plasma of rat (Keller et al., 1966), dog (Murphy, 1967), and human (Samuels, 1966; Sandberg et al., 1963; Nugent et al., 1964) contains a specific corticoid binding protein, an α globulin (Settlage et al., 1970) produced in liver. Affinity and saturation of these compounds depends upon species (Murphy, 1967). They have properties which have been characterized by Murphy (1967) for use in a competitive protein binding assay for glucocorticoids. Corticoids also bind with lower affinity to albumin (Keller et al., 1966). The bound steroid is protected from liver metabolism (Sandberg et al., 1963) and is otherwise biologically inactive (Slaunwhite et al., 1962). Free steroid is metabolized by kidney (Willoughby et al., 1959) and liver (Steenberg et al., 1960).

Half life of steroids depends upon species and specific steroid. Values in the dog range from 30 minutes (Gann et al., 1968) and 52 minutes for 17-hydroxycorticosterone (Kuipers et al., 1957) to 104 minutes for cortisol and 11-deoxycortisol

(Thomasson et al., 1965). Half life of cortisol in the dog is not altered by administration of small doses of ACTH or cortisol (Kuipers et al., 1957) or by hypophysectomy (Harwood et al., 1956) but may be prolonged with greater doses of ACTH (Kuipers et al., 1957) or under conditions of stress (Eik-nes et al., 1958; Steenberg et al., 1955).

E. Circadian Rhythm

ACTH and glucocorticoids undergo a circadian rhythm in mammals which has been extensively documented (Ganong, 1963). The rhythm can be altered by changing light and activity schedules (Orth et al., 1969). Preceded in time by a peak in plasma ACTH activity, cortisol levels peak upon waking in man (Berson and Yalow, 1968). A similar pattern is seen in the rat although corticosterone peaks late in the afternoon before waking of the nocturnal animal. The dog also has a steroid circadian rhythm; peripheral 11-hydroxycorticoid concentration ranged from a nadir of 24 micrograms/100 milliliters at 12 noon to a peak of 31 micrograms/100 milliliters at 8 a.m. (Everson, 1968). Plasma 17-hydroxycorticoids measured over a 7-hour period varied from 3.6 micrograms/100 milliliters at 9 a.m. to 1.9 micrograms/100 milliliters at 4 p.m. (Harwood et al., 1956).

Evidence for a CRF rhythm independent of steroid feedback as tested in male adrenalectomized rats has been provided by Cheifetz et al. (1969) and Hiroshige et al (1971). ACTH rhythm also persists in the adrenalectomized rat (Cheifetz et al., 1968).

Furthermore, Seiden et al. (1972) have shown that the CRF rhythm is also independent of ACTH feedback as tested in hypophysectomized rats; however, in the absence of ACTH and corticoids, absolute levels of CRF were elevated. Apparently, neural input to the hypothalamus is responsible for initiation and maintenance of the circadian rhythm, since interference with limbic structures modifies the rhythm and it is absent in humans with hypothalamic or temporal lobe disorders (Ganong, 1971) or in rats with anterior hypothalamic lesions (Slusher, 1964). In addition, the rhythm is abolished by morphine and central nervous system depressants. Although a diurnal rhythm is readily discernible in normal humans, the changing levels are maintained by episodic ACTH and corticoid secretion in man (Berson, 1968; Hellman et al., 1970; Orth et al., 1967, 1969).

F. Stress

Aside from diurnal variation, rises in CRF, ACTH and glucocorticoid secretion occur primarily in response to a variety of stimuli referred to as "stressful" stimuli. Whether a stimulus is stressful depends on the parameters one considers to be indices of stress. For the purpose of this thesis, a stressful stimuli is one which is known to provoke increased pituitary-adrenal function under the designated conditions. Testing an animal's ability to respond to a stress by measuring corticoid response has been used to assess the integrity of the hypothalamic-pituitary-adrenal system. Depending upon the nature and severity of the stress, graded amounts of hormones are released (Dallman et al., 1967).

G. Feedback Control

The classic approaches to the study of hormone control include pituitary or target organ ablation, transplantation, or hormone replacement. From such studies arose the concept of negative feedback whereby release of trophic hormone was inhibited by elevated levels of another compound secreted by the target organ on stimulation. As already mentioned, diurnal rhythms may be modified by circulating hormones but are largely independent of feedback. The role of negative feedback in modifying the response to stress is a different matter and has been under intensive investigation (Kendall, 1971).

Several questions pertaining to feedback control of the hypothalamic-pituitary-adrenal system in stress include: (1) how does pretreatment with steroids or ACTH affect the response to a stress; (2) where is (are) the site(s) of feedback; (3) how does the recent history of the system affect its response to a stress?

(1) Dexamethasone, cortisol, or corticosterone pretreatment in animals, including the rat, dog and man, effectively suppresses the secretory response to some kinds of stresses (see Chart 3).

(2) A negative short loop feedback involving ACTH inhibition of ACTH release from the pituitary (Kitay et al., 1959) or inhibition at the hypothalamus (Ifft, 1956; Motta et al., 1965) has been proposed. Although some evidence has been

provided for a short loop feedback of corticoids on corticoid secretion (Fekete et al., 1963) most attention has been focused on feedback sites of corticoids at the pituitary, hypothalamus, and other brain structures. Dexamethasone inhibits the rise in CRF activity in the medium eminence normally observed in rats two minutes after onset of laparotomy stress (Takebe et al., 1971). Hedge and Smelik (1969) suggest dexamethasone acts to inhibit CRF synthesis but not release. *In vitro* studies reveal that glucocorticoids can diminish pituitary sensitivity to CRF (Arimura et al., 1969); however, use of dexamethasone to suppress endogenous ACTH secretion in a rat used to assay CRF activity in median eminence extract indicates that dexamethasone does not totally inhibit the pituitary-adrenal system at the pituitary level. Nevertheless, the action of dexamethasone at the pituitary which leads to decreased response to CRF *in vivo*, like the action of glucocorticoids on induction of liver tyrasine amino transferase (Baxter, 1970), requires DNA-dependent RNA synthesis and therefore requires time to appear (Arimura et al., 1969).

Localization of tritiated glucocorticoids in the hypothalamus, septum, midbrain, and hippocampus (McEwen et al., 1969, 1970; Stevens et al., 1971) implicate these regions in possible feedback roles although one must keep in mind that glucocorticoids have many and varied functions and such localization might easily involve a function other than feedback control of ACTH secretion. Nevertheless, results of studies using implants or injections of glucorticoids into the

Chart 3.

Suppression of hypothalamic-pituitary-adrenal function by glucocorticoids

	Species	Corticoid Given	Route	Dose, Time Interval, and Provocation
1.	Human	Dex.*	Oral	1 mg @ 6 hrs over 24 hrs before insulin (10-20 units) induced hypoglycemia
2.	Human	Dex.	Oral	1 mg @ 6 hrs ending 2 hrs before insulin tolerance test (i) 2 mg total (ITT) (ii) 3 mg total (iii) 5 mg total
3.	Dog Pups	Dex.	I.P.	0.01 mg/kg 3 hrs before decapitation
4.	Dog	Dex.	S.C.	0.25 - 0.5 mg/kg 4-6 hrs before laparotomy
5.	Dog	Dex.	S.C.	4 mg/kg 3-24 hrs before test response to ovine CRF injected into putuitary
6.	Dog	Dex.	I.V.	100 µg/min over 4 hrs before 10-40 ml/kg hypovolemia
7.	Dog	Dex.	I.V.	5 mg/kg/hr starting 1 hr before adrenal venous cannulation
8.	Dog	Cortisol	I.V.	1 - 1.25 mg/kg/hr 1 hr before adrenal venous cannulation and ether exposure
9.	Rat	Dex.	I.P.	25 - 100 µg/100 gm body weight 4-7 hrs before histamine (various doses)
10.	Rat	Dex.	I.P.	400 µg + 600 µg/day 1/2-96 hrs before various doses epinephrine or vasopressin
11.	Rat	Dex.	S.C.	10 - 400 µg/100 gm body weight 4 hrs before ether + adrenal venous cannulation

Dex. = Dexamethasone
I.V. = Intravenous
I.P. = Intraperitoneal
S.C. = Subcutaneous

	INDEX MEASURED	SUPPRESSION OBSERVED	REFERENCE
1.	ACTH (RIA).	Yes	Berson et al. 1968
2.	Plasma 11-hydroxy-steroids	Yes; (i) 50% (ii) 80% (iii) 100%	Von Werder et al. 1971
3.	Plasma corticoids (resting level)	Yes; 50%	Meulheims et al. 1969
4.	Adrenal venous corticoid output	Yes; 75-100%	Boryczka et al 1973
5.	Adrenal venous corticoid output	Yes; only at 3-6 hrs	L'Age et al. 1969
6.	Adrenal venous corticoid output	Yes; only to hypovolemia <15 ml/kg	Gann et al. 1966
7.	Adrenal venous corticoid output	Yes	Egdahl 1964
8.	Adrenal venous corticoid output + plasma corticoids	Yes	Richards et al. 1956
9.	Plasma cortico-sterone	Yes	Dallman et al. 1967
10.	Plasma cortico-sterone	Yes; proportional to time, dose + stimulus intensity	Kendall et al. 1972
11.	Adrenal venous corticoid output	Yes	Kendall 1961

	SPECIES	CORTICOID GIVEN	ROUTE	DOSE, TIME INTERVAL AND PROVOCATION
12.	Rat	Dex.	I.P.	25-250 µg/100 gm body weight 1-20 hrs before laparotomy + intestinal handling
13.	Rat	Dex.	Oral	About 20 µg overnight in drinking water before 0.2 ml gelatin acid I.V.

Note: Although this chart presents several examples of suppression of the hypothalamic-pituitary-adrenal function by glucocorticoids, ut must be noted that glucocorticoids in certain doses and under specific experimental conditions have failed to suppress hypothalamic-pituitary function

	INDEX MEASURED	SUPPRESSION OBSERVED	REFERENCE
12.	CRF activity + plasma corticosterone	Yes; proportional to time and dose	Takebe et al. 1971
13.	Plasma corticosterone	Yes; proportional to stimulus intensity and time interval	Sirett et al. 1969

hypothalamus (Chowers et al., 1967), septum (Dallman et al., 1969) and pituitary *in vivo* suggest that all of these regions might be feedback loci. Microelectrophoretic application of dexamethasone has revealed steroid-sensitive cells in the hypothalamus (Ruf et al., 1967). In addition, dexamethasone administered to rats has been found to alter spontaneous firing activity of cells in various hypothalamic nuclei (Sawyer et al., 1968). Stimulation experiments also suggest that the brain contains several steroid-sensitive regions capable of inhibiting the adrenocortical system (McHugh et al., 1967).

(3) Prior history of the hypothalamic-pituitary-adrenal system appears to be an important variable modifying the response to further stiumlation of the system. Brennan and Yates, using a perfused dog adrenal, showed that a square wave infusion of ACTH (10 mU ACTH) elicited a rise in 17-hydroxycorticoid output which overshot before reaching a plateau by 30 minutes. This secretory pattern was repeatable only if a recovery period of at least 5 minutes was allowed following cessation of the first stimulus. The overshoot may be related to a readily releasable pool of corticoid, while the plateau was probably maintained by ongoing synthesis. Burthermore, no overshoot was observed if the second stimulating dose of ACTH was less than the first.

Handling of rats through weaning shortened the duration of elevated corticosterone levels in mature rats placed in a novel environment with electric shocks when compared to non-handled stressed controls (Ader, 1970). Differences in responses were unrelated to magnitude of electrical stimulation, but

adrenocortical reactivity of handled rats was less than that of controls, demonstrating modification of the stress response as a function of previous exposure to stresses.

Dallman et al. (1972) and Dallman and Jones (1973) have failed to show altered responsiveness of the pituitary-adrenal system following multiple stresses in rats; however, Gann and Cryer (1973) have clearly demonstrated both facilitation and inhibition of the pituitary-adrenal response to second hemorrhage stresses in dogs. Furthermore, Gann and Cryer suggest that both facilitation of the neural drive to ACTH secretion and inhibition of ACTH secretion by corticoids secreted in response to the first stress occurs and may cancel each other. This hypothesis could explain the failure of Dallman et al. (1972, 1973) to find inhibition (or facilitation) of a second stress response.

II. MATERIALS AND METHODS

A. Experimental Animals

Male mongrel dogs weighing 9-22 kilograms were obtained from dog vendors licensed in California by federal and county law. The dogs were maintained in rooms with windows, at a temperature of 70-74°F on a diet of Gaines Meal in the University of California San Francisco Animal Care Facilities at least 2-3 days before the morning of an experiment.

B. Protocols

I. Response of Circulating ACTH and Peripheral Corticoids to Multiple Laparotomy Stresses

Time (min)	Determination for Which Blood Drawn in Given Group A = ACTH; F = Plasma Corticoids			Manipulation
	(1)	(2)	(3)	
-80	A,F	A,F	A,F	Preanesthetic sample
-70	A,F	A,F	A,F	Post-anesthetic sample
-60	A,F	A,F	A,F	Post-femoral vein and artery cannulated
-1	A,F	A,F	A,F	PRESTRESS SAMPLE 2 min lap stress
+1	A,F	A,F	A,F	
+2	A,F	A,F	--	
+3	A,F	A,F	--	
+5	A,F	A,F	--	
+7	A,F	--	--	
+9	A,F	--	--	
+10		A,F	A,F	
+11	A,F	--	--	
+13	A,F	--	--	
+15	A,F	F	F	
+20	A,F	F	F	
+30	A,F	A,F	F	
+45	A,F	F	F	
+1 hr (+60)	A,F	A,F	A,F	

I. Response of Circulating ACTH and Peripheral Corticoids to Multiple Laparotomy Stresses (cont.)

Time	Determination for Which Blood Drawn in Given Group (1)	(2)	(3)	Manipulation
0				RESTRESS 2 min.
+1		A,F		
+2		A,F		
+3		A,F		
+5		A,F		
+10		A,F		
+15		F		
+20		F		
+30		A,F		
+45		F		
+2 hr	(+60)	A,F		
+3 hr	(+180)		A,F	
+5 hr	(+300)		A,F	PRESTRESS samples RESTRESS 2 min. lap.
+1			A,F	
+2			A,F	
+3			A,F	
+5			A,F	
+10			A,F	
+15			-	
+20			-	
+30			-	
+45			-	
+60			A,F	

II. Stress Response and Suppression by Dexamethasone Infusion

Time	Determination for Which blood Drawn A = ACTH; C = 170H Corticoids		Experimental Manipulation
	A	C	Anesthesia administered Femoral artery and vein cannulated; adrenal vein cannulated; Initial stress sample taken; Cannula placed in third ventricle;
-60			Saline infusion (0.01 ml/ min) begun into third ventricle; Cannulation of cephalic vein;
-10	A	C	Prestress samples drawn
-5			Laparotomy stress (approx. 2 min duration)
-4	A		First hour stress samples drawn

II. Stress Response and Suppression by Dexamethasone Infusion (cont.)

A = ACTH; C = 170 H Corticoids

0	C	Saline or dexamethasone infusion begun; four groups of dogs received; 1) control: saline infusion (15 ml/hour) 2) 0.03 mg dexamethasone/kg stat. + 0.06 (mg/kg/hr) 3) 0.05 " 0.1 4) 0.1 " 0.2

Time	Determination for Which Blood Drawn		Experimental Manipulation
+50 +170 +230	A	C	PRESTRESS SAMPLES
+55 +175 +235			Laparotomy stress (approx. 2 min duration)
+56 +176 +236	A		FIRST, THIRD, FOURTH HR RESTRESS SAMPLES drawn (only 3 dogs in group (4)
+60 +180 +240		C	underwent 1st and 3rd hr
+245			restresses i.v. bolus of 1 unit ACTH in 1 milliliter saline
+249		C	Sacrifice with overdose pentobarbital

C. Anesthesia

Dogs were anesthetized with 30 milligrams/kilogram sodium pentobarbital (Pentosol, Burns-Biotec, Laboratories, Inc., Oakland, California) administered into the cephalic vein (Miller, 1963) with a 12 milliliter syringe and a 20 gauge sterile needle. Hair was shaved from the areas of the femoral triangles, the right flank below the ribcage, the right forearm, and the head from the eyebrows back about 6 inches between the ears, as necessary for the experimental protocol. Further anesthesia was administered as necessary (0.5 - 1.0 milliliters at a time) during the experiment through a teflon cannula in a femoral vein. At the end of the experiment the animal was sacrificed with an overdose of pentobarbital.

In animals where preanesthesia samples were taken, blood was drawn from the cephalic vein into a plastic heparinized syringe. Anesthesia was then administered through the same needle.

D. Surgery

1. Femoral Cannulation

The right femoral vein and artery were cannulated using teflon tubing flushed with heparinized autoclaved saline (3 milliliters sodium heparin [Invenox, San Francisco, California; 1000 units/milliliter] per 500 milliliters saline) attached to a 3-way metal stopcock. The arterial cannula was attached to a Statham strain gauge and Grass Model 5 polygraph for continual monitoring of arterial blood pressure. Peripheral blood samples were drawn from the arterial cannula while anesthesia or 1 unit ACTH (ACTHAR, Armour Pharmaceutical Co., Chicago, Illinois) was administered via the venous catheter as called for.

2. Adrenal Vein Cannulation

The right lumboadrenal vein of some dogs was cannulated by the method of Hume and Nelson (1955). Polyethylene tubing, size PE200 (Intramedic polyethylene tubing, Clay-Adams, New York) was used for the adrenal vein cannula while both PE200 and PE50 were used for the choker. The first adrenal vein and peripheral blood samples were taken immediately upon placement of the choker. The incision was then sutured.

3. Cannulation of Third Ventricle

As a control for another experiment, some of the dogs received 0.01 milliliters saline per minute into the third

ventricle via PE20 tubing attached to a 20-gauge stainless steel cannula which was placed into the third ventricle using the stereotaxic instrument and coordinates described by Hume et al. (1956). Position of the cannula was determined by ventriculogram; 0.15 milliliters renographin-76 (Squibb Meglumine Diatrizoate Injection USP, E. R., Squibb and Sons, New York) was injected and an X-ray film developed.

4. Laparotomy Stresses and Restresses

A PE200 cannula filled with heparinized saline as above was inserted into a cephalic vein for infusion of saline or dexamethasone phosphate (DECADRON, Merck, Sharp and Dohm). For dexamethasone infusion, a loading dose equal to one half the hourly infusion rate was administered just prior to initiation of the constant infusion.

Laparotomy stresses consisted of making an incision with a scalpel approximately 3-5 inches long on the right flank (distinct from the incision of the adrenal vein cannulation if cannulation had preceded). Scissors were used to separate the muscular layers and to puncture the abdominal cavity. Fingers were used to stretch the incision and to manipulate the intestines. In the dexamethasone study the incision was retracted continually with a retracting instrument in protocol II. In the 2-minute laparotomy studies (protocol I) the incision was not continually retracted. In both cases the incision was covered with a saline-dampened gauze. Subsequent laparotomy stresses consisted of (protocol II) removing the retractor, further manipulating

and stretching of the intestines and incision with fingers and scissors for approximately 2 minutes and replacing the retractor; (protocol I) making a separate incision followed by intestinal handling to more closely approximate the stress involved in the initial laparotomy.

E. Blood Samples

1. Adrenal Venous Samples

Adrenal venous effluent blood was collected over a measured period of time into a 12-milliliter graduated conical-shapted test tube containing 5 drops of sodium heparin.

2. Peripheral Samples

Peripheral samples for ACTH and plasma corticoids were taken from the femoral artery (with the exception of pre-anesthesia samples which were taken from the cephalic vein) and collected into 15 milliliter, conical-shaped graduated plastic test tubes (Kimble disposable polystyrene, V.W.R. Scientific, Owens-Illinois, Toledo, Ohio) containing heparin. Because ACTH adheres to glass, plastic or paper products were used in handling ACTH samples wherever possible to minimize loss of ACTH. Post anesthesia samples were taken by femoral arterial puncture within 10 minutes after anesthesia was administered. These samples were collected into plastic syringes containing heparin and transferred to plastic tubes for centrifugation.

3. Processing Samples

All samples were kept on ice until centrifugation at 2000 rpm for 10-20 minutes at +4°C. Hematocrits were noted for adrenal venous samples only. Plasmas for ACTH determination were transferred to small plastic test tubes while adrenal venous plasmas were transferred to glass tubes. All samples were stored at +4°C until assayed.

F. Blood Measurements

1. Adrenal Venous 17-hydroxycorticoid Output

Concentration of 17-hydroxycorticosteroids in adrenal venous plasma was determined by the phenylhydrazine colorimetric method of Porter and Silber (1950) extracting 17-hydroxycorticosteroids from 1 milliliter of plasma. The values for 17-hydroxycorticosteroids concentration were determined from the standard curve using a computer program (for the Hewlett Packard 9100A desk top computer) (see Appendix 1) for determination of least squares line of best fit and for calculation of "y" from "x". Adrenal venous output in micrograms per minute was found from the product of adrenal venous plasma flow in milliliters per minute multiplied by the 17-hydroxycorticosteroid concentration in micrograms per milliliter.

2. Plasma ACTH Concentration

Plasma ACTH concentration was determined using Hane and Dallman's modification (manuscript in preparation) of

radioimmunoassays for ACTH (Rees, 1971; Berson, 1968; and Landon, 1968).

a. Antibody production

Antibody to ACTH was prepared by adapting the technique of Garra and Cendon de Bay Gorria (1959).

Ping pong balls drilled with many large holes to facilitate injecting and aspirating were implanted in the throat region of the rabbit, one ball on each side. Three weeks after implantation immunization was started. Corticotropin-zinc (Organon Inc., West Orange, New Jersey) was emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan); 2 milliliters of this mixture was injected into each ping pong ball, an equivalent of 40 USP units of ACTH in each ping pong ball. Injections were given at three-week intervals over a time period of 8 months. At the time of injection, the balls were aspirated and the exudate centrifuged at 20,000 rpm for 60 minutes (Spinco model 1 centrifuge, rotor #40). The supernatant was checked for antibody titer by plotting dilution curves. Only one animal out of four produced antibody with reasonable dilution and high avidity. Characterization of the antibody used in this study is being prepared for publication (Hane, Dallman, manuscript in preparation). The supernatant was frozen at +4°C in aliquots which were stable for at least 6 months. A stock solution was made for daily use from an aliquot of the antiserum reconstituted to a convenient dilution (1:25) with phosphate buffer (0.05 M; pH 7.6)

containing 0.015 M sodium azide (Matheson, Caleman and Bell, Norwood, Ohio) an antibacterioside, and normal human serum albumin (25%, Armour Pharmaceutical Co., Chicago, Illinois). This stock solution was found to be stable for 3 months.

Ping pong ball antibody was checked against antibody obtained from Kendall's laboratory since the Kendall antibody was used until an ACTH-specific antibody was obtained by the above method immunization procedure in our laboratory. Incubation time for the Kendall antibody was only 24 hours as opposed to 72 for the ping pong ball antibody. Use of either antibody gave comparable results on multiple samples assayed for ACTH concentration per milliliter plasma.

b. Preparation of ^{125}I ACTH

The method used for preparation of labelled ACTH was essentially that of Hunter and Greenwood with minor modifications (Hunter and Greenwood, 1962).

Two diluents were used: (1) diluent 1 was 0.05 M sodium phosphate buffer, pH 7.6; (2) diluent 2 was 0.05 M sodium phosphate buffer, pH 7.6 containing 2.5 milligrams per milliliter human serum albumin (25% human albumin solution, Cutter Laboratories, Berkeley, California) and 0.5% 2-mercaptoethanol (Matheson, Coleman, and Bell, Norwood, Ohio).

For labelling, 2 mCi high specific activity ^{125}I as sodium iodide (Iso-Serve Division, Cambridge Nuclear Corporation, Cambridge, Mass.) contained in the original flint glass shipping vial was used. Labelling procedure was carried out in a walk-in

cold room at 4°C. Twenty microliters of 0.25 M sodium phosphate buffer (pH 7.6) was added to the flint vial to concentrate the isotope to the tip of the vial. The following reagents were added rapidly in turn and mixed by pipetting up and down with disposopipettes following each addition; 2.5 micrograms synthetic 1-24 amino acid ACTH (Cosyntropin #26153, Organon Inc., West Orange, New Jersey) dissolved in 5 microliters 0.005 N HCl added and mixed by pipetting up and down 5 times, 20 microliters chloramine T (Eastman Organic Chemicals, Rochester, New York); mixed up and down 10 times, and 10 microliters sodium meta-bisulfite (Baker and Adamson #2212, General Chemical Division, Morristown, New Jersey) 2,3 milligrams per milliliter in diluent 1) mixed up and down 5 times. The complete mixture including the flint glass vial was immersed immediately in a 50-milliliter ground glass-stoppered centrifuge tube containing 20 milliliters diluent 2. Ten microliters were then transferred to a 15-milliliter polystyrene centrifuge tube (Kimble disposable polystyrene conical-shaped centrifuge tubes, VWR Scientific) containing 35 milligrams leached silica glass (Corning Glass Works, Corning, New York, #7930) for purification. The remainder was saved as a precaution against purification failure. The tube was capped with a hollow polyethylene stopper, contents were agitated for 10 seconds on a Vortex mixer, and rotated on a vertical turntable for 15 minutes to allow for absorption of undamaged labelled ACTH to the glass. After rotation, the sample was centrifuged at 2000 rpm for 10 minutes at room temperature, washed 3 times with 3-4 milliliters deionized

distilled water. The supernatant was aspirated and discarded. The glass precipitate was centrifuged and aspirated as above. ACTH was eluted with 2 milliliters of 40% acetone in 0.25 nanograms HCl mixture by rotation for 15 minutes, centrifugated and the supernatant carefully removed. The supernatant, containing undamaged labelled ACTH, was assessed for purity by chromatoelectrophoresis (Berson and Yallow, 1962) and stored at 4°C in a polystyrene tube.

For assay, aliquots were diluted with diluent 2 to yield 7-8,000 cpm per 200 microliters.

c. Preparation of cold ACTH for standard curves

Synthetic 1-24 amino acid ACTH (Cosyntropin #26153, Organon Inc., West Orange, New Jersey) was dissolved in 0.005 N HCl to a final concentration of 5 micrograms per 200 microliters. Two-hundred microliter aliquots were stored in polystyrene tubes at 4°C until needed for assay extraction or dilution procedure (see following). For the extraction procedure, one aliquot was brought to concentration of 2 nanograms ACTH per 10 micrograms with 4.8 milliliters diluent 2. For dilution procedure, 0.1 milliliters of the 2 nanogram ACTH per 10 microliters with an additional 1.9 milliliter diluent 2.

d. Extraction of ACTH from dog plasma

The method for extraction of ACTH from dog plasma was essentially that of Rees with some modification by Dallman and Hane (1973) as follows. All dog plasma samples were thawed,

along with an aliquot of dog plasma pool (obtained by bleeding out a dog which had received 1 unit ACTH at the end of the experiment) and a 6-milliliter aliquot of hypophysectomized dog plasma (obtained from a dog under sodium pentobarbital anesthesia hypophysectomized by the transbuccal approach and bled out one or more hours later). Plasma samples were centrifugated in a refrigerated centrifuge (5°C) for 5 minutes at 2000 rpm to concentrate the filbrin. The clot was removed with wooden applicators (Mulco Products Inc., Milford, Delaware). To 35 milligrams leached silica glass in labelled polystyrene tubes was added the following: (1) STANDARD (triplicate), 1 milliliter hypophysectomized dog plasma plus 2 nanograms 1-24 amino acid ACTH (2 nanograms per 10 microliters diluent 2); (1) BLANK (duplicate), 1 milliliter hypophysectomized dog plasma; (3) SAMPLES (singlicate), total plus recorded volume of plasma collected (usually 3-6 milliliters). NOTE: After centrifugation, ACTH concentration measured in the upper portion of the plasma differed markedly from that in the lower portion; therefore, it was imperative to use all the plasma in the vial or to mix plasma well before taking an aliquot. Furthermore, because ACTH concentration in dog plasma is fairly low, even after an ACTH release has been provoked by a stress such as laparotomy, several milliliters had to be extracted to achieve a concentration that would fall on the standard curve. This also meant that when multiple samples were taken from a dog, enough blood was drawn for one determination only in order to spare him from hemorrhage; (4)POOL, 0.5 milliliters

plasapool or that volume found to contain an amount of ACTH similar to that of the standard. All tubes were capped with plastic hollow caps. They were agitated on a Vortex mixer 15 seconds after glass was distributed throughout the plasma to allow for absorption of ACTH onto the glass. Tubes were centrifuged at 2000 rpm for 10 minutes and the supernatant aspirated with care not to remove any glass, or removed by pipet to labelled tubes for further analysis of plasma corticoids. Glass precipitate was washed with 3-4 milliliters deionized distilled water. Tubes were agitated, centrifuged 10 minutes at 2000 rpm and the supernatant aspirated. ACTH was eluted from the glass with one milliliter 40 percent acetone in 0.25 N HCl solution by 15 seconds of agitation on the Vortex mixer. Tubes were centrifuged as before the supernatant containing ACTH was carefully removed by pipet to labelled plastic tubes (VWR Scientific, Falcon plastics #2038, Division Becton, Dickinson & Co., Oxnard, California). The supernatant was dried down under nitrogen in a water bath at 50°C (approximately one hour). Extracts were covered and stored at 4°C until assayed. NOTE: Samples extracted and stored for one year were found to have the same concentration of ACTH as duplicates assayed immediately after extraction. Therefore, no loss of radioimmunodetectable ACTH occurred over that period of storage in the extracted condition.

e. Dilution

The method of dilution was essentially that of Rees.

Extracted ACTH was taken up in 400 microliters of diluent 2 and serially diluted using an Automatic Pipet (Micromedic Systems, Inc., Philadelphia, Pennsylvania, Model 25000). Extracted standards were serially diluted to 1/512th of the absolute amount originally existing in the first tube, while samples and pool were diluted to 1/64th and blanks to 1/16th their original concentrations.

Tube #		Final Volume	Fraction Amount ACTH Originally in tube #1
1.	400 µl diluent 2 + agitation	200 µl	1/2
2.	200 µl +200 µl diluent 2 + agitation		
		200 µl	1/4
3.	200 µl + 200 µl diluent 2 + agitation		
		200 µl	1/8

et cetera

Chart 4. Method of serial dilution

Two nanograms of 1-24 amino acid ACTH in 400 microliters diluent 2 was also serially diluted in duplicate. When the assay was completed and the data plotted, a comparison of this standard curve with the extracted standard curve was used as an indication of recovery of ACTH from the extraction procedure and also as an indication of parallelism between extracted and unextracted standards. Recovery of ACTH from the extraction procedure ranged from 90-100 percent.

For addition of ^{125}I-ACTH and antibody to tubes, two solutions were used. (1) solution A; diluent 2 containing approximately 8000cpm ^{125}I-ACTH per 200 microliters; (2) solution B:solution A containing ping pong ball antibody in dilution of 1:4000. Addition of 200 microliters of this solution to 200 microliters of assay material resulted in final antibody dilution of 1:8000.

Two-hundred microliter solution A was added to one set of extracted blanks (4 tubes serially diluted from the originally extracted milliliter of hypophysectomized dog plasma) and to two tubes containing 200 microliters diluent 2 (unextracted blanks). When the assay was complete, the percentage of radioactivity not absorbed to charcoal in these tubes during separation represented "damaged" labelled ACTH. Solution B was added to all other tubes and to 2 tubes of unextracted blanks.

f. Incubation

All tubes were incubated for one day when using the Kendall antibody and for three days when using the ping pong ball antibody at 4°C to allow for binding and equilibration of labelled and unlabelled ACTH to the antibody.

g. Separation by precipitation and assessment of radioactivity

Antibody-bound labelled ACTH and free labelled ACTH were separated by the addition of 200 microliters of a charcoal suspension of 3 grams Norit A activated charcoal (Pfanstichl

Laboratory, Inc.), 10 milliliters 0.5 molar phosphate buffer, 0.75 grams dextran T500 (Pharmacia Fine Chemicals), 60 milliliters horse serum (Grand Island Biological Company, Berkeley) diluted with deionized water to 100 milliliters, pH 7.6. All tubes were quickly agitated on a Vortex mixer, and centrifuged for 20 minutes at 2000 revolutions per minute at 4°C. The supernatant was aspirated, discarded, and the charcoal (containing free labelled ACTH) counted for one minute in a gamma counter (1185 Series Automatic Gamma Counting System, Nuclear, Chicago) set for the ^{125}I peak.

h. Calculations

Extracted and unextracted standard curves were drawn by plotting log concentration ACTH in serially diluted tubes against radioactivity (cpm) in the charcoal precipitate (see Table 1 and Figure 1).

A least squares line of best fit was calculated using a computer program (see Appendix 2) for the linear region of the extracted standard curve. Limits of linearity were determined by visual inspection. Radioactivity (cpm) in the charcoal precipitate from extracted and unextracted blanks incubated with labelled ACTH and without antibody, reflected the uppermost limit of sensitivity. Radioactivity (cpm) in charcoal from blanks incubated with both labelled ACTH and antibody reflected the lower limit.

Concentration of plasma ACTH in picograms per milliliter to samples was determined by taking the average of those values of the diluents which could be calculated by estimating x from

y over the linear portion of the standard curve (see Sample Calculation: Tables 1 through 3).

3. Peripheral Plasma Corticoid Concentration

Peripheral plasma corticoid concentrations were measured using the competitive protein binding method of Murphy (1967) which measures cortisol (F), corticosterone (B), and 11-deoxycortisol (S). Corticoids were extracted from 200 microliters aliquots, human (male) transcortin was used for the binding protein, and the standard durve was constructed using cold and tritiated cortisol.

SAMPLE ACTH CALCULATION (see corresponding curve on following page)

Cube #	cpm in charcoal (average of 3 extracted standards)	nanograms ACTH (here 4 nanograms were extracted so the first tube had 2 nanograms after dilution)
1	4915	2
2	4907	1
3	4522	.5
4	4197	.25
5	3642	.125
6	3071	.0625
7	2709	.03125
8	2380	.015625
9	2251	.0078125

Table 1. Summary data for extracted standard curve

Tube	Incubated with solution A	Solution B
extracted blanks	4300	2500
unextracted blanks	4650	2300

Table 2. Limits of sensitivity

Correlation coefficient was determined from computer program (Appendix 2) using linear points of the extracted standard curve (determined by visual inspection from sample graph) were y = nanograms ACTH and x = cpm in charcoal precipitate which fall within limits of sensitivity of the particular assay (Table 2).

Usable linear range = 4300 - 2500 cpm. Tubes #4-7 were therefore used to calculate "r". r = 0.995.

Tube#	cpm in charcoal precipitate	#milliliters plasma represented by ACTH in given tube	picograms ACTH per milliliter plasma
1-1	4568	2.1	--- (cpm above)
1-2	3581	1.05	104 sensitivity limit
1-3	3052	0.525	93
1-4	2771	0.2625	121
1-5	2447	0.13125	147
1-6	2284	0.165625	--- (cpm below sensitivity limit)
			$\overline{116}$ = average value

Table 3. Calculation ACTH concentration in sample unknown 4.2 milliliters plasma was extracted

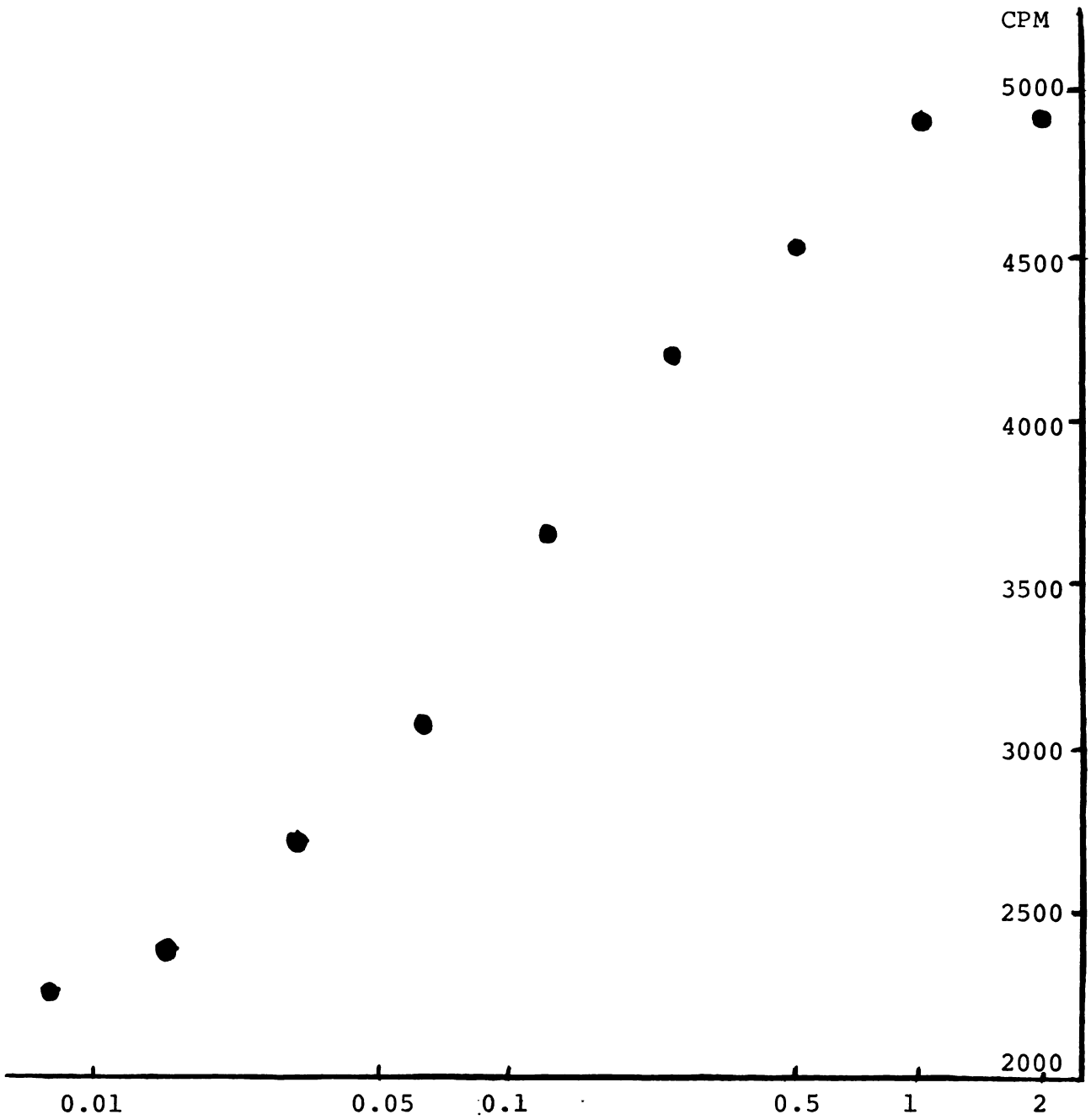


Figure 1.

The STANDARD CURVE in the ACTH radioimmunoassay consists of a semilog plot of nanograms ACTH in tubes of extracted ACTH standard plotted against radioactivity (cpm) of ACTH not bound to the antibody. Values plotted are from Table 1.

III. RESULTS

A. Rapid Response to Stress

To determine how rapidly the dog responds to laparotomy stress, plasma ACTH and peripheral corticoids were measured before and after induction of pentobarbital anesthesia and at frequent intervals following start of a two-minute long laparotomy stress. Table 4 contains the individual data from 6 dogs and meaned values are presented graphically in Figure 2. An increase in plasma ACTH concentration within three minutes after onset of the stress was seen in all dogs with the exception of dog #16-73 upon which the experimental protocol was performed twice with a week's time intervening, and which did not respond to laparotomy stress during either experiment. In the responding dogs, a rise in peripheral corticoid levels paralleled the rise in plasma ACTH concnetration with a lag of about five minutes.

Analysis of data from Tables 4-6 shows that no significant change in plasma ACTH concentration occurred by approximately ten minutes after induction of anesthesia as compared to ACTH concentration in the unanesthetized state. Circulating plasma ACTH concentration before and after induction of anesthesia (data from Tables 4-6) taken as a measure of "resting" ACTH levels, was found to be 49 ± 9 picograms per milliliter plasma. Furthermore, data from Tables 4-6 indicates that following onset of an initial laparotomy stress, concentration of plasma ACTH in the dog is frequently above prestress

levels within one minute and significantly elevated within two minutes ($p < 0.01$). Thus, the response of the hypothalamic-pituitary-adrenal axis to stress is a very rapid neuroendocrine response.

B. Multiple Laparotomies

To determine if corticoids released after an initial laparotomy stress can inhibit the ACTH response to a subsequent stress, dogs were subjected to two laparotomy stresses (each performed over a two-minute period) separated by one or five hours. Data is presented in Tables 4 and 5, and mean results are drawn in Figures 2 and 3, respectively.

The ACTH response to the second stress is similar to the first in both groups regardless of the time interval between application of the first and second stress. With the five-hour interval, the second ACTH stress response appears smaller than the first; however, the values, point for point, are not statistically different.

In both sets of experiments, peripheral corticoid levels paralleled plasma ACTH concentration closely. When laparotomies were separated by one hour, the rise in peripheral corticoid concentration was not significantly different from the rise in response to the first stress. When laparotomies were separated by five hours, the changes seen in peripheral corticoid concentration in response to the second stress were very similar to those seen in response to the first. Therefore, under these experimental conditions, inhibition of the second

Table 4.

Protocol I(1): Time course response of plasma ACTH concentration (A) and peripheral corticoid concentration (B) to one laparotomy stress. All dogs are from the 1973 series. Asterisk indicates dogs which received laparotomy incision 5 times larger than the others. Means exclude data on #16-73 since it was unresponsive in both experiments. Parentheses indicate means of less than four values.

DOG#	#12	#15	#16	#16	#37	#64*	#65*	Mean ± SEM
Kg. Wt.	16.4	16.0	19.6	19.2	19.8	12.0	9.6	14.7±
4A MINUTES	PLASMA ACTH CONCENTRATION (pg/ml)							
-80	-	58	131	-	37	32	75	51±9
Anesth.								
-70	49	53	104	90	60	38	46	49±3
Fem. Can.								
-60	95	79	55	74	65	136	187	112±20
-1	116	59	36	46	111	41	79	81±13
O STRESS								
+1	103	71	31	43	273	118	103	134±13
+2	-	-	-	-	204	195	102	(167±27)
+3	376	189	28	60	152	228	146	218±
+5	315	175	26	46	133	159	134	183±30
+7	283	147	17	38	144	186	166	185±26
+9	316	183	26	49	155	260	105	204±34
+11	263	172	29	46	147	242	122	189±24
+13	231	162	18	38	-	226	78	174±31
+15	252	137	19	49	161	217	62	166±29
+20	281	157	25	46	110	241	91	176±
+25	368	178	-	-	-	-	-	(273±67)
+30	353	178	21	49	115	278	115	208±42
+45	273	155	28	49	270	282	112	218±
+60	286	98	43	36	93	223	116	163±34

Table 4. (cont.)

DOG#	#12	#15	#16	#16	#37	#64*	#65*	Mean ± SEM
Kg. Wt.	16.4	16.0	19.6	19.2	19.8	12.0	9.6	
4B MINUTES	PERIPHERAL CORTICOID CONCENTRATION (µg/100 ml plasma)							
-80 Anesth.	4.0	3.5	5.1	3.6	5.0	5.2	4.6	4.5±0.3
-70 Fem. Can.	3.9	3.4	3.3	3.4	4.2	5.2	4.7	4.3±0.3
-60	4.5	5.5	3.2	3.3	4.4	6.6	4.4	5.1±0.4
-1	4.8	3.5	2.9	3.5	6.8	5.0	4.3	4.9±0.5
O STRESS								
+1	5.0	3.8	2.8	3.4	7.0	-	4.3	5.0±0.6
+2	-	-	-	-	7.0	-	4.4	(5.7±1.7)
+3	6.6	3.6	3.0	3.3	6.6	-	4.0	5.2±0.7
+5	8.0	5.4	3.1	3.3	7.0	5.4	4.9	6.1±0.5
+7	9.4	-	3.1	3.6	8.2	7.6	7.4	8.2±2.5
+9	9.8	7.7	3.0	4.2	8.6	8.3	8.6	8.6±0.3
+11	9.0	8.6	2.9	3.9	7.6	9.0	-	8.6±0.3
+13	11.1	9.0	2.2	3.5	-	9.6	9.9	9.9±0.4
+15	11.1	6.6	3.1	3.3	7.2	10.2	-	8.8±1.0
+20	12.9	9.4	3.1	3.3	6.8	11.2	6.4	9.3±1.1
+25	13.1	8.8	-	-	-	-	-	(11.0±
+30	15.6	8.6	-	3.2	6.4	12.6	5.2	9.7±1.7
+45	14.6	8.0	4.0	3.2	6.4	12.0	10.8	10.4±1.3
+60	-	6.0	-	3.	5.6	13.2	14.6	9.9±2.0

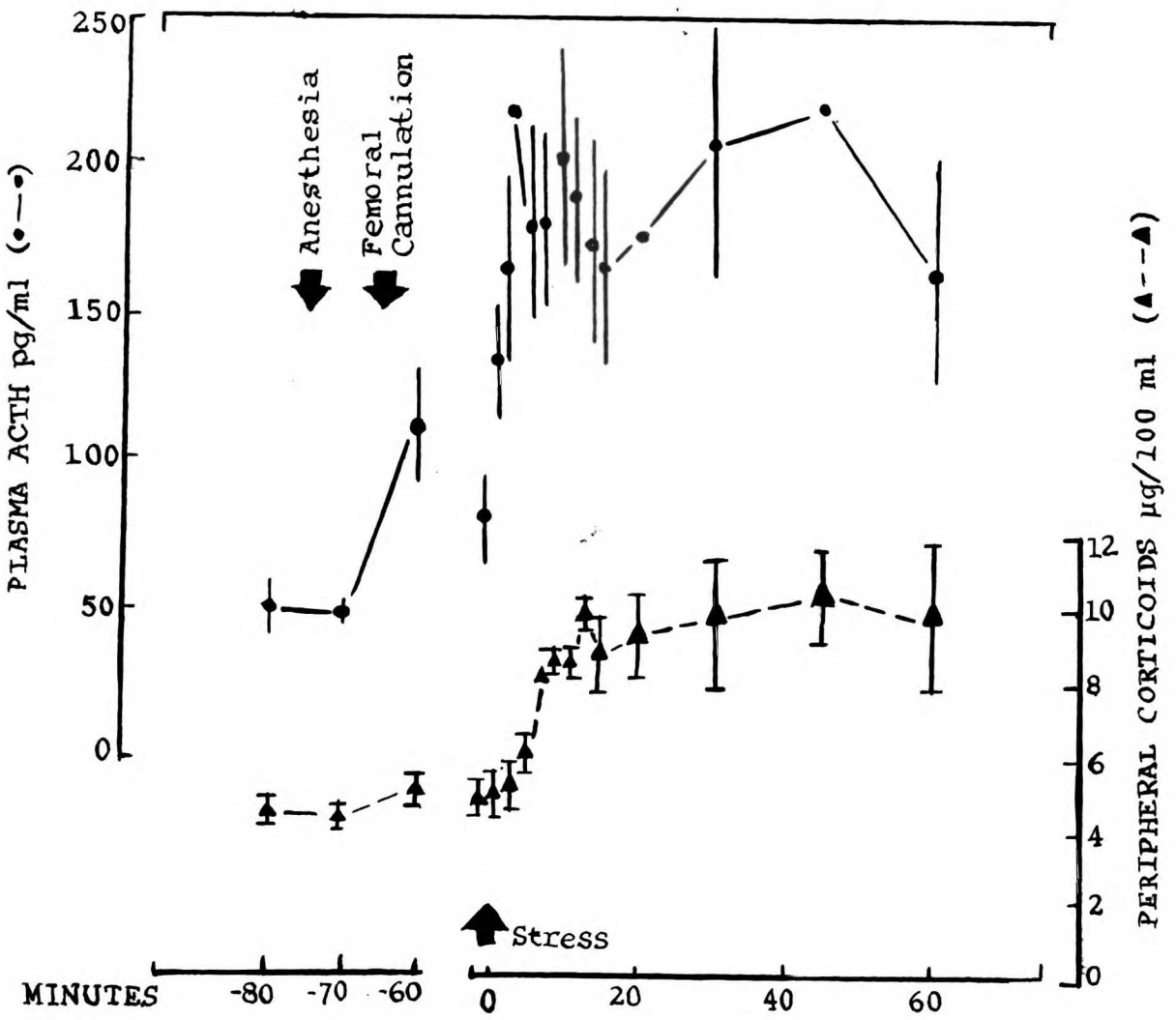


Figure 2.

Protocol I(1): Time course response of plasma ACTH concentration (•——•) and peripheral corticoid concentration (▲– –▲) to one laparotomy with frequent sampling over the first 15 minutes following onset of stress. Values plotted are means ± standard errors of the mean from Table 4. Note that values for dog #16 are not included in the meaned values.

Table 5.

Protocol I(2): Time course response of plasma ACTH concentration (A) and peripheral plasma corticoid concentration (B) to two laparotomies performed one hour apart

5A	Dog	#29*	#36*	#38*	#39	#53	#54	#62	#63	Mean ± SEM
	Kg. Wt.	19.0	18.0	12.0	12.4	17.6	13.0			
	Minutes			Plasma ACTH Concentration (pg/ml)						
	-80 Anesth.	76	59	--	--	21	36	36	23	42 ± 9
	-70 Fem. Can.	35	109	--	--	28	48	26	20	44 ± 14
	-60	159	62	--	--	122	57	44	160	101 ± 22
	-1	76	64	--	--	23	44	18	49	46 ± 9
	0 Stress									
	+1	72	52	--	--	31	--	10	64	46 ± 11
	+2	--	171	--	--	24	37	45	119	79 ± 28
	+3	68	145	--	--	29	--	146	84	94 ± 23
	+5	56	128	--	--	34	--	57	70	69 ± 16
	+10	73	102	115	103	23	66	45	77	76 ± 11
	+30	54	---	--	--	25	46	--	85	52 ± 12
	+60	39	78	--	--	42	--	94	46	60 ± 1
	Restress									
	+1	46	67	--	--	23	--	72	45	51 ± 9
	+2	44	145	--	--	31	50	139	69	80 ± 20
	+3	67	142	--	--	52	73	114	87	89 ± 14
	+5	61	187	--	--	62	84	134	96	104 ± 0
	+10	19	156	--	--	82	34	52	71	72 ± 24
	+30	--	---	--	--	119	49	--	125	(98 ± 24)
	+60	60	116	--	--	97	40	40	137	82 ± 17

* Indicate dogs in which the second stress was applied in the same incision as the first.
() Indicate means of less than four values.

All dogs are from the 1973 series.

5B

Dog #	#29	#36	#38	#39	#53	#54	#62	#63	Mean ± SEM
MINUTES	PERIPHERAL CORTICOID CONCENTRATION (µg/100 ml plasma)								
-80	3.6	4.0	3.3	3.8	-	4.3	3.4	4.4	3.8 ± 0.1
Anesth.									
-70	3.4	4.6	3.5	3.4	4.5	5.4	3.4	4.8	4.1 ± 0.3
Fem. Can.									
-60	3.6	3.9	3.2	3.8	5.0	4.8	3.0	4.2	4.0 ± 0.2
-1	3.9	4.0	4.8	4.2	4.6	4.8	3.0	4.2	4.2 ± 0.2
O Stress									
+1	-	-	-	-	-	-	3.4	4.7	(4.1 ± 0.5)
+2	-	-	-	-	-	-	3.2	4.5	(3.9 ± 0.5)
+3	-	-	-	-	-	-	3.0	4.7	(3.8 ± 0.6)
+5	6.3	4.8	5.6	6.7	4.2	5.6	3.6	4.8	5.2 ± 0.3
+10	4.2	6.0	7.8	11.4	4.7	11.2	3.6	4.8	6.7 ± 1.0
+20	3.8	5.4	7.8	5.2	4.4	10.0	4.2	4.4	5.8 ± 0.7
+30	3.4	4.8	4.7	4.4	4.0	7.2	4.8	6.2	5.0 ± 0.4
+45	3.3	4.0	4.8	4.8	4.0	7.2	5.4	4.7	4.8 ± 0.4
+60	3.8	3.8	4.0	8.6	10.9	6.6	6.0	4.7	6.1 ± 0.9
Restress									
+1	-	-	-	-	-	-	6.0	4.7	(5.4 ± 0.5)
+2	-	-	-	-	-	-	6.5	4.7	(5.6 ± 0.6)
+3	-	-	-	-	-	-	5.6	4.7	(5.2 ± 0.3)
+5	6.6	5.5	8.2	12.5	5.0	5.4	6.2	4.8	6.9 ± 0.8
+10	6.0	9.2	10.0	14.4	6.7	13.1	6.2	6.4	9.0 ± 1.1
+20	-	8.4	11.0	14.8	7.8	12.6	6.0	7.4	9.7 ± 1.1
+30	-	8.1	12.4	17.0	8.3	8.0	6.0	7.4	9.6 ± 1.3
+45	-	7.8	12.6	19.0	5.5	8.8	4.4	6.3	9.1 ± 1.8
+60	6.8	5.8	14.2	19.0	5.0	9.4	4.0	5.6	8.7 ± 1.7

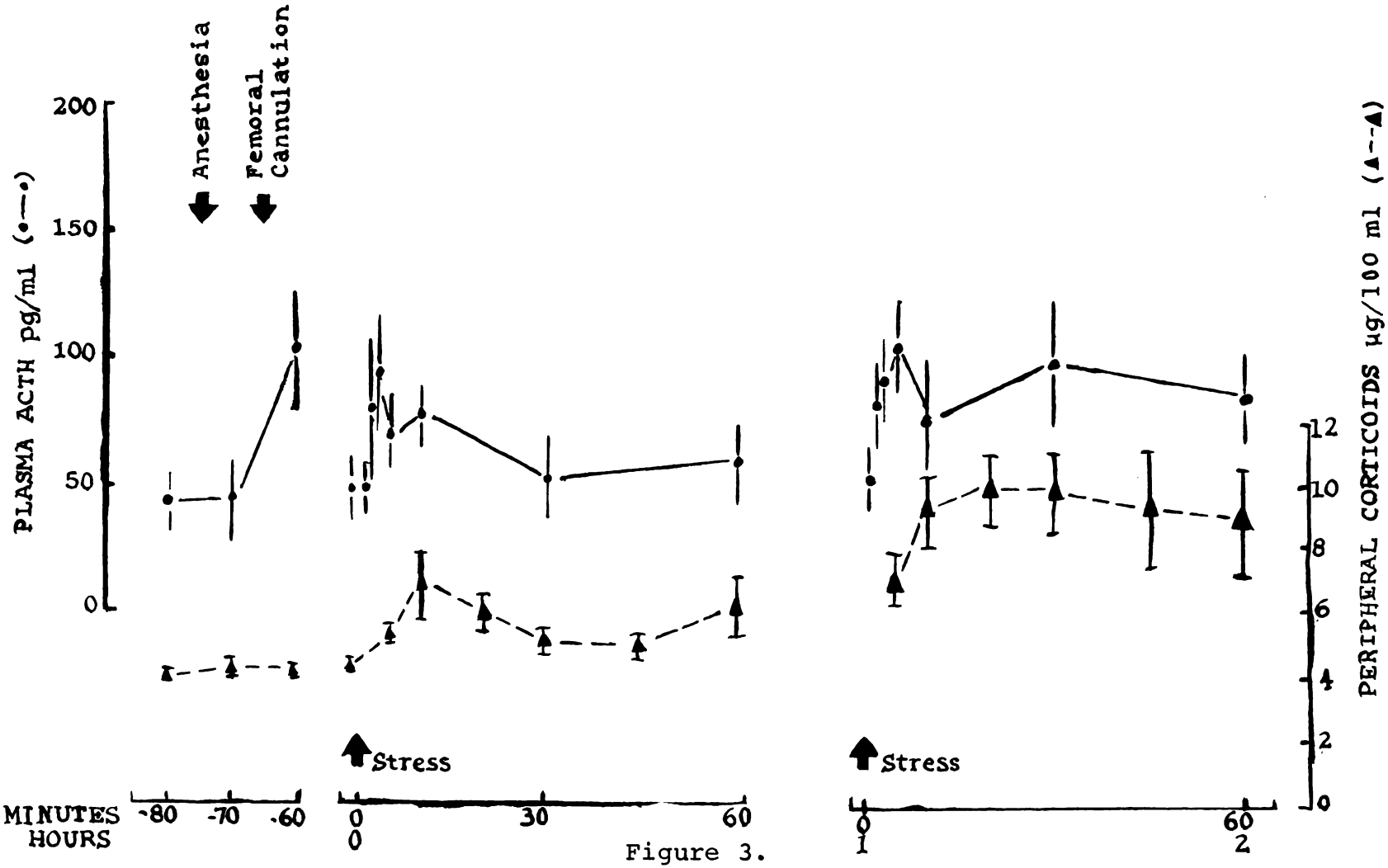


Figure 3.

Protocol I(2): Time course response of plasma ACTH concentration (●——●) and peripheral corticoid concentration (▲— —▲) to two laparotomies performed one hour apart. Values plotted are means ± standard error of the mean from Table 5.

Table 6.

Protocol I(3): Time course response of plasma ACTH concentration (A) and peripheral plasma corticoid concentration (B) to two laparotomies performed five hours apart. Asterisks indicate dogs in which the second stress was applied in the same incision as the first. All dogs are from the 1973 series. Parentheses indicate means of less than four values.

6A.

DOG#	#28*	#35*	#58	#59	#60	#61	Mean ± SEM
Kg. Wt.	13.7	12.4	12.3	12.0	18.0	20.0	13.1
MINUTES	PLASMA ACTH CONCENTRATION (pg/ml)						
-80	24	43	44	69	90	62	55 ± 9
Anesth.							
-70	15	32	23	32	30	59	32 ± 6
Fem. Can.							
-60	22	37	29	40	30	64	35 ± 7
-1	21	46	24	39	268	46	35 ± 5
O STRESS							
+1	37	65	16	45	38	39	40 ± 6
+2	-	-	19	172	28	61	70 ± 35
+3	60	167	23	144	34	123	92 ± 25
+5	60	189	14	238	37	169	134 ± 49
+10	118	217	20	238	37	169	133 ± 37
+60	159	342	37	164	65	114	145 ± 44
+180	143	199	-	-	28	79	112 ± 37
+300	77	55	22	65	31	61	52 ± 9
RESTRESS							
+1	59	27	21	80	38	68	49 ± 10
+2	181	-	89	63	91	110	107 ± 20
+3	137	75	150	120	99	102	114 ± 11
+5	145	67	-	141	30	69	90 ± 23
+10	125	56	69	189	105	107	109 ± 19
+60	159	-	29	56	28	79	70 ± 24

6B.

DOG#	#28*	#35*	#58	#59	#60	#61	Mean ± SEM
MINUTES	PERIPHERAL CORTICOID CONCENTRATION (µg/100 ml plasma)						
-80	3.6	4.0	6.0	4.2	4.4	4.0	4.4 ± 0.3
Anesth.							
-70	3.8	-	4.8	4.4	4.2	4.0	4.2 ± 0.2
Fem. Can.							
-60	3.6	3.6	4.6	4.0	4.2	4.0	4.0 ± 0.1
-1	3.7	-	4.1	4.4	4.8	4.0	4.2 ± 0.2
O STRESS							
+1	-	4.2	-	-	-	3.6	(3.9 ± 0.2)
+2	-	-	-	-	-	3.5	
+3	-	4.6	-	-	4.6	3.2	(4.1 ± 0.4)
+5	-	5.9	4.1	4.4	4.8	3.0	4.4 ± 0.4
+10	7.4	11.7	4.7	5.3	7.4	6.8	7.2 ± 0.9
+20	-	8.8	4.5	7.8	5.2	6.2	6.5 ± 0.7
+30	-	9.4	4.8	7.2	4.6	5.8	6.4 ± 0.8
+45	-	4.8	4.4	5.2	4.6	5.8	5.0 ± 0.2
+60	6.4	12.0	5.0	5.0	4.4	4.8	6.3 ± 1.1
+120	7.0	10.4	3.9	12.0	5.3	4.6	7.0 ± 1.1
+180	5.0	11.6	4.6	9.0	5.0	4.8	6.9 ± 1.3
+240	-	-	-	5.4	4.4	4.6	(4.8
+300	3.8	4.4	3.8	-	5.2	4.4	4.3 ± 0.2
RESTRESS							
+1	3.9	-	-	-	-	4.5	(4.2 ± 0.2)
+2	4.0	-	-	-	-	4.5	(4.3 ± 0.2)
+3	4.2	-	-	5.1	5.4	5.0	4.9 ± 0.2
+5	4.8	4.4	5.6	5.3	6.4	5.6	5.4 ± 0.3
+10	6.8	11.3	7.8	9.7	7.8	6.8	8.4 ± 0.7
+20	-	7.8	4.6	10.0	6.4	6.5	7.1 ± 0.8
+30	-	7.9	4.8	10.0	4.6	5.8	6.6 ± 0.9
+45	4.1	6.8	4.3	5.6	5.3	5.3	5.2 ± 0.4
+60	5.5	10.3	4.3	5.2	4.6	5.5	5.9 ± 0.8

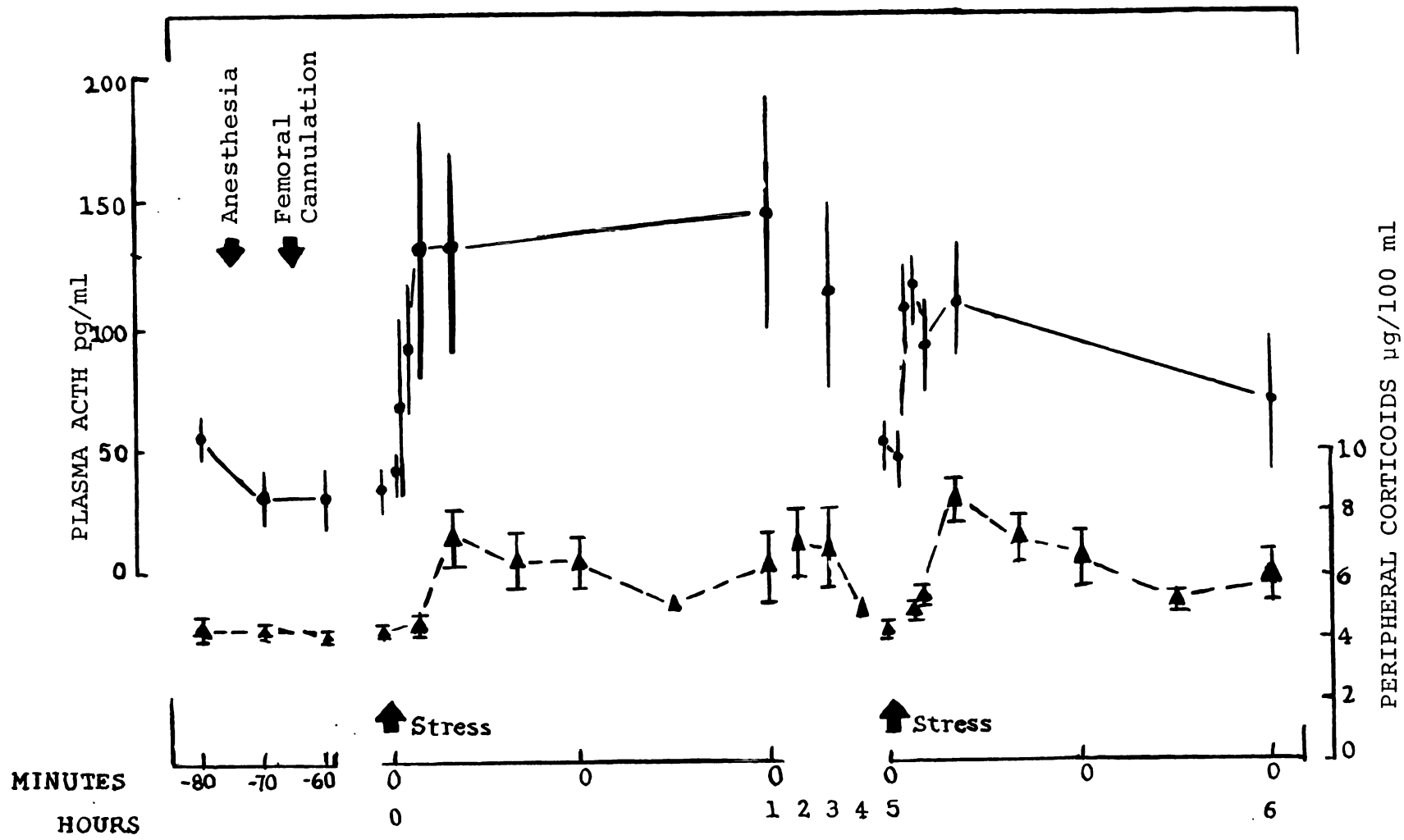


Figure 4.

Protocol I(3): Time course response of plasma ACTH concentration (●——●) and peripheral corticoid concentration (▲——▲) to two laparotomies performed FIVE HOURS APART. Values plotted are means ± standard error of the mean from Table 6.

stress response did not occur when the second stress followed the first by either one or five hours.

C. Adrenal Venous Output Correlation with Plasma ACTH Concentration

Rauschkolb et al. in 1954 found that in four out of five hypophysectomized dogs, maximal adrenal output of 17-hydroxycorticoids occurred four minutes after intravenous infusion of 40 units ACTH. One year later Nelson and Hume (1955) corroborated the finding that maximal 17-hydroxycorticosteroid secretion in hypophysectomized dogs occurs starting four minutes after administration of 1, 2, or 5 units ACTH, and that maximal stimulation by these amounts of ACTH lasts for four minutes. Because of these studies, plasma ACTH concentration determined one minute after onset of a laparotomy stress was correlated with adrenal venous 17-hydroxycorticoid output that occurred four minutes later. The data in the scattergram in Figure 5 shows that adrenal corticoid output rises to a maximum with increasing concentrations of plasma ACTH and then plateaus despite higher plasma ACTH levels because adrenal corticoid output is maximal. To further illustrate this relationship, mean 17-hydroxycorticoid output values were calculated for several ranges of plasma ACTH (Figure 5). Figure 5 clearly shows that 17-hydroxycorticoid output is proportional to levels of plasma ACTH up to about 150 picograms per milliliter. Thereafter, higher levels of ACTH could not elicit further corticoid output, because the

adrenals were maximally stimulated as is demonstrated by the fact that 1 unit ACTH administered intravenously could not further increase corticoid output. Furthermore, it is important to notice that endogenously-secreted ACTH frequently caused a rise in plasma concentration greater than that necessary to maximally stimulate corticoid output.

To see whether a correlation between ACTH and corticoid output could be observed at other sampling intervals, plasma ACTH concentration determined at different times before and after stress was correlated with 17-hydroxycorticoid output measured two minutes later (Figures 7 and 8) or at the same time (Figures 9 and 10). There is a fairly good correlation for either interval studied, and these data again demonstrate that plasma ACTH concentration rises above that necessary to maximally stimulate adrenal corticoid secretion.

D. Inhibition of Stress Response with Dexamethasone

To test whether glucocorticoids can inhibit stress-induced ACTH secretion, experimental protocol II was followed. Results are summarized in Tables 7-10 and in Figures 11 and 12. Plasma ACTH concentration and 17-hydroxycorticoid output were elevated at the time of completion of adrenal vein cannulation. The steroid output levels reached in response to the test laparotomy stress 55 minutes after start of the saline infusion into the third ventricle were approximately half those seen at the time of adrenal vein cannulation. From Table 7 it is clear that ACTH concentration did not change with onset of the

test laparotomy although levels at the time were considerably high compared to later values. The ACTH secretory response to laparotomy in the control dogs receiving saline was brisk and marked (Figure 11). The ACTH levels reached in response to laparotomy four hours after constant infusion of dexamethasone was markedly reduced in all animals. Corticoid output response to stress after four hours of constant dexamethasone infusion at the lowest rate was only partially inhibited although the ACTH level was not different from ACTH levels in other groups receiving higher doses of dexamethasone. The corticoid output response to stress in the groups receiving the larger amounts of dexamethasone over four hours were maximally suppressed. Also, despite infusion of saline or dexamethasone for four hours, the adrenals of all animals were capable of secreting steroids as shown by their response to one unit of exogenous ACTH administered intravenously. Corticoid output following exogenous ACTH was not as great, however, as at the time of adrenal vein cannulation. Urquhart (1965) demonstrated that corticoid output in the dog was best correlated with total ACTH (ACTH concentration x blood flow = total ACTH presented to adrenal) reaching the adrenal; however, the fall in corticoid output cannot be explained either by (1) a fall in ACTH concentration since one unit ACTH raised plasma ACTH concentration over 3000 picograms per milliliter; nor by (2) a fall in adrenal venous plasma or blood flow (Tables 9 and 10) since the flow was not significantly decreased after one unit ACTH as compared to flow at the time

of adrenal venous cannulation. Despite this decline in adrenal corticoid output, the ACTH and corticoid output responses of dogs receiving saline for four hours was still significantly greater than the response of dogs receiving dexamethasone. Furthermore, the data also indicates that suppression of the stress response is related to the amount of steroid administered.

Three dogs receiving the largest infusion rate of dexamethasone were subjected to repeated stresses at 1, 3, and 4 hours after onset of the dexamethasone infusion (Tables 7-10 and Figure 12). There was no suppression of the ACTH response to stress at one hour, partial suppression was suggested at three hours, and there was clearly maximal suppression after four hours of constant steroid infusion. Similarly, there was no suppression of the corticoid output response at one hour, partial inhibition was apparent at three hours, and maximal inhibition was again observed after four hours of constant steroid infusion. This data indicate that suppression of the stress response is also related to the time interval elapsing between onset of steroid administration and onset of stress. Furthermore, it appears that four hours of constant steroid administration at the rate of 0.2 milligrams per kilogram per hour is required for maximal suppression of the stress response in the dog.

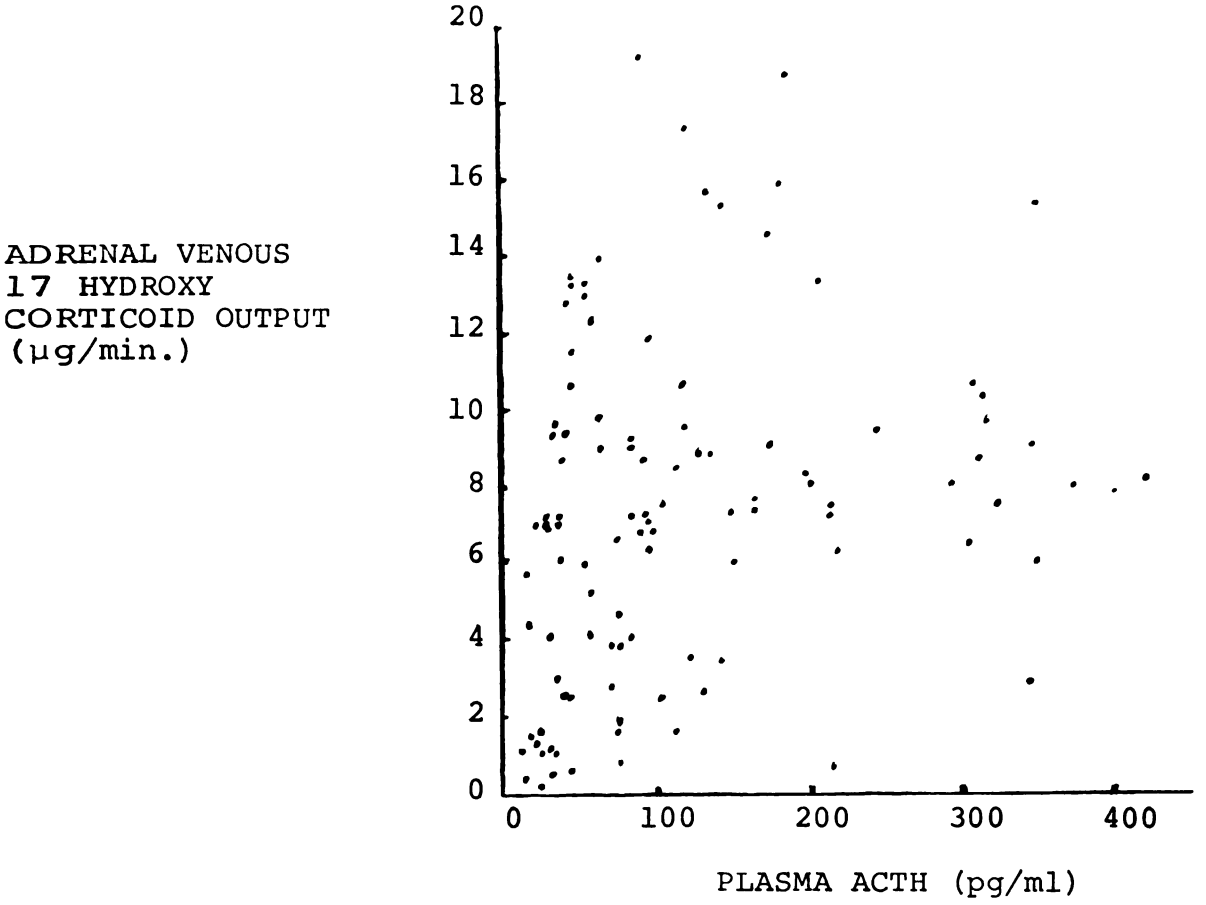


Figure 5.

Scattergram correlating plasma ACTH concentration which occurred after laparotomy stress with adrenal venous 17-hydroxycorticoid output that occurred four minutes later.

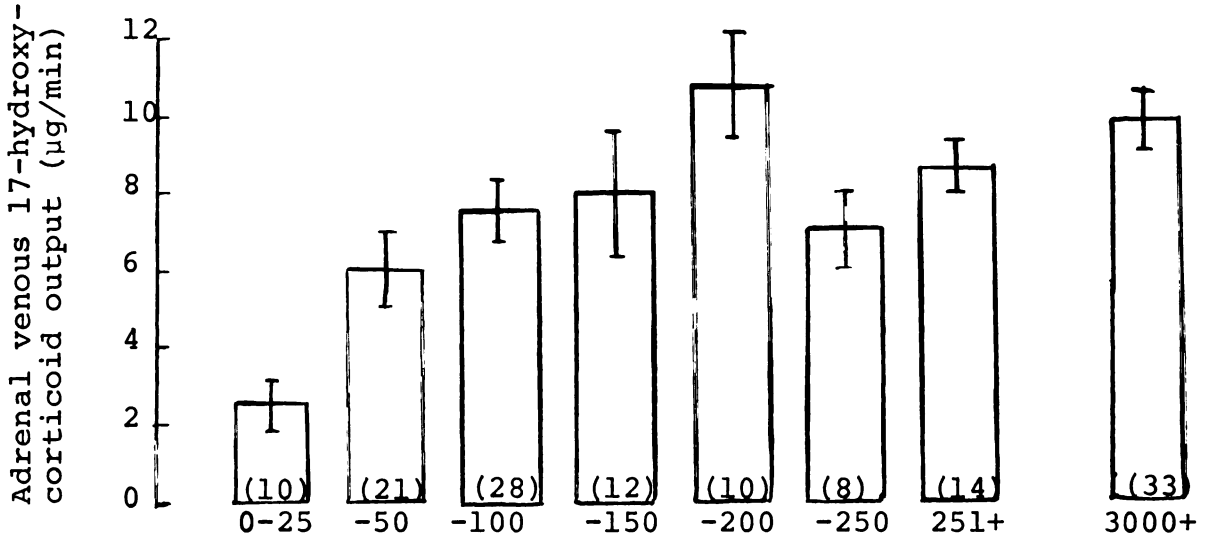


Figure 6.

Histogram correlating plasma ACTH concentration occurring one minute after onset of stress with 17-hydroxycorticoid output that occurred four minutes later. Final bar indicates values obtained following intravenous administration of one unit ACTH. Numbers within bars indicate number of values per indicated range of plasma ACTH concentration.

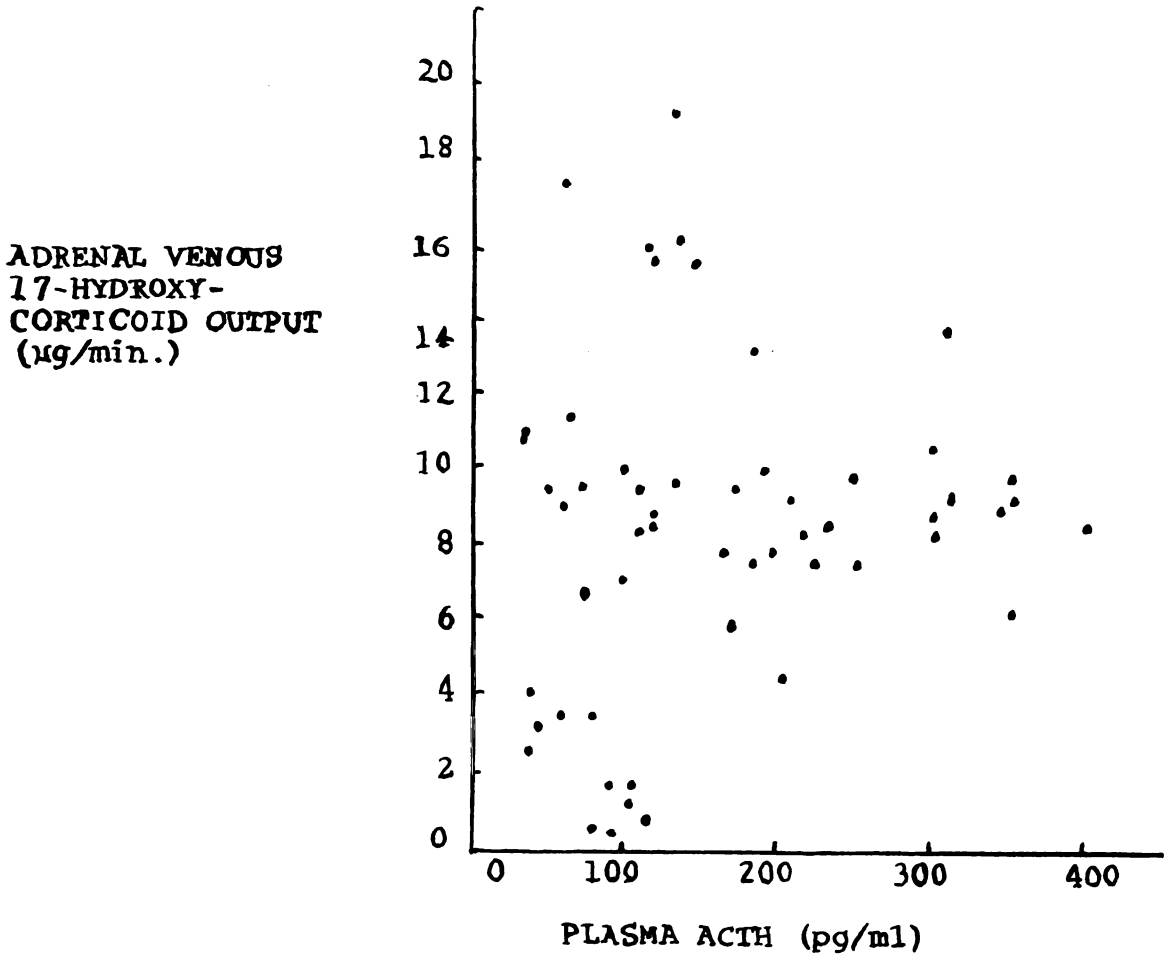


Figure 7.

Scattergram correlating plasma ACTH concentration with adrenal venous 17-hydroxycorticoid output that occurred two minutes later.

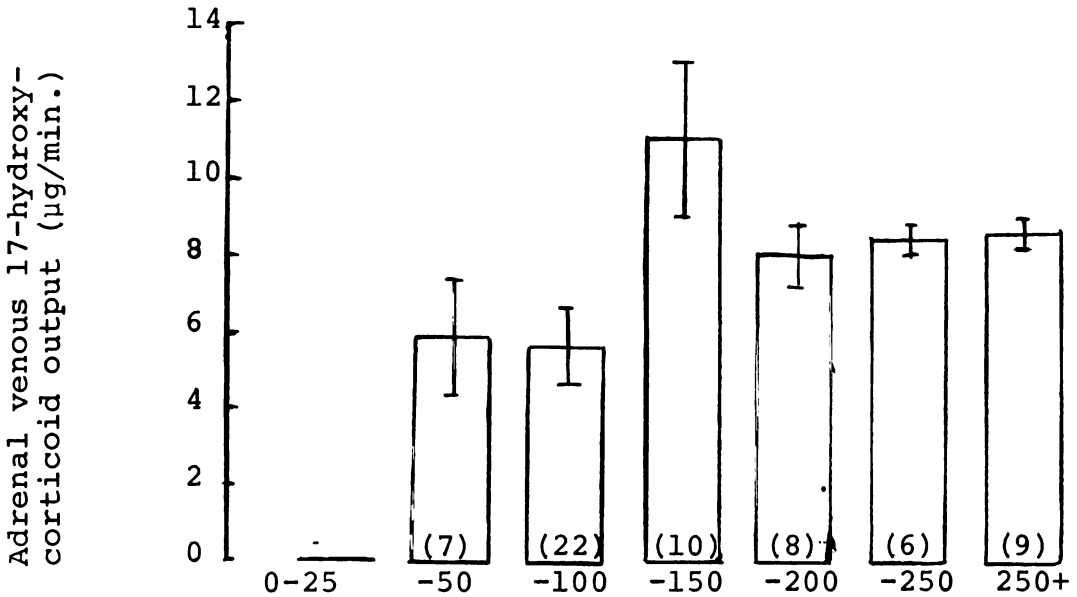


Figure 8.

Histogram correlating plasma ACTH concentration occurring after stress with adrenal venous 17-hydroxycorticoid output that occurred two minutes later. Numbers within bars indicate number of values within indicated range of plasma ACTH concentration

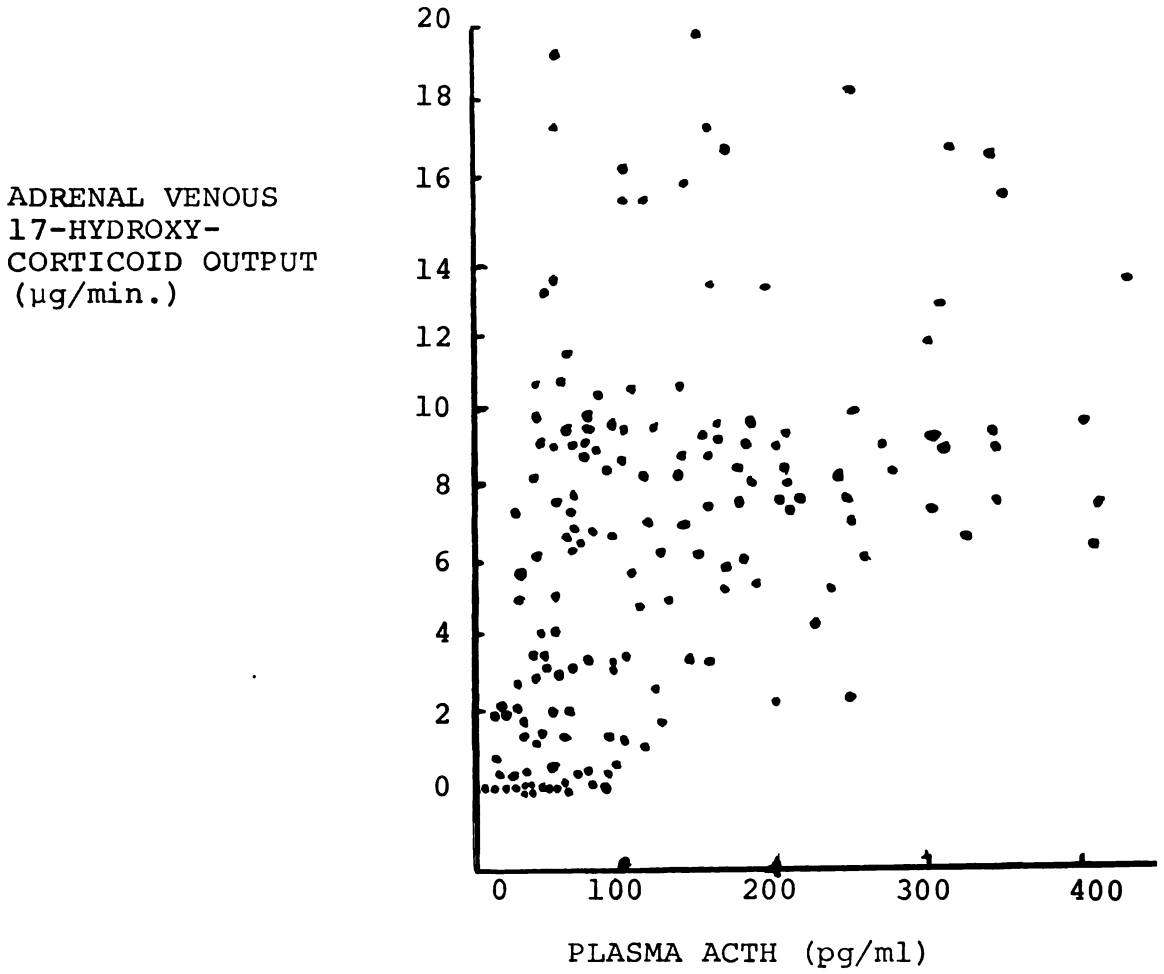


Figure 9.

Scattergram correlating plasma ACTH concentration with adrenal venous 17-hydroxycorticoid output that occurred at the same time.

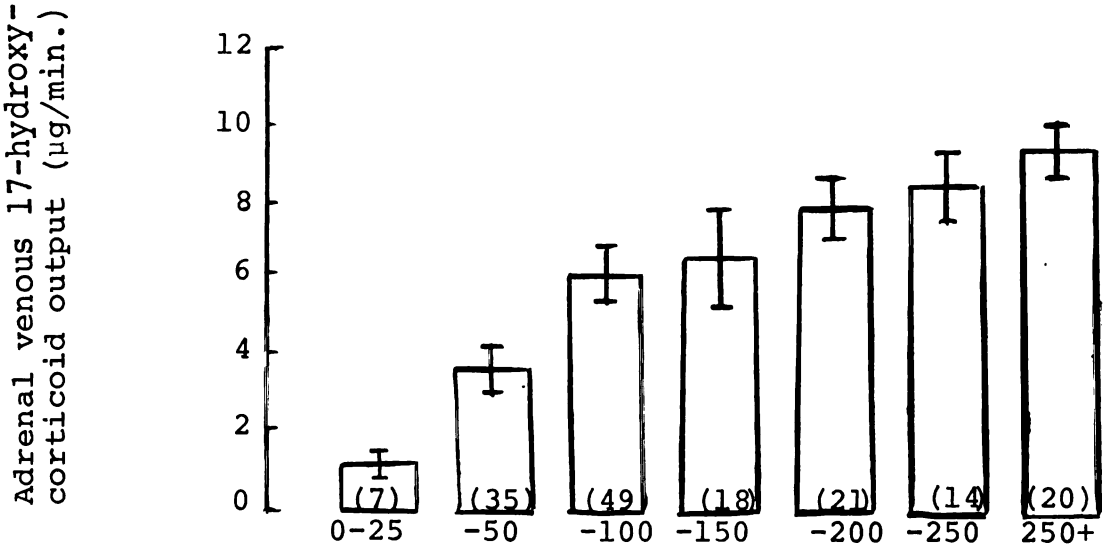


Figure 10.

Histogram correlating plasma ACTH concentration with adrenal venous 17-hydroxycorticoid output that occurred at the same time. Numbers within bars indicate number of values within indicated range of plasma ACTH concentration.

Table 7.

Protocol II: Plasma ACTH concentration in picograms per milliliter

INFUSION TREATMENT	DOG #	WEIGHT (kg)	CANN. STRESS	TEST STRESS PRE:	TEST STRESS POST:	INFUSION: ONE HR. PRE:	INFUSION: RESTRESS POST
1. SALINE CONTROL	206	11.5	163	126	216		
	208	18.6	-	162	97		
	210	14.0	181	-	-		
	211	14.0	178	-	76		
			174±6	144±18	130±44		
2. 0.06 mg/kg/hr†	92	11.0	298	-	62	41	44
	188	13.2	-	118	96		
	189	14.0	252	206	162		
	232	13.4	191	141	133		
	234	17.0	167	125	130		
MEAN ± SEM:			227±30@	129±24	116±17@	41	44
3. 0.1 mg/kg/hr	160	12.5	174	157	96		
	167	22.0	306	46	58		
	183	16.0	435	70	74		
	184	11.0	116	78	80		
	187	15.9	302	130	156		
MEAN ± SEM:			226±56	96±20	92±17		
4. 0.2 mg/kg hr	109	14.6	245	65	-	80	92
	115	20.6	338	68	114	86	218
	116	19.0	226	54	72	56	82
	137	12.2	415	-	151		
	140	11.5	-	-	52		
	155	20.5	80	-	56		
	56	15.0	338	(254)	186		
MEAN ± SEM:			274±48	62±4	105±22	74±16	131±44
TOTAL MEAN ± SEM:			245±23	110±13	105±22		

Cann. = adrenal venous cannulation
† = dexamethasone infusion rate. A loading dose equal to one half the hourly infusion rate preceded onset of infusion.
@ = significantly different from fourth hour post restress value ($p<0.05$)
= significantly different from all other "Total Means" ($p<0.05$)
* = significantly different from control value (Group 1) ($p<0.05$)
Levels of significance determined using Student's t test for paired or unpaired values as appropriate (Klugh , 1970)

Table 7. (cont.)

SALINE OR DEXAMETHASONE THREE HR. RESTRESS PRE:	POST:	FOUR HR. RESTRESS PRE:	POST:	ONE MINUTE AFTER 1 UNIT ACTH I.V.
		240	423	
		162	294	
		48	200	
		62	-	
		128±45	305±65	
31	42	20	20	>10,000
		30	38	>15,000
		49	53	
		34	50	
		33±6*	41±6*	
		-	(317)	
		68	46	>17,000
		20	19	> 9,000
		10	12	> 5,000
		62	20	>12,000
		40±15	24±7*	
28	35	28	35	> 3,000
-	106	82	72	>13,000
88	104	16	(346)	>10,000
		-	19	
		21	15	>15,000
		42	41	>10,000
		30	72	>12,000
58±42	82±23	37±10*	44±9*	

Table 8.

Protocol II: Adrenal venous 17-hydroxycorticoid output in micrograms per minute

INFUSION TREATMENT	DOG #	CANN. STRESS	TEST STRESS PRE:	TEST STRESS POST:	INFUSION: ONE HR. RESTRESS PRE:	INFUSION: ONE HR. RESTRESS POST:
1. SALINE CONTROL	206	13.2	1.9	1.7		
	208	12.2	8.8	7.0		
	210	9.7	6.3	7.4		
	211	6.1	0.2	0.8		
MEAN ± SEM:		10.3±1.6@	4.3±2.0	4.2±1.7@		
2. 0.06 mg/kg/hr	92	11.8	9.0	14.0	9.9	12.8
	188	12.5	8.7	6.4		
	189	9.9	8.7	7.4		
	232	13.3	3.5	8.9		
	234	16.7	2,4	3.6	___	___
MEAN ± SEM:		12.8±1.1@	6.6±1.5	8.1±1.7@*		
3. 0.1 mg/kg/hr	160	8.5	3.4	7.3		
	167	12.6	9.0	12.3		
	183	13.5	0.0	6.6		
	184	8.2	-	9.1		
	187	7.4	5.0	7.3		
MEAN ± SEM:		10.0±1.3@	4.4±1.9	7.6±1.4@		
4. 0.2 mg/kg/hr	109	8.1	0.2	8.1	0.7	8.8
	115	9.4	7.8	8.6	6.6	7.5
	116	4.2	0.7	2.9	2.2	4.1
	137	6.4	4.8	6.1		
	140	3.9	0.0	0.0		
	155	10.3	-	4.2		
	156	16.8	2.5	18.6	___	___
MEAN ± SEM:		8.4±1.7@	2.7±1.3	6.9±2.3@	3.2±1.8	6.8±1.4
		10.2±0.8	4.4±0.8	7.1±1.0**		

Symbols are the same as for Table 7 , unless indicated otherwise

** = value significantly different from cannulation stress value of 10.2±0.8 ($p<0.05$).

Table 8. (cont.)

SALINE OR DEXAMETHASONE THREE HR RESTRESS PRE:	POST:	FOUR HR. RESTRESS PRE:	POST:	ONE MINUTE AFTER 1 UNIT ACTH IV.
		7.7	8.3	6.5
		7.7	8.6	7.4
		3.0	9.6	8.9
		3.0	3.6	4.8
		5.4±1.3	7.5±1.3	6.9±0.9
5.8	7.1			9.0
		2.0	6.5	6.2
		1.3	2.5	6.1
		3.3	6.0	9.3
		0.0	1.1	11.4
		1.7±0.7	4.6±1.2	8.4±1.0
		0.6	0.5	9.3
		2.2	4.6	14.8
		0.4	4.4	9.7
		0.9	1.1	5.5
		0.8	1.6	18.0
		1.0±0.3*	2.0±0.5*	11.5±2.2@
2.9	3.0	0.3	1.6	8.7
6.6	7.7	0.9	1.8	4.8
1.5	2.5	0.7	2.9	4.8
		0.1	0.4	4.9
		0.1	0.2	6.2
		0.4	0.5	8.5
		2.1	3.9	10.6
3.7±1.5	4.4±1.7	0.4±0.1*	1.6±0.5*	6.9±0.9@
				8.2±0.8

Table 8. (cont.)

SALINE OR DEXAMETHASONE				
THREE HR RESTRESS		FOUR HR. RESTRESS		ONE MINUTE AFTER
PRE:	POST:	PRE:	POST:	1 UNIT ACTH IV.
		7.7	8.3	6.5
		7.7	8.6	7.4
		3.0	9.6	8.9
		3.0	3.6	4.8
		5.4±1.3	7.5±1.3	6.9±0.9
5.8	7.1			9.0
		2.0	6.5	6.2
		1.3	2.5	6.1
		3.3	6.0	9.3
		0.0	1.1	11.4
		1.7±0.7	4.6±1.2	8.4±1.0
		0.6	0.5	9.3
		2.2	4.6	14.8
		0.4	4.4	9.7
		0.9	1.1	5.5
		0.8	1.6	18.0
		1.0±0.3*	2.0±0.5*	11.5±2.2@
2.9	3.0	0.3	1.6	8.7
6.6	7.7	0.9	1.8	4.8
1.5	2.5	0.7	2.9	4.8
		0.1	0.4	4.9
		0.1	0.2	6.2
		0.4	0.5	8.5
		2.1	3.9	10.6
3.7±1.5	4.4±1.7	0.4±0.1*	1.6±0.5*	6.9±0.9@
				8.2±0.8

Table 9.

Protocol II: Adrenal venous blood flow over experimental collection period in milliliters per minute.

INFUSION TREATMENT	DOG #	CANN. STRESS	TEST STRESS PRE:	POST:	INFUSION: ONE HR. RESTRESS PRE:	POST:
1. SALINE CONTROL	206	5.6	3.75	4.0		
	208	4.8	4.1	4.5		
	210	5.3	4.2	4.9		
	211	2.35	2.45	2.6		
MEAN ± SEM:		4.5±0.7	3.6±0.4	4.0±0.5		
2. 0.06 mg/kg/hr	92	4.65	4.25	5.15	3.16	3.5
	188	4.4	4.27	4.4		
	189	5.28	3.93	3.77		
	232	3.6	3.65	3.93		
	234	5.95	3.93	5.6	____	___
MEAN ± SEM:		4.8±0.4	4.0±0.1	4.6±0.4@		
3. 0.1 mg/kg/hr	160	1.03	1.58	1.63		
	167	5.27	4.73	5.13		
	183	5.4	6.13	5.8		
	184	4.13	-	4.13		
	187	4.07	4.07	5.12		
MEAN ± SEM:		4.0±0.8	4.1±1.0	4.4±0.7		
4. 0.2 mg/kg/hr	109	5.12	3.0	3.3	3.3	2.6
	115	5.8	3.0	4.8	3.87	3.31
	116	1.49	1.11	1.3	1.65	1.66
	137	5.9	3.45	3.6		
	140	3.95	4.35	4.05		
	155	4.1	-	2.7		
	156	6.3	2.8	5.0		
MEAN ± SEM:		4.7±0.6@	3.0±0.4	3.5±0.5	2.9±0.7	2.5±0.5
TOTAL MEAN ± SEM:		4.5±0.3	3.6±0.3	3.5±0.5**		

Symbols are the same as for TAble 7 , unless otherwise indicated

** = significantly different from Pre-fourth hour restress ($p<0.05$)

Table 9. (cont.)

SALINE OR DEXAMETHASONE				
THREE HR. RESTRESS		FOUR HR RESTRESS		FOUR MINUTES AFTER 1
PRE:	POST:	PRE:	POST:	UNIT ACTH I.V.
		2.5	2.65	2.5
		3.1	3.3	3.5
		0.64	5.4	7.0
		3.1	3.3	5.0
		2.3±0.6	3.7±0.6	4.5±1.0
		-	-	2.96
		3.83	3.8	4.53
		1.74	2.13	4.4
		2.3	2.55	3.45
		3.15	3.2	5.0
		2.8±0.5	2.9±0.4	4.1±0.4[@]
		1.8	1.78	2.67
		3.6	3.25	7.3
		3.5	3.55	5.25
		2.65	2.7	4.33
		1.97	3.53	4.4
		2.7±0.4	3.0±0.3	4.8±0.8[@]
3.55	3.5	3.35	2.84	3.05
2.05	2.15	4.8	6.5	9.3
1.24	1.24	1.08	1.02	2.13
		1.68	3.55	4.25
		1.98	2.05	2.63
		1.25	0.8	3.3
		3.4	4.1	4.73
2.3±0.7	2.3±0.7	2.5±0.5	3.0±0.7	4.2±0.9[@]
		2.6±0.2	3.1±0.3	4.4±0.9

Table 10.

Protocol II: Adrenal venous plasma flow over experimental collection period in milliliters per minute

INFUSION TREATMENT	DOG #	CANN. STRESS	TEST STRESS PRE:	TEST STRESS POST:	INFUSION ONE HR. RESTRESS PRE:	INFUSION ONE HR. RESTRESS POST:
1. SALINE CONTROL	206	3,3	2.1	2.35		
	208	2.4	1.8	2.05		
	210	1.9	1.7	1.9		
	211	1.1	1.05	1.3		
MEAN ± SEM:		2.2 0.4	1.7 0.2	1.9 0.2		
2. 0.06 mg/kg/hr	92	2.35	2.2	2.5	1.48	1.6
	188	2.27	2.13	2.13		
	189	3.2	2.2	2.06		
	232	2.05	1.75	2.07		
	234	3.4	2.7	3.0	____	___
MEAN ± SEM:		2.7±0.3	2.2±0.2	2.4±0.2		
3. 0.1 mg/kg/hr	160	1.0	0.7	0.725		
	167	3.0	2.67	2.93		
	183	2.33	2.6	2.53		
	184	2.27	-	2.27		
	187	2.0	2.26	2.72		
MEAN ± SEM:		2.1±0.3	2.1±0.5	2.2±0.4		
4. 0.2 mg/kg/hr	109	2.2	2.2	1.8	1.75	1.4
	115	2.75	1.35	2.14	1.74	1.37
	116	0.7	0.56	0.53	0.7	0.7
	137	2.8	1.85	1.9		
	140	2.3	2.35	2.3		
	155	2.1	-	1.2		
	156	3.3	1.48	2.73	____	____
MEAN ± SEM:		2.3±0.4	1.6±0.3	1.4±0.3	1.4±0.3	1.2±0.2
TOTAL MEAN ± SEM:		2.4±0.2	1.9±0.1**			

Symbols are the same as for Table 7 unless otherwise indicated.
** = significantly different from Pre-fourth hour restress ($p<0.05$).

Table 10.(cont.)

SALINE OR DEXAMETHASONE THREE HR. RESTRESS PRE:	POST:	FOUR HR. RESTRESS PRE:	POST:	FOUR MINUTES AFTER 1 UNIT ACTH I.V.
		1.3	1.5	1.35
		1.3	1.35	1.5
		2.0	2.0	2.7
		1.5	1.6	2.8
		1.6±0.1	1.7±0.1	2.1±0.3
		1.35	1.25	1.4
		1.2	1.27	1.73
		0.89	1.07	2.33
		1.1	1.2	1.75
		1.55	1.65	2.67
		1.2±0.1	1.3±0.1	1.0±0.2
		0.78	0.7	1.13
		1.85	1.6	3.9
		1.3	1.3	1.9
		1.35	1.35	2.2
		1.07	2.24	4.4
		1.3±0.2	1.4±0.2	2.7±0.6
1.7	1.75	1.6	1.23	1.5
0.95	1.15	1.73	2.3	3.0
0.52	0.46	0.46	0.44	0.95
		0.82	1.95	2.05
		1.08	1.15	1.5
		0.4	0.38	1.5
		1.6	2.15	2.53
1.1±0.3	1.1±0.4	1.2±0.2	1.5±0.3	1.9±0.3
		1.3±0.1	1.4±0.1	

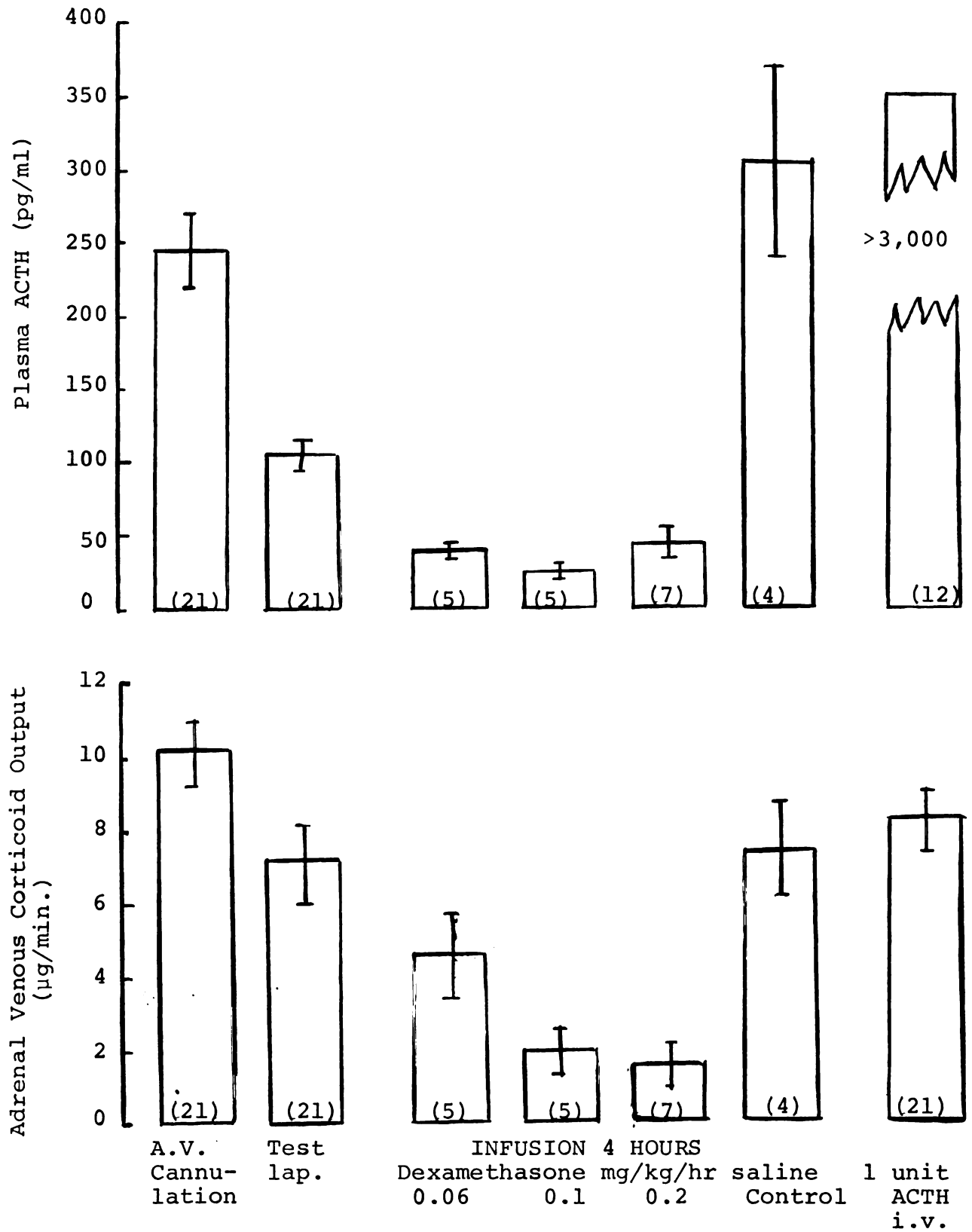

Plasma ACTH (pg/ml)
400
350
300
250
200
150
100
50
0
>3,000
(21)
(21)
(5)
(5)
(7)
(4)
(12)
Adrenal Venous Corticoid Output (µg/min.)
12
10
8
6
4
2
0
(21)
(21)
(5)
(5)
(7)
(4)
(21)
A.V. Cannulation
Test lap.
INFUSION 4 HOURS
Dexamethasone mg/kg/hr
0.06
0.1
0.2
saline Control
1 unit ACTH i.v.

Figure 11.

Figure 11.

Protocol II: Dexamethasone inhibition of stress response. First two bars indicate plasma ACTH concentration and adrenal venous corticoid output noted, respectively, at the time of adrenal vein (A.V.) cannulation and following a "test laparotomy stress'. Next 4 bars show that after 4 hours of constant infusion, different amounts of dexamethasone inhibit the rise in plasma ACTH and corticoid output that normally follows a laparotomy. Final bar indicates levels reached following intravenous administration of ACTH. Means and standard errors are from Tables 7 and 8. Numbers in parentheses indicate number of dogs per group.

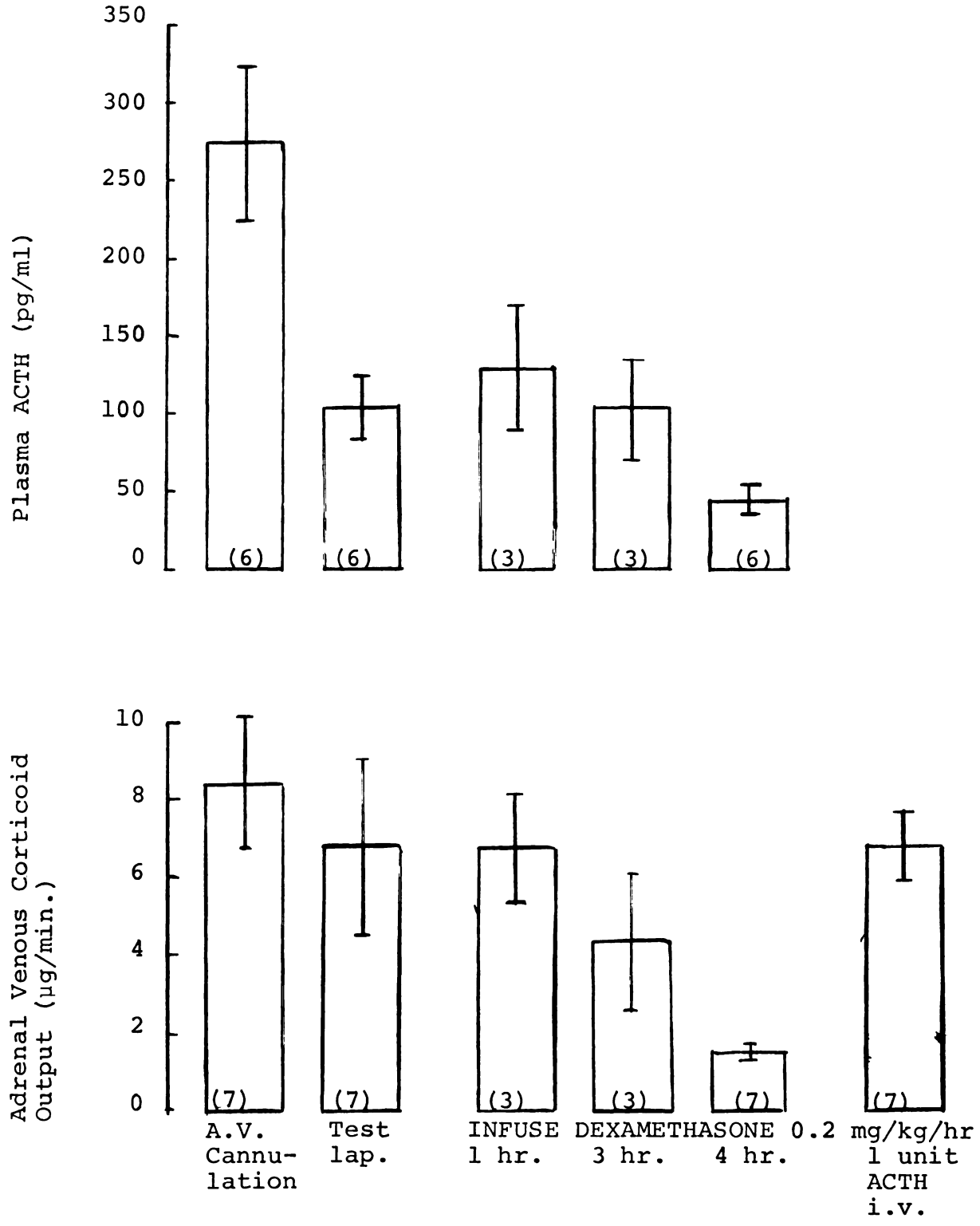

Plasma ACTH (pg/ml)
350
300
250
200
150
100
50
0
(6)
(6)
(3)
(3)
(6)
Adrenal Venous Corticoid Output (µg/min.)
10
8
6
4
2
0
(7)
(7)
(3)
(3)
(7)
(7)
A.V. Cannulation
Test lap.
INFUSE DEXAMETHASONE 0.2 mg/kg/hr
1 hr.
3 hr.
4 hr.
1 unit ACTH i.v.

Figure 12.

Figure 12.

Protocol II. Time course of dexamethasone inhibition. First two bars indicate plasma ACTH concentration and adrenal venous corticoid output noted, respectively, at the time of adrenal vein (A.V.) cannulation and following a "test laparotomy stress". Remaining bars show progressive inhibition of the stress response by dexamethasone when laparotomies were performed one, three, and four hours after start of the infusion. Means and standard error are from Tables 7 and 8. Numbers in parentheses indicate number of values per group.

IV. DISCUSSION

Plasma adrenocorticotropic hormone (ACTH) has never before been measured in the dog by radioimmunoassay although it has been measured in this animal by bioassay (Gold et al., 1963; Hume, 1958; McFarland et al., 1960; Nelson et al., 1955; Redgate, 1967). We found that levels in both the anesthetized and unanesthetized unsurgically stressed male dog (49 ± 6 picograms per milliliter plasma = 5 microunits ACTH per milliliter plasma) (Tables 4-6) were similar to levels determined by both bioassay (Dallman et al., 1972; 0.7 - 1.0 milliunits ACTH per 100 milliliters plasma = 70-100 picograms per milliliter plasma) and radioimmunoassay (Rees et al., 1971; 23-62 picograms per milliliter plasma) for the unanesthetized unstressed intact rat. Similarly low levels of circulating ACTH were found in both anesthetized and unanesthetized nonsurgically stressed humans (Berson et al., 1969; 22 picograms per milliliter plasma = 0.308 milliunits ACTH per 100 milliliters plasma) by radioimmunoassay.

Plasma ACTH concentration in the dog rose significantly within 2 minutes following onset of a laparotomy stress performed over 2 minutes. The rapidity with which ACTH is secreted following stress has been reported previously only for the rat. Farrell and McCann (1952) found that blood ACTH measured by the adrenal ascorbic acid depletion assay rose by one minute after intravenous injection of epinephrine in rats (levels rose from 0.26 milliunits per milliliter

blood to 0.64 milliunits per milliliter = 260-640 picograms per milliliter blood). Sydnor and Sayers (1954) found that ACTH, also measured by the adrenal ascorbic acid depletion method, while undetectable in blood from unstressed male rats, rose to detectable levels (1 milliunit per 100 milliliters blood = 100 picograms per milliliter blood) two minutes after exposure to ether plus scald stress. Hodges and Vernikos (1959), again using the adrenal ascorbic acid depletion method, could not detect resting ACTH levels in female rats, but they found a significant rise in plasma ACTH one minute after onset of ether plus adrenalectomy or sham-adrenalectomy stress which rose to a maximum of 20 milliunits per 100 milliliters blood (= 2000 picograms per milliliter blood) at 2 1/2 minutes after onset of stress. Rees et al. (1971) found that immunoreactive levels of ACTH in the intact rat rose from 23 picograms per milliliter plasma to 1250 picograms 2 1/2 minutes after onset of ether anesthesia. In humans, plasma ACTH concentration was markedly increased over resting levels 5 minutes following electro-shock therapy; however, onset of the rise may have occurred earlier (Yalow et al., 1969).

When comparing values for ACTH concentration, the following points must be kept in mind: (1) ACTH activity is absent from the cellular component of blood (Redgate, 1967); therefore, concentration of ACTH determined per milliliter plasma is approximately twice the concentration determined per milliliter whole blood: (2) using the Third International Reference Preparation of ACTH, the conversion factor for units to grams is

as follows: 100 International Units ACTH = 1 milligram, or 1 microgram = 10 picograms, or 10 picograms = 0.1 milliunits. However, Berson et al. (1968) use the following conversion factor: 140 Units ACTH = 1 milligram, or 20 picograms = 0.28 milliunits.

Following onset of stress in the dog, ACTH levels remain high for at least 20-60 minutes in all dogs studied. This prolonged elevation of ACTH differs from the response in the intact rat subjected to ether plus adrenalectomy of sham-adrenalectomy where plasma ACTH levels for pooled samples determined by bioassay (Dallman et al., 1972; Hodges et al., 1959) return to near prestress levels within 10-20 minutes. The maintained elevation of ACTH concentration in the blood over a period as long as 60 minutes may indicate (1) altered degradation with stress; (2) maintained ACTH secretion by the pituitary; (3) a measurement in the ACTH radioimmunoassay of ACTH fragments which were immunologically but not biologically reactive. One or more of the above must be operative to maintain the high ACTH concentration since the half life of ACTH in the dog, estimating from values in mammals as given in Chart 2 , is probably on the order of 5-10 minutes.

Gemzell et al. (1951) have demonstrated that ACTH is not inactivated by the adrenal since its half life was found to be the same in intact as in adrenalectomized rats. Furthermore, ACTH is known to be inactivated in the blood (Besser et al., 1971). The rate of its degradation (measured by bioassay or radioimmunoassay) in plasma can be reduced by (1) keeping

thawed plasma below 4°C; (2) adding to the plasma either the enzyme inhibitor, trasylol, or the chelating agent, sodiumedetat which binds divalent cations known to be necessary cofactors for enzyme reactions, or: (3) incubating the samples 30 minutes at 56°C, thus suggesting that ACTH is inactivated by enzymatic proteolysis. In addition, since ACTH disappears more rapidly in whole blood than from plasma, it appears that degradation depends in part upon some unidentified component of the cellular fraction of blood (Besser et al., 1971). How this unidentified process or any other involved in ACTH degradation is affected by stress has not been studied. Therefore (although direct evidence for this has not yet been presented), the maintenance of the high ACTH level might be at least in part attributed to altered rate of ACTH degradation during stress.

Evidence that maintained ACTH concentration might indicate maintained pituitary secretion rather than release of ACTH from other storage sites was reported by Gold et al. (1963) who demonstrated that the kidney did not contribute ACTH to the circulation following laparotomy and hemorrhage stress in the dog. Whether the liver or other parts of the body store a pool of ACTH for release during stress is untested but doubtful as the pituitary is unquestionably the major, if not the only source of ACTH in the body.

Since our assay measured the N-terminal portion of the ACTH molecule, and since this is the end which has been shown to contain biological activity, it is not altogether unreasonable

to expect that the ACTH measured in the dog is biologically, in addition to immunologically, reactive. However, since this relationship has not been directly tested, nor definitively shown for dog ACTH using the antibody here employed, it is possible that the observed prolonged elevation of plasma ACTH concentration following stress in these experiments reflects measurement of immunologically but not biologically active ACTH fragments. Evidence which suggest that this may be the case is provided by ACTH and peripheral corticoid data in some dogs (Tables 4-6) where the ACTH level rose in response to stress and remained elevated for various periods of time, while the peripheral plasma corticoid level rose only transiently. However, in contrast, the observation in some dogs that peripheral corticoid levels remained elevated with correspondingly elevated ACTH levels suggests a direct parallel in biologic and immunologic activity in these animals. The failure of peripheral corticoid levels to remain elevated in several animals might result from altered corticoid utilization during stress, although why such a phenomenon should be apparent in only some dogs is unclear. Furthermore, earlier reports indicate that corticoid utilization is decreased, not increased, during stress (Kuipers et al., 1958; Steenburg et al., 1955).

It is well known that glucocorticoids have a variety of functions. They promote mobilization of fat and protein peripherally and promote synthesis of proteins necessary for glyconeogenesis in the liver. In the liver the steroid enters the parenchymal cell nucleus to effect changes in protein synthesis

(Baxter, 1972). Corticoids also have anti-inflammatory activity, maintain vascular reactivity to norepinephrine and epinephrine, influence function of the nervous system in a complex manner, are necessary for normal kidney function, and finally, glucocorticoids are necessary for survival in the face of a variety of stresses, although the precise function in this last regard is still uncertain (Ganong, 1971; Baxter et al., 1972a).

Measurement of adrenal venous corticoid output in addition to measurement of peripheral plasma corticoid levels, should enable one to assess whether stress had altered peripheral corticoid utilization and degradation; however, adrenal venous corticoid output in Protocol I was not measured purposely to avoid subjecting the animals to an initial extended stress.

It appears, therefore, that all three factors discussed above may be important in explaining the prolonged elevation of circulating ACTH seen in many animals.

Figure 13 indicates the general pathway from the stimulation by laparotomy stress to secretion of ACTH and sampling from the femoral artery. When one considers that the total circulation time for the dog is 16 seconds (Prosser et al., 1961), then the circulation time from the pituitary to the sampling cannula in the femoral artery is probably about 8 seconds. In a dog whose plasma ACTH concentration rose dramatically within one minute of onset of stress, approximately 50 seconds remained for the neural stimulus to travel

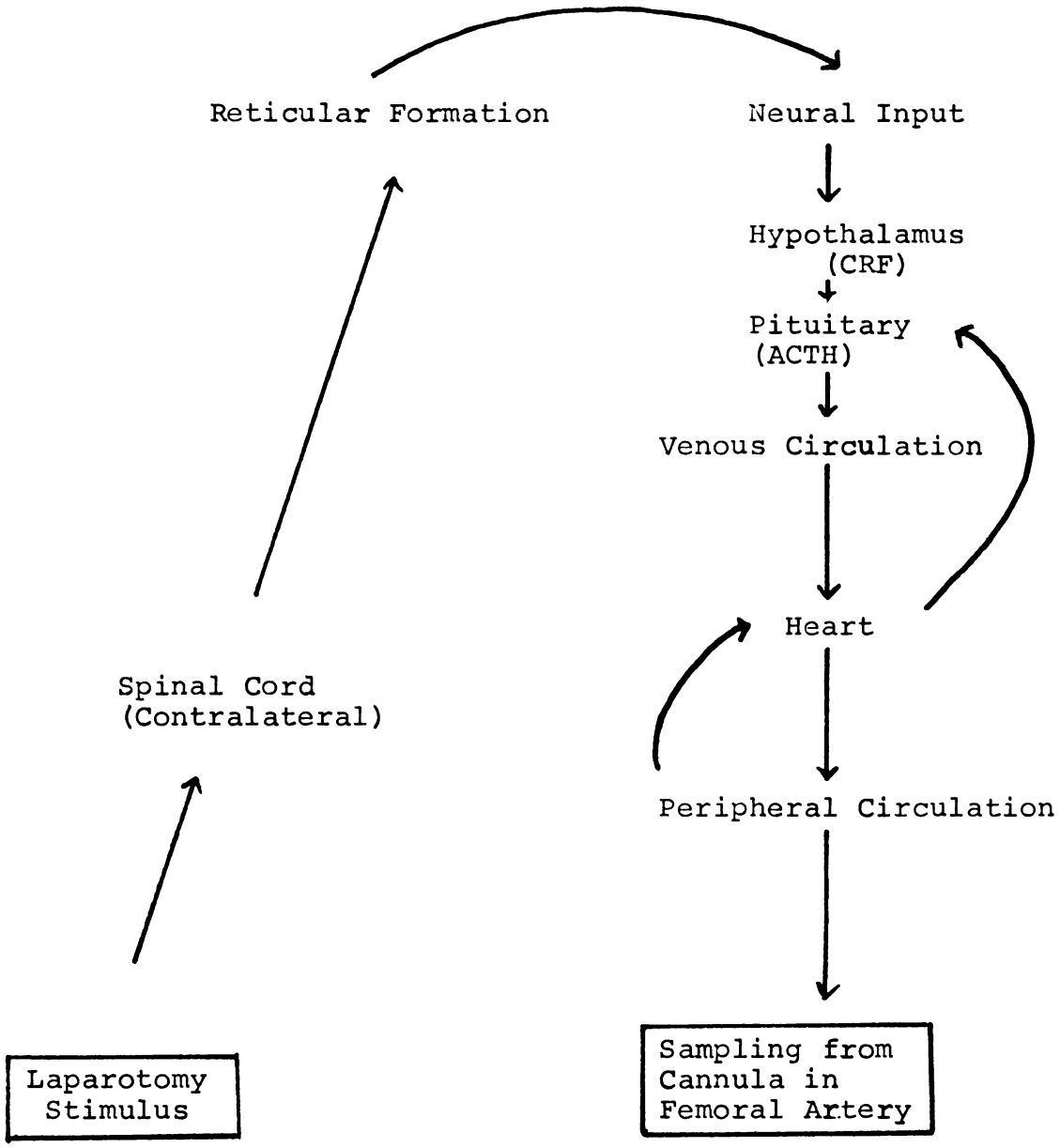


Figure 13.

Neural pathway toward activation of ACTH secretion and subsequent circulatory pattern

to the spinal cord, cross to the contralateral side, ascend through the pontine reticular formation of the hypothalamus (some ipsilateral fibers also reach the hypothalamus) (Greer et al., 1962; Gibbs, 1969a, 1969b) where CRF secretion into the portal vessels is stimulated, resulting in pituitary secretion of ACTH into venous portal blood which returns to the heart and general circulation. Pain fibers which carry the neural information regarding nature of the injury have a conduction velocity of 5-15 meters per second (Guyton, 1966). This means that the initial neural component of the stress response occurs very rapidly and that CRF and ACTH secretion can occur over the greater part of 50 seconds. This neuro-endocrine response to stress is remarkably rapid.

When examining sequential samples for ACTH concentration after stress in one animal, one sometimes finds fluctuations in ACTH concentration (Tables 4-6). If ACTH were secreted constantly one would expect to see a steady rise in plasma ACTH concentration without these fluctuations. However, if ACTH is secreted in pulsatile fashion as has been documented for human release of FSH and LH (Yen et al., 1972) and for ACTH (Berson et al., 1968), then the fluctuations seen in the dog might reflect pulsatile ACTH release from the pituitary. However, in the unanesthetized unstressed dog sampled every 2 1/2 minutes for a period of over 30 minutes, no pulsatile release of ACTH was observed (Dallman, 1973). That ACTH is secreted in pulsatile fashion in the human has been demonstrated by Berson and Yalow (1968) where 8 or more ACTH peaks were seen

over a 24-hour period with one-half hourly sampling frequency. Berson and Yalow attribute the transitory nature of the ACTH peaks primarily to distribution in extravascular spaces rather than to metabolic turnover alone; they argue that the observation that ACTH levels fall to very low levels soon after a peak indicates that immunochemically reactive metabolic products of ACTH do not return to the plasma in significant quantities. Pulsatile release of ACTH in response to the stresses of hypoglycemia, electroshock therapy, and histalog was not observed by Yalow et al. (1969); however their sampling interval in these studies was relatively large (5-10 minutes), and they may have missed this detail. It is also conceivable that different stresses elicit different secretory patterns.
In contrast to the above findings in humans, Dallman and Hane (1973b) have observed fluctuations in plasma ACTH concentration in humans with more frequent sampling (2 1/2-5-minutes intervals following insulin-induced hypoglycemia). Their data suggest that ACTH in the human is released in response to stress as a series of pulses which summate, resulting in an elevated ACTH level. Such oscillation was not observed in the unanesthetized dog whether unstressed or following insulin-induced hypoglycemia (Dallman, 1973).

From a close examination of the data in Tables 4-6 it should be apparent that this pulse phenomenon was certainly not evident for every dog in the present studies. Furthermore, part of the observed variation in ACTH concentrations may be attributed to error within the radioimmunoassay for ACTH. One

must remember also that the probability of observing the pulsatile phenomenon at all is limited since one cannot synchronize sampling with an unknown iscillatory frequency. Nonetheless, the data of Dallman and Hane (1973b) in the human suggest that pulsatile release of ACTH does indeed occur in response to stress in the human, while the data in the anesthetized and unanesthetized dog in these studies as well as in the Dallman (1973) studies strongly suggest that pulsatile secretion of ACTH does not occur in response to stress in the dog. In addition, Dallman suggests that species variation may account for the lack of pulsatile ACTH secretion in the dog as compared to the human.

The correlation between plasma ACTH and 17-hydroxycorticoid output shown in Figure 5-10 clearly shows that more ACTH is secreted in response to moderate to severe surgical stress in dogs than is necessary to maximally stimulate adrenal corticoid output. One might argue that since the ACTH measurements used for this correlation were taken one minute following onset of stress, they may represent "peak" ACTH pulse concentrations which are nonrepresentative of the concentrations effective at the adrenal level. This objection is probably invalid for the following reasons: (1) As already mentioned, plasma ACTH concentration was still rising at one minute following onset of stress in most dogs studied; (2) no conclusive evidence for a pulsatile release of ACTH was discovered in the dog as has just been discussed above.

In dogs, adrenal venous 17-hydroxycorticoid output declines

quite rapidly following hypophysectomy; output begins to fall within 15-30 minutes (Allison 1973) and reaches minimal levels within 2-3 hours (Sweat et al., 1954). In hypophysectomized dogs given 1, 2, or 3 units of ACTH intravenously, 17-hydroxycorticoid output declines within 10-20 minutes (Nelson et al., 1955). Increasing quantities of ACTH administered to hypophysectomized dogs increase adrenal venous corticoid output until a maximum output is reached; greater quantities of ACTH only prolong the duration of maximal corticoid output (Ganong, 1963). In view of these data, the data presented in Tables 4-6, and reasonable estimation of the half life of ACTH in the dog to be less than 10 minutes, it is clear that constant pituitary secretion is required to maintain adrenal corticoid output at high levels for extended periods of time in the intact dog.

Since ACTH is secreted in excess of that amount necessary to maximally stimulate adrenal corticoid output, one wonders if ACTH has any other functions besides prolonging the duration of maximal corticoid output. It is known from studies on isolated perfused dog adrenals, that corticoid output is directly related to the absolute amount of ACTH reaching the adrenal (concentration x blood flow) (Urquhart, 1965), and that a maximal level of corticoid output can be reached and maintained. One might wonder whether secretion of excess ACTH ensures adequate delivery of ACTH to the adrenal for stimulation of corticosteroid secretion during stress. This teliological reasoning is highly unlikely in view of Hume and Nelson's

findings (1954) that blood flow through the adrenal (range of 2-4 milliliters per minute) and minute output of 17-hydroxycorticoids (range of 4-24 micrograms per minute) was maintained in dogs in hemorrhagic shock until arterial blood pressure fell below 40 millimeters mercury and adrenal blood flow fell below 1.6 milliliters per minute. In the experiments reported in this thesis, arterial blood pressure never fell into the range associated with hemorrhagic shock, and almost without exception, adrenal blood flow was well over 1.6 milliliters per minute throughout the entire experiment (Table 9).

The question remains: what function, if any, is served by this excess amount of secreted ACTH? Mulrow et al. (1962) found that in hypophysectomized, nephrectomized dogs, 10 milliunits of ACTH produced maximal output of 17-hydroxycorticoids without affecting aldosterone secretion, while doses of 50, 100 or 1000 milliunits ACTH which were unable to cause a further increase in 17-hydroxycorticoid output, produced significant increases in aldosterone secretion. Others have also reported increases in aldosterone secretion in the dog following administration of ACTH (Farrell et al., 1955; Scian et al., 1959; Greenway et al., 1962).

Based on Hume's report (1958) of a mean value of blood ACTH of 7.4 milliunits per 100 milliliters blood (= 740 picograms per milliliter blood = 1480 picograms per milliliter plasma) in surgically stressed dogs, Ganong and Van Brunt (1968) calculated that 296 milliunits of ACTH would have to be administered to a 10-kilogram dog to elevate blood ACTH

concentration to these levels (7.4 mU/50 ml plasma x 2000 ml extracellular distribution volume in 10 kg dog) assuming a distribution volume of 20 percent body weight. The resulting value is six times greater than the ACTH dose required to stimulate aldosterone secretion in the hypophysectomized dog (Ganong et al., 1968; Mulrow et al., 1962), suggesting that ACTH may play a role in the control of aldosterone secretion.

In reassessing this relationship with the current data, let one assume the distribution volume for ACTH in the dog is 40 percent, as has been reported for the human (Wolf et al., 1965). Plasma ACTH concentration in dogs at the time of adrenal vein cannulation averaged about 300 picograms per milliliter plasma (3 mU/100 ml plasma = 1.5 mU/100 ml blood) (Table 7). This level of circulating ACTH was 2-3 times greater than necessary to elicit maximal adrenal venous corticoid output in these experiments. In order to raise plasma ACTH concentration to this high level in a hypophysectomized 10-kilogram dog, one would have to administer 120 milliunits ACTH (3 mU ACTH/ 100 ml plasma x 40% body weight x 10 kilograms [10,000 milliliters] body weight = 120 mU ACTH), not 298 milliunits as estimated by Ganong et al. (1968). The present value would be roughly one quarter that of the previous Ganong estimate if Ganong had also assumed a 40-percent distribution volume. Otherwise, the basic difference in the two estimates is that the present measurements of circulating ACTH concentration in stressed dogs (300 pg/ml plasma) is about one fifth (1/5th) the value obtained by Hume (1958) by bioassay (1480 pg/ml plasma). Note that this basic

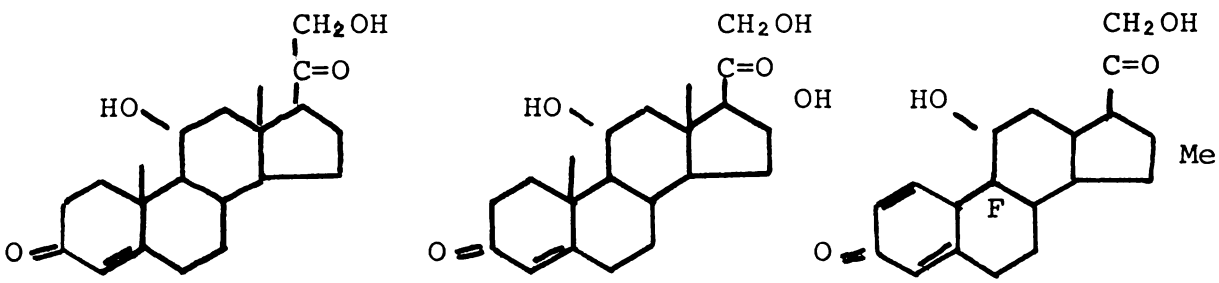
discrepancy suggests strongly that the antibody in the radioimmunoassay for ACTH is certainly not binding to non-ACTH-like nor non-steroidogenic ACTH fragments.

If one decreases the value of 120 milliunits ACTH to 40 or 60 milliunits ACTH to account for the observation that 3 milliunits ACTH per 100 milliliters plasma is really 2-3 times greater than that necessary for maximal stimulation of adrenal venous corticoid output, one arrives at an estimate of the amount of ACTH, which, when administered to a 10-kilogram hypophysectomized dog, just barely elicits maximal adrenal venous corticoid output. This estimate is 4-6 times greater than the minimal quantity of ACTH (10 mU ACTH) shown empirically by Ganong (1963) to just barely elicit maximal 17-hydroxycorticoid output in hypophysectomized dogs. It is highly tenable to suspect that the doses of ACTH given intravenously by Ganong reached and stimulated the adrenal before even distribution of ACTH throughout the normal distribution volume could occur, thus resulting in a falsely low estimate for the amount of ACTH necessary to elicit maximal adrenal venous corticoid output.

Nevertheless, since Mulrow et al. (1962) found that administration of 100 milliunits ACTH intravenously to hypophysectomized, nephrectomized dogs significantly increased aldosterone secretion, it is possible that the maximal ACTH concentrations reached in stressed dogs in the present experiments (300 pg/ml plasma calculated to be equivalent to 120 mU ACTH given intravenously), also significantly stimulated aldosterone secretion. Furthermore, an increase in aldosterone

secretion in surgically stressed dogs has been well documented (Ganong et al., 1959; Davis et al., 1961, Holzbauer et al., 1963). Concentration of circulating angiotensin II, a hormone which stimulates aldosterone secretion, is also elevated in dogs stressed by hemorrhage (Scornik et al., 1964). Thus, although other stimuli such as angiotensin II and changes in blood pressure or fluid electrolytes can increase aldosterone secretion, the present data demonstrate that physiologic levels of ACTH secreted in surgically stressed dogs maximally stimulate adrenal 17-hydroxycorticoid output and in addition may play an important regulatory role in the stimulation of aldosterone secretion.

It is well known that glucocorticoids administered by a variety of routes are capable of suppressing the response of the hypothalamic-pituitary-adrenal axis to a variety of stresses (Chart 3) . Dexamethasone, a synthetic glucocorticoid, cortisol (also called hydrocortisone), and corticosterone are commonly used in the suppression studies.



Corticosterone Cortisol Dexamethasone

Figure 14. Chemical structure of corticosterone, cortisol, and the synthetic glucocorticoid, dexamethasone

Effectiveness of steroid inhibition depends upon the nature of the stress (Dallman et al., 1967; Kendall et al., 1972), its duration or magnitude (Kendall et al., 1972, Sirett et al., 1969), the time interval between steroid administration and onset of stress (Arimura et al., 1969; Sirett et al., 1969; Takebe et al., 1971; Kendall, 1961), method of steroid administration (Sirett et al., 1969) and dose of steroid administered (Dallman et al., 1967; Kendall, 1961).

Boryczka et al. (1971) were able to suppress the adrenal venous 17-hydroxycorticoid output response to laparotomy stress in the dog 4-6 hours after a subcutaneous injection of 0.25-1.0 milligrams dexamethasone per kilogram body weight; suppression 75-100 percent using the response to one unit ACTH as a measure of maximal output. Richards and Pruitt (1956) suppressed the 17-hydroxycorticoid output response to exposure to 20 percent carbon dioxide and to surgical stress of adrenal vein cannulation with an infusion of 1.0-1.25 milligrams hydrocortisone per kilogram body weight beginning one hour before start of the cannulation procedure. Peripheral corticoid levels in their studies rose from control levels of 7 micrograms per 100 milliliters to 48 micrograms per 100 milliliters plasma. Egdahl (1964) was also able to partially suppress the response to adrenal vein cannulation with an infusion of 5 milligrams per hour of dexamethasone phosphate begun one hour before surgery; the "suppressed" response was still significantly above control levels. Some of the variability in Egdahl's results may be attributed to his failure to administer dexamethasone on a

per body weight basis, although one would expect such a large infusion rate to be effective even in larger animals. Egdahl also found that dexamethasone was ineffective in suppressing the 17-hydroxycorticoid output response to hemorrhage (500 ml removed over 20 minutes), endotoxin, and bilaterial decortication. In light of such studies, the ability of glucocorticoids to inhibit the response to stress in the dog has been questioned.

The present studies tested the effectiveness of three different rates of dexamethasone infusion to inhibit the response to laparotomy stress. After four hours of constant steroid infusion, all dose levels were able to suppress the ACTH response measured oneminute after onset of stress, while only the two greater rates of infusion were able to suppress the corticoid output response. This finding suggests that at the lowest rate of infusion, a rise in ACTH preceded the rise in 17-hydroxycorticoid output, although no indication of such a rise was noticeable at the time tested. Administration of dexamethasone may have altered the time course of the secretory response, although plasma ACTH concentration measured at frequent intervals after stress in two dogs which had been receiving an infusion of 0.06 milligrams dexamethasone per hour for 4 hours, did not fluctuate dramatically as shown in Table 11 below. In addition, since only four dogs were in the group, the results depicted in Figure 10 may have been caused by population variance insofar as some animals respond to a given amount of ACTH with a much larger change in adrenal venous corticoid output than others.

Table 11.

Plasma ACTH concentration after stress in two dogs receiving dexamethasone infusion for 4 hours at the rate of 0.06 mg/kg/hr

Minutes after stress	Dog #232-72	Dog #234-72
1	53	29
3	57	57
5	51	31
7	58	29

In these experiments the concentration of dexamethasone in the dog was presumably increasing with time, since the half life of dexamethasone is 200 minutes (3 1/2 hours) measured in the human (Peterson, 1959), and the infusion rate in these experiments more than compensated for this rate of metabolic loss. Kendall et al. (1972) reported a similar condition in their experiments. Despite this complication, the data in this thesis show a dose related suppression of the corticoid output response to stress and a time related suppression of both the pituitary-secretory response and the corticoid output response to laparotomy stress in the dog.

Dependence of corticoid suppression on time was also noted by Kendall et al. (1972). They found that onset of inhibition was a function of the nature and magnitude of the stressful stimulus and that inhibition increased with time starting as early as 1/2 hour after initial dose of dexamethasone. Chart 3 summarizes some experiments and indicates results as a function of a time variable. Von Werder et al. (1971) also found that dexamethasone suppression was a function of time, but

concluded from their experiments a lack of dependence on dose. In their studies, one milligram of dexamethasone was given every 6 hours over a period of 6, 12, or 24 hours ending two hours before insulin induced-hypoglycemia. Since the half life of dexamethasone is 200 minutes (Peterson, 1959), those subjects receiving dexamethasone over the longer periods of time may have had higher steroid levels at the time of the provocative test; thus, it is not clear that suppression is totally independent of dosage in thses studies. Furthermore, Kendall (1961) has clearly shown that in the rat, suppression by dexamethasone of the response to surgical stress is dose-dependent. In addition, Dallman et al. (1967) have claimed that in the rat, the response to a given stress may be only, for example, 50 percent suppressible regardless of increasing amounts of steroids administered. Takebe et al. (1972) have further demonstrated that increasing the amount of dexamethasone over that necessary to produce maximal inhibition in the system will maintain the suppression over longer periods of time. Obviously, if larger amounts of dexamethasone are administered and the half life remains the same, the effects of dexamethasone will be prolonged.

Both the ACTH and corticoid output levels seen at the time of adrenal vein cannulation in the dexamethasone studies (Protocol II) (Tables 7&8,) were higher than those seen one hour later. One reason for this may be the difference in magnitude and duration of the stresses. Adrenal vein cannulation took approximately one hour as compared to the laparotomy stress (flank incision plus intestinal handling) performed over two

minutes. If responses to these stresses can be graded as has been shown for different doses of norepinephrine (Kendall et al., 1972), vasopressin and histamine (Dallman et al., 1967), then it is also possible that the severity of the surgical stress can vary, producing different responses, particularly in this case where the apparent severity of the two procedures is so glaringly different. A comparison of plasma ACTH concentration measured at the time of adrenal vein cannulation (Table 7) with the maximum plasma ACTH concentration observed at (a) two minutes, or (b) any time after the initial two-minute laparotomy stress (Tables 4-6) shows that the responses were significantly different [(a); $p<0.02$; 40 degrees of freedom; (b) $p<0.05$; 41 degrees of freedom], suggesting that the surgical procedures were quantitatively different in terms of the pituitary-secretory response.

Another possible reason for the discrepancy is the difference in timing of the samples since ACTH measurements were made from samples taken one minute following onset of stress, with the exception of the initial samples which were taken at the time of completion of adrenal vein cannulation. It is possible that plasma ACTH concentration continued to rise after one minute following stress, and indeed data from Tables 4-6 shows such a pattern for many animals tested; however, this trend was not consistent in all dogs (Tables 4-6, 11).

These two observations make it clear that one cannot compare plasma ACTH concentration seen at the time of adrenal vein cannulation with the levels reached one minute after onset of stress. However, with the exception of the adrenal vein cannulation

stress, all subsequent stresses were similar and samples for hormone measurements were obtained at comparable times, it is valid to compare responses to the smaller laparotomies.

There remains one other explanation for the discrepancy between ACTH and corticoid output values at the time of adrenal vein cannulation and one hour thereafter. It is conceivable that steroids secreted in response to the stress of cannulation inhibited the response to a subsequent stress. This is likely in view of the findings of Gann et al. (1973) that 17-hydroxy-corticoid output response of the dog to a second hemorrhage stress (10 ml/kg) was reduced as compared to the response to the first hemorrhage of similar magnitude. It is difficult to compare Gann's experimental findings to the present data insofar as time intervals between stresses werenot included in the 1973 abstract. Nevertheless, the results of Kendall et al. (1972) and Dallman et al. (1972) indicate that negative feedback effects can be observed as rapidly as one half or one hour following administration of steroids. However, since Kendall et al. (1972) found that onset of inhibition does not occur for several hours in the face of "severe" stress, it is possible that steroids did not exert an inhibitory feedback effect on the response to surgery in the present experiments. Furthermore, the fact that the response to stress after one hour of infusion of dexamethasone at the highest infusion rate (Tables 7&8, Fig. 12) was the same as the response to the test laparotomy stress one hour earlier, demonstrates that no inhibition was occurring at this time. These results are in opposition to those

of Egdahl (1964) and Richards et al. (1956) who demonstrated suppression of the 17-hydroxycorticoid output response to surgical stress approximately 1 1/2-2 hours after onset of infusion of massive doses of dexamethasone or cortisol. These data suggest that onset of suppression might be dose related in the dog regardless of the severity of the stress.

Therefore, the difference between values for plasma ACTH concentration and 17-hydroxycorticoid output seen at the time of adrenal vein cannulation, and those values observed after the test laparotomy stress are probably due to differences in (1) severity of the stresses; (2) sampling time; and are probably not a result of (3) early onset of negative feedback.

To further test the possibility that steroids secreted endogenously in response to an initial stress could inhibit the response to a subsequent stress, dogs were subjected to two similar laparotomies, each performed over a two-minute period but separated by one or five hours. As seen in Figures 3 and 4, in both groups the second response for both plasma ACTH concentration and peripheral corticoid concentration was not different from the first. The lack of inhibition of the second response in dogs restressed one hour after the initial stress provides further evidence that inhibition does not occur rapidly in the dog. From the dexamethasone inhibition studies (Protocol II) it is clear that constantly elevated levels of glucocorticoids are, however, capable of inhibiting the stress response after four hours. The second response, as measured by plasma ACTH concentration in dogs restressed after five hours,

appears smaller than the first, although the reduction is not statistically significant.

Why is the second response so similar to the first when it has been demonstrated that dexamethasone, after four hours of constant infusion, inhibits a subsequent response? Possibly the amount of steroid secreted in response to the first response was not enough to cause inhibition; some readjustment in the drive to ACTH secretion may have occurred as has been suggested by Dallman et al. (1972, 1973c).

The first explanation is possible since Kendall (1961) found a dose"inhibition-of-response relationship in the rat, and these studies also show such a relationship (Protocol II). Although the present studies included no index of total steroid secreted, peripheral corticoid levels sampled periodically showed that steroid levels following stress were not markedly increased for very long in most animals studied. This condition differed from the artificial and prolonged elevation with dexamethasone, so apparent lack of inhibition of the second stress response especially at five hours may have been due to endogenous steroid levels.

Gann et al. (1973) found that an initial stimulus could leave a facilitatory trace although this could be offset by a negative feedback signal as a function of magnitude of the stimulus and quantity of steroid secreted in response to a previous stress. To determine if a facilitatory trace existed, one could mock the first adrenal response by administration of ACTH and then test whether a subsequent laparotomy, invoking

participation of the nervous system, would provoke a pituitary secretory response and an adrenal response of magnitude or pattern different from that seen following a second physical stress.

V. SUMMARY

In summary, the hypothalamic-pituitary-adrenal axis responds very rapidly to laparotomy stress. Plasma ACTH concentration frequently was above prestress levels within one minute following onset of stress and was significantly elevated within two minutes. Adrenal 17-hydroxycorticoid output begins to increase two minutes after the increase in plasma ACTH concentration (Nelson et al., 1955), and the rise in peripheral corticoid levels paralleled the rise in plasma ACTH concentration with a lag of about four to seven minutes.

In the anesthetized dog subjected to moderate to severe surgical stress, ACTH does not appear to be secreted in pulsatile fashion.

A correlation between plasma ACTH concentration and 17-hydroxycorticoid output four minutes later demonstrates that more ACTH is secreted in response to stress than is necessary to maximally stimulate adrenal secretion of 17-hydroxycorticoids. These elevated levels of ACTH may be responsible for the secretion of aldosterone observed in dogs responding to stress.

Suppression of the ACTH secretory response to stress in the dog is both time- and dose-dependent. Four hours of constant infusion of 0.1 or 0.2 milligrams dexamethasone per kilogram body weight per hour was required to maximally suppress the pituitary secretory response to laparotomy stress in the dog. No suppression of the rise in plasma ACTH concentration was observed one hour after the start of dexamethasone infusion at the rate of 0.2 milligrams per kilogram per hour; partial suppression was observed after three hours, and maximal sup-

pression was clearly evident after four hours. Dexamethasone infused at these rates did not prevent adrenal response to exogenous, and therefore, presumably to endogenous ACTH.

In dogs subjected to two laparotomies one or five hours apart, the second response, reflected by changes in plasma ACTH concentration and peripheral corticoid concentration, was not different from the first in either case. These observations are consistent with the hypothesis of Dallman and Jones (1973c) that there may be a compensatory increase in the drive to ACTH secretion after an initial stress. Furthermore, this compensatory increase in the drive to ACTH secretion may cancel out the inhibitory feedback effects of glucocorticoids secreted in response to the initial stress (Gann et al., 1973).

APPENDIX I

LINEAR REGRESSION AND CORRELATION COEFFICIENT

A computer program for the Hewlett Packard desk top computer was obtained for calculating the straight line of best fit for a set of data points by minimizing the sum of the squares of the deviations of these points. Data points from the "standard curve" were used for this calculation. The program was used to calculate "r", the correlation coefficient, "m", the slope, and "b", the intercept. From any unknown value of " " or " " and from the calculated values of "m" and "b", one could calculate a corresponding value for " " and " ", respectively. The defining equations follow below:

1. $$r = \frac{\sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y})}{\sum_{i=1}^{n} (X_i - \bar{X})^2 \sum_{i=1}^{n} (Y_i - \bar{Y})^2}$$

2. $$m = \frac{\sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y})}{\sum_{i=1}^{n} (X_i - \bar{X})^2}$$

3. $$b = \bar{Y} - m\bar{X}$$

4. $$Y = mX + b \qquad \text{or} \qquad X = \frac{X - b}{m}$$

APPENDIX II

COMPUTER CALCULATION FOR ACTH RADIOIMMUNOASSAY

For computing values for plasma ACTH concentration, radioactivity (counts per minute; cpm) was entered as "Y", the logarithm of nanograms of ACTH in the standard curve was entered as "X", and then "r", "m", and "b" were calculated from the same equations shown in Appendix I. However, in this case, one had to take the antilogarithm of the value of "X" (nanograms ACTH) calculated from "m", "b", and a known "Y" (cpm) in order to convert back from logarithms. This required a slight alteration of the computer program.

BIBLIOGRAPHY

Ader, R. 1970. The effects of early experience on the adrenocortical response to different magnitudes of stimulation. Physiol. Behav. 5:37.

Allison, J. 1973. Personal communication.

Arimura, A., C. Y. Bowers, A. V. Shally, M. Saito and M. C. Miller III. 1969. Effect of corticotropin-releasing factor, dexamethasone and actinomycin D on the release of ACTH from rat pituitaries in vivo and in vitro. Endocrinology 85:300.

Baxter, J. D. and P. H. Forsham. 1972. Tissue effects of glucocorticoids. Am. J. Med. 53:573.

Baxter, J. D., G. G. Rousseau, S. J. Higgins and G. M. Tomkins. 1972. Molecular basis of glucocorticoid response: Specific DNA binding of a steroid-receptor complex. J. Clin. Invest. 51:99.

Baxter, J. D. and G. M. Tomkins. 1970. The relationship between glucocorticoid binding and tyrasine aminotransferase induction in hepatoma tissue culture cells. Proc. Natl. Acad. Sci. 65:709.

Berson, S. A. and R. S. Yalow. 1962. Immunoassay of plasma insulin. Ciba Foundation Colloquia on Endocrinology 14:182.

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

Berson, S. A., R. S. Yalow, A. Bauman, N. A. Rothschild and K. Newerly. 1956. Insulin-^{131}I metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin-treated subjects. J. Clin. Invest. 35:170.

Besser, G. M., D. N. Orth, W. E. Nicholson, R. L. Byyny, K. Abe and J. D. Woodham. 1971. Dissociation of the disappearance of bioactive and radioimmunoactive ACTH from plasma in man. J. Clin. Endocrinol. Metab. 32:595.

Birmingham, M. K. and E. Kurlents. 1958. Inactivation of ACTH by isolated rat adrenals and inhibition of corticoid formation by adrenocortical hormones. Endocrinology 62:47.

Bloom, W. and D. W. Fawcett. 1968. A Textbook of Histology. W. B. Maunders, Co., Philadelphia, p. 466.

Boryczka, A. and W. F. Ganong. 1973. Unpublished.

Bush, I. E. 1953. Species differences in adrenocortical secretion. J. Endocrinol. 9:95.

Cats, A. and A. H. Kassenaar. 1957. The distribution of ^{131}I-labelled corticotropin preparations and proteins in rats after intravenous injection. Acta Endocrinol. (Kbh). 24:35.

Cheifetz, P. N., N. T. Gaffud and J. F. Dingman. 1968. Effects of bilaterial adrenalectomy and continuous light on the circadian rhythm of corticotropin in female rats. Endocrinology 82:1117.

Cheifetz, P. N., N. T. Gaffud and J. F. Dingman. 1969. The effect of lysine vasopressin and hypothalamic extracts on the rate of corticosterone secretion in rats treated with dexamethasone and pentobarbitone. J. Endocrinol. 43:521.

Chowers, I., N. Conforti and S. Feldman. 1967. Effects of corticosteroids on hypothalamic corticotropin-releasing factor and pituitary ACTH content. Neuroendocrinol. 2:193.

Colfer, H. F., J. deGroot and G. W. Harris. 1950. Pituitary gland and blood lymphocytes. J. Physiol. (London). 111:328.

Coutinho, H. B., B. L. Baker and D. J. Ingle. 1953. Effect of continuous injection of epinephrine on adrenal cortex and anterior hypophysis. Proc. Soc. Exptl. Biol. Med. 84:137.

Dallman, M. F. 1973. Unpublished (Dog ACTH data).

Dallman, M. F. and S. Hane. 1973a. Unpublished (ACTH radio-immunoassay).

Dallman, M. F. and S. Hane. 1973b. Unpublished. (Human ACTH data).

Dallman, M. F. and M. T. Jones. 1973c. Corticosteroid feedback control of ACTH secretion: Effect of stress-induced corticosterone secretion on subsequent stress responses in the rat. Endocrinology 92:1367.

Dallman, M. F., M. T. Jones, J. Vernikos-Danellis and W. F. Ganong. 1972. Corticosteroid feedback control of ACTH secretion: Rapid effects of bilateral adrenalectomy on plasma ACTH in the rat. Endocrinology 91:961.

Dallman, M. F. and F. E. Yates. 1967. Anatomical and functional mapping of the central neural input and feedback pathways of the adrenocortical system, in Mem. Soc. Endocrinol. (London) (eds., V. H. T. James and J. Landon). p. 39.

Davis, J. O., E. Anderson, C. C. J. Carpenter, C. R. Ayres, W. Haymaker and W. T. Spence. 1961. Aldosterone and corticosterone secretion following midbrain transection. Am. J. Physiol. 200:437.

Egdahl, R. H. 1964. The acute effects of steroid administration on pituitary adrenal secretion in the dog. J. Clin. Invest. 43:2178.

Eik-nes, K. and L. T. Samuels. 1958. Metabolism of cortisol in normal and "stressed" dogs. Am. J. Physiol. 63:82.

Estep, H., P. Franklin, R. Brown, K. Blaylock and E. Butts, 1967. Increased ACTH release without increased synthesis. Endocrinology 80:719.

Everson, R. A. 1968. Kinetics of ACTH inactivation in the dog. Ph.D. thesis, University of California, San Francisco.

Farrell, G. L. and S. M. McCann. 1952. Detectable amounts of adrenocorticotrophic hormone in blood following epinephrine. Endocrinology 50:74.

Farrell, G. and P. C. Royce. 1955. Secretion of aldosterone by the adrenal of the dog: Effects of hypophysectomy and ACTH. Am. J. Physiol. 182:269.

Fekete, G. and P. Gorog. 1963. The inhibitory action of natural and synthetic corticoids on adrenal steroidogenesis at the adrenal level. J. Endocrinol. 27:123.

Fleischer, N., R. A. Donals and R. W. Butcher. 1969. Involvement of adenosine 3',5'-monophosphate in release of ACTH. Am. J. Physiol. 217:1287.

Fleischer, N. F., G. S. Zimmerman, W. Schindler and M. Hutchins. 1972. Stimulation of adrenocorticotropin (ACTH) and growth hormone (GH) release by oubain; relationship to calcium. Endocrinology 91:1436.

Freeman, S., J. X. Wheeler and H. W. Hoegeneier. 1956. Free reducing and hydrocortisone-like steroids in human plasma. A.M.A. Arch. Intern. Med. 97:45.

Freidgood, H. B. 1970. The nervous control of the anterior hypophysis. J. Reprod. Fertility Suppl. 10:3.

Gann, D. S. and G. L. Cryer. 1973. Physiological feedback and facilitation in the adrenocortical response to hemorrhage. Endocrine Society Abstract.

Gann, D. S., L. E. Ostrander and J. D. Schoeffler. 1966. A finite state model for the control of adrenocorticosteroid secretion, in Systems Approach to Biology, (Mesarovic, M. D. ed.). Springer-Verlag Inc., New York, p. 185.

Ganong, W. F. 1963. The central nervous system and the synthesis and release of ACTH, in Advances in Neuroendocrinology, (Nalbandov ed.). University of Illinois Press, Urbana.

Ganong, W. F. 1971. Review of Medical Physiology, 5th Edition. Lange Medical Publishers, Los Altos, California.

Ganong, W. F., A. H. Lieberman, W. R. J. Daily, Y. S. Yuen, P. J. Mulrow, J. A. Luetscher and R. E. Bailery. 1959. Aldosterone secretion in dogs with hypothalamic lesions. Endocrinology 65:18.

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

Ganong, W. F. and E. E. Van Brunt. 1968. Control of aldosterone secretion in Handbook of Experimental Pharmacology, (H. W. Deane and B. L. Rubin, eds.). Springer-Verlag, Inc., New York, Vol. XIV, Chapter 9.

Garra, A. and G. Cendon de Bay Gorria. 1959. Producion local de anticuerpos. An. Fac. Med. Montevideo 44:544.

Garren, L. D., W. W. Davis, R. M. Crocco and R. L. Ney. 1966. Puromycin analogs: Action of ACTH and the role of glycogen. Science 152:1386.

Gemzell, C. A., D. C. Van Dyke, C. A. Tobia and H. M. Evans. 1951. Increase in the formation and secretion of ACTH following adrenalectomy. Endocrinology 49:325.

Gibbs, F. P. 1969a. Central nervous system lesion that blocks release of ACTH caused by traumatic stress. Am. J. Physiol. 217:78.

Gibbs, P. F. 1969b. Area of pons necessary for traumatic stress induced ACTH release under pentobarbital. Am. J. Physiol. 217:84.

Gold, E. M., W. W. Van Brunt, A. D. Daily, A. T. Boryczka and W. F. Ganong. 1963. ACTH content of dog kidneys. Proc. Soc. Exp. Biol. Med. 112:626.

Golder, M. P. and A. R. Boyns. 1971a. Distribution of (I^{131})-alpha$^{1-24}$ adrenocorticotropin in the intact guinea pig. J. Endocrinol. 49:649.

Golder, M. P. and A. K. Boyns. 1971b. The effect of dexamethasone on the distribution of ^{131}I-labelled 1-24 corticotrophin in the intact guinea pig. J. Endocrinol. 51:v.

Gordon, M. L. 1950. An immediate response of the demedullated adrenal gland to stress. Endocrinology 47:13.

Green, L. 1968. Mechanism of movements of granules in melanocytes of funulus heteroclitus. Proc. Natl. Acad. Sci. USA 59:1179.

Greenspan, F. S., C. H. Li and H. M. Evans. 1950. Disappearance rate of adrenocorticotropin hormone from rats' plasma after intravenous injection. Endocrinology 46:261.

Greenway, C. V. and E. B. Verney. 1962. The effect of adrenocorticotropic hormone on the secretion of corticosteroids by the isolated perfused adrenal gland of the dog. J. Physiol. (London) 162:183.

Greer, M. A., C. F. Allen, F. P. Gibbs and C. Gullickson. 1962. Pathways at the hypothalamic level through which traumatic stress activates ACTH secretion. Endocrinology 86:1404.

Greer, M. A., P. Parker and C. Rocke. 1967. Studies with puromucin on the mechanism of negative feedback inhibition of ACTH secretion by glucocorticoids. Endocrinology 81:14.

Guillemin, R., G. W. Clayton, J. D. Smith and H. S. Lipscomb. 1958. Measurement of free corticosteroids in rat plasma; physiological validation of a method. Endocrinology 63:349.

Guillemin, R., W. R. Hearn, W. R. Cheek and D. E. Householder. 1957. Control of corticotropin release; further studies with in vivo methods. Endocrinology 60:488.

Guyton, A. C. Textbook of Medical Physiology. 3rd edition. W. B. Saunders Co., Philadelphia.

Harwood, C. T. and J. W. Mason. 1956. Effects of intravenous infusion of autonomic agents on peripheral blood 17-hydroxy-corticosteroid levels in the dog. Am. J. Physiol. 186:445.

Hedge, G. A. and P. G. Smelik. 1969. The action of dexamethasone and vasopressin on hypothalamic CRF production and release. Neuroendocrinology 4:242.

Hellman, L., F. Nakada, J. Curti, E. D. Weitzman, J. Kream, H. Roffwarg, S. Ellman, D. K. Fufushima and T. F. Gallagher. 1970. Cortisol is secreted episodically by normal man. J. Clin. Endocrinol. Metab. 30:411.

Hiroshige, T. and M. Sakakura. 1971. Circadian rhythm of corticotropin-releasing activity in the hypothalamus of normal and adrenalectomized rats. Neuroendocrinology 7:25.

Hodges, J. R. and J. Vernikos. 1959. Circulating corticotropin in normal and adrenalectomized rats after stress. Acta Endocrinol. (Kbh.) 30:118.

Holzbauer, M. 1963. Aldosterone secretion during operative stress in relation to release of ACTH. J. Physiol. (London) 168:40.

Hume, D. M. 1952. The relationship of the hypothalamus to the pituitary secretion of ACTH. Ciba Foundation Colloquia on Endocrinology 4:87.

Hume, D. M. 1958. Hypothalamic localization of the control of various endocrine secretions, in Reticular Formation of the Brain (eds., J. H. Jasper, L. D. Proctor, R. S. Knighton, W. C. Nishay, R. T. Castello) p. 111-142, Little Brown Co., Inc., Boston.

Hume, D. M. and W. F. Ganong. 1956. A method for accurate placement of electrodes in the hypothalamus of the dog. Electroencephalogr. Clin. Neurophysiol. 8:136.

Hume, D. M. and D. H. Nelson. 1955. Effect of hypothalamic lesions on blood ACTH levels and 17-hydroxycorticoid secretion following trauma in the dog. J. Clin. Endocrinol. Metab. 15:839.

Hume, D. M. and D. H. Nelson. 1955. Adrenal cortical function in surgical shock. Surg. Forum, Proc. 40th Congr. Am. Coll. Surgeons. 1954 Vol. 5, p. 568. W. B. Saunders Co., Philadelphia.

Hume, D. M. and G. J. Wittenstein. 1950. Proc. First Clinical ACTH Conference 134. Blakiston Co., Philadelphia.

Hunter, W. C. and F. C. Greenwood. 1962. Preparation of iodine-^{131} labelled human growth hormone of high specific activity. Nature (London) 194:495.

Ifft, J. D. 1956-7. Further evidence for an "internal" feedback from the adenohypophusis to the hypothalamus. Neuroendocrinology 1:350.

Imura, H., L. L. Sparks, G. M. Grodsky and P. H. Forsham. 1965. Immunologic studies of adrenocorticotropic hormone (ACTH): dissociation of biologic and immunologic activity. J. Clin. Endocrinol. Metab. 25:1361.

Jutisz, M. and M. P. de la Llosa. 1970. Requirement of Ca^{++} and Mg^{++} ions for the in vitro release of follicle-stimulating hormone from rat pituitary glands and its subsequent biosynthesis. Endocrinology 86:761.

Keller, N., S. R. Sendelback, V. I. Richardson, C. Moore and F. E. Yates. 1966. Protein binding of corticosteroids in undiluted rat plasma. Endocrinology 79:884.

Kendall, J. W. 1961. Quantitative and temporal studies on effect of dexamethasone on corticosterone secretion in the rat. Proc. Soc. Exp. Biol. Med. 107:926.

Kendall, J. W. 1971. Feedback control of adrenocorticoptropic hormone secretion, in Frontiers in Neuroendocrinology. (eds., L. Martini, W. F. Ganong) Oxford University Press, New York, Chapter 7.

Kendall, J. W., M. L. Egans, A. K. Stott, P. M. Kramer and J. J. Jacobs. 1972. The importance of stimulus intensity and duration of steroid administration in suppression of stress-induced ACTH secretion. Endocrinology 90:525.

Kitay, J. I., D. A. Holub and J. W. Jailer. 1959. Inhibition of pituitary ACTH release; an extraadrenal action of exogenous ACTH. Endocrinology 64:474.

Klugh, H. E. Statistics: The Essentials for Research. John Wiley and Sons, Inc., New York. 1970.

Kraicer, J., J. V. Millingan, J. L. Gosbee, R. G. Conrad and C. M. Branson. 1960. In vitro release of ACTH: effects of potassium, calcium and corticosterone. Endocrinology 85:144.

Kuipers, F., R. S. Ely and V. C. Kelley. 1958. Metabolism of steroids; the removal of exogenous 17-hydroxycorticosterone from the peripheral circulation in dogs. Endocrinology 62:64.

L'age, M., A. Gonzales-Luque, and F. E. Yates. 1969. Inhibition by dexamethasone of CRF-induced ACTH release following intrapituitary injections of CRF in conscious dogs. Physiologist 12:280.

Lacy, P. E., S. Howell, D. A. Young and C. J. Fink. 1968. New hypothesis of insulin secretion. Nature (London) 219:187.

Landon, J. 1968. Basis of immunoassay with particular reference to adrenocorticotrophic hormone, in Recent Advances in Endocrinology. (eds., V. H. T. Landon, J. R. de Mowbray).

Landon, J., R. C. Greenwood. 1968. Homologous radioimmunoassay for plasma levels of corticotropin in man. Lancet 1:273.

Liddle, G. W., D. Island, A. P. Rinfret and P. H. Forsham. 1954. Factors enhancing the response of the human adrenal to corticotropin; is there an adrenal growth factor? J. Clin. Endocrinol. Metab. 14:839.

MacLeod, E. and H. Fonthan. 1970. Influence of ionic environment on the in vitro synthesis and release of pituitary hormones. Endocrinology 86:863.

McCann, S. M. 1971. Mechanism of action of hypothalamic-hypophseal stimulation and inhibiting hormones, in Frontiers in Neuroendocrinology (eds., L. Martini and W. F. Ganong) Chapter 8, pp. 209-236.

McEwen, B. S., J. M. Weiss and L. S. Schwartz. 1969. Uptake of corticosterone by rat brain and its concentration by certain limbic structures. Brain Res. 16:227.

McEwen, B. S., J. M. Weiss and L. S. Schwartz. 1970. Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rats. Brain Res. 17:471.

McFarland, L. Z., M. T. Cleff and W. F. Ganong. 1960. Concentration of ACTH in cavernous sinus and peripheral blood collected from unanesthetized sheep. Proc. Soc. Exp. Biol. Med. 103:438.

McHugh, R. P., and G. P. Smith. 1967. Negative feedback in adrenocortical response to limbic stimulation in macaca mulatta. Am. J. Physiol. 213:1445.

Matsukura, S. 1967. Folia Endocrinol., Japan. 43:1219.

Matsuyama, H., A. Ruhmann-Wennhold and D. H. Nelson. 1970. Biologic and immunologic similarities between rat and human adrenocorticotropin (ACTH). Endocrinology 87:756.

Matsuyama, H., A. Ruhmann-Wennhold, L. R. Johnson and D. H. Nelson. 1972. Disappearance rates of exogenous and endogenous ACTH from rat plasma measured by bioassay and radioimmunoassay. Metabolism 21:30.

Meakin, J. W., J. E. Bethune, R. H. Despointes and D. H. Nelson. 1959. The rate of disappearance of ACTH activity in the blood of humans. J. Clin. Endocrinol. Metab. 19:1491.

Meulheims, G. H., F. E. Francis and R. A. Kinselle, Jr. 1969. Suppression of the hypothalamic-pituitary-adrenal axis in the newborn dog. Endocrinology 85:265.

Miller, M. E. Anatomy of the Dog. W. B. Saunders Co., Philadelphia. pp. 401-402.

Motta, M., G. Mangili and L. Martini. 1965. A short feedback loop in the control of ACTH secretion. Endocrinology 77:392.

Mulrow, P. J., W. F. Ganong, G. Sera and A. Kuljian. 1962. The nature of the aldosterone-stimulating factor in dog kidney. J. Clin. Invest. 41:505.

Murphy, B. E. P. 1967. Some studies of the protein binding of steroids and their application to the routine micro and ultra-micro measurements of various steroids in body fluids by competative protein binding radioassays. J. Clin. Endocrinol. Metab. 27:973.

Murphy, S. S., R. A. Donald and J.D.N. Jabarro. 1969. The half-life of porcine corticotropin in pigs. Acta Endocrinol. (Kbh) 61:525.

Nelson, D. H. and D. M. Hume. 1955. Corticosteroid secretion in the adrenal venous blood of hypophysectomized dogs as an assay for ACTH. Endocrinology 57:184.

Nelson, D. H., H. Reich and L. T. Samuels. 1959. Isolation of a steroid hormone from the adrenal vein blood of dogs. Science 111:578.

Ney, R. L., W. W. Davis and L. D. Garren. 1966. Heterogeneity of template RNA in adrenal glands. Science 153:896.

Ney, R. L., E. Ogata, N. Shinizu, W. E. Nicholson and G. W. Liddle. 1964. Structure-function relationships of ACTH and MSH analogs. Excerpa Med. Int. Cong. Ser. 83:1184.

Nugent, C. A., H.R. Warner, V. L. Estergreen and K. Eiknes. 1964. The distribution and disposal of cortisol in humans. Proc. 2nd Internatl. Cong. Endocrinol., London, 83:257.

Ohtsuka, Y.,H. Ishikawa, T., Watanabe and F. Yoshimura. 1972. ACTH synthesizing and releasing activities of adenohypophyseal acidophils differentiating from the isolated chromophobes in a chemically defined medium supplemented with CRF. Endocrinol., Japan. 19:237.

Orth, D. N. and D. P. Osland. 1969. Light synchronization of the circadian rhythm in plasma cortisol (17-OHCS) concentration in man. J. Clin. Endocrinol. Metab. 29:479.

Orth, D. N., D. P. Osland and G. W. Liddle. 1967. Experimental alteration of the circadian rhythm in plasma cortisol (17-OHCS) concentration in man. J. Clin. Endocrinol. Metab. 27:549.

Peterson, R. E. 1959. Metabolism of adrenocorticosteroids in man. Ann. N. Y. Acad. Sci. 82:846.

Porter, J. C., R. S. Mical, P. R. Tippit and J. W. Drane. 1970. Effect of selective surgical interruption of the anterior pituitary's blood supply on ACTH release. Endocrinology 86:590.

Porter, C. C., R. H. Silber. 1950. A quantitative color reaction for cortisone and related 17, 21, dihydroxy, 20-ketosteroids. J. Biol. Chem. 185:201.

Prosser, C. and F. A. Brown, Jr. 1961. Comparative Animal Physiology. 2nd edition. W. B. Saunders Co., Philadelphia. p.396.

Rasmussen, H. and A. Tenenhouse. 1968. Cyclic adensoine monophosphate, calcium and membranes. Proc. Natl. Acad. Sci. USA 59:1364.

Rauschkolb, W. E., R. E. Rosnagel and G. L. Farrell. 1954. Secretion of 17-hydroxycorticosterone by adrenals of hypophysectomized dogs: Effect of ACTH. Proc. Soc. Exp. Biol. Med. 86:785.

Recant, D., D. W. Hume, P. H. Forsham and G. W. Thorn. 1950. Studies on the effect of epinephrine on the pituitary-adrenocortical system. J. Clin. Endocrinol. Metab. 10:187.

Redgate, E. S. 1967. Comparison of plasma corticotropin concentration in intact and adrenalectomized dogs and cats during hemorrhage. Endocrinology 80:741.

Rees, L. H., D. M. Cook, J. W. Kendall, C. F. Allen, R. H. Kramer J. G. Ratcliffe and R. A. Knight. 1971. A radioimmunoassay for rat plasma ACTH. Endocrinology 89:254.

Richards, J. B. and R. L. Pruitt. 1956. Hydrocortisone suppression of stress-induced adrenal 17-hydroxycorticosteroid secretion in dogs. Endocrinology 60:99.

Richards, J. B. and G. Sayers. 1951. Fate and excretion of adrenocorticotropic hormone. Proc. Soc. Exp. Biol. Med. 77:87.

Robinson, G. A., R. W. Butcher and E. W. Sutherland. 1971. Cyclic AMP and steroidogenesis,in Cyclic AMP. Academic Press, New York. Chapter 9, pp. 317-337.

Ruf, K. and A. Steiner. 1967. Steroid-sensitive single neurons in the rat hypothalamus and midbrain: identification by microelectrophoresis. Science 156:667.

Salcman, M., L.Peck and R. H. Egdahl. 1970. Effect of acute and prolonged electrical stimulation of the amygdala of the dog upon peripheral plasma concentration of corticosteroids. Neuroendocrinology 6:361.

Salmi, M. H. and I. I. Geschwind. 1968. Some effects of energy transfer inhibitors and of calcium-free or potassium-enhanced media on the release of luteinizing hormone (LH) from the rat pituitary gland in vitro. Endocrinology 82:225.

Samuels, L. T. Dynamics of steroid hormone distribution in the body particularly the distribution of cortisol, in Steroid Dynamics, (eds., G. Pincus, T. Nakai and J. P. Tait). Academic Press, New York. p. 385, 1966.

Sandgerg, A. A., W. R. Slaunewhite, Jr. 1963. Transcortin: a corticosteroid binding protein of plasma. V. In vitro inhibition of cortisol metabolism. J. Clin. Invest. 42:51.

Sawyer, C. W., M. Kawakami, B. Meuerson, D. I. Whitmoyer and K.K. Lilley. 1968. Effects of ACTH, dexamethasone, and asphyxia on electrical activity of the rat hypothalamus. Brain Res. 10:213.

Sayers, G., T. W. Burns, F. H. Tyler, B. V. Jager, T. B. S. Schwartz, E. L. Samuels and H. W. Davenport. 1949. Metabolic actions and fate of intravenously administered adrenocorticotropic hormone in man. J. Clin. Endocrinol. Metab. 9:593.

Sayers, M. A., G. Sayers, and L. A. Woodbury. 1948. The assay of adrenocorticotropin hormone by the adrenal ascorbic acid depletion method. Endocrinology 42:379.

Schally, A. V., R. N. Anderson, H. S. Lipscomb and R. Guillemin. 1960. Evidence for the existence of 2 corticotrophin-releasing factors, alpha and beta. Nature (London) 188:1192.

Scian, L. F., C. D. Westerman, O. R. Cruesi and J. G. Jilton. 1959. Effect of ACTH and vasopressin on aldosterone secretion. Federation Proc. 18:545.

Scornik, O. A. and A. C.Paladine. 1964. Angiotensin blood levels in dogs with experimental hypertension. Am. J. Physiol. 201:526.

Seiden, G. and A. Brodish. 1971. Physiologic evidence for "short-loop" feedback of ACTH on hypothalamic CRF. Neuro-endocrinology 8:154.

Seiden, G. and A. Brodish. 1972. Persistence of a diurnal rhythm in hypothalamic corticotrophin-releasing factor (CRF) in the absence of hormonal feedback. Endocrinology 90:1401.

Settlage, R. 1970. Quantitative analysis of serum proteins during treatment with oral contraceptive steroids. Contraception 1:101.

Silber, R. H. and C. C. Porter. 1954. The determination of 17, 21-dihydroxy-ketosteroids in urine and plasma. J. Biol. Chem. 210:923.

Sirett, N. E. and F. P. Gibbs. 1969. Dexamethasone suppression of ACTH release: effect of the interval between steroid administration and the application of stimuli known to release ACTH. Endocrinology 85:355.

Slaunwhite, W. R., Jr., G. N. Lockie, N. Back and A. A. Sandberg. 1962. Inactivity in vivo of transcortin-bound cortisol. Science 135:1062.

Slusher, M. A. 1964. Effects of chronic hypothalamic lesions on diurnal and stress corticosteroid levels. Am. J. Physiol. 206:1161.

Smelik, P. G. 1963. Failure to inhibit corticotrophin secretion by experimentally induced increases in corticoid levels. Acta Endocrinol. (Kbh) 44:36.

Sonnenberg, M., A. S. Keston, W. L. Money. 1951. Studies with labelled anterior pituitary preparations of adrenocorticotropin. Endocrinology 48:148.

Steenburg, R. W.and W. F. Ganong. 1955. Observations on the influence of extra-adrenal factors on circulating 17-hydroxycorticoids in the surgically stressed, adrenalectomized animal. Surgery 38:92.

Steenburg, R. W., L. L. Smith, W. C. Schoemaker and F. D. Moore. 1960. Observations on the role of liver hydrocortisone metabolism. Surg. Gynecol. Obstet. 111:697.

Stevens, W., B. I. Grosser and D. J. Reed. 1971. Corticosterone-binding molecules in rat brain cytosol: regional distribution. Brain Res. 35:602.

Sweat, M. L. and G. L. Farrell. 1954. Decline of corticosteroid secretion following hypophysectomy. Proc. Soc. Exp. Biol. Med. 87:615.

Sydnor, K. L. and G. Sayers. 1953. Biological halflife of endogenous ACTH. Proc. Soc. Exp. Biol. Med. 83:729.

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

Takebe, K., H. Kunita, M. Sakakura, Y. Horiuchi and K. Mashimo. 1971. Suppressive effect of dexamethasone on the rise of CRF activity in the median eminence induced by stress. Endocrinology 89:1014.

Thomasson, B. and R. W. Steenburg. 1965. Plasma clearance of cortisol and 11-deoxycortisol in dogs. Am. J. Physiol. 208:84.

Urquhart, J. 1965. Adrenal blood flow and adrenocortical response to corticotropin. Am. J. Physiol. 209:1162.

Vale, W. and R. Guillemin. 1967. Potassium-induced stimulation of thyrotropin release in vitro: requirement for presence of calcium and inhibition by thyroxin. Experientia 32:885.

Vernikos-Danellis, J. 1965. Effect of rat median emminence extract on pituitary ACTH content in normal and adrenal-ectomized rats. Endocrinology 76:240.

Vogt, M. 1952. Plasma adrenaline and release of ACTH in normal and demedullated rats. J. Physiol. (London) 118:588.

Voight, K. H., H. L. Fehm and E. F. Pfeiffer. 1971. Evidence for an ACTH binding protein in plasma. Horm. Metab. Res. 3:227.

Von Werder, K., S. Hane and P. H. Forsham. 1971. Suppression of the hypothalamic-pituitary-adrenal axis and growth hormone release with dexamethasone. Horm. Metab. Res. 3:171.

Wakabayashi, K. I., I. A. Kamberi and S. M. McCann. 1969. In vitro response of the rat pituitary to gonadotropin-releasing factors and to ions. Endocrinology 85:1046.

Wakabayashi, K. I., H. P. G. Schneider, S. Watanabe, D. B. Creighton, A. P. S. Dhariwal and S. M. McCann. 1968. Studies on the mechanism of action of the gonadotropin-releasing factors in the pituitary. Federation Proc. Abstract 27:269.

Wakabayashi, K. I.and B. I. Tamoaki. 1966. Biosynthesis of luteinizing hormone in the anterior pituitary. Protein (Tokyo) 11:1369.

Willoughby, H. W., C. Chen and S. Freeman. 1959. The metabolism of corticosterone-2-C^{14} in dogs. Endocrinology 65:539.

Wolf, R. L., N. Nendlowitz, L. J. Soffer and S. E. Gitlow. 1965. Metabolism of corticotropin in man. Proc. Soc. Exp. Biol. Med. 119:244.

Yalow, R. S., N. Varsano-Aharon, E. Echemendia and S. S. Berson. 1969. HGH and ACTH secretory responses to stress. Horm. Metab. Res. 1:3.

Yates, F. E. and R. D. Brennan. Study of the mammalian adrenocorticoid system by computer simulation. 1968.

Yen, S. S. C., C. C. Tsai, F. Naftolin, G. Vandenberg and L. A. Jabor. 1972. Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. J. Clin. Endocrinol. Metab. 34:671.